



2<sup>nd</sup> International  
Symposium on  
**Genomics of Plant  
Genetic Resources**

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*Final Program & Abstracts*

Bologna, Italy  
24-27 April 2010



AN EVENT

PASTATREND

THE GREAT PASTA EXHIBITION – 24-27 APRIL 2010 - BOLOGNA, ITALY



# Welcome to



## 2<sup>nd</sup> International Symposium on **Genomics of Plant Genetic Resources**

We are delighted and honored to welcome you to the 2<sup>nd</sup> International Symposium on Genomics of Plant Genetic Resources (GPGR2). The main theme of this 2<sup>nd</sup> edition will be "*Harnessing plant biodiversity for food security and nutritional quality*". We hope that this and the future GPGR congresses will provide a platform for presenting and debating critical issues and strategies relevant for the use of genetic resources to improve the well-being of humankind and the sustainability of agricultural practices.

Delegates representing 53 countries from all continents will evaluate and discuss how to best use genomics to harness the potential of plant genetic resources. In recent years, a truly impressive number of advances in genetics and genomics have greatly enhanced our understanding of the structural and functional aspects of plant genomes. These advances have led to new screening methods to select superior genotypes more efficiently and improve the decision-making process for more efficient breeding strategies. At the same time, the demand of agricultural production has changed in a dramatic way. World food production is being challenged by global climate change and a fast-increasing demand for food, feed, fiber and biofuel.

This symposium is particularly timely considering that 2010 has been declared as the "International Year of Biodiversity" by the United Nations. Never before has the importance of effectively harnessing the potential of plant biodiversity been more evident and urgent. More importantly, it has been estimated that food production will need to be doubled by 2050 in order to adequately feed mankind. Clearly, genomics research on plant genetic resources and genomics-assisted breeding have great potential to revolutionize world agriculture in both developed and developing countries.

We have made every effort to create a comfortable and hospitable environment for you at this conference. We hope that you will enjoy this conference and your stay in Bologna.

We wish to dedicate this Symposium to the memory of Norman Borlaug and Mike Gale.

The GPGR2 Congress Organizers

Emile Frison (Congress Chair), Bioversity International, Rome, Italy  
Andreas Graner (Congress Vice-Chair), IPK-Gatersleben, Germany  
Roberto Tuberosa (Congress Local Organizer), University of Bologna, Italy



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# *Many thanks to...*

The Congress Organizers wish to thank all the members of the Congress Committees for their help in the organization of this symposium.

The Congress organizers wish to thank Silvana Tamassia and Kay Stuart for their help in managing the abstracts and preparing this volume.

Many thanks to Patrizia Tazza for designing the GPGR2 logo.

As the local organizer, Roberto Tuberosa would also like to thank his colleagues at DISTA for taking over part of his responsibilities and duties during the preparation of this congress.



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# *Program at a Glance*





## 2<sup>nd</sup> Symposium on Genomics of Plant Genetic Resources

(24-27 April 2010, Bologna, Italy)

### Program at a glance

#### Saturday, April 24

08:30-09:45. Registration and poster mounting

09:45-12:00. Session 1: Opening session

12:00-13:00. Opening ceremony

*13:00-14:15. Lunch & Posters viewing*

14:15-18:45. Session 2: Harnessing plant diversity: From sequence to function

19:00-19:45. Congress keynote presentation

*20:00-21:30. Opening reception with local food and wine tasting; Posters viewing*

#### Sunday, April 25

08:45-13:00. Session 3. Harnessing plant diversity: Genomics-based applications

*13:00-14:15. Lunch, Posters viewing & LemnaTec presentation*

14:15-17:50. Session 4. Genomics of Triticeae genetic resources (COST Action FA0604 Tritigen)

18:00-19:30. Round table on "Educating a new generation of plant breeders"

#### Monday, April 26

08:45-13:00. Session 5. Genomics-assisted crop improvement for food security in developing countries

13:00-14:30. Lunch & Posters viewing

14:30-18:50. Session 6. Genomics for increased sustainability of Triticeae crops production (COST Action FA0604 Tritigen)

*20:00-22:30. Social dinner*

#### Tuesday, April 27

08:45-13:15. Session 7. Genetic resources for nutritional quality

*13:15-14:30. Lunch & Posters viewing*

14:30-16:40. Session 8. The next challenges

16:40- 18:20. Session 9. Closing session



# *Detailed Program*



Scientific program of  
2<sup>nd</sup> Symposium on Genomics of Plant Genetic Resources (GPGR2)  
24-27 April 2010, Palazzo dei Congressi, BolognaFiere, Bologna, Italy

Saturday, April 24

08:30-09:45. Registration and poster mounting

09:45-12:00. Session 1: Opening session

*Co-Chairs: Emile Frison (Bioversity International, Rome, Italy) and Roberto Tuberosa (University of Bologna, Italy)*

09:45-10:15. Welcome addresses

10:15-10:45. Opening keynote presentation

*Emile Frison (Bioversity International, Rome, Italy). Building a global plant genetic resources system*

10:45-12:00. Invited presentations

10:45-11:10. *Orlando de Ponti (International Seed Federation, Nyon, Switzerland). Germplasm exploitation and ownership: Who owns what?*

11:10-11:35. *Michele Morgante (University of Udine, Italy). From genomics to breeding: How to make the best use of genetic resources*

11:35-12:00. *Jerome Salse (UMR INRA-UBP, Clermont-Ferrand, France). Comparative genomics as a tool for trait dissection*

12:00-13:00. Opening ceremony

13:00-14:15. Lunch & Posters viewing

14:15-16:15. Session 2: Harnessing plant diversity: From sequence to function

*Co-Chairs: Suk-Ha Lee (Seoul National University, Korea) and Stefania Grillo (CNR, Italy)*

14:15-15:30. Invited presentations

14:15-14:40. *Suk-Ha Lee (Seoul National University, Seoul, Korea). Sequencing wild soybean genome reveals soybean crop domestication history*

14:40-15:05. *Riccardo Velasco (IASMA, San Michele all'Adige, Italy). Through the genome sequence: The origin of the cultivated apple*

15:05-15:30. *Scott Jackson (Purdue University, Lafayette, IN, USA). Genomic exploration of diversity in the genus *Glycine* (soybean) and related crop legumes*

15:30-16:15. Selected presentations

15:30-15:45. *Bicheng Yang (BGI-Shenzhen, Shenzhen, China). Whole genome resequencing for crop improvement*

15:45-16:00. *Sonia Negrão* (ITQB/IBET, Oeiras, Portugal). Use of EcoTILLING to identify natural allelic variants of rice candidate genes involved in salinity tolerance

16:00-16:15. *Tanveer ul Haq* (College of Agriculture, Dera Ghazi Khan, Pakistan). Spatial and temporal quantitative trait loci (QTLs) mapping of ions accumulation on chromosome-1 of rice (*Oryza sativa* L.) under salt stress

**16:15-16:45. Coffee break**

**16:45-18:45. Session 2: Harnessing plant diversity: From sequence to function**

*Co-Chairs: Masahiro Yano (NIAS, Japan) and Enrico Pe' (Scuola Superiore Sant'Anna, Italy)*

**16:45-18:00. Invited presentations**

16:45-17:10. *Masahiro Yano* (NIAS, Tsukuba, Japan). Genomics-assisted allele mining and its integration to rice breeding

17:10-17:35. *Richard Flavell* (CERES, Red Oaks, CA, USA). Creating new diversity using synthetic transgenes to improve crops

17:35-18:00. *Martin Ganal* (TraitGenetics, Gatersleben, Germany). The 50K maize Infinium chip - design and use for maize diversity analysis

**18:00-18:45. Selected presentations**

18:00-18:15. *Michael Deyholos* (University of Alberta, Edmonton, Canada). Whole-genome shotgun sequence assembly and other genomic resources for flax (*Linum usitatissimum*)

18:15-18:30. *Robert Henry* (Southern Cross University, Lismore, Australia). Whole-genome sequencing as a tool for plant genetic resource analysis

18:30-18:45. *Bhupendra Chaudhary* (Gautam Buddha University, Greater Noida, India). Expression partitioning of homoeologs during development and evolution of allopolyploid cotton (*Gossypium*)

**19:00-19:45. Congress keynote presentation**

*Gebisa Ejeta* (Purdue University, Lafayette, IN, USA). The sustenance of human livelihood via genetics and genetic resources

**20:00-21:30. Opening reception with local food and wine tasting; Posters viewing**

## Sunday, April 25

### 08:45-10:40. Session 3. Harnessing plant diversity: Genomics-based applications

*Co-Chairs: Jizeng Jia (CAAS, Beijing, China) and Antonio Blanco (University of Bari, Italy)*

#### 08:45-10:00. Invited presentations

08:45-09:10. *Jean Christophe Glaszmann* (CIRAD, Montpellier, France). Multiple scale analysis of genetic diversity in sorghum

09:10-09:35. *Albrecht Melchinger* (University of Hohenheim, Stuttgart, Germany). Genomics approaches to unravel the genetic basis of heterosis

09:35-10:00. *Richard Visser* (University of Wageningen, The Netherlands). Exploiting genetic resources for plant breeding in the 21<sup>st</sup> century

#### 10:00-10:45. Selected presentations

10:00-10:15. *Andrew Flavell* (University of Dundee, Dundee, UK). The genetic diversity and evolution of field pea (*Pisum*) studied by high-throughput retrotransposon-based insertion polymorphism (RBIP) marker analysis

10:15-10:30. *Jun Zou* (Huazhong Agricultural University, Wuhan, China). Impacts of interspecific hybridization on genome structure and agronomic traits via retrotransposon activation in *Brassica napus*

10:30-10:45. *Huaan Yang* (Department of Agriculture and Food Western Australia, South Perth, Australia). Development of molecular markers for large scale implementation for marker-assisted selection in lupin breeding

#### 10:45-11:15. Coffee break

### 11:10-13:00. Session 3. Harnessing plant diversity: Genomics-based applications

*Co-Chairs: Milena Ouzunova (KWS, Germany) and Elisabetta Frascaroli (University of Bologna, Italy)*

#### 11:10-12:00. Invited presentations

11:10-11:35. *Antoni Rafalski* (DuPont Agricultural Biotechnology Group and Pioneer Hi-Bred International, Wilmington, USA). Genomic tools for the analysis of genetic diversity

11:35-12:00. *Stefano Tartarini* (University of Bologna, Italy). Toward the fine mapping of Plum Pox Virus (Sharka) resistance QTLs from two different sources in apricot

#### 12:00-13:00. Selected presentations

12:00-12:15. *Pietro Piffanelli* (PTP, Lodi, Italy). EURIGEN: Characterization of European rice germplasm for stress response traits

12:15-12:30. *Le Hung Linh* (Agricultural Genetics Institute, Tuliem-Hanoi, Vietnam). Mapping QTL for spikelets per panicle and yield components using an NIL from an interspecific cross between *Oryza sativa* and *O. minuta*

12:30-12:45. *Silvio Salvi* (IASMA, San Michele all'Adige, Italy). LD estimation, analyses of diversity and domestication in apple

12:45-13:00. *Francis Ogbonnaya* (ICARDA, Aleppo, Syria). Recent progress in the utilisation of synthetics for wheat improvement

### 13:00-14:15. Lunch, Posters viewing & LemnaTec presentation (13:00-13:20; *Joerg Vandenhirtz*)

**14:15-16:00. Session 4. Genomics of Triticeae genetic resources (with the support of COST Action FA0604 Tritigen WG 1)**

*Co-chairs: Ikmet Budak (Sabanci University, Turkey) and Luigi Cattivelli (CRA, Italy)*

**14:15-15:30. Invited presentations**

14:15-14:40. *Karl Schmid* (University of Hohenheim, Germany). Towards natural selection mapping of useful genes in plant germplasm

14:40-15:05. *Alan H. Schulman* (University of Helsinki, Finland). Mining Triticeae genes with the aid of *Brachypodium*

15:05-15:30. *Eduard Akhunov* (Kansas State University, Manhattan, USA). Genome-wide patterns of SNP variation in polyploid wheat

**15:30-16:00. Selected presentations**

15:30-15:45. *Shiveta Sharma* (Christian-Albrechts University of Kiel, Germany). Mapping of QTL contributing to root lesion nematode resistance in barley

15:45-16:00. *Andreas Börner* (IPK, Gatersleben, Germany). Association mapping of wheat germplasm employing historical data

**16.00-16.30. Coffee break**

**16:30-17:50. Session 4. Genomics of Triticeae genetic resources (with the support of COST Action FA0604 Tritigen WG 1)**

*Co-chairs: Robbie Waugh (SCRI, UK) and Patrizia Galeffi (ENEA, Italy)*

**16:30-17:20. Invited presentations**

16:30-16:55. *Takao Komatsuda* (NIAS, Tsukuba, Japan). Functional diversification of transcription factors for the formation of barley spikes

16:55-17:20. *Robbie Waugh* (SCRI, Dundee, UK). Manipulating the resolution of whole genome association scans in barley using stratified germplasm collections

**17:20-17:50. Selected presentations**

17:20-17:35. *Song Weining* (Northwest A&F University, Yangling, Shaanxi, China) One site vs. the world: The genetic diversity of one natural population of wild barley compared to barley varieties from different countries

17:35-17:50. *Fedor Konovalov* (Vavilov Institute of General Genetics, Moscow, Russia). SSAP-based phylogenetic analysis reveals different amplification history of *BARE-1* and *Jel1* LTR retrotransposon families in A-genome diploid wheats

**18:00-19:30. Round table on "Educating a new generation of plant breeders"**

Moderator: *Chike Mba* (FAO, Rome, Italy)

Speakers: *Zoltan Bedo* (EUCARPIA), *Léon Broers* (KWS, Germany), *Sam Eathington* (Monsanto, USA), *Gebisa Ejeta* (Purdue University, USA), *Albrecht Melchinger* (Univ. of Hohenheim, Germany), *Alain Murigneux* (Limagrain, France), *Richard Visser* (Plant Research Institute, The Netherlands)



## Monday, April 26

08:45-10:40. Session 5. Genomics-assisted crop improvement for food security in developing countries  
*Co-chairs: Chike Mba (FAO, Rome, Italy) and Enrico Porceddu (University of Tuscia, Italy)*

### 08:45-09:40. Invited presentations

08:45-09:00. *Enrico Porceddu* (University of Tuscia, Viterbo, Italy). A doctoral program to harness the value of genetic resources in developing countries

09:00-09:15. *Edward Runge* (Texas A&M University, USA). The Monsanto's Beachell-Borlaug Initiative for food security in developing countries

09:15-09:40. *Sarah Davidson* (Cornell University, Ithaca, NY, USA). The Borlaug Global Rust Initiative: Reducing the genetic vulnerability of wheat to rust

### 09:40-10:40. Selected presentations

09:40-09:55. *Ahmed El Oualkadi* (INRA, Equipe «Diversité, Génétique et Génomique Vigne», Montpellier, France). Genetics resources of *Vitis vinifera* in the north of Morocco (Rif region): Characterization and conservation

09:55-10:10. *Yinghua Huang* (USDA-ARS Plant Science Research Laboratory, Stillwater, OK, USA) Toward safeguarding sorghum production against biotic stresses: Uses of worldwide germplasm collection and genomics-based approaches

10:10-10:25. *Zerihun Tadele* (University of Bern, Switzerland). Genomic tools for improving the cereal crop tef (*Eragrostis tef*)

10:25-10:40. *Alganesh Tesema Gellaw* (Institute of Biodiversity Conservation, Addis Ababa, Ethiopia). Molecular diversity in Ethiopian tetraploid wheat

### 10:40-11:10. Coffee break

11:10-13:00. Session 5. Genomics-assisted crop improvement for food security in developing countries  
*Co-chairs: David Bergvinson (Bill & Melinda Gates Foundation, USA) and Roberto Papa (University of Ancona, Italy)*

### 11:10-12:00. Invited presentations

11:10-11:35. *David Bergvinson* (The Bill & Melinda Gates Foundation, Seattle, USA). Leveraging plant genetic resources for Africa

11:35-12:00. *Rajeev Varshney* (ICRISAT, Hyderabad, India). Establishing genomics-assisted breeding foundation in chickpea for enhancing crop productivity in Africa and Asia

### 12:00-13:00. Selected presentations

12:00-12:15. *Khaled Masmoudi* (Plant Molecular Genetic Laboratory, Sfax, Tunisia). Ions transport activity related to plant adaptation to salt stress

12:15-12:30. *Fahriye Ertugrul* (TUBITAK Marmara Research Center, Kocaeli, Turkey). Use of expressed sequence tag resources to reveal multiplex quantitative gene expression profiles in diploid and polyploid wheat genotypes for salt stress tolerance

12:30-12:45. *Sundeep Kumar* (Sardar Vallabh Bhai Patel University of Agriculture and Technology, Modipuram, India). Molecular and pathological characterization of slow-rusting genes against leaf rust in wheat (*Triticum aestivum* L. em Thell)

12:45-13:00. *Rattan Yadav* (IBERS, Aberystwyth University, United Kingdom). Progress and prospects of increasing drought tolerance in pearl millet using genetics and genomics approaches.

13:00-14:30. Lunch & Posters viewing

14:30-16:30. Session 6. Genomics for increased sustainability of Triticeae crops production (with the support of COST Action FA0604 Tritigen WG 1)

*Co-chairs: Alan Schulman (University of Helsinki, Finland) and Nicola Pecchioni (University of Modena and Reggio Emilia, Italy)*

**14:30-15:45. Invited presentations**

14:30-14:55. *Jizeng Jia* (CAAS, Beijing, China). Core collection-based genomic stocks in wheat

14:55-15:20. *Peter Langridge* (ACPFPG and University of Adelaide, Australia). Genetic resources and genomic approaches to improve abiotic stress tolerance in cereals

15:20-15:45. *Tzion Fahima* (University of Haifa, Israel). Wild emmer wheat genetic resources: From genetic diversity to map-based cloning for increased sustainability of Triticeae crops

**15:45-16:30. Selected presentations**

15:45-16:00. *Nicola Pecchioni* (University of Modena and Reggio Emilia, Italy). Dissection of quantitative resistance to leaf rust (*Puccinia brachypodii*) in *Brachypodium distachyon*, the model plant for Triticeae

16:00-16:15. *Melda Kantar* (Sabanci University, Istanbul, Turkey). Identification and quantification of barley miRNAs and their targets in response to drought stress

16:15-16:30. *Stuart Lucas* (Sabanci University, Istanbul, Turkey). Subtleties of the wheat drought response displayed by a DRE-binding protein from *Triticum dicoccoides*

16:30-17:00. Coffee break

17:00-18:50. Session 6. Genomics for increased sustainability of Triticeae crops production (with the support of COST Action FA0604 Tritigen WG 1)

*Co-chairs: Beat Keller (University of Zurich, Switzerland) and Valeria Terzi (CRA, Italy)*

**17:00-17:50. Invited presentations**

17:00-17:25. *Beat Keller* (University of Zurich, Switzerland). Allelic diversity of fungal disease resistance genes in wheat

17:25-17:50. *Patrick Schweizer* (IPK, Gatersleben, Germany). Converging evidence for genes of basal defense in barley

**17:50-19:05. Selected presentations**

17:50-18:05. *Aaron Fait* (Ben-Gurion University of the Negev, Boqer Campus, Israel). Exploitation of diversity in nuclear-cytoplasm interaction using alloplasmic wheat lines

18:05-18:20. *Ciro De Pace* (University of Tuscia, Viterbo, Italy). Deployment of either a whole or dissected wild nuclear genome into the wheat gene pool meets the breeding challenges posed by the sustainable farming systems

18:20-18:35. *André Laroche* (Agriculture and Agri-Food Canada, Research Centre, Lethbridge, Canada). NextGen sequencing of the transcriptome of triticale

18:35-18:50. *Marco Maccaferri* (University of Bologna, Italy). Identification of agronomically valuable alleles in durum wheat through linkage and association mapping

18:50-19:05. *Enrico Francia* (University of Modena and Reggio Emilia, Italy). Towards physical mapping and sequencing the *FrH2* (*Frost resistance-H2*) region of barley chromosome 5H

20:00-22:30. Social dinner

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## Tuesday, April 27

### 08:45-10:50. Session 7. Genetic resources for nutritional quality

*Co-chairs: Joe Tohme (CIAT, Colombia) and Domenico La Fiandra (University of Viterbo, Italy)*

#### 08:45-09:35. Invited presentations

08:45-09:10. *Joe Tohme* (HarvestPlus; hosted at CIAT, Cali, Colombia). Strategies to develop and deploy biofortified crops

09:10-09:35. *Peter R. Shewry* (Rothamsted Research, Harpenden, UK). Improving the health benefit of wheat

#### 09:35-10:50. Selected presentations

09:35-09:50. *Marta Wilton de Vasconcelos* (Catholic University of Porto, Portugal). Effects of *AtFRO2* expression in the nutritional enhancement of soybean (*Glycine max* L.)

09:50-10:05. *Marion S. Röder* (IPK, Gatersleben, Germany). Exploiting the genomic sequences of rice and *Brachypodium* for delimiting a grain size QTL in wheat

10:05-10:20. *Simonetta Agostina Angioi* (University of Sassari, Italy). The genetic make-up of European landraces of common bean

10:20-10:35. *Owen Hoekenga* (Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca, NY, USA). Iron biofortification of maize grain

10:35-10:50. *Yamuna Rani* (University of Agricultural Sciences, GKVK, Bangalore, India). Biofortification of finger millet for high zinc

#### 10:50-11:20. Coffee break

### 11:20-13:15. Session 7. Genetic resources for nutritional quality

*Co-chairs: Francois Baulfourier (INRA, France) and Marina Carcea (INRAN, Italy)*

#### 11:20-11:45. Invited presentation

*Fabio Virgili* (INRAN, Rome, Italy). Nutrigenomics: New functions for "old" molecules

#### 11:45-13:15. Selected presentations

11:45-12:00. *Mario Motto* (CRA-Maize Research Unit, Bergamo, Italy). Gene discovery to improve grain quality-related traits in maize

12:00-12:15. *Qing-Yao Shu* (Zhejiang University, Hangzhou, China). Low phytate rice and soybean: Mutant generation, characterization and gene discovery

12:15-12:30. *Barbara De Nardi* (CRA-VIT – Research Centre for Grapevine Production, Conegliano, Treviso, Italy). Transcriptomic and metabolite analyses of genes implicated in traits relevant to grapevine quality

12:30-12:45. *Christoph U. Germeier* (Julius Kühn Institute, Quedlinburg, Germany). *Avena* genetic resources for quality in human consumption (AVEQ) – a European project on nutritional quality

12:45-13:00. *Søren K. Rasmussen* (University of Copenhagen, Denmark). Mutations in genes controlling the metabolism of inositol phosphates in cereals

13:00-13:15. *Luigi Cattivelli* (CRA-Genomic Research Centre, Fiorenzuola d'Arda, Italy). Exploitation of crop diversity to improve food quality at Agricultural Research Council (CRA) of Italy

#### 13:15-14:30. Lunch & Posters viewing

**14:30-16:40. Session 8. The next challenges**

*Co-chairs: Antoni Rafalski (DuPont, USA) and Chiara Tonelli (University of Milano, Italy)*

**14:30-16:35. Invited presentations**

14:30-14:55. *Maarten Koornneef* (Max Planck Institute, Koln, Germany). Arabidopsis natural variation as model for the use of plant genetic resources

14:55-15:20. *Ulrich Schurr* (Institute of Phytosphere Research, Julich, Germany). Plant Phenotyping: quantitative analysis of plant structure and function on mechanistic, high-throughput and field level

15:20-15:45. *Patrick Giavalisco* (Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany). Metabolomics: Tools and applications

15:45-16:10. *Serena Varotto* (University of Padova, Italy). Understanding epiallele formation and inheritance in response to environmental cues in plants

16:10-16:35. *Giorgio Valle* (University of Padova, Italy). Perspectives of plant genomes analysis

**16:40-18:20. Session 9. Closing session**

*Co-Chairs: Andreas Graner (IPK, Germany) and Francesco Salamini (IASMA, Italy)*

**16:40-17:20. Invited presentations**

16:40-17:00. *Carmen de Vicente* (The Generation Challenge Program; hosted at CIMMYT, Texcoco, Mexico). Capacity building in genomics of plant genetic resources within the Generation Challenge Program

17:00-17:20. *Annette Schneegans* (KBBE, EC, Brussels, Belgium). EU-funded research opportunities on genomics of plant genetic resources

**17:20-17:50. Closing keynote presentation**

*Andreas Graner* (IPK, Gatersleben, Germany). Exploiting the diversity of crop plants: Present state and future challenges

**17:50-18:05. Closing scientific remarks**

*Francesco Salamini* (IASMA, San Michele all'Adige, Italy)

**18:05-18:10. Presentation of the venue for the GPGR3 Congress**

*To be announced*

**18:10-18:20. Closing of the GPGR2 Congress**

*Roberto Tuberosa* (University of Bologna, Italy)

# *Invited Lectures*



## IL01 - Building a global plant genetic resources system

Emile Frison

Director General, Bioversity International, Rome, Italy

The greatest challenge facing humanity today is to feed tomorrow's population of more than 9 billion people. Productivity has to increase by about 70% with the additional uncertainties associated with climate change, against a background of less land and less water being available for agriculture. More than ever before, this will require the wise use of plant genetic resources.

Scientific advances such as high-throughput sequencing, marker assisted selection and direct manipulation of the genome have allowed breeders to identify traits and incorporate them into improved varieties more efficiently and more rapidly. The problem is that the genetic resources that are the foundation of these efforts are not being managed effectively.

Ex-situ collections are currently scattered across roughly 1600 genebanks, many of which are in poor physical condition and which continue to be degraded as a result of insufficient and insecure funding. Many of the accessions are duplicates, which is a waste of precious resources. There is little publicly available information about the accessions. Crop wild relatives, which are so important for resistance to biotic and abiotic stresses, are poorly represented in genebanks and in any case need also to be conserved in the wild so that they can continue to evolve in response to those stresses.

There is an urgent need to address all these issues by building an effective global system for the conservation and use of plant genetic resources. It will require close collaboration and partnership to ensure efficiency, which in turn will require a commitment to a global system of access and benefit sharing as foreseen by the International Treaty on Plant Genetic Resources for Food and Agriculture. It will require secure and sustainable funding so that we do not have to go through this process again every few decades. And it will require a global information system that guarantees access to much more useful information as well as to the accessions themselves.

The challenges are many and complex. As the paper will show, we have the means to meet them, if we engage strongly now, and if we do not we have little hope of feeding the future population adequately.

## IL02 - Germplasm exploitation and ownership: Who owns what?

Orlando de Ponti

International Seed Federation, Nyon, Switzerland

Apart from mastering modern technologies, success in plant breeding is based on access to a wide variety of genetic resources of natural and commercial origin. For thousands of years genetic resources were owned by the commons of farmers, who were undisputedly the owners of the genetic resources, which were freely available for anybody interested in the selection and development of improved seeds.

With the advent of targeted plant breeding this picture changed substantially by a cascade of conventions limiting access to natural and commercial germplasm, consequently limiting the freedom to operate. First the UPOV convention of 1961 regulated breeder's rights to secure return on investments for commercial breeding, while securing free access for further breeding. However, it also allowed farmers to save seeds of protected varieties for their own holdings, the so-called farmer's exception. Later on patents were allowed to protect specific traits and technologies. In most territories patent laws do not allow free access for further breeding, although this is currently lively discussed within industry and society.

Access to natural genetic resources, both *in situ* and *ex situ* were first regulated in a rather liberal way through the International Undertaking on Plant Genetic Resources (1983), based on the notion of world heritage. However, later on access was dramatically restricted by the Convention on Biological Diversity (1993), where access was regulated based on the notion of national sovereignty. For a number of agricultural crops the International Treaty on Plant Genetic Resources for Food and Agriculture (IT, 2004) created some relief, as access is regulated in a multilateral system by a regime for Access and Benefit Sharing through a Standard Material Transfer Agreement.

With the IT a lively discussion started on the issue of farmers' rights, not to be confused with the farmers' exception under the UPOV convention. Farmer's rights are related to ownership over seeds locally collected, selected and maintained by individual farmers. These seeds are mainly categorized as landraces.

However, one might also consider a different interpretation of farmer's rights, the right of fair access to the best genetics and the best seeds, connected to the human right on access to food.

"Who owns what?" creates moral dilemmas for those in the public and private sector engaged in research and development related to the development of better seeds for a growing world population.



### IL03 - From genomics to breeding: How to make the best use of genetic resources

Michele Morgante

Department of Agricultural and Environmental Sciences, University of Udine, Via delle Scienze 208, 33100 Udine, Italy  
Institute of Applied Genomics (IGA), Udine Scientific Park, Via J. Linussio 51, 33100 Udine, Italy

The genomics revolution of the last 15 years has improved our understanding of the genetic make up of living organisms. Together with the achievements represented by complete genomic sequences for an increasing number of species, high throughput and parallel approaches are available for the analysis of DNA sequence variation, transcripts, proteins. The use of genomic tools has allowed us to start to unravel the genetic make up of traits that are relevant to plant breeding. At the same time a deeper understanding of what natural variation is at the sequence level has also been achieved, allowing us to realize that nature can sometime have much greater fantasy and inventiveness than any laboratory scientist and that genetic variation is continuously created in crop species. The pace at which we can analyze natural sequence variation has recently been greatly accelerated thanks to the advent of new DNA sequencing technologies and today the bottleneck is still represented by our ability to genetically dissect complex traits and identify the genes underlying them. Finally, after more than seventy years of separation coincided with the development of quantitative genetics, plant breeding is being reunited to genes thanks to the opportunities offered by genomics for the identification of genes responsible for quantitative trait variation. A new phase in crop evolution of targeted modifications is on the horizon thanks to the progresses in genomics: we will describe the perspectives in this area using examples from different crop species.

#### IL04 - Comparative genomics as a tool for trait dissection

M Abrouk, U Masood Quraishi, S Bolot, C. Pont, J Salse

UMR INRA-UBP 1095, Domaine de Crouelle, 234 Avenue du Brézat, 63100 Clermont-Ferrand, France

In an attempt to unravel the structure and evolution of the cereal ancestor genome we have re-assessed the synteny and duplications of the wheat, barley, rice, maize, *Brachypodium* and sorghum genomes to identify and characterize shared duplications. We combined the data on the intra-genomic duplications with those on the colinear blocks and found duplicated segments that have been conserved at orthologous positions since the divergence of cereals. By conducting detailed analysis of the length, composition, and divergence time of the conserved duplications we identified common and lineage-specific patterns of conservation between the different genomes that allowed us to propose a model in which the grass genomes have evolved from a common ancestor with a basic number of five chromosomes (90 MYA) and then twelve chromosomes (60 MYA) through whole genome duplications (tetraploidization) and translocations followed by lineage specific segmental duplications, chromosome fusions and translocations (Salse et al. 2008, 2009a). Based on these data an 'inner circle' comprising 5 ancestral chromosomes with 9138 protogenes was defined providing a new reference for the grass chromosomes and new insights into their ancestral relationships compared to early marker-based macrocolinearity studies between the grass genomes that have led to arrange their chromosomes into concentric 'crop circles' of synteny blocks (Bolot et al. 2009).

The established cereal ancestor genome structure in term of chromosome structure and gene content offered the opportunity to study the impact of evolutionary shuffling events such as polyploidizations on (i) genome structure (with mechanism drives the diploidisation process); (ii) gene expression (role of epigenetics on neo/sub functionalisation); (iii) agronomical trait genesis (role of whole or segmental genome duplications on QTL epistatic interactions), that will be discussed in details (Throude et al. 2009). In addition to bringing new insight into genome evolution, the knowledge about the extent of conservation between the cereal genomes and the tools generated through the comparative genomics studies can be used to (i) define efficient strategies and for genetic studies and gene isolation through the design of conserved orthologous markers sets (Salse et al. 2009b).

Bolot S, Abrouk M, Masood-Quraishi U, Stein N, Messing J, Feuillet C, Salse J (2009) The 'inner circle' of the cereal genomes. *Current Opinion in Plant Biology*, 12:1-7.

Salse J, Abrouk M, Bolot S, Guilhot N, Courcelle E, Faraut T, Waugh R, Close TJ, Messing J, Feuillet C (2009a) Reconstruction of monocotyledonous proto-chromosomes reveals faster evolution in plants than in animals. *PNAS USA*, 106:14908-14913.

Salse J, Abrouk M, Murat F, Masood Quraishi U, Feuillet C (2009b) Improved standards and new comparative genomics tools provide new insights into grasses paleogenomics. *Briefings in Bioinformatics*.10:619-630.

Salse J, Bolot S, Throude M, Jouffe V, Piegu B, Masood U, Calcagno T, Cooke R, Delseny M, Feuillet C (2008) Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *Plant Cell*. 20:11-24.

Throude M, Bolot S, Bosio M, Pont C, Sarda X, Masood Quraishi U, Bourgis F, Lessard P, Rogowsky P, Ghesquiere A, Murigneux A, Charmet G, Perez P, Salse J (2009) Structure and expression analysis of rice paleo-duplications. *Nucleic Acids Research*, 37:1248-1259.

## IL05 - Sequencing wild soybean genome reveals soybean crop domestication history

S-H Lee<sup>1,2,3</sup>, MY Lee<sup>1,2</sup>, YJ Kang<sup>1</sup>, M Yoon<sup>1</sup>, G Jhang<sup>1</sup>

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<sup>3</sup> Plant Genomics and Breeding Institute, Seoul National University, Seoul, Korea

The genome of soybean (*Glycine max*), a commercially important crop, has recently been sequenced and is one of only five crop species to have been sequenced. We sequenced the genome of *G. soja* var. IT182932, which is the undomesticated ancestor of *G. max*. The nucleotide sequence of the *G. soja* genome, which contains 2.5 Mb of substituted bases and 406 kb of small inserted/deleted bases relative to *G. max*, is ~0.31% different from that of *G. max*. These data suggest that the *G. soja/G. max* complex is at least 0.25 million years ago (MYA), before the recent event of domestication, approximately 6,000-9,000 years ago. We collected 104 *G. max* and *G. soja* samples that were distributed from China, Korea to Japan for population genetic approach. To understand relationship between *G. max* and *G. soja* and soybean domestication history, 32 single-copy nuclear genes were selected and sequenced. Chloroplast genome of *G. soja* (var. IT182932) was sequenced and chloroplast sequence-specific primers were designed based on two chloroplast genome sequences of *G. max* (PI 437654) and *G. soja* (IT182932). Among our soybean samples, chloroplast nucleotide variation was also investigated. These nucleotide variations in nuclear and chloroplast sequences provided valuable information on the geographical divergence process of soybean and its wild relatives and soybean domestication history.

## IL06 - Through the genome sequence: The origin of the cultivated apple

Riccardo Velasco

Istituto Agrario San Michele all'Adige (IASMA), Trento, Italy

Apple (*Malus x domestica* Borkh.) is a very significant fruit crop, well known for its nutritional and health benefits. A high-quality draft sequence of the genome reveals a relatively recent whole genome duplication (WGD). Plant speciation in this tribe, which include the only species of this family with haploid chromosome number of 17, have occurred shortly after this WGD. This event may have been the basis for the emergence of the tribe Pyreae, including all extant maloid taxa. Fossils of Pyreae, the genus *Malus* included, are known from the Eocene, and inferences are made linking molecular data to the appearance of the family Rosaceae and tribe Pyreae. Our work provides estimates of relative molecular distances among accepted clades internal and basal to the family Rosaceae. The domesticated wild progenitor of apple is also identified. Traces of older WGDs provide support for the monophyly of the ancestral paleohexaploidy of dicots.

## IL07 - Genomic exploration of diversity in the genus *Glycine* (soybean) and related crop legumes

Scott Jackson

Department of Agronomy, Purdue University, Lafayette, IN, USA

Genetic diversity is the cornerstone of plant improvement. Soybean (*Glycine max*) cultivated germplasm has been shown to be genetically depauperate (Hyten et al., 2006 PNAS 103:16666-16671). Therefore, we are using genomic tools to exploit the recent soybean genome sequence to find diversity both in the undomesticated ancestor of soybean (*Glycine soja*) as well as perennial species located mostly in Australia. As part of this work, we are developing genomic libraries and genome sequence information to begin to understand the evolution and domestication of this genus and to exploit genetic variation to improve and protect yields in soybean. In particular, we are working on identifying genes in *G. soja* that provide resistance/tolerance to the soybean aphid which can result in yield losses as a vector of other diseases and because of feeding on the plant. In addition to soybean, we are developing genomic tools in other legumes species including common bean (*Phaseolus vulgaris*) to begin to better and more efficiently take advantage of genetic variation for crop improvement. In common bean, we are currently sequencing the genome using a whole genome shotgun approach and should have a draft sequence in late 2010. This will provide a framework to investigate the multiple domestications of common bean as well as to find valuable genes to improve seed quality and yield. I will discuss the value of these reference genomes in the identification and exploitation of genetic diversity for crop improvement.

### IL08 - Genomics-assisted allele mining and its integration to breeding in rice

Masahiro Yano, Shuichi Fukuoka, Yusaku Uga, Kaoru Ebana, Jun-ichi Yonemaru, Toshio Yamamoto

QTL Genomics Research Center, National Institute of Agrobiological Sciences, Tsukuba, Japan

Progresses on recent genomics in rice have provided a new tools and opportunities to enhance activity in crop improvement. Elucidation of the association between nucleotide and phenotypic changes is inevitable to this end and has been a big challenge in molecular genetics and breeding of rice. Toward this goal, we have been involved in the genetic dissection of natural phenotypic variations in rice and have identified several genes involved in complex traits, including heading date, shattering habit, pre-harvest sprouting, root morphology, disease resistance and eating quality. To enhance the power of genetic dissection of complex phenotypes, we are developing several mapping populations, such as recombinant inbred lines and chromosome segment substitution lines, which will allow us to extract the useful alleles from natural variants. Recently, QTL for durable resistance to rice blast has been cloned from Japanese upland rice. This finding has opened new opportunity to introduction of the unique blast resistance gene without a linkage drag of low eating quality. We have also detected a major QTL for deeper rooting on chromosome 9. This finding has open new opportunity to enhance drought avoidance in rice. To facilitate allele mining using novel plant materials, we have also embarked on the genome-wide discovery of single nucleotide polymorphisms (SNPs). In particular, to overcome a shortage of SNPs among temperate japonica cultivars, we have attempted whole-genome sequencing of several Japanese cultivars using next-generation sequencing approaches. This SNP discovery has led to the development of an array-based SNP genotyping system in Japanese rice cultivars. Large-scale genotyping of these SNPs has made it possible to visualize pedigree haplotypes of particular chromosome segments in the Japanese landraces and modern cultivars. These efforts in genomics have opened up new opportunities to accelerate not only the genetic dissection of complex traits, but also integration of genomics to breeding in rice.

### IL09 - Creating new diversity using synthetic transgenes to improve crops

Richard Flavell

CERES, Red Oaks, CA, USA

Crop improvement relies on genetic diversity but rarely is the diversity structured in ways that make it easy to use. We have attempted to discover the genetic basis of key traits and provide the genes in the form of transgenes that are easy to manage in a breeding program. Some of the results of this approach will be described together with their application in breeding C4 grasses for biofuel feedstocks. Projections of how such approaches could change plant breeding in the future and overcome the limitations imposed by today's genetic diversity will be made.

## IL10 - The sustenance of human livelihood via genetics and genetic resources

Gebisa Ejeta

Department of Agronomy, Purdue University, Lafayette, IN, USA

From time immemorial biodiversity has served humanity as the most fundamental staff of life. Long before the advent of the science of genetics, and even before the basis for trait expression was ever understood, genetic biodiversity was used to avert and overcome plant and animal epidemics and pandemics of immense proportions throughout human civilization. Solutions to so many plant and animal diseases were found, lives were saved, societal order was restored, and basic human livelihood was sustained as a result of keen observations and clever manipulations practiced on the generous endowment of the natural genetic variation in our planet. Developments in the science of genetics and associated disciplines have accelerated the rate and extent to which we could effectively exploit genetic variation to serve humanity. Plant, animal, and microbial genetics have improved human livelihood through food, feed, and energy production, enhancement of human nutrition, as well as health and therapeutic benefits thereby increasing the quality and quantity of life on earth. Examples will be drawn from plant biodiversity manipulations against major plant epidemics in the past, and more recent exploitations of natural genetic variation including the speaker's own research on sorghum [*Sorghum bicolor* (L.) Moench] to enhance discussion.

## IL11 - The 50K maize Infinium chip - design and use for maize diversity analysis

Martin Ganal<sup>1</sup>, Andreas Polley<sup>1</sup>, Gregor Durstewitz<sup>1</sup>, Mike Thompson<sup>2</sup>, Mark Hansen<sup>2</sup>

<sup>1</sup> TraitGenetics, Gatersleben, Germany

<sup>2</sup> Illumina, San Diego, CA, USA

In a collaborative effort between multiple partners (Illumina, USDA, Syngenta, INRA and TraitGenetics), an Infinium array has been designed that consists of more than 50000 maize SNPs. This array contains an average of two SNPs in approximately two-thirds of all maize genes and additional SNPs spread over most of the remaining maize genome. Through the analysis of a first set of maize lines, data will be presented regarding the quality and level of polymorphism of the SNPs on the array in a set of mainly European maize breeding lines. We will also present some data regarding the potential use of the array to generate high density genetic maps with many thousands of markers. This array also offers a tool to analyse diversity in the entire maize genome or chromosomal segments of the maize genome. Moreover, it offers the potential to perform diversity studies in many of the maize genes that contain SNP markers on the array.



## IL12 - Multiple scale analysis of genetic diversity in sorghum

Monique Deu<sup>1</sup>, Adeline Barnaud<sup>2</sup>, Clarisse Barro-Kondombo<sup>3</sup>, Sophie Bouchet<sup>1</sup>, de Alencar Figueiredo<sup>1,4</sup>, Fabrice Sagnard<sup>1,5</sup>, Jean Christophe Glaszmann<sup>1</sup>

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Crop species are characterised by their intimate association with human populations, their history and their practices and needs. In long domesticated crops, migration can have expanded gradually within continents and jumped between continents, shaping global patterns of diversity and adaptation. Sorghum (*Sorghum bicolor bicolor*) is one such case of very successful crop, which was domesticated in Subsahelian Africa and is now grown throughout the world. Here we review conclusions of recent studies conducted at various geographical scales (field, village, region, country, continent), sometimes including temporal variation, and practised at the level of morpho-agronomic traits, whole-genome molecular markers as well as selected candidate genes.

Local diversity in the area of sorghum origin is almost as large as whole-species diversity, with peripheral regions displaying specific genotypic combinations corresponding to distinct races, but very limited specific genic diversity. This pattern of diversity is accompanied with a generally low level of linkage disequilibrium, which is confined to genome segments within the Mb range.

A focus on certain genes involved in cereal grain quality revealed cases of novel alleles that appeared during the course of migration outside the centre of origin, being likely selected by the action of the farmers, highlighted the potential of neo-diversity for crop diversification.

The diversity of human groups acts together with the agro-ecological factors to shape the structure of sorghum genetic diversity. As detailed in a village in Cameroon, introgression occurs among weedy types and cultivated types, yielding an array of intermediates; farmers identify and name them, and actively select against certain morphotypes, but several practices unconsciously favour gene flow. Based on a study covering 79 villages in Niger, no genetic erosion occurred over a 26 year period; farmers' management can preserve the diversity despite recurrent and severe drought periods and major social changes.

The tremendous diversity maintained by farmers in traditional agroecosystems of Western Africa supports the development of crop improvement approaches making broad use of local germplasm in decentralized breeding programs.

## IL13 - Genomics approaches to unravel the genetic basis of heterosis

Albrecht E Melchinger

Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany

The exploitation of heterosis is of fundamental importance in breeding all types of plant cultivars except pure lines. In spite of intensive research efforts since its first description by Shull in 1908, the genetic and molecular bases of heterosis are not fully understood. Recent developments in genomics research have opened new avenues to better understand and exploit heterosis. This presentation will review the present status of heterosis research, focusing on the latest results obtained in maize. Comparison of QTL mapping studies by a meta-analysis revealed a high congruency of QTL for grain yield in maize, most of which are located in centromere regions. Together with insights obtained from the recently published maize genome sequence, the estimated QTL effects suggest that heterosis for grain yield in maize is most likely attributable to pseudo-overdominance as a result of the Hill-Robertson effect. Moreover, these findings suggest that different alleles have been fixed in various heterotic pools. Hence, introgression of new desirable alleles from germplasm resources by genomics approaches is urgently required to secure future progress in maize breeding.

These conclusions are also supported by the comparison of genome sequences of inbreds, which revealed ample variation in the genome structure due to presence/ absence variants (PAVs) and copy number variants (CNVs). Transcriptome, proteome and metabolome profiling of parents and hybrids showed that expression of the hybrids was in most instances either additive or within the range of the parents and transgression occurred rather infrequently. The prediction of heterosis and hybrid performance either by molecular markers (SNPs) or sequence information (PAVs, CNVs) or transcript profiles look very promising. Altogether, there is a great potential to apply new genomics approaches for better understanding the causes of heterosis and its efficient exploitation in plant breeding.

## IL14 - Exploiting genetic resources for plant breeding in the 21<sup>st</sup> century

Richard GF Visser

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With the availability of high quality genomic sequences of major crops and the possibility to obtain more and broader sequence information at affordable costs, gene assisted selection will come within practical reach for even the smallest crop. This will shift attention from the genotyping to high quality phenotyping. While this is, because of the nature of the trait, relatively easy for resistances it is a tremendous challenge for all kinds of quality related traits as well as a-biotic stress parameters. In many crops the search for resistances to major pests and diseases has lead to the identification of resistances in these wild relatives followed by the subsequent introgression into elite cultivars. This is however much less explored for minor diseases, a-biotic stresses and the majority of quality traits.

By using molecular tools in wild relatives of crop plants the door can be opened to a better use of these wild relatives when looking for improvement of traits. The ideal molecular marker for these traits seems to be SNP markers. The natural diversity in several candidate genes can be assessed by SNP analyses. This will provide a catalogue of DNA variation in the species. When and if traits can be phenotypically assessed these results may be combined with QTL data from segregating populations as well as QTL data from association panels and thus show that only certain unique (combinations of) alleles for different genes give rise to particular phenotypes.

## IL15 - Genomic tools for the analysis of genetic diversity

Antoni J Rafalski

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We now understand that many different types of DNA structural polymorphisms contribute to functional diversity of plant genomes, including SNPs, insertion of retrotransposons and DNA transposons, including Helitrons carrying pseudogenes and other types of insertion-deletion polymorphisms, many of which may contribute to the phenotype by affecting gene expression through a variety of mechanisms including those involving noncoding RNAs. These polymorphisms can now be probed with tools such as array comparative genomic hybridization and, most comprehensively, genomic sequencing. Rapid developments in next generation sequencing will soon make genomic sequencing of germplasm collections a reality. This will help eliminate an important difficulty in the estimation of genetic relationships between accessions caused by ascertainment bias. Also, it has now become obvious that epigenetic differences, such as cytosine methylation, also contribute to the heritable phenotype, although detailed understanding of their transgenerational stability in crop species is lacking. The degree of linkage disequilibrium of epi-alleles with DNA sequence polymorphisms has important implications to the analysis of genetic diversity. Epigenetic marks in complete LD with DNA polymorphisms do not add additional diversity information. However, epialleles in partial or low LD with DNA sequence alleles constitute another layer of genetic information that should not be neglected in germplasm analysis, especially if they exhibit transgenerational stability.

## IL16 - Toward the fine mapping of Plum Pox Virus (Sharka) resistance QTLs from two different sources in apricot

Tartarini S<sup>1</sup>, Dondini L<sup>1</sup>, Adami M<sup>1</sup>, Lain O<sup>2</sup>, Vendramin V<sup>2</sup>, Chiozzotto R<sup>3</sup>, Geuna F<sup>3</sup>, Bassi D<sup>3</sup>, Palmisano F<sup>4</sup>, Castellano Mariantonietta<sup>4</sup>, Savino Vito<sup>4</sup>, Boscia D<sup>5</sup>, Cattonaro F<sup>6</sup>, Wu Cheng-Cang<sup>7</sup>, Testolin R<sup>2</sup>, Verde I<sup>8</sup> and the International Peach Genome Initiative (IPGI)

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Sharka, caused by the *Plum Pox Virus* (PPV), is one of the most severe viral disease in apricot (*Prunus armeniaca* L.) and it determines large crop losses in many countries worldwide. Two PPV strains, PPV-D (Dideron) and PPV-M (Marcus), are particularly virulent in apricot, with the latter strain being the most threatening. Since twenty years, resistance sources have been identified in apricot germplasm and they have been widely used in breeding programmes to obtain new resistant high-quality cultivars but the genetics of such resistances is still under debate. Recent literature identified a major QTL for sharka resistance in linkage group 1 of several apricot varieties (Stark Earli-Orange, Goldrich, Lito and Harlayne) but most of the analyses have been conducted by using only the PPV-D strain for resistance phenotyping and the region where the QTL is located is not perfectly aligned among the different maps. For that reason two F1 apricot progeny derived from two different sources of resistance 'Lito' and 'Harcot' were used to map these QTLs for both strains, PPV-M and PPV-D. Results confirmed in both cultivars the QTL position in linkage group 1 as previously described but suggesting that Lito and Harcot represent two different sources of sharka resistance. Moreover additional markers for molecular selection of resistant seedlings were identified. These data were also used as a starting point for the fine mapping of this genomic region by using a random shear BAC library of the cultivar 'Lito' that comprises 33,366 clones for an estimated 10X genome coverage. As a first step, a number of clones carrying SSR marker alleles surrounding the region containing the main QTL for PPV resistance were isolated. Thanks to the high synteny between peach and apricot genomes, the availability of the whole genome peach sequence, released on April 2010, is a very powerful tool for further BAC screenings, "close the gaps" and isolate the Lito's resistance gene.

## IL17 - Towards natural selection mapping of useful genes in plant germplasm

Karl Schmid, Thomas Günther

Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Germany

The analysis of genetic variation is central to the management of genetic resources, genetic mapping and the selection of individuals in breeding programs. Usually, neutral genetic markers such as silent single nucleotide polymorphisms (SNPs) or simple sequence repeats (SSRs) are being used. Meanwhile, large-scale studies revealed a bewildering diversity of genetic polymorphisms in plants. Point mutations, repeat variants, polyploidization, transposable element insertions, and gene duplications are abundant and contribute to phenotypic variation in wild and crop plants. This diversity raises the question of whether markers can be classified into neutral, deleterious and advantageous polymorphisms to be used for different purposes. To identify deleterious amino acid polymorphisms, phylogenetic approaches implemented in the SIFT and MAPP programs were applied to simulated and real data sets to investigate the effect of bottlenecks and domestication events on the frequency of deleterious polymorphisms. We observed a significant increase of deleterious polymorphisms due to less efficient purifying selection in bottlenecked population and a high power to detect such bottleneck effects in large-scale sequence data. In contrast, the power to detect deleterious amino acid polymorphisms in single genes with known phenotypes is limited. We also investigated the power to detect positive selection during domestication. A new haplotype-based test, which controls for population structure, was applied to simulated and real data. The power to detect genes exposed to strong artificial selection during domestication is low for ancient domestication events. However, more recent selection can be detected with high power. As various resequencing approaches of crop plants are underway, the fine-grained characterization of genetic variation will lead to the identification of novel, useful genes and genetic variation in plant genetic resources.

## IL18 - Mining Triticeae genes with the aid of *Brachypodium*

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Gene order and chromosome relationships, known as colinearity and synteny, have been generally conserved during the evolution of the grasses. This same group of plants is also central nutritionally; rice, wheat, and maize alone provide 60% of the world's food energy, and grass fodder supports animal husbandry. The availability of whole genome sequences from the grasses, is therefore transforming genomics, gene isolation, and breeding for the key crops. The model plant *Brachypodium distachyon* is closely related to the core Pooids including wheat, barley, rye, oat, and the main fodder grasses; its compact genome has now been sequenced. Wild emmer wheat, *Triticum dicoccoides*, the tetraploid ancestor of all domesticated wheat cultivars harbours extensive resources for wheat improvement, and is a particularly promising source of resistance to the yellow rust disease caused by the fungus *Puccinia striiformis*. We are exploiting the synteny of *Triticum* to the sequenced *Brachypodium* and rice genomes in order to positionally clone *Yr15*, which confers a particularly high resistance to the yellow rust disease. SSR and RFLP markers assigned to chromosome 1BS formed the initial genetic map of the *Yr15* region and were used to assign *Yr15* to a 1BS deletion bin. Markers based on ESTs assigned to the bin enabled us to establish colinearity between the *Yr15* region and the rice and *Brachypodium* genomes. Using these genomes as mediators between the wheat and barley genetic maps matching the *Yr15* region, we developed further molecular markers and ultimately narrowed down the chromosome segment to collinear regions of 13 Kb in *O. sativa* and 28 Kb in *B. distachyon*. Comparative genomics and graphical genotyping with an F<sub>2</sub> mapping population of more than 2,000 individuals has brought us to a genetic interval of 0.2 cM and enabled isolation of wheat BAC clones for the region.

## IL19 - Genome-wide patterns of SNP variation in polyploid wheat

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Genome-wide set of SNPs is an essential tool for linkage and association mapping of agronomic traits and studying the genetic variation in populations of cultivated crop species and their wild ancestors. Recent advances in genomic technologies including the development of highly-multiplexed genotyping assays and next-generation sequencing technologies opened new possibilities for the analysis of plant genomes. We adopted these technologies to characterize the patterns of genetic variation in tetraploid and hexaploid wheat. A panel of 480 spring and winter US wheat cultivars was genotyped using 1536-plex Illumina OPA. The SNP genotyping data was used to infer the population structure and genome-wide patterns of genetic diversity and linkage disequilibrium in a collection of wheat cultivars and to assess the utility of an assembled hexaploid wheat panel for LD mapping. A strong effect of breeding on genetic differentiation of cultivars was demonstrated. The sequence-capture and next-generation sequencing approaches were used to re-sequence 1 kb fragments of 3,500 genes in tetraploid wild emmer and durum wheat. More than 3,000 SNP sites were used to investigate the distribution of mutations differentiating the genomes of wild and domesticated tetraploid wheat. Our study showed that this strategy is fast and very efficient method for the targeted analysis of the regions of interest in highly complex genomes, such as polyploid wheat. The potential impact of new genomic tools on utilization of plant genetic resources for breeding will be discussed.



## IL20 - Functional diversification of transcription factors for the formation of barley spikes

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The barley (*Hordeum vulgare* ssp. *vulgare*) spike is composed of three spikelets at each rachis node. Row-type of spike is determined by the allelic constitution at the *six-rowed spike 1 (vrs1)* locus on chromosome 2HL, with the recessive allele (*vrs1*) being responsible for the six-rowed phenotype. The *Vrs1 (HvHox1)* gene encodes a homeodomain-leucine zipper transcription factor. Here we show that the *Vrs1* gene evolved in the Poaceae via duplication, with a second copy of the gene, *HvHox2*, present at 27 cM away from the *HvHox1* in chromosome 2HS. Micro-collinearity and polypeptide sequences were both well conserved between *HvHox2* and its Poaceae orthologues, but *Vrs1* is unique to the barley tribe. A phylogenetic analysis demonstrated that *Vrs1* and *HvHox2* must have diverged after the separation of *Triticum* and *Hordeum*. *HvHox2* was expressed in all organs examined but *Vrs1* was predominantly expressed at the lateral spikelet primordia in immature inflorescence. The *HvHox1* protein lacks a short motif consists of eight amino acids at the C-terminal, which is conserved among the *HvHox2* orthologues suggesting a conserved function in it. From these analyses it is suggested that *Vrs1* acquired its new function, presumably a suppression of *HvHox2*, during the evolution of the barley tribe. The duplication and paralogous origin of *Vrs1* allowed diversity of the *Vrs1* shown by re-sequencing landraces and wild barley. Barley cleistogamy gene *cly1* also encodes a transcription factor, which contains two AP2 domains and a putative microRNA miR172 targeting site, an indication of orthology with *Arabidopsis thaliana* *AP2*. A perfect association between a nucleotide substitution at the miR172 targeting site and cleistogamy was established. The substitution was synonymous but cleavage of mRNA guided by miR172 was detectable only in a noncleistogamous background while no cleavage in cleistogamy barley. The miR172-derived down-regulation of *Cly1* promotes the development of the lodicules, although the single nucleotide change at the miR172 targeting site results in the failure of the lodicules to develop properly, producing the cleistogamous phenotype. Two alleles for cleistogamy have been detected: one originated from England and the other Western Mediterranean region through two independent evolutionary lineages. Cleistogamous spike are associated with reduced rachis internode length thus revealing the remarked *erectum* form of spike.

## IL21 - Manipulating the resolution of whole genome association scans in barley using stratified germplasm collections

Robbie Waugh, Joanne Russell, Ian Dawson, Luke Ramsay, Jordi Comadran, Bill Thomas, Dave Marshall, Xin Xu, AGOUEB Consortium\*, ExBarDiv Consortium\*\*, Andy Flavell

We have been successfully conducting whole genome association mapping studies for gene identification in the elite cultivated gene pool of *Hordeum vulgare* (Barley) using a population of *ca.* 1000 advanced breeders lines and cultivars and a collection of ~3000 bi-allelic SNP markers incorporated onto two 1536-plex Illumina™ Oligo Pool Assays. We have been doing this in collaboration with representatives from the NW European plant breeding community (AGOUEB\*) who are acutely interested in understanding the genetic variation that is segregating in this type of material. As would be predicted for an inbreeding species, we have observed that linkage disequilibrium (LD) can be extensive in certain regions of the barley genome in cultivated germplasm – especially in the rarely recombining regions around the genetic centromeres. We have therefore been exploring (in ExBarDiv\*\* and GCP\*\*\* projects) whether the use of stratified germplasm collections comprised of either landraces or wild species (*H. vulgare* ssp. *spontaneum*) can be used to manipulate the resolution of association studies while simultaneously accessing novel allelic variability. While LD will be less extensive in the latter gene pools, using this type of material for our envisaged purposes has raised a number of issues which need to be resolved before the approach can be routinely and successfully adopted. In this presentation I will summarise some of the results we have obtained when working within the cultivated elite gene pool and some of the hurdles we have faced when transitioning whole genome association studies to the more diverse landrace and wild species gene pools.

Members of the AGOUEB, ExBarDiv and GC consortia can be found at:

\*AGOUEB: <http://www.agoueb.org/>

\*\*ExBarDiv : <http://www.erapg.org/everyone/9587/18624/18614/18622>

\*\*\*GCP: <http://www.generationcp.org/research.php?da=0655608#genomic>

## IL22 - A doctoral programme to harness the value of genetic resources in developing countries

Enrico Porceddu

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Biological diversity has emerged in the past decades as a key area of concern for sustainable development, but crop diversity is rarely considered, in spite of the fact that it ensures a constant and varied source of food and raw material for all sorts of human population, as well as a critical source of genetic material for the development of new and improved varieties. This type of biodiversity is being lost in many parts of the world, at rapid pace. In fact there has been a tremendous decline in the number of cultivated species and their genetic diversity, brought about by the success of new varieties belonging to a few major species.

Concerns over this erosion has lead to efforts to assemble genetic resources in *ex situ* collections, an activity that needs to be followed by careful cataloguing and indexing both by trait and by genome such that breeders can easily access the deposited material. This is not the case in many gene banks, especially those in the developing countries, which lack educated and *ad hoc* trained staff.

With the aim of contributing to the enhancement of human resources capabilities in the utilization and management of genetic variation in agricultural and natural systems, in order to improve the sustainability of agricultural systems and the conservation of genetic resources for the wellbeing of present and future generation a Doctoral Programme in Agrobiodiversity is being supported by the Ministry of Education, University and Research, upon a proposal by the Italian National Academy of Sciences, and located at the Sant'Anna School for Advanced studies.

To fulfilling its mission the Programme provides education and training in the analysis of genetic variability in agricultural and forestry plants and their wild relatives, investigations in its amount in single genes and entire genome, mechanisms that control the ability of change in some genes or groups of genes, as those involved in resistance to pathogens and tolerance to geographical and climatic changes, analysis and promotion of functional biodiversity, and to planning and management of sustainable agro-ecosystems.

The programme has to curricula, one in Plant Genetic Resources, located in Roma, and the other in Functional biodiversity, located in Pisa.

An international scientific committee supervises the Programme to ensure research excellence and themes relevant to developing countries' agriculture.

The first call for six three years duration fellowships was issued in 2004 and the first course started in January 2005.

More than 600 applications from more than 40 countries have been received in answering the so far six application calls. At this date fifteen doctoral degrees have been issued; papers from doctoral theses are being published in peer reviewed scientific journals.

Examples of research results are provided in the presentation.

## IL23 - The Monsanto's Beachell-Borlaug Initiative for food security in developing countries

Edward CA Runge

Monsanto's Beachell-Borlaug International Scholars Program - Program Director  
Soil & Crop Sciences Department, Texas A&M University, College Station, Texas 77843-2474, USA

Monsanto's Beachell-Borlaug International Scholars Program honors two of the World's best known plant breeders who are widely credited for bringing the green revolution to rice and wheat production. The dramatic increase in rice and wheat production per unit area of land prevented starvation for billions of people. The MBBIS program is funded at \$2 million per year for 5 years for a total of \$10 million. The program provides complete support for PhD students in rice and wheat breeding. The MBBIS program was announced on Dr. Norman Borlaug's 95<sup>th</sup> birthday on March 25, 2009. The 1<sup>st</sup> round of application were evaluated by an internationally acclaimed panel of judges and 12 scholars were selected for support. Second round of funding closed on February 1, 2010. Applications for years 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> rounds of funding should be submitted between November 1, 2010, 2011 and 2012 and February 1 in 2011, 2012 and 2013. Funds are encumbered for the duration of the PhD program. Scholars must complete part of their PhD program in Australia, Canada, Western Europe or the United States and part in another country of the World. Students work with their advising professor or scientist who then submits the application for the student. To learn more about how to apply, please visit [www.monsanto.com/mbbischolars](http://www.monsanto.com/mbbischolars).

## IL24 - The Borlaug Global Rust Initiative: Reducing the genetic vulnerability of wheat to rust

Sarah Davidson, Gordon Cisar, Ronnie Coffman

Durable Rust Resistance in Wheat Project, IP-CALS Cornell University, Ithaca, NY

The Borlaug Global Rust Initiative (BGRI) is an umbrella organization with a portfolio of projects that all aim to reduce the world's vulnerability to stem, yellow, and leaf rusts of wheat. The organization advocates for and fosters a sustained international effort to minimize the threat of wheat rusts and advance wheat breeding to withstand future global threats to wheat. The BGRI partners are working to mitigate the threat of wheat rust by breeding for genetic resistance. Through the Durable Rust Resistance in Wheat project and other efforts of the Initiative, sources of stem rust resistance are being discovered in many close relatives of wheat. Both race-specific and non race-specific approaches are being pursued, with an emphasis on generating multigenic, adult plant resistant (APR) lines expected to confer more "durable" long-term resistance. As a result of massive screening efforts, BGRI member scientists have identified several Ug99-resistant lines that are candidates for varietal release, including 15 lines distributed to six at-risk countries. Responsible management and deployment of new sources of resistance remains a challenge and requires strategic global coordination.

## IL25 - Leveraging plant genetic resources for Africa

David Bergvinson

Senior Program Officer, Agricultural Development, Bill & Melinda Gates Foundation

The food crisis of 2008 brought into sharp focus the need to increase genetic gains in crop yields that are now less than 1% per annum for wheat and rice. The Gates Foundation recognizes the central role crop improvement, coupled with agronomic inputs, market opportunities and government policies, continues to play in achieving sustainable food production. For a second Green Revolution to occur we must include rainfed agriculture, develop broader and more innovative partnerships, and leverage science to meet future food, feed, fiber and fuel demands. All of this must be done while preserving our natural resource base. Crop improvement must leverage the wealth of genetic diversity that exists in global accessions to address the growing intensity of both abiotic and biotic stresses and the diverse consumer demands for nutritional quality, texture and taste for staple crops in Africa. In order to harness the wealth of functional genetic diversity in staple crops we must make better use of modern tools (e.g. geographical information systems, bioinformatics, molecular markers, and precession phenotyping), approaches (e.g. participatory variety selection) and effective partnerships (i.e. public-, private-sector and civil society) in order to meet the needs of smallholder farmers in sub-Saharan Africa. This talk will review the foundations strategy to leverage crop diversity to improve staple crops in Africa and our plan for a Molecular Breeding Platform that will enable the crop improvement community to accelerate genetic gains by more effectively using genetic resources to develop and deliver farmer-preferred varieties in Africa.

## IL26 - Establishing genomics-assisted breeding foundation in chickpea for enhancing crop productivity in Africa and Asia

Rajeev K Varshney

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502324, India & Genomics towards Gene Discovery Sub Programme, Generation Challenge Programme (GCP), c/o CIMMYT, Int APDO Postal 6-641, 06600 Mexico DF, Mexico

Chickpea, an important food legume is generally grown in marginal environments of semi-arid regions of Asia and Africa where the crop production is heavily challenged by abiotic stresses such as terminal drought and biotic factors like legume pod borer (*Helicoverpa armigera*), fusarium wilt and ascochyta blight. With an objective to tackle these production constraints through molecular breeding, in collaboration with several partners around the world, significant genomic resources have been developed recently (<http://www.icrisat.org/gt-bt/ICGGC/homepage.htm>). For instance, 1,655 novel SSRs have been isolated from the SSR-enriched library (311) and BAC-end sequences (1,344), a DArT array has been developed with >16,000 features and >20,000 ESTs from drought and salinity stress challenged tissues based on Sanger sequencing. Further, a set of 443,969 sequence tags were generated through FLX-454 sequencing from a pool of normalized cDNA assembled from developmental stages and abiotic stresses challenged tissues of a reference chickpea genotype (ICC 4958). Analysis of Sanger as well as FLX-454 sequence data provided 103,215 tentative unique sequences (TUSs). In parallel, transcriptomes of drought and *Helicoverpa* challenged tissues were sequenced using Solexa sequencing approach. Over 37 million drought responsive tags were obtained from ICC 4958 and ICC 1882 and their alignment with the TUSs provided 26,083 nucleotide variants (SNPs) between the two genotypes. Similarly, 81.2 million tags were generated from *Helicoverpa* challenged tissues of ICC 37 and ICC 506 genotypes and a total of 65,536 nucleotide variants were identified. In collaboration with University of California-Davis, a pilot Illumina GoldenGate assay for 768 SNPs has been developed. By using above mentioned resources, an integrated genetic map with >1534 marker loci has been developed based on an interspecific mapping population (*C. arietinum* ICC 4958 × *C. reticulatum* PI 489777). With an objective of identification of candidate markers associated with drought tolerance (root traits), the most challenging production constraint, two intraspecific mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) have been phenotyped for root traits in two environments and genotyped with >250 SSR markers. Marker-trait analysis has revealed several QTLs including one major QTL in both mapping populations that contributed up to 30% phenotypic variation for drought-tolerance component traits. This genomic region containing several QTLs for drought tolerance is being introgressed into three elite chickpea lines (JG 11, ICC 92318 and KAK 2), using marker-assisted backcrossing (MABC) approach, to develop superior cultivars with enhanced drought tolerance. It is therefore anticipated that developed genomic resources at large scale should facilitate genomics-assisted breeding that would lead to sustainable crop production of chickpea in developing countries.

## IL27 - Core collection-based genomic stocks in wheat

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One core collection (CC) and one mini-core collection (MCC) consisting of 1,160 and 231 accessions, respectively, were assembled from a collection of 23,090 Chinese wheat accessions. The CC and MCC collections captured more than 90 and 70% of the genetic diversity present in the initial collection, respectively. Four hundreds and eighty-seven accessions including the MCC, accessions introduced from across the world and synthetic wheats were crossed with Chinese commercial leading varieties followed by selfing and backcrossing. Consequently, 386 populations of recombination inbred lines (RILs) and 411 populations of introgression lines (ILs) were developed. The MCC and its derived RILs and ILs are identified as MCC-based genomic stocks. The MCC-based genomic stocks have been employed for gene discovery through linkage and association analysis, allowing us to detect hundreds of new agronomically important genes/QTLs, that affect yield components, grain quality, stress tolerance and disease resistance. Hundreds of elite lines selected from the ILs and RILs have been used as parents in Chinese wheat breeding programs and some of them have been released as new cultivars. Our results have demonstrated that the MCC-based genomic stocks are valuable for both functional genomics studies and breeding, and are expected to contribute significantly to wheat improvement in the near future.



## IL28 - Genetic resources and genomic approaches to improve abiotic stress tolerance in cereals

Peter Langridge

Australian Centre for Plant Functional Genomics, University of Adelaide, Glen Osmond, SA5064, Australia

Abiotic stresses such as extreme temperature, low water availability, high light intensity, high salt, and mineral deficiencies or toxicities can severely reduce crop plant productivity. In many cases, several types of abiotic stress challenge crop plants simultaneously. High temperatures, high irradiance, scarcity of water and nutrient deficiencies are commonly encountered under growing conditions but are frequently not amenable to management through traditional farm practices. Higher plants have evolved multiple, interconnected strategies that enable them to survive unpredictable environmental fluctuations. However, these strategies are not always well developed in the cereal cultivars grown by grain producers and most of the strategies are focused on plant survival at the expense of yield.

This presentation will focus on wheat and barley where the genetic control of traits determining yield in water limited and low yielding environments are generally expected to be of low heritability, polygenic and many of the key loci will show epistatic rather than additive effects. Current breeding and mapping techniques make it very difficult to detect and select for these types of loci. Know confounding factors, such as maturity, height, resistance or tolerance to soil diseases, and tolerance to related stresses such as boron, acidity, salinity and nutrient deficiencies must be taken into account. In many cases the genetic control of tolerance to these factors is known so that they could be fixed in both breeding and mapping populations.

In comparison to model organisms, wheat and barley have the advantages of extensive monitoring and archiving of genotypes and associated phenotypic data and the availability of unique populations adapted to specific environments and end-uses that have resulted from a long history of selective breeding. These advantages are becoming increasingly significant as analytic tools improve. Application of markers and genomics research in wheat and barley still faces a number of serious issues. In particular, many of the key traits influencing yield are poorly understood at the physiological level, hard to reliably phenotype and the genetic control is frequently poorly understood. However, whole genome approaches and systemic analysis of the molecular basis of stress tolerance responses are starting to reveal key pathways and process involved in maintaining yield in difficult environments. Results now coming out of genomics studies are providing new insights into stress responses and provide novel strategies to improve stress tolerance. A broad approach to using genomics techniques to tackle abiotic stress tolerance in wheat and barley will be presented with some specific examples of how these results can influence practical crop improvement.

## IL29 - Wild emmer wheat genetic resources: From genetic diversity to map-based cloning for increased sustainability of Triticaceae crops

Tzion Fahima<sup>1</sup>, Assaf Distelfeld<sup>1,2</sup>, Cristobal Uauy<sup>2</sup>, Daolin Fu<sup>2</sup>, Hanan Sela<sup>1</sup>, Dina Raats<sup>1</sup>, Elitsur Yaniv<sup>1</sup>, Roi Ben David<sup>1</sup>, Tamar Krugman<sup>1</sup>, Zvi Peleg<sup>1,5</sup>, Boulos Chalhoub<sup>3</sup>, Alan Schulman<sup>4</sup>, Abraham Korol<sup>1</sup>, Yehoshua Saranga<sup>5</sup>, Jorge Dubcovsky<sup>2</sup>

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Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (genome BBAA) is the tetraploid ancestor of domesticated durum and bread wheats. Wild emmer wheat offers a rich source of allelic diversity in many agronomic traits valuable for improvement of cultivated wheat. Nevertheless, traditional approaches for utilization of wild alleles are usually very slow. The advanced genomic technology available today may help to increase the efficiency of utilization of wild emmer wheat for crop improvement. Our studies are focused on unraveling the genetic basis of several qualitative and quantitative agronomic traits derived from wild emmer wheat. Genomic maps of wild emmer wheat enabled us to identify novel stripe rust and powdery mildew resistance genes, as well as to map QTLs responsible for drought resistance, grain protein content and yield components. Furthermore, transcriptome analysis was used to identify potential candidate genes for drought tolerance. Since the wheat genome is not sequenced yet, we are using synteny with rice and *Brachypodium* genomes as a model for positional cloning of several disease resistance genes (e.g. *YrH52*, *PmG3M*, and *Yr36*) and a high grain protein QTL (*Gpc-B1*) derived from emmer wheat. So far, we have completed the positional cloning of the high grain protein QTL, *Gpc-B1* (Science 314:1292) and the slow rusting gene, *Yr36* (Science 323:1357-60). *Gpc-B1* was shown to confer consistent increase in protein and mineral concentrations when introgressed into durum and bread wheat varieties. The ancestral wild emmer allele encodes a NAC transcription factor (*TINAM-B1*) that accelerates senescence and increases nutrient remobilization from leaves to developing grains, whereas modern wheat varieties carry a non-functional allele. *Yr36* is effective only under relatively high temperatures and provides partial resistance to stripe rust. A gene designated *WKS1* was found to be completely linked to *Yr36*. *WKS1* has a novel architecture with a kinase and a START lipid-binding domain. Mutation and complementation analyses confirmed that *WKS1* is *Yr36*. The absence of functional *Gpc-B1* and *Yr36* alleles in modern germplasm suggests a broad potential impact of these genes in breeding of cultivated wheat varieties and demonstrates the potential of the wild emmer wheat gene pool for improvement of cultivated wheat.

### IL30 - Allelic diversity of fungal disease resistance genes in wheat

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In the last hundred years, the development of improved wheat cultivars has led to the replacement of landraces and traditional varieties by modern cultivars. This has resulted in a decline in the genetic diversity of agriculturally used wheat. However, the diversity lost in the elite material is well preserved in crop gene banks. Therefore, the gene bank accessions provide the basis for genetic improvement of crops for specific traits and an insurance against possible future threats to agricultural production.

We have undertaken large scale molecular allele mining to isolate new alleles of the powdery mildew resistance gene *Pm3* from wheat gene bank accessions. The search for new *Pm3* alleles was carried out first on a set of germplasm identified by the focused identification of germplasm strategy (FIGS), and second on a geographically diverse set of 733 wheat accessions originating from 20 countries. *Pm3* specific molecular tools as well classical pathogenicity tests were used to characterize the accessions. Based on this strategy, we have isolated by now a total of nine new functional *Pm3* alleles. Previously, during hundred years of wheat resistance breeding, a total of seven *Pm3* alleles had been identified. The new resistance alleles were isolated from accessions from Turkey, China and Nepal. Thus, the repertoire of functional *Pm3* alleles now includes a total of 17 genes, making it one of the largest allelic series of plant resistance genes. The combined information on resistant and susceptible *Pm3* sequences will allow to study molecular function and specificity of functional *Pm3* alleles. This study demonstrates that molecular allele mining based on both FIGS defined germplasm as well as geographic definition of accessions are useful strategies to rapidly characterize the diversity of gene bank accessions at a specific genetic locus of agronomical importance. The identified wheat accessions with new resistance specificities can be used for marker-assisted transfer of the *Pm3* alleles to modern wheat lines.

### IL31 - Converging evidence for genes of basal defense in barley

Patrick Schweizer, Dimitar Douchkov, Annika Johrde, Axel Himmelbach, Götz Hensel, Jochen Kumlehn

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Quantitative pathogen resistance is of high importance to plant breeders due to its durability. However, it is usually controlled by multiple quantitative trait loci and therefore, difficult to handle in practice. Knowing the genes that underlie quantitative resistance would allow its exploitation in a more targeted manner. In order to identify genes that mediate quantitative resistance of barley to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*) we have combined functional-genomics approaches based on transcript profiling and transient-induced gene silencing (TIGS) with an association-genetic and a QTL-mapping approach. Starting with well over 1,000 gene candidates that are either up-regulated by pathogen attack, that have been mapped to one selected QTL region on chromosome 5H, or that belong to potentially important multigene families for disease resistance, we have obtained a first shortlist of approximately 10 candidates with converging evidence for an important role in quantitative disease resistance of barley. These candidates are being, or shall be, validated by larger-scale re-sequencing, QTL fine mapping, silencing in transgenic plants, TILLING, and by transient haplotype complementation assays. We think that the integration of functional-genomic with genetic approaches allow us to rapidly zoom into candidate-gene lists and genetic intervals of interest and hold the promise to accelerate the discovery of genes underlying complex, quantitative traits in crop plants.

## IL32 - Strategies to develop and deploy biofortified crops

Joe Tohme, Wolfgang Pfeiffer

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Micronutrient malnutrition, primarily the result of diets poor in bioavailable vitamin A and minerals such as iron and zinc, affects more than one-third of the world's population, especially women and preschool children who are at risk of premature death, lower cognitive capacity, and poor quality of life. The costs of these deficiencies in terms of lives lost, economic growth, and poor quality of life are staggering. To address such major global health problem, HarvestPlus, a global alliance of institutions and scientists, seeks to develop and deploy varieties of food staples, which are high in iron, zinc, and provitamin A through an alliance of scientific institutions and implementing agencies in developing and developed countries. HarvestPlus concentrates its efforts on staple foods consumed by the most of the world's poor in Africa and Asia; rice, wheat, maize, cassava, sweet potatoes, beans and pearl millet. The development and deployment of high iron wheat and rice using transgenic strategies with ferritin and iron transporters genes and high pro vitamin A cassava using a breeding strategy will be presented.

### IL33 - Improving the health benefits of wheat

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In addition to providing calories and protein, wheat is also an important dietary source of fiber, phytochemicals, vitamins and minerals. All of these components are concentrated in the outer layers of the grain which are removed as the bran on milling, and they are considered to be responsible for the health benefits associated with the consumption of wholegrain cereal products. The EU FP6 HEALTHGRAIN project has therefore focused on increasing the benefits of these components to consumers, through a combination of improved grain composition and innovative processing. My talk will focus on dietary fiber, and in particular on the arabinoxylans (AX) and  $\beta$ -glucans which are the major dietary fiber components in both whole grain and white flour, and will draw on work carried out by colleagues within the HEALTHGRAIN program as well as our own studies at Rothamsted.

In order to determine the range of variation in composition available to breeders we initially carried out a "diversity screen" of a collection of 150 wheat varieties of diverse origin. This showed substantial variation in the amount and composition of AX and further analyses of 26 varieties grown in six environments (site x year combinations) showed that this variation was highly heritable.

Further variation in composition is being generated by transgenesis, by isolating candidate genes for enzymes of  $\beta$ -glucan and AX synthesis and either suppressing their expression using RNAi or increasing their expression by inserting additional gene copies under the control of a strong endosperm-specific promoter.

Analysis of these lines has shown that changes in the amount, composition and properties of  $\beta$ -glucan and AX can be achieved, allowing the dietary fiber composition to be fine-tuned to improve its functional properties.

These studies should contribute to the development of novel wheats and wheat products with enhanced benefits for consumers.

## IL34 - Nutrigenomics: New functions for "old" molecules

Fabio Virgili

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The application of genomic technologies to nutrition has recently given rise to a new multidisciplinary scientific branch named *Nutrigenomics*, defined as the study of the interactions between dietary components and the entire genome. Vitamin E will be reported as a paradigm for an "omics" approach to nutritional sciences.

Vitamin E is a generic term used to indicate 8 different vitamers, namely  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocopherol (TOC) and tocotrienol (TT). This term and the related activity was originally based on the capacity of countering fetal re-absorption in deficient rodents. In humans, Vitamin E activity was initially considered to be merely related to the antioxidant properties of the tocolic chemical structure. There are now strong evidences for a large more complex spectrum of specific functions for each vitamer. In the last years, several reports have shown that TTs, but not TOCs, specifically inhibit proliferation and induce apoptosis in a large number of cancer cells, independent of their putative antioxidant properties. However, the molecular mechanism(s) involved in TTs action is partially unknown.

On the basis of a transcriptomic platform, we have recently demonstrated a novel mechanism for TTs activity that involves estrogen receptor (ER) signaling. *In silico* simulations and *in vitro* binding analyses indicate a high affinity of specific forms of TTs for ER $\beta$ , but not for ER  $\alpha$ . Moreover, we have demonstrated that specific TTs increase ER  $\beta$  translocation into the nucleus which, in turn, activates the expression of estrogen responsive genes (*MIC-1*, *EGR-1* and *Cathepsin D*) in breast cancer cells only expressing ER $\beta$  cells (MDA-MB-231) and in cells expressing both ER isoforms (MCF7). The binding of specific TT forms to ER $\beta$  is associated with alteration of cell morphology, caspase-3 activation, DNA fragmentation and apoptosis. Finally, we have indications that specific TT forms are able to activate an "endoplasmic reticulum stress response" in other cell lines (e.g. HeLa), independent of ER signaling.

Our studies indicate that specific forms of TTs have a distinct biological activity, significantly different from TOCs. We also propose that TTs should not be pooled together with TOCs within the broad term "Vitamin E" on the sole basis of their putative antioxidant properties. Finally, our studies confirm the potential of the nutrigenomics approach.

## IL35 - Arabidopsis natural variation as model for the use of plant genetic resources

Maarten Koornneef

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The species *Arabidopsis thaliana* has a wide geographical distribution and different accessions show considerable genetic variation for many traits, which we assume includes genetic variation related to adaptation. The analysis of this genetic variation can serve as an example of this type of analysis in crop plants. Because most of the genetic differences between accessions are of a quantitative nature, Quantitative trait loci (QTL) analysis is performed to detect the number of genes involved, as well as the map positions of the respective QTL. For this the Arabidopsis community developed various types of immortal mapping populations. A limitation of RIL populations is that only the variation between the two parents can be studied. To exploit the variation present within a species is genome wide association (GWA) mapping for which densely SNP genotyped natural accessions have been used. This genotyping will be replaced soon by the ongoing resequencing of genomes of many accessions. A compromise between biparental mapping and GWA is the use of mapping populations derived from multiple parental lines.

The final identification of the genes underlying natural variation requires in the fine-mapping of the QTL and their cloning which requires testing the effects of the different alleles using transgenic plants. However in case of recessive loss of function alleles, the absence of complementation when the recessive variant is crossed with a loss of function mutants is also a solid proof. The latter approach requires induced mutants that are available for almost all genes in Arabidopsis.

The possibility to identify natural genetic variation resulting in phenotypic variation is demonstrated by the large number of QTL that have been cloned. Examples of successful QTL cloning in Arabidopsis are the isolation of genes for developmental and physiological traits such as seed dormancy and flowering time. Complex traits such as growth differences under various environmental conditions, often show complicated inheritance due to epistatic interactions as is demonstrated by epistatic interactions that affect both growth and disease resistance. The use of natural variation to perform systems biology using the genetic co-location to identify the causal factor of networks, was demonstrated in studies using Arabidopsis in which RIL populations are analysed using various 'omic' technologies.



## **IL36 - Plant Phenotyping: Quantitative analysis of plant structure and function on mechanistic, high-throughput and field level**

Ulrich Schurr

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Plant Phenotyping is a key challenge to link gene function with performance in specific environmental settings. Increasingly technical setups are developed and become commercially available that allow quantification of relevant parameters. In parallel, new, mostly non-invasive technologies are developed and implemented into concepts that allow novel insights in the dynamic characteristics of plants above- as well as belowground. These concepts aim at all there scales of phenotyping - the high-resolution analysis for mechanistic understanding, the high-throughput approach for analysis of large numbers of genotypes as well as on field phenotyping. The talk will provide an overview on recent developments in technologies as well as the application of phenotyping platforms in the triangle between defined genotypes, sensors and relevant simulations of environments. An outlook will be presented on future technologies and the integration of non-invasive and invasive analysis of plants.

## IL37 - Metabolomics: Tools and applications

Patrick Giavalisco

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Metabolomics, the analysis of all small molecules in a given cell, is becoming an integral part of many life science studies. Mass spectrometry coupled to different chromatographic techniques is becoming more and more central to the analysis of these compounds. The different property of molecules, ranging from highly un-polar water insoluble lipids to highly polar water soluble primary metabolites, still requires the development of novel experimental strategies and bioinformatic tools. Nowadays two main strategies are used to tackle complex metabolomic mixture: One is the targeted analysis, where only a limited number of metabolites is extracted from the measured data. Such a selective, compound specific, approach is straight forward in the analysis since it does not suffer from annotation problems. Unfortunately a large part of the information contained in the extracts remains un-interpreted and un-exploited. The alternative approach, the un-targeted analysis, tries to overcome this problem by considering every measured signal. This more global strategy therefore has to cope with a number of problems, including accurate compound detection, discrimination of biological-, from contaminating, non-biological signals and last but not least, the proper annotation of peaks of interest. This presentation will provide results from a differential isotope labeling approach overcoming part of the aforementioned problems, enabling a straight forward analysis of complex metabolic mixtures, including primary, secondary and lipophilic metabolides derived from a single sample.

### IL38 - Understanding epiallele formation and inheritance in response to environmental cues in plants

Serena Varotto

Department of Environmental Agronomy and Crop Production, University of Padova, Italy

Epigenetics is defined as the study of heritable traits that do not depend on the primary sequence of DNA. The discovery of the importance of epigenetic mechanisms acting on chromatin to regulate global gene expression has revealed how heritable variation need not be sequence-based. Particularly, environmental factors can induce novel variation through the activation of specific epigenetic mechanisms that determine mutations of spatial and temporal pattern of gene expression. Plants have been shown to adapt to the changing environment by altering gene expression and by destabilizing the genome: the decrease in genome stability in response to environmental stress might be sequence independent. Moreover, there is sufficient evidence that environment can cause plants to grow differently and that the induced phenotypic changes are transmitted to the progeny and remain stable for several generations. In fact, increasing data suggests that an important fraction of phenotypic variability is of epigenetic origin. This evidence also indicates that the epigenetic component of phenotypic variation might have played an important role in the microevolution of natural population.

The aim of the European Project AENEAS that I am coordinating is elucidating the links between environmental stresses and epigenetic memory and in particular determining its stability at the transgenerational level. In fact the genetic improvement of crop plants not only depends upon generating new diversity in tolerance to abiotic stresses but it also requires that selected tolerance can be passed on from one generation to the next. The concept of the epigenetic allele (epiallele), that is an allele showing a heritable difference in expression as a consequence of epigenetic modifications, it is of particular interest in the context of plant improvement, where newly generated variability need to be heritable in order to be exploited. Although it is well known that environmental stresses can alter epigenetic states in plants, mechanistically by producing or removing epigenetic marks, we lack information on stability throughout mitosis and meiosis of the newly formed epigenetic states.

A wider understanding of how environmental induced acquired traits are inherited might show that there is much more to inheritance than genes and give a wider prospective to plant improvement.

### IL39 - Perspectives of plant genomes analysis

Elisa Caniato, Alessandro Vezzi, Alessandro Albiero, Davide Campagna, Riccardo Schiavon, Michela D'Angelo, Gianpiero Zamperin, Claudio Forcato, Nicola Vitulo, Giorgio Valle

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The advancements in DNA sequencing have opened the possibility to carry out *de novo* sequencing projects at a reasonable cost and in a short time. The technology is still evolving very rapidly as several "third generation" instruments have been announced, however at the moment two main types of DNA sequencers are available: 1) the Roche-454 that is based on pyrosequencing and produces about 1 million reads of "long" reads of 450 bases per run; 2) the Illumina-GA and the Applied Biosystems SOLiD that are based on different technologies and produce 150-300 million "short" reads of 50-75 bases per run.

*De novo* assembly of genomic sequences have been successfully achieved using either long or short reads; long reads are much easier to assemble and a relatively low coverage (such as 15x) is sometime enough to succeed. Unfortunately, the cost per base of long reads is much higher than short reads. On the other hand, the assembly of genomic sequences from short reads requires a very high coverage (such as 70x) and very powerful computing facility.

Here we propose a mixed approach that makes use 454 shotgun reads coupled with SOLiD "mate-pairs". The combination of the two types of data allows a considerable reduction in sequencing costs. Moreover, the assembling algorithm that we propose requires a relatively small computing facility.

Most shotgun assembly methods are based on two main steps. Firstly, overlapping sequences are assembled into contigs. Secondly, mate pairs (i.e. the pair of reads obtained at the end of the same library insert) are used to sort the contigs, giving them an order and direction into the "scaffold". This second step is not based on sequence alignment, but only on the relative position of the mate pairs within each other and within their respective contigs.

We use Newbler (V.2.3 - Roche) to obtain contigs from the 454 reads, while for the scaffolding we have developed a new algorithm that uses PASS (<http://pass.cribi.unipd.it>) to align the SOLiD reads on the 454 contigs and CONSORT, a new bioinformatic tool that we designed to sort contigs into scaffolds.

We applied CONSORT to integrate SOLiD mate pair data and 454 data for the assembly of the Tomato genome. The results are very satisfactory, showing a considerable improvement in the estimate of gap size as well as in the scaffolding of the contigs.

## IL40 - Capacity building in genomics of plant genetic resources within the Generation Challenge Program

Carmen de Vicente

The Generation Challenge Program; hosted at CIMMYT, Int APDO Postal 6-641, 06600 Mexico DF, Mexico

Since 2004, Generation Challenge Programme ([www.generationcp.org](http://www.generationcp.org)) of the Consultative Group on International Agricultural Research (CGIAR) has used advances in molecular genetics to understand and combine the wealth in global stocks of crop genetic resources and by doing so to reach its goal of setting the foundation for a new generation of plants that meet small-holder farmer needs. Noteworthy of this endeavour it is the development of genomic resources, especially for orphan crops, those less-endowed with research funding because they benefit smaller, not less important, communities. The presentation will summarize the progress achieved in generating genomic resources for food security crops in the course of the last six years. It will also introduce the molecular marker toolkit, which aims to provide easy and unlimited access to existing information on molecular markers used in breeding programs; the Genotyping Support Service (GSS), which offers cost-efficient genotyping services, both for fingerprinting and analysis of genetic diversity and for molecular breeding, acting as the much-needed bridge between laboratory and field research worldwide; and a plan for establishing Communities of Practice to promote and sustain the use of molecular markers among researchers working with crops in developing countries.

## IL41 - Exploiting the diversity of crop plants: Present state and future challenges

Andreas Graner

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Ex-situ conservation of plant genetic resources represents the major backbone to maintain the intra- and interspecific diversity of many important crop plant species. By the turn of the century than 6 million seed samples were stored in more than 1000 ex situ collections worldwide. Arguably, plant genetic resources (PGR) are required to develop improved cultivars that meet the manifold challenges agricultural production will be exposed to in the future. However, the vast diversity resting on the shelves of genebanks has been tapped into only marginally. Future challenges regarding the utilization of PGR will emerge in two areas: (i) improvement in phenotypic analysis and (ii) the generation and deployment of genetic information. As to phenotypic analysis, systematic screens of genebank collections so far remained restricted to few traits only, such as major resistance genes, that show high heritabilities and can easily be scored. The availability of new sensing and imaging technologies is expected to give access to large-scale analysis of quantitative traits or components thereof. Regarding the second field, the application of molecular genetic approaches opens many entry points for an improved utilization of PGR. Availability of a comprehensive set of SNP markers for major crop species such as barley allows for high density fingerprinting of large number of individuals and the genetic analysis of quantitative traits by performing genome wide association scans. The ever-increasing amount of genomic sequence data will facilitate the systematic exploitation of intergenomic information and accelerate the isolation of traits by map based cloning, even in complex genomes. Knowledge of the genes that underlie agronomic traits will help unveiling their allelic diversity by systematically mining genebank collections for novel alleles. Despite the improved access to genes and alleles, their deployment in breeding programs is frequently hampered by low levels of meiotic recombination. Here the application of GMO approaches may facilitate the rapid and targeted transfer of genes especially from wild relatives into adapted breeding lines.

# *Selected Lectures*





## SL01 - Whole genome resequencing for crop improvement

Bicheng Yang<sup>1</sup>, Xun Xu<sup>1</sup>, Xiaosen Guo<sup>1</sup>, Wen Wang<sup>2</sup>, Jinsheng Lai<sup>3</sup>, Ruiqiang Li<sup>1</sup>, Jun Wang<sup>1</sup>

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Obtaining the genome sequence of a wide range of individuals of a species will generate vast amounts of informative datasets and enable the rapid discovery of much greater genome-wide sequence variation than has been identified previously. With the decreasing cost of sequencing, the genetic maps of many species are getting increasingly dense, a great improvement for plant breeding and selection. Also, a wealth of knowledge will be gained from comparative genomic analyses within and across species, as how plants grow, function and survive different ecological conditions and various environmental stresses. Whole genome resequencing approach has been successfully used in rice and maize studies to identify evolution patterns during domestication and to develop efficient ways to discover domestication genes. We re-sequenced 25 representative cultivated rice and 25 wild rice, and developed the genome variation maps containing about 8.4 million SNPs. With a combination of conventional population genetic methods and a new tree-thinking method, about 500 genes were detected with strong selection signals in cultivated rice and thus could be candidate domestication genes. Many of them have functions related to growth, architecture, maturity, productivity or resistance and can be further applied in breeding programs. Similar study has been carried out in maize for whole genome resequencing of several maize inbred lines. A large number of SNPs and InDels were identified. Hundreds of genes that are present in one haplotype but absent in another were detected. More than 100 large chromosomal intervals with low-sequence-diversity represent putative selective sweeps which may be related with domestication. Limited amounts of intra-chromosomal recombination during pedigree breeding were identified. Whole genome resequencing will have far reaching implications for improving breeding strategies and plant varieties to meet the world's growing demand on plant production.

## SL02 - Use of EcoTILLING to identify natural allelic variants of rice candidate genes involved in salinity tolerance

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Soil salinity is a major problem affecting rice (*Oryza sativa* L.) productivity worldwide. To accommodate the growing demand for rice, more and poorly irrigated land is being brought into cultivation. Due to the increasing importance of salt stress in rice, the comprehension of the underlying molecular basis is nowadays crucial. The main objective of this work is to study the diversity of rice cultivars in order to find the correlation between allelic variation and contrasting phenotypes under salt stress. Our main goal is to find rice responsive alleles for salinity tolerance. We are using the EcoTILLING strategy to characterize natural alleles at specific *loci*, across approximately 400 germplasm accessions (of worldwide provenance). This mini-core collection is representative of the large morphological, physiological, and ecological variation available in domesticated rice. After DNA isolation from the 400 accessions, we pooled each sample with the respective reference variety 'Nipponbare' or/and 'IR64'. A crude extract of the endonuclease CEL1 was purified from celery (CJE- Celery Juice Extract) and used for digestion of mismatches and polymorphism detection in agarose gel electrophoresis. Primers were designed to amplify 1Kb fragments covering the whole gene region of 5 targets, namely *OsNHX1*, *OsHKT8*, *SaT*, *OsRMC* and *OsCPK17*. All these genes have been previously described and characterized as related to the increase of salinity tolerance in rice, through different mechanisms such as Na<sup>+</sup>/K<sup>+</sup> ratio equilibrium, signaling cascade and stress protection. The results obtained so far confirmed the existence of different haplotypes for all genes. Preliminary sequencing results proved the existence of SNPs (Single Nucleotides Polymorphisms) and small indels in *SaT*, resulting in aminoacid change in SALT protein. Phenotyping experiments are underway in order to identify putative correlations with the allelic variants. We will present our most recent data and discuss the utility of EcoTILLING and SNP discovery in breeding efforts for salt tolerance.

### SL03 - Spatial and temporal quantitative trait loci (QTLs) mapping of ions accumulation on chromosome-1 of rice (*Oryza sativa* L.) under salt stress

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World agriculture is at the risk of soil salinisation especially in arid and semi-arid regions. Identification of genomic regions, associated with complex traits such as tolerance of rice to higher concentration of salt is a key research issue in the world. A mapping population of 120 recombinant inbred lines (RILs) derived from the cross between Co39 (lowland, Indica) and Moroberekan (upland, Japonica) rice (*Oryza sativa* L.) cultivars, was used to map QTLs associated with leaf ions accumulation under salinity stress on chromosome-1. Na<sup>+</sup> and K<sup>+</sup> concentration and K<sup>+</sup>/Na<sup>+</sup> ratio in the sap of different parts of the plant were recorded after 7 and 21 days exposure to salt stress of 100 mol m<sup>-3</sup> NaCl + 5.0 mol m<sup>-3</sup> CaCl<sub>2</sub> in a flood bench system at Pen y Ffridd Research Station, Bangor University, UK. The integrated genetic map of rice chromosome-1, consisting of 45 molecular markers had a distance of 201.2 cM with an average interval of 4.57 cM between markers saturating a region that has previously been identified as a hot-spot for ions accumulation QTLs. QTL analysis identified several distinct regions containing QTLs for Na<sup>+</sup>, K<sup>+</sup> and K<sup>+</sup>/Na<sup>+</sup> ratio on chromosome-1. The highest Na<sup>+</sup> concentrations were recorded in the leaf sheaths. A total of 24 QTLs for ions accumulation were detected between 80 to 101 cM region of our genetic map. We identified separate regions that were active in controlling ion concentration during 21 days salt stress, suggesting that multiple genes were acting to regulate leaf sap ion concentrations in rice. The SSR markers such as RM8094, RM3412, RM10746 and RM493 on rice chromosome-1 were linked to salt stress related QTLs and can be used for marker assisted selection after their authentication in wide genetic backgrounds.

### SL04 - Whole-genome shotgun sequence assembly and other genomic resources for flax (*Linum usitatissimum*)

Michael K Deyholos, R McKenzie, D Pinzon-Latorre, S Khan, N Hobson, M Roach, M DePauw, A Badhan

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Flax (*Linum usitatissimum*) is a self-pollinating, diploid species that has been used historically as a model for developmental studies and can be grown at high density in the laboratory. Flax is also a major crop with interesting cell wall and seed oil properties and is a close relative of poplar. Because flax has a different evolutionary history than other models (e.g. *Arabidopsis*), it may be possible to obtain unique mutant phenotypes that are broadly informative about plant development. We are employing both forward and reverse genetics approaches in a 20,000 M2 family EMS population to characterize genes involved in vascular tissue development in the flax stem. In collaboration with Beijing Genomics Institute-Shenzen, we have also assembled 50X Illumina/Solexa sequence coverage of the 700Mb genome of flax, with a resulting N50 contig size of 20kb and a resulting N50 scaffold size of 320Mb. This Illumina-only assembly is highly similar to a random sample of fosmid and BAC sequence, and the ~44,000 predicted genes are also consistent with known ESTs for this species. The short-read WGS approach therefore appears to be a very efficient method for obtaining genomic sequence of the gene-rich component of the flax genome.

## SL05 - Whole-genome sequencing as a tool for plant genetic resource analysis

Robert J Henry

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Advances in DNA sequencing are providing opportunities to move beyond the conventional locus specific bar-coding of plants to the use of DNA sequence data from the whole genome. Sequencing of total plant DNA preparations permits analysis of the whole chloroplast genome from even a small data sample and the nuclear DNA from smaller plant genomes. Sequencing of fractions enriched in genes by PCR amplification, hybridization with specific probes or cDNA sequencing allows more targeted exploration of genetic variation especially in very large genomes. These strategies have been applied to define very large numbers of polymorphisms for genetic mapping and to conduct association genetic in large populations and with large numbers of genes. Examples of these approaches that will be discussed include sugarcane, rice, barley, wheat, almond, coffee, eucalypts and wild grasses.

## SL06 - Expression partitioning of homoeologs during development and evolution of allopolyploid cotton (*Gossypium*)

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Polyploidy is a vital enforcement in the evolution and species diversification of flowering plants. It provides raw material for the evolution of novelty by relaxing purifying selection on duplicated genes, and induces an extensive array of evolutionary responses. The genus *Gossypium* includes classic allopolyploids arising from a biological reunion of divergent diploids from different continents 1-2 MYA. This serendipitous genomic merger in the evolutionary history of cotton generated a spectrum of responses, including interference and concurrence of ancestral gene expression patterns. Using powerful and high-resolution technologies including genome-specific, mass-spectrometry technology and custom designed microarray platforms, we study global transcriptional changes with relative contribution of 'homoeologs' (pairs of genes duplicated by polyploidy) in differing tissues from synthetic and natural *Gossypium* allopolyploids and reconstructed F1 hybrid. Genomic merger and allopolyploid evolution followed by polyploid formation induces massive alteration in homoeologous-gene expression with transgressive expression patterns, although neither of the parental diploid genome shows genome-wide genomic dominance. Expression patterns in different tissues show transcriptional biases such that there is an unequal contribution of two genomes to the transcriptome, termed as subfunctionalization. Levels of instantaneous transcriptional subfunctionalization are substantial as in result for long-term retention of duplicates. Using a microarray that simultaneously distinguishes transcript levels for each homoeolog, we also demonstrate that the expression ratios change extraordinarily during fiber development. Interestingly, expression of the biased genes was shifted strongly toward the agronomically inferior parental D-genome. Direct comparison of wild and domesticated accessions highlighting the impact of domestication on homoeolog expression modulation reveals the temporal transition of transcriptional biases during polyploidy rather than domestication. These results provide a novel temporal perspective on expression partitioning and evolution of duplicate genomes adding to our understanding the importance of polyploidy and novel gene recruitment following genome doubling in plants.

### SL07 - The genetic diversity and evolution of field pea (*Pisum*) studied by high-throughput retrotransposon-based insertion polymorphism (RBIP) marker analysis

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The genetic diversity of crop species is the result of natural selection on the wild progenitor and human intervention by ancient and modern farmers and breeders. The genomes of modern cultivars, old cultivated landraces, ecotypes and wild relatives reflect the effects of these forces and provide insights into germplasm structural diversity, the geographical dimension to species diversity and the process of domestication of wild organisms. This issue is also of great practical importance for crop improvement because wild germplasm represents a rich potential source of useful under-exploited alleles or allele combinations. The aim of the present study was to analyse a major *Pisum* germplasm collection to gain a broad understanding of the diversity and evolution of *Pisum* and provide a new rational framework for germplasm core collections of the genus. 3020 *Pisum* germplasm samples from the John Innes *Pisum* germplasm collection were genotyped for 45 retrotransposon based insertion polymorphism (RBIP) markers by the Tagged Array Marker (TAM) method. The data set was stored in a purpose-built Germinate relational database and analysed by both principal coordinate analysis and a nested application of the Structure program which yielded substantially similar but complementary views of the diversity of the genus *Pisum*. Structure revealed three Groups (1-3) corresponding approximately to landrace, cultivar and wild *Pisum* respectively, which are resolved into 14 Sub-Groups, many of which correlate with taxonomic sub-divisions of *Pisum*, domestication related phenotypic traits and/or restricted geographical locations. Genetic distances calculated between these Sub-Groups are broadly supported by principal coordinate analysis and these, together with the trait and geographical data, were used to infer a detailed model for the domestication of *Pisum*. These data provide a clear picture of the major distinct gene pools of the genus *Pisum* and their spatial relationships with each other. Second, independent domestications of *P. sativum* ssp. *abyssinicum* and *P. sativum* are supported and new detailed models for them are proposed involving specific subsets of wild *Pisum*.

### SL08 - Impacts of interspecific hybridization on genome structure and agronomic traits via retrotransposon activation in *Brassica napus*

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Interspecific hybridization is a significant evolutionary force and a powerful approach for crop breeding. Extensive substitution of the AA subgenomic components in *Brassica napus* ( $A^nA^nC^nC^n$ ) with the *B. rapa* ( $A^rA^r$ ) subgenome by interspecific hybridization resulted in a sort of new type *B. napus* ( $A^rA^rC^nC^n$ ). In this study, genome-wide variations in sequence and chromosome structure between the A genomes of the two *Brassica* species were investigated. In addition to the introgression of  $A^r$  genomic components, a remarkable range of novel sequence variations, together with many chromosomal rearrangement events, were detected across the genome in a population of recombinant inbred lines (RILs) of the new type *B. napus*. The novel genome alterations occurred mainly during the early processes of genome stabilization after interspecific hybridization. Strong evidence was obtained that retrotransposons (RTs) were reactivated during the stabilization process and played an important role in the generation of the novel genomic variations. The RTs inserted preferentially into certain chromosomes such as A4 and A8. The novel genomic variations, together with introgression of the  $A^r$  subgenome, had a significant effect on the important agronomic traits of the RIL population. A considerable number of novel variations contributed to genetic enhancement of the new type *B. napus*. In particular, the activity of RTs might have a significant impact on the yield and yield-related traits of new type *B. napus*, and this could provide new insights into the basis of trait variation and interspecific heterosis for plant geneticists and breeders.

## SL09 - Development of molecular markers for large-scale implementation for marker-assisted selection in lupin breeding

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Marker-assisted selection (MAS) in plant breeding requires that the markers to be closely linked to genes of interest, be cost-effective, and applicable to a wide range of breeding germplasm. We applied the DNA fingerprinting technology called "microsatellite-anchored fragment length polymorphisms (MFLP)", which has proved to be highly efficient in generating candidate markers linked to genes of interest, and the markers could be easily converted into sequence-specific PCR markers desirable for routine implementation. To ensure the markers applicable to a wide range of crosses, we developed a strategy of generating multiple candidate markers followed by a validation step to select the best marker before conversion to an implementable form. By applying these techniques, we have developed a number of molecular markers linked to key genes of agronomic interest in lupin, including anthracnose disease resistance, phomopsis disease resistance, low alkaloid gene, pod non-shattering gene and soft seeded gene. In the last five years, about 100,000 lupin plants were tested and selected by molecular markers, representing one of the very few examples in the world of large-scale practical molecular plant breeding on legume crops. Examples are presented to illustrate the molecular strategies by which we successfully developed the implementable markers both for single gene controlled traits and for polygenic controlled trait.

## SL10 - EURIGEN: Characterization of European rice germplasm for stress response traits

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The general objective of the EU-funded EURIGEN project is the characterisation and exploitation of European rice genetic resources of the temperate rice growing area, to enhance competitiveness of Europe in rice production, and alleviation of biotic and abiotic constraints typical of the Mediterranean area. This goal is achievable by means of the acquisition, evaluation and conservation of existing rice accessions, and identification of new genetic materials targeted at sustainable agricultural systems, making use of the most updated genomic tools. The project has two major targets: i) identification and conservation of genetic resources and ii) identification of valuable sources of new genes and alleles for agronomic and quality traits relevant to breeding programs. The main platform of the project is the classification, maintenance and regeneration of the temperate rice germplasm bank. A panel of 455 rice accessions relevant for breeding programs in European growing areas were analysed at both phenotypic and genotypic level. A centralised seed bank of the collection was established and a DNA biorepository organised in bar-coded 96-well plates was created and made available to the EURIGEN partners. A core collection of 200 rice accessions was selected based upon phylogenetic analyses and phenotyped in the field and controlled conditions for adaptation to biotic and abiotic stresses including blast, reduced water availability and salinity. To identify molecular markers associated with the adaptation traits as well as alleles ensuring the best performance under stress, the 200 accessions were genotyped with 384 SNPs using the high-throughput ILLUMINA BeadExpress genotyping platform. The SNPs were selected in candidate genes involved in stress responses based on literature data and preliminary results from ongoing projects at international level. The integration of phenotypic and genotypic data will enable us to carry out association analyses and valorise the existing natural variation to devise novel strategies of rice improvement in EU countries. The EURIGEN actions pursue the general objectives in accordance with the assessments of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture, and the Council Regulation (EC 870/2004) establishing a *Community Programme on the conservation, characterization, collection and utilization of genetic resources in agriculture*.



### SL11 - Mapping QTL for spikelets per panicle and yield components using an NIL from an interspecific cross between *Oryza sativa* and *O. minuta*

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A near isogenic line (NIL), IL-34 developed by introgressing chromosomal segment from an accession of *Oryza minuta* (2n = 48, BBCC, Acc. No. 101141) into the *O. sativa* subssp. *japonica* cv. Hwaseongbyeo, showed significantly higher number of spikelets per panicle (SPP) than the recurrent parent Hwaseongbyeo. Quantitative trait locus (QTL) analysis in the F<sub>2</sub> generation derived from the cross between IL-34 and Hwaseongbyeo revealed that *spp7*, a QTL was located in the pericentromeric region of chromosome 7. Distribution of spikelets per panicle followed 3:1 ratio for single locus segregation. The additive effect of the *O. minuta* allele at the *spp7* QTL was 23 spikelets per panicle, and 43.6% of the phenotypic variance could be explained by the segregation of the SSR marker RM21596. To fine-map the *spp7* as a step for map-based cloning, we carried out fine-mapping with 3,700 F<sub>2</sub> plants derived from the cross between IL-34 and Hwaseongbyeo, and quantitative evaluation of panicle traits and grain yield was performed. One hundred and eighty-nine F<sub>2</sub> plants having informative recombination breakpoints within the region flanked by two SSR markers RM500 and RM21615 were identified and used for fine mapping of *spp7*. *spp7* was mapped between the SSR markers RM21596 and RM418 which was approximately 441-kb in length based on the physical map of the region. Of great interest, the QTL region also had effect on primary branches (PB), grains per panicle (GP) and grain yield (YD). These results are very informative for transferring or pyramiding *spp7* by molecular marker assisted selection in rice breeding programs and for cloning *spp7* by a map-base cloning.

### SL12 - LD estimation, analyses of diversity and domestication in apple

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For species with a long generation time such as apple (*Malus x domestica* Borkh), gene and/or QTL mapping based on experimental crosses can be very demanding in terms of time and costs. Marker-trait association based on linkage disequilibrium (LD) analysis across germplasm collections can provide an alternative. To implement this mapping strategy, the LD level, the existence of hidden population subdivisions, the transferability of the marker systems and the average nucleotide diversity should be investigated. We assembled a collection of about 200 apple accessions, including more than 100 cultivars and representing almost all the wild apple species. The collection was genotyped using several hundreds SNPs originally discovered within the FEM-IASMA Golden Delicious genome sequencing project and 35 publicly available SSRs. For portions of the collection, re-sequencing of 23 genic regions and several pairs of genes within distances of 60-200 Kb were also obtained. The average transferability of the Golden Delicious SNPs was 41% within the cultivated apple accessions whereas a much lower rate was observed with most of the wild species. LD level was shown to decay at distances longer than 1 cM, while significant level of *r*<sup>2</sup> was observed at closer distances. Molecular phylogeny of the genus *Malus* based on our molecular data broadly agree with the standard taxonomy of the genus and confirmed the tight relationships of *M. x domestica* with accessions of the wild apple species *M. sieversii*.

### SL13 - Recent progress in the utilisation of synthetics for wheat improvement

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Since early 2000, there have been significant national and international interests in the utilisation of synthetic hexaploids as sources of germplasm aimed at developing adapted, stress resistant and high yielding wheat cultivars. Of recent, yield improvement of wheat in rain-fed environment have plateaued accentuated by *the repeated occurrences of drought and heat giving impetus to the exploitation of natural diversity* in synthetic hexaploids as a means of improving productivity. Results from evaluation of synthetic backcross derived lines (SBLs) in diverse environment showed that there was consistent transgressive segregation for yield in all the locations with the magnitude of yield improvement over the recurrent parent ranging from 32 to 70% depending on the environment. The performance of the best SBLs to the recurrent parent, ranged from 26 to 58% depending on the environment. The molecular and physiological bases of the enhanced performances are being investigated. Further, we had previously shown that synthetic hexaploid wheat possess resistance to several different diseases. The feasibility of association mapping was tested on these. Three hundred and thirty synthetic hexaploids previously evaluated for resistance to root diseases – cereal cyst nematode (CCN, *Heterodera avenae* and two species of root-lesion nematode (RLN, *Pratylenchus neglectus* and *P. thornei*) were genotyped with DArT and linked molecular markers previously mapped to resistance loci for the three diseases for validation and discovery of potentially novel loci involved in conferring resistance to these root pathogens. Highly significant association of DArT markers with quantitative trait loci (QTLs) for resistance to CCN and the two RLN was detected. Clusters of disease resistance QTLs were located at multiple distinct genomic locations. There were coincident QTLs for CCN and *P. neglectus* on chromosome 1A, 1D, 2D, 3D, 5D, 6B and 7B; CCN and *P. thornei* on chromosomes 4B and 6A; *P. neglectus* and *P. thornei* on chromosomes 3B, 6B and 7A. Of significance is the identification of novel QTLs that are involved in the control of multiple root diseases in wheat. The linked DArT markers could be converted into diagnostic markers for use in marker assisted selection for selection of multiple disease resistance. The dataset provides valuable new information on the feasibility of using association genetics to position loci controlling multiple disease resistance in SHWs. The newly identified resistance loci will enrich the genetic basis of resistance in wheat breeding programs.

## SL14 - Mapping of QTL contributing to root lesion nematode resistance in barley

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Root-lesion nematodes of the genus *Pratylenchus* are significant pests in crop cultivation throughout many parts of the world. In the past years considerable yield losses have been recorded in German barley (*Hordeum vulgare* L.) production. This work aims at selecting barley accessions with resistance to the root-lesion nematodes *P. neglectus* and *P. penetrans* in a greenhouse resistance test and to develop molecular markers closely linked to the putative resistance genes. In the first step, 565 barley accessions encompassing cultivated (*Hordeum vulgare*) and wild species (*Hordeum spontaneum*) were screened for resistance against *P. neglectus* and 200 against *P. penetrans*. The number of nematodes per plant ranged from 375 for the most resistant accession up to 12000 for the most susceptible accession against *P. neglectus* infection and from 400 to 3400 against *P. penetrans* infection. In the second step, a detailed QTL analysis was conducted for these nematodes resistance in barley using 140 doubled haploid (DH) lines from an Igri x Franka cross. 551 DArT marker data were generated and subsequently used for QTL analysis. Previously available RFLP, SSR, SNP marker information on 70 DH lines was also utilized for QTL analysis. Nematode resistance screening was carried out in greenhouse in successive years. For *P. neglectus*, a total of eight QTLs, located on five (2H, 3H, 5H, 6H and 7H) linkage groups, were identified. Out of these, one QTL was found to have a LOD score of 8.7 and R<sup>2</sup> value of 21%. The remaining seven QTLs were classified as minor or moderate with a LOD score ranging from 2.1 to 4.7 and R<sup>2</sup> value ranging from 6 to 16. For *P. penetrans* resistance, three QTLs were identified located on three (2H, 6H and 7H) linkage groups. Out of these, one QTL was found to have a LOD score of 9 and R<sup>2</sup> value of 23%. The remaining two QTLs were classified as minor with a LOD score ranging from 2.3 to 4 and R<sup>2</sup> value ranging from 7% to 11%. These results provide a basis for establishing a marker test which shall replace the time consuming and expensive greenhouse test.

## SL15 - Association mapping of wheat germplasm employing historical data

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Association-based trait mapping is an innovative methodology in detecting genes and is based on linkage disequilibrium in a collection of unrelated plant material. Studies especially in wheat are rare. We demonstrate the usefulness of historical field data of a winter wheat collection, using a genome-wide assay with diversity array technology (DArT) markers. In total, 520 polymorphic markers were genetically mapped. Two subpopulations were identified by examining the population structure with the programme STRUCTURE. The collection was field trialed and phenotyped for 20 agronomic traits in up to eight different years. The associations and the extent of LD in the collection and the two subgroups were calculated with the programme Tassel 2.1. Correlations between traits across seasons were high in almost all cases. A total of 341 marker-trait associations were detected. The intrachromosomal location of many of these coincided with those of known major genes or quantitative trait loci, but others were detected in regions where no known genes have been located to date. These latter presumptive loci provide opportunities for further wheat improvement, based on a marker approach.



### SL16 - One site vs. the world: The genetic diversity of one natural population of wild barley compared to barley varieties from different countries

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Barley, *Hordeum vulgare* L., is one of the principle cereal crops in the world and is cultivated in all temperate areas around the world. Wild barley, *H. spontaneum*, has been considered to be a large reservoir of genetic diversity and represents the primary gene pool of cultivated barley (*H. vulgare*). The genetic diversity between one natural population of wild barley and barley varieties from different countries were investigated using PCR (Polymerase Chain Reaction) analysis with intron-splice junction (ISJ) primer and long random primers. A total of 101 DNA fragments across all sampled materials were scored; among them, 73 (72.3%) were polymorphic as indicated by their absence in at least one of the 29 accessions tested, which resulted in high variation at the DNA level. Pair-wise genetic dissimilarity ranged from 0.01 to 0.333. The result of this study suggests that the level of genetic diversity of one natural population of wild barley is much higher than that of a collection of barley varieties sampled from various locations around the world. Comparable data were found in an extension of this project with a different marker system, SSR, another wild barley population and more sampling of barley varieties from various parts of the world, lending more support to this conclusion. It is well-known that domestication and selection have resulted in drastic impoverishment of the gene pools of cultivars. Still, it is important to note that the genetic diversity among barley varieties in the entire world, as represented by this sampling, was found to be only about one-third of the genetic diversity in a wild barley population from a site in the Jordan Valley.

### SL17 - SSAP-based phylogenetic analysis reveals different amplification history of *BARE-1* and *Jeli* LTR retrotransposon families in A-genome diploid wheats

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Retrotransposons are the major component of many plant genomes. Current large-scale sequencing efforts reveal a wide diversity of transposable element structure, genomic distribution and evolutionary history; however, less is known about the natural factors affecting retrotransposon activity through the course of evolution. Varying activity of retrotransposon families during different periods in the past can affect the presence/absence polymorphism of retroelement insertions, which can therefore provide new insights into the concerted evolution of mobile elements and their hosts. We have explored the genetic diversity in diploid A-genome wheat (*Triticum boeoticum*, *T. urartu*, *T. monococcum*) using 441 polymorphic markers based on two LTR retrotransposon families: *BARE-1* (superfamily *Ty1/Copia*) and *Jeli* (superfamily *Ty3/Gypsy*). *Jeli* is an interesting example of an A genome specific family, being enriched in the A genome of hexaploid common wheat (*T. aestivum*) as the nulli-tetrasomic analysis demonstrated. The modified Sequence-Specific Amplification Polymorphism (SSAP) technique (Vaugh et al., Mol Gen Genet 1997) was employed for multiplex amplification of genomic regions flanking the retroelements' insertions in 49 diploid wheat accessions, and presence/absence data were scored for each polymorphic SSAP band. We have compared the phylogenetic trees and statistical data obtained for *BARE-1*-based and *Jeli*-based markers. Both retrotransposons provided a fair level of polymorphism and a clear separation of major inter- and intraspecific groups, supported by >75% bootstrap values. However, while the overall structure of phylogenetic trees was similar, *BARE-1* provided better detailed data for the relationships within the A<sup>0</sup>-genome species; *Jeli*, on the other hand, yielded a considerably higher proportion of species-specific markers distinguishing the A<sup>0</sup>-genome wheat *T. urartu* from the rest of the accessions. We conclude that a burst of *Jeli* amplification had taken place during *T. urartu* vs. *T. boeoticum* speciation, while *BARE-1* had been more active later during the intraspecific diversification of einkorn. The utility of retrotransposon-based markers in phylogenetic and diversity analysis is discussed.

## SL18 - Genetics resources of *Vitis vinifera* in the north of Morocco (Rif region): characterization and conservation

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A progressive reduction of the genetic diversity of crop plants is currently occurring. This phenomenon, called «genetic erosion» relates to many crop species and concerns the varieties (local autochthonous varieties, races, primitive cultivars) as well as wild compartments and species. In Morocco, the genetic erosion concerns many crops, including grapes in particular in the Rif region in the north of the country. This area supports a traditional agriculture, mainly based on food producing crops, but an old tradition of grapevine cultivation exists.

Within the framework of a cooperation program between Morocco (Ecole National d' Agriculture de Meknès and University of Tétouan) and France (INRA - Montpellier SupAgro) aimed at the estimation of genetic diversity still existing in the Rif region, a prospection of wild vines was carried out in the north of Morocco (Area of Rif). Approximately 170 individuals were identified in 18 populations distant from 10 to 50 km. The mean number of individuals per population was nine.

This sample was analyzed with 20 nuclear and 3 chloroplast SSR markers. The molecular data was compared with the data obtained for a group of 248 French wild vines (Lacombe et al. 2003), 116 cultivars of North Africa (El Oualkadi et al. 2010) and 92 European cultivars (Le Cunff et al. 2008). In order to check the level of diversity in wild grape in Morocco, its relationship with the cultivated grape and specificity compare to other genetic pools.

The genetic diversity of the wild vines of Morocco is high. Indeed, it is more important than that of the French wild grapevine and the cultivars from North Africa, but weaker than that of the European cultivars, selected to maximize genetic diversity at the 20 SSR markers.

Studies of structuring were then carried out. Within the wild vines, the French and Moroccan samples were well differentiated. The wild compartment (French or Moroccan origin) was also differentiated from cultivated, thus revealing the wild status of this indigenous material. Results detailed on diversity and the structuring will be presented and discussed in relation to the interest of this material for vine growing in Morocco.

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Le Cunff L, Fournier-Level A., Vezzulli S et al (2008) Exploitation of natural diversity in *Vitis vinifera* L.: construction of genetic core collections. BMC Plant Biol 8:31.

## SL19 - Toward safeguarding sorghum production against biotic stresses: Uses of worldwide germplasm collection and genomics-based approaches

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Sorghum, *Sorghum bicolor* (L.) Moench, is the fifth most important cereal crop grown worldwide. Successful development of new sorghum cultivars and hybrids to ensure sustainable production depends largely on the availability of genetic resources (i.e. germplasm bank) with desirable traits such as disease and pest resistance. Recently, one of our research efforts focused on evaluation of a large collection (over 40,000 accessions) of sorghum germplasm, leading to the identification of new sources of resistance to greenbug aphid, *Schizaphis graminum* (Rondani) which has been a major threat to sorghum production in the US and other parts of the world. New sources of resistance to smut head disease, caused by *Sporisorium reilianum* (Kuhn), has also been identified in sorghum using a similar approach. In addition, molecular markers were used to assess the genetic diversity and relatedness among those resistant accessions originated from different countries or geographic regions, which suggest a relatively diverse greenbug resistant sources exist in the sorghum germplasm collection as evidence by over 800 AFLP markers. Furthermore, a mapping project was executed more recently using an F<sub>2:3</sub> population containing 277 individuals in order to dissect the genetic resistance to greenbug into sorghum chromosomal regions. Single marker analysis suggests six SSR markers spread over five chromosomes are significantly linked to host response to greenbug feeding. Composite interval and multiple interval mapping procedures indicated that one major QTL and a minor QTL resided on chromosome 9 are responsible for resistance to greenbug attack. In summary, the newly identified resistant accessions are valuable sources for developing new resistant parents and breeding greenbug resistant commercial hybrids. The molecular markers closely-linked to the respective resistance QTLs will facilitate early selection of breeding lines through marker-assisted selection.

## SL20 - Genomic tools for improving the cereal crop tef (*Eragrostis tef*)

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Tef is among the most important cereal crops in the Horn of Africa particularly in Ethiopia, where it is the number one in terms of acreage. The crop is tolerant to many biotic and abiotic stresses prevalent in the region. In general, tef plays a vital role in food security, nutrition, and income generation to resource poor farmers. Despite its importance tef produces the lowest yield compared to other cereals due mainly to the widespread use of landraces and cultivars lacking desirable agronomic traits such as lodging resistance. Tef is considered as understudied or orphan crop since it is largely neglected by the world scientific community and limited improvement with limited resources has been achieved up till now. A pilot genome sequencing we have recently made from tef has convincingly shown that complete sequencing of this small genome crop is technically feasible and cost-effective. Hence, we have initiated the Global Tef Genome Sequencing Project and currently the sequencing is progressing at the Functional Genomic Center Zürich (FGCZ) in Switzerland, and soon to begin at the Biosciences for Eastern and Central Africa (BecA) in Kenya. The sequencing is performed with the Next Generation Sequencing Platform using different libraries: a fragment library, mate-pair libraries, and BAC libraries. Once the sequencing is completed, the project will focus on sequence analysis and annotation. The Global Tef Genome Sequencing Consortium is being established with members from Ethiopia, Germany, Kenya, Switzerland and USA. The genome sequencing has immediate applications in our other projects particularly in the TILLING Project in elucidating genomic sequences for the genes of interest. TILLING is a non-transgenic and a high-throughput method used to improve plants by identifying novel genetic variations in genes that affect traits of choice. The technique has been successfully applied in major crops such as maize and rice. We implement TILLING to tackle the major yield limiting factors in tef production: lodging and drought. The tef TILLING population consists of about 6,000 M<sub>2</sub> families to be screened for the genes of interest. Two semi-dwarf candidate lines obtained from the screening are being evaluated in the field in Ethiopia.

## SL21 - Molecular diversity in Ethiopian tetraploid wheat

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Ethiopia has the second largest wheat growing area in Sub Saharan Africa, having 877,000 ha of arable land occupied by wheat. A total of 30 populations of 790 individuals of *Triticum durum* originated from ten regions were assayed using a set of 14 wheat microsatellites, representing 7 markers from genome A and 7 from genome B located on 14 different chromosome locations. A total of 347 alleles were detected with average alleles of 24.8 per locus. The highest number of alleles per locus was detected in the A genome with 34, 32, 31, 27, 25, 21, 20 alleles per locus, compared to 30, 29, 16, 12 alleles per locus for genome B, respectively. The highest genetic variation exists in the non-centromeric regions than in the centromeric regions of chromosomes, in both Genome A and B short arms. The mean of polymorphism of all over region ranged from 100 to 92.86%. The analysis of molecular variance, revealed that 72% of the total variation was found within population, 12% among population and 16% among regions. High levels of genetic diversity were existed among regions (17%) in genome A long arm. The highest variation of among populations were detected in Shewa, Bale and Wello (21, 15, 14%), whereas in Hararghe, Gojam & Tigray among populations were low (9%). But, the within populations were existed in high level of diversity in (Tigray, Gojam, Hararghe), Gonder and Arsi, (91, 90, 88%). The highest grand mean of observed heterozygosity were detected in Tigray, Hararghe, Shewa and Wellega (8, 7, 6, and 6%). The highest grand mean diversity was found in Gojam, Wello, Hararghe, Arsi and Wellega (4.1, 4.0, 3.9, 3.8, 3.8), while in Shewa was lowest (2.8). In general, in one or in another way of parameters, every region exhibited significantly higher polymorphism.

## SL22 - Ions transport activity related to plant adaptation to salt stress

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Salinity is one of the most severe environmental stresses affecting plant productivity worldwide. In many plant species, salt sensitivity is associated with the accumulation of sodium (Na<sup>+</sup>) in photosynthetic tissues. Adaptation of plants to salt stress (i.e. resumption of growth after exposure to high soil salinity) requires cellular ion homeostasis. Sodium efflux from root cells prevents accumulation of toxic levels of Na<sup>+</sup> in the cytosol and transport of Na<sup>+</sup> to the shoot. The Na<sup>+</sup>/H<sup>+</sup> antiporter localized to the plasma membrane (SOS1) is the only Na<sup>+</sup> efflux protein from plants characterized so far. We have identified a plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchanger from durum wheat (*TdSOS1*) that catalyses Na<sup>+</sup> efflux and regulates its root/shoot distribution. Heterologous expression of *TdSOS1* in yeast knockout strain AXT3K showed complementation phenotype in medium containing high concentrations of NaCl, when coexpressed with the Arabidopsis protein kinase complex SOS2/SOS3. *TdSOS1* was able to detoxify yeast cells by exclusion of sodium. *In vitro* phosphorylation of *TdSOS1* with the Arabidopsis hyperactive form of SOS2 (T/DSOS2Δ308), showed the importance of two essential serine residues at the C-terminal hydrophilic domain. Mutation of these two Serine residues to Alanine decreased consequently the phosphorylation of *TdSOS1* with T/DSOS2Δ308. In addition, C-terminal deletion at the Serine residues generated a hyperactive form of *TdSOS1* which has a sodium exclusion activity independent from the signalling SOS complex proteins. This could be easily explained by the elimination of the auto-inhibitory domain at the C-terminus part essential for its activation by protein Kinase SOS2. Transgenic *Arabidopsis* plants overexpressing SOS1 have lower Na<sup>+</sup> in the xylem transpirational stream and in shoots compared with wild-type plants. These plants also show enhanced salt tolerance, measured in terms of their growth, ability to flower at high salt concentrations (50-200 mM NaCl), while control plants became necrotic and have failed to flower.

## SL23 - Use of expressed sequence tag resources to reveal multiplex quantitative gene expression profiles in diploid and polyploid wheat genotypes for salt stress tolerance

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Excess amount of salt in the soil adversely affects plant growth and development which directly causes decline in the yield and the quality of produce. Consequently, much effort has been directed toward understanding the molecular and cellular mechanisms of salt stress tolerance in plants. In this study, new generation quantitative expression analyses of salt-stress related genes were investigated in Turkish bread wheat (*Triticum aestivum* cvs. Alpu01, ES14), durum wheat (*Triticum durum* cvs. Ç1252, Meram) and *Aegilops tauschii* (Ae45, Ae95) genotypes. These genotypes were subjected to salt stress at 150 mM NaCl and leaf and root samples were harvested at 8th and 27th hour of stress treatment. Expressed sequence tag (EST) analysis is an effective method in discovering novel genes and investigating gene expression in different plant organs and tissues. In our studies, 136 seedling and 268 root contig tags were assembled from salt stressed *Triticum aestivum* EST database ([http://wheat.pw.usda.gov/cgi-bin/westsq/est\\_lib.cgi](http://wheat.pw.usda.gov/cgi-bin/westsq/est_lib.cgi)) and all contig tags were analyzed using BLASTX algorithm for functional annotation. Twenty-three different functional categories were identified for root and seedling tissues. Under each category, genes with high sequence similarity to contig tags were tabulated. Ten genes for root and four genes for seedling tissues were selected from cell rescue and defense group and their quantitative expression analyses were carried out by multiplex quantitative polymerase chain reaction. The multiplex primer panels were designed together with housekeeping genes for each tissue type by using GeXP Genetic Analysis System's software. The expression of each gene were determined by running fluorescently-labeled fragments on capillary electrophoresis system and quantified precisely. Salt tolerance in *Aegilops* species is well-known and has been associated with D-genome. In the same way, expressions of stress-related genes in root and leaf tissues of Ae95 genotype were meaningful in our studies, 7/10 genes in root and 3/4 genes in leaf tissues were found up-regulated. Interestingly, the expression of MYB80 transcription factor in Ae95 genotype was not detected in measuring range under stressed and unstressed conditions. In bread and durum wheat genotypes, expression profiles of stress-related genes assigned comparatively and generated informative data to be used in breeding programs.

## SL24 - Molecular and pathological characterization of slow-rusting genes against leaf rust in wheat (*Triticum aestivum* L. em Thell)

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Rust diseases specifically leaf rust caused by *Puccinia recondita* f. sp. *tritici*, is globally important fungal of wheat (*Triticum aestivum* L. em. Thell) that is responsible for significant yield losses; up to 40% worldwide. Due to rapid change of pathogen races, single gene resistance is short lived in wheat cultivars. Alternatively, a more durable form of resistance is attributed to slow leaf rusting for which certain genotypes have been identified and characterized. Genetic studies indicate that slow rusting resistance is under polygenic control with moderately high heritability. Such resistance is controlled by a number of minor genes also referred to as adult plant resistance (APR) genes. Although 10-12 slow rusting genes are known to be present in CIMMYT spring wheat, only two genes Lr34 and Lr46 have been characterized for slow rusting. Fifteen wheat genotypes including twelve CIMMYT entries, two elite Indian wheat cultivars i.e., HUW 234 and HUW 468 and one known leaf rust susceptible cultivar i.e., Agra Local were included in the present study. These lines were first evaluated under field conditions for disease severity %, latent period and incubation period. Then, evaluated under controlled laboratory conditions where, a detached leaf assay techniques was employed with three virulent pathotypes. In the present study, we used 29R45 (12-5), 121R63-1 (77-5) and 21R55 (104-2) pathotypes to screen all the fifteen wheat lines under controlled conditions as well as field conditions for characterizing the effect of different slow rusting genes /their combinations on leaf rust resistance. Genotypes, G-5, G-11, G-12 and G-13 showed least disease severity, were very close to near immunity and showed comparatively a higher level of resistance against all the three pathotypes. In addition, ten tightly linked microsatellite markers were also used to characterize all the 15 genotypes for the presence of different slow rusting/ durable rust resistance genes. This was an indirect selection for desirable allele, to exploit the advantages of the durability of slow rusting and to develop a better understanding about its mechanism that might be of much promising in the development of durable rust resistant cultivars.



## SL25 - Progress and prospects of increasing drought tolerance in pearl millet using genetics and genomics approaches

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Pearl millet is a staple cereal grain and fodder crop grown by subsistence farmers in the hottest, driest regions of sub-Saharan Africa and the Indian subcontinent. Post-flowering drought stress is one of the major factors reducing its yield and yield stability drastically. This presentation will review progress made so far towards identification, characterisation and breeding of drought tolerance quantitative trait loci (QTLs) in pearl millet using genetic resources adapted to conditions of Africa and Asia. It will particularly focus on the fine-mapping of a validated major quantitative trait locus (QTL) for terminal drought tolerance mapping to linkage group 2, which explained up to 32% of variation in grain yield under multi-environment terminal drought screening using mapping population testcrosses of F2:3 segregants from two independent crosses. Results will be presented on the genetics and physiology dissected of this QTL, as well as on the successes of its marker-assisted backcross transfer into elite pearl millet hybrid parental lines. Data will be presented on the added advantage offered by this drought tolerance QTL in saline and alkaline stress conditions. Current efforts being taken towards fine mapping and towards developing gene-based markers for targeted saturation mapping of this major drought tolerance QTL will be discussed. Genetic stocks (QTL-NILs, high resolution genetic cross, and inbred germplasm panel for association genetics studies) and genomic resources (gene sequences, gene-based markers and comparative genomics information) currently assembled for these purposes will be discussed in length.

## SL26 - Dissection of quantitative resistance to leaf rust (*Puccinia brachypodii*) in *Brachypodium distachyon*, the model plant for *Triticeae*

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The model plant *Brachypodium distachyon* (L.) Beauv. has been employed to dissect its quantitative resistance to leaf rust. An F2 mapping population generated between the two *B. distachyon* diploid inbred lines Bd1-1 and Bd3-1 was used to develop a molecular marker linkage map. The map was initially populated with 192 AFLP marker loci. SSRs and conserved orthologous sequence (COS) markers have also been added to the AFLP framework to provide anchor points for comparative genomics studies with other *Brachypodium* and *Triticeae* maps. To locate quantitative resistance loci on the map, the F2 plants were evaluated for their reaction to the leaf rust *Puccinia brachypodii*. To improve and validate the dissection of the trait, F2-derived F3 families were tested for resistance to leaf rust in two additional independent experiments. Disease evaluations showed continuous, quantitative and transgressive segregation. Interval mapping and MQM mapping were performed on the data of the different experiments by using the software MapQTL 5.0 and then QTL positions were compared. Two major genomic regions involved in resistance to leaf rust were detected and are here discussed according to recent increase of genomic knowledge in *Brachypodium*. Together they accounted for about 40-50% of the observed phenotypic variation. Our results suggest that leaf rust resistance in *B. distachyon* is a polygenic trait influenced by few major genes with large effect as observed in the *Triticeae*. A search for candidates has been started through the *Brachypodium distachyon* 8x released genomic sequence, to obtain and map candidate gene-derived markers in the QTL intervals.

## SL27 - Identification and quantification of barley miRNAs and their targets in response to drought stress

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Micro-RNAs (miRNAs) are tiny (21-24-nt) non-coding RNAs taking regulatory roles in several organisms. A number of plant miRNAs have been detected in various species but a limited number were surveyed for their function. In this work, we were interested in the identification of conserved miRNAs in *Hordeum vulgare* (barley) focusing on a possible role of miRNAs in drought stress. Using computational approaches, 29 new barley miRNAs belonging to 18 distinct miRNA families were identified in *H. vulgare* using NCBI Expressed Sequence Tag (EST) databases. Detailed nucleotide analyses of these barley miRNAs revealed that preliminary miRNAs (pre-miRNAs) are in the range of 46-114 nucleotides and mature miRNAs are in the range of 20-24 nucleotides. Mature miRNAs contained mostly uracil (U) at the first position and were found to be located both in 5' and 3' positions of precursor RNAs. With quantification of miRNAs using real-time PCR (qRT-PCR), we have validated the existence of selected barley miRNAs and measured their expression level differences in response to drought stress. In this study, we have shown that the expression level of some predicted miRNAs change in shock drought stressed samples. Hvu-MIR156, Hvu-MIR166, Hvu-MIR171, Hvu-MIR408 were detected as drought stress responsive barley miRNAs. Additionally, using detected 29 new miRNAs as queries 445 potential target mRNAs were *in silico* predicted in *H. vulgare* using NCBI EST database. Computationally proposed Hvu-MIR156, Hvu-MIR171 and Hvu-MIR408 target transcripts were also measured in dehydration shocked barley leaf and root tissues. A positive correlation was detected between increase in levels of Hvu-miRNA156, Hvu-miRNA171 expression and suppression of their target mRNA transcripts in response to drought stress in leaf. With a modified RNA ligase-mediated 5' rapid amplification of cDNA ends (5' RLM-RACE) procedure, seven miRNA cleaved sequences were retrieved from drought stressed leaf stress samples. For these seven sequences, 15 potential protein homologues were found in *Viridiplantae*. Using computational approaches, 15 potential miRNAs which can target these transcripts were also proposed. The majority of the computationally and experimentally identified target mRNAs encode transcription factors and proteins involved in metabolic processes, signal transduction and stress response.

## SL28 - Subtleties of the wheat drought response displayed by a DRE-binding protein from *Triticum dicoccoides*

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Water scarcity is one of the major stresses affecting cereal crop production. One major group of stress-responsive plant genes are those containing the Dehydration Response Element (DRE). First identified in *A. thaliana*, DRE-regulated genes were upregulated by drought, cold and salt stress independently of abscisic acid signalling (Yamaguchi-Shinozaki, 1994, Plant Cell 6: 251-264). Subsequently members of the DREB (DRE-binding) transcription factor family have been shown to induce DRE genes. By simultaneously regulating multiple stress-responsive genes, DREB proteins have a major role in determining plant endurance under stress. This study aimed to understand the role of a DREB from wild emmer wheat in the greater drought tolerance of this species compared to cultivated wheats. We used a putative *Triticum aestivum* DREB sequence to identify and clone a DREB gene from wild emmer wheat (*Triticum turgidum* ssp *dicoccoides*), named *TdWdreb2*. Highly homologous genes in barley and cultivated wheats are drought-responsive and express multiple mRNA transcripts by alternative splicing. We therefore used an RT-PCR approach to analyse the expression of this gene in both drought-tolerant and drought-sensitive cultivars of *T. dicoccoides*. *TdWdreb2* was strongly induced by both rapid drought shock and slow drought stress, and produced multiple transcripts on induction. Under drought shock it was only upregulated in root tissue, but after a longer period of drought was expressed highly in both root and leaf. Interestingly, the roots of the drought-tolerant but not the sensitive cultivar expressed a *TdWdreb2* transcripts constitutively. We also expressed the DNA-binding domain of *TdWdreb2* as a recombinant GST-fusion protein and analysed its binding to DRE by electrophoretic mobility shift assays (EMSA). Point mutations of the DRE consensus sequence defined in *A. thaliana* (TACCGACAT) showed that this DREB binds preferentially to a slightly different sequence. EMSA of nuclear extracts from drought-stressed plants found that DREB expression increased in drought-stressed roots, correlating with the RT-PCR results, but not in leaf, showing that further regulation occurs at the protein level. These results show that, in wheat, the DREB-DRE interaction is subtly regulated at several levels. Drought-tolerance is conferred not by the presence of DREB genes, but the kinetics of their activation.

**SL29 - Exploitation of diversity in nuclear-cytoplasm interaction using alloplasmic wheat lines**Crosatti C <sup>1</sup>, Quansah L <sup>2</sup>, Atienza SG <sup>3</sup>, Marè C <sup>1</sup>, Fait A <sup>2</sup>, Cattivelli L <sup>1</sup><sup>1</sup> CRA-Genomic Research Centre, via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy<sup>2</sup> The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Israel<sup>3</sup> Plant Breeding Department, IAS-CSIC, Apdo. 4084, Cordoba, 14080, Spain

Nuclear genome has a predominating role on the inheritance of most plant traits, nevertheless cytoplasmic factors and cytoplasm × nucleus interactions are also important and still largely unexplored. Since preliminary evidences suggested that phenotypic and metabolic variations are associated with different combinations of nuclear-cytoplasm genomes (Atienza et al., 2008 *Euphytica*, 159: 325-331), parallel transcriptomic and metabolomics analysis were carried out on three alloplasmic lines designed to investigate the effect of *H. chilense*, *Ae. uniaristata* and *Ae. squarrosa* cytoplasm on nuclear-cytoplasm interaction in the euplasmic genetic background of *T. aestivum*. GC-MS metabolic profiling of leaves revealed significant differences in the primary metabolism of the alloplasmic lines. For instance, the cytoplasm of *Ae. squarrosa* led to a decrease activity of the TCA cycle when introduced into a *T. aestivum* nuclear background, while the cytoplasm of *Ae. uniaristata* induced a significant accumulation of fructose and raffinose. When the transcriptome of each alloplasmic line was compared to the correspondent euplasmic line, significant variations in the mRNA steady state levels were detected. More than 500 genes modified their expression when *H. chilense* cytoplasm was introduced into a *T. aestivum* nuclear background, while only few dozen of genes altered their expression due to the introgression of *Ae. squarrosa* or *Ae. uniaristata* cytoplasm. As expected, most of the genes whose expression level was modified in alloplasmic lines encoded for mitochondrion/chloroplast localized proteins. A sequence corresponding to the mitochondrion/chloroplast localized maturase related proteins is the most up regulated mRNA in all three alloplasmic lines. The alignment of metabolic and transcriptomic data underlined that the amino acid biosynthetic pathways are strongly dependent from nuclear-cytoplasm interaction. The results provide a genomic overview of the nuclear-cytoplasm interaction and offer an additional level of investigation on genetic diversity.



### SL30 - Deployment of either a whole or dissected wild nuclear genome into the wheat gene pool meets the breeding challenges posed by the sustainable farming systems

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The rate of yield increase for the global cereal grain production should be maintained at about 1% every year, in order to meet predicted demands for the next forty years. Cereal breeding may occur through advanced mutagenesis, interspecific introgression, and genetic engineering methods. These approaches could provide varieties expressing trait-enhancing genes and adaptations suitable for yield progress, especially when supported by genomic (MAS) and precision phenotyping selection tools. Resilience of gene expression is a requirement when the selected varieties will experience year-to-year fluctuating climatic conditions and implementation of sustainable use of environmental resources in farming systems. Many dominant genes for adaptation have been lost during cereal crop domestication, but they have been retained in the genome of the wild components of the Triticeae gene-pools. In natural habitat, wild Triticeae species such as *Dasypyrum villosum* (*Dv*), whose genome was exposed to over hundred million years of climatic and environmental changes, are now expressing increased heading earliness, density stands, and plant biomass. Deploying whole and dissected *Dv* nuclear genome in the homoeologous wheat genetic background through interspecific hybridization and introgression, is a lower-cost and effective option to help wheat breeders to select the proper germplasm to sustain the needed yearly grain-yield increase. Combining the *Dv* and *T. turgidum* var *durum* nuclear genomes, several hexaploid amphiploids have been produced displaying typical adaptive traits of *Dv* such as high resistance to diseases (caused by *Tilletia tritici*, *Blumeria graminis* f. sp. *tritici*, *Puccinia triticina*, and *P. graminis* f. sp. *tritici*), fortified caryopses (high protein and micronutrient contents), heading earliness, and good water and nitrogen-use efficiency. The dissection of the *Dv* genome by either "*Triticum aestivum* cv Chinese Spring (CS) x hexaploid amphiploid" or "(CS x *Dv*) x CS" hybridization and backcrossing, provided wheat introgression breeding lines (IBL) showing one or more of the *Dv* adaptive traits. Molecular analyses revealed that either cryptic or GISH-detectable *Dv* chromatin introgression occurred in those IBLs. Interestingly, the IBLs, after three years of genetic analyses and low-input field tests (in Italy and Hungary), showed dominance and genetic stability of the adaptive traits. The expression in some IBLs of genes for improved nutritional value and end-use quality, was an added benefit of the *Dv* chromatin.

### SL31 - NextGen sequencing of the transcriptome of triticale

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Triticale possesses favourable agronomic attributes originating from both its wheat and rye progenitors including high grain and biomass yields. Triticale, primarily used as animal feed in North America, is an excellent candidate for production of industrial bio-products. Little is known about coordination of gene expression of rye and wheat genomes in this interspecific hybrid but significant DNA losses from the parental genomes have been reported in triticale. In order to clarify the regulation of gene expression in triticale, we carried out 454 sequencing of cDNAs obtained from root, leaf, stem and floral tissues in different cultivars of triticale exhibiting different phenotypes and employed Newbler Assembler (454 Life Sciences) and SeqMan NGen (DNASTAR) to assemble reads into contigs. Challenges to the data assembly were the absence of reference genomes for triticale or its wheat and rye parental lines and the paucity of the rye sequences in GenBank or other public databases. Consequently, we have sequenced cDNAs libraries from roots, seedlings, leaves, floral tissues and immature seeds from a single rye cultivar to facilitate the identification of triticale sequences originating from rye. A database of 20,000 non-redundant full length wheat CDS genes based on existing databases and contigs has been verified against protein sequences from the grass genomes of *Brachypodium*, rice, sorghum and maize. To date, more than 40% of triticale 454 transcriptome sequences have been aligned against sequences from the full length databases. We also identified genes related to cell wall development in triticale.

### SL32 - Identification of agronomically valuable alleles in durum wheat through linkage and association mapping

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At DISTA and PSB, in collaboration with European and West-Asian and North African (WANA) research Institutions, CIMMYT and ICARDA, a program for mapping useful genetic variation in durum wheat through a both linkage and association mapping approaches has been undertaken since 2003. Up to now, three RIL populations derived from Kofa x Svevo (Maccaferri et al., 2008, Genetics 178:489-511), Meridiano x Claudio and Colosseo x Lloyd (each including ca. 180 RILs) and a germplasm collection of 240 elite durum wheat cultivars (DISTA durum panel) have been characterized with molecular markers in a genome-wide approach and phenotyped under a range of environments for adaptive and agronomically relevant traits, kernel quality traits and response to the major wheat diseases such as leaf rust, powdery mildew and soil-borne cereal mosaic virus. The DISTA durum panel largely covers the genetic variation present in the main durum breeding programs. Population structure analysis showed the presence of five main subpopulations, corresponding to important breeding lineages and durum ideotypes. A linkage disequilibrium (LD) survey based on 180 mapped SSR loci showed high LD levels ( $r^2 > 0.20$  and  $D' > 0.60$ ) within a 5-10 cM (maximum) inter-marker distance (Maccaferri et al., 2006, Plant Gen. Res., 4:79-485). Examples of the use of the germplasm collection for QTL dissection studies include: a major gene for leaf rust resistance on chr. 7BL (identified in the Colosseo x Lloyd mapping population), two major QTLs for grain yield and related traits on chrs. 2BL and 3BS (Kofa x Svevo) and one major QTL for soil-borne cereal mosaic virus on chr. 2BS (Meridiano x Claudio). Additionally a comprehensive analysis of the genetic basis of kernel yellow pigment content and micronutrient content (Fe, Zn, Cd) in the three populations and in the germplasm collection allowed us to evaluate allelic variation and germplasm diversity at some of the major QTLs controlling these important quality traits. Collectively, our results indicate the suitability of the DISTA durum panel to investigate the genetic basis of agronomically valuable traits to improve the sustainability and quality of durum wheat production.

### SL33 - Towards physical mapping and sequencing the *Fr-H2* (Frost resistance-H2) region of barley chromosome 5H

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*Fr-H2* is one of two major QTL, located on chromosome 5H, affecting freezing tolerance and winter hardiness of barley. Of the 14 genes encoding *CBF* transcription factors that map coincident with *Fr-H2* it is still not definitely demonstrated in barley which is the molecular basis of the QTL effect. The resistant phenotype could be either the result of a single *CBF*, or of a gene copy number variation of *CBF* elements among genotypes, or an effect of other sequences independent from the *CBFs*. As a first step towards *Fr-H2* physical mapping we generated a large mapping population (2,849 F2 plants) from the 'Nure' (frost tolerant) x 'Tremois' (frost susceptible) cross. Recombinants between seven out of the 14 *CBFs* have been identified showing that the *CBF* cluster spans 0.81 cM and that there is recombination among *CBF* subclusters. A genomic BAC library of barley cv. 'Morex' has been screened via PCR with six *CBF* markers mapping at *Fr-H2* and the first BAC clone addresses were obtained for all markers assayed. To create anchor points between the genetic and physical maps of the region, a High-Information-Content Fingerprinting of the selected BACs has been performed and clones have been assembled into contigs. Additional BACs belonging to the contigs detected were then PCR-screened for the presence of all available *CBFs* and the more interesting clones have been sequenced using Roche 454 FLX platform. Software MIRA has been used for 454-sequence assembly. The construction of a single physical contig encompassing *Fr-H2* will provide useful information for detailed comparative analyses of the genomic organization of the locus in other barley cultivars, like 'Nure' and 'Tremois'. Enlargement of contigs to obtain a complete physical map of the locus is in progress.

### SL34 - Effects of *AiFRO2* expression in the nutritional enhancement of soybean (*Glycine max* L.)

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Soybean, whose earliest evidence of cultivation dates back to 1000 BC, remains one of the key plant sources of nutrients worldwide. Iron (Fe) is one of the most important micronutrients in human and plant nutrition, and adequate iron nutrition in plants is central in providing adequate concentrations of this important mineral in harvested plant products for human food and animal feed. The first step in the absorption of iron by many plant species is the reduction of Fe(III) to Fe(II). This is an obligatory step for iron uptake by all dicotyledonous plants. Most studies of this process focus on the root reduction of iron, and not on the role of leaf iron reduction in seed loading of iron and other nutrients. Soybean [*Glycine max* (L.) Merr.] constitutively expressing the *FRO2* iron reductase gene from *Arabidopsis thaliana* was analyzed for leaf reductase activity, and the effect on seed nutrient concentrations was assessed. It was found that protoplasts isolated from the transgenic leaves had three (3)-fold higher reductase activity, and that the seed iron levels also were increased by 10%. However, leaf and pod wall iron concentrations increased as much as 500% in the transgenic plants, suggesting that other factors are limiting the translocation of the excess iron into the seeds. It was found that *ferritin* expression levels were higher in the transgenic leaves than in the control. This suggests that the excess iron may be stored as *ferritin* in the leaves and therefore unavailable for phloem loading. Finally, concentrations of Mn, K, P and Zn had significantly higher concentrations in the leaves, pod walls, root and xylem sap of the transgenic plants, and that Zn concentrations also were higher in the transgenic seeds. This suggests a more ubiquitous role of the iron reductase in plant mineral dynamics.

### SL35 - Exploiting the genomic sequences of rice and Brachypodium for delimiting a grain size QTL in wheat

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The previously described QTL for thousand-grain weight *QTgw.ipk-7D* associated with microsatellite marker *Xgwm1002-7D* was originally detected in a BC<sub>2</sub>F<sub>3</sub> advanced backcross population of the German winter wheat variety 'Prinz' and the synthetic wheat line W-7984 (lab designation: M6) (Huang et al, 2003). We developed nearly-isogenic lines (NILs) carrying introgressions of M6 in the genetic background of 'Prinz' with varying size on chromosome 7D. The BC<sub>4</sub>F<sub>3</sub> NILs had a 10% increased 1,000-grain weight compared to the control group and the recurrent parent 'Prinz' and 84.7% of the phenotypic variance could be explained by the segregation of marker *Xgwm1002-7D*.

By using homozygous recombinant lines it was possible to delimit the QTL *QTgw.ipk-7D* to an interval of approx. 1 cM flanked by the markers *barc126*, *wmc405* and *gwm44* on chromosome arm 7DS. From a chromosome arm 7DS specific BAC library BACs positive for each of these markers were isolated and their sequences were obtained by 454 sequencing. A good synteny to the genomic sequences of rice and Brachypodium was observed. By adding further markers based on wheat ESTs from the respective genomic region we have now delimited the interval of the QTL to ca. 420 kb of the rice genomic sequence on rice chromosome 6 containing 53 predicted genes and a syntenic region of ca. 820 kb in the Brachypodium genome with 55 predicted genes. Most genes are collinear between rice and Brachypodium, however, ca. 20 Brachypodium genes are homologous to other rice chromosomes. We are in the process of exploiting the available sequence information of rice and Brachypodium to generate more closely linked genetic markers in wheat and further delimit the interval of the QTL in recombinant wheat lines.

In general, our data support the concept of using nearly isogenic introgression lines for validating and dissecting QTLs into single Mendelian genes and open the gateway for map-based cloning of a grain size QTL in wheat.

### SL36 - The genetic make-up of European landraces of common bean

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The pathways of dissemination of common bean into and across Europe were very complex, with a number of introductions from Americas that were combined with direct exchanges between European and other Mediterranean countries. We analyzed a large collection of European landraces of *P. vulgaris* with six chloroplast microsatellite (cpSSR) loci and two unlinked nuclear loci (for phaseolin types and *Pv-Shatterproof1*). We compared the genetic structure and the level of diversity of this collection with a group of American individuals representative of the Andean and Mesoamerican gene pools. The results show that the majority of the European common bean landraces are of Andean origin. Moreover, bottleneck due to the introduction of *P. vulgaris* into the Old World, was very weak for chloroplast analysis but of greater intensity for nuclear analysis. Finally, a relatively high proportion of the European bean germplasm has derived from hybridization between the Andean and Mesoamerican gene pools. Based on the analysis of the distribution of genetic diversity and hybrid individuals across European countries, we suggest that the entire European continent can be regarded as a secondary diversification centre for *P. vulgaris*. Lastly, we outline the relevance of these inter-gene-pool hybrids for plant breeding.

### SL37 - Iron biofortification of maize grain

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Mineral nutrient deficiencies are a worldwide problem that is directly correlated with poverty and food insecurity. The most common of these is iron deficiency; more than one-third of the world's population suffers from iron deficiency-induced anemia, 80% of which are in developing countries. The consequences of iron deficiency include increased mortality and morbidity rates, diminished cognitive abilities in children, and reduced labor productivity, which in turn stagnates national development. The developed world has made tremendous success in alleviating nutrient deficiencies through dietary diversification, food product fortification, improved public health care, and supplementation. In developing countries, these strategies are often expensive and difficult to sustain. Poverty is the most common cause for dietary deficiency in developing countries, as consumers' dietary choices are limited as regards the quality, quantity, and diversity of foods consumed. The resource-poor typically consume what they grow and are dependent upon a small number of staple crops for the vast majority of their nutrition. Therefore, genetic improvement of staple crops (biofortification) is the most cost effective and sustainable solution to this global health problem. Here we describe an integrated genetic, physiological and biochemical analysis of iron nutrition in maize grain, to discover the genes and compounds that influence grain iron concentration and bioavailability. Multiple quantitative trait loci (QTL) for each trait have been identified and validated via multi-year and/or multi-location testing. QTL for iron bioavailability have been isolated in near isogenic lines, which were provided to collaborators in five states for planting in summer 2008. Efficacy of these QTL in multi-location trials will be discussed. We will also present the results from poultry feeding experiments that validate iron nutritional quality predictions made from the QTL model and in vitro bioassays for iron nutritional quality. Our results indicate that biofortification of maize grain with iron is an achievable goal.

### SL38 - Biofortification of finger millet for high zinc

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Zinc (Zn) is an essential micronutrient for both plants and humans. Zn deficiency ranks fifth amongst the health risk factors and three billion people suffer from Zn and other micronutrients deficiency throughout the world. Biofortification relies on plant breeding and biotechnological approaches to increase the micronutrients in crops is now been considered as a long lasting solution to supplement crops with micronutrients. Similar biofortification approach was followed in finger millet. We characterised finger millet, staple food crop of southern India, germplasm lines for variability in zinc content and expression of Zn transporters; and the finger millet genotype with high Zn content was engineered to further enhance its Zn content by independently transforming OsZIP1 transporter driven by constitutive (CaMV35S) or endosperm specific (Bx17) promoter. The Zn content ranged from 1 mg to 7 mg/100 g among the 333 finger millet genotypes screened. Molecular characterization of Zn transporters was carried out in three high and three low Zn types contrasting in their seed Zn content (7.86 and 2.81 mg/100g, respectively) grown under three levels of soil Zn content. Semi-quantitative and quantitative expression studies of seedlings grown under different levels of soil Zn status showed differential induction of the ZIP family Zn transporters in leaves and roots. ZIP1 transcript levels were higher under Zn deficient conditions in both high and low Zn types. However, Zn dependent enzyme, carbonic anhydrase, showed enhanced expression under high Zn nutrition. Constitutive expression of OsZIP1 resulted in enhanced leaf Zn content compared to untransformed wild type plants but they were unable to increase their seed Zn content when both plants were grown under moderate levels of Zn nutrition. However, under similar soil Zn nutrition transgenic plants expressing OsZIP1 under control of endosperm specific promoter resulted in significant increase in seed Zn content compared to untransformed wild type plants. It is clear that although the leaf Zn content increases it does not necessarily result in higher seed Zn content. Therefore, depending on the economic importance of the plant part or tissue consumed it would be better to express Zn transporters under either constitute or tissue specific promoters.

### SL39 - Gene discovery to improve grain quality-related traits in maize

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Maize is one of the most important crop in the world. Its grain provides feed, food, and a resource for many industrial products. Developing plants with improved grain quality traits involves overcoming a variety of technical challenges inherent in metabolic engineering programs. Advances in plant genetics and in technologies for genome-wide studies and for large-scale gene expression analysis are contributing to the acceleration of gene discovery for products development. To increase our understanding of the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and proteins, we have assayed a series of endosperm mutants by evaluating protein, amino acid composition, and transcriptome profiling. Specifically, for the *o2* and *o7* mutations we found that the overall amino acid compositions of these mutants appeared similar. Each mutant had a high Lys and reduced Glx and Leu content with respect to wild-type. Gene expression profiling, based on a Unigene set, composed of 7,250 ESTs, allowed us to identify a series of mutant-related up-regulated (17.1%) and down-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to gene encoding enzymes involved in amino acid synthesis, carbon metabolism (TCA cycle and glycolysis), in storage protein and starch metabolism, in gene transcription and translation processes, in signal transduction, and in protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrated that *o2* and *o7* mutants are pleiotropic and play a critical role in several endosperm metabolic processes. Pleiotropic effects were less evident in the *o7* mutant, but severe in the *o2* and *o2o7* backgrounds, with large changes in gene expression patterns, affecting a broad range of endosperm-expressed genes involved in several metabolic pathways. Although, work is required to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized to date give us an intriguing insight into the mechanisms underlying endosperm metabolism.



## SL40 - Low phytate rice and soybean: mutant generation, characterization and gene discovery

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Phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate, PA) is the most abundant phosphorus (P) compound in cereal and legume seeds, accounting for about 75% of seed total P. PA and its salt form phytate in food and feed is generally considered to be problematic since the P and mineral elements in PA and phytate are biologically not available to human and mono-gastric animals. The indigested P when leaked into environment becomes an important source of P pollution. We have generated a dozen soybean and rice low phytic acid (LPA) mutant lines with reduction of PA content from 35-60%. The effect of LPA mutations on agronomic and quality characters has also been extensively investigated resulting in some novel findings, e.g. the negative effect of most LPA mutations on seed viability and tolerance to storage, increase content of sucrose and isoflavonoids. Comparative metabolite profiling not only identified the chemical differences between LPA mutants and their corresponding parental genotypes, but also gave an important clue to the causative gene that resulted in the LPA mutation in certain instances. Using map-based cloning six different LPA genes are cloned, three are genes with known function in PA biosynthesis, and others are with yet unknown function in PA metabolism including two putative transporter genes. Our study not only generated valuable LPA germplasm for breeding LPA rice and soybean varieties, but also significantly extended our understanding of the phytic acid metabolism and genetic control; the identified genes also provide new targets for developing LPA crops through genetic engineering.

## SL41 - Transcriptomic and metabolite analyses of genes implicated in traits relevant to grapevine quality

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Many genes influencing grape and wine quality are expressed during ripening. Recently, grape genome sequencing projects provided new candidate genes implicated in traits relevant to grape quality, such as secondary metabolites content. Unfortunately, most currently available grape microarray platforms are not up-dated or are expensive. In this work, a flexible high-quality, completely customizable, electrochemical array was designed to overcome these limitations. This original platform was used to investigate fruit ripening in *Vitis vinifera* L. cv Muscat of Hamburg. Probes (35-40mers) were designed on approximately 6000 genes carefully selected from fruit EST libraries, *in silico* gene expression analysis, scientific literature and earlier experimentation. Gene expression profiles obtained from berries at five developmental stages, from pre-veraison to ripening, were analyzed in two growing seasons. Real-time PCR analyses were also performed to validate microarray data. Moreover, the evolution of organic acids, sugars, and free and glycoside aromatic compounds was monitored on the same berry samples, using GC/MS and HPLC.

A total of 920 genes were modulated in almost one season (15% of the probes). More than 30% of them corroborated previously reported findings, while a considerable amount of transcripts showed for the first time variation at the transcriptional level. They are involved in a wide spectrum of biochemical changes such as fruit softening, chlorophyll degradation, decreased acidity, accumulation of sugars, biosynthesis of carotenoids, anthocyanins, essential oils, and flavor and aroma components. Many genes active in the biosynthesis of tartrate and malate showed good correlation with tartaric and malic acid contents during ripening. Interestingly, three of the four pathways proposed for ascorbate (tartaric acid precursor) biosynthesis were well represented, supporting the theory of myo-inositol as a possible entry point into the ascorbate biosynthesis. Many transcripts involved in terpenoid, benzenoid and C13 norisoprenoid biosynthesis showed differences in transcription profiles in at least one ripening stage. Moreover, a modulated transcripts dataset involved in other aromatic compounds biosynthesis, such as phenolic- and aminoacid-derived volatile chemicals, was reported. Our work indicates the reliability of such platform for transcriptomic analyses and the utility of metabolic data to support gene expression studies.

## SL42 - *Avena* genetic resources for quality in human consumption (AVEQ) – a European project on nutritional quality

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In a cooperative project with fifteen partners from nine European countries genebank material and current commercial varieties (600 accessions in total) are evaluated for traits considered important for future oat breeding for a premium market in Europe. These are quality traits related to the increasing health food market, resistance to contamination by mycotoxins and tolerance to cold. The latter reflect the fact, that oat growing may be affected by future climate changes leading to more winter cropping and higher *Fusarium* disease pressure with increasing precipitation in the Northern European regions.

In 2008 and 2009, field experiments were performed widely distributed all over Europe to sample harvest material for quality analysis. These were laid out in augmented designs with eleven standard cultivars – mainly modern varieties bred in different European countries. In three locations artificial inoculation with a mixture of *Fusarium* isolates was performed to analyse *Fusarium* infection and mycotoxins contamination. For comparison a wheat variety was included into these trials. Analytical work is currently ongoing. It covers the following traits: 1) Analysis for protein, fat and minerals. In this work package also *Avenin* patterns are determined. 2) Analysis for total dietary fibre, total starch, total and soluble  $\beta$ -glucan. 3) Analysis for antioxidants (tocopherols, tocotrienols and avenanthramides). 4) *Fusarium* and mycotoxin analysis.

Large differences in yield and technological quality (e.g. seed weight) are observed as a result of genotype and environmental influences. Modern hexaploid cultivars are superior to obsolete cultivars, wild or diploid types. Overall less diversity with a trend to higher yield and higher seed weight was found in the field experiments for modern cultivars compared to the other types. Mycotoxin analysis confirmed low contamination of oats compared to wheat for deoxynivalenol, but higher susceptibility for T2/HT2 toxins. Significant differences were observed in the standard cultivars. Fluorescence is measured for detecting low temperature stress-induced injury on photosynthesis. These results correspond with field results during winter cropping in Bulgaria.

All project results will be made available to the genetic resources community by the European *Avena* Database (EADB). Web applications are being developed for an oat crop portal supporting management of large cooperative projects on characterisation and evaluation of genetic resources.

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### SL43 - Mutations in genes controlling the metabolism of inositol phosphates in cereals

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Phytic acid (PA) is the primary storage compound of phosphorus in seeds. PA poses a number of challenges in husbandry fodder and for staples in human nutrition. PA reduces the bioavailability of iron and thus contributes to the 'hidden hunger', while people still obtain sufficient energy from the starchy cereal grains. In the case of feedstuff, the main issue is phosphate management in animal production systems. PA cannot be dephosphorylated in the digestive system of monogastric animals, and excreted phytate might contribute to environmental phosphate pollution with eutrophication of aquatic ecosystems as a result. The biosynthesis and accumulation of PA can be detected a few days after anthesis and it seems to continue during seed development until maturation. The first step in PA biosynthesis is the formation of Ins(3)P by conversion of glucose 6-phosphate. This is then followed by a sequential and ordered phosphorylation of the remaining five positions of the inositol ring by a number of kinases, resulting in PA. Identification of low-PA mutants in cereals, legumes and *Arabidopsis* are instrumental for resolving the biosynthetic pathway and identification of genes controlling the accumulation of PA. Mutations in seven genes involved in the metabolism of PA has been identified and characterized among five plant species using induced mutagenesis and insertion elements. Understanding the biosynthetic pathway and genes controlling the accumulation of PA in plant seeds and how PA may balance the free phosphate is of importance for molecular breeding of crop plants, particularly cereals and legumes. We are currently exploring ecoTILLING and TILLING in wheat, barley and *Brachypodium* in order identify useful mutations.

### SL44 - Exploitation of crop diversity to improve food quality at Agricultural Research Council (CRA) of Italy

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The Agricultural Research Council of Italy (CRA) is a national research organization dedicated to promote the innovation in agriculture. Collection, characterization and exploitation of genetic resources is a key strategy in the current CRA vision to improve crop yield and quality. The CRA holds large germplasm collections for many crops (cereals, vegetables, fruit trees, etc) and a number of projects are dedicated to identify new useful alleles for crop improvement. This presentation will highlight some significant examples where the germplasm has been exploited to improve quality traits in cultivated varieties. In peach, several QTLs related to fruit quality (solid soluble content, fruit colour) have been localized in a linkage map. The availability of the genome sequence, recently obtained by an international consortium, will speed up the exploitation of favorable alleles in the large peach germplasm collection available and already phenotyped for several fruit quality traits. 35 accessions, chosen for their broad variability, are being resequenced using an Illumina platform for variants discovery (SNPs, small indels and structural variants). In durum wheat a large germplasm collection has been characterized for all quality traits, then a specific work was carried out to investigate all alleles at the loci controlling the pasta yellow pigment content. The colour of semolina products is the result of the natural carotenoid pigments present in the grains, of their residual content after storage and milling, and of the oxidative degradation mainly due to lipoxygenase (LOX) activity during pasta processing. Several QTLs for carotenoid accumulation, candidate genes for some of these QTLs as well as loci and alleles encoding for LOX enzyme were described using the germplasm collections available. Molecular and biochemical studies highlight that the differences in pasta yellow colour observed between samples produced with different genotypes can be explained by the different allele composition either at the loci controlling pigment biosynthesis during seed development or at the loci encoding for lipoxygenase.



# *Posters*

## *Session 2*

### *Harnessing plant diversity: From sequence to function*



## P2.01 - Functional analysis and characterization of miR393 in *Arabidopsis thaliana*

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In this research project, miRNAs which are involved in glyphosate stress response and/or resistance are wanted to be functionally characterized. From the evaluated miRNA profiling results of glyphosate treated *Festuca arundinacea*, ath-miR393a was found to be upregulated in 5% glyphosate treated samples whereas they were downregulated in 20% treated when compared to control samples. Mir393 is a conserved miRNA species that targets F-box proteins TIR1 and AFB1/2/3 transcripts. F-box motif mediates protein-protein interactions and mostly found in proteins as components of SCF ubiquitin-ligase complexes. For this reason they have very important roles in the maintenance of protein stabilities inside the cell. TIR1 and AFBs have also been associated with auxin signaling pathways as auxin receptors. To functionally characterize mir393 in *Arabidopsis thaliana*, mir393 overexpressing and also mir393 resistant AFB3 overexpressing *Arabidopsis* lines are aimed to be generated. It is anticipated that increasing the number of mismatches between the AFB3 mRNA and miR393 from two in wild-type to six in mutant version will make it resistant for mir393. Without altering the amino acid sequence of the encoded AFB3 protein its functionality will be preserved. It is predicted that the induction of miR393 expression will result in decreases in TIR1 and AFB mRNA levels, which possibly leads to less proteolysis of E3 ubiquitin ligase targeting proteins (probably regulators or determinants of glyphosate tolerance). In the mir393 resistant AFB3 overexpressing phenotype the opposite results are expected. Until now we have successfully cloned mir393 and mutant AFB3 into pEarleyGate100 destination vectors. Now *Arabidopsis* is on the way of being transformed by the method of floral dip Agrobacterium-mediated transformation. On the verge of understanding the functions of miRNAs, this project would be a good starting point for exploring and elucidating the miRNAs associated with regulation of plant responses to glyphosate.

## P2.02 - Nucleotide sequence of the 5.8S rRNA in *Ficus carica*: A conserved motif and analysis of secondary structure

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The nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) region has become an important nuclear locus for molecular systematic investigations of angiosperms at the inter-specific and intra-specific levels. Universal PCR primers are positioned on the conserved rRNA genes (18S, 5.8S, and 26S) to amplify the entire ITS spacer region. PCR products were directly sequenced and their identity was verified with the Blast (NCBI) as an appropriate programme. Results show that the 5.8S rRNA gene, a part of the large subunit rRNA complex, is highly conserved in length (162-165) for the all cultivars studied except for the caprifig 'Jrani' ones. In the other hand, few changes were observed due to the mutations observed in the aligned sequences. This report describes a conserved 14 base pair (bp) motif in the 5.8S rRNA gene that can be used to differentiate between thirty one Tunisian fig cultivars. In addition, sequence analysis of this gene showed the presence of the conserved convenient *EcoRV* restriction site in most cultivars. The 5.8S rRNA of *Ficus carica* shows homology of 100% with the corresponding gene of the genus *Ficus* species and 97% to 98% of other plants. In addition, the proposed secondary structure of the 5.8S rRNA is similar of seventeen cultivars studied which have 100% homology in their sequence of 5.8S gene, except sequences that have undergone some mutations, their secondary structure has different.

### P2.03 - Investigation of the phylogenetic relationships within the genus *Ficus* (*Moraceae*) using plastid *trnL-trnF* sequences

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The phylogenetic relationships within the genus *Ficus* were analyzed by comparing the *trnL-trnF* intergenic spacer sequences of the chloroplast DNA (cpDNA). The size of the sequences of the 76 species ranged from 398 base pairs (bp) to 476 bp. The base contents were estimated and results demonstrate an average of GC content of 36.3% and a relatively high average AT values of 63.6 % in the *trnL-trnF* spacer of chloroplast DNA of fig species. The transition/transversion rate ratio was calculated and shows that transition are most frequent ( $R = ti/tv = 1.285$ ). Of the 497 aligned positions in this region of these species, 107 sites were variable (21.52%). The evolutionary distances were computed using the Maximum Composite Likelihood (MCL) method exhibited an average value of 0.023. The Disparity index were calculated and exhibited an average value of 0.009 suggesting that the fig species studied are characterised by high level of polymorphism at the cytoplasmic DNA level. Tajima's Neutrality tests rejected the neutrality assumption in the total sample ( $D = -2.07$ ) and suggests a recent demographic expansion of these species. A phylogenetic tree was generated based on Neighbour-joining (NJ) analysis of *trnL-trnF* sequences data from 76 fig species. The consensus tree contained two clusters. The First one (I) is composed only by *Ficus Tambourissa* whereas the second group (II) is composed of the remaining species. Our result proved that the *trnL-trnF* spacer of chloroplast DNA is useful for discrimination as well as for investigation of patterns of variation in fig species.

### P2.04 - TILLMore: A forward- and reverse-genetics resource for the identification of root morphology-related mutants

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At DiSTA, University of Bologna, a TILLING resource has been developed following chemical treatment with sodium azide ( $\text{NaN}_3$ ) on barley (cv. Morex). The resource, named TILLMore, can be considered for both forward- and reverse-genetics analyses (Talamè et al., 2008, Plant Biotechnol J. 6: 477-485).

The TILLING approach has been used to analyze four barley genes involved in root development (*Brx1*, *Rpd1*, *HvExpβ1* and *Miz1*). This procedure allowed for the identification of 25 mutants corresponding to an average of six alleles per gene. Almost all the detected mutations were CG-to-TA transitions, as expected using sodium azide as mutagen agent, and several of them were missense, implying a change in amino acid sequence. In parallel, a forward-genetics analysis was performed on a portion of the mutagenized population to single out root morphology alterations by comparison with Morex wild-type. For this purpose a simple paper-roll approach was performed allowing for the identification of phenotypic variants at the seedling stage. The analysis was completed on ca. 1,000  $M_3$  families and the phenotypic evaluation was repeated only for the putative mutants identified during the preliminary screening. The screening for root phenotype at the seedling stage allowed us to identify a total number of ca. 70 lines with altered root morphology, corresponding to ca. 7% of the families. A more accurate phenotypic characterization of the mutant lines is currently in progress.

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## P2.05 - A highly conserved gene island of three genes on chromosome 3B of hexaploid wheat: Diverse gene function and genomic structure maintained in a tightly linked block

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Recent publication of the physical map of hexaploid wheat (*Triticum aestivum* cv. Chinese Spring) chromosome 3B has enabled the establishment of genome sequencing studies investigating biological features contained in the wheat genome. In this study, we sequenced and assembled a group of 15 wheat BACs from the chromosome 3B physical map FPC contig *ctg1034* into a 783,553 bp genomic sequence. This *ctg1034* sequence was annotated for biological features such as genes and transposable elements. A three-gene island was identified among >80% repetitive DNA sequence. Using bioinformatics analysis there were no observable similarity in their gene functions. The *ctg1034* gene island also displayed complete conservation of gene order and orientation with syntenic gene islands found in publicly available genome sequences of *Brachypodium distachyon*, *Oryza sativa*, *Sorghum bicolor* and *Zea mays*, even though the intergenic space and introns were divergent. We propose that *ctg1034* is located within the heterochromatic C-band region of deletion bin 3BL7 based on the identification of heterochromatic tandem repeats and presence of significant matches to chromodomain-containing *gypsy* LTR retrotransposable elements. We also speculate that this location, among other highly repetitive sequences, may account for the relative stability of the gene island.

## P2.06 - Collections of mutants for functional genomics in the legume *Medicago truncatula*

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Three complementary collections of the model species *Medicago truncatula* (Porceddu et al., 2008) were produced in the frame of the Italian functional genomics project MIUR-FIRB 'Post-genomics of Forage Legumes'. A wide range of variation in plant and plant organ morphology was found in the TILLING collection formed by about 1900 lines with DNA (M<sub>2</sub> generation) and seed (M<sub>3</sub>/M<sub>4</sub> generation). The estimated rate of mutation, on the basis of the screening for three genes of interest, was 1 mutation/Kbp/400 plants.

Two of these genes are involved in the biosynthetic pathway of plant secondary metabolites (triterpenic saponins and trypsin inhibitors) displaying physiological, agronomical and industrial interest. In particular, two segregant mutant lines of the 6 found for the orthologue of the trypsin inhibitor gene *MstI* from *M. scutellata* were examined but no significant variation in trypsin inhibition was found compared to the full sib wild type. Two lines carrying different point mutations in the *MPHY1* gene, coding for an extracellular phytase, were also studied, one of them showing different ( $P = 7.5\%$ ) enhanced phytase activity with respect to the control line.

Transposon mutagenized lines were also produced (approx. 1500 R0 plants) according to the strategy of d'Erfurth et al. (2003). Several mutants were isolated also regarding plant architecture (for example leaf and flower morphology) and secondary compounds (tannins). Results will be presented on few selected mutants that have been characterized more extensively.

D'Erfurth I, 2003, Plant J. 34: 95-106

Porceddu A et al., 2008, BMC Research Notes, 1: 129.

**P2.07 - Differential expression of the functional marker candidate *AOX* is related to stress-induced reprogramming of growth behavior in a primary culture of *Daucus carota* L.**

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Alternative oxidase (AOX) is a mitochondrial protein encoded by the nuclear genome. In higher plants *AOX* genes form a small multigene family mostly consisting of the two subfamilies *AOX1* and *AOX2*. Recently, four *AOX* genes (*DcAOX1a*, *DcAOX1b*, *DcAOX2a* and *DcAOX2b*) were identified in carrot (*Daucus carota* L.) by our group (Costa et al., 2009, Plant Physiol Biochem, 47:753-759). AOX is suggested to play a crucial role in efficient cell reprogramming under stress (Clifton et al., 2006, Bioch et Bioph A, 1757:730-741 and Arnholdt-Schmitt et al., 2006, Trends Plant Sci., 11 (6):281-287). In order to focus on the dynamics of gene expression during development and growth, a carrot primary culture experimental approach was chosen. AOX expression is being studied under controlled temperature conditions after inoculation of differentiated secondary root phloem explants in a cytokinin-containing nutrient solution that induces tissue redifferentiation and callus growth. RT-PCR analysis revealed differential expression of carrot *AOX* genes in this system (Campos et al., 2009, Physiologia Plantarum, 137:578-591). Studies by qRT-PCR in order to search for *AOX* differential gene expression between individual plants will be presented. Additionally, the results of a validation of several housekeeping genes are reported.

**P2.08 - Gene expression analysis in grapevine plants during *Uncinula necator* infection revealed differential responses between resistant and susceptible cultivars**

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European *Vitis vinifera* cultivars usually show high susceptibility to the pathogenic fungus *Uncinula necator*. 'Trincadeira' and 'Aragonez' are two of the most important Portuguese cultivars for red wine production. Since they are very sensitive to powdery mildew, entire crop plantations are lost, representing a dramatic decrease of the economic profits. So, grapevines with improved disease resistance would be welcome, especially if important agronomic traits are not altered. The application of genetic engineering techniques may have a real impact by making possible the genetic improvement of these grapevine cultivars, due to its ability to directly transfer a single trait into a grapevine variety while leaving unchanged its distinctive characteristics of the variety.

In genetic transformation, the crucial point is to have cells that are both able to regenerate and to be transformed. An efficient and reliable protocol to regenerate plants of both cultivars by somatic embryogenesis has been developed (Cardoso et al., 2009, Acta Horticulturae, 812:305-311). The improvement of the genetic transformation using these embryogenic cultures was done using particle bombardment gene delivery (Cardoso et al., 2004, Acta Horticulturae, 652:407-413) and gene transfer by *Agrobacterium tumefaciens*. For development of fungi resistant transgenic grapevines it is necessary to achieve early and high expression of several pathogenesis-related proteins. Several genes related to plant response to pathogenic fungi were isolated from the *V. vinifera* cv. 'Regent' (resistant) and the cv. 'Riesling' (susceptible). The expression profile of these genes was studied through qRT-PCR in resistant and susceptible cultivars under *U. necator* infection conditions. The results of this study are here presented and discussed

## P2.09 - Sugarcane mosaic virus (SCMV)-tolerant maize obtained by RNAi technology

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Today corn is one of the most cultivated cereal in the world (155 million ha). Brazil is the third largest producer, behind only the U.S. and China. Among the great losses faced by agriculture are the pests and diseases in corn such as Mosaic (SCMV) and streak virus (MRFV). The effects caused by mosaic in maize plants are greater if the infection occurs earlier, where experiments can show reduction up to 50%. A search for cultivars more productive, disease resistant and adapted to different conditions can be accelerated with the use of techniques such as gene manipulation and transformation. Thus, the purpose of this research is to test the efficiency to develop a tolerance corn to SCMV by expression the coat protein of this virus with the RNAi technology. From the 250 transgenic events seed were obtained only from 82. Of these, 47 events were germinated, and four seeds per event were tested against the virus. The 15 day-old seedlings were inoculated with the virus (carborudum Bioglobal mesh 600) for 4 consecutive weeks, one injection per week. Of a total of 142 plants that had been tested 26 asymptomatic. It has been also observed a decrease in symptoms in some of the plants tested in the weeks after the first infection. Southern blot, PCR and test with the herbicide were done to confirm the presence of the transgene. The phenotyping of some F3 population has shown that SCMV tolerant maize plants have been obtained by the RNAi technology. These plants have been self pollinated and Southern blot used to identify single copy transgene. The expression of the gene construction and virus quantification have been followed by Real Time PCR.

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## P2.10 - Methylation profiling at the maize flowering time locus *Vgt1*

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The *Vegetative to generative transition 1 (Vgt1)* locus is one of the most commonly identified flowering time QTLs in maize. *Vgt1* was positionally cloned on chrom. bin 8.05 after its Mendelization (Salvi et al., 2007, PNAS, 104: 11376-11381) and shown to correspond to an upstream (70 kb) non-coding regulatory element of *ZmRap2.7*, an Ap2-class transcription factor known to influence flowering time. A transposon (MITE) insertion was identified as a major allelic difference within *Vgt1*. One of the hypotheses is that *Vgt1* might function by modifying *ZmRap2.7* chromatin structure/function through an epigenetic mechanism. Therefore, we decided to investigate the methylation state at multiple regions of ca. 250 bp each, within *Vgt1* and the promoter of *ZmRap2.7*. Following digestion with McrBc, an endonuclease that acts upon methylated DNA, real-time PCR analysis was performed on genomic DNA from near-isogenic maize lines carrying different combinations of late and early alleles at both loci. DNA was extracted from leaves and shoot apices sampled at several stages of development. Preliminary results showed a reduction in methylation from the first through the fifth leaf stage irrespectively of the genotype at both *Vgt1* and *ZmRap2.7*. Additionally, the C22-4/Gaspé allele/haplotype showed a reduced methylation in comparison to N28. The region closer to the MITE insertion showed a constant and very dense methylation level throughout leaf development and for both alleles. Additional genotypes and stages of development are currently being investigated.



**P2.11 - Identification of differentially expressed genes during flower development in *Olea europaea* L. by suppressive subtractive hybridization and transcript analysis**

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Olive (*Olea europaea* L.) inflorescences contain both hermaphrodite and staminate flowers, and the fruit set is correlated to the proportion of perfect flowers. A mature tree produces up to 500,000 flowers and only 1-2% of them set fruits that reach maturity, thus the flower abscission represents a serious yield-limiting factor. Both, ovary abortion and flower abscission, are influenced by nutritional factors, environmental conditions and genetic background, since different cultivars behave in different ways.

In order to isolate up- and down-regulated genes between two selected flower developmental stages in the cv Leccino, large libraries of differentially expressed gene sequences were generated through suppressive subtractive hybridization (SSH). A total of 1,127 sequences were computationally annotated, leading to the identification of 232 ESTs. Time-course transcriptional profiles of genes involved in carbohydrate and lipid metabolism, C-fixation, energy metabolism and cellular processing were evaluated. Results demonstrated that transcript levels of most of the analysed genes decrease during flower bud development. Moreover, when validating these data on cultivars exhibiting abundant flowering and low fruit set, such as cv. Dolce Agogia, transcript levels were shown to be quantitatively modulated in comparison with cv. Leccino which showed a remarkable fruit set capacity.

Our data widen the knowledge on the regulation of molecular changes that occur during flower development and suggest that carbon deprivation, as well as modification of all carbohydrate pathway, induce ovary abortion through programmed cell death (PCD). This hypothesis is supported by the analogy of biological events that occur in cascade, such as consumption of initial carbohydrate reserves, growth arrest, breakdown of proteins and lowered expression of many enzymes involved in glycolysis.



## P2.12 - Cloning SLG and SRK genes as candidates for self-incompatibility in olive (*Olea europaea* L.): Domains organization and expression studies

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*Olea europaea* L. is one of the oldest agriculture tree crops and, in spite of its great cultural and economic importance, a few studies have been carried out on the reproductive barriers in this species, the main one being the self-sterility of most cultivars. The aim of this research was that of studying the self-incompatibility system in olive from cyto-histological and bio-molecular standpoints. Self-incompatibility is one of the most important systems adopted by many flowering plants to prevent inbreeding, maintaining so diversity within the species. A deep comprehension of this reproductive constrain is crucial because it may help to implement breeding strategies useful to obtain a higher yield of fruits. The available literature on the topic reports that olive trees adopt a Gametophytic Self-Incompatibility (GSI) system. According to this knowledge, a preliminary research approach was started to search genes responsible for GSI, such as S-RNase (S-locus ribonuclease) and SLF (S-locus F-box containing protein). At the same time, cyto-histological analyses by means of stain-clearing and aniline blue staining of pollinated and non-pollinated pistils, were also conducted in putative self-compatible and self-incompatible cultivars (*i.e.* Leccino and Frantoio, respectively). Both cyto-histological observations and bio-molecular results led us to suppose a Sporophytic Self-Incompatibility (SSI) system occurring in olive. Consequently, the main genes known to play a crucial role in SSI were searched, like SLG (S-locus Glycoprotein), SRK (S-locus Receptor Kinase), and SCR (S-locus Cysteine Rich protein). In order to obtain messengers encoded by these genes, either degenerated and non-degenerated primers were designed and tested on consensus sequences, belonging to SSI-related species (*e.g.* *Brassica* spp.), obtained by multiple alignments of records retrieved from the NCBI database. Different approaches based on the use of both cDNA and genomic DNA as templates allowed us to recover the full-length of an SLG-like gene and four sequences putatively encoding for SRK-like proteins. A differential expression of these genes among different olive tissues and organs (*i.e.* flowers of self-compatible and self-incompatible cultivars at different developmental stages, and samples deriving from buds, leaves, branches, fruits and roots) was then tested by means of Real-Time PCR analysis using different subdomain-specific primer combinations.

## P2.13 - GreenPhyl: Phylogenomic resources for comparative and functional genomics

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With the increasing number of plant genomes being sequenced, a major objective is to transfer accurate annotation from characterized sequences to uncharacterized sequences. GreenPhyl (Conte et al., 2008, *Nucleic Acid Research*, 36: D991-8) is a tool for plant comparative genomics that predicts the function of genes based on their evolutionary relationship with genes of known function. The database (version 2) comprises protein sequences of 16 plant species fully sequenced including socio-economically important crops like rice, sorghum and maize that were grouped into gene families using similarity-based methods. GreenPhyl contains approximately 13,000 gene families being annotated, computational analyzes and external cross-references (InterPro, KEGG, Swiss-Prot, Pubmed) related to all gene members. Once manually annotated (*i.e.* properly named and classified), gene families are finally processed by phylogenetic analyses to distinguish orthologous and paralogous gene. Orthologous genes descend from the last common ancestor through speciation and most probably encode proteins with a similar function in different species. In addition, the website offers a range of user-friendly tools to query the data. These resources will be particularly helpful to molecular biologist for gene discovery and gene function inference. We believe that a better understanding of genome evolution will contribute to elucidate the genetic basis of important agronomic traits and therefore facilitate ongoing plant breeding efforts.

## P2.14 - Identification of novel transposon sequences in *Ruta graveolans* genome

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Lately, much interesting is focusing about potential anti-inflammatory and anticancer action of *Ruta graveolans* extract for human therapeutic purposes. However, until today, *Ruta* and related genera are poorly differentiated offering a primary example of discordant systematic conclusions

Knowledge of the *Ruta graveolans* genome or is currently restricted to the 62 sequences available in Gen-Bank (nucleotide, February 2010). It is well known that it is likely that the acquaintance of novel repetitive sequences involving transposable elements could increase the amount of molecular data for understanding the genomes evolution.

Transposable elements are found throughout the plant kingdom representing about 50% of the total DNA in some species; they can mediate genomic rearrangements, including insertions, deletions, inversions and duplications, are potentially associated with or subsequent to speciation events.

To improve our understanding on *Ruta graveolans* genome, for the first time, we have scanned its genome using PCR targeting of the reverse transcriptase and ribonuclease H domains of retrotransposons. The multiple alignments showed that we have isolated novel types of *Copia*-like reverse transcriptase sequences from *Ruta graveolans* related to *Citrus* retrotransposons. To clarify the origins of these retrotransposon sequences, we studied their evolutionary relationships with those of other organisms.

The analysis of transposable element sequences and genomic distribution might contribute to our understanding of their potential impact on *Ruta* evolution and diversity and to set up molecular markers for phylogenetic studies.

## P2.15 - Regulation of flower colour in azalea assessed by qPCR of candidate genes and eQTL mapping

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Flower colour in azalea is a semi-qualitative trait, mainly determined by anthocyanin and flavonols pigment type and content. A two-gene model explains the major phenotypic variation between white, brick red and carmine red colour, however, the regulatory network behind is still unclear. To identify key driver genes, conventional genetics were combined with gene expression profiling, resulting in eQTL mapping. So far, mainly micro-array expression profiling has been used, limiting the technique to large scale projects. Nevertheless, qPCR can be a good and cost efficient alternative.

In a crossing population (250 plants) segregating for flower colour a genetic map was constructed (De Keyser *et al.*, 2010. BMC Molecular Biology 11:1). Besides anonymous AFLPs and SSRs, functional EST markers for key-enzymes in the flavonoid biosynthesis pathway and *Myb*-profiling, generating dominant markers functionally related to the *Myb* gene family, were applied. Flower colour was determined by image analysis; colour measurement values were input to QTL mapping. Gene expression profiles of five genes coding for key enzymes of the flavonoid biosynthesis pathway were generated in the petals of a subset of 70 flowers of the crossing population. Normalized data were subjected to Box-Cox transformation in order to get a Gaussian distribution prior to QTL mapping. Both Kruskal-Wallis rank sum tests and Interval Mapping were used (MapQTL5).

For the candidate genes mapped as EST markers, we checked for both *cis* and *trans*-acting eQTLs e.g. *DFR* appears to be local-*trans* regulated. eQTLs for multiple genes were co-located with *Myb*-markers, suggesting a combinatorial transcriptionally regulation of these genes. Interestingly, the expression of *FLS* is mapped at the Q-locus (responsible for carmine colour). Indeed, *FLS* leads to the production of co-pigments (flavonols), which are only visible in the presence of anthocyanins and differentiate between carmine red and red flowers. This correlation proves the precision of our qPCR results and their potential for eQTL mapping.

**P2.16 - Molecular adaptation of the chloroplast *matK* gene in *Nymphaea tetragona*, a critically rare and endangered plant of India**

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Sustainable utilization of plant genetic resources for food and agriculture has been increasingly discussed at both national and international forums. Besides exploitation, conservation of plant genetic resources has become an integral part of these discussions. Conservation aims at maintaining the diversity of living organisms, their habitat and the interrelationship between organisms and their environment. For achieving such goals, appropriate conservation strategies has to be adopted. Determining the genetic makeup of a particular plant species is of critical importance when planning a suitable conservation strategy. In this study, we sequenced the nrDNA ITS region, chloroplast *trnK* intron, *matK* and *rbcl* gene aimed at understanding the rarity of *Nymphaea tetragona*, a critically rare and endangered plant of India found at only one location. We extended our investigation to other *Nymphaea* species namely *N. alba* var. *rubra*, *N. caerulea*, *N. x marliacea*, *N. nouchali*, *N. pubescens* and *N. rubra* that are commonly available throughout India. Interestingly, *matK* gene of *N. tetragona* revealed a high number of non-synonymous substitutions. Such adaptive changes at the DNA and protein sequence level of *matK* gene may have been associated with the colonization of *N. tetragona*, suggesting that it would have migrated from China. Appropriate measures for conservation of *N. tetragona* and relevant findings are discussed.

**P2.17 - Molecular biology taking twists from cereal endosperm to development in general of all plants? Controlled cell divisions determine cell identity, body pattern and meristem function**

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Evolutionary studies and increasing whole genome sequence information, are expected to give us new insight into fundamental questions in biology like how plants became multicellular, and stem cell/meristem control. We will present results from cereals and Arabidopsis on how DEFECTIVE KERNEL1 (DEK1) might be a key to understand both these fundamental questions. DEK1 is the exclusive plant Calpine identified, membrane anchored, highly conserved, single copy gene (of about 24 kb) in all sequenced annotated plant genomes and expressed in all actively dividing cells (Lid et al. 2002 PNAS; Tian et al. 2007 Plant Cell). This suggests it is a fundamental gene for plant function. It is known to be necessary for epidermal identity and this might be how organisms could distinguish self from non-self and outside from inside. We have recently shown that all effects of DEK1 are via controlled cell divisions and deposition of cell walls (unpublished). How this might cause *dek1* embryos to arrest at globular stage and further affect meristem organization will be discussed. Seed development is controlled by organized anticlinal and periclinal cell divisions and corresponding cell wall depositions. Genes and signaling pathways are further directing pattern formation by secure apical-basal and radial symmetry of the embryo, and meristem functions developing all parts of the adult plant. Exciting different expression of body pattern markers like *PIN4*, *AtML1*, *STM*, *WUS*, *CLV3*, *REV* and *WOX* in *dek1* embryos will be shown by *in situ* hybridization, and how this might give new suggestions of how they are functionally related to change plant development. Dissecting the long protein sequence of 240 kDa, has shown the membrane part to be involved in regulation of DEK1 function and the CALP part to rescue the *dek1* mutant phenotype. Some of our working hypotheses on DEK1 function and possible interactions with central genes in meristem function will be presented. Finally we will discuss progress in understanding gene function by evolutionary studies. Like discussing possible roles of DEK1 in the moss *Physcomitrella*, not depending on L1 identity when growing as single cell files yet depending on controlled cell divisions and cell wall deposition.

## P2.18 - Expression analysis of genes involved in the iron deficiency of two citrus rootstocks using the CombiMatrix platform

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Low Fe bioavailability is a primary constraint to plant growth in calcareous soils. In these soils iron inefficient citrus rootstock genotypes often induces symptoms of iron deficiency stress with evident symptoms in particular way in the leaves (interveinal chlorosis). Our aim was the identification of candidate genes and related processes for further experimentation to increase our understanding of citrus response to iron deficiency stress. In this study microarray analyses was used for the identification of genes involved in citrus rootstocks response to iron stress. We evaluated the expression level of genes involved in the iron chlorosis using Tarocco Scirè orange [*Citrus sinensis* (L.) Osbeck] grafted on Swingle citrumelo (*C. paradisi* x *Poncirus trifoliata*), high sensitive to chlorosis and on Carrizo citrange (*C. sinensis* x *Poncirus trifoliata*), medium sensitive. Citrus trees were grown in plastic pots using a volcanic soil, completely absent of active lime (0%) and a calcareous one, containing 9% of active lime. The expression analysis was performed on the CombiMatrix 90K platform at the University of Verona, on a root citrus chip carrying 7,969 specific probes derived from clustering and assembly of ESTs related to *Citrus* and its rootstocks (released April 16, 2009) using the TGICL software. The comparison of three biological replicates, each with 4 technical replicates (two rootstocks and two different soils) for a total of 12 hybridizations, evidenced genes whose expression levels exceeded a two-fold difference using a *P*-value  $\leq 0.05$ . Most of them are involved in stress response, salt induction, pathogen resistance, and some other are not annotated. The expression level of some of differential genes (oxalate oxidase, glutathione peroxidase, germin-like) were validated and confirmed through the Real time PCR.

## P2.19 - The cloning and sequence analysis mRNA of putative 2-oxoglutarate dependent dioxygenase (As-GA3-oxidase) from *Avena sativa* L. cv. Pal

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The hexaploid oat (*Avena sativa* L.) is a cereal whose importance is increasing. Gibberellins are well-known phytohormones which play a key role in a large number of plant development processes. Currently it is known more than 120 gibberellin metabolites in plants, fungi and bacteria. Biologically active gibberellins GA<sub>4</sub> and GA<sub>1</sub> arise in plants through conversion from GA<sub>9</sub> and GA<sub>1</sub>. Reactions are catalysed by 2-oxoglutarate-dependent dioxygenase enzymes, specified as GA<sub>20</sub>-oxidase and GA<sub>3</sub>-oxidase. Presence of these key enzymes was documented in nearly all of commercial important crops except for common oat. Using the cDNA analysis of GA<sub>3</sub> oxygenases from common wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and rice (*Oryza sativa*) we designed degenerate primers to identify the coding sequence of GA<sub>3</sub>-oxygenase of oat. The used primer sequences were following: FORWARD-1-5'-ATGCCACGCCGKCGCACYT-3', FORWARD-2-5'-GRACCGDCTRCTTCGA-3', REVERSE-1-5'-CBRKGTSPTYGCGCGC-3', REVERSE-2-5'-CTRCGACRAGAKSASGTCGT-3'. Analysed samples were taken from hexaploid oat, cultivar Pal. RNA was isolated from cotyledons. PCR products were cloned and both DNA strands were sequenced. The final fragment of 1119 bp covered all the coding region of mRNA. Comparing the final fragment sequence with database <http://blast.ncbi.nlm.nih.gov/Blast.cgi> we found this sequence to code probably the GA<sub>3</sub>-oxygenase and basing on these facts we named this sequence as putative As-GA<sub>3</sub> oxygenase (Genebank accession code GU727617) Our sequence showed the highest level of homology with cDNA GA<sub>3</sub> oxygenase from common wheat (Appelford et al., 2006, Planta, 223:568-582) where the sequence identity matrix reached 0,89. According to the ORF analysis of the putative amino acid sequence we proved, that the new sequence encodes probably GA<sub>3</sub>-oxygenase. Putative GA<sub>3</sub>-oxygenase has identical active site amino acid composition and metal binding sites. The homology of oxidoreductase activity responsible domain is 95%. In oats so far only individual GA-metabolites were identified and reduced-height mutants and their sensitivity to gibberellin addition are known.

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## P2.20 - Two different sources of CMS in beets and their distribution in the germplasm collection

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Although several sources of cytoplasmic male sterility (CMS) have been described, the so-called Owen type is the only cytoplasm used to produce sugar and garden beet hybrids. Due to its importance for breeding, the molecular basis of Owen CMS has been carefully studied. We determined the complete nucleotide sequences of the mitochondrial genomes from normal fertile and Owen CMS plants. An in-depth sequence comparison of the two mitochondrial genomes, together with an in organello translation assay and Western blot analysis, indicated that the presequence of *atp6* (designated preSatp6) encodes a variant 35kDa protein that is possibly related to Owen CMS. A second source of CMS, called I-12CMS(3), was derived from wild beets collected in Pakistan. Interestingly, the 35kDa preSATP6 protein was missing in the sterile anthers carrying the I-12CMS(3) cytoplasm, which instead expressed a CMS-correlated protein of 12kDa. This 12kDa protein proved to be encoded by an unusual mitochondrial gene designated *orf129*. The association of *orf129* with CMS was further confirmed by the observation that this sequence caused pollen sterility in transgenic tobacco. We also verified that *orf129* is entirely absent from the Owen mitochondrial genome. Beets thus appear to maintain distinct CMS cytoplasms, each capable of conferring male sterility by an apparently different mechanism. Garden and leaf beet germplasm accessions were evaluated for the presence of normal fertile and male sterile cytoplasms using polymorphisms in the mitochondrial minisatellite loci. Eleven mitochondrial haplotypes were identified, of which two were associated with Owen and I-12CMS(3) cytoplasms, and two others with normal cytoplasms. The results indicated that normal cytoplasm predominated in both the garden and leaf beet gene pools, whereas Owen cytoplasm rarely occurred. In addition, I-12CMS(3) cytoplasm was sporadically found in the beet germplasm accessions examined.

## P2.21 - New expression system for transient assay of gene function in lettuce

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*Agrobacterium*-mediated transient expression is a useful tool for assessing gene expression constructs in plants. In this study an efficient, scalable transient expression system has been developed and characterized for production of recombinant gene in lettuce.

The transient expression system utilizes lettuce (*Lactuca sativa* L.) agroinfiltrated with *Agrobacterium tumefaciens* bearing the reporter gene encoding thermostable lichenase (LicBM2) cloned into pBIN vector, under control of different regulatory elements.

The optimal transient expression system for LicBM2 gene in lettuce was chosen among the constructed and tested vectors by obtaining highest level expression LicBM2 gene for p35S-LicBM2-ER vector.

Compared to previously described systems; this is a transient expression system with easy-changing gene interest and regulatory elements for successful gene expression and protein accumulation in endoplasm reticulum of lettuce cells. Thus, it is a useful evaluation of gene expression system in lettuce before transformation. The results demonstrate that the transient expression of recombinant gene in lettuce by this system is very rapid, efficient, inexpensive and scalable.



## P2.22 - Evolution of MIR159/319 microRNA genes and their post-transcriptional regulatory link to siRNA pathways

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MicroRNAs (miRNAs) are prevalent and important endogenous gene regulators in eukaryotes. miR159 and miR319 are highly conserved miRNAs essential for plant development and fertility. Despite high similarity in sequence, mature miR159 and miR319 have distinct expression patterns, targets and functions. Thus, MIR159/319 appears to be a miRNA gene family with ancient origin and considerably diverged clades. Both MIR319 and MIR159 precursors produce multiple miRNAs in a phased loop-to-base manner. The phylogeny of MIR159/319 genes and why such unusual style of miRNA production has been conserved during evolution is not well understood. We reconstructed the phylogeny of MIR159/319 genes and analyzed their mature miRNA expression. The phylogeny suggests that the MIR159/319 genes have formed at least ten extant early-branching clades through gene duplication and loss. A series of duplications occurred in the common ancestor of seed plants lead to the original split of flowering plant MIR159 and MIR319. The results also indicate that the expression of MIR159/319 is regulated at post-transcriptional level to switch on the expression of alternative miRNAs during development in a highly spatio-temporal specific manner, and to selectively respond to the disruption of defensive siRNA pathways. Such intra-stem-loop regulation appears diverged across the early-branching clades of MIR159/319 genes. Taken together, the MIR159/319 genes have evolved from ancestral phased stem-loop small RNA into post-transcriptionally regulated product-variable miRNA genes that might integrate development programs with genome defense.

## P2.23 - Prospecting AP2 family genes from resistant rice genotypes for improving drought tolerance

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Rice (*Oryza sativa*) is the most important food crop world wide, and its productivity is affected by abiotic stresses. Crop improvement under stressful condition is a challenging task because of the complexity of the traits. Many regulatory genes such as transcription factors (TFs) are upregulated under stress which can activate set of downstream genes. Amongst many TFs, the proteins belonging to the family AP2/ERF, which is involved in key developmental steps, such as flower organogenesis and seed development, have been reported to be important. Overexpression of the AP2/EREBP factors CBF1, DREB1A and CBF4 resulted in drought/salt/cold tolerance in Arabidopsis. Transgenic Arabidopsis overexpressing OsDREB1 showed salt, cold and drought tolerance. Similarly, over expression of wax-related SHN1, an AP2 type TF displayed drought resistance. The major goal of the present study is to prospect candidate TFs belonging to AP2 family from resistant rice genotypes for improving drought tolerance in aerobic rice cultivars. Since stress adapted plants have efficient mechanisms to manage stress, it is believed that candidate genes from such plant types are ideal. Identification of stress responsive genes from adapted crops might be even more advantageous since the growth rates of these species are superior compared to resurrection plants. With this concept, we evaluated rice genotypes for the variability in drought resistance and short-listed 15 wild genotypes for detailed analysis. These genotypes were screened under salinity (NaCl stress ranging from 100, 200, 300 and 400 mM) and dehydration stress (PEG stress from -4, -6, -8, -10 and -12 bars) at seedling stage. Based on growth analysis, a few genotypes (Doodiga, Kesari, Nanibanta, Nereguli, Kirwana and Mantalaga) were selected as resistant types and one of the tolerant types, Doodiga was used for cloning of the AP2 family protein SHINE (SHN1). Full-length of SHN1 was amplified from genomic DNA, sequenced and annotated. Since there is differences in the gene between *Zea mays* and *Oryza*, attempts are being made to clone SHN1 from contrasting rice genotypes to examine the variations if any in this protein, which may contribute for differential response in stress tolerance.

## P2.24 - Targeted association analysis for tolerance to salinity in rice using SSR markers

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Rice is considered as a crop sensitive to salinity; however, it is grown mainly in deltaic areas with salt problems all over Europe. European rice breeding programs (France, Greece, Italy, Portugal and Spain) have established an European Rice Genetic Resources Collection (ERGR) of some 450 accessions, mainly temperate *japonica*, extensively characterised for agronomic traits and maintained by CIRAD. The main goal of the present study is to identify within this collection, a set of best performing genes and alleles for salinity tolerance, as well as the associated donors and molecular markers for use in breeding programs. In order to obtain the general organisation structure of the ERGR we assessed its genetic diversity through a Bayesian analysis of genotypic data over 26 SSR loci. A sub-sample of 200 accessions maximizing simultaneously allele number and allelic associations was then extracted for association analysis. The sub-sample was phenotyped for salinity tolerance at an early vegetative stage under controlled conditions, being leaf Na<sup>+</sup>/K<sup>+</sup> ratio the most discriminating trait. Based on literature review, we assembled a list with more than 100 rice candidate genes for salt tolerance, which are involved in signaling, ion homeostasis, stress tolerance, transcription regulation, general metabolism and unknown functions. With this information, we developed a database of rice QTLs and candidate genes for salinity tolerance ([http://tropgenedb.cirad.fr/html/rice\\_QTL.html](http://tropgenedb.cirad.fr/html/rice_QTL.html)). We selected 16 of these candidate genes for association analysis. SSR markers were used for the association analysis. A first set of 58 common SSR ([www.gramene.org](http://www.gramene.org)) covering these particular genes was used. In addition, we designed 320 SSR markers covering 100 kb up and downstream each candidate gene. In the end, we selected four of the designed SSR markers, the two closest to the gene and one in each end of the linkage disequilibrium region (100 kb each gene side). Results of association analysis between 16 target areas (using 60 SSR) and a dozen salinity tolerance traits are presented. Methodological constraints stem from the use of multi-allelic markers are discussed.

## P2.25 - Involvement of alternative oxidase (AOX) in adventitious rooting of *Olea europaea* L.

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AOX was proposed as a functional marker candidate for efficient adventitious rooting of olive (*Olea europaea* L.) shoot cuttings (Arnholdt-Schmitt et al., 2006, Proceedings of the 2nd International Seminar Olivebioteq, Marsala, Mazara del Vallo, Italy, Vol I, pp 249–254; Arnholdt-Schmitt et al., 2006, Trends Plant Sci., 11 (6):281-287). Recently, this hypothesis was strengthened by results showing the involvement of AOX activity in adventitious rooting in semi-hardwood olive cuttings of the easy-to-root cultivar 'Cobrançosa'. Additionally, a high degree of sequence polymorphisms and 3'-UTR length variability had been identified for *OeAOX2* as potential sources for differential gene regulation (Santos Macedo et al. 2009. Physiologia Plantarum 137:532-552). The cultivar 'Galega vulgar' is a bad rooting olive genotype presenting average rooting rates in shoot cuttings of only 5-20%. However, under optimized *in vitro* culture conditions this cultivar demonstrates the high rate of 60–75 % adventitious rooting. After 5-7 days in culture, some cells from the cortex and also from the sub-epidermal tissue reveal a dense cytoplasm and present high mitosis rates and the first morphogenetic root fields are observed after 12-16 days in culture. Root primordial, become visible after 20 days. (Peixe et al. in preparation). To study the general relationship of AOX transcript accumulation to rooting, RT-PCR analyses of *OeAOX2* have been performed with cv. *Galega vulgar* in both optimized systems for olive rooting, in shoot cuttings and micro shoots. The results will be discussed in view of the potential of *OeAOX2* as functional marker candidate for efficient root induction.

## P2.26 - The influence of vernalization and daylength on expression of flowering-time genes of winter wheat and barley

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Responses to prolonged low-temperature treatment of imbibed seeds (vernalization) were examined in winter wheat and barley. These occurred in two phases: the perception of prolonged cold, which occurred gradually at low temperatures, and the acceleration of reproductive development, which occurred after vernalization. Expression of the VERNALIZATION1 gene (*VRN1*) increased gradually in germinating seedlings during vernalization, both at the shoot apex and in the developing leaves. This occurred in darkness, independently of VERNALIZATION2 gene (*VRN2*), consistent with the hypothesis that expression of *VRN1* is induced by prolonged cold independently of daylength flowering-response pathways. After vernalization, expression of *VRN1* was maintained in the shoot apex and leaves. This was associated with accelerated inflorescence initiation and with down-regulation of *VRN2* in the leaves. The largest determinant of *VRN1* expression levels in vernalized plants was the length of seed vernalization treatment. Daylength did not influence *VRN1* expression levels in shoot apices and typically did not affect expression in leaves. In the leaves of plants that had experienced a saturating seed vernalization treatment, expression of *VRN1* was higher in long days, however. *FT1* (VERNALIZATION3 gene) was expressed in the leaves of these plants in long days, which might account for the elevated *VRN1* expression. Long-day up-regulation of *VRN1* was not required for inflorescence initiation, but might accelerate subsequent stages of inflorescence development. Similar responses to seed vernalization were also observed in barley. These data support the hypothesis that *VRN1* is induced by cold during winter to promote spring flowering in vernalization-responsive cereals.

## P2.27 - Towards the understanding of Ubc2 in plant disease resistance

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Ubiquitylation is a type of post-translational modifications during plants lifecycles. Poly-ubiquitylation of proteins is used for selective turn-over. Ubiquitin (Ub) is a tag used as a recognition signal and is covalently attached to target proteins for selective proteolysis. Recent findings indicate strong links between ubiquitin-mediated protein degradation and regulation of plant defense responses against wide range of pathogens. There are three main components in this system: Ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2, Ubc2) and ubiquitin-ligase enzymes (E3s). However, there is a lack of information about the regulatory role of Ubc2 in plant disease resistance. We aimed to find out the role of Ubc2 in response to pathogen attack in plants. *Arabidopsis thaliana* has three *Ubc* gene homologs (*ubc1-1*, *ubc2-1*, *ubc3-1*) in its genome. The detected *ubc* mutants were subjected to pathogenesis tests. Stable *Ubc2* over-expressor *A. thaliana* Col-5 lines are produced to measure the change in pathogen response. Additionally mutants will be complemented with stable transformation for gain of function analyzes. To confirm results, Ubc2 gene was silenced in *Nicotiana benthamiana* via TRV mediated VIGS. Sub-cellular localization of Ubc2 protein was detected in onion cells which indicates it localized in cytoplasm and nucleus. The results will be discussed in detail.



## P2.28 - DREB gene family in drought tolerant maize lines

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The evolutionary and the economic performances of the plant are affected directly by reducing its survival in the natural environment and its productivity in agriculture. Plants respond to water stress by biochemical and physiological modifications that may be involved in tolerance or adaptation mechanisms. The molecular bases of water stress tolerance remains unknown. Candidate genes induced by water-deficit stress in plants relatively sensitive to cellular dehydration have been identified and characterized, mainly in the model plant *Arabidopsis thaliana* (Vinocur and Altman, 2005; Verslues et al., 2006) such as the DREB gene family. DRE (*Dehydration Responsive Element*) is present in one or multiple copies and has been reported to promoter expression of many genes related to drought stress (Yamaguchi-Shinozaki and Shinozaki, 1994; Kasuga et al., 2004). Many of these genes seem to be related to the maintenance of structure and basic cellular function during water deficit, low temperatures and high salinity (Shinozaki and Yamaguchi-Shinozaki, 1994). Considering that, studies of DREB gene family in the tolerant and sensitive maize genotypes at different time course and in different maize genotypes have been performed. These results will help associate the effect of the DREB gene in drought tolerant maize lines. Parallel to this study experiments with a specific DREB gene from JIRCAS has also initiated recently to demonstrate its effect in transgenic plants.

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## P2.29 - Expression Quantitative Trait Loci analysis of two genes encoding rubisco activase in soybean (*Glycine max* (L.) Merr.)

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Ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA) catalyzes the activation of Rubisco *in vivo*, and plays a crucial role in photosynthesis. However, until now, little is known about the molecular genetics of RCA in soybean (*Glycine max* (L.) Merr.). Here, we cloned and characterized two genes encoding the longer isoform and the shorter isoform of soybean RCA (*GmRCA $\alpha$*  and *GmRCA $\beta$* , respectively). The two corresponding cDNAs are divergent in both the translated and 3'-untranslated regions. Analysis of genomic DNA sequences suggested that the corresponding mRNAs are transcripts of two different genes, and not the products of one single alternatively splicing pre-mRNA. Two additional possible -form RCA encoding genes, *GmRCA03* and *GmRCA14*, and one additional -form RCA encoding gene, *GmRCA11*, were also isolated. To examine the function and modulation of RCA genes in soybean, we determined the expression level of *GmRCA $\alpha$*  and *GmRCA $\beta$* , Rubisco initial activity, photosynthetic rate ( $P_N$ ) and seed yield in 184 soybean recombinant inbred lines. Correlation of gene expression levels with three other traits indicates that RCA genes could play an important role in regulating soybean photosynthetic capacity and seed yield. Expression quantitative trait loci (eQTL) mapping revealed four *trans* eQTLs for *GmRCA $\alpha$*  and *GmRCA $\beta$* . These results could provide a new approach for the modulation of RCA genes to improve  $P_N$  and plant growth in soybean and other plants.

## P2.30 - Genetic transformation of durum wheat with *HVA1* gene of barley using biolistic approach

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In Morocco, drought is the most important environmental stress affecting cereal production and results in an alarming reduction of the yield. Most effective way of managing this stress is through use of drought tolerant wheat cultivars. In addition to traditional approaches, the direct introduction of one or few genes from diverse species by plant transformation (genetic engineering) showed an improved tolerance to drought stress in various plant species including wheat and therefore offers suitable alternative and rapid approach for improvement of drought tolerance. In this study, we aimed to transform Moroccan durum wheat varieties with *HVA1* gene from barley using biolistic approach in order to improve drought tolerance. Transformation protocol suitable for Moroccan varieties had been developed and was used for transforming the *HVA1* gene. Five Moroccan varieties of durum wheat ('Irden', 'Marzak', 'Marouane', 'Isly' and 'Chaoui') were used for this study. The immature zygotic embryos were collected placed on induction medium at 25 °C in the dark for 3-5 days to induce embryogenic callus. The selected callus were placed in the osmotic MS medium supplemented with 15% mannitol for 4 h, and bombarded with 1 µm gold particles coated with plasmid DNA containing *HVA1* gene construct. The embryogenic tissue was placed in selection medium containing basta and the percentage of survival was 44, 37 and 19% respectively for 'Chaoui', 'Marouane' and 'Isly'. This percentage is very low in the case of 'Irden' and 'Marzak' varieties (around 4%). PCR analysis with primers bar gene and 35S promoter sequences confirmed the integration of transgene in the variety 'Irden'. Further studies on physiological characterization of the transgenic plants for drought tolerance are in progress.

# *Posters*

## *Session 3*

### *Harnessing plant diversity: Genomics-based applications*



### P3.01 - Differential allele expression in closely related clementine revealed by Denaturing High-Performance Liquid Chromatography analysis

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The analysis and characterization of genetic diversity are fundamental for any strategy of conservation and breeding as well as in the management of plant genetic resources (GR). The differentiation of varieties within species of high economic interest would be of great importance not only to rationalize the management of the collections of the GF but also to protect the rights of breeders, growers and nurseries.

In citrus, most crop species show close genetic proximity. In clementine mandarins (*Citrus clementina* Hort. Ex Tan.) or sweet oranges (*Citrus sinensis* (L.) Osb.) for example, almost all varieties have originated by spontaneous mutations or somaclonal variation in the field which, probably, only affect one or a few genes, making it very difficult to characterize molecularly the varieties by conventional methods.

Denaturing High-Performance Liquid Chromatography (DHPLC) represents a highly sensitive and automated method for DNA variant detection. In the present work DHPLC was applied to a systematic search of SNPs in 56 clementine mandarin varieties from the IVIA Germplasm Bank. The selection of the genes to be analysed was based on the DNA sequence information obtained from the citrus EST database (<http://bioinfo.ibmcp.upv.es/genomics/cfgpDB/>) and on previous results of our group. Among 14 analyzed genes, we have been able to identify 22 heterozygous positions. In seven of these positions, there is differential expression of alleles in the analyzed clementines. Based on the allele expressed by each variety, we have clustered the studied clementines into five groups: a major one with 34 varieties, three groups of two, nine and nine varieties respectively, and a final group of three varieties that bear most of the discriminative changes and therefore is the most distant from the remaining four groups.

For each SNP found in the clementines, we have analyzed the alleles which are present in Mandarin (*Citrus reticulata* Blanco) and Pummelo (*Citrus maxima* (L.) Osb), the taxa they have originated from, with the aim of tracing the origin of each allele expressed for each variety of clementine, and study the possible dominance of one of the parental genome over the other.

### P3.02 - Mapping of Quantitative Trait Loci (QTLs) associated with domestication characteristics using a recombinant inbred population derived from a cross between wild and cultivated cowpea (*V. unguiculata* (L.) Walp.)

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Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume commonly grown and consumed in many parts of the tropics and subtropics. We have constructed a genetic linkage map using simple sequence repeat (SSR) markers and a recombinant inbred (RI) population (159 individuals) derived from a cross between the cultivated cowpea cultivar 524B (a California Blackeye type) and 219C-01, a perennial wild isolate. Out of a total of 912 SSR primer pairs tested, 202 SSRs (22.14%) were found to be polymorphic between the two parents and were placed on the map which contains 11 linkage groups and spans 2991 cM, with an average interval distance between markers of 14.5 cM. Yield and three traits associated with domestication (seed weight, testa and pod size) were analyzed together with the genotypic data. About four-six QTLs for each trait were revealed with the phenotypic variation ranged from 5.6-26.1%. A marker associated with resistance to the parasitic plant *Striga gesnerioides* was also placed on the map. The present study reports the construction of the first SSR based genetic linkage map for cowpea and also demonstrates its utility for molecular mapping of QTLs controlling yield as well as domestication related traits. In general the map and these markers will be useful for the development of tools for marker-assisted selection in cowpea breeding.

### P3.03 - Maize gene bank as a source of drought tolerance

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Maize Research Institute gene bank is one of the largest maize collections in the world. New sources of drought tolerance were searched among the MRI gene bank accessions. The entire collection (6371 accessions) is consisted of local and introduced material and together with some commercial inbred lines and hybrids was subjected to water stress in Egypt. This material was divided into five groups, according to the duration of the growing season: extra early, early, medium, medium late and late. The main goal of the core collection is to represent the genetic diversity of a crop species and its relatives with a minimum of repetitiveness. Five groups of the experimental material were sown separately and were irrigated until the appearance of the first tassels. Before harvesting the best accessions were recorded by scoring stay-green, total appearance of the plant, ASI, percent of plants with seed set, percent of seed set and percent of grain filling for further experiments. The same traits were also observed next year on the selected 672 accessions. Trials were performed on three locations - Egypt, Macedonia and Serbia/Zemun Polje. The chosen accessions (51) were crossed in Chile (winter nursery) to elite inbred testers from three heterotic groups (Lancaster, BSSS and independent source). Accessions were used as female components, and at least 50 plants from each population were crossed to encompass the genetic variability. The criterion for the selection of good test-crosses was the yield (not significantly different from the control) or the performance index (based on grain yield and grain moisture at harvest) that was over 100% in comparison to the check. It is important to underline that seven accessions had good CA with all observed heterotic sources. They enable broadening of genetic variability and these accessions can make a completely different source of favourable germplasm. They will be further characterised by SSR markers. They could be used for new generations of maize ZP hybrids of higher yielding potential and adaptability.

### P3.04 - Comparison of the genetic variability present in Occidental and Chinese peach germplasm

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Peach is the most important fruit crop of the genus *Prunus* that include also other fruit crops such as plum (*P. domestica* and *P. salicina*), apricot (*P. armeniaca*) and cherry (*P. avium* and *P. cerasus*). It originated in China and from there expanded to central Asia and later to Europe and to the American continent. During centuries, peach was cultivated and selected for different agronomical characters, giving place to locally adapted populations. About 75 years ago, North American breeders started to produce a new wave of varieties based on a small number of founders. These breeding programs were extremely successful and nowadays, most commercial varieties grown in America and Europe are descendant of them. Genetic analyses have demonstrated a narrow genetic variability present in such germplasm. To explore new sources of variability we have analyzed 90 Chinese peach cultivars with 50 SSRs genome-wide distributed, and we have compared the results with the obtained in occidental cultivars analyzed with the same SSR set. Chinese varieties showed higher levels of variability measured as number of alleles (A) and observed heterozygosity ( $H_o$ ). Genetic distance and population structure analysis based on SSRs separate Chinese and Occidental cultivars into two different clusters. The obtained results indicate that Chinese germplasm can be a source of new genetic diversity for the Occidental breeding programs.

### P3.05 - Identification of QTLs for leaf traits in *Brassica rapa* L. in Russia

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Genetic basis of wide variation for morphological and developmental traits in *Brassica rapa* is largely unknown. QTL mapping of the morphologies in segregating populations derived from crosses between different morphotypes has been investigated in trials in St. Petersburg, Russia.

Three major QTLs were determined for the flowering time, 12 QTLs for plant habit and weight, 32 QTLs for leaves, five for flower and seven for seedpods. The QTLs for plant diameter, height, weight, leaf type, petiole length and width, lamina length, width, shape and surface, surface tissue, lamina edge, colour and hairiness were identified generally on top of R01 and R03, and middle part of R07 and their positions did not depend from environments, that confirms an importance of these loci contribution in development of whole plant. QTLs for clubroot resistance were found on 2, 5, 10 linkage groups, QTLs for ascorbic acid – on 3, 4, 5, and 7 linkage groups. Our studies indicate the usefulness of linkage mapping to determine QTLs for important agronomic characters.

### P3.06 - The use of simple sequence repeats marker for detection of genetic diversity and genetic relationships in lentil germplasm

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Assessment of genetic diversity of germplasm at gene bank has a strong impact on plant breeding and conservation of genetic resources. It is particularly useful in the characterization of individuals, accessions, and cultivars in determining duplications in germplasm collections and for selecting parents. The objective of the study was to assess the level of genetic diversity and genetic similarity among lentil germplasm collection of Central Asia and Caucasian (CAC) origin using simple sequence repeat (SSRs) marker. SSR data were generated using 10 primer pairs on 95 lentil landraces and improved cultivars. The observed allelic frequencies ranged from 0.005 to 0.71, the mean allelic frequency was 0.15. 31 alleles (59.6%) appeared with the frequencies of 0.1 or lower, while 4% (2 alleles) was detected in most of lentil accessions and was characterized with frequency of higher than 0.5. Range and average of genetic diversity index for all SSR loci were 0.43-0.83 and 0.68, respectively. There was a positive correlation ( $r = 0.94$ ;  $P < 0.5$ ) between gene diversity index and the number of accessions from each region. The lowest and the highest genetic diversity were for markers SSR 199 and SSR 213, respectively. Genetic Similarity indices between pairs of 95 accessions ranged from 0.21 to 1.0, with average of 0.61. Cluster analysis revealed four major groups in CAC lentils. Although there were both genetically distant and close genotypes in each geographic group, clear association between genetic similarity and geographic distance among landraces was observed. Hybridization or crossing between distantly related genotypes of the present study could be an appropriate strategy for landrace improvement programs.

### P3.07 - Multiplex SSR sets for variety identification in sunflower

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Germplasm characterization in crop species is essential for diversity conservation and utilization as well as for the protection of intellectual property. Due to their high polymorphism and reliability, microsatellites are considered the most suitable markers for those purposes; moreover, multiplex PCR analysis of SSR loci as a way to further increase informativeness and efficiency of testing, has already been reported for several species including sunflower. The discriminatory power of some SSR multiplex sets developed by other authors was here evaluated in order to establish a system for a rapid variety identification in sunflower. One hundred and twenty-one inbred lines of different origin, obtained from US, French and Italian public institutions, and representative of a wide range of genetic variability, were considered. All lines were male-fertile, and included both restorer and maintainer genotypes. In order to account for heterogeneity, for each accession DNA was extracted from five seedlings. Three six-plex combinations, with a total of eighteen SSR loci representing all chromosomes, were used to genotype all accessions. The number of alleles of individual markers ranged from 3 to 9, showing an heterozygosity (PIC) ranging from 0.34 to 0.83, with a mean of 0.65; the maximum average PIC value per set was 0.71. Each multiplex set allowed to distinguish accessions in more than 99% of the pairwise comparisons. The data indicate that this approach could represent an interesting tool for variety identification in sunflower.

### P3.08 - Current status of the exploitation of the olive genetic resources

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The genetic heritage of olive, one of the oldest agricultural tree crops of the Mediterranean basin with remarkable economic and cultural importance, includes the cultivated form (cultivars), the wild tree (oleaster) populations and numerous related subspecies. The richness of the cultivated germplasm represents an unusual case among horticultural crops, as a consequence of tree longevity and lack of turnover with new genotypes. More than 1,200 varieties, collected in 79 international and national repositories, are still actively cultivated, and genuine oleaster populations have been demonstrated to survive as important components of the Mediterranean maquis. Wild olive trees represent an interesting source of variability for olive breeding, bearing in mind that they may carry traits, such as pest resistance, adaptation to adverse environments and low plant vigor, rarely found within olive cultivars.

We report the initiatives currently in progress in Spain and Italy, under a collaborative perspective, to exploit the natural genetic variation of wild and cultivated olives by means of functional genomics, genetic and association mapping, molecular assisted breeding.

A revision of the relationships between the two forms is being carried out, as well as an extensive collection of oleaster samples from populations established at different sites representing adverse and heterogeneous ecological conditions. They are being characterized at molecular and phenotypic level and the possibility to introgress new and superior alleles into cultivated varieties is under exploration.



### P3.09 - Variability of wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) analyzed by SSR markers and agro-morphological traits

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Wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) represent a distinctive element of Mediterranean flora and an important genetic source for resistance to biotic and abiotic stresses in olive selection and breeding. In this study, genetic diversity among 48 wild genotypes sampled from four different sites from two provinces of Andalusia (Cadiz and Jaen), Southern Spain, was evaluated by means of SSR markers and agro-morphological traits. SSR markers revealed high genetic variability among the genotypes. The cluster analysis based on their polymorphism indicated a certain grouping of wild olives according to their sampling sites. The 20 agro-morphological traits evaluated showed a high variation between genotypes and significant correlation coefficients were obtained among the values recorded in two consecutive harvest seasons, 2007/08 and 2008/09 ( $r$ : 0.59-0.78). Traits such as pit shape, fruit symmetry (position A) and oil content on dry basis were very useful in genotype discriminating. The average values obtained in wild olives for fruit size and oil content were lower than the previously reported in cultivated material. However, it is worth mentioning that individuals with fruit weights (1.3 g) and olive oil percentage in dry matter (33.8%) comparable to the values found in some olive cultivars, were also found. With both DNA based and agro morphological descriptors, higher levels of variability were found within each site than between sites. Genetic variation observed among the wild olive germplasm at the DNA level was higher than the agro-morphological traits, indicating the efficiency of SSR markers for detecting genetic diversity among wild olive genotypes and their relationships. Molecular data obtained by SSR markers together with *in situ* morphological and agronomical characterization of wild olive trees confirmed the high diversity found within the wild populations evaluated. These results indicate the richness of wild olive genetic resources in Andalusia.

### P3.10 - Contrast genetic diversity and differentiation among Tunisian cultivars of (*Prunus* spp.): A study using mt DNA RFLP

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Phylogenetic relationships among 20 accessions of Tunisian plums (*Prunus* spp) are investigated using variation in mitochondrial DNA (mt DNA). For this purpose, genetic diversity was analysed using PCR-RFLP. Appropriate primers were used to amplify the corresponding targeted regions *nad1*/B-C and *nad4*/1-2. The PCR products amplified from these regions were digested with different restriction enzymes: *Hae*III, *Rsa*I, *Hind*III, *Hin*I, *Eco*RI and *Mse*I for the *nad1b-nad1c* region and *Taq*I, *Hin*I, *Mse*I and *Eco*RI for the *nad4*(1/2) region. The results showed that, the polymorphism obtained was detected only in the region *nad1b-nad1c*. A genetic distance matrix and UPGMA dendrogram were generated to analyse genetic relationships among cultivars. In addition, the study of the phylogenetic tree showed the distribution of cultivars undependably from their geographic origin, and closeness among local and introduced varieties was observed. The PCR-RFLP approach could be informative to detect cytoplasmic polymorphism. Knowledge of the genetic diversity and relationships among the cultivated species of *Prunus* is important to recognizing local gene pools and to develop effective conservation and management strategies.

### P3.11 - Estimation of DNA contents in Tunisian plum cultivars (*Prunus* spp) using flow cytometry method

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Flow cytometry is now among the most promising optimization technique. Based on DNA estimation, this method is convenient, reliable, and rapid and does not require expensive reagents. Forty five plum cultivars originating from diverse geographic regions of Tunisia were analysed. Plum is belonging to the genus *Prunus* including many species with different level of ploidy: diploid, tetraploid and hexaploid. Flow cytometry was used in order to identify the local germoplasm and to specify the DNA content for each cultivar. Our results show that among the investigated plums only three of them were hexaploid belonging so to *Prunus domestica* and all the remaining accessions were diploid and seems correspond to cultivars of *Prunus japonica*. This result shows that only diploid and hexaploid plums were cultivated in Tunisia.

### P3.12 - Exploring molecular variation in maize landraces from Central Italy

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Landraces are domesticated local plant varieties that did not experience a deliberate and intensive selection during a formal breeding program. They are subjected to the combined actions of natural and human factors which determine the agro-ecosystem. Thus landraces represent a very important source of genetic diversity to exploit for conservation, plant breeding, and for evolutionary studies.

In Italy flint maize landraces are still cultivated in order to produce traditional food, for which the dent corn is not suitable from a quality point of view. Here, using 21 SSR and 168 AFLP molecular markers, we compared two landrace flint maize collections from Central Italy obtained in two different periods, spanning 50 years: an 'old' collection undertaken during the 1950s, thus before the introduction and spread of hybrid varieties and a 'recent' collection (2000-2005). For comparison a sample of improved germplasm including hybrids and inbred lines was also used. The population structure and divergence analysis showed that the introduction of hybrid varieties led to a significant amount of introgression from hybrid varieties into the recent landrace collection. Selection tests were performed in order to disentangle the effects of migration and selection in determining the introgression seen. Overall the effect of selection was small and on average favoured the introgression from modern maize into landraces. Interestingly the outlier loci identified suggested a selection both acting between the flint and dent gene pools, and for changing environments or in favour of new alleles introduced by migration from hybrids over the last 50 years. Overall, these results show the potential of landraces to be exploited as models for studies aimed at the detection of loci that control important adaptive variants and agronomic traits.

### P3.13 - High-throughput gene and SNP discovery in *Cucurbita* spp.

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The new world genus *Cucurbita* is one of the most variable within the Cucurbitaceae family. It includes several cultivated species (commonly referred to as gourds, squashes and pumpkins) considered minor crops within Cucurbits. However, the *Zucchini* and other "Summer squash" morphotypes of *C. pepo* rank among the highest-valued vegetables in Europe and USA, and many "Winter squash" types, belonging to *C. pepo*, *C. maxima* and *C. moschata*, are food staples and rich sources of vitamins in developing countries. *Cucurbita* spp. are also important as rootstocks for overcoming soilborne diseases in cucurbits. There exist increasing genomic resources available in the three major cucurbits (watermelon, cucumber and melon, (<http://www.icugi.org/>), high density molecular maps, new mapping populations, TILLING and EcoTILLING platforms, ESTs collections and even whole genome sequence. However, in *Cucurbita* species similar genomic tools are still lacking. The high-throughput and cost-effective next generation sequencing methodologies are being successfully applied to large-scale EST sequencing in model and non-model species. We have used 454 GLX Titanium sequencing to generate EST sequences from two normalized cDNA libraries (prepared from RNA pools of leaf, root and ovary tissue). cDNA collections were generated from two accessions, representing the main cultivar groups in each one of the two subspecies in which *C. pepo* is divided: *C. pepo* subsp. *pepo* cv. *Zucchini* Mu16 and *C. pepo* subsp. *ovifera* cv. *Scallop* UPV196. These two accessions have contrasting phenotypes for plant, flowering and fruit traits and are being used as parentals of a RIL mapping population. 392.370 and 407.723 sequences (average length 252 bp) were generated by half 454 run for each parental. Raw sequences were clustered into non-redundant sequences or unigenes. These were used to identify potential SSRs markers. By aligning the sequencing reads from the two genotypes, we detected putative SNPs. Functional classification of the unigenes was carried out following the Gene Ontology scheme. This is the first broad survey of gene sequences and allelic variation in *C. pepo* where limited prior genomic information existed.

### P3.14 - Molecular and morphological diversity in Japanese rice germplasm

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The use of rice genetic resources available at gene-banks is an important strategy for incorporating genetic variability into rice breeding programs. Molecular and phenotypic characterization of germplasm is fundamental to its utilization. The diversity within 192 Japanese rice accessions was analysed with respect to agro-morphological traits and SSR markers. Twenty-two descriptors, 13 quantitative (Mahalanobis's distance) and 9 qualitative (Jaccard's distance) were evaluated. The quantitative characteristics which more contributed to genetic divergence and important agronomic traits were evaluated in two consecutive years to consider the effects of environment-genotype interaction. Twenty-four SSR markers were used to determine Rogers-W's distance. A total of 181 alleles were detected, 38 of which were exclusive. The number of alleles per marker ranged from 2 to 16, with an average of 7.54 alleles per locus. The PIC ranged from 0.01 to 0.80 with an average of 0.43 and the  $H_e$  ranged from 0.01 to 0.82 with an average of 0.46. Tocher's method applied to a total distance matrix was used to determine cluster formation. The total distance matrix was the result of the standardization and sum of four distance matrices (two Mahalanobis's distance matrices, one for the first year and another for the second year; one Jaccard's distance matrix and one Rogers-W's distance matrix). Thirteen diversity groups were determined by Tocher's method. The most part of the accessions (81%) were clustered in the same group, while eight accessions (Kyuushuu, Eika Ine, Ishiwari Mochi, Col/Fukui/1965, Ookuma Nishiki, Suzume Shirazu, Iwate Ryon 1 and Toga) were not clustered with other accessions, originating clusters with only one accession (clusters VI to XIII). Fourteen accessions were grouped in cluster II, nine in cluster III and two in clusters IV and V. The inter-cluster distance ranged from 8.192 (between clusters I and X) to 11.5324 between cluster IV (Hitachi Nishiki and Nourin Mochi 6) and X (Ookuma Nishiki). Accessions of cluster IV have longer cycle, shorter grains and lesser yield than Ookuma Nishiki, which was the most similar accession to accessions of cluster I. The accessions showed diversity at phenotypic and molecular level and some of them also showed good agronomic performance.

### P3.15 - Opportunities of genetic association studies in pepper: Estimation of the genetic structure and linkage disequilibrium in a germplasm sample

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Genetic association studies permit to dissect complex traits, but the resolution depends on the extent of the linkage disequilibrium (LD). When LD declines rapidly around a target locus, locus scans help to identify candidate genes. To avoid false positive associations, the germplasm genetic structure has to be considered. To evaluate opportunities of genetic association in pepper (*Capsicum* spp.), we investigated the LD extent and the genetic structure in a germplasm sample, at two levels: the whole-genome and a 1-Mb target locus. INRA maintains a pepper collection of 1,322 accessions from five cultivated and six wild species. We randomly sampled 381 accessions from the five cultivated species (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, *C. pubescens*) and one wild species (*C. eximium*). We genotyped them with seven SSR markers from seven chromosomes and six SNP markers within the target locus. The genetic diversity of the sample was higher at the genome-wide level ( $H_e = 0.48$ , expected heterozygosity, Nei, 1973) than at the target locus ( $H_e = 0.39$ ). However, SNPs are generally less polymorphic than SSRs. The SSR data revealed that the sample was structured into two major groups. Group1 mostly contained *C. annuum* accessions, while group2 consisted in a mixture of the other species. As expected, group2 presented more allelic diversity than group1. Group2 was itself structured into three sub-groups, roughly splitting the accessions from *C. pubescens*, *C. baccatum* and *C. frutescens*. At the whole-genome level, the LD was high ( $r^2$  mean = 0.28) compared to other species (0.06 for potato). This is favourable for genome-wide association studies. A high LD could be due to the sample structure or related to the limited number of markers. At the target locus, the LD extended up to 300 kb ( $r^2$  mean = 0.9) in group1, and was also high compared to other species (7 kb for maize, 200 kb for lettuce). But it was specifically low ( $r^2$ mean=0.09) in group2. A high LD is unfavourable for locus-specific association studies. To highlight genotype-phenotype fine associations on pepper, we will focus on haplotype analysis with more markers and within a larger germplasm sample, in considering especially accessions from group2.

### P3.16 - Genetic diversity in intron1 of carrot *AOX2b* – a useful tool to develop a functional marker?

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Recent knowledge on the regulatory functionality of intronic regions and sequence variabilities identified in intron3 of the *AOX2a* carrot gene from diverse carrot genotypes and breeding lines (Cardoso et al., 2009. *Physiologia Plantarum*, 137:592-608) made it reasonable to investigate intron variability also in other *AOX* carrot genes. The complete cDNA sequence of the *DcAOX2b* is recently available (Campos et al., 2009. *Physiologia Plantarum*, 137:578-591). This allowed us to investigate the diversity in this gene among genotypes in view of potential future applications in carrot plant breeding. In the presented study we focus on sequence variations at genomic DNA level. The use of gene specific primers designed in 5' and 3'-untranslated regions revealed the typical genomic organization of *AOX* genes in plants characterized by four exons interrupted by three introns. However, the total *DcAOX2b* gene size can vary significantly depending on the variable length of intron1 near the 5'-UTR (intron length polymorphism – ILP). PCR analysis led to the identification of several fragment patterns between genotypes, nevertheless, no more than two fragments were identified per genotype suggesting two different alleles. Allelic variation could be used as a tool to discriminate between single plant genotypes in cv. Rotin but also between individual wild carrot plants. Repetitive patterns of intron length variation have been observed that allows a grouping of genotypes. Sequence analysis in the region of intron1 of polymorphic but also of obviously identical PCR-fragments revealed underlying high levels of sequence polymorphisms between alleles and genotypes. Variability was due to insertion/deletion (InDel) events and intronic single nucleotide polymorphisms (ISNPs). The results suggest that the high *AOX2b* gene diversity in *D. carota* can be explored for the development of functional markers related to agronomic traits related to stress-behavior (see Special Issue of *Physiologia Plantarum* number 137). Further, mapping of *DcAOX2b* to the linkage group 4 in the segregating F2 of a DM19 population using the ILP methodology will be shown.

### P3.17 - Chloroplast SSR diversity in Portuguese grapevine cultivars from "Vinhos Verdes" DOC region

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Chloroplast genome has a lower evolutionary rate than the nuclear genome. Mononucleotide repeats in the chloroplast genome represent useful markers to understand the genetic structure and variation within and among populations, to study crop plant evolution and domestication and for phylogeny reconstruction.

*Vitis vinifera* L. chloroplast genome is 160,928 bp in length and is maternally inherited. Ten consensus microsatellite loci have been widely used in chloroplast genome studies in different *Vitis* species and in wild and cultivated *Vitis vinifera* L. These loci have shown the existence of a reduced number of chlorotypes, which could show specific geographic distributions. "Vinhos Verdes" is one of the principal Portuguese DOC regions. Grapevine culture in this region has unique characteristics, namely as concerns the form of guiding. The vines are planted next to tall growing trees and they interlace with the tree branches.

An analysis of chloroplast DNA variation at three microsatellite loci (ccmp3, ccmp5 and ccmp10) of 14 grapevine cultivars recommended for wine production in the Portuguese "Vinhos Verdes" DOC Region was performed.

The three cpSSR loci were polymorphic, two different alleles were found in each *locus*. Allele variants of the three loci combined in two different haplotypes. One chlorotype revealed to be more representative of this group of cultivars since it was present in 85.7% of them. This chlorotype corresponds to the previously reported as the most frequent in Iberian Peninsula and Occidental Europe.

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### P3.18 - Genetic diversity of cultivated Macedonian tobacco varieties

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Tobacco (*Nicotiana tabacum* L.) is an important agricultural crop plant for the economy of many countries. Assessment of the genetic diversity of cultivated tobacco varieties is of importance for long-term tobacco improvement. Microsatellite markers are currently the marker system of choice for genetic analysis of allopolyploid plants and total of 282 highly polymorphic microsatellite markers have been reported for identification of closely related varieties of tobacco.

Cultivated tobacco varieties in Macedonia are highly polymorphic with wide range of morphological types and are mainly used in cigarette manufacturing. There are three main groups of cultivated tobacco in Republic of Macedonia: oriental, semi-oriental and broad-leaf. Their classification until now was based only on morphological, biological and technological characteristics such as selection based on pest resistance, morphological traits and leaf quality parameters.

In this study, we evaluated the use of 30 microsatellite markers for the identification of 10 varieties of cultivated tobacco in the Republic of Macedonia. Among microsatellite markers used, 24 (80%) were polymorphic. A total of 90 alleles were detected among 10 tobacco varieties and the number of alleles per locus ranged between one and six with an average of three alleles per locus. On average, 70% of the varieties shared a common allele at any given location. PIC values for each of the 30 SSR loci varied widely among loci and ranged from 0.0 to 0.76 with an average value of 0.39 per locus. We found 24 of microsatellite markers to be sufficient for identification of these tobacco varieties. Cluster analysis showed that Macedonian tobacco varieties are classifiable into three distinct groups.

The obtained data could be used for reducing duplicate collection of germplasms and therefore be of great use in tobacco breeding programs in the Republic of Macedonia. This study also provided additional information in the area of *N. tabacum* population genetics and aided in expanding the knowledge about the genetic variation within diverse gene pools of cultivated tobacco.



### P3.19 - *In silico* comparative analysis of SSR markers in ancestral and model plants

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The adverse environmental conditions impose extreme limitation to growth and plant development, restricting the genetic potential and reflecting on plant yield losses. The progress obtained by classic plant breeding methods aiming at increasing abiotic stress tolerances has not been enough to cope with increasing food demands. New target genes need to be identified to reach this goal, which requires extensive studies of the related biological processes. Comparative analyses in ancestral plant groups can help to elucidate yet unclear biological processes. In this study, we surveyed the occurrence patterns of expressed sequence tag-derived microsatellite markers for model plants. A total of 13,133 SSR markers were discovered using the *SSRLocator* software in a non-redundant EST database for all eleven species chosen for this study. The dimer motifs are more frequent in lower plant species, such as green algae and mosses, and the trimer motifs are more frequent for the majority of higher plant groups, such as monocots and dicots. With this *in silico* study, survey results validated with several bioinformatics tools suggest that comparative studies of EST-SSR markers among all plant lineages is well suited for plant evolution studies as well as for future studies of transferability of molecular markers.

### P3.20 - An SSR-based assessment of diversity in wild species of potato

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In a project aiming at the development of molecular markers for alleles conferring resistance to potato wart (*Synchytrium endobioticum* (Schilbersky) Percival), potato genetic resources from the IPK Genebank were also included in order to mine them for novel resistance alleles.

Twelve species from nine taxonomic series of tuber-bearing potatoes were selected because of earlier publications on their resistance to potato wart. In a first step, tubers from appr. 800 individual genotypes coming from 82 GLKS accessions were produced and tested for resistance against selected *Synchytrium* races at JKI Kleinmachnow according to Glynne-Lemmerzahl. Simultaneously, leaf material of all individuals was harvested and used for the extraction of genomic DNA.

In an experiment designed at elucidating the applicability and power of resolution of microsatellite markers from different sources (genomic DNA/EST sequences) of cultivated potato *Solanum tuberosum* L. subsp. *tuberosum*, SSR analyses were conducted. Here, 14 SSRs were employed in four multiplex PCR reactions and separated on an automated fragment analysis system using fluorescence labeling. Samples were loaded as two combined multiplexes containing amplification products from six or eight SSR primer pairs, respectively.

The evaluation of the generated banding patterns led to insights into the level of diversity within accessions - e.g. in selfers like *S. acaule* Bitter ssp. *acaule* or *S. demissum* Lindley vs. outcrossers like *S. pinnatisectum* Dunal or *S. sparsipilum* (Bitter) Juz. & Bukasov - and to a certain extent also within and between species. In combination with resistance data for the respective genotypes (partially still to be generated), this diversity assessment provides the basis for the subsequent identification of novel alleles for potato wart resistance

### P3.21 - Genetic polymorphism of tobacco varieties based on ISSR and IRAP markers

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*Nicotiana tabacum* L., the most well-known *Nicotiana* species, is a natural amphidiploid ( $2n = 48$ ) thought to have arisen by hybridization of wild progenitor species. Tobacco is one of the most important non-food crops being cultivated in more than 100 countries around the world. Numerous types of tobacco are grown and are classified to a large extent by region or area of production, method of curing and use in manufacturing, as well as by some distinct morphological characters and chemical differences. At the present time only limited information is available on the relationship between morphological variability and genetic diversity in *N. tabacum*. In the present paper Inter-Simple Sequence Repeat (ISSR) and Inter-Retrotransposon Amplified Polymorphism (IRAP) molecular markers were used to reveal the genetic polymorphism among *N. tabacum* varieties belonging to different tobacco types. Genomic DNA of 36 tobacco varieties and of six wild *Nicotiana* species, including the presumed progenitors of tobacco, was extracted and amplified utilizing nine ISSR and ten IRAP primers. PCR products were separated on agarose gel, stained with ethidium bromide and visualized by UV light. The electrophoretic banding patterns were scored in terms of presence or absence of the bands. For both molecular markers, amplification profiles revealed a high degree of polymorphism as 99% of amplified products were polymorphic among the *Nicotiana* species examined. The amount of genetic polymorphism present among *N. tabacum* lines examined was limited as evidenced by the high degree of similarity in ISSR and IRAP profiles. From the cluster analysis performed the thirty-six tobacco varieties showed a similarity index higher than 90% and the major number of cultivated tobacco lines were not resolved, for both molecular markers. The data obtained are consistent with our previous findings on genetic variability in *N. tabacum* by RAPD analysis confirming the narrow genetic diversity among tobacco varieties. These results put an intriguing question about the high degree of existing phenotypic variability among tobacco lines, in comparison to the limited variation at level of nucleotide sequence of DNA revealed by the different types of molecular markers.

### P3.22 - RAPD and ISSR markers for study of genetic variation among parsley (*Petroselinum crispum* (Mill.) Nym.) accessions

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Many commercial cultivars and breeding accessions of parsley (*Petroselinum crispum* (Mill.) Nym.) are currently included in trait test and quality characteristics. It is known that parsley plants have a great variation that sometimes depends on environment.

To arrange the breeding material and to engage new genotypes the precise classification should be done based on DNA marker variation. The application abilities of PCR-based RAPD and Inter-simple sequence repeat (ISSR) variations have been clearly demonstrated in many plant species. In this study both these methods have been utilized for parsley genotype classification.

Thirty-three accessions of parsley of various origin including root, curly-leaf and common forms were taken for the study. Five selected RAPD primers out of 20 and six SSR primers out of 13 were selected to discriminate all accessions of parsley. In the total, 51 RAPD and 66 ISSR bands were generated with chosen primers. Jaccard's coefficient was applied and the dendrogram based on the matrix data was drawn with the group average method. The amplification with ISSR primers produced more bands per reaction as compared with RAPD profiles on average 11 and 10.2, respectively. In each case RAPDs and ISSRs were polymorphic in the same accessions i.e. were correlated. The genetic diversity among root parsley accessions was higher than in leaf forms with indexes 0.68 and 0.72, respectively.

The two clusters of leaf and root plant forms were the most distant at similarity 0.33 from each other. Consequently, root form-specific markers were observed. Not all flat leaf and curly-leaf forms have been clearly separated, but some of curly-leaf accessions formed their sub-cluster.

It is quite necessary to compare agronomic and taxonomic data with variation of different DNA markers, it serves unique genome sequences that can be very probably connected with new plant characteristics for each genotypes or/and some close related ones.

### P3.23 - The use of RAPD markers for the study of genetic polymorphism in *Brassica* A and C genome accessions

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The objective of this research was to estimate the genetic diversity and relationship among 27 accessions of *B. oleracea* (C genome, n=9) and 18 accessions of *B. rapa* (A genome, n=10) using RAPD markers. The C genome was represented by entries of head cabbage *Brassica oleracea* convar. *Capitata* (L.) Alef. var. *capitata* L. f. *alba* DC., red cabbage (convar. *Capitata* (L.) Alef. var. *capitata* L. f. *Rubra* (L.) Thell.), ornamental kale (convar. *acefala* D.C.), broccoli (var. *cymosa* Duch), Savoy cabbage (convar. *Capitata* (L.) Alef. var. *sabauda* L.), kohlrabi (convar. *Acefala* (DC.) Alef. var. *gongyloides* L.), and for A genome Chinese cabbage *Brassica rapa* ssp. *chinensis* (L.) Hanelt., bird rape (ssp. *oleifera* (DC.) Metzger f. *biennis*), Mizuna and Mibuna salad green (ssp. *nipposinica* (Bailey) Hanelt) were taken from All-Russian Research Institute of Vegetable Breeding and Seed Production. The selected nine primers produced 128 (96.2%) polymorphic bands, which were then used to discriminate all *Brassica* accessions. The size of amplified DNA fragments scattered from 200-2000 base pairs. Jaccard's coefficient was applied and the dendrogram was drawn with the group average method.

The variation in RAPD patterns estimated was higher in A genome (74%) than in C genome (50%).

All *Brassica* entries fell into two clusters corresponding to A and C genome with index 0.2 between them.

The A genome cluster itself consisted of three sub clusters where accessions of Chinese cabbage, bird rape and Japanese salad green formed their own small sub-clusters with similarity index 0.65 between them. However, there is no clear a difference was discovered within a Japanese salad green between Mizuna and Mibuna varieties. Chinese cabbage accessions were much closer to Japanese salad green genotypes than to bird rape genotypes.

The C genome cluster itself consisted of two sub clusters with index 0.75 between them where all 19 accessions of head cabbage formed their own sub-cluster. Accessions of second sub-clusters were divided into five groups; each group corresponded to varieties presented in the study.

12 A genome-specific and 16 C genome-specific markers were found. These markers can be useful for interspecific hybrid purity test and genetic diversity estimation in *Brassica*.



### P3.24 - Analysis of genetic diversity in a potato (*Solanum tuberosum* ssp. *tuberosum*) collection using microsatellite markers

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Thanks to favorable climatic conditions in Western Brittany, the UMR APBV INRA team maintains, in healthy conditions, a large collection of potato and relatives genetic resources. This collection, which is maintained by vegetative propagation, consists of French, European and American varieties, breeding lines, dihaploid genotypes, clones belonging to 32 different related species and interspecific hybrids. This collection has been characterized for a great number of morphological, agronomical or technological traits. We are now evaluating its genetic diversity using microsatellite markers. This study aims at evaluating the genetic diversity of part of the cultivated potato (*S. tuberosum* ssp. *tuberosum*) collection. Using the phenotypic data that are available in the Europotato database ([www.europotato.org](http://www.europotato.org)), 350 varieties or breeding lines have been chosen in order to have a good representation of the phenotypic variability. Four genotypes of each of the two diploid wild species *Solanum sparsipilum* and *Solanum stenotomum*, and 14 genotypes of the tetraploid species *Solanum tuberosum* ssp. *andigena*, the different species which are at the origin of the cultivated potato (Hawkes, 1990), have been included in this study. A set of 26 SSR markers has been selected in the literature (Kawchuk et al., 1996; Milbourne et al., 1998; Ghislain et al., 2004; Feingold et al., 2005) according to their polymorphism, their position in the genetic map and their pattern quality. The SSR genotyping has been performed at the high-throughput genotyping platform in INRA Clermont-Ferrand using an ABI PRISM® 3100 Genetic Analyser. The fragment analysis, performed with GeneMapper software, led to the detection of 324 different alleles. The mean allele number per marker is 12.5 and the mean diversity index is 0.84. Using DARwin software, a distance matrix has been calculated and used to perform a Principal Coordinate Analysis and to construct a dendrogram. Even if the *S. tuberosum* ssp. *andigena* and *S. sparsipilum* genotypes grouped separately from the *S. tuberosum* ssp. *tuberosum* genotypes, these analyses revealed no clear genetic structure in the analysed gene pool. The following step will be to perform a population structure analysis based on a Bayesian approach implemented in the program Structure.

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### P3.25 - Elucidating the genetic basis of seed traits using an interspecific tomato introgression line collection

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Natural variance provides a powerful means to study complex traits such as abiotic stress responses and seed quality traits. To foster a better understanding of the genetics of variability in seed traits, we investigated the metabolic diversity of seeds in a collection of introgression lines originating from interspecific crosses between the cultivated *Solanum lycopersicum* and the wild species tomato *Solanum pennellii*. By employing a GC-MS based approach we analyzed the metabolite profiles of seeds collected from plants of the homozygous lines of the collection grown in Akko, Israel, across two seasons. Based on the tomato chromosome maps and the recently completed tomato genome sequence we identified (a) the chromosome regions associated with a quantitative variation in the metabolic traits (mQTL) and (b) the candidate genes potentially responsible for the recorded diversity. Next, we searched for associations between metabolic data measured in the seeds and metabolic data from previous profiling of fruits (pericarp) and yield associated traits (YAT) measured in the same plants. The analysis revealed that several metabolic traits in the seed were conserved with metabolic traits in the fruit, and with YAT. We finally employed correlation based metabolic network analysis to study the regulation of metabolic processes in the seed and the fruit and highlighted differences and commonalities in the regulation of metabolic processes.

### P3.26 - Study of genetic variation in yarrow accessions from Iran

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To study the genetic diversity of 37 *Achillea millefolium* accessions we used RAPD and ISSR markers. Nine RAPD primers and seven ISSR primers have shown good polymorphic. The Jaccard's similarity indices (J), based on RAPD and ISSR profiles, were subjected to complete linkage analysis. The dendrogram generated revealed six groups. The principle coordinate analysis (PCoA) data confirmed the results of the clustering. Application of Mantel's test for cophenetic correlation to the cluster analysis indicated the low fitness of the accessions to a group ( $r = 0.6$ ). The results of the clustering showed that *A. millefolium* subsps *elbursensis* is separated from other genotypes in the dendrogram, this subsps is endemic of north of Iran. Essential oil obtained from dried plants of this subsps was more than (0.31%) other genotypes. The results of the clustering analysis, based on RAPD and ISSR markers, corresponded closely with the geographical origins of the genotypes. The results of the present study will provide the basic information for effective conservation of these genotypes for selection and breeding programs.

### P3.27 - Discrimination of Portuguese and Spanish olive cultivars using microsatellite markers

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Olive cultivation is one of the oldest agricultural activities in the Iberian Peninsula. Nowadays, Spain and Portugal contain a high diversified genetic patrimony within this species. The correct identification of the olive cultivars is very important for the conservation of genetic resources, and for the development of a competitive and sustainable olive production system. In addition it also serves research and scientific purposes.

In this work, four microsatellite markers previously described as highly recommended for olive cultivar identification, have been selected to carry out SSR screening on 22 olive accessions. Among the above mentioned accessions, 11 are originals from Spain, and 11 are originals from Portugal. A CTAB-based protocol has been employed for DNA extraction from young leaves. For the detection of alleles, microsatellites were amplified by PCR and analyzed on a capillary automatic sequencer. Genetic relationships were studied by observing the UPGMA tree obtained after calculating Dice genetic distances. Results showed that the selected microsatellites markers were able to discriminate among the studied accessions. Cultivars were separated through four important clusters while 'Arbequina' cultivar was completely separated from the remaining accessions showing a very low similarity coefficient. Furthermore, three accessions have presented an identical allelic profile and were considered as the same cultivar. The factorial analysis of correspondence has demonstrated that there is no separation between Portuguese and Spanish cultivars according to its origin, and this is probably due to the geographical continuity of the Iberian Peninsula.

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### P3.28 - Study of symptoms and gene expression in four *Pinus* species after pine wood nematode infection

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Pine wilt disease, caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, is originating severe infections in pine trees. The disease is detected when external symptoms appear (e.g. needles chlorosis), but trees could remain asymptomatic for long periods and serve as a long-term hosts.

The primary goal of this work was to assess the effect of the inoculation with an avirulent isolate of *B. xylophilus* (C14-5) on different *Pinus* spp. seedlings (*P. sylvestris*, *P. nigra*, *P. pinea* and *P. pinaster*). At the same time, seedlings were also inoculated with a virulent strain, HF, in order to compare the phenotypic and genomic results of the two types of inoculations. The effect of inoculation was determined in terms of expression of various *Pinus* genes potentially involved in the response to the disease.

The results suggest that *P. pinea* and *P. nigra* are more resistant to infection by the nematode than *P. sylvestris* and *P. pinaster*. The phenotypic and genetic differences were more marked among *P. pinea* and *P. pinaster*.

### P3.29 - Identification, characterization and utilization of unigene derived microsatellite markers from *Medicago truncatula*

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This study was conducted to identify microsatellite or simple sequence repeats (SSR) markers linked to biotic and abiotic stress genes in different plant species (Cereals and *Medicago*) and examine their cross-applicability in different genetic backgrounds and also to assess the genetic variability. In recent years, expressed sequence tag (EST) projects have generated a vast amount of publicly available sequence data from plant species and these data can be mined for microsatellites. These sequences are among the best marker technologies applied in plant genetics and breeding. These markers are valuable because of their higher level of transferability to related species, and they can often be used as anchor markers for comparative mapping and evolutionary studies. Unigene derived microsatellite markers identified from publicly available sequence database have the advantage of assaying variation in the expressed component of the genome with unique identity and position. In this direction, a total of 18,098 *Medicago truncatula* unigene sequences were selected from databases, and SSR were fished out. A total of 7,607 SSR were recovered and these SSRs will be further used for designing primers for their application in plant genetics.

This work was supported by Generation Challenge Programme (GCP) project.

### P3.30 - Genome-wide variation from the wild to the cultivated and its implications to study on germplasm of soybeans

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After the domestication of cultivated soybean (*Glycine max* (L.) Merr.) in China from its annual wild relative *Glycine soja* Sieb. & Zucc., thousands and thousands of landraces were developed by the ancient Chinese farmers during the long history, based on which more than 1300 improved cultivars were released in China since 1923. From studies on large samples of wild accessions, landraces and released cultivars by using genome-wide SSR markers, it was found that the genetic diversity in terms of genetic richness and genetic dispersion decreased obviously from the wild to the cultivated landraces and in turn to the released cultivars, which showed obviously two stages of bottlenecks, but with a great number of new alleles emerged during human being's artificial improvement. In fact, the parental materials used in breeding programs are mainly released cultivars or related breeding lines which provide more than 90% of the germplasm to the newly released cultivars. Therefore, to evaluate the genome-wide genetic structure of breeding target traits of the released cultivars should be the number one task in the study of soybean germplasm. By means of linkage mapping and association mapping the elite genes and their alleles for traits related to yield, quality and tolerance to abiotic and biotic stresses in the released cultivar population were detected and evaluated. The results imply that for a better utilization of the elite germplasm, genes and alleles, the pyramiding technology of genes and alleles needs to be further studied since to pyramid individual major gene(s) with the help of marker-assisted selection is usual, but to pyramid a number of minor genes is rather difficult.

### P3.31 - Analysis of genetic diversity and population structure of *Citrus* Germplasm using nuclear (SSRs, INDELS) and mitochondrial markers

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Previous studies with molecular markers (ISSR, RAPD, SCAR, AFLP and SSR) have shown that most of the genetic diversity of cultivated *Citrus* (except *C. aurantifolia*) comes from the recombination between three main species: *C. medica* (citron), *C. reticulata* (mandarin) and *C. maxima* (pummelo). However, the precise contribution of these basic species to the genome constitution of secondary species (*C. sinensis*, *C. limon*, *C. aurantium*, *C. paradisi*) and recent hybrids is not known. In this study, 58 nuclear and four mitochondrial markers were used to investigate the genetic diversity among 106 *Citrus* accessions, representing the three main ancestors groups, secondary species and several hybrids from the 20<sup>th</sup> century breeding programs. For the nuclear analysis, 50 simple sequence repeats (SSRs) developed from genomic libraries and ESTs databases were used. Moreover, 10 Insertion-Deletion (INDEL) markers were developed from genomic sequences of some primary and secondary metabolites determining the citrus fruit quality (sugars, acids, flavonoids and carotenoids). All the SSR markers and one INDEL are included in a consensus genetic map of clementine and pummelo Chandler and are distributed along the nine linkage groups, representing positively the global genome of *Citrus*. Genetic diversity statistics were calculated for each SSR and INDEL marker, within the entire population and within and between the different specified *Citrus* groups. The organizations of the genetic diversity among all the accessions were determined by constructing neighbor-joining trees for the different sets of primers. INDEL markers are less polymorphic than SSRs, display a higher organization of genetic diversity and appear to be better phylogenetic markers to trace the contribution of the three ancestral species. Population structure was studied using the Structure software, version 2.2.3, (<http://cbsuapps.tc.cornell.edu/structure>) which implements a model-based clustering method for inferring population structure using genotype data. The relative proportion of ancestral taxa genomes in the secondary species and recent hybrids was assigned. Mitochondrial markers revealed a maternal phylogeny of citrus germplasm accessions in agreement with previous studies with chloroplastic markers. This analysis allowed a better understanding of the genetic diversity organization among citrus cultivars, opening the way for a better management of citrus germplasm banks and breeding programs.

### P3.32 - DNA sequence polymorphism discriminated Mesoamerican and Andean gene pools in common bean (*Phaseolus vulgaris* L.)

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Common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world. It is an important source of calories, proteins, dietary fibres, minerals and vitamins for millions of people in both developing and developed countries. Common bean was originated and domesticated in the New World and has two major gene pools, the Andean and the Mesoamerican, based on their centers of origin in South and Central America, respectively. The existence of Andean and Mesoamerican gene pools is supported by molecular marker analyses (seed storage protein, isozymes, RFLPs, RAPDs, AFLPs, and SSRs). These can be complemented by DNA sequence data that provide a complete and unambiguous genotype of all specific genomic regions. In this study we assessed the frequency of SNPs in a fragment of *P. vulgaris* DNA localized on the B8 linkage group. This fragment was obtained from genomic DNA. We conducted a sequence analysis of 80 European domesticated landraces, belonging to a European core collection, 147 American wild and domesticated common bean genotypes, that had been previously assigned to the Andean or Mesoamerican gene pools using phaseolin patterns and as a control, 10 genotypes of *P. coccineus*, *P. lunatus*, *P. acutifolius* and *P. polyanthus*. Including indels, sequence lengths was 393 bp. In *P. vulgaris*, one 46 bp indel and four SNPs distinguished unequivocally the Mesoamerican and the Andean gene pools. The population structure identified in this study is generally consistent with the current hierarchical scheme of gene pools. This DNA sequence displays five polymorphic markers that discriminate very clearly the two gene pools of *P. vulgaris*.

### P3.33 - Molecular characterization of the ancient sweet cherry germplasm from Romagna

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The CRA-FRF has started a programme of individuation, recovering and characterization of the ancient cherry germplasm of Romagna (Southern Po Valley area, Italy), to preserve from erosion its interesting agronomic and adaptive traits, as well as its cultural value. The origin of some genotypes dates back centuries and different ecotypes were found in local farms. To explore the genetic diversity and unravel homonyms/synonyms in the germplasm collected, 22 accessions (referable to 11 different names) were screened with 16 cherry-derived and 2 peach-derived microsatellite (SSR) primer pairs. Eight reference accessions were also included in the set of genotypes analysed. All the reference accessions and 16 of the 18 SSRs were chosen by the European Collaborative Programme for Genetic Resources *Prunus* Working Group to standardize allele scoring and allow easier comparison among molecular datasets of European Cherry Collections. The DNA, extracted from young leaves, was amplified and separated in a 6% polyacrilamide gel silver stained. The bands obtained were visually scored using 25, 50 and 100 bp ladder (Invitrogen) by Gene Tools software. To confirm fragment scores, the four most polymorphic primers pairs were also fluorescently labelled to perform gene fragment analysis by ABI PRISM 3130 Sequencer (Applied Biosystems). The set of SSRs used in this study revealed 17 unique fingerprints. Six out of 10 accessions named "Cornetta" differed for 1-2 alleles, showing similar but not identical profile. This historical variety, cultivated in Romagna since XVIII century, has presumably differentiated into ecotypes with similar fruit characteristics but genetically diverse. A more accurate pomological characterization of all the "Cornetta" accessions is needed to ascertain which genotype is more correspondent to the original ideotype. Another case of homonymy was solved for two accessions named "Gemella". Alleles per SSR locus ranged from 2 to 9, with a mean of 5. Fragment lengths in our germplasm resulted mostly within the range reported for the 8 references. New allelic scores were also found. As standardized with that of ECPGR reference accessions, this dataset is comparable with other cherry datasets harmonized to the reference values and could contribute to the development of the European Cherry database.



### P3.34 - QTL mapping for root characteristics at the seedling level based on a maize introgression library

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A maize introgression library (IL) developed from the cross between B73 and Gaspé Flint was studied in order to elucidate the genetic control of seminal root number. The IL collection includes 75 lines, most of which retain one single chromosome introgression of the donor genome (Gaspé Flint) of ca. 40 cM. It has been estimated that ca. 70% of the Gaspé Flint genome is represented within the collection. The two parental genotypes showed contrasting seminal root architecture. The IL lines were evaluated for root characteristics by applying two different methodologies, i.e. a paper-roll based protocol and a pot-growing system (seedlings grown until the fourth-leaf stage in sand/clay pebble pots). Particularly striking differences were observed between the two parental lines and among the IL lines for the number of seminal roots developing from the scutellar node. B73 produced an average of 2.8 seminal roots per plant while Gaspé Flint did not develop any seminal root. Among the IL lines, a few showed a Gaspé-like phenotype for seminal root number, implying that the QTLs controlling this trait are localized on the corresponding introgressions. A major QTL for number of seminal roots (*Seminal root 1, Sr1*) was localized on chromosome 1S, ca. 10-15 cM away from the root architecture locus *Rtcs1* (Taramino et al., 2007, Plant J. 50:649-659). Phenotypic expression and fine mapping indicate that the two loci do not coincide. Positional cloning of *Sr1* is underway.

### P3.35 - Advances in conservation of potato germplasm in India

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The national repository of potato germplasm maintained at the Central Potato Research Institute, Shimla, India has more than 3800 accessions belonging to cultivated as well as more than 120 wild species. This collection is being maintained in field as well as in *in vitro* gene banks, and the wild species are conserved mainly as true seeds. Studies were conducted for slow-growth in-vitro conservation of germplasm. Different combinations of osmoticums (mannitol and sorbitol in combination with sucrose; concentrations 2-4%), and temperature regimes (6 and 24 °C) were tested. The results showed that the best protocol was to culture nodal cuttings on MS medium supplemented with 2% sucrose and 4% sorbitol under 16 h photoperiod at 6 °C. Following this method a culture could be maintained without sub-culture for 12-18 months depending on the genotype. Use of microtubers was also explored for increasing the conservation period. However, efforts to prolong the dormancy of the microtubers for this purpose by supplementing the medium with known dormancy promoting growth regulator abscisic acid proved to be counterproductive under in-vitro conditions. The collection is also regularly checked for their freedom from viruses and viroids. For virus elimination from the infected accessions, studies were conducted on meristem-tip culture following different combinations of thermotherapy (37 °C), chemotherapy (Ribavirin 20 mg/l) or both. The results showed that mericlone following combined chemo- cum- thermo-therapies for 5 weeks of the infected plants was effective even against the most difficult viruses PVX and PVS. The results and protocols developed will be discussed.

**P3.36 - Nucleotide diversity analysis in wild and domesticated *Phaseolus vulgaris* L. from Mesoamerica**Goretti D<sup>1</sup>, Bitocchi E<sup>1</sup>, Bellucci E<sup>1</sup>, Rossi M<sup>1</sup>, Nanni L<sup>1</sup>, Attene G<sup>2</sup>, Papa R<sup>1</sup>

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The common bean (*Phaseolus vulgaris* L.) is a diploid ( $2n = 2x = 22$ ), annual species that is predominantly self-pollinating and is the most important grain legume for direct human consumption. For *P. vulgaris*, many aspects of its molecular and phenotypic diversity, migration dynamics and population structure are well known. To date, in contrast, little information is available on the level and extent of its nucleotide diversity. The common bean was domesticated independently in Mesoamerica and in the Andes, and the largest diversity of its wild and domesticated forms is found in Mesoamerica, where a single domestication event is believed to have occurred. The main aims of the present study were to develop SNP markers and to identify genes and genomic regions that are related to the adaptive processes during domestication of *P. vulgaris*. We developed 30 primer combinations to amplify and sequence the orthologous counterparts of genes previously studied in wild and domesticated soybean. All of the primer combinations were used for a preliminary selection of 10 loci. A sample of 24 genotypes was developed to represent the wild and domesticated Mesoamerican populations (18), including six additional genotypes from the Andean and phaseolin I gene pools. Here, we present and discuss the results from the sequencing of 30 gene fragments (including five loci previously identified as potentially under selection during the domestication process in Mesoamerica).

**P3.37 - Development of a bandscoreing plug-in tool and its application for molecular trueness-to-type testing**Goris J, Michielsens D, Janssens K, Vauterin L

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In the last two decades, the use of DNA fingerprinting techniques has revolutionized plant breeding (marker-assisted breeding). We have developed a plugin tool for the BioNumerics® software to facilitate scoring of DNA markers (e.g. AFLP, RAPD), run on either slab gel or capillary electrophoresis systems. The tool allows for binary band scoring (presence/absence), as well as quantitative scoring (zygosity) via a sophisticated iterative algorithm. Normalization of the profiles enables reproducible comparison between different runs. This feature and the possibility to store all data in a central and powerful relational database (SQL Server, Oracle, MySQL) allows the construction of large databases over long periods of time. Here, we illustrate the use of the Bandscoreing plugin to streamline the workflow for trueness-to-type testing via fluorescent AFLP. Marker sets were developed for a base set of hybrids, which involved automated band detection using thresholds, followed by a manual refinement of the band scoring through the graphical user interface. The marker sets were then compared to AFLP patterns of seedlots to be tested. From a statistical analysis, the trueness was evaluated. We conclude that the Bandscoreing plugin is a convenient and versatile tool for semi-automated high throughput scoring of molecular markers in trueness-to-type testing.



### **P3.38 - Development of a software tool that performs automated cluster calling and data management for SNP genotyping assays**

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With an ever-increasing number of available genome sequences, single-nucleotide polymorphism (SNP) genotyping using Taqman or related technologies is expected soon to become the preferred method for genomics-assisted breeding and germplasm characterization for all but the smallest crops. Being a high-throughput method, massive datasets are generated, requiring sophisticated analysis and storage solutions. We developed a plugin application for the BioNumerics software, which performs a fully automated cluster calling and provides a flexible user interface for visualization and manual fine-tuning of the calls. The plugin performs its automated cluster calling using a multi-seed partitioning algorithm, with the ability to store optimal seed points per SNP marker. Confidence values are reported per batch and for each individual SNP call. Thresholds for calling, algorithm parameters and report options can be specified by the user. All data, including calls, plant sample information and experiment settings, are stored in an industry-standard relational database (e.g. SQL Server, Oracle or MySQL), effectively allowing the construction of large genotyping databases over long periods of time. Additionally, users can take advantage of the sophisticated backup and user management tools available in the database management system and integration with existing storage platforms can be achieved. SNP scores can be further analyzed in BioNumerics using clustering and dimensioning reduction techniques (PCA, MDS and discriminant analysis) or with statistical tools such as multivariate analysis of variance (MANOVA). These analysis tools greatly facilitate common tasks in plant breeding, such as finding the hybrid that is most similar to the recurrent parent during backcrossing and comparing seed lots with representatives of known cultivars in trueness-to-type analysis. In addition, SNP genotyping results can be compared in the same software with results from other techniques such as SSRs, AFLP, Multiplex Ligation-dependent Probe Amplification (MLPA), high-resolution melting curves and phenotypic characterizations.

### **P3.39 - Marker-assisted selection of superior planting materials for accelerated coconut replanting in the Philippines**

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Coconut production in the Philippines has been declining due to decreasing hectareage and increasing stands of senile palms. Replanting with improved varieties is the most cost-effective technology intervention. This study aimed to develop and use microsatellite (SSR) markers to fast track the identification and utilization of outstanding tall populations and hybrids of coconut as superior planting materials for the nationwide replanting program. To date, putative hybrids have provided the bulk of superior planting materials. Nine varietal crosses have been developed but only three were confirmed hybrids based on morphological markers. Ten diagnostic SSR markers were used to validate the morpho-hybridity detection. Using at least one diagnostic SSR marker, all the nine varietal crosses were confirmed genuine. However, non-hybrid trees from most varietal crosses were also detected. For Batangas and Quezon provinces, 11 outstanding Tall populations of coconut were selected based on nut, copra, and oil yield. Selected SSR markers were used to assess the genetic diversity and relationship of these coconut selections. Based on Nei's dissimilarity index calculated from the proportion of shared alleles, two Tall populations from each province were found similar while the others were relatively diverse. The SSR marker technology has proven useful in accurately identifying superior planting materials using the Tall coconut populations. Based on the established genetic distance, strategic replanting using the Tall coconut selections can be recommended to preserve the inherent genetic diversity of the crop in the target areas.

### P3.40 - Genetic diversity in maize local populations assessed by morphological traits and molecular markers

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Maize Research Institute Zemun Polje genebank maintains the collection of local populations of maize from ex-Yugoslavia territories, as well as the collection of introduced material. Local populations are classified into 18 agroecological groups based on pedigree and morphological data. In an ongoing project a subset of 54 local populations, carefully chosen to have some desirable traits (early flowering, drought tolerance, yield potential...), which are representatives of each agroecological group, are being subjected to morphological evaluation and SSR analysis with the aim to develop genetic fingerprints for their characterization, identification and classification, as well as for estimation of their genetic diversity. Herein, we present the first results of the experiment, which include eight populations. Six populations belong to the local germplasm (three to Derived flints agroecological group and three to Dents type of USA Corn Belt dents agroecological group), while two introduced populations originating from China were used as a check since they are expected to be genetically distant from local populations. Based on average values of 15 morphological traits and their standard deviations cluster analysis was performed using square Euclidean distance and complete linkage method. The square Euclidean distance was further used for correspondence analysis in order to capture a global overview of the analyzed populations from the aspect of continuous variability. Statistical analyses were performed using program package SPSS 15.0. Both cluster and correspondence analyses grouped all flint with two dent populations and one dent population with China germplasm. SSR analysis is being done with 80 primer pairs on bulked DNA samples for each population. Genetic distances will be determined using the Modified Rogers Distance and cluster analysis will be performed using Unweighted pairgroup method (UPGMA). The results of marker analysis together with comparison between molecular and phenotypic descriptions will be presented on the poster. This comparison will allow evaluating the best way to combine information in a comprehensive way.

### P3.41 - Study on genetic diversity of cabbage (*Brassica oleracea* L.) breeding lines for genetic mapping of black rot and club root disease resistance and development of molecular breeding system

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*Brassica oleracea* L. is one of the most important crops which show very diverse morphological difference. The major devastating disease that poses huge crop losses on cultivated *B. oleracea* is black rot disease. Cultivation of resistant varieties is the most effective method of disease control. Resistance to black rot (*Xanthomonas campestris* pv. *Campestris*) in *B. oleracea* L. has been reported as quantitative in nature, involving varying numbers of genes. Genetic mapping is a reliable tool for discerning complex traits and identifying quantitative trait loci (QTLs). In order to construct genetic map and to develop molecular markers of resistant genes to black rot and club root disease for development of molecular breeding system for cabbage, we inspected 16 *B. oleracea* breeding lines, which consisted of five and nine lines resistant and susceptible to black rot, respectively, and the other two accessions resistant to club root disease, to select best parental combinations. To develop genome-wide sequence-based markers, we have used comparative genomics information of related *Brassica* species such as intron-based polymorphism (IBP) and SSR markers in *B. rapa* and *B. napus* genome maps as well as markers in *B. oleracea*. And we have collected all of the EST sequences containing NBS domain and tried to develop disease resistance gene related markers. Using a total of 105 polymorphic SSR, IBP and NBS primers, we estimated the genetic diversity among 16 *B. oleracea* lines. Based on the phylogenetic tree, we selected two parental combinations, IMO-005 x IMO-010 combination for black rot resistance and IMO-010 x IMO-018 combination for clubroot resistance. Both combinations showed approximately 0.6 coefficient similarity.

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### P3.42 - Wheat genetic transformation as approach to increase crops genetic resources and biodiversity

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Elaboration of new germ-line biotechnology (genetic transformation by germ elements – pollen, ovary, embryo, seed) for wheat transgenic plants creation enhance possibility of simple introduction of new valuable genes for plant disease and abiotic stress resistance, improvement of grain quality, increasing of microelements and vitamins, genetic modification of photosynthesis, yield crop improvement, increasing of crops genetic resources and biodiversity. We have elaborated method of wheat genetic germ-line transformation by *Agrobacterium* Pipetting into ear stigma. Method is based on unique for wheat mechanism of pollen distance transfer which provided by high level of flavonol glucosides which act as inducers of the *vir*-zone of Ti plasmid. Biotechnological protocol includes several stages: *Agrobacterium* culture grown and optimization; improvement of technique for *Agrobacterium* pipetting into wheat ears stigma; determination of optimal stage of plant development; screening of putative transgenic seeds on antibiotics which coded gene were included into gene construct of valuable gene as selectable markers (for example, *nptII* encoded kanamycin resistance and *hptII* encoded hygromycin resistance); biochemical screening of putative transgenic plants in T<sub>1</sub> progeny, in case of PEPC gene introduction – PC-assay, activity of PEP-carboxylase or transgene encoded protein expression; molecular biological detection of transgenes in wheat genome in T<sub>1</sub> and T<sub>2</sub> - generations by PCR, Real Time PCR, Northern and Southern Blotting, yield analysis. Elaborated biotechnology could be implemented to wide number of wheat genotypes; it except waste time and money steps of tissue culture and regeneration, simple, economic and effective (1.8-2.3%). New method converts wheat genetic transformation into routine process, which might be successfully used by many breeders and investigators for increasing of wheat genetic resources and biodiversity.

### P3.43 - Marker-aided selection of field resistance gene to blast in rice

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We identified the QTL for field resistance to rice blast using 117 BC<sub>3</sub>F<sub>5</sub> lines from a cross between two *japonica* cultivars. Genotypes were determined for 117 BC<sub>3</sub>F<sub>5</sub> lines by 134 simple sequence repeat (SSR) markers. These 117 lines were evaluated in blast nurseries at four locations for two years. QTL analysis identified two QTLs on chromosomes 4 and 7 for resistance to blast nursery tests. One QTL, *bn4* on chromosome 4 was detected at all locations in both years explaining from 16.8 to 35.9% of the phenotypic variance. Genetic analysis of the blast phenotypic data of the F<sub>2</sub> and F<sub>3</sub> population from a cross between a BC<sub>3</sub>F<sub>5</sub> line harboring the target region on chromosome 4 and the recurrent parent, indicated that a major dominant gene designated as *Pi45(t)*, was conferring resistance to blast nursery test. Linkage analysis indicated that *Pi45(t)* was located in the interval RM5709- RM3687, a region of approximately 577kb. Twelve lines with/without *Pi45(t)*, were assayed in the greenhouse using a sequential planting method in seven cycles using 29 virulent isolates in Korea. Lines with the *Pi45(t)* gene showed less than 20% diseased leaf area, which was significantly below the threshold level of 40% considered for durable blast resistance. Five promising lines nearly isogenic to the recurrent parent expect for blast resistance were evaluated at the preliminary yield trial. These lines with enhanced blast resistance did not show differences from the recurrent parent in amylose content and 1,000-grain weight. Our study based on a new method of sequential planting test indicated that the resistance gene *Pi45(t)* would be effective for durable blast resistance breeding in rice.

### P3.44 - Genetic variation of miRNA sequence in grapevine cultivars

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MicroRNAs (miRNAs) are 21-25 nucleotide long non-coding RNAs that are involved in post-transcriptional regulation. miRNAs are transcribed by specific nuclear genes (MIR genes) and for the most part they are the final products of a two step sequential processing: i) the generation in the nucleus of precursor-miRNAs (pre-miRNA) from a primary transcript (pri-miRNA) by the DCL1/HYL1 complex, and ii) the production of mature miRNAs from pre-miRNAs by DCL1, which in plants occurs again in the nucleus. Sequence variation in/and around the processing sites and in the mature miRNAs may have profound effects on miRNA biogenesis and function and may influence several biological processes.

To-date information regarding the position and extent of polymorphisms in plant MIR genes or in target genes is still very scant. In this study we present an analysis of sequence variation in the miR172 family, known to be associated with flowering time and developmental processes in plants. This family comprises four members, produced by four distinct MIR genes, and it has been shown that their mature products target the APETALA2-like transcription factor family. Specifically, we performed a single nucleotide polymorphism (SNP) analysis of these genes in 15 *Vitis vinifera* cultivars and in 35 different accessions belonging to several species of the genus *Vitis*. Sequence and genomic location of grapevine miRNAs genes were obtained from Rfam website (<http://rfam.sanger.ac.uk>). A genomic region of 1 kb, centered on each of the predicted pre-miRNA172 was amplified, cloned and sequenced. Our preliminary data show the presence of SNPs both in pri-miRNA, in the pre-miRNA- as well as in the mature miRNA. A number of SNPs have an influence on the predicted secondary folding structure, which might have strong implications in the subsequent miRNAs maturation process. The relevance of such polymorphisms on miRNA biogenesis and stability and their correlation with inflorescence architecture and flowering time will be addressed.

We are currently utilizing re-sequencing data available on a number of grapevine cultivars to extend genome-wide our analysis of sequence variation in known grapevine MIR genes.

### P3.45 - Progress on genetic mapping and genome sequencing of *Panax ginseng*

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We are trying to construct genetic map and sequence the genome of *Panax ginseng* (the Korean ginseng) which is a most famous medicinal herbs in Araliaceae family. Even though its medical efficacy have been studied for long time, little study was conducted on its genetic and genomics. Up to date, we produced large amounts of EST sequences from three ginseng cultivars; 37 and 49 Mb from Gopoong and Geumpoong, respectively, using GS-FLX and 5 Mb EST sequences (5,731 reads with an average of 860 bp high quality sequence) from normalized full length enriched cDNA library of a cultivar Chunpoong. We are developing large volume of SSR and SNP markers from the gene sequences using PAGE and high resolution melting (HRM) analysis. These markers will be genetically mapped using a F2 population of a cross between Chunpoong x Yunpoong. Further study for identification of large volume of SNP markers by comparing EST sequences derived different cultivars will be followed for construction of high resolution genetic map. To obtain the snapshot of the large ginseng genome structure estimated as 3 Gbp for its haploid genome consisting 24 chromosomes, we have sequenced 23 BAC clones, three of them are repeat-rich BAC clones containing redundant ginseng unique-repeats to characterize its heterochromatin structure and other 20 more BAC clones derived from euchromatin region to understand the ginseng genome.

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### P3.46 - Identification and characterization of variability in *Sus4* homologues in *Solanum tuberosum* varieties

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Sucrose synthase catalyses reversible conversion of sucrose and UDP to UDP-glucose and fructose in higher plants. Sucrose synthase is involved in a number of important processes such as starch synthesis, cell wall synthesis, regulation of sugar import and anaerobic and cold stress response.

Sucrose synthase genes (*Sus* genes) have been isolated from many starch-, sucrose- and hexose-storing monocot and dicot plants. The majority of characterized *Sus* genes belong to a small gene family. In *Solanum tuberosum* only two members of *Sus* gene family *Sus3* and *Sus4* are known that differ in expression profiles. In addition *Sus4* gene is expressed predominantly in developing tubers.

In the present study *Sus4* variability in 39 *S. tuberosum* varieties was characterized. The chosen varieties differ in cold/drought stress response and starch content. Selective primers were designed which enabled to discriminate and amplify only *Sus4* homologues.

In total 149 SNPs were identified both in exons and introns. 27 SNPs found in exons were specific and 26 were present in several potato varieties. In analyzed sequences, beside SNPs several indels were detected in introns. The most variable intron V was characterized by the presence of two insertions and a 5-bp insertion was detected in intron IV.

A total of 24 (45%) out of 53 SNPs that have been identified in analyzed *Sus4* exons result in amino acid substitutions, 16 of them were non-synonymous, 19 of detected amino acid substitutions were specific, and five were found in several potato varieties.

Identified SNPs can be used as potato genotype markers. Interdependence between starch content, abiotic stress resistance and indels and SNPs found is discussed

### P3.47 - Soybean and peanut in Turkey: Genetic diversity revealed by ISSR and SRAP analysis

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Developing successful strategies to ensure future increase in yield of soybean and peanut crops hinges in part, on improving the genetic basis of the cultivars. Knowledge of genetic relationship in crop breeding programs provides valuable information that can be used by plant breeders as parental line selection tool. So far, a throughout analysis of genetic diversity among the soybean and peanut genotypes grown in Turkey had not been attempted at the DNA level. In this study, inter simple sequence repeats (ISSR) and sequence related amplified polymorphism (SRAP) markers were used to evaluate genetic diversity among 21 soybean and 32 peanut cultivars and breeding lines adapted to different regions of Turkey. The ISSR analysis performed with 46 primers in soybean and 47 primers in peanut, yielded 31 and 26 polymorphic bands respectively, while 26 and 17 polymorphic amplicons were amplified by 34 and 36 SRAP primer combinations in soybean and peanut respectively. Unweighted pair group method with arithmetic means dendrograms (UPGMA) based on Jaccard similarities were obtained from the combined ISSR + SRAP data for both soybean and peanut. In soybean, UPGMA dendrogram clustered all soybean cultivars into same group except 'Ye ilsoy', whereas, in peanut, it separated cultivars southwest runner and spantex from all other cultivars, breeding lines and plant introductions. In light of narrow germplasm base of soybean and peanut genotypes grown in Turkey, a renewed emphasis should be placed on introduction of new sources of germplasm into breeding pool in order to enhance genetic variability to permit continued progress in developing high yielding cultivars and lead to greater gains for selections. The results obtained from this study will be helpful for soybean and peanut breeders in Turkey to have information about genetic diversity and will facilitate them to make a future strategy for broadening the genetic basis of these crops.



### P3.48 - Molecular characterization of the Latvian apple (*Malus*) genetic resource collection based on microsatellite (SSR) and scab resistance gene *Vf* analysis

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Apple varieties are an integral part of the Latvian State Program for Preservation and Sustainable Use of Genetic Resources for Food and Agriculture (PGR). According to PGR system requirements, the fruit crop collections have two priority groups: i) national plant genetic resources of limited number, mainly local origin accessions, and ii) working collection for research, variety testing and breeding. A total of 109 apple varieties nominated as National Plant Genetic Resources were analyzed using a set of eight selected microsatellite (SSR) markers recommended by the ECPGR *Malus/Pyrus* working group and a marker for scab resistance gene (*Vf*). All SSR loci exhibited a very high level of polymorphism - 12 to 39 alleles, 18.75 in average, with high observed heterozygosity ( $H_o$ ) ranging from 0.64 to 0.89 and a mean of 0.78. The gene diversity (PIC value) for tested loci varied from 0.79 to 0.90, with 0.86 in average. Results of genotyping showed high presence of rare or unique alleles, 38 and 26%, respectively. All varieties in the collection could be distinguished with the tested set of SSR loci. For the *Vf* gene, all three possible genotypes (*VfVf*, *Vfvf* and *vfvf*) were detected for one, six and 102 varieties, respectively. Cultivar Prima was used as a positive control for apple scab resistance gene (*Vf*) detection. Cultivars with *VfVf* and *Vfvf* genotypes have been selected as valuable sources for further apple scab resistance breeding. Internal relationships of apple varieties were evaluated using SSR data based dendrogram created using Nei and Li genetic distances and UPGMA tree construction method. Cluster analysis did not reveal a clear pattern of clustering with well-defined variety groups, but confirmed known relationships based on putative pedigree and accession collection information. The analysed Latvian apple germplasm showed high genetic diversity, particularly landraces, while the cultivars developed within the modern breeding program clustered mainly into two groups. Analyzed plant material included also two widely grown cultivars 'McIntosh' and 'Prima', which allowed for checking the accuracy of allele scoring and comparing the results of this study with those presented in previously published studies.

### P3.49 - Investigation of genetic diversity in Russian collections of raspberry and blue honeysuckle

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The N.I. Vavilov Institute for Plant Industry (VIR) holds and maintains collections of various crop plants among the largest and oldest worldwide. Among them, small berry trees have gained attention because of their potential for human health. Small berries, usually containing various valuable compounds such as vitamins or antioxidants in significant quantities, could be used for easily improving human diet. Subsets of VIR collections of raspberry (*Rubus idaeus* L.) and blue honeysuckle (*Lonicera caerulea* L.) were investigated for genetic diversity. Ninety-five raspberry accessions were genotyped with eight nuclear SSR (microsatellite) markers. Results indicated a fair level of genetic diversity, but also a structure of three main groups in the collection. Blue honeysuckle accessions were genotyped with five ISSR markers, yielding more than 1100 polymorphic fragments across the 194 accessions. Statistical analysis of these data showed that the subspecies level was key in explaining blue honeysuckle diversity. This study shows that the collections constitute important resources that could be used for either direct consumption goals or breeding of new cultivars. Results may also be used to establish recommendations for efficient conservation of these genetic resources.

### P3.50 - A pilot study of estimating genetic diversity of wild soybean (*Glycine soja* S. and Z.) from Korea, China and Japan using molecular markers

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Wild relatives of crop species are an essential source for introducing useful genes into cultivated crops. Knowledge of wild germplasm diversity, therefore, has a significant impact on the crop breeding and conservation of genetic resources. Wild soybean is the wild progenitor of cultivated soybean and generates viable vigorous F1 seeds with cultivate soybean. However, genetic diversity of wild soybean is little estimated. The objective of this study was to evaluate and compare the genetic diversity of 354 wild soybean accessions originating from Korea, China and Japan using seven simple sequence repeat (SSR) markers as a pilot study. Seven SSR markers generated a total of 189 polymorphic alleles with an average of 28.3 alleles per locus. Alleles range from 86 to 394 bp. The mean of gene diversity was relatively high with 0.7456, 0.8692, and 0.9079 from Korea, China, and Japan, respectively. The highest gene diversity of Japanese accession might be due to high degree of heterozygosity of the population. Approximately, 55.1% of Korean accessions were clustered in a clade with only one accession from Japan and seven from China. Nearly all of other accessions were scattered into several clades resolving few insights into the genetic structure among three countries in the UPGMA dendrogram.

### P3.51 - Shotgun proteomic analysis for detecting differentially expressed proteins in the reduced culm number (rcn) rice

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To survey protein expression patterns in the reduced culm number (RCN) rice, a comparative shotgun proteomic analysis was conducted. For large-scale protein identification, multi-dimensional protein identification technology (MudPIT) coupled with pre-fractionation of plant shoot proteins led to identification of 3,004 non-redundant rice proteins. By statistically comparing relative amounts of 1,347 reproducibly identified proteins between the RCN rice and the normal rice, 44 differentially expressed proteins were detected, where 42 proteins were increased and two proteins were decreased in the RCN rice. These proteins appear to have roles in glycolysis, TCA cycle, secondary metabolism, nutrient recycling, nucleotide metabolism and repair, and sugar signaling. Consequently, we hypothesized that the RCN rice might fail to maintain sugar nutrient homeostasis and we observed that the RCN rice showed a hypersensitive response to exogenous sucrose treatment.



### P3.52 - Population structure and genetic diversity in elite sugar beet germplasm investigated with SSR markers

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The assessment of genetic diversity and structuration of germplasm is essential for the efficient organization of breeding material. The objectives of the study were to (i) examine the population structure of elite sugar beet germplasm, (ii) investigate genetic diversity within and among subgroups of elite sugar beet germplasm, and (iii) assess the extent of linkage disequilibrium (LD) within elite sugar beet germplasm. A total of 111 and 178 inbred lines from the seed and pollen parent heterotic gene pools, respectively, were genotyped with 23 SSR markers in this study. Two distinct subgroups were detected within the entire germplasm set by STRUCTURE and principal coordinate analysis (PCoA). This observation was not expected because the SP heterotic pool of sugar beet was developed out of the PP heterotic pool in the late 1970s. Our observation of high LD in elite sugar beet germplasm suggests that association mapping will be possible in the examined germplasm set using a relatively low numbers of markers. However, to reduce the problem of false-positive marker-phenotype association, it might be necessary to examine the subgroups separately or apply association mapping methods which take into account this structure.

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### P3.53 - Domestication of transposable elements into microRNA genes in plants

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Transposable elements (TE) usually take up a large portion of eukaryotic genome. Activities of TEs can cause genome instability that is harmful or even disastrous to the host. However, TEs contribute to gene and genome evolution at many aspects. Part of miRNA genes in mammals have been found to derive from transposons while convincing evidences are absent for plants. We found that a great number of previously annotated plant miRNAs are identical or homologous to transposons, which include a small number of bona fide miRNA genes (TE-MIR) that conform to acknowledged plant miRNA annotation rules, and hairpin derived sRNAs (TE-hsR) likely to be pre-evolved miRNAs. Analysis of these TE-hsR and TE-MIR indicate that transitions from the medium to high copy TEs into miRNA genes may undergo steps such as inverted repeat formation, integration into transcription unit, sequence speciation and adaptation to miRNA biogenesis. We also identified initial target genes of the TE-hsR/TE-MIR, which contain homologous sequences in their CDS as consequence of cognate TE insertions. Interestingly, most of these TE insertions span boundaries between coding and non-coding sequences indicating their incorporation into CDS through alteration of splicing or translation start or stop signals. Taken together, our findings suggest that TEs in gene rich regions can form foldbacks in non-coding part of transcripts that may eventually evolve into miRNA genes and be integrated into protein coding sequences to form potential targets in a 'temperate' manner. Thus, transposons may supply as resources for the evolution of miRNA-target interactions in plants.

### P3.54 - High-throughput genotyping and high-resolution phenotyping for a comprehensive QTL mapping related to apple fruit crispness

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In apple fruit quality crispness is certainly the major actor. Crispness, which is associated to the cell wall disruption mechanism and internal turgor pressure, is perceived as emitted sound during compression.

Besides its sensorial perception, a crispy apple is generally more appreciated by consumers because its higher flavour and aroma release.

In this context we performed a pilot study aimed to discovery the QTLs putatively involved in the control of the "crispy" phenotype in apple. To perform our investigation we genotyped two mapping populations: Fuji x Delecta and Fuji x Pink Lady, with two types of molecular markers. First, a series of SSR (CH and Hi series) were extended in a multiplex system with an ABI 3730 DNA analyzer, to build up the maps scaffold, necessary for linkage groups comparison with other reference maps.

The second category was represented by a set of SNP markers (ad hoc identified between the two haplotypes of the heterozygous Golden Delicious genome), genotyped in high throughput using SNPlex™ (Applied Biosystem) and Golden Gate genotyping assay (Illumina).

High resolution phenotyping, addressed to dissect most of the fruit flesh complexity, was carried out analyzing a series of acoustic and mechanical parameters via a TA.XT Texture Analyzer instrument (Stable Micro Systems) equipped with an acoustic detector. The preliminary QTL mapping study identified significative genomic regions on these two populations possibly involved in the control of fruit crispness and firmness. These regions will be further explored in order to identify the gene set included in the QTL interval, with the final aim to investigate the allele mining of these future candidate genes in a wider apple collection.

### P3.55 - Molecular diversity of the tomato chromoplast specific lycopene $\beta$ -cyclase (CYCB) gene and development of PCR-based markers distinguishing its *Beta* alleles

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The *Beta* (*B*) locus encoding the chromoplast specific lycopene  $\beta$ -cyclase (CYCB) enzyme in tomato (*S. lycopersicum*) increases fruit  $\beta$ -carotene content at the expense of lycopene, resulting in orange-pigmented fruit (Ronen et al., 2000. Proc. Natl. Acad. Sci. USA, 97:11102-11107). Orange-fruited accessions carrying introgressed chromosome segments of *S. habrochaites*, *S. cheesmaniae*, *S. pimpinellifolium*, *S. chilense*, and *S. chmielewskii* and containing high concentrations of  $\beta$ -carotene have been described and widely used for breeding of high  $\beta$ -carotene tomatoes (Zhang and Stommel, 2000. Theor. Appl. Genet., 100:368-375). To study molecular diversity of the CYCB gene and develop allele-specific markers we have sequenced the entire coding regions of the CYCB genes from four lines carrying different *Beta* alleles introgressed from wild tomato taxa: *S. pennellii* (LA4062), *S. chilense* (LA0348 (*og*)), *S. habrochaites* (LA2374), and *S. cheesmaniae* (LA3899). Multiple polymorphisms was observed when the *S. lycopersicum* CYCB gene sequence was compared with wild type ones (20 SNPs and 10 amino acid (AA) changes in *S. pennellii*, 14 SNPs and eight AAs in *S. habrochaites*, 11 SNPs and seven AAs in *S. chilense*, and four SNPs and three AAs in *S. cheesmaniae*). The only two SNPs, A<sup>406</sup>/G (Asn<sup>136</sup>/Asp) and G<sup>868</sup>/A (Asp<sup>290</sup>/Asn), distinguish all studied wild-type sequences from the *S. lycopersicum* one. Selected SNPs were used to develop a collection of specific PCR-based DNA markers differing alleles of the CYCB gene. The reliability of those markers was then validated on a set of wild tomato accessions. The markers developed enable an early genotypic selection of high  $\beta$ -carotene tomatoes and can be used for breeding purposes or to enhance screening of genebank accessions.

**P3.56 - Genetic diversity of *Corylus avellana* L. in Portugal revealed by chloroplast microsatellite marker**Martins S<sup>1</sup>, Simões F<sup>2</sup>, Mendonça D<sup>2</sup>, Matos J<sup>2</sup>, Silva AP<sup>3</sup>, Carnide V<sup>1,4</sup><sup>1</sup> Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal<sup>2</sup> Molecular Biology Group (GBM)/Genetic Resources, Ecophysiology and Plant Improvement, INRB, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal<sup>3</sup> Centre for the Research and Technology of Agro-Environment and Biological Sciences, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal<sup>4</sup> Institute for Biotechnology and Bioengineering, Centre of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

The genus *Corylus*, a member of the birch family *Betulaceae*, includes several species that are widely distributed throughout temperate regions of the Northern Hemisphere. The development of microsatellites or SSRs for non-coding regions of the chloroplast genome and their higher sequence variation compared with coding regions has provided a higher resolution tool for the study of closely related taxa. These chloroplast polymorphisms provide a marker system to evaluate the genetic structure of plant populations. This study describes the level of genetic diversity and differentiation of 35 genotypes of *Corylus avellana* in Portugal, divided into three groups (10 old, 3 commercial and 13 wild hazel trees). Variation at four chloroplast microsatellite (cpSSR) loci was examined. Eleven haplotypes based on length variants at the four cpSSR loci were identified. A good level of genetic diversity was observed at genotype level with the number of alleles per locus (A) ranging from 2 to 4 (average 2.75), the proportion of polymorphic loci (P) equaling 66.7%, and the diversity index (H) ranging from 0.111 to 0.440 (average 0.192). AMOVA results indicated that about 53% of variation in the data set was from genotypic variations within genotypes and the remaining 47% to differences among genotypes indicating a good degree of population structure. Considering the allele variants at the four loci, eleven different chlorotypes were detected and their relationships were analysed under a network model. The most frequent chlorotype (chlorotype A) is mainly associated with old and commercial groups of hazel trees.

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**P3.57 - Researching rice drought tolerance via multiple approaches focusing on a typical upland genotype**Mei HW<sup>1</sup>, Shu LB<sup>1</sup>, Feng FJ<sup>1</sup>, Ma CF<sup>2</sup>, Xu GW<sup>2</sup>, Liu HY<sup>1</sup>, Ding W<sup>1</sup>, Luo LJ<sup>1</sup><sup>1</sup> Shanghai Agrobiological Gene Center, Shanghai 201106, China<sup>2</sup> Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

An upland variety, IRAT109, was selected as a representative genotype of high level drought resistance in rice. It used as a parental line to develop mapping populations like RILs, and derived QTL NILs. Candidate genes were cloned from marker intervals hosting QTL for multiple traits. A set of  $\gamma$ -ray irradiation induced mutants were also developed from this variety. Among them, a few mutants had varied reactions to drought stress while most others had mutations in morphological or developmental characters.

IRAT109 was also widely used in our breeding program as a donor of drought tolerance. As an example, a CMS maintainer (Huhan 2B) was developed by the strategy based on backcrossing together with severe stress screening. The new line looked very similar to the recurrent parent (Hanfeng B), but had much better drought resistance. Whole genome marker survey showed very low proportion of introgressed genetic components from the donor parent.

Proteomic analysis, together with cDNA and metabolite profiling, was used to investigate the reactions to drought stress in this upland rice variety. Integrated analysis of multi-omics data showed strong regulation in the flow of substances and energy. Following the idea of partial root-area drying (PRD), osmotic stress were applied to partial roots (PROS) and whole roots (WROS). It was found that PROS did not produce severe damage to leaf tissues but generated stress signals like increased ABA content. Differentially expressed proteins were detected by 2-DE and MALDI-TOF/TOF. The number of such proteins was much larger under WROS than that under PROS. Stress defence and photosynthesis were the most involved categories under both treatments while some categories were detected under WROS only.

### P3.58 - Effects of mutation and drought stress on protein, oil and fatty acid content in sunflower

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Oil and protein content of sunflower seed and their quality are influenced by genetic and environmental conditions. In order to find the effects of mutation and drought stress on oil, protein and fatty acid content of seeds in sunflower, nineteen M6 mutant lines which were produced using gamma ray from AS613 were planted in field under two full- and limited-irrigation conditions. Thousand-seed weight, oil content, protein, and contents of palmitic acid, stearic acid, oleic acid and linoleic acid were determined for each mutant in both conditions. The genome of mutant lines was scanned using 175 AFLP markers. Analysis of variance revealed significant differences among mutants for all traits. Some of the mutant lines showed significant superiority to AS-613. Drought stress caused significant decrease in all traits except for palmitic acid content. Irrigation x genotype interaction was present for protein, oleic and linoleic acid contents. Despite the small sample size, Student's t-test indicated several markers potentially linked to one or more traits.

### P3.59 - Seed longevity in canola (*Brassica napus* L.) – genetic variation and QTL mapping

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Research on seed longevity was performed in canola (*Brassica napus* L.) maintained in the seed store of the 'Satellite Collections North' of the German *ex situ* genebank, IPK Gatersleben. A high intraspecific variation was detected in those natural aged accessions. After 26 years of storage, germination ranged between 0 and 90%. The accessions investigated came from a seed multiplication performed in the same year at the same place. Furthermore they were handled in the same way during/after harvest (threshing, cleaning) and stored under identical conditions in the cold chamber. Therefore, the differences in germination percentage discovered must be due to genetic variation in seed longevity.

In addition, a doubled haploid canola mapping population (Ye2-DH) being artificial aged was investigated to study the inheritance of seed longevity. Quantitative trait loci were detected on different linkage groups. The mapping positions of the loci were compared with syntenic regions in *Arabidopsis* and discussed with respect to putative genes which may determine the longevity of seeds.

### P3.60 - *Phaseolus coccineus* L.: Genetic diversity and structure of a world-wide collection

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*Phaseolus coccineus* L. is closely related to *P. vulgaris* and is the third most important cultivated *Phaseolus* species. In this work, a representative collection of its world-wide diversity was developed. The collection includes wild forms (WFs) and landraces (LRs) from Mesoamerica (the crop domestication area), as well as LRs from Europe. The collection and a sample of *P. dumosus* LRs were studied by using 12 SSR molecular markers. The genetic diversity, population structure and phylogenetic relationships were investigated. Data from coding and non-coding regions were compared to make inferences about the evolutionary history of the crop. The results indicate that a) the European and Mesoamerican gene pools are clearly differentiated, b) the European gene pool is rich and a limited reduction of diversity occurred with introduction into Europe c) the Mesoamerican LRs (*P. dumosus* included) and WF are closely related and are connected by a high gene flow and d) the differentiation of the European gene pool from the Mesoamerican one is due to a different balance of evolutionary forces. Inferences on the domestication sites and role of different species in the domestication process of *P. coccineus* are also presented. The genetic diversity of both WF and LR is an important source for *Phaseolus* spp. breeding programs and deserves to be preserved *in situ* and *ex situ*.

### P3.61 - Identification of essentially derived varieties in durum wheat

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Essentially derived are considered those varieties (hence EDV) that, being obtained from an original variety by means of methods that do not modify substantially its genetic structure, should be considered dependent from it. The identification of EDV is therefore an important aspect of the protection of Breeder's Rights. It requires the definition of a threshold value of genetic conformity between the new and the original variety that, if exceeded, would provide a clue of derivation. Molecular markers-based protocols estimating genetic similarities have been proposed for EDV identification in some species, whereas no information was available for durum wheat.

A set of 60 genotypes (F8- or F9- lines and their parental varieties) representing different levels of relatedness, were genotyped using 14 AFLP primer combinations (*Sse8387+2-MseI+2*) and 109 SSR loci evenly distributed in the genome. Jaccard similarities were calculated for all the pair-wise comparisons among entries. For both markers an EDV threshold, defined as the 95 percentile of the distribution of similarity estimates among independently developed progenies, allowed the detection of all pairs of closely related lines branched in advanced generations (F7-F8), indicating a rather good agreement of the two marker systems in evidencing cases of suspected derivation. Nevertheless, for their superior informativeness compared to SSR, AFLP appear more suitable to assess essential derivation.



### P3.62 - Genetic and morphological assessment of traditional varieties of common bean (*Phaseolus vulgaris*) from Lazio, Italy

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The common bean (*Phaseolus vulgaris* L.) was introduced into the Mediterranean region from Latin America between the 15<sup>th</sup> and 16<sup>th</sup> centuries. The species has since diversified into numerous varieties locally adapted to areas within the region, which is now considered a secondary centre of bean diversity. The Italian varieties possess a wide range of seed colour, size, growth habit and agro-ecological adaptations, some of which are globally recognized varieties, such as the cannellini and borlotti beans. The Lazio region of Italy is geographically diverse, ranging from the Apennine Mountains in the east to the plains of western Lazio. In many of the less industrialized areas, local varieties have been maintained through the use of traditional farming methods and home gardens. The work presented here focused on 30 landraces obtained from local farmers as seed and planted at experimental field sites of the University of Tuscia, Viterbo, Italy. The landraces were evaluated morphologically using IPGRI (now Bioversity International) descriptors such as seed (bean) size, weight and colour, flower and seed pod morphology, as well as assessment of leaf form and plant growth. Results showed that some varieties were clearly distinct but others could be reclassified as a single variety, especially those that originated from the same province. The genetic component of this study involved the extraction of DNA from young leaves and diversity assessed using microsatellite markers. High levels of diversity were found both within and among the populations and levels of gene flow were also assessed.

### P3.63 - Study of genetic diversity of *Sulla* in the northwest of Morocco

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The *Sulla* genus (*Hedysarea*) is represented by nine species in the Mediterranean Basin and has a wide distribution in the North African countries (Choi et al. 2003). Due to its good forage quality characterized by a high level in protein content and almost no anti-nutrition substances, this culture has recently received growing interest either to satisfy the increased local demand for forages or to be used for soil protection and pastoral production (Trifi-farah et al. 2002; Issolah et al. 2006; Houda et al. 2007; Annicchiarico et al. 2008). In the northwest of Morocco, this species is used mainly as forage grazed or chopped on natural sites where it is subject to severe genetic erosion due to overgrazing, the decline in agricultural areas and advancing urbanization. A recent agronomic study of five Italian varieties of *Sulla* in the Tangiers area shows a satisfactory yield (six tones dry matter per hectare) and a good adaptation to local conditions of rainfall agriculture (personal data).

In view of preserving and valorizing the local germplasm of *Sulla*, this study aims to assess the genetic diversity and capitalize on this variability through breeding programs and agronomic operations. In this sense, a study of exploration and collection was conducted in the Tangier's area in summer 2009 based on morphological criteria such as size of panicles, the precocity and the general behavior of plants. To understand this morphological diversity, the ecotypes collected and the Italian variety *Irpina* were analyzed by molecular markers technique RFLP. The prospection has permitted to collect ten different accessions based on the criteria above and the molecular analysis revealed a great genetic variability in relation with the vegetative aspect that affects the agronomic development. Thus, creeping ecotypes show an important asset for improving pastoral production and soil conservation in this region subjected to severe erosion while upright ecotypes are to be used for forages operated as green fodder or silage.

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### P3.64 - Cold-modulated expression of genes encoding for key enzymes of the sugar metabolism in spring and autumn cvs. of *Beta vulgaris* L.

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Autumn sugarbeet (*Beta vulgaris* ssp. *vulgaris*) cultivars are sown in early fall in several regions of Central and Southern Italy; the different growth cycles imply different selection strategies and capability of the plants to adapt to the environment. It is likely that these differences include differently modulated responses in the levels of gene expression of key metabolic enzymes. We compared the modulation of expression of nine sugarbeet genes of relevant importance for development and sugar metabolism of plantlets of the autumn cv. Franca and of the spring cv. Bianca, in response to low temperature (LT) treatments induced in controlled environment. TCs (tentative consensus, *Beta vulgaris* Gene Index-TIGR) were employed when homologous sequence information for these genes was missing. Real-time PCR was used to analyze gene expression profiles during and after low temperature treatments, with a time course up to 1 week post-stress. Data were analyzed by the comparative Ct method and normalized to the geometric mean of two housekeeping genes specifically selected either in LT-treated leaves and LT-treated roots of sugarbeet, and finally expressed as fold-change compared to unstressed conditions. The transcriptional responses to LT of the two sugarbeet cultivars were correlated with the LT-induced electrolyte leakage measure of the tissue damage, in both roots and leaves of the plantlets. Important stress-induced alterations in the level of transcripts were detected in the two cultivars, suggesting an induction at the transcriptional level of sucrose synthases and a reduction of carbohydrate partitioning during and after cold stress. It was found that in the autumn cultivar the cold stress, irrespective of temperature or exposure time, induced profound and fixed changes, especially in 1,6-biphosphatase gene expression.

These differential features of LT-response expression profiles in cultivars selected for different climates and response to LT, constitute first possible hints of the basis of the markedly different response of sugarbeet cultivars not only to LT, but also to other environmentally regulated differences between autumn and spring cvs, such as resistance to bolting.

### P3.65 - Allele mining in the gene pool of orphan *Solanum* species for homologues of late blight resistance gene *RB/Rpi-blb1*

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A CC-NBS-LRR gene *RB/Rpi-blb1* initially isolated from *S. bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* and is currently employed in potato breeding for durable late blight (LB) resistance. *RB/Rpi-blb1* homologues were reported from *S. bulbocastanum*, *S. papita*, *S. stoloniferum* and *S. verrucosum*; some of them retained defence function when introgressed into potato cultivars by cisgenesis. *RB*-mediated resistance has not been defeated in the field tests as yet; however, Champouret et al. (2009) reported on two Mexican *Phytophthora* isolates virulent on *RB* potato. Pyramiding several *RB*-like genes from various *Solanum* species in potato cultivars would promote durable LB resistance. Here we report an early evidence on *RB*-like sequences in the wide range of orphan *Solanum* species section *Petota*. The panel of 141 accessions of 19 *Solanum* species was screened with the *RB/Rpi-blb1* locus-specific marker RB-629 spanning intron and second exon fragments and with the RB-226 marker (modified from Colton et al., 2006) tagging the active allele of *bulbocastanum RB*. RB-629 was present in 57% of accessions representing 15 *Solanum* species whereas RB-226 was present only in 16% of accessions from eight *Solanum* species. LB resistance of *Solanum* species was independently assessed using field and detached-leaf assays. Neither RB-629 nor RB-226 was associated with higher LB resistance. It is therefore evident that RB-226 cannot universally diagnose the active *RB* allele even in resistant *S. bulbocastanum* accessions. Comparative analysis of RB-629 sequences from 16 accessions of 12 *Solanum* species revealed 69 SNPs and a *S. polytrichon*-specific insertion. The RB-629 sequences were grouped into 14 polymorphic haplotypes. Their phylogenetic analysis using maximum likelihood and parsimony produced four distinct clusters: cluster I of *S. bulbocastanum*-like haplotypes, cluster II of pseudogenes, cluster III specific for *S. polytrichon* and cluster IV combining other putatively active *RB* homologues. It is noteworthy that the RB-226 marker was found only in the cluster I genotypes. The pattern of polymorphisms was not species- and series-specific, and we conclude that duplication and evolution of *RB*-like loci preceded *Solanum* speciation. Remarkably, the duplicated *RB*-like genes in *Solanum* species retained the conservative intron. Supported by the ISTC-USDA-ARS project 3714p.



**P3.66 - Morphological and molecular diversity analysis for quantitative traits in minicore collections of groundnut (*A. hypogaea* L.)**

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The genetic diversity analysis involving 182 accessions from minicore subset belonging to different habit groups was carried out using Principal Component Analysis and Hierarchical Cluster analysis for 10 morphological traits to know the genetic diversity prevailed in groundnut and to isolate parents for hybridization. The RAPD analysis involving 25 genotypes and 20 primers was also being carried out to know the existence of genetic variability at the molecular level. The results revealed that all the ten characters except growth habit contributed to multivariate polymorphism. The hierarchical cluster analysis revealed eight distinct clusters indicating genetic diversity. The RAPD analysis revealed eight accessions as resistant, which belonged to mainly Virginia bunch and Virginia runner habit group (Holbrook and Dong, 2005) and none of them belonged to Spanish bunch. As many as eighteen accessions were promising for rust, which belonged to the entire habit groups except Spanish bunch. The test weight ranged from 24.45 to 61.15 g and genotypes with high seed mass belonged mainly to Virginia runner, which helped for isolating parents with confectionery traits. The oil content ranged from 41.3 to 48.85% and appeared to be low. The RAPD analysis involving selected accessions for both foliar disease resistance and seed quality traits indicated greater polymorphism with as many as 15 primers out of 20 used (Nalini et al., 2005 and Yugandhar 2005) low to moderate polymorphism reported (Mondal et al., 2005). Further, the similarity coefficient ranged from 0.56 to 0.93 indicating wider variability in the minicore subset indicating representative variability constituted in the minicore collections. The greater diversity was observed between ICG 11219 and a susceptible cultivar JL 24 (Sij value 0.56) followed by ICG 11219 and resistant checks GPBD 4 (Sij value 0.66). Although, dendrogram revealed five distinct clusters they appeared to be overlapped with subspecies indicating independence of molecular diversity with morphological diversity. Thus, the present studies enable to choose parents with minimum repetitive variability for the characters of interest either based on morphological or molecular diversity evaluation for genetic enhancement for foliar disease resistance and confectionery traits.

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### P3.67 - Sequence characterization of mitochondrial genome-derived BACs from four lines, two CMS and two MF lines, of *Raphanus sativus*

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Cytoplasmic male-sterility (CMS) which is efficient tool for F1 hybrid production is a trait controlled by a gene in mitochondrial genome with interaction with nuclear genomes. To identify novel CMS gene and understand mitochondrial genome evolution in *Raphanus sativus*, we constructed and analyzed organelle genome-specific BAC libraries from two male-sterile and two fertile radish lines. For construction of physical map representing mitochondrial genome for each genotype, we performed BAC-end sequencing and fingerprinting using SNaPShot analysis against 192 BAC clones for each library. We obtained 8, 6, and 4 fingerprints-based contigs for DCGMS, Ogura and DBRMF2 libraries, respectively. BLAST-x using BAC-end sequences showed 6, 13, and 7 clones have significant similarity with mitochondrial genes in DCGMS, Ogura and DBRMF2 libraries, respectively. Consequently, 31, 14, and 9 BAC clones were selected for sequencing by GS-Titanium and comparative analysis from DCGMS, Ogura and DBRMF2 libraries, respectively. As the results, we get the mitochondrial contigs, and the sequence of DCGMS and Ogura contigs were shown the colinearity with mitochondrial genome of *Brassica napus* by PipMaker. To make the phase 3 sequence, gap filling of contigs is processing. Further sequence-based information will be discussed later.

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### P3.68 - An innovative multi-parental population for high-resolution mapping of complex traits in maize

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Advanced cross designs might provide unprecedented and powerful resources of information for the dissection of the genetic bases of complex traits, including heterosis.

Here we describe the ongoing development of such a population in maize (*Zea mays* L.), namely an 8-way Recombinant Inbred lines (8W-RI) population aimed at genetic and molecular dissection of heterosis and other complex traits. To this purpose, eight maize inbred lines, selected to include a wide genetic and phenotypic variability, were first intercrossed by an 8 x 8 half-diallel cross. All twenty-eight 2-way F<sub>1</sub> hybrids were further intercrossed in a so called "disjoint diallel" scheme (i.e. allowing crosses only between entries with no parents in common). The so obtained two-hundred and ten 4-way hybrids were bulked in 70 pools, each composed by the three 4-ways hybrids bearing the same alleles in all possible parent-of-origin cis combinations (e.g. AB x CD, AC x BD, AD x BC). Thirty-five 8-way hybrids were then obtained by paired crossing of complementary 4-way hybrids pools in a second disjoint diallel. Production of 8W-RI lines followed by single-seed descent of weighted random samplings of the thirty-five 8-way hybrids. Currently, seeds of the fourth selfing generation (8W-RI F<sub>4</sub>) are being harvested from about 3,600 8W-RI F<sub>3</sub> plants selfed at a winter nursery location in Yangon (Myanmar). The 8W-RI F<sub>5</sub> mapping population will be available by fall this year. According to what recently estimated in mouse, such a material should allow mapping QTL with effect size > 5% of the total variance to an interval of 0.5 cM using fewer than 1,000 lines.

All 2-, 4- and 8-way hybrids plus the parental inbred lines will also be available for phenotypic evaluation of heterosis in maize.

### P3.69 - Agropolis Resource Center for Crop Conservation, Adaptation and Diversity (ARCAD): A new open multi-function platform devoted to plant agrobiodiversity

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ARCAD is an initiative supported by Agropolis Fondation and the Region Languedoc Roussillon (France). ARCAD aims at setting up a new open multi-function (conservation, research and training) platform devoted to the assessment and better use of plant agrobiodiversity in Mediterranean and tropical regions. The programme's scientific agenda will prioritize the study of history and patterns of crop domestication and adaptation as well as the analysis of key parameters underpinning adaptation and diversity structure, at various time scales, through studies of evolutionary genomics, population genetics and social sciences. The research will focus on Population comparative genomics, Crop adaptation to climate change and Cereal crops in Africa. These activities will be complemented with technological and methodological components for the conservation (DNA bank, cryopreservation) and analysis (bioinformatics, linkage disequilibrium) of crop diversity. A major objective of the programme is also to set up a demand-oriented capacity building platform, based upon the educational facilities offered by universities in Montpellier and the development of specific training modules. The ARCAD programme is jointly developed by CIRAD, INRA, IRD, Montpellier SupAgro and University of Montpellier 2, in partnership with numerous South and international institutions. As an open platform, ARCAD will continuously seek the involvement of interested partners.

### P3.70 - Genetic diversity assesment of Italian common fig (*Ficus carica* L.) using microsatellite markers

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The common fig (*Ficus carica* L.) is native to the western Asia and was later dispersed to the Mediterranean area. The fig was one of the first plants ever to be cultivated by humans. Kislev *et al.* (2006) published in *science* that fig trees could have been the first domesticated plant of the Neolithic Revolution, preceding cereal domestication by about one thousand years. Archaeology guide, K. Hirst (2006), says figs were domesticated "five thousand years earlier" than millet or wheat. With this historical background, fig attracts the scientific researchers to characterize using molecular tools to unravel the genetic wealth it possesses.

Aimed at the analysis of genetic diversity in common fig collected from Italy, we characterized around 88 accessions using 37 polymorphic microsatellite markers.

There is generally a paucity of information available for diversity pattern among the Italian common figs and in particular till date no molecular work has been made with Italian common fig genotypes. Henceforth this work was made to analyse the Italian common fig using microsatellite markers and this assessment made possible to unravel the genetic diversity for which there was null information so far.

Both morphological and molecular characterizations were made and will be precisely presented in the poster.

### P3.71 - Development and application of EST-SSRs for diversity analysis in Ethiopian grass pea

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*Lathyrus sativus* L. is a leguminous crop widely cultivated in Ethiopia, covering about 9% of total pulse growing area of the country. The crop has desirable properties which enable it to survive when other crops fail. These include the ability to grow in wide soil types, drought tolerance and ability to grow in land subjected to flooding.

DNA markers have proven to be very useful for a variety of purposes in genetic studies of plants. The availability of sequence data from complete or partial genes makes it possible to develop molecular markers based on these sequences. Expressed Sequence Tags (ESTs), offer an opportunity to identify simple sequence repeats (SSR) and develop EST-SSR markers. To date limited molecular knowledge and molecular tools are available on legumes in general and on grass pea in particular. Although the amount of publicly available sequence information for grass pea is very limited, these along with sequence information and molecular markers developed for model legumes could serve as a useful resource for developing molecular markers that can be useful in molecular studies in the species.

Here we present our research activity which aims at assessing the genetic diversity of grass pea using EST-SSR markers developed from *L. sativus* EST sequences and transferable molecular markers from *Medicago truncatula*. Diversity study was done on 240 genotypes of grass pea collected from different geographical regions of Ethiopia. The different variation measures applied showed higher within population variation, and about 14% of the total genetic variation in grass pea is found among populations, suggesting that each population has a unique genetic property which makes it a significant unit for conservation efforts and breeding purposes.

### P3.72 - Development and characterization of 110 novel microsatellite markers in foxtail millet [*Setaria italica* (L.) P. Beauv.]

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Foxtail millet [*Setaria italica* (L.) P. Beauv.] is still the staple food to a large section of people in the semi-arid regions of Asia and Africa. The availability of microsatellite markers and saturated genetic linkage map has been limited in foxtail millet, though it is considered as a model drought tolerant crop and close relative to several important biofuel crops. Hence, development of microsatellite markers will have a major impact on genetic analysis and breeding of foxtail millet and should serve as an excellent surrogate genome to assist future study and improvement of closely related biofuel crops. In this study, we described development of 110 new microsatellite markers from two genomic libraries enriched for the CA/GT and ATG/TAC repeat motifs in *Setaria italica* cv. 'Prasad'. As a result of sequence analysis of 1358 clones, 202 unique sequences were identified and were searched for SSR motifs. Out of the 202, 172 (85.1%) sequences contained 114 di- and 58 tri-nucleotide SSR motifs and 110 (64%) SSR markers were successfully developed from them. The above developed 110 SSR markers were validated in a set of five foxtail millet accessions. To assess the usefulness of the SSR markers developed, 23 SSR markers were randomly chosen to analyze genetic diversity of 40 foxtail millet accessions. A total of 37 alleles were detected with an average of 1.6 alleles per locus. The Polymorphic Information Content value for each locus ranged from 0.14 to 0.50 with an average of 0.35 and the mean Marker Index of all 23 microsatellite markers was 0.60. The dendrogram generated on the basis of UPGMA algorithm grouped the 40 foxtail millet accessions into six major clusters in which all the *S. italica* accessions grouping was largely consistent, while the other *Setaria* species tended to be grouped together. We found CA/GT repeats were relatively present in higher number than ATG repeats in our target SSR motifs. The SSR markers developed in the present study will be further used for germplasm characterizations, comparative mapping and construction of SSR-based molecular genetic linkage map for QTL discovery in foxtail millet including other millets, cereals and forage grass species.

### P3.73 - Assessment of the genetic variability among Sri Lankan rice varieties by fluorescent AFLP markers

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Traditional rice (*Oryza sativa*) varieties are one of the important components of biodiversity of Sri Lanka. Farmers cultivated traditional varieties due to their great adaptability to local climatic conditions, soil types and pest and disease problems. However, with the introduction of high yielding foreign varieties, traditional varieties became extinct from cultivation. Some of those varieties are still conserved *ex-situ* and as seeds in gene banks. This may be an important gene pool for rice breeders in developing new rice varieties. However, no records on genetic diversity of those rice varieties are available. FAFLP was carried out to genetically differentiate 47 traditional rice varieties, five wild species, 28 cultivars and one sample of *Hygroryza* sp. One of the site-specific primers is fluorescently labeled (HEX, TMR and FAM). Fragments were separated through a capillary on automated MegaBACE 1000 DNA sequencer using ROX 400 as size marker. The electropherograms were analyzed by genetic profiler software and used for phylogenetic analysis. Ten primer combinations generated a total of 784 fragments sizes ranging from 30 to 500 base pairs long. Of these 772 were polymorphic and 12 fragments were monomorphic. UPGMA clustering of the 81 rice accessions showed four major groups. The most susceptible, dwarf variety

“Taichung Native 1” was uniquely separated. All five wild rice varieties showed clear separation from other accessions and are found at the same cluster. *O. nivara* and the traditional rice variety Kombili are originated from the same branch and both are morphologically very similar. A sample from genus *Hygroryza* was also included in this study to find out the reliability of data and analysis and it was clearly separated. Up land and low land varieties were separated clearly into two different clusters. There are morphological similarities between the plants found at the same clusters, e.g. height, grain colour, etc. Knowledge of the genetic diversity at molecular level enriches the information on pool of traits available to plant breeders and enhances the rice improvement programs. This genetic diversity data will be useful for plant breeders and useful in conservation of traditional rice genetic resources.

### P3.74 - Brassica and relatives, phylogeny based on SNP analyses

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Domestic *Brassica* species and their wild relatives from the primary, secondary and tertiary gene pools were analysed for phylogenetic diversity with 10 SNPs validated for polymorphism in *B. rapa*. These were amplified across 11 domestic accessions (*B. carinata*, *nigra* and *rapa* subsp.) and 14 species (including subspecies) of wild relatives represented by 29 accessions, from the Australian Temperate Field Crops Collection. Genetic distances were calculated from SNP flanking sequence data for construction of a dendrogram showing phylogenetic relationships. The most closely grouped accessions were usually of the same species/subspecies complex, and these species tended to cluster into groups according to ‘nigra’ and ‘rapa/oleracea’ lineages, but with some exceptions. Clustered with *B. nigra* were the *Hirschfeldia*, *Sinapis*, *Crambe* and *B. carinata*, while clustered with *B. rapa* were *Brassica* sp; *barrelieri*, *oxyrrhina*, *tournefortii*, *incana*, *montana* and *ruvo*, and the genera *Eruca*, *Diplotaxis*, *Capsella* and *Matthiola*. However *B. deflexa* clustered with the ‘nigra’ lineage though morphologically aligned with the ‘rapa’ lineage. New information is presented for *B. ruvo* placing it in the ‘rapa’ lineage, which phenotypically has seedless beaks in fruits, in contrast to seeded beaks for the ‘nigra’ lineage. However, phenotypically some genera such as *Diplotaxis* are represented by species in both lineages.



### P3.75 - Utilization of genetic diversity potential in rice varietal improvement

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The frequent use of genetically similar rice breeding lines may narrow the genetic base of modern cultivars. Thus, a diverse collection of parent germplasm in breeding programs is very essential in crop improvement. The International Network for Genetic Evaluation of Rice (INGER) has facilitated the evaluation and exchange of diverse breeding lines worldwide since 1975. Over 600 released varieties have been produced from 17,000 crosses involving INGER entries and more than 300 varieties have been directly released in 60 countries. Thousands of unique breeding lines were distributed in 85 countries for the past three decades. This study aimed to determine the extent of genetic variation among INGER entries either directly released as varieties or utilized as parents in rice breeding programs worldwide. Forty-five breeding lines directly released as varieties and used to hybridize with locally-adapted germplasm in different countries were analyzed with 20 polymorphic SSR markers. The Dice coefficient was used to compute genetic similarity and clustering was based on unweighted pair group method with arithmetic mean (UPGMA). At 31% genetic similarity, two major branches were observed which roughly represented indica and japonica subspecies of rice. An average gene diversity of 0.60 and an average genetic relatedness of 0.077 were calculated, implying a fair degree of diversity in the germplasm assayed. The utilization of these lines in different countries may thus have contributed to the maintenance of diversity in the different breeding programs. Moreover, they also served as valuable sources of tolerance genes to various biotic and abiotic stresses.

### P3.76 - Assessment of genetic variation in an artichoke European collection by means of molecular markers

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The globe artichoke [*Cynara cardunculus* var. *sativa* Moris, syn.var. *scolymus* (L.) Fiori], is native of the European Mediterranean Basin. Italy (474,253 Mt), Spain (212,400 Mt) and France (50,662 Mt) are now the leading European countries for globe artichoke production. A core collection constituted of accessions from former germplasm collections present in Italy (Tuscia, ENEA, CNR IGV, CNR ISAFOM), France (GEVES), and Spain (ITGA) has been built up, thanks to the respective partners of the CYNARES European project (action 063 receives financial support from the European Commission, Directorate-General for Agriculture and Rural Development, under Council Regulation (EC) No 870/2004) which are acknowledged. The core collection include germplasm from all the four artichoke typologies, i.e. "Petit Violet de Provence", "Catanese", "Spinoso Sardo", and "Romanesco"; as well as cardoon, for a total of 134 accessions of artichoke and 14 for cardoon. The core collection has been analyzed at the morphological, biochemical and molecular level to assess the diversity present, to measure the genetic distance among accessions and to cluster them. Genomic DNA was extracted from 477 genotypes belonging to the artichoke germplasm collection, and 72 belonging to cardoon. Material was analysed using different molecular markers typologies (i.e., ISSR, SSR, AFLP). In present paper are reported the analyses, at the molecular level using four ISSR and four AFLP primers combination, carried out by the Tuscia University Unit. The data obtained from all markers were scored in 0-1 matrices for absence-presence of amplification bands. The matrices are used to detect genetic diversity within and between accessions, existence of private bands (i.e. band belonging to a single accession) and genetic distance among accessions. The different markers were able to detect different levels of polymorphism, in particular ISSR detected a lower variation within the populations and a higher variation between the populations. The majority of genetic diversity was for within accession rather than between accessions. Accessions were clustered based on their genetic distance; moreover, discriminant analysis was undertaken to detect the correct assignment of each genotype with respect to country of origin and artichoke typologies.

### P3.77 - Sugarbeet genetic and genomic resources for disease resistance in the western United States

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In 2009, 29,445,000 short tons of sugarbeet (*Beta vulgaris* L.) were grown on 1,172,900 acres in the United States, fulfilling 56% of the domestic sucrose production. Sucrose is used by drinks and processed food manufacturers and the pharmaceutical industry. Plant disease is a major constraint to sugarbeet production due, in part, to its narrow germplasm base. Sugarbeet has only been specifically selected and bred for sucrose production for the past two centuries, beginning from open-pollinated selections of fodder beets grown in Caulsdorff, Germany. The bulk of the United States germplasm is derived from this European material, probably from a limited number of introductions selected for curly top virus resistance and *Cercospora* leaf spot resistance beginning in the late 1920s. In the western United States, diseases caused by nematodes and fungal and viral pathogens are important. Sugarbeet's narrow germplasm base raises concerns about the ability to increase or simply sustain crop yield and quality in the face of such dynamic stresses. While host-plant resistance may be the only long-term, economical, and environmentally sound way to produce sugarbeet, most likely that resistance will be found outside the primary gene pool. The National Plant Germplasm System's Germplasm Resources Information Network (GRIN) database contains 454 *Beta vulgaris* subsp. *maritima* (Bvm) accessions from 22 different countries. Bvm is the only wild relative that will successfully cross with sugarbeet. Here we demonstrate how our project, by developing populations and genetic stocks for mapping qualitative and quantitative sources of disease resistance, evaluating wild and ancestral relatives of sugarbeet both phenotypically and genotypically for novel traits, and integrating genomic tools, is developing new germplasm with improved agronomic and quality characteristics and resistance to biotic and abiotic stresses.

### P3.78 - Identification of synthetic *cis*-acting DNA elements responsive to biotic phyto-stimuli by a combination of a functional *in vivo* assay, massive parallel sequencing and bioinformatics methodologies

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The identification of *cis*-acting DNA elements responsive to biotic/abiotic stresses are of importance both for basic gene regulation studies and for applied purposes. Delineation of *in vivo cis*-acting DNA elements is tedious and requires extensive functional dissection of promoter regions. We have developed a novel screening method that allows the isolation of synthetic *cis*-acting DNA elements based on their *in-vivo* activity in plant cell cultures and on their responsiveness to biotic as well as to abiotic stresses. This screening is coupled with Massive Parallel Sequencing and with a Bioinformatics Pipeline to analyse millions of sequence data. The novel screening method exploits the fact that a phosphorylated form of the RNA polymerase II (RNAPol-II) correlates with the transition from transcriptional initiation to elongation and mRNA capping. Using an available monoclonal antibody specific for the phosphorylated form of RNAPol-II, fragments of cross-linked RNAPol-II-chromatin, representing actively transcribed genes can be immunoprecipitated. Randomized oligonucleotides, an unbiased source of synthetic *cis*-acting DNA elements, were used to construct libraries linking the synthetic elements upstream of a minimal promoter driving luciferase gene expression. By this means, synthetic elements taking part in transcription were immunoprecipitated with the RNAPol-II antibody from a population of parsley protoplast transformed with the library of randomized synthetic elements following a PAMP (pathogen associated molecular pattern) treatment with Pep25, a *Phytophthora sojae*-derived peptide. Libraries and pools of chromatin immunoprecipitated synthetic elements containing putative PAMP-responsive DNA elements were sequenced using Solexa-Illumina technology. To identify known and novel synthetic *cis*-acting DNA elements involved in PAMP-mediated transcriptional activation, custom bioinformatics tools have been created to analyze the large sequencing data set. Selected synthetic *cis*-acting DNA elements are currently being validated in transfected protoplasts as well as in planta. Several of them show responsiveness to the applied biotic stress.



### P3.79 - Selection signatures during the independent domestications of common bean in the Andes and Mesoamerica

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The aim of our study was to identify genomic regions related to adaptive processes during domestication of the common bean *Phaseolus vulgaris*. *P. vulgaris* was domesticated independently in the Andes and in Mesoamerica, and thus the molecular signature of selection during domestication can be followed in two independent gene pools. We analyzed 183 wild and domesticated genotypes chosen to represent the evolutionary histories of *P. vulgaris*. We used mainly AFLPs, most of which genetically mapped in two recombinant inbred populations. Our data reflect the previous knowledge of the occurrence of two major wild gene pools of *P. vulgaris*, from which two independent domestication events originated. Departure from neutral expectation was estimated using four different  $F_{ST}$ -based methods for both the Andean and Mesoamerican gene pools. Mapped markers were classified according to their proximities to known genes and domestication QTLs. Our data indicate that a large fraction of the genome appears to have been subjected to the effects of selection during domestication, and, in most cases, that the same genomic regions were affected by selection during the two independent domestication events. An excess of inter-chromosomal linkage disequilibrium (LD) was found in the domesticated populations of both gene pools, which suggests an important role for epistatic selection during domestication of *P. vulgaris*. This role was confirmed by separately comparing the LD analysis for putative selected and putative neutral loci. Finally, our data highlight some key aspects of the evolutionary history of *P. vulgaris*, including the Mesoamerican origin of this species.

### P3.80 - Analysis of variability R-genes and resistance gene analogues (RGAs) in pea (*Pisum*)

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Pea (*Pisum sativum* L.) is the second most important food legume worldwide after common bean. Information about pea genome diversity especially in resistance genes is an important aspect for the design of optimal breeding strategies for continued progress in pea improvement.

For the first time a variability of resistance genes and their analogues (RGAs) in wild and cultivated pea varieties has been investigated by using the NBS profiling techniques (van der Linden et al., 2004), which is based on the usage of PCR primers designed to the conserved motives of the NBS domain of R-genes. Most fragments that are obtained by NBS technique originate from genes harboring the targeted domain; therefore, polymorphisms in the banding pattern are most likely to be associated with the function of the conserved motif. In the current study 120 wild and cultivated pea accessions presented *P. sativum* with different genome type (Ab, As, T, S), origins, pathogen resistance status and characters of responsiveness to arbuscular mycorrhizal fungi and bacteria from the Russian germplasm collection have been taken into analysis. As a result for each pea genotype unique RGA-specific patterns have been revealed. Groups of pea accessions/varieties with similar RGA profiles were determined. In total 171 polymorphic bands presumably associated with the pathogen resistance have been obtained. Based on the obtained RGA profiles the genetic distance indexes (GD) were calculated and the levels of genetic variability in an analyzed gene pool have been estimated. Rather high polymorphism levels of pea RGAs were shown. Unweighted pair-group method arithmetic average cluster analysis and principal coordinate analysis were performed that result in grouping of the cultivated varieties separately from the wild accessions. These data point out the potential of wild accession gene pool in pea breeding programs.

A number of variety-specific markers were identified in the current study, which could be useful for variety identification and source of resistance genes.

### P3.81 - Identification *Sus* gene homologues in pepper species

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Sucrose synthase is a key enzyme in plant sucrose catabolism. Sucrose synthase genes form a small gene family, which include 2-6 members, coding a number of isoforms with different expression patterns. In whole expression activity of *Sus* genes correlates with sugar import, cell wall synthesis, sink strength and abiotic stress resistance in different monocot and dicot plants.

In the present study pepper *Sus* gene fragment coding the main domain of sucrose synthase enzyme have been isolated from 11 wild and cultivated *Capsicum* species. Nucleotide and amino-acid sequence polymorphism have been characterized. The level of whole sequence variability among *Capsicum* species is 6.3%. The most differences concentrate in intron III (17.6%) and exon V (4.6%). In total, among *Capsicum* species 72 SNPs have been identified, 20 of them localized in coding sequences and only five of them result in amino acid substitutions. The transitions/transversions ratio in coding region is 3.3. In the most conservative intron V (0.8% SNPs) a 23-bp deletion is detected, which is specific to *C. cardenasii* and *C. pubescens* *Sus* homologues. Out of seven SNPs identified in *C. annuum* sequences, only one is located in the coding region and is specific to cultivar California Wonder. Comparison of *Sus* sequences reveals specific patterns of nucleotide polymorphisms not only for each of the analyzed pepper species but also for *Capsicum* phylogenetic complexes: *annuum*, *baccatum*, *pubescens*.

Out of five amino acid substitutions discriminating *Capsicum* species two are non-synonymous. One (E/K<sub>114</sub>) is specific for *C. pubescens* *Sus* homolog, and one (K/R<sub>188</sub>) –for *C. annuum* cultivar California Wonder. Other specific substitutions are detected in *C. galapagoense* (L/I<sub>70</sub>) and in *C. annuum*, *C. chinense* (K/R<sub>72</sub>) *Sus* homologous.

Identified polymorphism in *Sus* homologous may be useful for genotype identification of pepper species and cultivars.

### P3.82 - SPAR and SP-SSR marker-based genetic relatedness and interspecies diversity revealed in Acacias of western Rajasthan

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Insights into the relative genetic relatedness between the *Acacia* species can be useful for designing strategies for gene introgression and selective breeding programmes to produce desired recombinant hybrid genotypes with effective expression of quantitative trait loci (QTL). In this study we focused on the species which forms an important part of agroforestry of Rajasthan in order to analyze their molecular genetic relatedness. Genetic diversity of *Acacia* species of Western Rajasthan was assessed using SPAR and SP-SSR markers. Genetic similarity between *Acacia* species were evaluated on 121 SPAR, 113 SP-SSR and combined 234 (SPAR and SP-SSR) loci. Using 12 SPAR primers, 121 bands were produced with an average of 11 bands per primer. A total of 113 scorable bands were generated from 9 SP-SSR primers. Polymorphisms between *Acacia* species were high, with 84.85% for SPAR and 91.77% for SP-SSR. Resolving power is used as a parameter for primer selection. Out of 12 SPAR primer screened, SPAR-1, SPAR-4 and SPAR-5 had very high resolving powers. Similarly in reference to SP-SSR analysis, SP-SSR-9 and SP-SSR-1 depicted much higher value and could therefore potentially be used for identifying an *Acacia* species from any mixed population. The pairwise value of Jaccard's similarity coefficient ranged from 0.06024 to 0.47458 in case of SPAR, 0.1428571 to 0.48 in case of SP-SSR and 0.21469 to 0.50704 in case of combined (SPAR and SP-SSR) approach. The dendrogram generated using Unweighted Pair Group Method using Arithmetic Averages (UPGMA) from SPAR and SP-SSR grouped *Acacia nilotica* ssp. *indica* (A1), *Acacia tortilis* (Forsk.) (A3) and *Acacia jacquemontii* Benth, Will (A4) as a single major cluster whereas the *Acacia senegal* (L.) (A2) forming the second major cluster suggesting a close association between A1, A3 and A4. Our results have shown that statistical analyses of combined data were feasible in revealing molecular relationship among *Acacia* species.

### P3.83 - Exploitation of kiwi germplasm and use of genomic tools for the development of the new cultivar cv. Tsehelidis

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Kiwifruit is a newly commercialized fruit crop, native to southern China. It belongs to the genus "*Actinidia*", which includes over than 70 species, with a wide variation in growth habit, morphological traits and fruit characteristics. However, despite all that genetic variance available, the international market is dominated by a single cultivar, "Hayward" (*Actinidia deliciosa*). It becomes clear that such exclusive use of a single cultivar implies both possible hazards of genetic vulnerability, and more important poor utilization of the available genetic potential. Thus, the development of new cultivars, of high commercial value, is of crucial importance in kiwifruit.

"Tsehelidis" is a new kiwifruit cultivar of *Actinidia deliciosa* species. The cultivar was developed in northern Greece, after sporophytic selection in 'Hayward', supported by molecular assisted selection with RAPD markers. Thus, the new cultivar was developed from one seedling, selected for its plant vigor, growth habit and the large size of fruits. The variety was evaluated for its plant and fruit characteristics. The genetic identity of the new cultivar was determined using molecular DNA analysis, based on SSR molecular markers. The results of the analysis supported the uncontested differences between the two genotypes (Tsehelidis vs. Hayward), pointing out the utility of such markers in distinguishing different genotypes.

The new kiwi cultivar gets its official registration by C.P.V.O. (Nov. 2007) and becomes commercially available in Europe and South America. The agronomical behavior of cv. "Tsehelidis" is rather stable and exceeds in earliness, yield potential, fruit characteristics (size, weight) and physiochemical parameters, the cv. "Hayward". In case of this new kiwi cultivar cv. Tsehelidis, was proved that the use of genomic tools (SSR and RAPD markers) could help the effectiveness of selection but their medium accuracy sometimes could be proved very useful for included in selection, unpredictable and new desirable genetic events.

### P3.84 - Genetic structure of *Musa acuminata* populations in Sri Lanka revealed by SSR markers

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*Musa acuminata* is a wild ancestor of most cultivated banana and plantain. The species has great value for banana improvement programmes because of its rich genetic diversity and the presence of viable seeds. *Musa acuminata* occurs in areas of elevations from 179–1500 m in the forests in 'Knuckles Matale' (population 1) 'Knuckles Kandy' (population 2) and 'Saptha Kanya' and Yatiyantota (population 3) in Sri Lanka. Information on genetic diversity and genetic structure is of great value for the *in-situ* conservation, germplasm collection missions, setting up core collections, evaluation of germplasm and for banana breeding activities. SSR analysis was carried out using five primer pairs for samples representing the three populations. SSR loci visualized on silver stained 8-10% denaturing PAGE gels were analyzed using GenAEx 6 and SPSS 10. Percentages of polymorphic loci for populations 1, 2, and 3 were 20, 40, and 60%, respectively. The AMOVA resulted 19% of variance among and 81% within populations. Nei's Genetic distance increased along with the increased geographic distance. The mean Ho (0.2727) and mean He (0.1943) indicates that out-breeding occurs in *M. acuminata* populations. Bats and bees are the pollinators. However, the presence of few fruits in bunches of *M. acuminata* reflects that there is a barrier for cross pollination which limits the seed formation and thereby seed propagation. Lower level of allele migration rate ( $Nm = 0.784$ ) and higher  $F_{st}$  (0.242) was observed between distantly located populations 1 and 3 (aerial distance about 69 km) in contrast to the closely located (aerial distance about 24 km) populations 1 and 2 ( $Nm = 1.640$  and  $F_{st} = 0.132$ ).

Kaemmer, D., 1997. Molecular breeding in genus *Musa*: a strong case for SSR marker technology. *Euphytica* 96: 49-62.

Chandrarathna, M. F. 1951. The origin of cultivated races of banana. *Indian Journal of Genetics and Breeding* 11:29-33.

### P3.85 - Implementation of AFLP and SNPs to characterize the CrocusBank World Collection of Saffron and Crocus Genetic Resources, CROCUSBANK

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The World Collection of Saffron and Crocus Genetic Resources, CROCUSBANK, has been recently created (EU co-funded) with the aim to preserve, characterize, utilize and exploit the endangered saffron crop and its allies. The bank compiles saffron accessions and *Crocus* wild species from native and cultivation areas world wide. This work contributes to the molecular characterization of the bank, thus being applicable in identification of genetically linked characteristics (yield, drought and salt tolerance or disease resistance) and also in species or varieties determination for proper rationalization of the bank. AFLP and SNPs are the main sources of molecular markers. The AFLP methodology allowed us to discriminate between species but not between *C. sativus* from different origins. SNPs' inspection was done by genomic DNA high-fidelity PCR of a subset of *Crocus* accessions, using primers from 50 *C. sativus* ESTs derived from a stigma library. Forty primers generated specific and reproducible PCR products, which were cloned and sequenced. The average of candidate SNPs found in a single fragment ranged from 0 to 6, and only 1-2 per fragment were further assayed with TaqMan Genotyping probes in a broader set of accessions. This approach allowed us to evaluate markers present in *C. sativus* and a few of its wild *Crocus* relatives, despite the inherent difficulties to analyze a triploid genome and the generalized heterozygous state in many accessions. All together, these data have proven useful for the evaluation of the bank and it also provided additional evidence for the intriguing issue of determining the hybrid origin of *C. sativus*.

### P3.86 - Agronomic and molecular analysis of heterosis in alfalfa

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Double 'free-hybrids' (DH) in alfalfa, a polyploid, allogamous and perennial forage crop, were obtained by crossing in a diallelic scheme six simple hybrids (SH), multiplied for two generations (2S<sub>2</sub>Syn3), derived from four partly inbred (S<sub>2</sub>) constituents (Rotili et al., 1999). Specific Combining Ability (SCA) source of variation for dry matter yield (DMY) resulted highly significant and larger than General Combining Ability (GCA) component and supported heterosis values of DHs vs the best parent of +45% on average, ranging from +76 to +5%. The investigation at the molecular level was carried out by means of SSR marker analysis on the 6 parental SHs and the 15 DH progenies and by comparison of gene expression profiles of a single DH and the respective parental plants.

The parental genetic diversity, estimated by SSR markers, showed a significant relationship with heterosis and SCA effects ( $r = 0.70$  and  $0.76$ , respectively). The variation of heterozygosity estimates of the DHs explained a little part (about 20%) of their variation in DMY ( $r = 0.45$  n.s.) while the number of alleles was significantly related to DM performance ( $r = 0.61$   $P < 0.05$ ).

A microarray analysis of the transcriptome was conducted in a highly performing hybrid (DH) compared to its parents (2S<sub>2</sub>Syn3). T-test enabled to identify genes with additive/non additive (according to the nomenclature as reviewed in Hochholdinger and Hoecker, 2007) value of expression in the hybrid compared to the parents ( $P < 0.05$ , Benjamini and Hochberg False discovery rate). Q-PCR validated the level of expression from microarray analysis for a few selected genes. In our experiment, most of the variation in gene expression was additive (87%). Interestingly among the genes with non-additive pattern of expression the greater proportion of probe sets (86%) fell outside the parental range, namely above/below the level of expression of the high/low parent; among such genes ontology classes highly represented are metabolism and genetic information processing. Prevalent additive mode of gene expression has been observed in a few expression analysis of heterotic hybrid vs. parents in different organisms (maize, Arabidopsis).

Rotili P, 1999, [www.naaic.org/TAG/TAGpapers/rotili/rotili.html](http://www.naaic.org/TAG/TAGpapers/rotili/rotili.html).

### P3.87 - Genetic diversity among Italian melon *inodorus* (*Cucumis melo* L.) germplasm revealed by ISSR analysis and agronomic traits

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Melon (*Cucumis melo* L.) is an important horticultural crop in Italy. The *inodorus* melon landraces, commonly known as "winter melons" for their capability to be preserved during the winter time, are traditionally cultivated in the Mediterranean area and they have a relevant importance in the economy of the Italian southern regions. (Ficcadenti et al., 2007). A great difficulty in the cultivation of these genotypes is that they are often identically named in the same cultivation area determining the onset of homonymies and synonymies that produce confusion in the recognition of the same population (Sestili et al., 2008). The genetic relationships among 13 melon *inodorus* populations, collected from various parts of South Italy, were assessed by using 100 ISSR primers and 15 morphological traits. The dihaploid line (DH) Nad-1 and the cultivar Charentais-T, both belonging to the botanical variety *cantalupensis*, were employed as reference accessions in the molecular and phenotypic analysis. A total of 358 polymorphic bands obtained from 39 out of the 100 ISSR primers used and 15 phenotypic traits scored were utilized for genetic similarity (GS) calculation and cluster analysis. The cluster analysis (UPGMA) based on the ISSR data grouped the *inodorus* melon accessions into two clusters on the basis of the skin colour of fruits.

The similarities among the *inodorus* accessions used for the molecular analysis were also established on the basis of their phenotypic traits. The binary data matrix obtained was used to generate the dendrogram that grouped the accessions sharing similar agronomic features. In particular the genotypes were distinguished on the basis of their skin colour in accordance with the molecular data analysis.

The distance matrix obtained from the phenotypic data was further compared with the matrix obtained from the ISSR data. Mantel's test revealed a good correlation between morphological and molecular data in their ability to detect genetic relationships among melon ecotypes.

Results obtained confirm the efficacy of this approach and opens new perspectives to reveal a possible molecular association with the phenotypic traits analysed.

### P3.88 - Japonica rice mutants as a resource for plant genomics and breeding

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Rice breeding is an endless procedure of creating desirable haplotypes expressing better performances of agronomic traits. Even a certain desirable allele type having effective breeding value was evaluated in other subspecies or wild relatives, practical utilization of it for japonica also demands additional tedious breeding procedures to remove most deleterious linkage drags after crosses between the donor and recipients. Transgenic tech would be a possible option for alleviating that, technical and practical circumscriptions have been still remained. Under assumptions of 1) screening to find new favorable allele types would be conducted under the current cultivation circumstances, 2) identified useful alleles are supposed to be introduced into the current elite lines promptly, and 3) the alleles found would be potential materials for functional genomics by using advanced DNA marker systems as well as the rice pseudomolecules, high yielding japonica cultivar, Namilbyeon was treated with sodium azide and ethyl methanesulfonate, and now a total of 7,500 mutant lines were established at M8 generation. Excepting few lines showing extreme morphological and physiological characteristics, most of mutant lines are highly similar to their original cultivar. Through conducting several mass screenings, various mutant types were identified for grain shape, endosperm characteristics, and resistance to biotic and abiotic stresses. Those mutant lines are under intensive fine evaluations and field trials and also were subjected to be used as donor lines to improve other elite japonica cultivars as well as genetic studies to tag the target locus by using advanced biotech tools.



### P3.89 - Importance of marker-assisted selection for the improvement of genetic resources of grain legumes in India: An overview

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India is known as a center of diversity hosting various flora and fauna. Traditional farmers living in the country highly varied agro-ecological zones have developed various farming systems that are characterized by the high degree of inter- and intra-specific crop diversity across space and time. A wide range of crop diversity has been maintained by traditional farming societies in a sustainable way through the accumulated experience and interaction of farmers with their natural environment and without the need for technical scientific knowledge or external commercial inputs. The pulses are one of them and are the part of said cropping systems all over the country because these crops fit in well in the crop rotation and crop mixture followed. The pulse crops are widely grown in varying conditions with low and irregular yields. The grain legumes have a larger seed size with higher protein content than cereals but are lower yielding due to the lack of progress in grain legume improvement due to the lack of genetic variability, improper technological packages of improved high input seeds, inorganic fertilizers, pesticides and herbicides. Susceptibility of the legumes crops to various biotic and abiotic stresses, seriously eroding genetic diversity and restricted distribution of progenitors has limited yield potential of the grain legumes for years. Therefore, breeders dealing with grain legumes throughout the country and world focus on increasing yield potential by pyramiding genes for resistance/ tolerance into elite

germplasm through marker assisted breeding or markers assisted selection. The potential benefits of using markers linked to genes of interest in breeding programmes for grain legumes, thus moving from phenotype based towards genotype-based selection have been obvious for many decades. However, realization of this potential has been limited by the lack of markers. Plant disease resistance characters controlled by multiple genes have historically been difficult to study. The complex nature of host-parasite interactions, complicated further by several resistance loci, has made it extremely difficult to analyze oligogenic and polygenic disease resistance (Geiger and Heun, 1989). Gene number and the effect of heterosis have been examined for complex resistance to wheat leaf rust (*Puccinia recondita* f. sp. tritici) (Lee and Shaner, 1985), and the level of dominance involved in resistance to powdery mildew of barley (*Erysiphe graminis* f. sp. hordei) has been estimated (Jones et al., 1982). Nevertheless, these studies did not pinpoint specific genomic regions involved in resistance or characterize the effects of individual loci on disease response. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest, thus allowing MAS finally to become a reality.

Efforts to employ MAS have been initiated, however, the saturation of genomic areas of interest with markers, and their polymorphism in different genetic backgrounds; the mapping of resistance genes for biotic stresses not sited in current genomic maps which require the development of new RIL populations; the integration of genes or QTL controlling resistance already located in different genetic resources while simultaneously considering the importance of pathotype differentiation for the pathogen and mapping and integration into the

current genomic map of genes or QTL controlling tolerance to abiotic stresses such as drought, earliness, chilling, freezing and salinity, in addition to molecular markers tagging these genes is of prime importance. The genetic make-up and genome organization of related species of grain legumes is often sufficiently conserved, allowing alignments of the genomes. Genome alignment enables research communities to predict the presence of genes, build physical maps, and conduct comparative genome analysis among and between species. The recent genome sequencing of various organisms has enhanced the rate of new gene identification, annotation, and functional validation. Genome information available in the public domain has been used extensively in comparative genome studies with the help of bioinformatics tools. The implications of these technologies the challenges and projection, the particular functions of diversity in sustaining production, the need for conserving and utilization of landraces and marker assisted selection (MAS) through precision breeding play an imperative role in the improvement of grain legumes.



### P3.90 - SCAR markers of the *R*-genes and germplasm of wild *Solanum* species for breeding late blight-resistant potato cultivars

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Potato late blight (LB) caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is among the most important crop diseases. For several decades, breeding for LB resistance has heavily relied on germplasm introgression from wild *Solanum* species, primarily *S. demissum*, the source of race-specific resistance genes (*R*-genes). New pathogen races are known to rapidly defeat such resistance, and therefore the breeders search for new sources of durable resistance, such as *S. bulbocastanum* comprising *R*-genes of broad-spectrum specificity. Nonetheless, the presence of the SCAR marker for the major *demissum* gene *R1* in potato cultivars was associated with higher field indices of LB resistance (Gebhardt et al., 2004; Beketova et al., 2006). Such evidence corroborates the participation of race-specific *R*-genes in plant defense response (Stewart et al., 2003; Tan et al., 2008). We developed six SCAR markers recognizing the race-specific genes *R1* and *R3a* of *S. demissum* and *S. stoloniferum* and the broad-spectrum resistance gene *RB* of *S. bulbocastanum* and the germplasms of these species. The markers for *R*-genes in wild *Solanum* species and potato cultivars were also validated by sequencing. In addition to *S. demissum* and *S. stoloniferum*, homologues of *R1* and *R3a* were found in few accessions of *S. cardiophyllum*, *S. hougasii*, *S. iopetalum*, *S. pinnatisectum*, and *S. polytrichon*. In contrast, *RB* homologues were frequently found in these species. By screening wild *Solanum* species and potato accessions reportedly free from wild *Solanum* germplasm (Chilotanum forms and heirloom cultivars), we demonstrated that the markers reliably discerned between germplasms of *Solanum tuberosum* ssp. *tuberosum* and wild sources of LB resistance. Following introgression, only some markers of wild *Solanum* germplasm crossed several meiotic generations and were maintained at high frequencies in modern potato cultivars. Screening about 200 potato cultivars demonstrated that the *R1* marker was associated with field and laboratory indices of LB resistance (Mann-Whitney test) presuming that *R1* contributes to high LB resistance of potato cultivars; such relationship was less evident for the *R3a* marker. The described markers would help maintaining germplasm collections and promote breeding for LB resistance, especially when screening cross and backcross segregants. Supported by the ISTC-USDA-ARS project 3714p.

**P3.91 - Genetic resources of the fruit crops in southern Russia and opportunities of their utilization for breeding**

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The voluminous genetic fund of the fruit crops is concentrated in the south region of Russia. Collection of genetic resources includes about 12,000 genotypes. It consists of species and varieties belonging to genus: *Prunus* (ca. 7,000 accessions); *Malus* (ca. 3,000 accessions); *Pyrus* (ca. 1,200 accessions). The majority of the varieties belong to species *Prunus domestica*, *Prunus cerasifera*, *Prunus persica*, *Prunus armeniaca*, *Prunus avium*, *Prunus cerasus*, *Malus domestica*, *Pyrus communis*. Specific diversity is also presented in the large scale. There are classes of donors of different traits as well as traits complexes among represented species and varieties. Breeding collection includes intervarietal, interspecific hybrids, mutant forms, spontaneous and induced polyploids. There are about 17,000 advanced breeding forms among all accessions in breeding collection. The genetic stock material is used for development adaptive, technological and high quality varieties. The common directions of the fruit crops breeding in the south Russia is the breeding for low temperature tolerance during winter and spring period and drought and high temperature tolerance during summer period. Created varieties have traits mentioned above. Resistance to fungal and bacterial pathogens is current importance in the breeding for biotic stress resistance. The main breeding directions in this respect are following: for apple – resistance to *Venturia inaequalis*, *Podosphaera leucotricha*; for pear - resistance to *Venturia pirina* and *Erwinia amylovorum*; for cherry – resistance to *Monilia cinerea* and *Coccomyces hicmalis*; for plum – resistance to *Clasterosporium carpophilum*. We apply marker assisted selection to introduce scab resistance gene *Vf* in the breeding scheme for apple scab resistance, disease caused by *Venturia inaequalis*. DNA-analysis is also used to identify allelic polymorphism of apple self-incompatibility gene in the Russian apple germplasm. Besides that, study of genetic diversity of different fruit crops through SSR and ISSR markers is underway at present time. Molecular genetic investigations are supported by Russian Foundation for Basic Research (projects : 09-04-96552, 09-04-99139, 09-04-96601, 09-04-99134, 09-04-99126).

Large-scale introduction of genetic resources of fruit crops in the breeding gave opportunity to breed sets of different varieties suitable for intensive gardening.

### P3.92 - A high-throughput, cost effective, and all-stage DNA extraction protocol for sorghum (*Sorghum bicolor*)

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A new DNA extraction protocol has been optimized for field studies of sorghum. The protocol uses the Whatman FTA™ plant saver card and/or cards made of Whatman ordinary filter paper. Both types of cards were tested for extracting DNA from mature wild sorghum plants in the field and from seedlings in the greenhouse. This method was optimized for sampling wild sorghum populations in remote areas of Ethiopia for studies of genetic diversity at microsatellite loci. A solution eluted from a disc of 6mm diameter, punched from a well soaked card, is adequate to run up to 50 PCR amplifications. The resulting DNA was run on a 3% agarose gel stained with ethidium bromide and viewed under UV transilluminator. Both types of cards were equally effective for extracting genomic DNA for PCR-based analyses. Extracting genomic DNA from mature plants *in situ* is particularly attractive for sampling at sites that are far from the laboratory where samples are analyzed. Moreover, highly skilled personnel are not required to collect DNA samples using this protocol. The Whatman FTA™ card is much more expensive than the ordinary Whatman filter paper. Therefore, without compromising efficiency, the lower cost filter paper is recommended for DNA extraction, especially for institutions in developing countries.

### P3.93 - The use of protein markers to characterize and classify Iranian olive (*Olea europaea* L.) germplasm resources

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Assessment of genetic diversity of olive and study of genetic flux and introgression are the promised strategies in maintaining genetic resources of this plant.

In this study, olive seed storage proteins (SSPs) of different Iranian olive cultivars had been compared to elucidate their purity, diversity and homogeneous.

Olive SSPs are soluble in aqueous alcohol, with limited solubility in water and dilute salt. At present little is known about olive SSPs. However they will play an important role in future biotechnology. Protein extracted from the olive seeds were characterized by SDS-PAGE analysis and banding pattern was used to compare cultivars of different Iranian geographical areas. The clusters of several olive cultivars in more cases were overlapped for providing low diversity in protein allelic pattern. Native analysis and bi-dimensional electrophoresis provided more discriminative results. Simple Sequence Repeats analysis on the same cultivars is in progress with the aim of supporting achieved aforementioned data.

### P3.94 - The study of direct and indirect defenses of common bean cultivars against two-spotted spider mite (*Tetranychus urticae*)

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Two-spotted spider mite, *Tetranychus urticae*, is one of the most important pests of common bean. In order to study of direct and indirect defenses in different common bean genotypes, we evaluated resistance of 19 common bean genotypes (were obtained from the main Iranian common bean genetic resource) against the mite in greenhouse. We calculated a resistance index as  $1/xyz$  for each genotype that x, y and z are the mean number of mites, mean of number of eggs and damage score per leaf, respectively. Genotype that had the largest index was introduced as the most resistance genotype that is called Naz.

Then expression ratio of some defense genes that involve in indirect and direct defenses compared in Naz by quantitative real time PCR (QRT-PCR). The results of QRT-PCR exhibited that expression of Chitinase and for chitinase genes were increased but expression of S-adenosylmethionine (SAM) synthetase (SAMS) (Arimura et al., 2002. The Plant Journal, 29:87-98) and (E)- -ocimene synthesis (Arimura et al., 2004. Plant Physiology, 135:1976-1983) were down-regulated in the mite infested versus non-infested leaves of Naz. It concluded that Naz has good direct defense response but doesn't succeed in indirect defense.

### P3.95 - Assessment of genetic diversity among wormwood (*Artemisia austriaca*) germplasm from wild populations

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The main focus of this research was to analyze the data and describe the genetic diversity and similarity of wormwood by using randomly amplified polymorphic DNA (RAPD) technology. For this aim, we have collected wormwood from eight wild populations of the northeastern part of Turkey. This study provided a first detailed analysis of genetic diversity among wild wormwood populations. It has been determined high genetic polymorphism among studied wormwood accessions based on RAPD primers. Cluster analysis of the cornelian cherry genotypes was performed based on data from polymorphic RAPD bands, by using Jaccard's similarity coefficient and the unweighted pair-group method with arithmetic average (UPGMA) clustering method. The genetic relationship based on similarity degrees showed a clear linkage between genetic relations among accessions and their geographical regions of origin.

### P3.96 - *Avena* genetic resources phenotyping for *Fusarium* resistance

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In the cooperative project "Avena Genetic Resources for Quality in Human Consumption" (AVEQ) a wide oat germplasm collection have been evaluated for traits related to quality and safety in human consumption. More in details, a total of 652 oat accessions, including genebank materials belonging to primary and secondary gene pools and current commercial varieties, have been characterized for genetic variability in resistance to *Fusarium* spp. and to toxin accumulation. *Fusarium* head blight is not usually apparent on oats in the field, but high levels of mycotoxins can be observed. More information is necessary about the genetic variability for resistance to *Fusarium* spp. and toxin accumulation in wild and cultivated oats. In the frame of the AVEQ project, screening of oat genotypes is carried out under conditions of inoculation with selected *Fusarium* species at three sites across Europe (Czech Republic, Germany and Romania); at the fourth location, in Italy, mycotoxin content is evaluated after natural *Fusarium* infestation. At each location, 11 modern, currently grown oat varieties from different parts of Europe are used as standards. In the two years, 2008 and 2009, 323 and 329 entries were evaluated for phenotypic traits including the resistance to *Fusarium*. DON and T-2 toxin, two important *Fusarium* mycotoxins from trichothecene group, were chosen to be analysed in a selected set of 250 entries. DON is now regarded to be the most important *Fusarium* mycotoxin and its content in cereals for human consumption is controlled by the European law (Regulation (EC) 1881/2006). The mycotoxins T-2 and HT-2 are of special importance in oat because oat grain is often naturally infected by the *Fusarium* species producing T-2 and HT-2. Mycotoxins analyses are carried out in four laboratories using two methods, reference LC-MS/MS and immunochemical ELISA method. Moreover, qPCR based approaches are used for the identification and quantification of *Fusarium* species on a subset of oat samples. The generated data will be made available to the genetic resources community in the European *Avena* Database (EADB).

This project is funded by the European Commission, DG AGRI within the framework of Council Regulation 870/2004 and co-funded by Peter Koelln KGaA, Elmshorn, Germany, Emco Limited, Prague, Czech Republic, and Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP), Bonn, Germany.

**P3.97 - Allelic diversity of *Md-ACS1*, *Md-ACO1* and *Md-Exp7* genes of apple cultivars (*Malus × domestica* Borkh.)**

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Good storability and shelf life of apple fruits are importance commercial characteristic. The genes *Md-ACS1*, *Md-ACO1* and *Md-Exp7* are associated with fruit ethylene production and firmness. Allelic diversity of these genes was determined for 130 apple cultivars and hybrids growing in Belarus. The set of accessions included local and introduced as old and modern apple cultivars characterized by different storability and shelf life. The ACS1-2 homozygote producing lowest ethylene content was detected in the cultivars 'Alkmene', 'Discovery', 'Elise', 'Gala must', 'Otawa', 'Pinova', 'Rebristoye', 'Relinda' and 'Topaz'.

The ACO1-1/1 genotypes associated with lowest ethylene production were detected in accession X1924 and hybrid *M. sieboldii* 25/175. No cultivars were detected to be homozygous for both ACS1-2 and ACO1-1. A total of 41 tested genotypes contained the combination 198/202 of Md-Exp7<sub>SSR</sub> markers, which is favourable one for apple breeding. The complemented alleles 212, 210, 206 and 200 bp were found for Md-Exp7<sub>SSR</sub> marker among old and modern cultivars. However, alleles associated as low and high ethylene production were detected in cultivars as with short and long shelf life. Some tested cultivars having long shelf life even did not contain the favourable alleles of the *Md-ACS1*, *Md-ACO1* and *Md-Exp7* genes. This is the evidence of presence other factors, which have essential impact on shelf life of apple cultivars tested. The results of genotyping apple cultivars can be use in marker-assisted selection for breeding apple cultivars for better storability and long shelf life.

**P3.98 - Molecular marker-based genetic diversity analysis of kumquat (*Fortunella* spp.) species**

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Kumquats (*Fortunella* spp.) are species of subtribe Citrinae tribe Citreae subfamily Aurantioideae family Rutaceae. They are widely cultivated for ornamental purposes with small and attractive fruits indoor or garden use. These species are member of 'true citrus fruit trees' group as Citrus relatives. In this study we designated of genetic diversity of five kumquat species using sequence related amplified polymorphism (SRAP) markers. Twenty-one primer combinations were used and 116 bands were obtained and 62 of them were polymorphic. Band numbers per primer combinations varied from 3 to 11. Average polymorphism information content (PIC) value determined as 0.31 for primers. Data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 2000). The unweighted pair group method arithmetic average (UPGMA) analysis demonstrated that the accessions had a similarity range from 0.69 to 1.00. Round kumquat (*Fortunella japonica*) were the most distant species with similarity value of 0.69. Genetic variation for the remaining four species were very low and three of them were identical. Our results showed that kumquats have a low level of genetic diversity except for round kumquat.

Rohlf, F.J. 2000. NTSYS-pc, numerical taxonomy and multivariate analysis system, version 2.11. New York, Exeter, Setauket.



### P3.99 - Molecular genotyping of genetic resources: Anonymous vs. targeted markers

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Genomic variation affecting traits of adaptive significance can be considered as the prime diversity to optimize in genetic resources collections. However, which of the currently available genotyping methods is the most appropriate to estimate this variation remains an unanswered question. Ideally, information should be generated from many functional genomic regions that collectively constitute a representative sample from the total expressed DNA. Genetic diversity in a selected set of 80 *Lactuca* accessions was estimated using the anonymous marker systems SSR, AFLP and SSAP, and the targeted marker systems SRAP, TRAP and NBS profiling. The accessions were also described morphologically, and all characterization methods were evaluated against the genetic diversity assessed by a panel of three crop experts. The morphological data appeared weakly associated with the molecular data, and did not outperform the molecular data in relation to the expert-based assessments. In comparison with the anonymous markers, no added value was observed for the targeted markers when marker performance was related to the expert-based assessments. It could be concluded that markers that are targeted to specific gene sequences may still behave as anonymous markers if their relationship with the phenotypic variation has not been established. Furthermore, the type of marker system appeared irrelevant at low taxonomic levels if a clear genetic structure is absent, for example as a result of intensive breeding activities.

### P3.100 - Water stress in *Beta vulgaris*: Gene expression and metabolic profiling of ssp. *vulgaris* vs. ssp. *maritima*

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*Beta* is an economically important genus, but it also comprises wild subspecies such as *B. vulgaris* ssp. *maritima*, source of agronomically important traits. "*maritima*" germplasm includes accessions that were shown to have mechanisms of escape from water shortage (e.g. osmotic adjustment) more effective than the cultivated *B. vulgaris* ssp. *vulgaris*.

An experiment was carried out comparing, under rain shelters, the response to a progressive water shortage in two subspecies of *Beta*. Water limitation was applied to adult plants of both accessions, in such a way that the osmotic potential at the end of the season was different in the stressed (av. -2.0 MPa) and control plants (av. -1.4 MPa). During the onset of water shortage, periodic samplings of leaf tissue from control and stressed plants of the two accessions were carried out, and osmotic potential and relative water content measured, as it was the soil water potential. At the end of the season, root samples were also recovered. From all samples, the levels of soluble sugars was measured by HPLC-ELSD, and gene expression of key enzymes of the sugar metabolism and of the response to drought were determined by qPCR.

In HPLC-analysed samples from individual roots after 4 months, as expected, we found that sucrose concentration was 40% of dry weight. The HPLC profiles appeared more complex both in the roots and in leaves of *B. maritima* compared to *B. vulgaris*.

In the leaves, the results show that choline monoxygenase and sucrose synthase 1 (mainly controlling sucrose cleavage) are expressed at constitutively higher levels in *B. maritima* than in *B. vulgaris*, while no differences between the subspecies were recorded for sucrose phosphate synthase 2, the enzyme mainly involved in sucrose synthesis in sugarbeet.

In the *B. maritima* leaves, the sucrose synthase 1 appeared to be up-regulated simultaneously with an increased glucose concentration detected at the latest stages of growth, in stressed conditions.

In the roots of *B. vulgaris*, the gene for choline monoxygenase 1 was found to be strongly up-regulated at the later stages of growth in stressed plants compared to control ones.



### P3.101 - Assessing the genetic variability of grape clones

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Grapevine (*Vitis vinifera* L.) is a long-living and woody plant grown worldwide. Its perennial nature and vegetative propagation over long periods favours accumulation and fixing of mutations within individual genotypes, which might exhibit altered phenotypes. Clonal selection, as a procedure of crop improvement, takes advantage of the identification of sports with agronomically and enologically important traits, which are vegetatively propagated to raise new grape clones. Given the high economical value, as reflected by related patenting and legal issues, their identification is of great relevance. While cultivar identification in grapevines is traditionally based on ampelographic descriptors and on microsatellite (SSR) profiles, clone discrimination is not possible with such tools. To allow true genetic identification of clones, new specific molecular markers have to be implemented. Given the availability of the Pinot Noir (clone ENTAV 115) genome sequence, it is timely to use techniques exploiting the polymorphism information of unique coding and non-coding regions along with approaches based on specific genomic sequences of interest, such as DNA transposons and retrotransposons. Transposable elements (TEs), which possess the capability to change their genomic location, are indeed potential source of mutations leading to clonal variation.

In this study we focus on the application of two genome sequence based approaches, SNPlex™ Genotyping System and Transposon Display, to fathom clonal genetic variability within six wine grape cultivars. We have analysed the state of 573 putative (electronic) SNPs, identified in coding and non-coding regions of the mentioned grape genome, in 141 genotypes. This sample set refers to three biological replicates (plants) of 47 clones (both registered and biotypes) belonging to the Pinot Noir, Pinot Gris, Pinot Blanc, Meunier, Teroldego and Gewürztraminer cultivars. The same set of clones was tested using 17 primers targeting specific regions (LTRs, LTR downstream or upstream, ORF) of six different TE families. Here we report preliminary results of the identified polymorphisms enabling molecular characterization of clones within international and local grape varieties.

### P3.102 - Adaptive changes in pearl millet landraces in Niger revealed by the genetic comparison of their *ex situ* and on-farm genetic resources

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Cultivation of Pearl millet in West Africa is still mainly traditional. During the past decades, important human and climatic changes have occurred in Sahelian countries. In Niger, the cultivated area and human population have doubled in 25 years. Moreover isohyete 400 mm has moved southwards by 200 km in the west and by 100 km in the east of the country. The impact of these changes on genetic diversity and adaptation of pearl millet landraces is still unknown. In this study we analyzed samples of pearl millet landraces collected in the same villages in 1976 (*ex situ* collections) and 2003 (on-farm collections) throughout the entire cultivated area of Niger. The diversity assessed with microsatellite loci did not display significant changes between the 1976 and 2003 collections. We tested the change in allele frequency at the flowering locus *PHYC*. The results suggested a positive selection for the earliness allele, which was consistent with the phenological trends towards earliness observed between the 1976 and 2003 collections by comparing them over three crop seasons in a common garden experiment. In the context of a changing climate, the shortening of the life cycle is an adaptive change in landraces that can cope with climatic change by flowering earlier in drier environments. This study shows how genomics-based diversity studies boost the usefulness of *ex situ* collections of crop genetic resources as baselines to monitor the dynamics of evolutionary changes in agroecosystems, and to identify traits and loci involved in adaptation to changing environmental conditions which can then be targeted by varietal improvement programs.

### P3.103 - Genes associated with multiple disease resistance identified through a multivariate mixed model association genetic analysis

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Plants are attacked by pathogens representing diverse taxonomic groups, such that genes providing multiple disease resistance (MDR) would likely be under positive selection pressure. We examined the hypothesis that there is a pleiotropic genetic basis for MDR in maize in the context of naturally occurring genetic variation. To do so, we extended structured association genetic mapping to a multivariate statistical framework. We found high positive genetic correlations between resistances to three different diseases in a public panel of diverse maize inbred lines with linkage disequilibrium that decays over very short physical distances (sometimes within genes). The positive correlations suggested that functional allelic variation at specific genes for MDR do exist in plants. We also used the multivariate approach to test multitrait-marker associations and identified glutathione *S*-transferase as a putative MDR gene. The gene's documented general role in cytoprotection, including defense against pathogen infection, provided biological plausibility for the association in terms of shared aspects of pathogenesis for the pathogens studied. Glutathione *S*-transferases and other proteins involved in detoxification are potentially an important component of quantitative variation in disease resistance.

**P3.104 - Development of an efficient genetic framework for studying genomic responses to artificial selection**

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Response to selection is fundamental to plant breeding, prompting the development of a range of techniques for population improvement of crop species. To gain insight into the genetic basis of responses to artificial selection, we are developing a novel genetic analysis approach, using pre-existing or custom-made recurrently selected populations, to simultaneously map loci controlling specific traits associated with population improvement and characterize the allele frequency response at those loci. Using maize as our model system for combining association and selection mapping methods, we are investigating the genomic response to selection for quantitative disease resistance (three generations of selection) as well as the adaptation of a tropical population to a temperate environment (ten generations of selection). The concept of our approach and current results for both populations under study will be presented. Our aim is to provide a new experimental approach for the analysis of quantitative traits applicable to many crop species to gain a broader and deeper understanding of genomic responses to selection.

**P3.105 - Application of chloroplast simple sequence repeats (cpSSRs) in identifying cytoplasm diversity in *Brassica* and its close relatives**

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Cytoplasmic genome consist of chloroplast and mitochondrion genome, in which many functional genes, eg. photosynthesis and fertility, are located. However, plastid genome tend to have low mutation rates, as a result limited cytoplasmic variation are detectable at sub-species levels. Broadening genetic diversity in cytoplasmic genome of *Brassica* crop species is thus necessary for better yield and quality. The family Cruciferae includes approximately 340 genera and 3350 species, of which the model plant *Arabidopsis thaliana* and domesticated species such as radish, mustard, and the *Brassica* crops are perhaps most familiar. *Brassica napus*, *B. rapa*, *B. juncea* and *B. carinata* provide about 12% of the worldwide edible vegetable oil supply. The diversity exist in the family Cruciferae provide a good base for exploiting germplasm with diverse cytoplasm for breeding of crop Brassicas. To identify and screen a set of genetic resources of diverse cytoplasm, 57 accessions of Crucifer germplasm belong to two tribes, seven genera and 13 species were selected for analysis of cytoplasmic genetic diversity, 19 primer pairs of chloroplast simple sequence repeats (cpSSRs) were used to assess the genetic variation of chloroplast genome, a total of 87 alleles were generated in the experiment, and 30 different haplotypes were detected among the 57 accessions tested. Phylogenetic relationship among the 57 materials were confirmed. High level of cytoplasmic genetic diversity were detected at inter-tribe, inter-genus and inter-species levels. And different level of intra-specific variation were found in the four major crops species, in *B. napus* and *B. oleracea*, only one haplotype was identified respectively, in *B. juncea*, two haplotypes were verified, the highest diversity was found in *B. rapa*, four haplotypes were detected. The results lay a good foundation for exchange utilization of crucifer germplasm with diverse cytoplasm.

### P3.106 - "Genomics for Agricultural Innovation" - Japan's national research project for utilization of genomics in innovative crop improvement

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The world population is estimated to reach 9 billion in the middle of the 21<sup>st</sup> century and climate change induced by global warming will widely affect food production that may lead to severe food shortage. Therefore, breeding of cereal crops with higher yield and adaptation to a hostile environment is crucial in maintaining a stable world food supply.

In the last 20 years, the extensive Rice Genome Research Program led by the National Institute of Agrobiological Sciences (NIAS) has provided the foundation for understanding the structure and function of the rice genome. More importantly, it has paved the way for the successful decoding of the entire rice genome in 2004 by an international sequencing consortium led by Japan. Subsequently, many agronomically important genes associated with high yield and resistance to both biotic and abiotic stresses were identified.

The "Genomics for Agricultural Innovation" project was launched in 2008 by the Ministry of Agriculture, Forestry and Fisheries (MAFF) in order to focus on practical utilization of the fruits of extensive rice genome analysis for agricultural purposes and to serve as a core program for improving cereal crops through the advancement of basic and applied biotechnologies for finding useful genes, identifying gene functions, controlling gene expression, and introducing novel genes into widely cultivated varieties. To enrich our understanding of many agronomically important traits based on the genomic information of rice and other cereals, wide range of researches are being undertaken. The project consists of 16 individual research programs from basic technology such as bioinformatics and development of research resources, to applied breeding technology such as production of novel crop varieties using transgenic approach or marker assisted selection. These programs are organically integrated in order to attain maximum results that will provide new insights for effective application of genomics in innovative crop improvement programs. With this project, we hope to make a significant contribution in the worldwide efforts to develop novel cereal crops that may solve the problems associated with food shortage as well as those associated with the environment and energy.

### P3.107 - Application of molecular based PCR markers for development of hybrid rice

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The wild abortive cytoplasmic male sterility (CMS-WA) system, an ideal type of sporophytic CMS in indica rice, is used for the large-scale commercial production of hybrid rice. Searching for restorer genes is a good approach when phenotyping is very time-consuming and requires the determination of spiklet sterility in testcross progeny. So in 60 Iranian local rice cultivars and lines, two STS makers, RG140(linked with  $Rf_3$ ) digested with *EcoRI* locus on chromosome 1 and S10019(linked with  $Rf_4$ ) digested with *FnuDII* locus on chromosome 10 and one SSR marker, RM6344 that is linked to  $Rf_1$  locus on chromosome 7 were used to screen restorer lines. After all the other Microsatellite markers (RM1108, RM258, RM171 that are linked to  $Rf_4$  locus on chromosome 10, RM443 and RM1 that are linked to  $Rf_3$  locus on chromosome 1) were used to confirm the previous results. The results of these primers confirmed the last results and studying the first three primers were more suitable than the others, especially two first STS markers. Molecular analysis of these three primers, compared with sterility phenotyping test. The results showed that 5 lines were without any fertility restorer genes that are useful for producing the cytoplasmic male sterility lines. Meanwhile 6 lines were fertile in phenotyping test, and compare with molecular data, these lines had all three fertility restorer genes( $Rf_1$ ,  $Rf_3$  &  $Rf_4$ ). So these lines can be used as restorer lines in hybrid seed production. But the other lines with less than %85 field fertility that had two or one of these restorer genes can not be used as restorers in fact.

### P3.108 - Molecular characterization of 187 pomegranate genotypes by SRAP

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There are different types and forms of pomegranates in Turkey because it is located in its native spreading areas. As the result of several selection programs many promising genotypes have been chosen. The selections have been examined in Aegean and Mediterranean ecological conditions where they originated. Alata Horticultural Research Institute has one of largest pomegranate genetic resources in Turkey. This collection contains 187 pomegranate genotypes. The collection consists of Mediterranean, Aegean, South Eastern, and Bitlis region genotypes, and one US, one Turkmenistan and two Spanish cultivars. The aim of this study was to characterize 187 pomegranate genotypes by SRAP markers. SRAP molecular analysis with 15 primers generated a total of 80 reproducible bands; 20% of which were polymorphic. The results indicate a low level of genetic diversity present amongst the pomegranate genotypes.

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### P3.109 - Factorial and genetic dissection of rice grain yield using a rice mini core collection

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Grain yield is composed of several complex traits, such as panicle number, grain number and grain size. Using a representative rice mini core collection, which contained 300 cultivated rice varieties (*Oryza sativa* L.) and retained 70% of the genetic variation in the whole rice germplasm resources all over the world, we investigated the morphological and genetic architecture of rice yield. Seven yield traits including panicle number, grain number, 1,000-grain weight, panicle length, number of primary branch, grain length and grain width were evaluated in five trials (one in Hainan in 2006, two in Hainan in 2009, and two in Beijing in 2009). Three principal yield traits, panicle number, grain number and grain size, took on different correlations under different environments. The morphologically factorial architecture of seven traits was established through the structural equation modeling provided in amos7.0 of SPSS. GLM with population structure in TASSEL revealed that 11-57 SSR markers were significantly associated with each of seven yield traits (with  $P < 0.001$ ).  $R^2$  for majority of them (about 67%) is higher than 10%, with the maximum of 34%. Only one marker associated with grain width in all five trials, and approximately half of the markers could be detected in more than two trials for panicle length, grain length and grain width; however, majority of the markers associated with panicle number, number of primary branch and 1,000-grain weight could be detected only in one trial. Based on a multifactor dimensionality reduction method and the structural equation modeling, we revealed a complex genetic network controlling rice yield and each yield trait, and investigated its interaction with environment. Finally, several genomic regions were labeled as the potential resources to identify genes involved in rice yield; and a set of materials with novel high-yield genotypes were screened as the potential parents to develop super-rice.

# *Posters*

## *Session 4*

### *Genomics of Triticeae genetic resources*





#### P4.01 - On the road to a high-density genetic linkage map of wheat chromosome 5A

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A high density genetic map is needed for anchoring BAC contigs during the construction of a physical map and for DNA sequence assembly. The International Wheat Genome Sequencing Consortium is dedicated to the development of physical maps of individual chromosomes as the first step towards the whole genome sequencing of hexaploid wheat. To undertake this challenge for wheat chromosome 5A, we rely on two mapping populations and different parallel approaches for marker development. A Chinese Spring x Renan (CSxR) F2 population is being used while an F2 population derived from CS x CS-*Triticum dicoccoides* disomic substitution line for chromosome 5A is under creation. For marker development, a Diversity Array Technology (DArT) platform containing about 4,000 new probes specific for the short and long arms of wheat chromosome 5A has been established using DNA from flow sorted chromosomes, also including more than 6,000 non-chromosome specific wheat probes. Besides the DArTs, a set of SSR (chosen from databases and literature), specific for 5AS and/or 5AL chromosome arms, have been selected. Sequencing and annotation has been undertaken for sorted DNA of 5AL and 5AS. To date the one-fold coverage aided in thousand of SSR and ISBP marker development. After the assignment to chromosome 5A, performed using CS deletion and aneuploid lines, the markers are being tested for polymorphism between the parents of the two mapping populations. Polymorphic DNA fragments, specific for 5A, have been mapped in CS x R population. The resulting genetic linkage map of the wheat chromosome 5A will be presented.

#### P4.02 - Molecular characterization of an Old Portuguese durum wheat collection using IRAP markers

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The Inter-Retrotransposon Amplified Polymorphism (IRAP) methodology (Kalendar et al. 1999) has been reported as a feasible tool for DNA fingerprinting. Recently, this methodology proved to be useful for assessing the diversity and for the establishment of genetic relationships among Old Portuguese bread wheat cultivars (Carvalho et al. 2010). Additionally, IRAP has been used for evolutionary studies, DNA fingerprinting, genetic mapping linkage and detection of rearrangements induced by polyploidisation (Kalendar et al. 1999; Bento et al. 2008). In the present study, we aimed to use IRAP markers for the molecular characterization of 51 Old Portuguese durum wheat cultivars, in order to infer about their genetic diversity. The IRAP markers were achieved with five combinations of LTR primers, which produced a total of 93 bands, 61 of them being polymorphic, resulting in 65.59% IRAP polymorphism. On average, 18.6 IRAP bands were amplified per combination of LTR primers. The bands ranged in size from 450 to 3100 bp. The 51 durum wheat cultivars belong to the species *Triticum turgidum* L. subsp. *turgidum* (syn. *T. turgidum* L.) and *Triticum turgidum* subsp. *durum* (syn. *T. durum* (Desf.) Husnot, and were classified as belonging to 27 botanical varieties. However, IRAP markers failed in the clustering of cultivars by the species or botanical variety criteria, since cultivars from different species or different infraspecific *taxa* shared common IRAP patterns. Nonetheless, this study showed that IRAP markers could be reliable for assessing genetic variability at the individual level, being useful for determining the relationships among genotypes, providing useful information for future wheat breeding programs.

A. Carvalho et al., (2010) Annals of Applied Biology ISSN 0003-4746. doi:10.1111/j.1744-7348.2010.00390.x (*In Press*)

M. Bento et al., (2008) PLoS ONE 3, e1402. DOI:10.1371/journal.pone.0001402.

R. Kalendar et al., (1999) Theoretic and Applied Genetics 98:704–711.

Work supported by PTDC/AGR-GPL/65876/2006 project financed by the Portuguese Foundation for Science and Technology (FCT).

#### P4.03 - Specificity of wheat and rye microsatellite markers in triticale

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Microsatellite markers available for wheat and rye can serve as references in the process of construction of genetic maps of triticale. However, SSR markers differ in respect to number and distribution of detected loci thus particularity in intraspecific transfer may require prior validation studies to verify genome specificity. Homeology of wheat, rye and triticale genomes, can provide a source of additional variability leading to improper annotation of linkage groups formed during process of mapping. In order to avoid these problems we found it necessary to analyze the allelic variability of 102 SSR markers in groups of random genotypes of wheat, rye and triticale. The research material consisted of 15, 6, and 6 cultivars of triticale, rye, and wheat, respectively. Markers were tested on pooled DNA samples gathered from 30 plants per genotype. PCR products were analyzed on denatured polyacrylamide gel with silver staining. We obtained 582 polymorphic bands that clearly distinguished rye, wheat and triticale on dendrogram according to taxonomic division. Rye genotypes were characterized by the greatest internal similarity (Dice similarity of 0.667), while higher variability was observed in wheat (0.614). Triticale constituted the largest group with the highest genetic variation (0.484). The analysis on restricted genotypes including parents of mapping population allowed us to select the reference markers for each of the seven chromosomes of genomes A, B and R, with the exception of chromosome 1R. SSR markers developed for rye generated polymorphism in A, B and D wheat genomes and vice versa. A total of 40% of wheat SSRs from series *barc*, *wmc* and *wms* amplified additional alleles from rye genome. Homoplasmy played a minor role (3%) in the transferability of information within wheat, rye, and triticale.

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#### P4.04 - Segmental chromosomal duplications harbouring group IV *CONSTANS*-like genes in cereals: Implications on cereal evolution and domestication

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Due to the large genomes of barley and wheat, map-based cloning remains an expensive and time-consuming prospect. Accordingly, comparative genetic approaches are routinely used, predominantly utilizing the sequenced genomes of rice (and more recently, *Brachypodium distachyon*), for development of genetic markers and establishing candidate genes for traits of interest in cereal crops. Understanding the structural organisation of grass genomes has implications for the effective utilization of comparative approaches in map-based cloning. Using published genomic sequence and genetic mapping, we investigate a region of the intra-specific chromosomal duplication between rice chromosomes Os3 and Os10, and their colinear relationships with brachypodium chromosomes Bd1 and Bd3, and the barley/wheat group 4 and 1 chromosomes. We show these regions contain segmentally duplicated *CONSTANS*-like (*CO*-like) genes in barley, one of which is known to underlie the collinear series of *VRN-2* flowering-time loci in temperate crops. Although *CO*-like genes are not present at collinear regions in rice and brachypodium, we show that by taking into account intra-genomic duplication, comparative genetics could have been used to successfully highlight a *CO*-like gene as a candidate during the cloning of *VRN-A<sup>m</sup>2* in *Triticum monococcum*. Furthermore, this work highlights how the differential evolutionary fate of members of the segmentally duplicated *CO* group IV genes has had profound effects on grass evolution: presence of *ZCCT* genes has resulted in the evolution/retention of vernalization requirement loci in many temperate cereals, while absence of this locus is associated with a lack of vernalization requirement in rice and brachypodium accession BD21. After the divergence of the Oryzoideae and Pooideae, complete deletion of *ZCCT* genes in barley contributed to the domestication of spring-sown types that lack a vernalization response, while deletion of *Os*/in rice has been shown to be associated with its spread into temperate agri-environments. The effects of differential retention/deletion of *CO*-like genes on the post-domestication spread of barley and rice, highlights the importance of improved characterisation of the 'dispensable genome' within cereals. The continued development of an integrated model describing the structure of grass genomes that takes into account chromosomal duplication and intra-specific variation, will provide an increasingly detailed inter- and intra-genome cross-referencing framework with which to dissect agronomic traits.

#### P4.05 - Protein disulfide isomerase family in wheat: Genomic structure, synteny conservation and phylogenetic analysis

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The Protein Disulfide Isomerase (PDI) gene family encodes several PDI and PDI-like proteins containing thioredoxin domains and controlling diversified metabolic functions, including disulfide bond formation and isomerization during protein folding. Genomic, cDNA and promoter sequences of the three homoeologous genes encoding the "typical" PDI had been cloned and characterized in a previous study. The purpose of the present research was the cloning and characterization of the genes encoding PDI- and PDI-like proteins in bread wheat and the comparison of their genomic structure with those of homologous genes isolated in other plant species. Fourteen wheat cDNA sequences of PDI-like genes were amplified and cloned; eight of them were relative to distinct PDI-like genes, whereas six corresponded to homoeologous sequences. Also the genomic sequences of the eight non-homoeologous genes were amplified and cloned. Phylogenetic analyses, which included the eight PDI-like genes cloned in this research and the typical PDI gene, assign at least one of them to each of the eight major clades identified in the phylogenetic tree of the PDI gene family of plants. Although not probable, the presence of additional wheat genes of the PDI family can not be ruled out. The genes of the wheat PDI family were located in chromosome regions syntenic with the chromosome locations of their rice homologs, confirming their close syntenic relationships. Within the same phylogenetic group a high level of conservation, in terms of sequence homology, genomic structure and domain organization, was detected between the wheat sequences and those of the compared plant species. Phylogenetic analysis showed that the complete set of PDI and PDI-like genes was already present in *P. patens* and that extended phenomena of duplication events have characterized the evolution of this gene family in different plant taxa. The comparison of the exon/intron structure showed a very similar genomic organization across the analysed species, including *P. patens*, whereas the alga *C. reinhardtii* showed a different intron/exon structure. The high level of conservation of sequence and genomic organization within the PDI gene family, even between distant plant species, might be ascribed to the key metabolic roles of their protein products.

#### P4.06 - Protein Disulphide Isomerase (PDI) promoter sequence analysis of *Triticum uratu*, *Aegilops speltooides* and *Aegilops tauschii*

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Protein disulphide isomerase is an abundant oxidoreductase enzyme in the lumen of the endoplasmic reticulum accomplishing several metabolic functions, the most important of which consists in disulfide bond formation and isomerization during the folding of secretory proteins. In hexaploid wheat cultivar Chinese Spring (AABBDD) the genomic, cDNA and promoter sequences of the three homoeologous gene encoding PDI had been cloned and characterized in a previous study (Ciaffi et al., 2006) revealing high levels of conservation. The identity ranged between 96.0 and 97.5% in the ORFs, between 94.5 and 92.0% in the genomic sequences while the three putative promoter regions showed an overall identity of 89%. The promoter sequences of the three homoeologous PDI genes possessed some regulatory motifs typical of genes with endosperm specific expression and consistent with their putative regulatory function. The purpose of the present research was the characterization of the variability in a 700 bp region comprising 600 bp of 5' upstream putative promoter region and 100 bp of the first exon of the typical PDI gene in the diploid wheat progenitor. In particular eight plants per accession, five accessions each from *Triticum uratu* (AA), *Aegilops speltooides* (BB) and *Aegilops tauschii* (DD), from diverse origins of Middle East countries were analyzed and compared to the available sequences from cultivar Chinese Spring. Comparative analysis of sequences indicated large variation among species, close similarity within each species and overall conservation of regulatory motifs conferring the endosperm specific expression. The highest percentage of similarity was observed between the sequence located on chromosome 4D of CS and those isolated from the *Aegilops tauschii* (DD) progenitor.

#### P4.07 - Identification, molecular-genetic and physical mapping of genes that control the inflorescence development in bread wheat (*T. aestivum* L.)

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Spike morphology is influential in the processes of pollination and seed formation, and thus plays a role in the determination of grain yield. Identification and characterization of genes involved in these processes will add to our understanding of how spike development and architecture is regulated. The wheat spike normally bears one spikelet per rachis node, and the appearance of supernumerary spikelets (SS) is rare. The locus responsible for the 'multirow spike' or MRS trait in wheat was mapped by genotyping F<sub>2</sub> populations with microsatellite markers. The MRS trait is under the control of a recessive allele at a single locus. The *Mrs1* locus is located on chromosome 2DS, co-segregating with the microsatellite locus *Xwmc453*. The placement of flanking microsatellite loci into chromosome deletion bin 2DS-5 (FL 0.47–1.0) delimited the physical location of *Mrs1* to the distal half of chromosome arm 2DS, within the gene rich region 2S0.8. Use the COS - SSCP approach (Quraishi et al., 2009) and *in silico* mapping of sequenced RFLPs linked to the mutant locus allowed us to identify the syntenic chromosomal region on the rice chromosome 7. The region hosts the *Fzp* (*FRIZZY PANICLE*) gene, whose mutation altered the identity of the spikelet meristem, causing inflorescence branching (Chuck et al. 2002). The wheat orthologues of the gene were isolated. Allelic relationships of *mrs* and other genes determined the SS/branched spike" trait were tested using bread wheat material of different origin. The practical importance of the MRS spike is that it produces more spikelets per spike, and thereby enhances the sink capacity of wheat, which is believed to limit the yield potential of the crop.

#### P4.08 - Genetic variation of naked barley (*Hordeum vulgare* L.) assessed by biochemical and molecular markers

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The genetic variation existing in 63 naked barley (*Hordeum vulgare* L.) accessions originating from ICARDA was investigated using random amplified polymorphic DNA (RAPD), monomeric prolamins and hordein polymorphism. The RAPD-based genetic similarity ranged from 0.221 to 0.81, with the mean of 0.481. Cluster analysis based on Jaccard Similarity Coefficient divided genotypes into eight different groups. In the analysis of the hordeins, no polymorphism was observed in the area D hordein. However, 10 patterns in the area C hordein, and 13 patterns in the area B hordein were observed; and in total 32 bands and 32 patterns were observed. The average genetic diversity index for these proteins was calculated as H = 0.856. In the analysis of the monomeric prolamins, which was performed with the Acid-PAGE method, 15, 9, 24, and 20 patterns were observed for the , , , and areas, respectively. The average of the genetic diversity index for these proteins was H=0.889, and, totally 33 bands as well as 57 patterns were observed. The average of genetic diversity index for RAPDs and storage proteins were compared and showed that mean of genetic diversity index was lesser for RAPDs than storage proteins. With regard to the fact that monomeric prolamins enjoy a greater diversity than hordeins and RAPD markers and are more powerful in identifying samples and regarding the simple, in addition to the low cost of conducting the analysis, it can be used in a variety of genetic studies such as genetic diversity assessment, identifying genotypes and determining the phylogenetic relations in naked barley.

#### P4.09 - An overlooked cause of seed degradation underlines the need to capitalize on genomics

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Divergent selection for yield in the absence of interplant competition within the green revolution soft wheat cultivar Siete Cerros ended in high and low yielding lines which were compared in RCB trials under: pure stand, mixed stand with S. Cerros, and absence of competition, allowing the estimation and establishment of a high significant negative correlation ( $r = -0.94$ ) between yielding and competitive ability. This correlation, also confirmed in trials within the local barley landrace cv. Athenaida, is the cause of at least two detrimental effects revealed under dense stand. The first effect translates to a diminished ability to select for high yield on a single plant basis. The second is the gradual seed degradation under dense stand due to the proliferation of high competitors at the expense of high yielders. Genomic analysis of seeds from both high competitors and high yielders will reveal the molecular basis of seed degradation, a process which is shown to: (1) necessitate maintenance breeding, (2) provide an additional explanation for the practice of the "inexplicable" seed replacement among traditional farmers, (3) render indispensable the practice of "nonstop" selection, and (4) shed further light on issues of genetic resource preservation.

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#### P4.10 - An incremental approach for association mapping of useful traits in barley - The EXBARDIV Project

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We are working in barley towards establishing an incremental association mapping approach based on different population types for the discovery of new gene alleles in wild and/or landrace barley, which can be exploited for crop breeding. Our approach builds upon the strong genomics base of barley and applies association genetics concepts pioneered in humans and *Arabidopsis* to test the efficiency of the association genetics approach for identifying gene alleles in *Hordeum* that are needed by the breeder. The results of our first year's analysis for grain weight (1,000-kernel weight) will be presented.



#### P4.11 - Evaluation of near isogenic lines for heterotic QTL in maize

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Heterosis, or hybrid superiority, has been extensively exploited in maize (*Zea mays* L.), but its genetic basis is not fully understood yet. As a contribution to its study, we undertook a long term research aimed at providing a framework of comprehensive quantitative trait locus (QTL) phenotyping. We first applied a combined QTL analysis following a North Carolina III (NCIII) mating design on genetic materials originated from single cross B73 × H99. For agronomic traits, several heterotic QTL were detected which were characterized by dominant or overdominant gene action, whereas non-allelic interaction proved to be of minor importance. For six of those heterotic QTL, pairs of NILs differing only for alleles at QTL flanking markers were produced, starting from Residual Heterozygous Lines (RHL). The present study was aimed at validating QTL additive and dominance effects for complex traits, as grain yield and its components, and at getting inside into their reactivity to different genetic backgrounds and to mildly stressful environmental conditions. NILs were thus evaluated (i) as near isogenic triplets (the two members of a NIL pair and their cross), (ii) as crosses with the related inbred lines H99 and B73, and (iii) as crosses with unrelated testers. The results showed that: (i) QTL effects were in accordance with the effects detected in previous analyses, (ii) effects were consistent in different genetic backgrounds and inbreeding level, and (iii) heterotic effects varied depending on stress conditions and (iv) were more pronounced for complex traits.

#### P4.12 - Towards the construction of an interspecific introgression line library in einkorn using *Triticum urartu*

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Among *Triticum* species two different A<sup>u</sup> and A<sup>m</sup> genomes derived from *Triticum urartu* and *Triticum monococcum*, respectively, were recognized. A<sup>u</sup> and A<sup>m</sup> genomes share similar chromosomal organization, notwithstanding, significant molecular divergences have been assessed using sequence data and AFLP markers, supporting chimeric A<sup>u</sup>/A<sup>m</sup> chromosomes as a tool to dissect their genetic differences.

Interspecific introgression line (IL) libraries have been used extensively in plants as their power to detect QTLs with small effects is higher compared to mapping populations having whole genome fragments segregating. In addition, interspecific ILs are a valuable tool for adding foreign DNA in "elite" varieties to improve their agronomic traits. Sterility as well as low levels of viability can affect the development of interspecific ILs despite that, they were successfully obtained for tomato, watermelon and barley.

Offsprings obtained from crosses between *T. urartu* and *T. monococcum* were reported to be sterile, but rare fertile F<sub>1</sub> plants were obtained by crossing the "elite" einkorn accession L118 with *T. urartu* ID388. From these fertile genotypes, ILs were developed backcrossing F<sub>1</sub> hybrid lines for several times with *T. monococcum* L118 as recurrent parent. Hundreds of mapped codominant markers in einkorn are currently used to characterize the exotic DNA fragment in each IL and to construct a panel of ILs carrying overlapping chromosome fragments of *T. urartu*.

As *T. monococcum* L118 and *T. urartu* ID388 differ in the grain content of Zn, Ca, carotenoids, tocopherols as well as in other important agronomic traits, the panel of ILs will be a valuable tool to gain insights on the key loci controlling micronutrient content of kernel in diploid wheats.

The phenotyping of the IL panel for some important traits associated with micronutrient content in kernel as well as statistical analyses to identify contingent or transgressive QTLs are currently underway.

#### P4.13 - Study of *glutamine synthetase* genes through a “linkage disequilibrium analysis” in a collection of wheat germplasm

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Seed storage proteins are directly related to the nutritional and technological value of the derived products. Several studies have attested the key-role of the glutamine synthetase enzyme in plant nitrogen metabolism. *Glutamine synthetase* gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutammate, essential compounds in aminoacid-biosynthetic pathway. High protein content is a very important quantitative trait controlled by several genes located on wheat chromosomes. *Glutamine synthetase* genes are located on the homeologous chromosomes 2A, 2B, and 2D where several authors reported major QTL for protein content. The goal of the present study was to assess the linkage between *GS* gene and the QTL for protein content through a linkage disequilibrium analysis in a collection of 75 wheat germplasm evaluated for grain protein content in two different environments. For this purpose, 10 marker were designed on the bases of the nucleotide sequence of *glutamine synthetase* gene acc. DQ124214 available in public data bases (<http://www.ncbi.nlm.nih.gov/>). The primers were physically mapped using the nulli-tetrasomic and ditelosomic lines of Chinese Spring, and a stock of 58 deletion lines dividing the A and B genome chromosomes in 94 bins. The two couple of primers named “GS2” and GS-46 mapping of chromosomes 2A and 2B identifying the *glutamine synthetase* gene were selected and analysed. The analysis carried on a collection of 75 different wheat genotypes identified three alleles for the marker GS2 and four different alleles for the marker GS-46. The regression analysis carried out between markers and grain protein content character showed a positive significant correlation with the allele of 173 bp ( $P < 0.03$ ) related with GS2 marker and a negative significant correlation with the alleles of 480 bp ( $P < 0.02$ ) detected with the marker GS-46. The present study represents the first step for the identification and sequencing of GS2 alleles increasing grain protein content, which could be employed in breeding programs aimed to increase grain protein content commercial cultivars. Moreover, *Gs-B2* and *Gs-A4* represents functional markers that could be also efficiently used in marker assisted selection (MAS) programs and map-based cloning.

#### P4.14 - Genetic and environmental factors affecting grain hardness in cultivated and wild tetraploid wheat

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Exaploid common wheat (*Triticum aestivum*) cultivars can be classified into three hardness classes based on their SKCS (Single Kernel Characterization System) values, i.e. soft (SKCS index = 15-50), medium hard (55-70) and hard (71-95). In tetraploid durum wheat (*T. turgidum* ssp. *durum*) kernels are characterized by extra-hard texture with SKCS index > 80 due to the absence of puroindolines, encoded by the *Pina-D1* and *Pinb-D1* loci on the short arm of chromosome 5D and claimed to be the principal determinant factor of endosperm texture. Kernel hardness is a main determinant in end product quality because of its strong effects on milling condition, flour and semolina yield, granularity of flour, starch granule integrity and water absorption. Variation in kernel hardness amongst common wheat cultivars are largely assignable to allele composition at *Pina-D1* and *Pinb-D1* loci and puroindoline sequence variation controls the majority of wheat grain texture variability. Nevertheless, cultivars of durum wheat also vary measurably in grain hardness. Here this important characteristic of wheat grain is analyzed in 240 wild and cultivated tetraploid genotypes (*T. turgidum* ssp.) grown in southern Italy in 2007-08 and 2008-09. Quality analysis were performed on mature kernels from two replications. Protein content of kernels ground on a Cyclotec 1093 mill (0.5 mm screen) was determined by the microkjeldahl method (Nx5.7), and expressed on a constant 12% moisture basis. Grain hardness was estimated by the SKCS 4100 using a sample of 300 kernels, with values ranging from 40 to 95. Each sample was analyzed for 1000-seed weight as well. The objective is to identify regions of the genome that contribute to affect tetraploid wheat-endosperm texture in a QTL and association mapping analysis.



#### P4.15 - Establishing the barley EST DNA repair database (bEST-DRD) as a tool for identification of barley genes involved in genome maintainance

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The biological impact of any DNA mutagenic agent used for the creation of genetic diversity is a combined function of the chemical nature of the induced lesions and the efficiency and accuracy of their repair. Although much has been learned from microbes and mammals about both the repair of DNA damage and the biological effects of the persistence of these lesions, much remains to be learned about the mechanism of repair in plants. Recently performed studies conducted with the use of bioinformatic tools enabled outlining the list of genes participating in different pathways of DNA damage repair in *Arabidopsis thaliana*, however information regarding DNA repair mechanisms in crop plants is very limited. Arabidopsis sequences involved in DNA repair, identified so far, serve as a basis for retrieval of ESTs collected in other species databases in order to identify homologous genes. In the presented work barley EST DNA repair database – bEST-DRD was established ([www.best.us.edu.pl](http://www.best.us.edu.pl)) where sequences of 128 Arabidopsis sequences (together with encoded polypeptides) are used as queries for browsing barley EST repository. Deposited barley ESTs are derived and compiled from three databases TIGR, The IPK Crop EST (CR-EST) and Computational Biology and Functional Genomics Laboratory. The database contains more than 3000 annotated alignments and is used as a tool for identification of two barley coding sequences *UvrD* and *MLH1*, involved in excision repair and mismatch repair, respectively. The database will soon be enriched with 61 Arabidopsis genes encoding factors participating in DNA replication, which will be used as queries. Correspondingly, barley ESTs repository will also be enlarged to encompass sequences sharing similarity with Arabidopsis genes. This will enable the use of bEST-DRD database as a tool for identification of barley sequences involved in DNA metabolism.

#### P4.16 - Altitude cline of variation in Ethiopian barley landraces identified by molecular analysis

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To determine the level and pattern of genetic variation in barley (*Hordem vulgare* L.) landraces from North Shewa zone, in the central highlands of Ethiopia, the genetic variability at seven nuclear microsatellite loci was examined. Analysis was carried out on a total of 106 landrace populations sampled in two growing seasons (Meher and Belg, the long and short rainy season, respectively), across three districts (Ankober, Mojanawadera and Tarmaber) and, within each district, all along an altitudinal gradient (from 1798 to 3324 m a.s.l). Genetic variation has been ascribed to differences between altitudinal classes ( $F_{ST} = 0.10$ ) more than between seasons or among districts ( $F_{ST} = 0.02$ ). The most relevant outcome of the experiment is that altitude level largely overrides geographical distance as main cause of divergence among individual plants. Moreover, results also suggest that the patterns of clinal variation among districts and seasons are inconsistent with a simple model drift and dispersal (seed exchange). They suggested instead a role for historical patterns of colonization, or, alternatively, present-day selective forces acting on some of the SSR analysed.

#### P4.17 - Three single nucleotide polymorphisms (SNPs) in the *Wx-B1* gene are associated with increased grain yield and total starch content in hexaploid wheat (*Triticum aestivum* L.)

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The biosynthesis of starch is the major determinant of yield in wheat. Starch is composed of approximately 75% amylopectin and 25% amylose. The *Waxy* (*Wx*) gene encodes granule-bound starch synthase I (*waxy* protein) for amylose production. Three *Wx* loci, *Wx-A1*, *Wx-B1* and *Wx-D1*, are located on chromosome arms 7AS, 4AL (translocated from 7BS) and 7DS, respectively. The genomic sequences of the three *Wx* genes consist of 11 exons and 10 introns. Association mapping based on sequences from the first intron to the ninth intron of the *Wx-B1* gene indicated that the nucleotide T at a SNP (C/T) in the sixth exon was significantly associated with increased grain weight per plant and thousand kernel weight and total starch content in two spring and five winter cultivars. The T allele explained about 16.8, 13.1, and 10.9% of phenotypic variation for increasing grain weight per plant, thousand-kernel weight and total starch content, respectively, in a population of 68 wheat genotypes that originated from Canada, USA, Germany and UK. However, this SNP did not cause an amino acid substitution. To further investigate the cause of the association between the SNP and thousand kernel weight, new primers were designed from wheat ESTs to amplify the promoter region and the first exon of the *Wx-B1* gene. Sequence analysis showed that three SNPs, T/C in the promoter region, G/A in the first exon, and C/T in the sixth exon, formed a distinct haplotype (C-A-T) and displayed a very strong linkage disequilibrium. The G/A SNP in the first exon led to an amino acid substitution from Alanine (GCC) to Threonine (ACC) at the first position of N-terminal sequence of mature *Wx-B1* protein. High-throughput, cost-effective and co-dominant SNP markers were developed using temperature-switch PCR (TSP) and can be used for marker-assisted selection of high grain yield and starch content lines in the wheat breeding programs.

#### P4.18 - Potential of nuclear and cytoplasm variability in *Hordeum chilense* for wheat breeding

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*Hordeum chilense* Roem. Et Schultz. is a diploid wild barley native to Chile and Argentina. The high crossability of this species with other members of the Triticeae tribe promoted the development of the new species × Tritordeum Ascherson et Graebner (Martin & Sánchez-Monge Laguna, 1982). Hexaploid tritordeum was developed from the hybrid derived from the cross between *H. chilense* (used as female parent) and durum wheat. However, the interest of *H. chilense* is based in the presence of traits potentially useful for wheat breeding, including high endosperm carotenoid content (Atienza *et al.*, 2004; Atienza *et al.*, 2007a; Atienza *et al.*, 2007b) or septoria tritici blotch resistance. Besides, the variability at cytoplasm level is also important in this species. The development of common wheat-*H. chilense* alloplasmic lines (nucleus from wheat cytoplasm from *H. chilense*) results in both fertile and male sterile genotypes, depending of the accession donating the cytoplasm (Atienza *et al.*, 2007c; Martin *et al.*, 2008a; Martin *et al.*, 2008b; Martin *et al.*, 2009). Furthermore, these alloplasmic lines constitute an ideal system for deepening our knowledge on nuclear-cytoplasm interactions (Atienza *et al.*, 2007c; Atienza *et al.*, 2008).

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#### P4.19 - Genetic markers based on 'Angela-like' subfamily of LTR retrotransposon *BARE-1* in barley (*Hordeum* sp.) and their applications

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The genome of barley (*Hordeum vulgare*) contains a large proportion of repetitive DNA, primarily retrotransposons, which are becoming increasingly well characterized in the recent sequencing efforts. The highly multiplex genetic marker system that utilizes retrotransposon insertional polymorphism – the Sequence-Specific Amplification Polymorphism (SSAP) method – was also originally developed in barley (Vaugh et al., Mol Gen Genet 1997). The SSAP procedure relies on autoradiography or fluorescent detection of PCR products, allowing the analysis of complex and high-copy retrotransposon families like *BARE-1*, which occupies more than 12% of barley genome (Manninen and Schulman, Plant Mol Biol 1993; Wicker et al., The Plant J 2009).

We have developed a modified SSAP procedure for barley which utilizes the insertion site polymorphism of a certain subfamily of *BARE-1* with a 5' LTR (long terminal repeat) sequence similar to *Angela* retroelement in wheat. Due to more limited presence of this subfamily in barley genome compared to the total *BARE-1* population, it is possible to obtain 20-30 clearly distinguishable PCR products in a SSAP reaction with five random selective bases added to 3'-end of LTR primer and to separate the PCR products in denaturing polyacrilamide gels with silver staining or via agarose electrophoresis. The number of PCR products obtained in a single reaction varies in different *Hordeum* species: 20-30 in *H. vulgare* and *H. spontaneum*, about 50-80 in *H. bulbosum* (4x) and *H. murinum* (6x), about 20 in *H. chilense*. If different selective bases are used, the number of *Angela*-like SSAP markers in each species can theoretically reach several thousands. The proportion of polymorphic bands revealed by our marker system in F<sub>2</sub> mapping population "Multiple Dominant Marker line 'DM' (Wolfe and Franckowiak, Barley Genet Newslett 1990) x Dzhau Kabutak" was over 55%. We have also tested the ability of SSAP marker system to distinguish the closely related malting barley cultivars with similar spectra of seed storage proteins (hordeins). The possible practical applications of retrotransposon-based markers are discussed.

#### P4.20 - Molecular and bioinformatic analysis of mutations induced by MNU in barley genome

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M<sub>2</sub> population of barley (*Hordeum vulgare*) obtained after mutagenic treatment with N-methyl-N-nitrosourea (MNU) was used in the study. Doubled haploid line 'H930-36' was applied for mutagenic treatment to avoid heterogeneity, often observed in cultivars. Chemical mutagenesis was conducted with employment of three doses of MNU: 0.5; 1.0 and 1.5 mM/3h. The scanning of the whole genome for amplified fragment length polymorphism (AFLP) was applied to estimate the frequency of DNA changes induced by MNU. Two restriction enzymes: *EcoRI* and *MseI* and seven primer combinations were used. In all employed doses of MNU, M<sub>2</sub> plants with changes in AFLP profiles were identified. The AFLP fragment sizes ranged from approximately 50 to 500 bp. In total, 6,821,000 bp for 920 M<sub>2</sub> plants obtained after MNU treatment were scanned. Assuming that each polymorphic band (65 total) results from a single nucleotide change, the frequency of mutations induced in barley genome after treatment with MNU was established as 9.5 for 1 Mbp or 1 mutation per 105 kbp. The longest polymorphic AFLP bands (32 in total) were extracted from the polyacrilamide gels, cloned in pGEM-T Easy Vector and sequenced. The majority (94%) of bands were heterogenic, with 2-8 different AFLP products present in one amplicon. The polymorphic AFLP products were characterized in terms of their similarity to the records deposited in databases: GenBank, EMBL and DDBJ using program BLASTN. For the majority of AFLP products, the annotations in databases were found, with the repeated sequences (54%) and among them LTR retrotransposons (36.7%) being the most frequent types. The coding sequences (5.1%) and gene flanking sequences (11.2%) were also identified among polymorphic AFLP products. The bioinformatic analysis demonstrated that MNU-induced mutations can be found in all types of barley sequences and AFLP markers scan the whole barley genome. The types of sequences in which mutations were identified reflected the organization of barley genome.

#### P4.21 - Comparison of genetic maps of durum wheat using SSR and DARt markers

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The construction of genetic maps based on molecular markers represents the first step for the dissection of genetic basis of complex traits and for the identification of closely associated molecular markers useful to transfer the favourable alleles into elite cultivars by MAS programs.

A number of technologies are available to increase the abundance of DNA markers and contribute to developing high resolution genetic maps suitable for genetic analysis. Diversity array technology (DARt) allows simultaneous typing of several hundred polymorphic loci spread over a genome without any previous sequence information about these loci. Hundreds of DARt markers have been mapped in bread wheat, by using anchor markers as SSR, and more recently a dedicated DARt genotyping platform has been produced for durum wheat.

In this work, the comparison between two intervarietal genetic maps of durum wheat based on SSR and DARt markers is reported. The two maps were developed on two RIL populations derived from the crosses Creso x Pedroso and Ofanto x Cappelli. A total of 609 loci were utilized to assemble 24 linkage groups, giving a total map length of 1673.6 cM for the Creso x Pedroso map, while 639 loci were assigned to 31 linkage groups which formed the Ofanto x Cappelli map, with a total length of 2210.9 cM. A total of 716 individual DARt markers were identified in the two genetic maps, with about 30% of markers in common. The comparison of the two maps revealed common and peculiar features in terms of marker order, clustering of DARt markers and segregation distortion. The Creso x Pedroso and Ofanto x Cappelli maps allowed to determine the chromosomal location about 300 DARt markers in respect to SSR markers. Map data obtained in this work were also compared with those available in literature in order to study the locus-specificity and stability of DARt versus microsatellite markers.

#### P4.22 - LTR retrotransposon family *Jeli* provides multiple genetic markers for the A genome of common wheat (*T. aestivum*)

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The sequence-specific amplified polymorphism (SSAP) technique (Vaughn et al., 1997, Mol Gen Genet, 253, 687-694) designed to analyze the insertion polymorphism of high copy number LTR (Long Terminal Repeat) retrotransposons in plant genomes can be used as a powerful tool of experimental genomics that can be applied to molecular mapping, marker-assisted selection, diversity analysis and evolutionary studies.

We have studied the distribution of retrotransposon insertions belonging to *gypsy*-like family *Jeli* in the common wheat (*Triticum aestivum* L.) genome using nullisomic-tetrasomic chromosome substitution lines obtained from common wheat cv. Chinese Spring (Sears, 1954, Missouri Agr Exp Res Bull, 572,1-59). In total, 168 SSAP bands representing the insertion sites of *Jeli* were scored and assigned to the individual wheat chromosomes. The insertions were mostly found in A genome (71% of bands scored), with substantially lower quantities in B genome (18%) and in D genome (11%). We have also mapped the polymorphic *Jeli* insertion sites in the recombinant inbred lines (RIL) mapping population Opata85 x Synthetic W-7984. The mapping experiments demonstrated about the same general distribution of *Jeli* between three wheat genomes as the nulli-tetrasomic analysis. No tight clustering of *Jeli* was observed, although some chromosomes contained a higher number of *Jeli* polymorphic insertions than the others. *Jeli* family, therefore, can be considered an A-genome specific dispersed repeat sequence. Such an unequal distribution of the retrotransposon in wheat genomes can be explained by a burst of *Jeli* amplification in the diploid A genome donor (*Triticum urartu* Thum. ex Gandil.), with a lower retrotransposition activity in B and D genome donors. The SSAP system based on *Jeli* family can be used for a targeted analysis of A genome in evolutionary studies, genetic mapping and polymorphism screening. It can also be a promising A genome identification tag when used as a probe in fluorescent *in situ* hybridization (FISH).

#### P4.23 - HRM technology for the identification and characterization of INDEL e SNPs mutations in genes involved in drought and salt tolerance of durum wheat

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Salinity stress is one of the major factors inhibiting cereal yield throughout the world. Tolerance to salinity stress can be considered to contain three main components: Na<sup>+</sup> exclusion, tolerance to Na<sup>+</sup> in the tissues and osmotic tolerance. Therefore, plants have developed a complex and elaborate signaling network that ensures their adaptation to salinity. Understanding these mechanisms is of fundamental importance for breeding programs. Recently, numerous transcription factors, able to considerably enhance tolerance for salt and drought stresses, have been identified. Conserved portions of DREB1, DREB2, AP2/EREB, WRKY and HKT1 transcription factors (TFs) have been utilized, through multi-alignments in different species of wheat, rice and *Arabidopsis*, to design specific primers to obtain amplification fragments not longer than 100 bp. High Resolution Melting (HRM) technology represents one of most recent and powerful tools for SNPs and INDEL mutations analysis. Here, HRM technology has been employed to detect the presence of SNPs and INDEL mutations into the TFs in durum wheat cultivars differently tolerant to salt and drought stresses. Seeds of Cham I (moderately salt tolerant), Jennah Khetifa (salt tolerant), Belikh 2 (moderately salt tolerant) and Trinakria (salt susceptible) accessions have been germinated in hydroponic solution containing CaSO<sub>4</sub> 10 mM. After germination, plants have been grown with a nutritive solution containing micro- and macro-elements and Fe<sup>++</sup> for 7 days. After which, NaCl at different concentrations, 0 M (as a negative control), 0.75 M and 1.5 M, has been added to the nutritive solutions. RNA has been extracted from root and leaf materials, converted in cDNA used in HRM amplifications. By the analysis of the different profiles of resulting melting curves, some amplification products have been chosen, sequenced and aligned with the corresponding sequences present in genes databases in order to identify and characterize potential SNPs and INDEL mutations.

#### P4.24 - Morphological, yield and molecular characterization of an Old Portuguese bread wheat collection

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Characterization of wheat genetic resources such as old cultivars or landraces is an important feature for breeding purposes. In this study, we characterized an Old Portuguese bread wheat collection composed by 48 cultivars, belonging to nine botanical varieties, using morphological, yield and molecular markers. Eleven morphological and yield characters (length of the main culm, length of the main spike, length of the first and second internodes, number of tillers, number of spikelets in the main spike, number of seeds in the main spike, weight of the seeds of the main spike, number of seeds per spikelet, number of seeds of the secondary spikes and weight of the seeds of the secondary spikes), were evaluated in a total of 480 plants. Molecular characterization was performed with RAPDs on 48 individuals.

Morphological and yield characterization showed high variability, as revealed by the statistical significant differences detected for all characteristics among populations. High positive correlations were detected between number of seeds from secondary spikes and weight of seeds from secondary spikes (0.806); number of seeds and number of spikelets from main spike (0.695), and between number and weight of seeds of the main spike (0.659), revealing the high yield potential of these populations. The RAPD markers also revealed that these populations presented a high genetic variability. Nine RAPD primers (OPA15, OPA17, OPA20, OPE12, OPE18, OPE20, OPH7, OPH12 and OPH14) produced a total of 118 bands, 84 of them being polymorphic (71.32% RAPD polymorphism). Five unique bands were also amplified. An UPGMA dendrogram of genetic similarity based on RAPDs was constructed. The Jaccard coefficient ranged from 0.61 to 0.91, indicating high genetic similarity among cultivars despite their morphological variability. The cultivars were clustered in three main groups, with Group 1 being exclusively constituted by the four cultivars from the botanical variety *millurum*. In general, this Old Portuguese bread wheat collection constitutes an interesting germplasm repository to our country and worldwide and showed potential to be used in further wheat breeding purposes.

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#### P4.25 - Simple Sequence Repeats (SSRs) banding patterns representing core collection of *Hordeum vulgare* germplasm

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Redundant materials in germplasm cause great hurdles in their management. To alleviate such discrepancies, present study was conducted to evaluate similarities of the simple sequence repeats (SSRs) banding patterns for 404 *Hordeum vulgare* landraces collected from Pakistan, India, Iran, Nepal, Iraq, Turkmenistan, Uzbekistan and Kazakhstan. In total, 50 alleles were detected for seven chromosomes of barley using one simple sequence repeats marker specifically for one chromosome. Number of alleles observed was highest for Bmag0023 (11) followed by HVLOX (9), HVM54 (8), Bmag0382 (7), Bmag0500 (5), Bmag0490 (5) and HVID (5).

Similarities in the banding patterns for SSR markers was determined using Jaccard's similarity index for each barley landrace which showed 42 patterns for 404 barley landraces including 14 unique and 28 frequent patterns. Their frequencies were observed in different regions in the order of Pakistan > India > Nepal > Turkmenistan > Iraq > Iran > Uzbekistan > Kazakhstan. Un-weighted Pair Group Method with Arithmetic mean (UPGMA) clustering clustered them into two groups (group A and group B) while Principal Component Analysis (PCA) grouped them into three groups. The overall results revealed that differentiation of patterns with UPGMA clustering and PCA was in accordance with the distribution of geographic regions. Furthermore, it was concluded that similarities in the banding patterns of SSR markers was observed useful strategy to reduce redundancies in the germplasm and develop core collection for future gene bank management.

#### P4.26 - Genetic distances revealed by morphological characters, storage proteins and RAPD markers in wheat doubled haploids

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Genetic diversity among 102 doubled haploid wheat (*Triticum aestivum* L) accessions originating from CIMMYT was investigated using morphological traits, gliadin patterns and random amplified polymorphic DNA (RAPD) variation. Among the morphological traits under study, the highest amount of diversity was related to yield of grain per plant, number of total tillers and number of fertile tillers. Principle components analysis and cluster analysis for morphological traits could effectively classify the samples. Based on these analyses, three genotypes with maximum yield and the related traits were determined. In the analysis of gliadins, 48 bands and 47 different patterns were detected. The average of genetic diversity index for these proteins was calculated as H=0.75. The mean of genetic diversity index was more for RAPDs than gliadins (H=0.83). Although during statistical reviews one pattern in the  $\omega$  area was found to have relations with the trait of spikelet per spike, no relationship was found between morphological, storage proteins and RAPDs data. As a result, it seems that applying only one of these methods is not sufficient to estimate the genetic diversity and in order to have a clearer picture of the status of genetic diversity in different populations of bread wheat it is recommended that all the three methods be applied simultaneously.

#### P4.27 - Protein disulfide isomerase family in wheat: protein structure and expression analyses

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PDI and PDI-like proteins are responsible for multiple metabolic functions, including secretory protein folding, chaperone activity and redox signalling. Most studies on their diversified metabolic roles have been carried out in mammals, whereas in plants the knowledge on the structural and functional features of these proteins and of their encoding genes is much less extensive. The purpose of the present research was to characterize the genes of the PDI family in wheat and to compare the structure of their deduced amino acid sequences and their expression with those of homologous genes from other plant species. Former studies in wheat had been restricted to the genes encoding the typical PDI, which is of special interest for its potential involvement in determining the technological properties of flour.

The deduced amino acid sequences of 17 PDI and PDI-like cDNAs of wheat assigned to nine homoeologous groups were searched for conserved motives by comparison with characterized sequences in different protein databases. The protein sequences of the wheat PDI-like genes of different homoeologous groups showed a high level of structural similarity with the protein sequences of genes clustered into the same phylogenetic group. The proteins of five groups (I-V) have two thioredoxin-like active domains and show structural similarities to the corresponding proteins of higher eukaryotes, whereas those of the remaining three groups (VI-VIII) contain a single thioredoxin-like active domain. The expression analysis of the nine non-homoeologous wheat genes, which was carried out by quantitative real time RT-PCR (qRT-PCR) in a set of 29 samples including tissues, developmental stages and temperature stresses, showed their constitutive, although highly variable transcription rate. Highly diversified expression rates and patterns were detected even between close paralogous genes. The present research confirmed the very high expression of the gene *TaPDIL1-1* (typical PDI) in developing caryopses, which is consistent with its hypothesised role in the folding, aggregation and deposition of seed storage proteins. The comprehensive structural and expression characterization of the complete set of *PDI* and *PDI*-like genes of wheat performed in this study represents a basis for the functional characterization of this gene family in the hexaploid context of bread wheat.

#### P4.28 - Interaction of three barley ear/flower homeotic mutants and prospects of using their hybrids for ornamental purposes

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Since olden times in the Vilnius region of Lithuania, specific dry bunches (called 'verba') are very popular. A significant part of them are produced from dry Poaceae plants. Several homeotic barley mutants, especially *Hooded*, are very ornamental themselves. However, although all *Hooded* mutants have 305 bp duplication in the regulatory IV intron of *HvKnox3* gene (Müller et al., 1995; Roig et al., 2004), they are very polymorphic as regards their spike structure and an additional flower instead of an awn or on it. The two other groups of mutants *laxatum-a*, partially *lax-c*, and *tweaky spike* are characterized, first of all, by lodicules conversion to stamens. The *lax* type mutants have also sparse spikes, and *lax-a* was attributed to class B of flower organ identity genes (Laurie et al., 1996). The *tw* mutants have been characterized genetically, but they have a specific spike structure, and their lodicules may be also converted to pistils. We have sequenced the IV intron of *HvKnox3* gene of *tw* mutants and showed that it has no 305 bp duplication, but single nucleotide variations are present.

Double and triple hybrids which had an interesting spike structure and combined, at least two specific spike characteristics were selected from the hybrid population. We believe that such hybrids and homeotic barley mutants may increase the amplitude of usage of barley genetic resources.

Laurie DA et al., (1996) Theor Appl Genet 93, p. 81-85.

Müller KJ et al., (1995) Nature, 374, p. 727-730.

Roig C et al., (2004) Genetics, 167, p. 439-448.



#### P4.29 - Variability analysis of *MATE* gene sequences in several species of *Secale*

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Aluminum (Al) toxicity is a major constraint for crop production in acid soils that comprises about 30–40% of world arable lands. Al-tolerant cultivars contribute to increase the productivity and to widen the areas of crop production. Variability to Al tolerance, between species or among plants of the same species, is found in several crops. Among cereals, rye (*Secale cereale*) has been described as the most tolerant species. Genus *Secale* comprises, besides the cultivated rye, several weedy and wild types that can show higher tolerant phenotypes against environmental stresses. Therefore, these species may be a good resource to isolate high-tolerant genes and/or mechanisms against these stresses. The knowledge of the mechanism and the genes that control Al-tolerance is an important goal that will provide fundamental information to increase Al tolerance in other related species.

A role for organic acid exudation as a mechanism of Al tolerance has been highlighted by the recent cloning of Al-tolerance genes that encode aluminum-activated root malate and citrate exudation (*ALMT* and *MATE*).

In this work, screening of Al tolerance was done in different species of *Secale*: wild species (*S. kuprijanovii*, *S. segetale*, *S. ancestrale*, *S. vavilovii* and *S. montanum*) and cultivated rye *S. cereale* (three rye European varieties JNK, D. Zlote and Imperial- and one regional rye population Alvão). The most tolerant and non-tolerant plants were selected to obtain the sequences of two genomic DNA exons of the *ScMATE* gene.

Using genomic DNA and primers designed for *Secale cereale*, sequences of the exon 1 and exon 4 were obtained. It was observed that these sequences are conserved for these species presenting several SNPs (12 in exon 1 and seven in exon 4) and INDELS (three in exon 1 and one in exon 4). The data will be discussed according to the sequences obtained in all the species and in the tolerant and non-tolerant plants and an evolutionary analysis will be done.

#### P4.30 - A multiplex SSR system for variety identification of Italian bread wheat

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The availability of accurate methods for variety identification is relevant for quality control in the seed sector, as well as for traceability in the food chain production. Due to their high discriminative ability and reproducibility, microsatellites (SSR) are commonly used for genotyping in research and breeding. One relevant aspect for their wide adoption in routine testing is the availability of highly informative multiplex sets, which would allow the cost-effective processing of numerous samples. The aim of this work was to establish a rapid identification system for Italian bread wheat cultivars based on the use of multiplexed SSR.

Candidate markers, pre-selected on the basis of available literature for their polymorphism, molecular weight and genomic distribution, were tested for suitability to be combined in multiplex analyses. A set of seven highly informative SSR loci, containing di- and tri-nucleotide repeats and mapping on different chromosomes, was finally identified that could be co-amplified and easily scored after electrophoresis. The discriminatory power of the set was assessed on a panel of 51 bread wheat genotypes including the most prominent varieties currently grown in Italy as well as some old cultivars and recently-developed experimental lines. The multiplexed markers allowed the complete discrimination of all entries: of the 1,275 possible pairwise comparisons, just eight were distinguishable based on one marker only, and for just one of them alleles differed for only two nucleotides. The system seems to be a valuable tool for efficient variety identification in bread wheat.

#### P4.31 - Molecular characterization of the *Psy2* gene for the carotenoid biosynthesis in durum wheat germplasm

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Carotenoids are a large class of isoprenoid-derived pigments that are synthesised *de novo* by many organisms. In plants, carotenoids are important for a number of biological functions such as photosynthesis and photoprotection. In human, carotenoids, in particular  $\beta$ -carotene, are an important source of provitamin A. Carotenoid pigment content in durum wheat has a particular importance for commercial and nutritional characteristics conferred to end-products like pasta.

The first committed step in carotenoid synthesis is the formation of the first C40 compound phytoene by condensation of two molecules of geranylgeranyl diphosphate (GGDP). This reaction is catalysed by phytoene synthase (*Psy*). The objective of the research reported in this thesis was to isolate and characterize the *Psy2* gene in durum wheat and to verify if this gene is associated with quantitative trait loci (QTL) affecting carotenoid accumulation in seeds.

The isolation of the cDNA coding for *Psy2* from durum wheat by RT-PCR amplification and the development of functional markers useful for the genetical and physical mapping of the homoeologous loci of the gene are described. Using this strategy the *Psy-B2* gene was localized on the 5B chromosome.

The tentative involvement of the *Psy-B2* gene with the carotenoid accumulation in the kernels by two different approaches was carried out: QTL analysis and association mapping by the candidate gene approach. According to the results of the QTL analysis on chromosome 5B, we observed a different localization of the *Psy-B2* gene. The *Psy-B2* gene was found to be not associated to the accumulation of carotenoid in the seeds. The association mapping also confirmed the absence of linkage between the phytoene synthase 2 locus and the carotenoid content.

#### P4.32 - Triticeae genomics for the advancement of essential European crops

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The TriticeaeGenome project: Genomics for Triticeae improvement

Europe faces the challenge of delivering safe, high-quality, and health-promoting food and feed as well as bio-products in an economical, environmentally sensitive, and sustainable manner across environments that face climatic change and increasing abiotic and biotic stresses. Triticeae cereals (wheat, barley and rye) are essential in human and domestic animal nutrition and are arguably the most important crops for European agriculture. Existing germplasm resources and current breeding methods alone are insufficient for understanding the mechanisms underlying important traits and for catalysing a quantum leap in yield, sustainability and quality improvement. Major advances in crops will require a broad suite of direct genomics approaches, built on relevant data from model plants (rice, *Brachypodium*). Such a strategy is massively complex and can only be carried out efficiently at the international level. The COST Action will coordinate, focus and strengthen national and pan-European Triticeae genomics to improve sustainability and value of the crops.

#### P4.33 – TriticeaeGenome: Genomics for Triticeae improvement

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For many years the size and complexity of the wheat and barley genomes have hampered the development of genomics and its application to produce Triticeae crops with improved composition and characteristics. Recently, however, new and more efficient scientific capabilities and resources have been developed that allows robust genomic programs to be established for the Triticeae. The TriticeaeGenome European FP7 project (Genomics for Triticeae improvement) is designed to achieve significant progress in Triticeae genomics and to support efficient breeding of improved varieties for the European agriculture by:

- (i) Constructing anchored physical maps of wheat and barley chromosomes of group 1 and 3, which carry a large number of important agronomic traits;
- (ii) Isolating five genes and QTLs (Quantitative Trait Loci) underlying disease resistance, yield and quality traits in wheat and barley;
- (iii) Identifying and exploiting new alleles for the isolated genes through the use of mutant populations and genetic resources;
- (iv) Supporting the development through molecular breeding of new varieties that meet farmer, processor, and consumer needs;
- (v) Developing new bioinformatics tools to integrate and display large-scale genomics data;
- (vi) Coordinating and integrating TriticeaeGenome research through interactions with other projects at National, European and International levels, for enhanced efficiency and mutual benefit;
- (vii) Providing training in emerging technologies, disseminate the results and transfer know-how to industry and users.

TriticeaeGenome mobilizes scientists from 14 European research institutes and 2 industrial partners from 9 countries\* for a duration of 4 years (2008-2012) and a budget of 5.3 M Euros.

\**TriticeaeGenome Pls and Task Leaders*: Catherine Feullet, Delphine Steinbach, Etienne Paux, Philippe Leroy, and Helene Berges (INRA, France), Nils Stein (IPK, Germany), Jaroslav Dolezel (IEB, Czech Republic), Klaus Mayer (HMGU, Germany), Laura Rossini and Francesco Salamini (UMIL, Italy), Tzion Fahima and Abraham Korol (HU, Israel), Robbie Waugh (SCRI, UK), Hikmet Budak (SU, Turkey), Andy Greenland (NIAB, UK), Michael Bevan (JIC, UK), Beat Keller (UZH, Switzerland), Emmanuelle Lagendijk (IT, France), Sebastien Praud (BGA, France), Viktor Korzun and Burkhard Schinkel (KWL, Germany), Michele Morgante (IGA, Italy), Roberto Tuberosa and Maria C. Sanguineti (Italy).

#### P4.34 - QTL and meta-QTL analysis of kernel shape and weight in wheat

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Differences in kernel weight between genotypes are affected by the kernel shape, i.e. kernel length, kernel width and other factors (Campbell et al. 1999; Dholakia et al. 2003). Kernel shape and size have emerged as important breeding objectives, but due to its complex nature, little is known regarding its genetic control and is suggested to be quantitative. Genetic maps have greatly facilitated the investigation of the genetic basis of complex quantitative traits. The aim of this study was to identify genomic segments controlling kernel shape and weight bread wheat. A recombinant inbred lines (F<sub>6</sub>) population, comprising 114 individuals, produced from a cross between two cultivars 'Arrehane' and 'Sardari', was used to construct a linkage map based on 189 microsatellite molecular makers. Parental lines and progeny were planted for two seasons and phenotyped for four traits: kernel length, width, thickness and weight. Seven significant QTL mainly on 1D, 2A, 2D and 5B chromosomes were identified in the studied population. Obtained results and literature review showed most relevant genomic regions involved in kernel weight and shape on homeologous chromosome groups 1, 2, 5 and 7. These effects were condensed using meta-QTL analysis and projected onto a consensus SSR map of wheat.

#### P4.35 - *In silico* analysis of wheat, corn and barley whole submitted uni-genes by employing simulation- based system of cDNA-AFLP

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One of the major drawbacks in various functional genomics methods such as cDNA-AFLP, is the failure of targeting whole expressed genes which lead to losing chance of studying favorite genes. In the standard cDNA-AFLP method as a useful differential display technique, transcriptome coverage depends on effectiveness of two restriction enzymes. In order to evaluating various combination of restriction enzymes to introduce more effective combination, *In silico* based simulation system was employed to analyzed whole wheat, barely and corn unigenes which up to know has been submitted in NCBI website . Analysis of virtual gels with bioinformatics softwares showed that different combination of enzymes work well for each given plants which was *MseI/TaqI*, *NlaIII/AclI* and *HpaII/Tsp509I* for wheat, barely and corn, respectively. In order to compare result of simulation to real lab experiment, standard cDNA-AFLP with fluorescent labeled primers was conducted in order to studying differential gene expression post inoculation wheat leaf rust pathogen. Analysis of estimated data from simulation and observed one from lab experiment showed great correlation between them. Therefore simulation could be eligible to choose drastic enzymes before conduction of cDNA-AFLP.

#### P4.36 - Next-generation genotyping of polyploid wheat

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Wheat was domesticated about 10,000 years ago; polyploidization has been crucial in the establishment of wheat as a major crop. Consequently an understanding of this process is crucial both for gain a better insight into the evolution and domestication of wheat. We utilized next-generation genotyping by Illumina® Golden Gate Assay using 480 plants belonging to tetraploid (both wild and cultivated forms) and hexaploid wheat species derived from diverse geographical locations. The adopted strategy includes profiling of 384 SNPs that covers the whole genome. The result of the profiling and the key issues dealt during the analysis will be presented.

#### P4.37 - Discovery of SNPs in the transcriptome of durum wheat by using next generation sequencing

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Durum wheat is an allotetraploid species having the genome size of about 11.2 Gbp. In the aim of the AGROGEN project an attempt has been made to sequence the transcriptome of eight durum wheat inbred lines namely Cappelli, Ciccio, Creso, Ofanto, Molise, Pedroso, Simeto, and Svevo, in order to identify putative allelic Single Nucleotide Polimorphisms (SNPs). The Illumina mRNA-seq protocol was used to create libraries for cluster generation from 2 week-old plants. The number of short reads produced with Illumina Genome Analyzer (GAII) from these inbred lines ranged from 53 to 89 million. Due to the unavailability of the genome or complete transcriptome sequence from durum wheat and due to the fact that no computational approaches are available for SNP detection directly between Solexa datasets, the NCBI Unigene set of 40,349 genes (28,5474.76 bp) from hexaploid wheat (*T. aestivum*) has been used as a reference to align the Illumina reads. A bioinformatic pipeline able to call, after alignment, SNPs with respect to the reference, to filter between allelic and inter-homeologue SNPs and finally to compare the SNP calls among varieties is currently under implementation at the Institute of Applied Genomics (IGA) in Udine (Italy). As an initial step to check the pipeline, some putative SNPs were manually inspected by using Mapview and validated by PCR and Sanger sequencing. A preliminary and rough estimate of about 1 SNP per 1,000 bp in expressed sequences has been made. The strategy for identification of SNPs from entire transcriptome among the eight inbred lines will be discussed.

#### P4.38 - Variation of seedling root traits in wild barley (*Hordeum vulgare* L. ssp. *spontaneum*) germplasm

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Improved root architecture of cultivated barley is likely to improve yield in drought prone area. Seedlings of 315 accessions from the Wild Barley Diversity Collection (WBDC) were grown under hydroponic conditions for eight day and then root characters were analyzed. The seminal root number (SRN) ranged from two to six, root length (RL) from 15 to 188 mm and root fresh weight (RFW) and root dry weight (RDW) from 1.1 to 14.2 mg and 0.1 to 0.85 mg, respectively. Root water content (RWC) ranged from 0.10 to 13.20 mg/mg and specific root length (SRL) from 3.85 to 66.67 cm/mg, while specific fresh root weight (SFRW) and specific dry root weight (SDRW) ranged from 0.21 to 3.5 mg/cm and 0.015 to 0.26 mg/cm, respectively. One hundred seed-weight (100-SW) was positively correlated with all root characters except RWC. A negative correlation was found between 100-SW and SRL. RDW was positively correlated with most of the traits except RWC and SRL. The variation of root traits for geographical origin showed that accessions from each region had its own distinctness and was grouped independently through PCA analysis. Accessions WBDC266, WBDC302, WBDC286 and WBDC241 had the longest RL, highest RW, SDRW and SRL, respectively, and may be useful in drought condition for the improvement of these root traits in cultivated barley. This work was supported by a grant (Code # 20070301034043) from BioGreen 21 Program, Rural Development Administration, Republic of Korea and the Lieberman-Okinow Endowment at the University of Minnesota.

#### P4.39 - A first survey of the wheat chromosome 5A composition through a next-generation sequencing approach

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Within the International Wheat Genome Sequencing Consortium, Italy is in charge of Chromosome 5A. As a part of this project, here we present a preliminary shotgun sequencing analysis performed with 454 technology. As a starting material we used the short and long arm of chromosome 5A, isolated by flow cytometry. The DNA from the sorted chromosome arms was amplified by GenomiPhi DNA Amplification Kit by GE Healthcare, processed for DNA fragment analysis and run on a Roche 454-Titanium sequencer, producing a coverage of more than 2x. More than two-thirds of the 454 reads exhibit significant homologies to known Triticeae repeat sequences (TREP database). Several transposable element families poorly represented in a close related species such as barley appear to be abundant in wheat chromosome 5A. The results reveal the presence of a number of putative genes and of several putative microRNA-related sequences (microRNA coding genes or microRNA target genes). Numerous Insertion site-based polymorphism (ISBP) candidate (more than one per twenty 454 reads) were identified. Beside their intrinsic interest, these data can be useful for the construction of the genetic and physical maps of chromosome 5A.



#### P4.40 - Localization of QTLs for androgenic responsiveness in doubled-haploid mapping population of triticale (*x Triticosecale* Wittm.)

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Incorporation of doubled haploids (DHs) into breeding programs and other research areas as molecular studies and genetic engineering could be very advantageous in comparison with traditional methods. The instant production of totally homozygous lines connected with more effective selection and trait evaluation could save time, space and work consumption. However, deployment of DHs depends mainly on its production effectiveness, which is for many species, among them – triticale, not enough for practical purposes. It was shown that the production of DH is controlled by at least three independent genetic systems. Identification of its genome localization could bring significant progress in DHs utilization. Quantitative trait loci (QTLs) for androgenic responsiveness were mapped in the population of DH lines derived from F1 hybrid of triticale variety 'Modus' and 'Saka 3006'. Molecular marker linkage map for this population composed of 90 DH lines has been constructed previously by Biotechnology Group (State Plant Breeding Institute, University of Hohenheim, Stuttgart), then supplemented (research project in the frame of COST Action 860 SUSVAR) and now contains 1,518 markers (DArT, AFLP and SSR) located in 21 linkage groups. Screening of androgenic responsiveness among mapping DH lines population proved a great variation in all components of this feature: androgenic embryo-like-structure (ELS) induction, total plant regeneration and green plant regeneration ability. SMA (Single Marker Analysis) and CIM (Composite Interval Mapping) methods with the help of Windows QTL Cartographer version 2.5 software was used for all calculations. Threshold LOD scores were calculated by 1,000 permutations and accepted when the LOD score was greater than 3.5. Analyses showed that major QTL for ELS induction and QTL determining the final androgenetic response were localized on chromosome 4R; whereas QTL for ELS regeneration ability was found on chromosome 4B. However, these results need further confirmation.

The research was supported by the COST Action FA0604 Triticeae genomics for the advancement of essential European crops (TritiGen) research project.

#### P4.41 - Genotyping of barley revertants developed from genetically unstable mutants *tweaky spike* by RAPD and ISSR methods

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The barley pleiotropic monogenic recessive spike structure mutants *tweaky spike* were characterized primarily also by genetic instability and reversion to WT. Several revertants are of economic value and were characterized by not a complete reversion to WT according to quantitative characters (Ranelis et al., 2004; Vaitkėnienė et al., 2008). In the present work, revertants are characterized by two methods of DNA analysis – RAPD and ISSR. The latter technique was successfully applied to characterize barley cultivars (Fernandez et al., 2002). Both methods were applied to two allelic mutants, *tw<sub>1</sub>* and *tw<sub>2</sub>*, 17 revertants and cv. 'Auksiniai II' from which *tw* mutants arose as WT. The RAPD and ISSR methods gave not fully identical results, but both methods, as it may be expected, revealed a low level of DNA polymorphism. The ISSR method showed a higher polymorphism in comparison with the RAPD method (47.4 and 19.4%, respectively). However, five revertants were identified, which differ more significantly from the rest of the group. Among this group, there are two revertants characterized by a higher resistance to lodging; one of them is also more resistant to *Puccinia graminis*. Some polymorphic RAPD and ISSR bands were cloned and sequenced. In some cases BLAST search results showed a significant homology with sequence entries from the GenBank.

Fernandez M. et al., (2002) Theor Appl Genet, 104, 845-851.

Ranelis V. et al., (2004) In: Genetic Variation for Plant Breeding. Eds. J. Vollmann, H. Grausgruber, H. Ruckebauer, Vienna: EUCARPIA and BOKU, p. 219-222.

Vaitkėnienė V. et al., (2008) In: Modern Variety Breeding for Present and Future Needs. Proc. 18 EUCARPIA Gen. Cong. Valencia, Spain, p. 485-489.





# *Posters*

## *Session 5*

### *Genomics-assisted crop improvement for food security in developing countries*



### P5.01 - Random Amplified Polymorphic DNA analysis for discriminating *Azolla* genotypes to further enhance their utility for sustainable agriculture

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A preliminary analysis of molecular polymorphism in four distinct species of *Azolla* using RAPD markers was conducted. The studies led to clear understanding of their unambiguous differentiation and revealed the phylogenetic relationships. A total of 227 distinct DNA polymorphic bands ranging from 0.1 to 2.0 kbp revealed wide range of variability among the species. Similarity index of pair-wise comparisons estimated on the basis of all the 20 primers ranged from 0.2434 to 0.4839. *Azolla microphylla* showed highest similarity with *Azolla filiculoides* (0.4839) while *Azolla filiculoides* showed least similarity with *Azolla pinnata* (0.2434). The data revealed two main distinct groups viz. groups I and II. Group I consisted of only one species of *Azolla pinnata* and exhibited less species diversity and group II exhibited broader species diversity and consisted of three species such as *Azolla microphylla*, *Azolla filiculoides* and *Azolla rubra*. Our studies indicate the enormous possibilities of employing RAPD-PCR analysis in the efficient differentiation of *Azolla* species. Identification of *Azolla* is cumbersome due to the observed time lag in sporulation of different species and difficulty in identifying the sporocarps. In a germplasm collection cross contamination and differentiation of strains is another constraint. These are the major stumbling blocks in the development of appropriate technology involving efficient region specific species of *Azolla*. The results may be exploited further by evaluating the biomass production and nitrogen fixing potential to develop a sound and viable biofertilizer technology.

### P5.02 - Efficiency of ISSR and RAPD markers in DNA fingerprinting and classification of drought tolerant and non-tolerant wheat cultivars

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This research was conducted to evaluate ISSR (Inter Simple Sequence Repeat) and RAP (Random amplified polymorphism DNA) marker systems for their ability to detect genetic diversity within a set of 26 bread wheat cultivars (15 tolerant and 11 susceptible to drought stress) spanning both the Drought tolerant and susceptible wheat gene pools and to compare the efficiency of these two marker types in the classification of cultivars according to their response to drought stress. Cluster analysis using the average-linkage (UPGMA) method was performed by NTSYS program, with the similarity matrix as input data, based on Jaccard coefficient genetic distance. The dendrograms generated by cluster analysis with both markers revealed two-three major clusters, which were identified as the tolerant and non-tolerant genotypes. Accurate classification of wheat germplasm into the two major clusters (tolerant and susceptible) and many sub-clusters can provide essential information for selecting parents in the development of inter-cluster crossing program. Results revealed that ISSR and RAPD markers could be efficient in differentiation of the wheat cultivars and recognition of markers associated with tolerant and non-tolerant genotypes. When comparing the groups formed using ISSR and RAPD markers, there were similarities in the combinations of genotypes from the same drought stress response. Correlation between genetic distances obtained through RAPD and ISSR markers was relatively high (0.34), indicating that both marker systems are efficient for evaluating DNA fingerprinting in the genotypes of bread wheat that we evaluated.

### P5.03 - Polymorphism of 3D chromosome SSR-loci among genotypes that are potential recipients of alien resistance gene to powdery mildew (*Pm*) from *Aegilops* species

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Introgression of vertical powdery mildew resistance genes from wild *Triticeae* species to common wheat genetic pool is the most effective genetic protection way for varieties that are grown as a monoculture [1]. A closely linked with gene of interest genetic marker is necessary to track the resistance gene transfer from resistant donor line to genome of modern commercial variety. Therefore, the development of resistant line with alien gene must be accompanied by modern wheat genotypes studying in order to find potential recipients that demonstrate a polymorphism in the closely linked with resistance gene genetic marker. The objective of this research is to study the microsatellite loci polymorphism among present diversity of common wheat genotypes in order to find the potential recipients in crosses with resistant introgressive lines, which possess alien powdery mildew resistance gene on the Aurora variety genetic background. Plant material: 12 modern common wheat varieties *Triticum aestivum* L. (A<sup>u</sup>A<sup>u</sup>BBDD) of Ukrainian breeding, genome-substituted forms Aurosis (A<sup>u</sup>A<sup>u</sup>BBS'S<sup>l</sup>, S<sup>l</sup> – genome from *Aegilops sharonensis*), Aurolata (A<sup>u</sup>A<sup>u</sup>BBUU, U – genome from *Ae. umbellulata*), and Aurodes (A<sup>u</sup>A<sup>u</sup>BBSS, S – genome from *Ae. speltoides*), which join in their genomes tetraploid component of common wheat variety Aurora (A<sup>u</sup>A<sup>u</sup>BB) and the genomes of diploid *Aegilops* species [2]; hexaploid common wheat lines that were developed from crossing Aurora variety with Aurosis [3]. The PCR analysis of plant material with six primer pairs to microsatellite loci located on 3D chromosome was performed. According to our previous studies [4], the introgression from *Ae. sharonensis* and *Ae. speltoides* concerns 3D chromosome. The polymorphism in six SSR-loci between 12 common wheat genotypes and Aurora, as well as genome substituted forms Aurosis, Aurodes, and Aurolata that are donors of powdery mildew resistance genes, was found. It allowed us to develop several hybrid combinations between resistant introgressed lines and modern common wheat varieties of Ukrainian breeding. It is possible to apply BSA method with one studied microsatellite locus when working with descendants of these segregating populations. The common wheat genotypes that could be used as recipients in crosses with introgressed lines that possess powdery mildew resistance gene *Ae. sharonensis* are determined.

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4. Zh Vdovychenko, 2005, Cytology and Genetics (Tsitologiya i Genetica), v. 39, 21–30.

#### P5.04 - Investigations on tolerant and susceptible wheat (*Triticum aestivum* L.) cultivars for yellow rust disease with AFLP markers

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Yellow rust (YR) is one of the most devastating diseases of wheat throughout the world. The aim of this work is to determine the molecular markers genetically linked to yellow rust disease resistance which is caused by a fungal pathogen, *Puccinia striiformis* f. sp. *tritici*, in Turkish wheat genotypes. For this purpose, 34 AFLP primer combinations based on cutting enzymes *Pst*I and *Mse*I were used to screen the parents and F<sub>2</sub> populations from the cross zgi2001 (resistant male parent) x ES14 (susceptible female parent) at seedling and adult stage. The most resistant and susceptible F<sub>2</sub> populations selected by yellow rust scoring, were used to perform bulk segregant analysis in order to find out molecular markers linked to yellow rust resistance. Selective primers exploited in AFLP assay generated total 154 polymorphic fragments. The 31 primer pairs (91%) amplified polymorphic fragments and remaining three primer pairs (9%) amplified monomorphic fragments. M-ACG, P-GAC primer combination amplified a DNA fragment of 170 bp that was present in the resistant parent and resistant bulk. The 170 bp fragment was present in 29 out of 30 individuals in resistance bulk at seedling plant stage. At adult plant stage, the 170 bp fragment was present in 26 out of 30 individuals in resistance bulk but it was absent susceptible ones both at seedling and adult plant stage. The presence of AFLP marker that is associated with yellow rust resistance may significantly enhance the marker assisted selection studies for yellow rust resistance in Turkey's wheat breeding programs. Conversion of this AFLP marker into a sequence characterized amplified region (SCAR) anchor marker is under investigation and it will allow us to assess sequence conservation of polymorphic region in different wheat germplasms.

#### P5.05 - Identification and mapping of QTLs determining agronomic important traits in *Triticum aestivum* L. grown in different ecological zones in Russia

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For the first time a set of 110 recombinant inbred lines of the „International Triticeae Mapping Initiative“ mapping population was grown in plots at two different ecological zones in Russia: Pushkin Experimental Branch of VIR (St.-Petersburg) and Moscow Experimental Station (Mikhnevo, Moscow Region) during the seasons 2005-2008. In each of environments, 48 characters were evaluated. All agronomic important traits were scored according to broad utilised classifiers of the genus *Triticum* L. adopted at VIR. In total 412 QTLs with a LOD threshold of > 2.0 (minor QTLs) were detected of which 126 reached a LOD score of > 3.0 (major QTLs). Often QTLs were detected in comparable positions in different experiments. The analysis of variance showed that strong differences exist between genotypes for all 48 analysed quantitative traits. The interaction with environments was much smaller. The correlation coefficients for the single traits between pairs of experiments were used as rough estimates of heritability in the experiments. Evaluated quantitative traits formed two distinct groups: group of productivity and group of plant architecture. Thus characters as flowering time, 1,000-grain weight, number of seeds and spikelets per spike and some others were highly correlated with the length of vegetative period. But plant height, flag-leaf position, length and width of leaf blade, leaf waxy bloom (outer), stem waxy bloom, stem-length of upper internode, stem-node size and different spike characters formed the other group of positively correlated traits. No correlation was found between these groups. Homologous and homoelogous relationships of the detected QTLs and already described major genes or QTLs determining the same traits in wheat or other Triticeae member are discussed.

### P5.06 - Towards triticale's powdery mildew resistance via QTL analysis

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With production of 3.7 million tons yearly, Poland is a top producer of triticale (x *Triticosecale* Wittm). Since 2004, powdery mildew (*Blumeria graminis*) has become an important factor limiting triticale's yield. There are new triticale-specific strains. Polish breeders would like to maintain their position by improving cultivar's resistance to powdery mildew attack. In such a situation DNA markers linked to genes controlling powdery mildew resistance can be useful in molecular breeding approach. The studies aim at identifying QTLs controlling triticale resistance/tolerance to the fungus infection. The F<sub>2</sub> population Lamberto × Moderato (LM) was developed with Lamberto as the susceptible parent. LM reaction to pathogen was field tested in provoked infection conditions (Adult Plant Resistance, APR). Genetic linkage map based on genotyping the populations with SSR, RGA and DArT markers was constructed. Triticale genomes were represented by balanced chromosome, and 908 mapped markers were unequally distributed across genomes A (171 markers), B (312 markers), and R (425 markers). The presence of structural rearrangements was also detected. Kruskal-Wallis and interval mapping analyses revealed two main, stable QTLs on chromosome 1R. Saturating of the map with EST-SSR markers and additional QTLs analyses are still in progress.

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### P5.07 - Mapping QTLs for powdery mildew seedling-stage resistance in triticale

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Powdery mildew caused by fungus *Blumeria graminis* is one of the most harmful triticale diseases. Triticale (x *Triticosecale* Wittm) is an important cereal for Polish breeders and producers (growing area: 1.44 million hectares). Search for molecular markers associated with response to *B. graminis* seems to be a key challenge in the fight against the pathogen. Bidirectional F<sub>2</sub> populations between 'Lamberto' (susceptible) and 'Grenado' (resistance) were developed and common linkage map was constructed. Inoculation tests with triticale-specific isolates revealed that on LG, and GL populations, powdery mildew is controlled by a single, and two recessive genes, respectively. Common genetic linkage map of 1,650 cM length for pooled LG, and GL population consisted of 475 DArT markers. We found that in the resultant triticale map, chromosomes 2B, 3B, and 7A were represented by double linkage groups instead of respective rye chromosomes. A single linkage group was made of fragments of chromosomes 3B and 5B. Preliminary QTL analysis suggests that resistance to powdery mildew depend on cytoplasm. In population GL, reaction to *B. graminis* was determined by two independent loci located on distal parts of chromosome 1R. Main gene responsible for the resistance in LG population was located on chromosome 6R.

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#### P5.08 - Assessment of genetic variation in Brazilian rice: Novel traits and novel techniques

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Rice is one of the three major cereals cultivated worldwide. It is also a staple food for over half of the world's population. Additionally, the complete sequence of its genome has been used as a gold standard for the genetic dissection of traits in other cereals and orphan crops. Rice is among the six major crops in Brazil, reaching an annual production of over 13 million tons. Our previous results have shown that the genetic basis for the lowland Brazilian germplasm is narrow and derives from few parental genotypes. Efforts to enlarge the germplasm basis have to cope with keeping standards for farming and cooking quality. Since 1994, we have used strategies to create, evaluate and select genetic variants of rice with better alleles for root development and abiotic stresses. Differences between japonica and indica, as well as upland and lowland genotypes have indicated that these variations can be successfully introgressed into elite lines. Genomics and Proteomics tools have revealed many iron response genes shared by pools of cultivars and many that can be potentially introduced to improve the current germplasm. Potential useful germplasm, tools and strategies are discussed.

#### P5.09 - Analysis of QTLs for yield components and chlorophyll *a* fluorescence parameters in wheat under three levels of water availability

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Drought is one of the major factors limiting wheat yields in many developing countries worldwide. Parameters of chlorophyll *a* fluorescence kinetics under drought stress condition have been used to characterize dehydration tolerance in wheat.

In the present study, a set of 94 doubled haploid lines obtained from Chinese Spring x SQ1 (CSDH), mapped with 450 markers (Quarrie et al., 2005. *Theor Appl Genet* 110: 865-880), was evaluated for yield (dry weight of grains per main stem), number of grains per main stem (NG) and chlorophyll *a* fluorescence parameters (FC) under moderate (MD) and severe drought (SD) stress, and compared with results for well-watered plants, as a control. Both drought conditions were imposed for 4 weeks during the late vegetative stage, to be relieved at around the time of flowering. QTLs were identified using Windows QTL Cartographer version 2.5 software and results were analysed using single-marker analysis (SMA) and composite interval mapping (CIM). QTL analysis showed similar results using SMA and CIM methods. The genetic control of yield, NG and FC varied considerably between drought-stressed and non-stressed plants. Both methods identified 1 QTL under MD on chromosome 6D (LOD = 3.3) and 2 QTLs under SD on chromosomes 2B and 5D for yield, with  $R^2$  values 11.2, 17.7 and 12.0% respectively. QTLs for NG were identified on chromosomes 5A and 6B only under moderate drought. SMA identified additional major QTLs on 5A for yield and NG for both drought treatments. Mapping QTL for FC identified a total of 20 additive QTLs: eight QTLs detected under well-watered, one QTL under moderate and thirteen QTLs under severe drought. The results for FC suggest that several major loci (LOD > 5.0) for severe drought stress were located on chromosome 2A ( $R^2$  from 17 to 23%). Genetic analysis of these traits provided an excellent tool to understand better the mechanisms regulating responses of wheat to drought stresses.

These studies were supported by COST FA0604 (479/N-COST/2009).

### P5.10 - Genetic diversity of a DREB-related gene in durum wheat and its wild relatives

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The genetic diversity of various accessions of durum wheat, *Aegilops speltoides*, *Triticum urartu* and different *Aegilops* species has been investigated by analysing the structure of the *DRF1* gene, which belongs to DREB-family and is involved in the abiotic stress response. We have cloned and fully characterized the *DRF1* gene in about 100 durum accessions and more than 200 wild ancestor accessions. The alternative splicing mechanism that characterizes the *DRF1* gene expression in wheat was also observed in its wild relatives.

SNP, SSR and insertion/deletion were detected and analysed by various bioinformatic tools in all the obtained sequences, in view of gaining more knowledge about the intra- and inter-specific variability of this gene, of its pseudogenes and of its homeologous copies.

Furthermore, we have identified a novel transposon encompassing intron1-intron3, thus including exon2 and exon3. Due to its characteristics, it was used as a tool for investigating the genesis of the DREB-genes.

An interactive database was built to store, access and compare all the derived information.

### P5.11 - Genetic variability study of Septoria fungus "*Septoria tritici*" on wheat in Morocco using AFLP technique

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*Septoria tritici* leaf blotch (STAB) caused by *Mycosphaerella graminicola* (anamorph *septoria tritici*), is one of the most important foliar diseases of wheat worldwide. Significant losses from this disease have been reported around the globe. In Morocco, the yield production losses can exceed 35%. This study had as objective to evaluate the molecular genetic diversity of *S. tritici* populations on wheat in Morocco where the climatic conditions are favorable for fungus development. Amplified fragment length polymorphism (AFLP), using *Pst*//*Mse* restriction enzyme, was used for this study. Three regions were studied: Gharb region, Saïss-Zear and moyen Atlas region and nord-Tangeroi region. Sixteen *Pst*//*Mse* primers combinations were tested and three of them were used for the analysis of genetic diversity. 436 polymorphic loci were obtained. The results indicated high levels of genetic variability between isolates and between regions.

## P5.12 - Multiple sources of resistance to insects pests -Russian wheat aphid, Hessian fly and Sunn pest in synthetic hexaploid wheat

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Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), Sunn pest, *Eurygaster integriceps* (Puton), and Hessian fly, *Mayetiola destructor* (Say) are among the most important insect pests of wheat in North Africa and West and Central Asia. Host plant resistance is the most economical and practical means of controlling these insects. Through field and greenhouse screening, several sources of resistance to Hessian fly, Russian wheat aphid, and Sunn pest have been identified in wheat and its wild relatives.

To further broaden the genetic base of resistance to these pests, 914 synthetic hexaploid wheat (SHW) commonly designated as primary synthetic wheat were evaluated for resistance to Russian wheat aphid, Hessian fly and Sunn pest. The initial screening for Russian wheat aphid and Sunn pest were carried out in the field at Tel Hadya, the main experimental station of the International Center for Agricultural Research in the Dry Areas, Aleppo, Syria, and for Hessian fly in the greenhouse. Promising accessions from the initial screening for Hessian fly and Russian wheat aphid were evaluated in replicated trials in the greenhouse for confirmation. Sixteen lines were found highly resistant to Hessian fly and four showed only a moderate level of resistance to this pest. The level of resistance to Russian wheat aphid in SHW was considerably much lower; only two had high level of resistance and four were moderately resistant. One hundred and forty five lines from the initial field screening for Sunn pest resistance are being evaluated this season (2009/2010) in a replicated trial in the field under artificial infestation in cages. Crosses between the resistance sources and elite bread wheat have been initiated. Further ongoing genetic and genomic studies using these accessions are ongoing to identify and characterize the resistance genes and reveal potentially new resistance genes for deployment in ICARDA's breeding programs to develop wheat germplasm with multiple resistances to these key pests.

## P5.13 - Marker-assisted selection for rusts resistance, grain quality and water-use efficiency traits in bread wheat

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Several traits of agronomic interest have been tagged with molecular markers in bread wheat. Such markers can be readily used for marker-assisted selection that could increase the genetic gain, efficiency and effectiveness of a specific breeding strategy. In this paper, we report integration of molecular markers and doubled haploid technique in conventional backcross breeding program aimed at improving rusts resistance, water use efficiency, and grain protein content. Molecular markers were used to screen a BC<sub>1</sub>F<sub>1</sub> population produced from a cross between the recurrent parent 'Tilila' (a well adapted variety to Moroccan condition with 1RS translocation) and the donor parent 'Yecora rojo-GPC' (with high grain protein content and yellow rust resistance) for the presence of high grain protein content (GPC)/Yr genes. The anthers of selected BC<sub>1</sub>F<sub>1</sub> plants were used for developing haploid plants. These haploids were screened for presence of 1RS, GPC and Yr genes and selected, and the selected plants were diplotized using colchicine. The results with respect to genetic improvement for the rust resistance and water use efficiency were discussed. The integration of marker-assisted selection for specific target genes, particularly at the early stages of a breeding program, is likely to substantially increase genetic improvement in bread wheat.

#### P5.14 - Genetic analysis and cloning of *STOP1* - A candidate gene for aluminium tolerance in wheat

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Plant response to high levels of aluminium toxicity under acidic condition differs widely between species and within the same species. Molecular characterization of these mechanisms is the key process to develop efficient breeding programs. In order to increase variability in modern cereal cultivars, nowadays, old cultivars, wild types and landraces represent additional value due to the income of new genes, principally if they have already been incorporated in crop improvement. Recently, a Portuguese wheat landrace (Barbela 7/72/92) was identified as a good source of Al tolerance revealing higher level of Al tolerance than BH1146 the International tolerant tester. However, several major genes associated with Al tolerance and involved in organic anion transporters and in exclusion tolerance mechanism have been cloned in wheat. Recently in Arabidopsis, in addition to *ALMT* (for Aluminum-Activated Malate Transporter) and *MATE* (for Multidrug and Toxic Efflux transporter) genes, *STOP1* (for sensitivity to proton rhizotoxicity) gene was found to be a key factor involved in the signal transduction pathways regulating both *ALMT* and *MATE* gene families. In the present study, we cloned the *STOP1* gene in the bread wheat cultivar Anahuac (sensitive tester) that represents 1681 bp. In addition, cloning and sequencing of *STOP1* gene in Barbela 7/72/92 and some genetically diverse wheat cultivars is in progress. The sequences will be presented and compared.

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#### P5.15 - An assessment of EST-SSR markers for yellow rust disease resistance on Turkish bread wheat genotypes

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Wheat yellow rusts caused by *Puccinia striiformis* f. sp. *tritici* is important diseases of wheat in Turkey as well as in many parts of the world. With the use of molecular markers and a genetic linkage map, various wheat genes that control yellow rust resistance have been successfully tagged. In this study, we have used the bulk segregant analysis technique in combination with EST-SSRs to identify potential molecular marker(s) associated with yellow rust resistance in wheat. The EST-SSRs have great potential as a genetic marker because of their physical association with coding regions of the genome and these features provides significant information for agronomically important loci. In wheat, 5,425 EST-SSRs are already available in the public domain and are being put to a variety of uses. In this study, EST-SSRs, derived from A and B genomes of wheat, were used to determine the molecular markers genetically linked to yellow rust disease resistance in Turkish wheat genotypes. For this purpose, 113 EST-SSR primer pairs were used for screening of parents and F<sub>2</sub> plants from the cross Izgi01 (resistant male parent) x ES14 (susceptible female parent) and zgi01 x Aytin98 (susceptible female parent) at seedling and adult plant stage. The most resistant and susceptible F<sub>2</sub> plants selected by yellow rust scoring, were used together with their parental lines for bulk segregant analysis. One of the used EST-SSRs (BU099658) which was present in the resistant parent and in the resistant bulk, but absent in the susceptible parent and in the susceptible bulk, was identified. BU099658 marker can potentially be used to select yellow rust resistant wheat germplasm.

P5.16 - Defense-related multiplex gene expression in wheat in response to interaction with *Puccinia striiformis*

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a major constraint in wheat production and is a serious threat to food security worldwide. In this study, EST-based multiplex gene expression analyses were performed by using yellow rust infected susceptible and resistant wheat varieties. In the first part of the study, 1549 ESTs from yellow rust infected *Triticum aestivum* cDNA library (TA117G1X) were chosen to assemble 39 contigs and 96 singletons matched with *Triticum aestivum* proteins which were assigned to 8 functional groups namely protein synthesis, photosynthesis, metabolism & energy, stress proteins, transporter proteins, protein breakdown & recycling, cell growth and division and reactive oxygen scavengers. Only stress and stress related contigs and singletons were selected for multiplex gene expression analyses. In the second part, the genes responsible for the disease resistance, were determined and the mRNA sequences of the disease resistant genes were subjected to GenomeLab GeXP Genetic Analysis System for primer designing. We designed GeXP primers for 9 contigs, 20 singletons and 10 genes for disease resistance to be used gene expression analyses by performing 4 multiplexes. Multiplex gene expression analyses were conducted using RNA samples from yellow rust infected and control plants. 5 different time points (0h, 8h, 12h, 24h and 48h) were chosen to mimic the conditions, trigger disease resistance mechanism. The time-course allowed for discrimination between early (8 h after inoculation hai), intermediate (12–24 hai) and late (48 hai) induction or repression. Six bread wheat genotypes (*Triticum aestivum* L. cvs. PI178383, Izgi01, Sönmez2001- yellow rust resistant cultivars and *Triticum aestivum* L. cvs. Harmankaya99, ES14, Aytın98- yellow rust susceptible cultivars) were used for the gene expression analyses. PR5 (Pathogenesis related gene5), PR2 (BlastX homolog of singleton CA598181), GRAB2 (BlastX homolog of singleton CA597983) defense response genes exhibited high level of gene expression level in inoculated plants while there was no expression in the controls. In summary, we demonstrate that EST approach combination with multiplex quantitative PCR has an unprecedented power for studying gene expression in plants with known nucleotide sequence data.

### P5.17 - A whole genome transcriptomics approach to identify candidate genes associated with drought resistance of wild emmer wheat germplasm

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Low water availability is the major environmental factor limiting crop productivity. This problem is expected to increase with the projected global climatic change and the rise in food demand for the world population. Wild emmer wheat (*Triticum dicoccoides*), the progenitor of domesticated wheat, has been found to harbor wide genetic diversity for various agronomic traits, including drought tolerance (Nevo et al., 1984). Screening of 110 wild emmer wheat genotypes representing various habitats in Israel, for their responses to drought stress under field conditions revealed a wide diversity for yield related traits, morpho-physiological traits, and phenology (Peleg et al., 2005). In the current study, a whole genome transcriptomics approach was used to identify candidate genes involved in drought resistance of wild emmer wheat germplasm and to provide insights into the molecular networks that are involved in adaptation to drought. Comparative analysis was conducted using leaves and roots of wild emmer wheat genotypes contrasting in their productivity and yield stability under drought stress. Gene expression patterns in roots and leaves of the drought resistant (R) and drought susceptible (S) genotypes revealed that multi-level regulatory and signaling processes were enriched among the drought induced transcripts, in particular in the R genotype. Further analysis was focused on 221 transcripts in leaves and 118 transcripts in roots that were uniquely expressed or showed higher abundance in the R genotype in response to drought, as potential candidate genes involved in drought resistance. Annotation analysis revealed that 26% of the candidate leaf genes and 43% of the candidate root genes are involved in multilevel regulation, including: transcriptional regulation, DNA and RNA binding and metabolism, kinase activity, and calcium and hormone signaling. Some of these genes showed similar pattern of regulation both in leaves and roots, such as the transcription factors, phospholipid and calcium signaling. Some of these candidate genes were previously shown to be associated with drought adaptation pathways in other plant species (e.g. cell wall adjustment, cuticular wax deposition, lignification, osmoregulation, and dehydration protection). These results demonstrate the potential of wild emmer wheat as a source for candidate genes for improvement of drought resistance in domesticated wheat.

Nevo et al. 1984. *Euphytica* 33: 717-735.

Peleg et al. 2005. *Plant Cell Environment* 28:176-191.



**P5.18 - Identification and functional analysis of autophagy related gene, *Atg8*, in wild emmer wheat under osmotic/drought stress condition**

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Autophagy, literally self eating, is an evolutionary conserved catalytic process for vacuolar degradation of intracellular components, previously examined in yeast, mammals and plants. Autophagosomes, double membraned vesicles containing cytoplasmic macromolecules and organelles, are formed and targeted to vacuole or lysosome for break down and recycling upon induction of autophagy. Among 30 identified autophagy genes, one of the most widely studied genes, *Atg8*, has been used for monitoring autophagy in a variety of organisms. Abiotic stress factors, including nutrient starvation, oxidative stress, salt stress and osmotic stress have been previously reported to induce autophagy in plants. This study is the first to identify *Atg8* gene from *Triticum dicoccoides* (*TdAtg8*). Examination of *TdAtg8* expression patterns indicate that *Atg8* expression was immensely upregulated with osmotic stress treatment, especially in the roots. Monodansylcadaverine (MDC), a convenient marker to monitor autophagy in plant cells, has been utilized to observe autophagosomes and autophagy was revealed to be constitutively active in *T. dicoccoides*. Moreover, with MDC staining, autophagy was determined to be more active in plants exposed to drought stress when compared to plants grown under normal conditions. *T. dicoccoides Atg8* gene was demonstrated to complement *atg8Δ::kan MX* yeast mutants grown under starvation conditions in a drop test assay. For further functional analysis, ATG8 protein from *T. dicoccoides* were expressed in yeast and analyzed with western blotting. We conclude that, *TdAtg8* gene might play a role in regulation of *T. dicoccoides* under osmotic stress conditions.

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**P5.19 - Exploring genetic diversity of core-set rice germplasms using their physiological responses against salinity**

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A core-set of rice germplasm collections was developed based on their identical genetic backgrounds. Those core-set germplasms were tested for the genetic diversity of salt tolerance using key physiological parameters, especially, related to osmotic and ionic relations. Long-term moderate salinity imposed to young seedlings in the water culture within a growth chamber, providing an optimal plant growth environment except salt stress. In order to understand the physiological responses of rice seedlings in the presence of long-term moderate salinity, this study set precise physiological assessment protocol for the rice genotypes using the relative water content of shoots, osmotic potentials and ion concentration of the expressed cell sap and the simultaneous water uptake and transpiration of intact plants. This study revealed that the core-set germplasm showed a great diversity in each physiological parameter in response to salinity, as compared with cv. 'Pokkali', which is a well-known salt-tolerant genotype, and cv. 'IR29', which is a reference genotype for salt-sensitive. The genetic diversity could enhance the utilization of the genetic resources for further studies on functional genomics, metabolomics as well as conventional breeding. This presentation also includes the potential of their utilizations.



## P5.20 - Integrative analysis of wheat-rye translocations lines for wheat improvement

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Wheat-rye translocation lines are useful sources for wheat improvement. Translocated rye chromatins such as 1RS and 2RL have conferred an important genetic source of pest resistance and superior performance in the unfavorable environment for wheat production. Elucidation of structure-function relationships of translocated rye chromatins in wheat is vitally important for understanding wheat-rye translocations. The goals of our research are to: (1) develop and introduce new cultivars and germplasms having improved yield, pest resistance, and end-use quality and (2) elucidate resistance mechanism of translocations in the form of 2BS.2RL. To achieve above research goals, integrative technologies encompassing molecular genetics, genomics, proteomics, and conventional breeding methodology that contribute to the development of high quality and stress tolerance wheat cultivars were applied. In molecular genetics approaches, ESTs-based 2RL specific markers that would provide a basis for the development of ESTs-derived markers for detecting wheat-rye translocations were developed. These markers could be employed in elucidating functional analysis of genes on 2RL. Proteomic analysis on wheat-rye translocations have been conducted for the elucidation of metabolomic systems responsible for the pest resistance. This study demonstrated that integrative technologies could elucidate the near-isogenicity and pest resistance in wheat-rye translocations and enhance the usefulness of rye chromatins for wheat improvement.

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## P5.21 - How to screen barley genotypes for the resistance to net blotch disease using molecular markers

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Barley (*Hordeum vulgare* L.) is important crop species grown especially for malting and for feeding in the Czech Republic. One of diseases that threaten the yield and as well as the quality of barley is net blotch caused by fungus *Pyrenophora teres* Drechs. Although the fungicides are commonly used, the better way is to breed new lines resistant to this disease. The first problem is to find good donors of resistance and the second is to be successful with crossing and lines evaluation. This study deals namely with the first problem. We used a set of microsatellites of which several are localised nearby QTLs associated with the resistance to *P. teres* previously detected in several mapping populations. For 43 barley microsatellite loci (12 from QTLs) and 67 barley genotypes, 640 alleles were detected. The average number of alleles per locus was 14.9. Further, retrotransposons in *Mlo* locus were used also to screen barley lines and varieties that could be introduced into net blotch resistance breeding program. In total, 27 alleles were detected using 16 primer pairs localised in *Mlo* locus. Cluster analysis based on data showed that diversity in studied file of barley accessions can be explained mainly by pedigree and do not correspond to their resistance level to *P. teres* even though microsatellite markers were chosen to be localised nearby QTLs associated with the resistance to net blotch. Further analysis showed the only weak association between markers and field evaluation of *P. teres* attack.

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## P5.22 - Characterization of small RNA transcriptome in wild barley, *Hordeum spontaneum*, at 'Evolution Canyon' I, Mount Carmel, Israel

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Small RNAs have been recognized as important regulatory molecules involved in both gene expression and epigenetic regulation of the genome. Subsets of miRNAs and natsiRNAs play important roles in response to various stresses. However, how the small RNA repertoire evolves and contributes to the adaptation to different environment has not been investigated. 'Evolution Canyon' (ECI) at Lower Nahal Oren, Mount Carmel, Israel, is an optimal natural microscale model for unraveling evolution in action highlighting the twin evolutionary processes of adaptation and speciation. A major model organism in ECI is wild barley, *Hordeum spontaneum*, the progenitor of cultivated barley, which displays dramatic interslope adaptive and speciation divergence on the 'African' dry slope (AS) and the 'European' humid slope (ES), separated on average by 200 m. We performed high-throughput small RNA sequencing for wild barleys from the two opposing slopes of ECI. Sequencing scales of the four samples are all beyond one million reads. We found that the expression of some miRNAs, including those highly conserved, vary greatly in different genotypes of wild barleys. In addition, 30 novel barley miRNA candidates were identified in our study. All of them are specific to one genotype. These results reveal that the expression of small RNAs within wild plant genetic populations living in a natural environment can have great diversity.

## P5.23 - Identification of proteins which potentially improve drought tolerance during grain filling in a series of exotic barley introgressions

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Drought stress during grain filling results in reduced grain fill and subsequent loss in grain yield. This project aims to identify novel exotic proteins associated with improved drought tolerance during grain filling in barley. To achieve this aim a set of spring barley introgression lines (S42-ILs, Schmalenbach et al., 2008) that originate from the cross Scarlett (*Hordeum vulgare* ssp. *vulgare*) x ISR42-8 (*H. vulgare* ssp. *spontaneum*) were screened for drought tolerance during grain filling. In total 49 S42-ILs and Scarlett as the control genotype were grown in the glasshouse using an automated irrigation system that provided well watered and drought growth conditions during grain filling. Plants were phenotyped for drought related physiological traits and this data were then used for genotype by trait association studies to identify QTLs. The analysis identified exotic alleles associated with increased and also decreased plant performance under drought stress. Furthermore, we could confirm several QTLs detected in previous field experiments using this S42-IL population. To understand the molecular mechanism controlling identified QTLs a proteomics study is currently being performed. Grain samples were collected during grain development from selected drought tolerant and intolerant S42-ILs and Scarlett that were grown under well-watered and drought stress conditions. Quantitative 2D gel electrophoresis is being used to detect differently expressed proteins. Identified proteins associated with improved drought tolerance can potentially be used as diagnostic bio-markers to assist in the selection of higher yielding barley lines under drought conditions. Furthermore, this research will give a greater understanding of the genetic and biochemical mechanisms that control drought tolerance in barley.

Schmalenbach, I., Körber, N. and Pillen, K. (2008). Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. *Theoretical and Applied Genetics* 117, 1093-1106.

**P5.24 - A genetic linkage map of durum wheat segregating for drought, heat and salt tolerance**

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A set of 114 recombinant inbred lines derived from the cross, Omrabi 5 x Belikh 2, were utilized to construct a genetic linkage map for durum wheat. Omrabi 5, a cultivar which combines drought tolerance with yield and yield stability, developed in particular for the Mediterranean drylands and Belikh 2, the second parent, is a heat and salt tolerant cultivar; both parents were developed in ICARDA, Syria. The population was genotyped with 275 microsatellite markers comprising of 161 GWM, 64 BARC and 50 WMC markers. The present map will be used for identifying QTLs associated with phenotypic traits for drought, heat and salt tolerance. This work is part of ICARDA/ CIMMYT wheat improvement program, for developing drought and heat tolerant germplasm for the dry areas of North Africa and West Asia. Phenotypic characterization of the mapping population will be carried out in different locations of the partner countries as organized by ICARDA/CIMMYT.

**P5.25 - Genetic variation for and QTL analysis of salinity tolerance in barley (*Hordeum vulgare*)**

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The genetic variation of the response to salinity stress in barley was studied using a diverse set of 24 genotypes. Seedlings at 2-leaf stage were exposed to four different salt treatments (0, 100, 200 and 300 mM NaCl) for a period of 3 weeks on hydroponics. Shoot and root growth were measured three times with an interval of one week. The resulting dried root and shoot samples were used to collect data on salt stress-induced changes over time in mineral composition. Salinity induced a strong adverse effect on growth that increased with salt concentration and duration. Large genotypic differences were observed under salinity stress of growth response and Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup> and SO<sub>4</sub><sup>2-</sup> content in roots and shoots. Tolerant genotypes showed significantly less dry matter reduction under salt stress than susceptible genotypes. The variation between tolerant and sensitive genotypes in ion content was larger in the shoot than in the root fraction of the plants. Tolerant genotypes tended to have a clearly higher K/Na ratio and phosphate concentration and significantly lower concentrations of Cl<sup>-</sup> and sulphate in their shoots. These genotypes mostly had a relatively low Mg<sup>2+</sup> and Ca<sup>2+</sup> concentration in the root fraction. It was concluded that a salinity level of 200 mM NaCl for 3 weeks on hydroponics can be most effectively used for studying salinity tolerance of a large number of plants. This protocol was used to screen salinity tolerance in relation to mineral content of the Steptoe x Morex DH population. We identified more than 20 QTLs (range for LOD scores: 3-20) controlling variation for various salt-stress response traits scattered over the barley genome. Two specific regions on chromosomes 2H and 3H showed co-localization of several strong QTLs for ion contents and growth both for control and saline conditions indicating a clear, perhaps even causal relationship between these stress traits. This interdependency suggests that genes underlying the QTLs may control ion transport and affect ion homeostasis and proton pump activities.

## P5.26 - SSR-marking of genes that control awnedness in durum wheat (*Triticum durum* Desf.)

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Genes that promote awn development in *Triticum* species are of considerable interest. First of all, awned plants demonstrate greater adaptability than awnless ones. Then, genes that promote awn development are homeotic [1], and their identification and localization are important for further fundamental studies. The objective of this research is to locate genes that control awn development in several durum wheat genotypes by means of SSR-markers. Three *T. durum* Desf. genotypes that differed in a degree of awnedness were used for mapping populations development. Two F<sub>3</sub> families were developed from different crosses by means of SSD method. The segregation in these hybrid combinations is determined by two genes [2]. In the combination between awnless Mutico italicum (MI) and awned Leucurum these genes are awn inhibitor and awn promoter, and in the combination MI x Rubrum, which is weakly awned, the segregation is determined by awn inhibitor. For these durum wheat genotypes we propose that awnless genotype possesses *B1* gene on the 5 chromosome, awned one has awn-promoting gene *Awn<sup>P</sup>* on the 6B. The weakly-awned genotype is a recessive homozygote for both genes. Two F<sub>3</sub> populations were studied by means of SSR loci, specific to 4A, 5A, and 6B chromosomes. In cross combination MI x Leucurum the observed segregation into awnless, weakly-awned and awned plants has fitted to the expected one 15:34:15 respectively, and in combination MI x Rubrum the observed segregation into five awnless : three weakly-awned has fitted the expected one as well. The alleles of microsatellite loci have segregated into two homozygous and one heterozygous classes in an expected for F<sub>3</sub> 3 : 3 : 2 ratio respectively in all cases but one. In the combination MI x Leucurum the segregation of *barc178-6B* alleles did not match theoretically expected one. Among awned plants significantly ( $\chi^2 = 15.5$ ,  $P < 0.05$ ) prevailed individuals with allele of awned parental form Leucurum. The linkage evaluation by means of maximum-likelihood formula between SSR-locus and awn promoter *Awn<sup>P</sup>* is 34.7 cM. Therefore, on the basis of linkage with located on the 6B chromosome SSR-locus, awn promoter *Awn<sup>P</sup>* is located on this chromosome 34.7 cM far from SSR-locus *barc-178*.

1. S Takumi, 2000, *Gene*, v. 249, 171-181.

2. D Prokopyk, 2009, *Cytology and Genetics (Tsitologiya i Genetika)*, v. 43, 3-9.

## P5.27 - Aluminum tolerance and molecular characterization of diploid and tetraploid *Secale* species

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The phytotoxicity of soluble aluminium (Al<sup>3+</sup>) is a major limitation to crop growth in acid soils. Plant roots development is affected by aluminum toxicity, resulting in a decrease of water and nutrient supply hindering the plant development. The achievement of new Al tolerant cultivars is an important goal to increase world crop production. There is a wide genetic diversity in the response of Al tolerance between and within plant species. Among cereals, rye is the species that shows greater Al tolerance.

The cultivated rye (*Secale cereale* L.) is a diploid species originated from wild species as a result of natural selection and chromosomal mutations that confer favourable agronomic characteristics. Polyploid species are obtained from diploids by hybridization and chromosome doubling.

In this work different species of *Secale* (diploid and tetraploid wild and cultivated species), were evaluated to aluminum tolerance. Genetic variability by ISSR markers was also studied. Results of aluminum screening didn't indicate a pattern of Al tolerance in diploid and tetraploid rye species, with no major differences between them. Seven ISSR primers amplified 92 reproducible bands of which 80 were polymorphic and the polymorphism percentage was 86.96%. Cluster analysis (UPGMA) separated diploid and tetraploid ryes in two main groups. The importance of this material, according to the ploidy level and agricultural status, for improved sustainable production will be discussed.

**P5.28 - Genetic diversity of winter wheat (*Triticum aestivum* L.) germplasm for resistance to yellow rust**

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Yellow rust caused by the obligate biotroph fungus *Puccinia striiformis* f. sp. *tritici* is among the most destructive of pathogens, causing substantial losses to wheat production annually on a global scale. Characterization of wheat (*Triticum aestivum* L.) germplasm for yellow rust resistance by means of DNA fingerprinting techniques provides a tool for precise germplasm identification and a quantitative estimate of genetic diversity. In this study, 65 SSR (Simple Sequence Repeat), eight ISSR (Inter Simple Sequence Repeat) and 18 SRAP (Sequence-Related Amplified Polymorphism) markers were used to assess genetic diversity and mean genetic distance among the resistant and susceptible Turkish wheat genotypes for yellow rust disease. In this frame, bread wheat genotypes that are resistant (PI178383, zgi2001 and Sönmez2001) and susceptible (Harmankaya99, ES14 and Aytın98) to yellow rust were screened with different types of molecular marker techniques based on polymerase chain reaction. Resulted polymorphic band patterns between genotypes were obtained with 37 out of 65 SSR markers, seven out of 8 ISSR markers and 15 out of 18 SRAP markers for combinations. The genetic similarity coefficient for all the accessions ranged from 0.6515-0.7748, 0.7184-0.8738 and 0.6955-0.8235 for SSRs, ISSRs and SRAPs, respectively. The results indicated that SSR, ISSR and SRAP markers can be used for genetic diversity studies as highly valuable sources and they were useful in the identification of suitable parents for the development of mapping populations for tagging disease resistance genes.

**P5.29 - Dissecting QTLs for yield under water-stressed environment in bread wheat**

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The availability of water is a major determinant of world-wide crop yield in many regions of the world. Although there is genetic variability for drought tolerance and yield stability, these are complex and multigenic traits with a large environmental component which makes breeding for improved performance under drought difficult. In order to better understand the relationship between genotype, component traits, and environment over time, QTL mapping is as one of necessary steps. Previous studies with 112 bread wheat recombinant inbred lines (RILs) from the cross of two parental lines trialled over 2 years identified major yield quantitative trait loci (QTLs) on 7As and 7AL, expressed mainly under stressed-water condition. Sardari, Iranian tolerant landrace, contributed alleles increasing yield on 7AS and 7AL. Additional results which focus on the 7As and 7AL yield QTL are presented here. The yield QTL on 7AL have been reported by several studies (Quarrie et al., 2005 & 2006) and is known to be expressed more frequently under stressed conditions, particularly drought. The strategy adopted in the present study was to compare the location of the 7AS and 7AL yield QTLs with the location of QTLs for traits might be contributed to variation in final grain yield, measured at different stages of development. In this way, it would be possible to assess the likely process involved in the regulation of yield at the 7AS and 7AL QTLs. Comparative mapping with barley, rice, maize and brachypodium identified orthologous regions corresponding to the wheat 7AS and 7AL QTLs. The orthologous sequenced regions, could be used to develop markers such as COS (Conserved Orthologous Set) for fine mapping on advanced populations.



**P5.30 - Specific resistance genes in a wheat Chinese landrace 'Wangshuibai' against two Iranian *Mycosphaerella graminicola* isolates**

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*Septoria tritici* blotch, caused by the fungus *Mycosphaerella graminicola*, is currently the major foliar disease of wheat world-wide, and new sources of resistance and their genetic control are needed to improve breeding for resistance against this disease. An F<sub>10</sub> recombinant inbred population from a cross between the Chinese landrace 'Wangshuibai' and the susceptible cultivar 'Seri82' was tested at seedling stage under controlled greenhouse conditions. Isolate-specific resistance genes to the Iranian *M. graminicola* isolates IPO08002 and IPO08003 were mapped on Chromosomes 2BL and 7DS, respectively. Both genes were derived from cv. 'Wangshuibai', at positions where *Stb9* (on 2BL) and *Stb4* (on 7DS) were reported previously. The two genes are closely linked to microsatellite markers, which can be used for marker-assisted selection. 'Wangshuibai' may therefore be a valuable source of resistance to septoria tritici blotch for wheat breeding, especially in Mediterranean environments.

**P5.31 - Virus-Induced Gene-Silencing approach in studying drought related genes in *Ae. tauschii***

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RNA silencing of endogenous plant genes can be achieved by virus-mediated, transient expression of homologous gene fragments. This powerful, reverse genetics approach, known as virus-induced gene silencing (VIGS), has been demonstrated widely in dicot plant species and recently in some monocot plants such as wheat and barley, where it has become an important tool for functional genomics. Comparing to RNAi and artificial microRNA, VIGS allows rapid characterization of gene function, does not require development of stable transformants, and offers the potential to silence either individual or multiple members of a gene family. Barley Stripe Mosaic Virus (BSMV), a tripartite positive RNA virus has a wide range of hosts, including important cereals as barley, wheat, oat, maize and even some dicots. To apply BSMV-VIGS to *Ae. tauschii*, the D genome progenitor of wheat, four different accessions were analyzed. The present study demonstrated the viral vectors containing 185-bp fragment of phytoene desaturase (PDS) gene succeeded in interfering with the carotenoids biosynthesis pathway, exposing photobleaching in *Ae. tauschii* plants, indicating that BSMV-VIGS can be used in gene functional analysis in *Ae. tauschii*. Moreover, this VIGS system was successfully applied to silence *Dreb2* a member of DREB (Dehydration Responsive Element Binding protein) family involved in abiotic stress signal transduction indicating BSMV-VIGS can be used in functional analysis of *Ae. tauschii* genes including those of signal transduction pathway.

**P5.32 - Allele variation in loci for adaptive response and yield traits in Bulgarian wheat**

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This study comprised the period of the modern Bulgarian wheat breeding started in the beginning of last century. The significant progress in wheat breeding practices started was assisted with the introduction of large number of imported varieties in the selection programs. Some very important productive and reproductive traits of wheat have been introduced in Bulgarian germplasm. Here we study the variation in the microsatellite locus Xgwm261, linked to *Rht8* gene, *Ppd-D1(Ppd1)*, *Vrn-A1*, *Vrn-B1* and *Vrn-D1* loci in order to assess the temporal and geographic distribution of semi-dwarf, photoperiod and vernalization response genes in Bulgarian cultivars and advanced breeding lines released in the the period 1911-2007. Eight allele variants in locus Xgwm261 were identified in the analyzed germplasm collection among which only the 192bp allele is supposed to be referent diagnostic for the presence of *Rht8* gene. The *Ppd-D1a* and *vrn-A1*, *vrn-B1* and *vrn-D1* alleles were determined in almost all wheat genotypes illustrating the relationship between the photoperiod and vernalization response and the adaptability to the regional environments. In this study the association between specific alleles at the *Rht-B1*, Xgwm261, *Ppd-D1*, *Vrn1* loci and plant height, heading time and some yield components are also statistically examined.

**P5.33 - DNA methylation pattern in Turkish bread wheat (*Triticum aestivum* L.) genotypes with CRED-RA analysis**

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Environmental and genetic perturbations induce the novel genetic and epigenetic changes that trigger methylation. Methylation polymorphisms are likely an important source of novelty for crop improvement. In order to evaluate the extent of genome methylation, resistant (*Triticum aestivum* L. cvs. zgi01, Sönmez2001, PI178383) and susceptible (*Triticum aestivum* L. cvs. Aytin98, ES14, Harmankaya99) Turkish wheat genotypes were used as plant material. PI178383 x Harmankaya99, zgi01 x ES14 and Sönmez2001 x Aytin98 crosses for yellow rust resistance variability currently have been using in wheat breeding program of the Anatolian Agricultural Research Institute - Turkey. For the detection of methylation, a pair of restriction endonucleases *HpaII-MspI* was used. All genomic DNA's were screened by 123 RAPD primers and of these 103 primers were showed polymorphism between resistant and susceptible wheat genotypes of crosses. Totally, 729 polymorphic bands were observed in all crosses. Furthermore, all primers used for RAPDs (Random Amplified Polymorphic DNAs), were also tested by CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification), a method to detect DNA methylation, and they produced 796 polymorphic amplification products. Our results showed that epi-marker proportion ranges were 5.2, 4.9, and 2.5% for PI178383 x Harmankaya99, zgi01 x ES14 and Sönmez2001 x Aytin98 combinations, respectively. Resulted data enabled us to evaluate potential epigenetic markers for contribution to appropriate selection of genotypes in wheat breeding programs.



### P5.34 - Association mapping of stress resistance QTL in a European spring barley germplasm

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Barley (*Hordeum vulgare*) is an excellent model system to unravel the genetic bases of plant response to abiotic and biotic stresses. Within the ERA-PG funded project ExBarDiv (Genomics-assisted analysis and exploitation of barley diversity) three different populations - namely cultivar, landrace and wild (*Hordeum spontaneum*) germplasm collections - have been assembled in order to test the efficiency of an incremental association mapping approach for identifying new useful gene alleles. Here we report the evaluation of 230 spring 2-rowed barley cultivars for their resistance to freezing conditions and to leaf stripe disease, caused by *Pyrenophora graminea*. In a first experiment, eight first-leaf stage plants for each accession have been cold acclimated for 4 weeks (3 °C, 8 h light and 2 °C, 16 h dark), then exposed to a temperature of -12 °C for 10 h. To evaluate the effect of freezing on the functionality of the Photosystem II reaction centers, the maximum quantum yield of the PSII photochemistry has been measured by the ratio of variable (Fv) to maximal (Fm) fluorescence in a dark-adapted state (Fv/Fm), after a 24 h recovery time. In a second experiment, for each line, sixty seeds have been surface-sterilized and incubated in three Petri dishes between two Potato Dextrose Agar layers colonized by an actively growing mycelium of the *P. graminea* isolate Dg2. After 20 days of incubation in the dark at 6 °C, the emerged seedlings have been transplanted to pots and grown in the greenhouse (20 °C, 14 h light and 12 °C, 10 h night). Resistance has been assessed as the percentage of plants showing leaf stripes symptoms. The same germplasm collection has been genotyped with 1,536 gene-based SNPs using the Illumina™ OPA (oligo probe assay) high throughput marker technology. Molecular marker information has been used to determine the underlying population structure and perform association analyses between the phenotype and genotype data sets. Broad genomic regions containing potentially useful gene alleles for barley stress resistance will be presented.

### P5.35 - Identification of quantitative trait loci responsible for tolerance to *Microdochium nivale* in triticale

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*Microdochium nivale* is the most widespread component of snow mould disease that attacks winter triticale (*Triticosecale* Wittm.). Due to low level of resistance present in triticale, it is important to identify genetic factors that can be exploited to increase natural resistance in marker assisted selection. There is a little know about genetic determination of triticale response to *M. nivale* and understanding the infection process in all cereals is poor. Thus, there is a need to perform studies to elaborate efficient breeding strategies towards resistance against pink snow mould. A population of 90 DH lines derived from F<sub>1</sub> hybrid of triticale variety 'Modus' and 'Saka 3006', mapped with 1,518 markers (DArT, AFLP and SSR) was used for identifying QTLs. Plants were inoculated with soil-borne mycelium and imitation of snow cover was applied. Control plants were treated the same way except inoculation. All plants were incubated in darkness in the cold chamber. Six parameters connected with morphology and physiology, were selected to measure plant response to *M. nivale*. Principal component analysis showed that overall variation of 95.7% can be explained by interaction of nine independent factors. Single Marker Analysis and Composite Interval Mapping approaches revealed that triticale reaction to snow mould is determined mainly by wheat genome, and respectively nine candidate QTLs on chromosomes 2A, 3A, 5A, 1B, 3B, 5B, 6B, 7B, and 7R were identified with LOD > 3.

The present study was financed by COST/254/2006 and partially by COST Action FA0604.

**P5.36 - Genetic and phenotypic variation in *Eruca sativa* (Brassicaceae) - Searching for adaptive traits in wild populations of crop relatives in Israel**

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Populations of the self-incompatible *Eruca sativa* in Israel cover a narrow distribution range, and grow in diverse habitats ranging from arid environments in the south to mesic environments in the north. An AFLP analysis of nine representative populations revealed two main geographical clusters. One cluster included six populations from the southern and central parts of the distribution range ( $F_{st}=0.048$ ), and the second cluster ( $F_{st}=0.059$ ) consisted of two northern populations. While the differentiation between the two clusters was quite large ( $F_{st}=0.0125$ ), gene flow between the two clusters explains the admixed nature of a population located between them. Expected heterozygosity values ( $H_e$ ) showed similar magnitudes of genetic diversity within all populations. In contrast to these results, in common garden experiments populations of *E. sativa* from the south and from marginal populations in the north showed early bolting and flowering, whereas the others had higher biomass production due to their longer vegetative growth. Ecological and environmental characteristics indicated that in marginal habitats populations of *E. sativa* grow in relatively harsh environments different from those from favorable habitats in the central distribution area. The phylogeographic pattern detected with AFLPs can plausibly be explained as reflecting the existence of Irano-Turanian (south group) and Mediterranean (north group) elements in the study area. Nevertheless, the results show that a full understanding of patterns of genetic and phenotypic intraspecific variation can only be achieved through the combination of molecular and experimental approaches. Moreover, the adaptive differentiation in populations of *E. sativa* point to the potential of the Israeli flora when searching for environmental adaptive traits in wild relatives of crop plants.

# *Posters*

## *Session 6*

### *Genomics for increased sustainability of Triticeae crops production*



### P6.01 - Fine-mapping of genomic regions associated with Philippine downy mildew resistance in maize (*Zea mays* L.)

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Maize is the Philippines' second most important cereal crop next to rice. The crop is the source of livelihood for one-third of the total Filipino farmers, 80% of whom live below the poverty level. Downy mildew caused by *Peronosclerospora philippinensis* Weston (Shaw) is one of the most important diseases limiting maize production in the country. The use of resistant varieties remains the most effective control measure against downy mildew. Bulk segregant analysis (BSA) coupled with simple sequence repeat (SSR) and resistance gene analog (RGA) marker analyses were employed to saturate the quantitative trait loci (QTL) for downy mildew resistance (DMR) and to identify tightly linked SSR/RGA markers. Nine RGA markers were mapped in DMR QTL regions especially in chromosomes 2 and 3, and in the major QTL region in chromosome 8. RGA marker *srga3* mapped exactly within the interval of the major QTL, which is flanked by RFLP markers *umc150* and *asg52*. Four EST-derived SSRs were also mapped in this region and confirmed 100% linked to DMR by BSA. The *srga3* fragment in maize is currently being cloned for sequencing. This is to characterize this putative plant resistance gene ortholog, develop DMR-specific DNA markers for marker-assisted selection, and to isolate the underlying R genes via a QTL map-based approach. RGA *srga3* marker is derived from a plant disease resistance gene sequence of soybean.

### P6.02 - Towards the fine mapping of two major QTLs for grain yield in durum wheat

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Two major QTLs for grain yield and related morpho-physiological traits have been mapped on chromosomes 2BL and 3BS following the analysis of 249 RILs (Kofa x Svevo) tested across 16 field environments characterized by a broad range of water availability (Maccaferri et al., 2008, Genetics 178:489-511). For *QYld.idw-3B*, high map resolution has been obtained by exploiting the sequence information produced from the construction of the physical map of chr. 3B of bread wheat. A total of 30 markers (SSR and ISBP markers) were added in the interval, with a final resolution of ca. 1 cM. All markers were anchored to the physical map, which allowed us to position the QTL peak in the chromosome interval identified by contigs 954 and 918. Further increase in the genetic resolution will be possible through the evaluation of additional progeny derived from the cross of two near-isogenic lines for *QYld.idw-3B* and carrying a recombination event in the target region. To this end, ca. 5,000 F<sub>2</sub> plants have been obtained and are presently being analysed with markers flanking *QYld.idw-3B*.

For *QYld.idw-2B*, markers enrichment has been undertaken exploiting synteny information among cereals. A total of 32 COS (conservative orthologous sequence) markers, from syntenic analysis between wheat and rice, were analyzed. Four COS markers were mapped in the 2BL genetic map. The synteny analysis indicates a good conservation of genes order between the QTL interval on 2BL and chr. 4 of rice. Additional markers are being developed exploring the collinearity between wheat and genes annotated in the distal region of chr. 4 of rice (ca. 700 Kbp) identified by the position of two COS markers mapped in the QTL region.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under grant agreement FP7-212019.

### P6.03 - Exploiting genetic resources for developing rice germplasm with eco-efficient water use

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The Cirad/CIAT rice collaborative project focuses on the development and enhancement of rice composite populations (CPs) through recurrent selection (RS) breeding. Our goal is to develop and diffuse improved material for various rainfed ecosystems in Latin America and the Caribbean (LAC). Our breeding strategy is based on the development of broad-base populations; their improvement through RS and the exploitation of their genetic wealth for line development.

From a base population, four CP were derived, some subjected to selections under acid soils conditions (savannas of Colombian Llanos), others selected under the conditions of Bolivian rice agriculture. We used 16 SSR loci to assess the genetic diversity within these populations and estimate genetic differentiation between them. We applied large scale phenotyping method adapted to field conditions to evaluate the response to drought. Through thermographic infra-red technology (IR) we screened S<sub>1</sub> progenies extracted from each population which were exposed to a 15-day drought period at flowering stage.

Allelic variability measured in the four CP revealed high levels of neutral diversity. The genetic diversity expressed in terms of number of observed alleles per locus ( $N_a$ ) and Shannon diversity index ( $H$ ), was high within the populations ( $N_a > 3.133$ , and  $H = 0.693$  to  $H = 0.800$ ). Significant allelic and genotypic differentiations were found at most loci. A total of six alleles were found with frequency  $> 5\%$  and unique to a particular population. IR screening showed that the CPs hold favorable alleles for resilience to drought. S<sub>1</sub> lines with cool canopy temperatures during the dry period indicated good capacity to maintain transpiration and thus sustained growth under water stress conditions. These lines are potential progenitors to develop a new population with increased eco-efficiency to water use.

Besides showing the genetic wealth retained in these CP, our work presents an advance towards the integration of high-throughput phenotyping and use of molecular markers for improving RS breeding strategy. Our objective is not limited to integrating disciplines; the outcomes of the project, both methodologies and germplasm, are shared among our network of rice breeders from LAC.

#### P6.04 - Application of next generation sequencing for developing large-scale genomic resource in chickpea (*Cicer arietinum* L.)

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Chickpea, an important semi-arid legume crop is seriously challenged by abiotic stresses- drought and salinity. Enhancing genomic resources (molecular markers and candidate genes) is a pre-requisite to facilitate breeding for improving tolerance against these stresses. In this context, a set of 20,162 drought- and salinity-responsive ESTs from four genotypes using Sanger sequencing and 435,018 transcript reads from a normalized cDNA pool derived from 30 different tissues/stages of chickpea cultivar ICC 4958 using 454/FLX sequencing have been generated. Cluster analysis of these sequence data has provided a set of 103,215 tentative unique sequences (TUSs) that is being used for functional annotation, gene structure prediction and genomic synteny exploration. Furthermore, with an objective to develop large scale SNP marker resources in chickpea, mRNA for four parental genotypes of two mapping populations (ICC 4958 x ICC 1882 and ICC 506 x ICC 37) has been sequenced by using Illumina/Solexa sequencing approach. As a result, a total of 118.9 million reads of 36-bp length have been generated. These reads are being aligned with the TUSs of chickpea and > 10,000 SNPs have been identified across the parental genotypes of mapping populations. These resources will be used to develop large scale Illumina GoldenGate assays that would supplement the recently developed pilot GoldenGate assay detecting 768 SNPs based on conserved gene orthologs. In summary, these transcriptomic resources open new avenues for functional genomics in chickpea and comparative genomics across the legume species.

#### P6.05 - C<sub>3</sub> to C<sub>4</sub> photosynthesis engineering strategy in wheat for increasing sustainability of Triticeae crop production

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Modification of genes encoding C<sub>4</sub> enzymes in ancestral C<sub>3</sub> plants to allow their high-level expression in green leaves must increase sustainability of crop production and biodiversity. Genetic improvement of wheat photosynthesis for increased yield by application of plant transformation technologies could be considered as a first step to wheat transformation for increased yield, metabolic engineering and metabolomics. Simple natural germ-line transformation technique by pipetting *Agrobacterium* into the spikelets of wheat before anthesis has been elaborated. Using this method allowed us to produce putative transgenic wheat plants expressing two important maize genes: phosphoenolpyruvate carboxylase (PEPC) and orthophosphate pyruvate dikinase (PPDK). High level of expression of these genes in transgenic wheat plants was determined by assaying the activity of PEPC in leaf protein extract, followed by gel electrophoresis, western, southern blot and PCR analyses. High activities of the enzyme were correlated with the amounts of enzyme protein in the leaves. Most transgenic wheat plants exhibited an enhanced photosynthetic capacity. All transgenic lines showed superior photosynthetic performance under different water regimes. Transgenic wheat lines expressing maize genes produced higher grain yields (25-30%), especially under adverse conditions.



### P6.06 - Phenotyping for drought tolerance in selected chickpea (*Cicer arietinum* L.) germplasm in semi-arid areas of Kenya

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Due to persistent food shortages in semi-arid tropics, screening for alternative drought tolerant crop varieties could provide reliable solution to reducing these adverse effects. Chickpea is a good alternative food-security legume crop since there is more drought. This study was aimed at phenotyping for drought tolerance and yield performance in reference sets of selected chickpea germplasm to be grown in marginal rainfall areas of Kenya. The study was conducted in one dryland site in Kenya (Koibatek) for two seasons during the short rains and long rains (October-March 2008 and March-Aug 2009). The trial evaluated 289 lines for drought tolerance, harvest index (HI) and yield in a lattice design with two replicates, spaced at 40 x 10 cm, 2 m long. The findings showed significant difference ( $P < 0.05$ ) in measured traits among the test genotypes; with days to flowering and maturity ranging between 44-74 and 82-144 days, respectively. Mean HI ranged from 0.18-0.69 (mean 0.45). Genotypes ICCV 10, ICCV 7272, ICCV 92311, ICC 3362, ICC14595 had greatest HI (0.63-0.69) and moderate grain yields  $> 3$  tons ha<sup>-1</sup>. Genotypes ICCV 7272, ICCV 92311 and ICC14595 had high 100-seed weight (23-35 gm) which could have contributed to high yield ( $r = 0.65^{**}$ ). Highest yielding genotypes were IG 6905, ICC 2580, ICC 7315, ICC 3631, IG 10500, ICC 7255 and ICC 8855, though they had moderate HI values. From these findings several genotypes, with acceptable quality traits will be evaluated further and released as commercial chickpea varieties for growing in semi-arid lands of Kenya under TLII activities. Our study showed high potential for chickpea as an alternative food-security legume for ASALS of the ESA region.

### P6.07 - Tilling and mutagenesis, a new approach for the elucidation of the mechanisms of tolerance to dryness and the development of adapted genotypes

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Durum wheat is the one of strategic and important crop cultivated in Morocco. It occupies the second place after bread wheat in term of area and production. In spite of its importance, this culture knows fluctuations and/or apparent regressions dues to the problems of dryness which are sometimes intense, unforeseeable and variables from one year to another. The development of tolerant varieties requires the comprehension of the mechanisms of tolerance. In this objective, Mutagenesis and Tilling (Target Induced local lesions) was developed. A Tilling population of 6,000 lines was produced from Cham1 variety. Seeds were irradiated by the use of chemical radiations using Ethyl Methane Sulfane 0.6% (EMS 0.6%). The population was advanced in generation M5/M6 and M7. An evaluation with respect to the biotic stress (leaf rust, septoria, the powdery mildew and other diseases) and in unfavourable Bours conditions was carried out. The population show high variable and contrasting result for leaf rust and septoria disease. For dryness aspect, the evaluation in unfavourable conditions at the maturation stage show high contrasting result of fluorescence content. Agronomics traits (number of spikes, tillers, 1,000-seed weight) are also variable between lines of tilling population and performed lines were selected. The reverse genetics (Tilling), new technique, is developing on M3 generation for 2,500 lines for genes as the dehydrins, Ga20ox, known for their association with the dryness.

## P6.08 - Mapping QTLs for powdery mildew resistance in durum wheat

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To dissect the genetic basis of the resistance to powdery mildew (Pm) carried by Claudio, a population of 181 RILs from the cross Claudio (resistant) × Meridiano (highly susceptible) has been profiled with 207 SSR and 716 DArT<sup>®</sup> markers and evaluated in two replicated field trials carried out under artificially inoculated conditions during 2007 and 2008. A genetic map of 2487 cM in total was used to carry out multiple interval mapping QTL analysis. The QTL analysis evidenced a complex genetic control of the powdery mildew response. Major QTLs were located on chr. 6BL (*QPm.ubo-6B*) and 7BL (*QPm.ubo-7B*) with the resistance alleles contributed by Claudio. In both years, the effect of *QPm.ubo-7B* against Pm infection declined as the disease progressed, while *QPm.ubo-6B* was undetectable in the early stage of the disease cycle and increased its effectiveness as the disease progressed. At the seedling stage, most of the phenotypic variance among RILs was accounted for by *QPm.ubo-7B*. Additional minor QTLs with lower and less consistent effects across years were found on chrs. 2BS, 2BL, 6AS and 7AS, with both parents contributing the resistance alleles. *QPm.ubo-7B* is positioned in the distal end of chr. arm 7BL (*Xbarc340*, *Xgwm146* and *Xgwm344*) and possibly located in the homoeologous position of *Pm1*. *QPm.ubo-6B* is positioned near *Xwmc539*, *Xbarc79*, *Xgwm1682* and *Xgwm889*. Molecular marker enrichment for the two chromosome regions is being carried out using RGA profiling and the Conserved Orthologous Sequences (COS markers) information from the wheat, rice and *Brachypodium* synteny analysis. Up to now, 13 RGA polymorphisms and two COS markers have been mapped in the *QPm.ubo-6B* QTL region. As to the COS marker approach, a major limitation is represented by the low polymorphism rate in sequence variation observed between the two parents (1bp polymorphism / ca. 2,000 bp). The two major QTLs are valuable targets for marker-assisted selection in durum wheat with the aim of increasing the resistant response to powdery mildew.

Research carried out with the financial contribution of the European Union (Sixth Framework Programme, Contract no. 513959, BIOEXPLOIT Integrated Project)

## P6.09 - Genetics of *Soil-borne cereal mosaic virus* (Sbcmv) response in durum wheat

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*Soil-borne cereal mosaic virus* (SBCMV) is a Furovirus transmitted by *Polymyxa graminis* Led. that causes an important wheat disease widespread in the main wheat-growing areas of the world including Italy. In this study, a recombinant inbred population of 181 lines (RILs) obtained from the cross between the medium-resistant cv. Meridiano and the susceptible cv. Claudio plus a germplasm collection of 111 cultivated durum accessions suitable for association mapping (AM) analysis were characterized in field experiments carried out in 2007 and 2008 near Bologna, Italy, under severe and uniform SBCMV infection. A genetic linkage map of 2487 cM including 207 SSR and 716 DArT<sup>®</sup> markers, has been assembled from the RIL population. A wide range of disease reaction (as estimated by symptomatology and DAS-ELISA) was observed for both RILs (transgressive segregation) and germplasm accessions. At least 12 QTLs accounted for most of the phenotypic variation observed. A major QTL (*QSbm.ubo-2B*) identified in the distal 2BS chromosome region accounted for most of the SBCMV response in the Meridiano × Claudio RIL population (QTL  $R^2$  of 91.6 and 85.3% in 2007 and 2008, respectively). The major QTL mapped in 5 cM region coincident with the location of *Sbm2* locus, identified as a minor SBCMV response QTL in bread wheat. The AM analysis confirmed the role and location of the major QTL. *QSbm.ubo-2B* appears as a good candidate for marker-assisted selection and for positional cloning.

Research carried out with the financial contribution of the Emilia-Romagna Region and the CARISBO Bank Foundation, project "Genomica Grano Duro".

**P6.10 - Genetic characterization and relationships of 60 clones of sweet potato cultivated in Mozambique**Maquia I<sup>1</sup>, Muocha I<sup>1,2</sup>, Neves L<sup>1,3</sup>, Andrade M<sup>4,5</sup>, Ribeiro A<sup>6</sup><sup>1</sup> Centro de Biotecnologia, Universidade Eduardo Mondlane, Maputo, Moçambique<sup>2</sup> Faculdade de Agronomia e Engenharia Florestal, Universidade Eduardo Mondlane, Maputo, Moçambique<sup>3</sup> Centro Internacional dela Papa, Lima, Peru<sup>4</sup> Instituto de Investigação Agrária de Moçambique, Maputo, Moçambique<sup>6</sup> Instituto de Investigação Científica Tropical, Portugal

Sweet potato is a significant food crop in developing countries ranking as the seventh most important crop in the world, and the fifth in developing countries after wheat, rice, maize, and cassava. In Mozambique it is considered the third most important crop, mainly as a dry season staple for lower income population. The wealth of diversity in sweet potato present in Mozambique remains largely untapped due to lack of knowledge. Characterization of germplasm diversity and the genetic relationships among cultivars and genotypes could prove useful in the implementation of breeding programs. In this study, we used RAPD markers (Random Amplified Polymorphic DNA) to genetically characterize sweet potato clones from various origins. The sampling consisted of 60 clones collected in four provinces in Mozambique (40), as well as clones from South Africa (6), Zimbabwe (3) and Kenya (1), Uganda (1) while some samples also came from USA (1) or were obtained from CIP (Potato International Center) (6) and IITA (International Institute of Tropical Agriculture) (2). RAPD markers were generated using five random primers. Data were recorded manually, and only clear polymorphic bands were scored for either presence (1) or absence (0). A total number of 80 scored polymorphic fragments with a mean of 11.4 bands per primer were generated. Analyses were performed (using the NTSYS.pc 2.0 software) to investigate the relationships amongst the different clones by determining the DICE coefficient of similarity and constructing phylogenetic trees. The primers used were able to distinguish all the 60 clones. The phylogenetic trees obtained showed that the several clusters were divided according to the collection site of the clones. The clones originated from USA and CIP belonged to the same cluster. The South African clones were quite close to the Mozambican ones, except for one (Tacna) that was highly distant from the others, and incidentally happened to show the highest resistance to water deficit. This typing method developed thereby should provide a useful tool in programs aimed at improving the quality of the crop, particularly as drought-resistance is concerned.

### P6.11 - Molecular diversity and variation of *Sub1* gene expression in relation to submergence tolerance in rice

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Breeding for improving submergence tolerance in rice cultivars is a major objective for flood prone areas of Asia. A cluster of three ERF like genes at the SUB1 locus (chromosome 9) has been identified from a rice variety, FR13A that confer tolerance to complete submergence for about 14 days. Genetic diversity is one of the important issues to find out additional sources of tolerance to submergence. Further, SUB1 gene-expression differences contributing to phenotypic variation are useful to understand the mechanisms of submergence tolerance. In the present investigations, SSR-based clustering among 160 flood-prone rice accessions and expression studies of *Sub1A* and *Sub1C* loci (conferring submergence tolerance) were undertaken, using semi-quantitative RT-PCR method. Microsatellite cluster analysis at 30 SSR loci grouped the varieties into four major clusters (based on geographical origin) and four sub-clusters. SSR-based clustering and submergence screening indicated that a diverse collection of accessions could provide additional diversity and submergence tolerance sources for further studies. SNPs study generated three patterns at *Sub1A* locus and four patterns found at *Sub1C* locus. Of the eight haplotypes (combination of SNPs), A1C1 haplotype was most tolerant than other haplotypes, indicating major role of *Sub1A1* and *Sub1C1* alleles for the tolerance. Haplotype specific expression of two ERF genes indicated that allelic expression variation affected the tolerance level of different haplotypes, differentially. In tolerant varieties (IR40931), the highest expressed gene was *Sub1A*; while expression level of *Sub1C* was low. In susceptible Fulkari, the highest-expressed gene was *Sub1C* and the *Sub1A* was not expressed. In moderately tolerant varieties (Motorsail and Kottamali) both *Sub1A* and *Sub1C* transcripts were up-regulated by submergence. These studies also demonstrated that two hybrids had unequal expression of *Sub1A* and *Sub1C* alleles that also affected the elongation patterns differentially. Selection of hybrid 2 (Kalukanda/Swarna *Sub1*) might led to improvement of tolerance in presence of moderate level of elongation having balanced level of *Sub1A* and *Sub1C* transcripts. These finding could be used as a new innovation to develop intermediate tall improved rice varieties with submergence tolerance for flood prone rice areas of south-east Asia, where adaptation of semi-dwarf rice varieties are limited.

### P6.12 - Maize genetic transformation with the *SbMATE*, aluminum tolerance gene isolated from sorghum

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The toxicity of aluminum (Al<sup>3+</sup>) is a major limiting factor for plant growth in acid soils which represent 68% or 250 million hectares of Brazilian territory and nearly 50% of arable land on the planet. Recently, in sorghum was identified and isolated by positional cloning technique and comparative genomics, a major aluminum tolerance gene, called *SbMATE*. This gene encodes a member of a membrane transporters family responsible for efflux of citrate in roots of sorghum. The present research test the hypothesis of the use of this gene for the generation of transgenic maize cultivars with higher levels of adaptation to acid soils. Callus of maize line Hi-II, were transformed via *Agrobacterium tumefaciens* with the gene *SbMATE*. The gene construction used *SbMATE* gene under the control of the ubiquitin promoter and bar gene driven by CaMV35S promoter. Transformed cells were selected in media containing the herbicide ammonium glufosinate. From the five transgenic plants containing the bar gene four also had the *SbMATE*. The presence of these genes has been shown by PCR specific primers. The high expression in these four plants has been shown by Real-time PCR analysis comparing non-transgenic plants of the same genotype. Results have demonstrated an effect of the sorghum *SbMATE* gene in maize which would bring a great impact in the acidic soils agriculture around the world.

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### P6.13 - Dehydrin genes diversity and their expression in response to water deficit in ecotypes of cowpea from Mozambique

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Cowpea (*Vigna unguiculata* (L.) Walp) is a tropical grain legume, playing an important nutritional role in developing countries of the tropics and subtropics. Its production is limited by a lot of environmental stresses of which drought appears one of the most critical. It has been reported that cowpea includes in its genome genes associated with environmental stresses. One of the most prominent groups of proteins belongs to a family called dehydrins. They are expressed at the last stages of embryogenesis and in response to various stresses, such as drought, high salinity, low temperature or to abscisic acid application. It has been suggested that dehydrins act through stabilizing large-scale hydrophobic interactions, such as membrane structures and hydrophobic patches of proteins. As their expression increase in response to dehydration, we proposed to use dehydrins as marker to drought tolerance. For this purpose, fifteen ecotypes of cowpea collected in different sites of Mozambique, six of which were wild and nine cultivated, were used. Two approaches were taken. First, a fragment of dehydrin was amplified and used to analyse the variability of dehydrin genes in cowpea. This revealed a high level of diversity in this protein family, with a high identity to the *V. unguiculata* dehydrin genes associated with chilling tolerance. We also used semi-quantitative RT-PCR (Reverse transcription PCR) to evaluate dehydrin gene expression in plants subjected to water stress. We found that the expression was higher on the wild ecotypes evidencing a greater adaptation to water deficit in this group of ecotypes as compared to the related cultivated ecotypes.

### P6.14 - Development and agronomic evaluation of contiguous segment substitution lines (CSSLs) in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Pearl millet is a highly cross-pollinated diploid C4 cereal, grown for grain and dry fodder in the semi-arid and arid tropics. Pearl millet productivity is severely constrained by drought stress, a regular occurrence in these regions. Application of molecular markers and genetic linkage maps using conventional mapping populations has identified specific genomic regions controlling polygenic traits, termed 'Quantitative Trait Loci' (QTLs), in all major crops. Resolution power of QTL identification is limited in conventional mapping populations by overshadowing effects of major QTLs, preventing effective estimation of 1) numbers of independently segregating minor QTLs and 2) interactions between pairs of unlinked QTLs. Considering utility of introgression lines for identification of QTLs responsible for grain and stover yield, the present study developed and evaluated a set of pearl millet substitution lines from advanced backcross populations derived from elite seed parent ICMB 841-P3 and *Iniari* landrace-derived seed parent 863B-P2, known to differ for downy mildew resistance, drought and salinity tolerance, straw ruminant nutritional quality, grain Fe and Zn concentration, and grain and stover yield components. Over 1,090 advanced backcross progenies, expected to provide coverage across most of all seven pearl millet linkage groups, were genotyped using 75 marker loci (49 SSRs, 21 SSCP-SNPs and 5 STSs) to identify 134 CSSLs (12 for LG1, 10 for LG2, 10 for LG3, 41 for LG4, 32 for LG5, 12 for LG6 and 17 for LG7). Fifty were unique introgressions from 863B-P2 in ICMB 841 background. Testcross evaluation of CSSLs for LG3, LG4 and LG5, using genetically diverse, elite inbred pollinators H 77/833-2, PPMI 301 and RIB 3135-18, was conducted under terminal drought stress and fully-irrigated conditions. Twelve phenotypic characters related to grain and stover yield were recorded and line × tester analysis was performed estimating general combining ability (GCA) of each CSSL in each environment and across these environments. Several CSSLs had significant positive GCA for grain yield and stover yield. Results suggested presence of a straw yield drought tolerance QTL on LG3 that was not detected in earlier studies using testcrosses of a small biparental F2-derived F4 mapping population based on these same parental lines.



## P6.15 - iMAS 2.0: A unified computing and decision support system for marker-aided breeding

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Over the years, molecular marker technology has become an integral part of any breeding programme due to its advantages over conventional breeding programs including study of multiple genes, late expression of trait, seasonal and geographical considerations. An ideal molecular breeding programme involves selection of parental lines segregating for target traits, development of mapping population, genotyping of segregating population with molecular markers and phenotyping of the test units in appropriate environments. The phenotyping and genotyping data obtained are subjected to sophisticated algorithms to obtain phenotypic mean and to construct linkage map. Once the linkage map and mean are available, several advanced methodologies can be used to detect quantitative trait loci (QTL). QTL-flanking markers are then used to transfer linked loci to improve the trait value of existing parental lines.

All these analyses require understanding of different software at each step of the analysis as none of them are able to perform all these analyses at one go. The presently available software require researchers to manually prepare the required input data files for these software and do each analysis one by one, as they move from one software to the other. This process is highly time consuming, tiring and fraught with errors. To overcome these potential bottlenecks, iMAS project was initiated.

iMAS is a single, unified computing and decision support platform to facilitate marker-aided selection and breeding through integration of a number of freely available open-source quality computing tools. The iMAS system frees the user from the painful, time-consuming and error-prone manual preparation of input data files required by these software by automating the whole process.

The system comprises of six modules: *Data Validation*, *Phenotyping*, *Linkage Map Building*, *QTL Analysis*, *Genome Display* and *MABC Sample Size*. Salient features of the system are integration of different computing tools into one single platform, extensive but simple-to-use online decision guidelines and a manual which allows the user to use iMAS and interpret results correctly. Because of these features iMAS has also emerged as an excellent tool for teaching and training purposes and till date over 150 breeders have been trained in the use of iMAS system.





# *Posters*

## *Session 7*

### *Genetic resources for nutritional quality*



### P7.01 - Citrus genetic resources of Pakistan and nutritional analysis of some potential cultivars for diversification of citrus industry

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The citrus industry of Pakistan is monopolized by Kinnow (*Citrus reticulata* Blanco), it contributes over 70% of the total citrus production. Citrus germplasm plays key role in evolving new high yielding, nutritionally improved and disease resistant varieties for sustainable development and diversification of citrus industry. A study was initiated to document the indigenous and exotic germplasm of existing citrus species/cultivars in Pakistan. Various research centers/institutes of the country (Punjab, NWFP, Sindh, Baluchistan and Federal areas) were visited to collect detailed information of existing citrus species/cultivars. About 210 citrus cultivars were recorded out of which 154 were scions and 56 were rootstocks. Maximum collections of citrus cultivars (210) were recorded in Punjab province followed by Federal areas (80), NWFP (46), Sindh (9) and Baluchistan (8). Among different groups of citrus, 54 cultivars were of sweet oranges, 28 cultivars of lemons and limes, 27 cultivars of mandarins, 15 cultivars of grapefruits, and 27 hybrids were observed along with 56 type of rootstocks and two cultivars of kumquats. Important cultivars of citrus as Sweet Oranges (Succari, Pineapple, Hamlin, Valencia Late, Salustiana, Musambi, Blood Red) Mandarin (Kinnow, Feutrell's Early, Wilking, Freemont, Honey, Fairchild) Grapefruit (Marsh Seedless, Duncan, Shamber) along with Eustis lime were tested for physiochemical properties. Results indicated that among sweet orange cultivars Salustiana produced the heaviest fruit (218.20 g) and maximum juice percentage (54.05%). Maximum TSS (11.51) and minimum acidity (0.2%) was observed in Succari. Among mandarins maximum fruit weight (189.6 g) was observed in Kinnow followed by Fairchild, Wilking, Honey, Feutrell's Early and Freemont. Maximum juice percentage (54.90) and TSS (12.09) was observed in Freemont. Lowest acidity (0.70%) was observed in Feutrell's Early while maximum amount of vitamin C (43.37 mg) was found in Wilking. Among grapefruit cultivars Marsh Seedless was heaviest with maximum acidity and vitamin-C. Results showed that the sweet orange cultivars and mandarins other than Kinnow have much higher potential for sustainable industry development in Pakistan. We believe this information will contribute towards researchers/academicians in understanding citrus genetic resources in Pakistan and their better utilization in the national and international citrus improvement research programmes.

### P7.02 - Variety-dependent variations in phytonutrient compounds in seeds of adlay (*Coix lacryma jobi* L.) most commonly cultivated in South Korea

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To characterize health beneficial effects of adlay (*Coix lacryma jobi* L.), eight adlay varieties commonly cultivated in Korea were selected and their unpolished and polished seeds were used for quantification of various nutraceutical compounds: tocopherol, tocotrienol, squalene, phytosterols, and fatty acid composition. Gamma tocopherol and gamma tocotrienols were two major forms of vitamin E of unpolished adlay seeds with an average value of 1.47 and 1.42 mg/100g, respectively. In the case of unpolished seeds squalene, campesterol, stigmaterol and sitosterol contents ranged from 2.01 to 7.44, 5.49 to 10.23, 7.6 to 18.04, and 20.72 to 40.26 mg/100 g, respectively, with an average of 4.12, 8.04, 13.11 and 31.34 mg/100 g, respectively. No prominent differences could be observed between polished and unpolished seeds in most tested varieties in most tested compounds, with an exceptional case of variety 'Johyeon', which showed the lowest total vitamin E content (3.172 mg/100 g) in unpolished case, but the highest total vitamin E (5.136 mg/100 g) in polished case compared to other tested varieties. In both polished and unpolished cases, oleic acid and linoleic acid were two major fatty acids consisting of 46 and 38%, respectively.

### P7.03 - Starch metabolism mutants in barley: A TILLING approach

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At DiSTA (University of Bologna), a sodium azide-mutagenized barley (cv. Morex) population of ca. 5,000 M<sub>3</sub> families, named TILLMore, has been developed for identifying mutants at target genes using the TILLING procedure (Talamè et al., 2008, Plant Biotechnol J. 6: 477-485). We utilized the TILLING approach to identify mutants for genes related to starch metabolism in barley. Starch is the major reserve of plants and serves as primary carbohydrate component in human and livestock diets and has also numerous industrial applications. Mutants for biosynthetic or regulatory genes of starch metabolism often produce starch granules with abnormal morphological and molecular features that could be of interest also for technological applications. Molecular screening of TILLMore for mutations has already been completed for five starch-related genes (*Limit dextrinase1*, *GBSSI*, *Bmy1*, *SSI* and *SSI1*) with a total number of 20 mutants identified. Almost all the mutations detected were CG-TA transitions and several (ca. 60%) implied a change in amino acid sequence and therefore possible effects on phenotype. In four cases, we identified non-sense or splice junction mutations which affect drastically the protein function. Characterization of the mutants identified so far is under way.

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### P7.04 - Nutritional quality of einkorn wheat (*Triticum monococcum* L.) kernels

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Einkorn (*Triticum monococcum* L.) is a hulled wheat, widely cropped and eaten in prehistoric times but largely neglected today. Current trends towards low-impact and sustainable agriculture as well as an increase in the consumption of healthy and functional food suggest that it may still play a role in human consumption. Therefore, einkorn potential for human consumption was assessed by analysing the chemical composition of several *T. monococcum* accessions, as well as *T. turgidum* and *T. aestivum* controls, grown at S. Angelo Lodigiano, Italy.

A broad within-einkorn variation for all the traits analysed was observed. Compared to the controls, on average einkorn seeds had high protein content ( $18.2 \pm 1.48\%$ ), high ash content ( $2.35 \pm 0.165\%$ ), low total starch ( $65.5 \pm 2.56\%$ ) and average amylose ( $25.7 \pm 1.23\%$  of total starch). Carotenoids and tocopherols (lipophilic antioxidants) were more concentrated in *T. monococcum* than in *T. turgidum* and *T. aestivum*: carotenoids, mostly lutein, averaged  $8.4 \pm 1.40$  mg/kg, and several accessions showed significant amounts of carotenes (above 25% of total carotenoids); tocopherols, mainly  $\gamma$ -tocopherol, averaged  $78.0 \pm 8.73$  mg/kg dm. Einkorn had also high contents in fructan ( $1.90 \pm 0.19$  g/100 g) and microelements: Zn ( $69 \pm 9$  mg/kg), Fe ( $49 \pm 4$  mg/kg), Mn ( $43 \pm 4$  mg/kg) and Cu ( $8 \pm 0.4$  mg/kg). Furthermore, lipid content was abundant ( $4.2 \pm 0.40$  g/100g) and 14 compounds were identified, mainly linoleic (50.8%) and oleic (24.8%) acids; 80.6% of total fatty acids were unsaturated.

The analysis of several chemical parameters across a representative sample of *T. monococcum*, *T. turgidum* and *T. aestivum* confirmed the superior nutritional value of einkorn.

#### P7.05 - QTL mapping for micronutrient content in brown rice

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Fe, Mn, Zn, Cu are essential trace elements for human health. In the present study, two *indica* black pericarp rice cultivars, Xiangheino 195 and Zhonghuazixiangnuo were used to construct a F<sub>2</sub> population which were employed to construct a linkage map containing 132 SSR markers and to perform QTL mapping for Fe, Mn, Zn, Cu content in brown rice grain. The Fe content of the F<sub>2</sub> population showed a continuous distribution and biased to the parent with lower content, while Mn, Zn, Cu content displayed a normal distribution. Correlation analysis showed a significant positive relation between the four trace elements. 14 QTLs controlling Fe, Mn, Zn, Cu content in the brown rice grain were identified on chromosomes 1, 2, 4, 7, 8, 10, 11. There are 7 QTLs for Fe content with a total additive contribution of 44.09% and a total dominant contribution of 22.04%. QTL for Fe content on chromosome 1 flanked by RM6464 and RM6340 had the largest additive contribution of 17.63% and the largest dominant contribution of 8.82%. One QTL for Mn content was detected on chromosome 8 and located between RM6850 and RM547, explaining a variation of 22.71%. Three QTLs for Zn content were located on chromosomes 4 and 8 with a total additive contribution of 32.73% and a total dominant contribution of 25.16%. We also found three QTLs for Cu content distributing on chromosomes 8 and 10. Among of them, *qCu8* had an additive contribution of 27.68%, while *qCu10-2* had an over-dominant contribution of 35.43%. A total of 19 pairs of epistatic QTLs were identified affecting Fe, Mn, Zn, Cu content, explaining a phenotypic variation of 52.82, 6.83, 29.82 and 28.01%, respectively. The QTL mapping results suggested that the content of the four trace elements was controlled by multiple genes and influenced by different genetic factors. QTLs with main additive effect would be useful in marker assisted breeding program.

#### P7.06 - A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype

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Rice genotypes with pigmented caryopses have received increased attention because of their antioxidant properties. Previous works evidenced that the kernel of red rice is characterized by the presence of proanthocyanidins, whereas black rice is characterized by the presence of anthocyanins. At the CRA-GPG the antioxidant properties of a set of Italian rice varieties (three white, two black and five red ones) were evaluated to establish the existing genetic variability for these parameters. To this aim, the total antioxidant capacity and the concentration of the main classes of antioxidant compounds (tocols, g-oryzanol, proanthocyanidins, anthocyanidins and total polyphenols) were studied. We also characterized the allelic variants responsible for kernel pigmentation.

The pigmented rices, on average, had a TAC four times higher than the white ones. As expected, red-grained genotypes contained no detectable anthocyanins and one black rice contained no detectable proanthocyanidins. However, the black-grained cv. Artemide had large amounts of both proanthocyanidins and anthocyanins. This genotype was also characterized by the highest TAC and polyphenol content: its TAC was about twice the TAC of the other pigmented rices, and it had a polyphenol content 2–3 times the content found in the other pigmented rices. Pigmented genotypes are confirmed to be very interesting to breed rice for high polyphenol content and TAC. Furthermore, the possibility to select for genotypes accumulating both anthocyanins and proanthocyanidins provides a way to substantially increase the polyphenol content and TAC of the rice caryopsis.

### P7.07 - Diversity of seed storage protein patterns of Slovak accessions in jointed goatgrass (*Aegilops cylindrica* Host)

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Variations in seed storage protein patterns for 12 accessions of *Aegilops cylindrica* populations collected in Slovakia within the framework of the bilateral Co-operation in Science and Technology between the Slovak Republic and Hungary was investigated. The present study covered the populations of jointed goatgrass collected from the southwestern (localities: Sered, Dunajská Streda), southern (localities: Chaba, Kamenica nad Hronom) and southeastern (localities: ierna nad Tisou, Dobrá) parts of Slovakia. Seed storage protein patterns were analyzed using acid polyacrylamide gel electrophoresis (A-PAGE) method. Electrophoreogram for each population was scored. Electrophoretic analysis has revealed appreciable polymorphism in the number of gliadin bands. The most variation in gliadin bands among the populations was observed from Dunajská Streda. Small differences were detected among the populations from ierna nad Tisou, Dobra, Kamenica nad Hronom and Sere. The lowest variations were detected in populations from Chlaba. The result from comparison with protein types of Hungarian populations reveal that protein type from Kamenica nad Hronom population contain similarity bands with Bokros 4 populations from Hungary. The present investigation showed that the jointed goatgrass populations collected from Slovakia exhibit valuable genetic resources for wheat crop improvement programs.

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### P7.08 - Characterization of durum wheat (*Triticum durum* Desf.) quality from gliadin and glutenin protein composition

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Glutenin polymers are formed by high (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The aim of the studies was the electrophoretic characterization of evaluation of gliadin and glutenin proteins in kernels of *Triticum durum* Desf. All 108 accessions originating from different geographical areas of world were evaluated for high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW) composition using SDS-PAGE and A-PAGE. The data indicated the prevalence of the null allele (81%), allele 1 (8%) and allele 2\* (8%) at the *Glu-1A* and five alleles, namely 7+8 (36%), 6+8 (33%), 13+16 (10%), 20 (9%) and 17+18 (4%) represented at the *Glu-1B*. Protein subunit *Glu-1A1* was correlated positively with improved dough strength as compared to subunit null. On the chromosome *Glu-1B* subunit 6+8 was associated with slightly stronger gluten type than 7+8 and 13+16, whereas subunit 20 was associated with weak gluten properties. On the basis of electrophoretic separation of gliadin fraction it was found that 87 genotypes contained -45, 3 genotype -42 and 18 genotypes another one. Cultivars having the low molecular weight (LMW) glutenin allele LMW-2 (or gliadin band  $\gamma$ -45) generally gave stronger gluten than lines with allele LMW-1 (or gliadin band  $\gamma$ -42). The combined better alleles at *Glu-B1* (coded bands 6+8, 13 + 16, 7 + 8) and *Glu-3* (patterns LMW-2) showed linear cumulative effects for dough strength. These results could provide a more complete understanding of the studied collections diversity on high molecular subunits and it will be useful to breeders who now possess a tool to formulate crosses by choosing varieties with appropriate characters.

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### P7.09 - Molecular characterization of *Glu-Ay* gene from *Triticum urartu* for its use in quality wheat breeding

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The endosperm storage proteins of wheat are widely associated with the bread making quality. These proteins are divided in two main groups (gliadins and glutenins) according to their molecular characteristics. Glutenins are also divided in high molecular weight (HMWGs) and low molecular weight (B-LMWGs and C-LMWGs) subunits. Among these proteins, the HMWGs coded by genes at the *Glu-1* loci located on the long arm of group-1 homoeologous chromosomes are the best studied. Each locus contains two tightly linked genes that encode for two types of HMWGs, called  $x$ - and  $y$ -type. In cultivated wheats, the *Ay* genes are not usually expressed. These genes are expressed in wild diploid as *Triticum urartu* Thun. *ex Gandil* ( $2n = 2x = 14; A^uA^u$ ).

In a previous study, 169 accessions of this species were analysed for the seed storage composition. Up to 17 allelic variants for the *Glu-A1* locus were detected. All alleles showed the expressed *Ax* subunit, but only 9 of them were active for the *Ay* subunit. The aim of the current study was the molecular characterization of these *Ay* genes (active or not) for its possible use in the wheat breeding. The *Ay* sequence of these 17 allelic variants were analysed by PCR amplification. Both the entire coding sequence and the central-repetitive domain were amplified with specific primers. The results showed that the size differences were always due to the size of the central-repetitive domain. So, for obtaining information on the internal structure of each gene, the PCR products were digested with two endonucleases (*DdeI* and *PstI*). This showed the presence of two different gene families according with the restriction pattern, which has not similar to the patterns showed in the cv. Cheyenne and Alaga, using as standards. Because of many studies have suggested that the presence of active *Ay* genes could increased the bread-making quality in durum and common wheat, these new active alleles could be an useful tool for enlarging the quality genetic pool of the modern wheat.

### P7.10 - Polymorphism of waxy proteins in Spanish hulled wheats

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Hulled wheats are the wild or cultivated species of the *Triticum* genus, this denomination makes reference to that their glumes remain adhered to the grain after threshing. In Spain, three species of cultivated hulled wheats were widely sown until the late 1960s: einkorn (*Triticum monococcum* L. ssp. *monococcum*;  $2n = 2x = 14, AA$ ), emmer (*T. turgidum* ssp. *dicoccum* Schrank;  $2n = 4x = 28, AABB$ ) and spelt (*T. aestivum* ssp. *spelta* L. en. Thell.;  $2n = 6x = 42, AABBDD$ ); although nowadays only emmer and spelt wheat survive in marginal farming areas of Asturias (North of Spain). Fortunately a part of the biodiversity than once existed is currently conserved in Germplasm Banks. These collections have showed to be a useful gene reservoir for quality improvement in breeding programs, independently of their development as crops in modern agriculture that demands new products. With respect to quality, starch is the main component of the wheat endosperm and consists of two types of glucose polymers, the essentially linear amylose and the highly branched amylopectine, in a ratio of 22-35%/68-75%. The *waxy* proteins are the enzymes responsible for amylose synthesis during the seed filling. Since some starch properties such as gelatinization, pasting and gelation depend on the amylose/amylopectine ratio; consequently, the *waxy* protein is crucial for flour quality. The aim of this survey was to analyse the polymorphism of the *waxy* proteins in a wide collection of Spanish hulled wheats. The *waxy* proteins were extracted from purified starch and separated by SDS-PAGE. Several alleles were detected in the three species, including *null* alleles in emmer and spelt wheat and a new allele with different electrophoretic mobility not described previously in einkorn wheat. The *null* alleles were confirmed by two dimensions electrophoresis (IEF×SDS-PAGE). In conclusion, the current study showed that the evaluation of these traits could be very important for the genetic diversity conservation of these old and renewed crops, together with its possible use for enlarging the gene pool of *waxy* proteins in the modern wheats.



### P7.11 - Genetic diversity of nutraceutical compounds in 394 Korean rice landraces

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To characterize phytonutrient property of Korean rice, 394 landraces were selected and their brown rice contents of alpha (α)-, beta (β)-, gamma (γ)-, and delta (δ)- tocopherol (T) and tocotrienol (T3), squalene (SQ), campesterol (CA), stigmasterol (ST) and sitosterol (SI) were determined. The average contents of αT, βT, γT and total T were 8.3, 0.3, 1.1 and 9.7 μg/g, respectively and αT3, γT3, δT3 and total T3 were 6.5, 17.4, 0.3 and 24.3 μg/g, respectively. The average contents of SQ, CA, ST, SI, and total phytosterols (TP) were 33.9, 25.1, 43.2, 94.6, and 162.8 μg/g, respectively. Among tested compounds, γT which ranged from 0.15 to 7.67 μg/g exhibited the highest coefficient of variance (68.7%), while αT showed the lowest variation (CV 27.9%). The variety and its content (in μg/g) which showed the highest value for each nutraceutical compounds were as followings: αT, Hwaseongbatchal (18.0); γT, Sun (7.7); αT3, Arongbyeon (28.2); γT3, Joseokjo (34.8); SQ, Kunjo (91.3); CA, Sun (64.5); ST, Hwanghaedo (96.6), and SI, Nokdudo (223.4).

### 7.12 - Biochemical characterization of the starch by analytic methods

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The lack of information of the varietal characteristics of common wheat requires a biochemical characterization of the starch of these corns by identifying these two compounds in particular the amylose and the amylopectin which playing a determinant role in the functionality of starch and derivatives. The aim of the present study is the biochemical characterization of the starch. It was conducted to evaluate various amylose determination methods. Samples were analyzed using high-performance size-exclusion chromatography (HPSEC), the asymmetrical flow field flow fractionation (AFFFF) and iodine binding procedure. The incapacity of the clear separation between amylose and amylopectin using SE-HPLC with two columns conducted us to use AFFFF. The coupling between flow field-flow fractionation, multi-angle laser scattering and differential refractometer index provides a promising technique for fractionation of starch polysaccharides. The affinity of amylose for iodine is a fundamental characteristic allowing the separation between these two fractions of the starch. Our results using this biochemical technique showed a significant effect of fertilization and place of culture on the content of amylose fraction.

### P7.13 - Assessment of genetic diversity of red rice (*Oryza sativa* L.) genotypes carrying good grain quality traits using molecular markers

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An effective breeding program, based on knowledge of genetic diversity of the cultivars, is needed to broaden the genetic bases of rice germplasm. In this study, we used a set of 20 simple-sequence-repeat (SSR) markers distributed across the rice genome to assess the genetic diversity of 90 red rice genotypes. The PIC value ranged from 0.08 (RM 234) to 0.83 (RM 555) with an average of 0.44. The dissimilarity coefficient ranged between 2% (IET-11865 and IR - 138436 -14-1-2-3-3) and 30% (Honasu and Jolaga). A total of 55 polymorphic alleles were detected, the number of alleles per marker ranged from 2 (RM 212) to 7 (RM 555), with an average of 2.75. UPGMA-cluster-analysis based on genetic distance coefficients clearly separated all the genotypes and grouped them into five clusters. Diversity clustering based on Mahalanobis D<sup>2</sup> statistics and Tocher method, for physico-chemical characters grouped the genotypes into 11 clusters, the highest number of genotypes were found in cluster VII followed by cluster II, while cluster I and cluster XI were farthest. The cluster VII had genotypes which had relatively high value for most of the characters viz., Andrewsai for high phenols content and high crude protein content of grain, NM-Batta for high Zinc content, P-Kirwana for high Iron content and KHRS-47 for high Kernel L:B ratio hence, genotypes from this cluster can be used to enhance grain quality and nutritional traits. The study revealed that SSR markers facilitated the classification of genotypes according to their genetic relatedness.

### P7.14 - Breeding for quality in flavouring and aromatic crops

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At All-Russian Research Institute of Vegetable Breeding and Seed Production more than 20 years the plant collection of aromatic crops have been maintained and breeding program has been proceeded to develop new varieties that can be a necessary source of functional food for human health. These new varieties as a plant product can be widely utilized as food additives, flavouring agents and raw material for food, perfumery, cosmetic and pharmaceutical industries. The plant collection of salad and scented crops includes more 1000 accessions belonging to such plant families as Apiaceae, Portulacaceae, Polygonaceae, Chenopodiaceae, Lamiaceae, Asteraceae, Fabaceae, Rutaceae, and etc. This collection consists of local population, varieties, breeding accessions, wild genotypes and accessions from Vavilov Institute of Plant Industry. The breeding program for new variety selection was carried out on the base of multi-year selection of unique plant genotypes with their progenies in the different environmental conditions. Analytical breeding, clonal breeding with hybridization are involved in selection during successive generation. Basically, the breeding program is focused on development of varieties with such desirable traits as high yield capacity, fast maturation, and high storage characteristics, high nutritional value identified by biochemical characteristics, essential oil and its component contents. Moreover, these varieties are selected to possess resistant to abiotic stresses and fungal infection and to be suitable for mechanized harvesting. By selection during successive generation the Makovey fast maturation variety of Purslane (*Portulaca oleracea* L.) with vitamin C content up to 50 mg per 100 g in foliage has been developed. Rhubarb variety Malakhit and Gribovchanin variety of Tarragon (*Artemisia dracunculus* L.) were marked by their high nutritional early maturation characteristics. A number of cultivars have been released in annual species: *Ocimum basilicum* L. (Karamelny variety), *Satureja hortensis* L. (Gribovskiy 23 variety), *Dracocephalum moldavicum* L. (Albion variety), *Trigonella caerulea* L. (Gurman variety), and in perennial species *Origanum vulgare* L. (Rhea variety), *Hyssopus officinalis* L. (Inei variety), *Melissa officinalis* L. (Zhemchuzina variety), *Nepeta cataria* L. (Barkhat variety), *Lophantus anisatum* Benth (Snezhok variety), *Ruta graveolens* L. (Kruzhevnitza variety) and *Levisticum officinale* Koch. (Lider variety). The mentioned varieties have all required vegetable, aromatic, medicinal and ornamental characteristics.

### P7.15 - Rice seed development is regulated by ascorbate peroxidase OsAPxb

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Ascorbate peroxidases (APXs) are enzymes detoxifying peroxides such as hydrogen peroxide using ascorbate and that exist as isoenzymes distributed in distinct cellular compartments such as the cytosol, mitochondria and peroxisomes. APXs play essential roles in scavenging ROS and protecting cells against these toxic effects in higher plants, algae, euglena and other organisms. APXs also respond to environmental stresses such as salinity and drought. Rice ascorbate peroxidase b (OsAPxb) participates in salinity tolerance and its function in relation to salinity tolerance is more important than that of OsAPxa. We found that OsAPxb also plays an important function in seed development including fertilization. In developing rice seed, mRNA and protein amount of OsAPxb are much higher in low eating quality cultivar than high eating quality cultivar. Height and grain size of *OsAPxb* mutant are almost the same with those of wild type. But, approximately 50% of the seeds are sterile in *OsAPxb* mutant although they normally flower. Our results suggest that ascorbate peroxidase functions as a regulator of flower and seed development as well as a scavenger of ROS.

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### P7.16 - Molecular and biochemical studies of opium poppy (*Papaver somniferum* L.) – a basis for enhancing the quality of food and pharmaceutical products

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Opium poppy (*Papaver somniferum* L.) is one of the oldest crops which has been used since the Neolithic. Through a long time of men's selection a high variability within the species occurred. Nowadays, two contrary breeding purposes, on one hand the low alkaloid content for the food industry, e.g. bakery products, and on the other hand the high alkaloid content for the pharmaceutical industry exist. Beside, some morphological characters like flower colour and seed colour are of interest. Based on the large poppy collection of the German genebank a core of 300 different accessions were selected and cultivated under field conditions in Gatersleben in three consecutive years to study the biodiversity in opium poppy. In order to classify the accessions the composition and contents of the five main alkaloids morphine, codeine, thebaine, papaverine, and noscapine were investigated with high performance liquid chromatography (HPLC). A high variation could be detected with respect to the quantitative composition of the main alkaloids within all 300 accessions. In nearly all accessions morphine was the main alkaloid, the content ranged from 978.1 to 22,575.2 µg/g dry in one year. A highly significant correlation between total content of alkaloids and morphine ( $r = 0.926$ ,  $P < 0.001$ ) could be determined, while the other four alkaloids showed no clear pattern. For comparison of the biochemical analysis with molecular data the amplified fragment length polymorphism (AFLP) fingerprint technique was used to assess genetic diversity and relationships between the accessions. For analysing the data 267 polymorphic fragments out of three primer pairs were used. Within the accessions one group with a noticeable higher papaverine content could be clearly separated by molecular markers. The differentiation between variable morphine contents will be discussed. The studies presented are the basis for enhancing the quality of food and pharmaceutical products.

### P7.17 - Genetic diversity of the genus *Citrus* analysis aimed by soluble sugars and organic acids contents from fruit pulp and sequence polymorphism analysis of genes involved in primary metabolism

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Sugar and acidity levels are the main criteria of general fruit quality and citrus juices pulp in particular. For Clementine, these parameters were also used to determine the degree of fruit ripeness as the acidity decreases during ripening while the sugar level increases. Although the overall fruit acidity is used to classify into two groups the cultivated citrus species, the constituents of the acidity (organic acids) and the sweetness (glucose, fructose and sucrose) have never been used to study the genetic diversity of the genus *Citrus*. By the end of January, we evaluated by HPLC the content of 87-variety juice compounds belonging to the eight major *Citrus* species, grown under the same environmental and cultivation conditions. Using, Principal Component Analysis, (PCA) we could displayed that the biochemical diversity is strongly correlated with the molecular diversity, confirming recent hypotheses about the phylogenic relationships between species of this genus. Three groups corresponding to the ancestral species (mandarin, pummelo sans citron) were clearly distinguishable. As expected, the secondary species were closely related to their putative species genitors except for *Citrus aurantium* which was apparently intermediate between mandarin group and lemon group while it's supposed to be a combination between mandarin and pummelo. Three months later, we repeated the experiment to assess the potential changes in sugar content and acidity. Clear variations occurred in all mandarin varieties and their closest related citrus, including clementine and oranges, and not in the other studied citrus species. In fact, it enforced the differenced between taxonomic groups. In order to identify putative genetic factors of the observed biochemical diversity, we investigated by SSCP the sequence diversity of 6 candidate genes encoding for key enzymes of sugars and organic acids metabolic pathways. We observed the same genetic organization of genus *Citrus* than produced with molecular markers (SSR). But the polymorphism of single gene does not corroborate the biochemical diversity. This might signify that the observed sequence diversity of the 6-studied genes is not involved into sweetness and acidity regulation. We could suppose that the regulation of sugar and acid contents is localized at the control of gene expression level.

### P7.18 - Induced mutagenesis for yield and nutraceutical traits in sesame

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Sesame [*Sesamum indicum* (L.)] has been valued throughout history for its contributions to diet, medicine and household uses. Modern research reveals that, this long prized plant offers a wealth of health benefits. Sesame and its lignans-fibrous compounds that may act as antioxidants and influence hormone metabolism may be valuable therapeutic tools in modulating cardiovascular risk through their numerous actions in the body. Sesame oil has been widely used as a domestic ayurvedic remedy in India. In the present study an attempt has been made to evaluate the mutants for nutraceutical traits, yield potential and quality. Ethyl methane sulphonate (EMS 0.5%) was found to be more effective in inducing greater number of mutants (22) in the first formed capsules than the later formed ones. Among the radiation doses treated, 400 Gy was found to be more effective in inducing higher number of mutants (9) followed by 300 Gy. The frequency of transgressive mutants indicated that, chemical mutagens are more effective in inducing maximum number of mutants (25) when compared to radiations (23). ANOVA indicated very highly significant variation for lignan profiles in the mutants. The mutants with superior lignan profiles were further assessed for their yield potential and seed quality. Mutant No.699 (1481 kg/ha) and mutant No.949 (1449 kg/ha) had high seed yield compared to its parent DS-1 (810 kg/ha). However, they had 6.42 and 7.56 g/kg oil sesamin content with a test weight of 3.75 and 3.66 g, respectively. Mutant No. 1022 had higher sesamin (10.46 g) content compared to parent DS-1 (3.59 g) and recorded high test weight (seed quality) of 3.92 g with a seed yield of 1266 kg/ha. However, these promising mutants need to be tested over locations to confirm their superiority. The material constitutes an excellent source for elucidating the genetic control and molecular characterization of nutraceutical traits in sesame.

### P7.19 - Genetic enhancement of nutritional quality traits with induced mutagenesis in groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) is the third most important source of vegetable protein and fourth most important source of edible oil in the world, since the kernels of groundnut contain high quality edible oil (45%) and easily digestible protein (24%). However, the limited accessible genetic variability for these quality traits restricts the genetic improvement of the crop. An attempt made to induce genetic variability through physical (gamma-rays) and chemical (EMS) mutagenesis with two Spanish bunch cultivars (TPG41 and GPBD4) resulted in significant increase in genetic variability for these nutritional quality traits at Main Agricultural Research Station of University of Agricultural Sciences, Dharwad.

The protein content of kernels has been enhanced from 23.8 to > 28% in three mutants of TPG41 and from 30.75 to > 34% in seven mutants of GPBD4. Oil content has been increased from 45.7 to > 48% in three mutants of TPG41 and marginal increase of 1% in GPBD4 from 48.8 to 49.8%. However, there has been significant enhancement in oil quality with increase in oleic acid from 50.3 to > 60% in seven mutants of GPBD4 and from 59.8 to > 67% in four mutants of TPG41 that resulted in increased O/L ratio from 1.66 to > 3.3 in five GPBD4 mutants and from 2.82 to > 4.75 in four TPG1 mutants.

The significant increase in seed size was also achieved from 37.4 to > 47 g/100 seeds in seven mutants of GPBD4 and from 63 to > 72 g/100 seeds in four mutants of TPG 41 These new mutants isolated for the above quality traits could add to the genetic resources kit of the crop for further genetic improvement to mitigate malnutrition and health problems in semi – arid tropics of Asia where groundnut crop is predominantly grown for edible oil and protein.

### P7.20 - Isolation and characterization of polymorphic microsatellite markers from the saffron, *Crocus sativus*, and their cross-species amplification in the *Crocus* genus

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Saffron (*Crocus sativus* L.) is a crop of primary economic importance and exceptional therapeutic properties. The numbers of SSR markers and their utilization have not been determined and investigated as extensively in *Crocus* species as compared to other crop species. The current report presents 56 new SSR markers in *Crocus sativus* and their application to related species in the genus *Crocus*. Of the 56 SSRs, 15 polymorphic SSR markers were detected and utilized in a genetic diversity analysis of a cultivated and wild saffron population consisting of 87 accessions of diverse origin. These results demonstrated that wild genotypes are maximally different from the cultivated gene pool and could readily be distinguished. The diversity information obtained using these new SSRs and their cross-transferability to related *Crocus* species will increase our understanding of genetic structures and species relationships within the *Crocus* genus.

### P7.21 - Fine mapping and candidate gene analysis of the floury endosperm gene, *FLO(a)*, in rice

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In addition to its role as an energy source for plants, animals and humans, starch is also an environmentally friendly alternative to fossil fuels. In rice, the eating and cooking quality of the grain is determined by its starch properties. The floury endosperm of rice has been explored as an agronomical trait in breeding and genetics studies. In the present study, we characterized a floury endosperm mutant, *flo(a)*, derived from treatment of *Oryza sativa* ssp. *japonica* cultivar Hwacheong with MNU. The innermost endosperm of the *flo(a)* mutant exhibited floury characteristics while the outer layer of the endosperm appeared normal. Starch granules in the *flo(a)* mutant formed a loosely-packed crystalline structure and X-ray diffraction revealed that the overall crystallinity of the starch was decreased compared to wild-type. The *FLO(a)* gene was isolated via a map-based cloning approach and predicted to encode the tetratricopeptide repeat domain containing protein, *OsTPR*. Three mutant alleles contain a nucleotide substitution that generated one stop codon or one splice site, respectively, which presumably disrupts the interaction of the functionally conserved TPR motifs. Taken together, our map-based cloning approach pinpointed an *OsTPR* as a strong candidate of *FLO(a)*, and the proteins that contain TPR motifs might play a significant role in rice starch biosynthetic pathways.

### P7.22 - Exploitation of genetic resources for improving maize grain quality

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The introduction of hybrids into cultivation brought up a significant increase in maize (*Zea mays* L.) grain yield, and a better resistance to pathogens. On the other hand, the possibility to help maintaining good health through a convenient diet has recently focused the interest in food plants on the nutritional quality issue. A recent survey of maize germplasm from several countries revealed the existence of a wide genetic variability for the main components of the grain (Berardo et al., 2009). In particular, Italian germplasm was found to be rich in some bioactive compounds, e.g. carotenoids. With the aim to find new sources of alleles to improve the nutritional quality of maize hybrids, a set of old inbreds were grown in the field during two years and selfed. The chemical composition of the grain was investigated by NIR spectroscopy in terms of protein, oil, starch and carotenoids. A preliminary test of resistance to *Fusarium verticillioides* ear rot was also carried out testing the inbreds under kernel artificial field inoculation. The severity of *Fusarium* attack, evaluated using ratings based on the percentage of kernels with visible symptoms of infection, showed in most of the inbreds a medium – low susceptibility; fumonisin accumulation was also determined. The use of hyperspectral imaging for distinguishing between inoculated, non-inoculated, open and self pollinated maize kernels was evaluated. Hyperspectral imaging is a powerful technique that could be used for cereal grains characterization in order to produce localized information. Because of its high speed of analysis, it could be of great value when thousands of samples need to be analyzed for resistance to fungal pathogens in a maize breeding program. Moreover, non-destructive analysis of single kernels composition could help identifying outlying individuals both for breeding and for industrial seed sorting applications, selecting and propagating single seeds with desirable composition traits. The genetic diversity among these genotypes is currently being described by molecular markers.



### P7.23 - Proso millet - A promising 'nutricereal'

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Proso millet (*Panicum millaceum* L.) is an important millet by virtue of its nutrient composition and it is quite comparable or superior to major cereals with respect to protein, minerals and vitamins with good biological value. Grain contains about 65-70% carbohydrate, a high proportion of which is in the form of non starchy polysaccharides and dietary fibre. It has higher protein content (> 12 %) than wheat and rice and grain protein is rich in essential amino acids. Proso millet is also rich in minerals and trace elements like iron, zinc, copper and manganese. It is the staple food for millions in many parts of the world and provides cheap source of proteins, minerals and vitamins for the unprivileged people in the hilly regions. In spite of its superior nutritive value of grains their use is largely confined to rural areas and has received far less research and development attention so far. Identification and evolution of nutritionally superior varieties of proso millet would be a logical approach to the prevention of malnutrition especially in small, marginal and tribal farms where it is used as staple food. Germplasm is the basic raw material in any crop improvement programme. Hence characterizing these resources is a prerequisite for their efficient use in crop improvement. Conscious screening for better nutritional value has not been reported so far in this crop and the available genetic diversity for nutrient contents has not been evaluated and utilized properly. So an attempt was made for screening the grain nutrient characteristics viz, total carbohydrate, protein, iron and zinc of 364 proso millet germplasm accessions maintained at Small Millet section of Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India. Total carbohydrate was determined using anthrone and phenyl sulphuric acid method, protein estimation by Lowry's method, Prussian blue staining for iron and 1,5 Diphenyl thiocarbazon staining for zinc content. Wide range of variability was observed for carbohydrate content in the material studied. Significant differences in protein content were found among the evaluated germplasm accessions. They were categorized in to low, medium and high based on iron and zinc content. Large genetic variability existed for these traits indicated good opportunities to select proso millet genotypes with high nutrient content. For further utilization of this variability, studies on heritability, genetic advance and gene action have to be under taken.

### P7.24 - Seed storage protein diversity in wheat (*Triticum aestivum* L.) varieties

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Storage proteins are convenient biochemical markers for identification and registration of wheat cultivars, analysis of their purity. The major seed storage proteins of wheat are glutenin and gliadin. Glutenin is composed of high molecular weight (HMW) subunits and low molecular weight (LMW) subunits. The HMW glutenin subunits are encoded by homoeologous loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which are located on the long arms of homoeologous group-one chromosomes. It has been demonstrated that good bread-making quality is firmly associated with the presence of specific HMW glutenin subunits. Since the storage protein composition shows association with bread-making quality, databases of wheat varieties and their HMW-glutenin subunit compositions may offer breeders the prospect of further advancement by combining good HMW glutenin subunits. The objective of our study was to determine the composition of HMW-glutenin subunits in 120 cultivars of common wheat (*Triticum aestivum* L.) originating from 10 European countries and USA. Ten of the analyzed wheat accessions (8.3%) were observed to be heterogeneous in their glutenin profiles. Five cultivars were heterogeneous at one locus, two cultivars at two loci and another three varieties were heterogeneous at all three *Glu-1* loci. Fourteen alleles and 34 allelic compositions were detected using the sodium dodecyl sulphate polyacrylamide gel electrophoresis. The most frequent HMW-GS alleles at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci were null (57.1%), 7+9 (42.2%), and 5+10 (62.4%), respectively. However, also low frequented HMW-GS, such as 13+16, 20, 21, 7, 18 encoded by the *Glu-B1* locus and 4+12 encoded by the *Glu-D1* locus were observed. The wheat-rye 1BL/1RS translocation was identified in 25 cultivars using the acid polyacrylamide gel electrophoresis.

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## P7.25 - Nutraceutical properties in artichoke germplasm and the control of chlorogenic acid synthesis

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Artichoke has beneficial effects on human health, since it possesses anticarcinogenic, anti-HIV, antioxidative, cholesterol-lowering, bile expelling, hepatoprotective, and diuretic properties. These nutraceutical qualities are mainly due to polyphenolic compounds, particularly mono- and dicaffeoylquinic acids (e.g. chlorogenic acid, cynarin), caffeic acid and flavonoids (e.g. luteolin-7-O-glucoside, naringerin), with chlorogenic acid (CGA) being the most abundant.

The content of polyphenol compounds, especially chlorogenic acid and cynarin, was measured in various tissues (leaves, bracts and receptacles) and physiological stages of artichoke plants, and in different artichoke genotypes belonging to the CNR-IGV *Cynara* world collection. A variation among tissues and genotypes was observed, and these variations were quite reproducible among years.

On the same tissues, transcript levels of key genes for the synthesis of chlorogenic acid were measured by means of real time PCR. In particular, *hqt* genes, coding for HQT enzymes (acyltransferases of the BAHD family), have been shown to play a fundamental role in the synthesis of CGA in some plant species. Recently, *hqt1* and *hqt2* genes, possessing two exons and one intron each, have been isolated from artichoke, starting from Asteraceae EST sequences. Coding sequences were heterologously expressed in *E. coli* and the crude extract was used for enzyme characterization and substrate specificity. Both HQT1 and HQT2 were able to synthesize CGA *in vitro* and showed a remarkable preference towards quinate over shikimate, which distinguishes HQT enzyme class from HCT class (preferring shikimate). Based on the available crystallized structures of two BAHD enzymes, modeling and docking analyses were used to assume structural models for our HQT enzymes and to predict their potential binding sites.

The content of CGA in the various tissues and genotypes analysed was more directly correlated with *hqt1* expression levels. Moreover, transient and stable expression of HQT1 in *Nicotiana benthamiana* and in *N. tabacum* respectively, produced an increase in the content of CGA and cynarin, a derivative of CGA. Our findings indicate that both *hqt1* and *hqt2* are involved in the synthesis of CGA, but possibly at different steps of the metabolic pathway, and according to the plant exigencies. Moreover, the synthesis of cynarin (which is still unclear) might take place starting from CGA.

### P7.26 - Mapping pearl millet [*Pennisetum glaucum* (L.) R. Br.] QTLs for Fe and Zn grain density

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Pearl millet is a multipurpose grain/fodder crop of the semi-arid tropics, feeding many of the world's poorest and most undernourished people. They depend on cereal- and legume-based diets, as they have limited access to meat and dairy products, fruits and vegetables.

Genetic variation among adapted pearl millet inbreds and hybrids suggests it will be possible to improve grain micronutrient concentrations by selective breeding. We mapped QTLs for grain [Fe] and [Zn] using replicated samples of 106 pearl millet RILs derived from ICMB 841-P3 x 863B-P2, which segregate for these and many other traits. Skeleton-mapping with 104 SSR markers detected seven linkage groups covering 1557 cM (Haldane). Self-pollinated grain samples were collected from plots of RILs, their parents and additional controls in a 120-entry, 3-replication alpha-design field experiment sown on an Alfisol at ICRISAT-Patancheru late in the 2009 rainy season. Atomic Absorption Spectroscopy determined mineral composition of ground grain samples. Plot data for Fe and Zn concentrations, 50% flowering time (FT), plant height (PH), panicle length (PL), and thousand grain mass (TGM) were analyzed by Residual Maximum Likelihood. Repeatabilities for all traits exceeded 0.7. Among RILs, grain mineral concentration ranges were 34.3-99.5 ppm for [Fe], and 36.3-90.3 ppm for [Zn]. Similarly, ranges for FT (36.9-53.2 d), PH (79-130 cm), PL (14-22 cm), and TGM (6.13-11.70 g) were substantial. Correlation between [Fe] and [Zn] was significantly positive (+0.86\*\*), but both mineral concentrations exhibited significant negative correlations with TGM (-0.4\*\*).

RIL BLUPs employed for QTL detection (by Composite Interval Mapping using PlabQTL) detected seven putative QTLs for grain mineral concentration — five for [Fe] (LG2, LG3, LG5, LG6 and LG7) explaining 6, 23, 10, 14 and 11% of observed phenotypic variation, respectively (39% adjusted R<sup>2</sup>), with two overlapping those for [Zn] (LG3 and LG6) explaining 34 and 13% of observed phenotypic variation, respectively (40% adjusted R<sup>2</sup>). Four favorable alleles were from Inari landrace-derived 863B and one (LG6) was from ICMB 841-P3. Adjusted R<sup>2</sup> values for putative QTLs for FT (7), PH (4), TGM (3), and PL (2) were 54, 43, 40 and 21%, respectively. The LG3 QTL region will be validated by marker-assisted backcrossing.

### P7.27 - Feedback inhibition resistance and regulation of amino acids by their respective analogs and their *in vitro* selection in rice

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Regulation of LYS, THR and MET biosyntheses under feed back inhibition levels of analogs of the three amino acids in the aspartate family were examined for making *in vitro* selection of resistant mutants showing enhanced levels of amino acids. Pathways in the callus and chlorophyll syntheses indicated inhibitory effects of the feedback levels by slow rate of growth and development. Feedback inhibition was observed by the contents of the end metabolite amino acids in callus and leaf. But, to most of the inhibitory levels, solo or dual, resistance was observed by desensitized or increased activities of the regulatory enzymes. Cross resistance was also observed. LYS or THR resistance enhanced MET content showing common regulation of AK, HDHS, TS and altered, escaping or resistant form of MATS for the biosynthesis of the three amino acids. The regenerants of the resistant cell lines carried forward these enzymic mutation forms of enhanced and product contents to the advanced selfed generations.

### P7.28 - Genetic erosion in 2/12 regions of Ethiopian durum wheat

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Ethiopia is an important centre of genetic diversity for *Triticum durum*. Genetic diversity is an indispensable resource for improving and stabilising crop yield. The objective of this study was to estimate the amount of genetic diversity loss. A total of 200 plants of durum wheat belonging to eight landrace populations, were analysed by two microsatellite markers per chromosome. Samples were collected at 15 years interval in two regions of Ethiopia, Tigray and Shewa, the first severely affected by drought at different times, the other located close to a business area. The average number of alleles per locus ranged from 2 to 10 with a mean of alleles per locus of 4.35 in Koraro, Tigray, 1965; 15 years later the number ranged from 7 to 1 with a mean of 3.64, indicating that about one allele per locus got lost in that period. The number of alleles per locus was higher in Shewa samples and an apparent lack of erosion was noticed. The number of alleles per locus was higher in genome B than in A. The heterozygosity was higher at the beginning of the period analysed in Tigray (10 vs. 5%) than in Shewa (7 vs. 5%) material; however the difference was statistically significant only in the Tigray material and not the Shewa. In Tigray, the first component accounted for 25.98% and the second for 20.93% of the variation, whereas in Shewa the values were 27.09 and 21.63%, respectively. In conclusion, the presence of allele erosion in both regions, and erosion was more severe in Tigray than in Shewa.

### P7.29 - Genetic diversity of protein subunits in Myanmar cowpea and its cross compatibility with the subgenus *Ceratotropis*

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Cowpeas are one of the most important food legume crops in the world because of its nutritional values. This study was conducted to detect the genetic diversity of 68 cowpea (*Vigna unguiculata*) accessions mainly from Myanmar on the basis of protein subunit variations. In total 18 resolution bands, of which 14 were polymorphic and the stained gel was divided into four regions. The cluster constructed using UPGMA method revealed 2 major clusters on the basis of protein banding patterns. Cluster analysis revealed random grouping of different colored genotypes that indicated no response for discriminating cowpea for seed colored differential types. The accessions collected from the different regions of Myanmar exhibited the considerable variations although its magnitude was limited through SDS-PAGE 11.25 slab gel. To be broadened the genetic diversity of cowpea, interspecific hybridization between cowpea and the Asia *Vigna* were conducted. The intermediate band patterns of F<sub>1</sub> hybrid were detected from the cross between mungbean and cowpea. It is suggested that SDS-PAGE is as a promising tool to detect genetic relationships of *Vigna* interspecific hybrids because differences were found between known genetic similarities of both parents.

### P7.30 - Genetic variability and evolutionary peculiarity of isoflavone content and its components in soybean germplasm from China

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To characterize the genetic variability and evolutionary peculiarity of the soybean germplasm from both cultivated soybean (*Glycine max* (L.) Merr.) and wild soybean (*Glycine soja* Sieb. et Zucc.), 895 accessions, including 580 landraces, 106 released cultivars, 209 wild materials from various eco-regions in China, with 88 cultivars from abroad as reference, in a total of 983 accessions were tested for their 12 isoflavone contents by using rapid High Performance Liquid Chromatography technique. (1) There showed large variation in total isoflavone (TISF) and its components both in cultivated and wild soybeans in China. The ranges of TISF in wild accessions, landraces and released cultivars were 927.29~7932.94, 259.38~7725.45 and 547.49~5735.15  $\mu\text{g g}^{-1}$ , with their averages of 2994.51, 3241.33, and 2704.83  $\mu\text{g g}^{-1}$ , respectively. (2) On average, with the long term artificial selection, the total genistin group content (TG) and total glycitin group content (TGL) increased, while total daidzin group content (TD) decreased obviously, which led TISF in released cultivars lower than that in wild soybean. (3) There existed also great variability of isoflavone contents in wild and cultivated soybeans within each eco-region as was that in the whole country. The TISF of cultivated soybeans negatively correlated both with longitude ( $r = -0.264$ ) and latitude ( $r = -0.380$ ) at  $P < 0.01$  significance level, while no such correlation found in wild soybeans, which indicated that the differential directions of artificial selection acted on the cultivated soybeans among geographic regions caused the correlation between genotypes and geographic sites in the cultivated soybean different from that of the wild soybeans. (4) From the 983 accessions, elite ones, such as ZYD3621 (TISF 7932.94  $\mu\text{g g}^{-1}$ ), N3188 (TISF 7725.45  $\mu\text{g g}^{-1}$ ), N20793 (TGL 5122.21  $\mu\text{g g}^{-1}$ ), etc. were screened out for isoflavone breeding. The evolutionary peculiarity of isoflavone content and its components from the wild species to cultivated landrace and to the released cultivars of soybean in China elucidated that the average of TISF, TG and TGL of *G. max* were higher than that of *G. soja*, while the average of TD of *G. max* was lower than that of *G. soja*.

### P7.31 - SNPs mining and adaptive evolution of *amy2* genes in wild barley

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-Amylases hydrolyze internal -1,4-glucosidic bonds in starch and related dextrans and oligosaccharides, playing an essential role in the germination and malting process by hydrolyzing the stored starch granules present in the endosperm of barley. The sequences of *amy 2* genes encoding the low-pI alpha-amylase I from wild barley *Hordeum spontaneum*, the progenitor of cultivated barley, were characterized. A comparison of 109 sequences obtained from 11 Israeli populations revealed three types of *amy2* genes (including X05166, M17128, and M17127, respectively). All of the *amy2* genes had four exons and three introns. A total of 36 single nucleotide polymorphisms (SNPs) were detected in the mature protein coding sequence with 26 (7 in introns), 8 (1 in intron), and 2 SNPs in Type I, Type II, and Type III *amy2*, respectively. Population-specific SNPs were detected for Type I gene, including 66A/C, 72C/T, 109C/A, 760G/A, and 1192G/A. The 45T/G SNP was absent from the xeric (dry) climate population at Sede Boqer, and 825A/G and 1288A/G were found in populations at the northern slope of "Evolution Canyon" I, and at Meron and Maalot, which have mesic climates. Some population specific SNPs were also predicted to make amino acid changes in the functionally important protein domains of Type I genes. These protein domains are important in determining enzyme-binding efficiency for starch granules. Remarkable interslope Type I *amy2* SNP diversity was found at the model microsite of "Evolution Canyon" I at Mount Carmel, Israel. Half of the SNPs were slope-specific and only one SNP was shared between the xeric-savannoid "African" slope and the mesic-forested "European" slope. Most of the slope-specific SNPs were correlated with water and temperature factors. The high level of polymorphism is likely to modify the amylase hydrolyzation unequally distributed between the slopes and populations at "Evolution Canyon" I. SNPs in *amy2* were subjected to selective pressure and adaptive to environmental changes. This is the first step towards understanding amylase, amylase-inhibitor adaptive coevolution, and the ecological driving forces associated with the differential dormancy level in Israeli wild barley.

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### P7.32 - Cultivated and wild *Solanum* species as a potential source for health promoting quality traits: Results of a 3-year experiment

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Wild potatoes are of increasing interest as gene pool in breeding, with the goal of upgrading the nutritional and health value of potatoes. In this study, different accessions/genotypes of cultivated and wild *Solanum* species were examined for total soluble phenols and proteins as well as their antioxidant activity measured as ascorbic acid (ACE) and trolox equivalent (TXE). In tuber tissue of *S. tuberosum* subsp. *andigena* (adg), *S. phureja* (phu), *S. bulbocastanum* (blb), *S. chacoense* (chc) and *S. pinnatisectum* (pnt) accessions the antioxidant activity (ACE) ranged between 0.06  $\mu\text{g mg}^{-1}$  fresh weight (phu) and 4.22  $\mu\text{g mg}^{-1}$  fw (pnt). Interestingly, pnt had on average multiple higher quantities of antioxidants in its tissue than the other four *Solanum* species, and in pnt tissue, the high antioxidant potential coincided with a similar high level of phenols and proteins. These last two components were significantly correlated with the antioxidant capacity. An involvement of *S. pinnatisectum* in breeding could be profitable to improve the antioxidant potential and with it, the health value of new potato cultivars. Moreover, it could be helpful to increase the biodiversity. However, in case of pnt, a Mexican diploid wild potato species, it will be necessary to overcome the crossing barriers.

### P7.33 - Genetic diversity resources improving maize and sorghum digestibility for food and feed

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One of the greatest factors affecting the utility of a crop plant as a food source is the bioavailability of its nutrients. In maize, where energy release from starch is the primary nutritional benefit, the rate of starch digestion is important in determining how effective a variety is. Rapid digestion in raw grain is desirable for animal feed to improve weight gain, while rapid digestion of cooked flour is desirable for providing calories to undernourished people and slow digestion of cooked flour is of benefit to diabetic patients and in obesity-related disorders. In sorghum, protein is only about 89% as digestible as protein from an equivalent amount of maize, and its nutrition absorbed less well, when consumed in its raw form. Cooked forms of sorghum consumed by humans have even lower digestibility (only ~60% that of cooked maize) due to extensive cross-linking among proteins. A mutant sorghum line with both increased protein digestibility and increased starch digestibility (Ejeta et al, 1987; Zhang et al, 2006) indicates that improvement of this trait can be achieved through genetics. Recent data indicate that the increased digestibility in this mutant appears to be a quantitative trait (Winn et al, 2009), that the induced mutation probably interacts with additional genes and that some alleles of these additional genes improve digestibility still further. Screens of natural variation and EMS-induced variation in maize have identified lines that produce flour with significantly increased and lines with significantly decreased starch digestion rates. We have initiated similar screens of natural and induced variation in sorghum to identify lines with improved protein and starch digestibility. Using genetic diversity association mapping resources will allow rapid identification of genes impacting digestibility in these two major grain crops and facilitate breeding efforts to provide better nutrition to those who need it.

### P7.34 - The protective role of butanolic extract from *Paronychia argentea* L. against chlorpyrifos-induced toxicity: *In vivo* and *in vitro* studies

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Natural products of plant origin are still a major part of traditional medical systems in developing countries. All parts of Algerian plant *Paronychia argentea* L. were phytochemically studied in the *n*-butanolic extract. In the present study, the antioxidant properties and protective effect of the butanolic extract isolated from aerial parts plant were investigated *in vivo* and *in vitro*.

*In vitro* antioxidant activity of the extract were conducted by determining the , -diphenyl- -picrylhydrazyl (DPPH) and 2,2 - azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activities. The antioxidant activities of plant extract was evaluated *in vivo* in terms of its inhibition of lipid peroxidation as well as its protective effect against chlorpyrifos (CE) toxicity. Pregnant Wistar Albino rats were used in this study, pesticide (20 mg/kg) and plant extract (200,100 and 50 mg/kg) were administered daily by gavages from the 6<sup>th</sup> to the 15<sup>th</sup> day of gestation. The present findings established that CE can cause a strong induction in plasma and liver LPO and enhanced the production of super oxide anion. While treatment by plant extract reduced /or protect CE toxicity. The decrease in serum enzymes and LPO levels and the increase in GSH and SOD enzymes activities revealed the antioxidant property of this extract. The plant extract completely prevented the toxic effect of CE on the above serum parameters. A significant *in vitro* antioxidant activity of plant extract was reported.

In conclusion, the present data provide further evidence for an important role of the butanolic extract of *Paronychia argentea* L. against CE toxicity. It might be regulated liver function.



*Miscellaneous*

*Posters*





## PM01 - Exploring and collecting of potato (*Solanum tuberosum* L) for morphological parameters in different regions of Kosovo

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Potato (*Solanum tuberosum* L.) in Kosova is the an important crops after wheat and maize and a major component of human food. Based on Statistics of Kosovo (2008-2009) potato was grown on a total area of 8000-10000 ha<sup>-1</sup> with average yield 7-8 t ha<sup>-1</sup>. The main objective of this expedition was to describe the potato landraces, included collection and conservation. A field study was conducted in the different parts of Kosovo. The field survey was carried out during 2009. Our expedition-investigation has identified 21 accessions of potato. The accessions were found at coordinates and different altitude from 441 to 1033 m.a.s.l. Collected material of plants was based with design a randomized complete block (RCBD) Split-plot method with three replications. Farmer was chosen randomly. 3-5 kg seed assembled (collected) from farmers and brought to the National Gene Bank of Kosovo (NGBK). Based measurements we prepared passport for each accession according to the IPGRI descriptions for potato landraces. The survey was structured and is made with more than 20 farmers. Under the growing conditions of these expedition to determine bio-morphological data per plant; Size of tuber (ST), tuber weight (TW), colours of tuber (CT), shape of tuber (SHT). The results from our expeditions show significant results for different traits. The experimental average value for tuber size of all potato landraces was 8.45 cm, with genetic variability 38.57%. For tuber weight (TW) average value was 101.44 g per accession. The total genetic variability was significantly higher 108.50%. With longer shape of tuber was identify 12 genotypes or in relative value 57.14%, while with ovale shape only seven genotypes or 33.33% and on longer-ovale shape were two genotypes or 9.52%.

## PM02 - Identification of wheat genotypes resistant to heavy metals

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Kazakhstan is characterized by the presence of mining and metallurgical industries, highly polluting with heavy metals, polluting the agricultural land in particular. The use and development of cultivars that are tolerant to pollutants, especially heavy metals, is a component of environmentally clean technologies that allow producing clean agricultural products on the polluted soil. At first stage of this process it is necessary to study the genotypes of cultivated and wild plants and to segregate resistant plants genotypes and donors that accumulate the minimum quantity of ecotoxicants. We screened ten genotypes of wheat as widely cultivated crop in Kazakhstan. The experiments were carried out on seven-day-old seedlings grown in nutrient solution containing ions of Pb or Zn at a concentration of 200 and 400 mg/liter. Growth parameters, content of the Pb and Zn and the percentage of electrolyte leakage in the aboveground organs and roots of wheat seedlings were determined. Heavy metals content was determined by atomic absorption method. The electrolyte leakage analysis was performed according to the Dexter method. Investigation of the accumulation of lead and zinc in roots and aboveground organs, as well as the growth parameters of wheat seedlings of different genotypes, allowed revealing genotypes that are the most sensitive and genotypes that are the most resistant to Zn and Pb. The plasma membrane is a direct target of heavy metal-induced cellular injury. The occurrence of cell membrane injury as a function of stress-induced leakage of electrolytes from wheat tissue was investigated to reveal some of physiological events that contribute to the differential responses of wheat genotypes to heavy metal. Based on the results of the percentage electrolyte leakage obtained for sensitive genotype MK-3745 and tolerant cultivar Mironovskaya – 808, sensitivity to Pb and Zn in genotype MK-3745 is accompanied by more extensive disruption of cellular membranes then that in tolerant cultivar Mironovskaya – 808. This fact indicates that the resistance of plants in general can be attributed to the resistance of their cell membranes to the influence of stress.

### PM03 - Applications of bioinformatics in genetic resources conservation and management – Nigeria's case study

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Global conservation standards in most of the about 1,500 collection holding of more than 6 million crop germplasm exist in the world today. This is not encouraging ;especially among developing countries where it has fallen grossly below expectations and in non-conformity with the binding conservation agreements and treaties such as the convention on biological diversity, global biodiversity information facility, the global plan of action and the international treaty on plant genetic resources for food and agriculture of the United Nations.

The National Centre for Genetic Resources and Biotechnology, Nigeria is the country's focal point for genetic resources conservation and use having about 15,000 collections in both short and the long- term storage facilities. It however needs an improvement in the conservation facilities for a thorough harmonisation of the nation's genetic resources and collections as well as the development of manpower and use of novel biotechnology tools in its programs.

Wholistic approach in germplasm conservation and use must include the application of biodiversity informatics system and computer linkages to ensure adequate integration and knowledge about each others collections, traits detection especially biotic and abiotic factors and other characterisation information. This is a necessity among developing nations and particularly Nigeria, towards ensuring its facilities upgrade to meet global conservation standards.

### PM04 - Study of genetic variation among Iranian Iris species using morphological characteristics

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*Iris* is one of the most important cut flowers in Iran and around the world. Number of chromosomes of Iranian *Iris*, varies between  $2n=2x=18-48$ . Identification genetical potentials of *Iris* germplasm in Iran and their uses in creation of new cultivars, is the main aim of *Iris* breeding. Genetic diversity of 15 *Iris* species collected from different parts of Iran was studied using 10 quantitative and 15 qualitative traits that were evaluated on the basis of UPOV guidelines. The experiment was carried out at Research Station of Ornamental Plant Center at Mahalat in 2009. Statistical methods including correlation coefficients, means, principle component analysis (PCA), and cluster analysis by UPGMA algorithm were applied for the quantitative traits. Qualitative characters were also used for species grouping according to cluster analysis. The results showed genetic variations among different species with considering various characters. Branch flowering thickness with crown thickness and crown thickness with leaf width had the highest positive significant correlation and the perianth tube length with pistil width of bridge had the lowest negative correlation. Mean while, *I. germanica*, *I. iberica*, *I. paradoxa* and *I. spuria* almost had the highest quantitative traits. Results of principle component analysis presented that 77.39% of total variations are defined by four components. Cluster analysis based on quantitative traits, divided all species into two main groups. One group is tall *Iris*es with rhizomatous type and grows naturally under humid conditions and the other short *Iris*es having rhizomatous or bulb and is usually found in dry conditions. Cluster analysis based on qualitative traits, divided all *Iris*es in to three main groups varying in flower color, bud flower color, and perfume. No relationship was found between genetic diversity and geographical classification.

#### PM05 - Study of the genetic diversity of Iranian Iris species using RAPD markers

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Iris is one of the most important cut flowers in Iran as well as in the world. Number of chromosomes of Iranian Iris varies from  $2n=2x=18-48$ . Characterization and identification of genetic potentials of *Iranian Iris* germplasm is very important for the development of new varieties and breeding purposes. Genetic diversity of 18 *Iris* species collected from different parts of the country was studied using 10 arbitrarily primers in a RAPD markers. A total of 255 RAPD polymorphic bands were detected. Polymorphic marker per primer combination ranged from 19 (RAPD3) to 31 (RAPD2). The highest and the lowest PIC scores detected for arbitrary primer combinations of OPC-9 and OPB-12 were 0.152 and 0.193, respectively. In general, a high degree of genetic diversity was revealed by RAPD markers. Cluster analysis of 18 species with 10 primers produced six groups. Principal component analysis based on the first and second components indicated that *I. Spuria* species can be easily identified from the other species. Classification of species had no clear relationship with their geographical distribution.

#### PM06 - The characterisation of collected blackdisk medick (*Medicago orbicularis* (L.) Bartalini) populations from the Middle Black Sea Region, Turkey

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Annual medic species are common in natural flora of Turkey. The aim of this study was to determine similarities and differences regarding some characteristics variation of blackdisk medick (*Medicago orbicularis* (L.) Bartalini) genetic resources collected Middle Black Sea Region (35°01'27"-38°03'23" E; 40°06'59"-41°33'39"N; h=18-1190 m) in 2008-2009. In this research, 34 populations of blackdisk medick were collected from Samsun, Tokat, Amasya and Çorum provinces in this region. The selected characters were included in the description form developed for medics by The International Board for Plant Genetic Resources (IPBGR). Cluster analysis were performed to determine relationship among populations. Cluster analysis based on 17 variables identified 9 groups in the current study. The dendrogram was prepared to evaluate similarity between blackdisk medick populations. This evaluation can assist geneticists and breeders to identify populations with desirable characteristics for inclusion in variety breeding programs.

**PM07 - Combining ability of F<sub>1</sub> generation from diallel cross of bread wheat for some quality traits**

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The inheritance of quality characteristics of wheat is complex due to polygenic nature of these traits. Therefore, this study was carried out at research and implementation area of department of Field Crops, University of Cukurova, under East Mediterranean conditions of Adana/Turkey, for determining the inheritance pattern of important quality characteristics of bread wheat. Five parents, namely Genç99, Balatilla, Sagitario, Pandas and Adana99, were selected because of diversity of their quality characteristics, and they are widely grown in most wheat growing area of Turkey. Analysis of variance for combining ability showed equal role of both additive and non additive gene actions for all of the studied traits. Magnitude of general combining ability (GCA) values were higher than specific combining ability (SCA) values for sedimentation volume, protein contents, wet and dry gluten contents, falling number, gluten index value and test weight, whereas, SCA value was higher for only 1000- kernel weight. The effect of general combining ability was higher for all traits except 1000-kernel weight in accordance with ratio of GCA: SCA. Reciprocal effect was also significant for all traits, indicating possible maternal effects. Sagitario was best general combiner for sedimentation volume, protein contents, dry and wet gluten, whereas pandas was also good general combiner for falling number dry gluten, gluten index value, sedimentation value, protein contents. Genç99 and Adana99 were best general combiners for 1000-kernal weight and test weight. Crosses having high good specific combining ability were also between mid-parent value and better parent and a mid-parent heterosis existed and moreover, one of the parents involved in some crosses had better general combining ability (Pandas and Sagitario), indicating that these combinations would yield desirable transgressive segregants. These results can be used to design selection strategy for improving the bread wheat quality for providing better nutrition to the human beings.

**PM08 - Effect of salinity on growth and membrane integrity of three varieties of durum Wheat (*Triticum durum* Desf.)**

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This study was carried out with three varieties of durum wheat cultivated in Tunisia: Om Rabii, Karim and Razzek. These varieties were treated with three concentrations: 0, 3.5 and 7 g/l of NaCl and the analysis was realised at physiologic maturity of the plants at the stage of 4<sup>th</sup> leaf completely spreaded.

The results showed that the presence of salt in the medium generated a reduction of fresh and dry weight of Razzek variety. The variety Om Rabii was indifferent even to a treatment of 7 g/l which has affected considerably the production of fresh matter at the varieties Karim and especially Razzek, the latter would be most sensitive. Om Rabii seems to be most resistant. The results obtained on the membrane integrity measured by the efflux of electrolytes after rehydration of the squares of limb pilot and treated by the PEG 400 (osmotic agent exerting a stress equivalent to 7 NaCl g/l, but in the short run), showed that Razzek variety presented the percentage of the highest damage. The variety Om Rabii was shown more resistant and Karim variety was very slightly affected.

### PM09 - Effect of water deficit on drought tolerance indices on wheat (*Triticum durum* Desf.)

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Two hundred forty-nine F<sub>7</sub> Recombinant Inbred Lines (RILs), developed from a cross between the two durum wheat cultivars Kofa and Svevo, were tested through the comparison of yield components for adaptation to Mediterranean environments in both irrigated (GYi) and rainfed (Gyp) conditions. Six drought tolerance indices involving: Stress Tolerance Index (STI), Stress Susceptibility Index (SSI), Mean Productivity (MP), Geometric Mean Productivity (GMP), Yield Stability Index (YSI) and Stress Tolerance (TOL) were used to identify high yielding and drought tolerant RILs. MP explained GYi while GMP and STI explained GYp. TOL under favourable and SSI or YSI under water deficit discriminate between tolerant genotypes to stress. Greater values of GMP, STI and MP indices were associated with higher yielding RILs under both growing conditions. Inversion of RILs ranking was obtained for SSI and TOL as compared to YSI. Higher TOL and SSI values were associated with significant grain yield reduction in stressed environment suggesting higher stress responses of RILs. Significant positive associations between MP, STI and GMP and negative between YSI and SSI were noted. The former three indices were independent from YSI, SSI and TOL. Highly significant correlations were shown positive between STI and GMP and negative between YSI and SSI; relations were demonstrated between these indices in twos. PCA results revealed that the first axis PC1 definite the high potential yielding and drought tolerance axis whereas PC2 can be considered as high stress tolerance axis. High PC1 values associated with low PC2 values provide upper yielding and stress tolerant RILs.

### PM10 - Variation of indigo precursors in woad (*Isatis tinctoria* L.) accessions growing in Europe

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Woad (*Isatis tinctoria* L.), one of the oldest known sources of indigo, has been cultivated in Europe since the Middle Ages. Despite the current commercial availability of the synthetic equivalent dye, plant-derived indigo surprisingly retained a contemporary importance because of the global popularity of indigo-dyed blue jeans, the consumer's increasing awareness of the provenance of daily basis products, and the concerns about environmental impact and sustainability. In contrast to most natural dyes, indigo is not produced in appreciable amounts by any plant. Instead it is formed in the course of the extraction process from the precursors accumulated in the woad leaves. During extraction, water-soluble isatan B (indoxyl ketogluconate) is hydrolysed via fermentation to give indoxyl, which will further react with another indoxyl molecule producing indigo. In order to analyze the economic viability of future crops for indigo production in Europe, ten woad accessions from different countries were studied under field conditions. Isatan B and indoxyl content, as well as residual indigo amount were determined by HPLC-DAD. Reciprocal interactions between these precursors and fresh leaf weight, were compared through genetic diversity, with significant differences in isatan B (0.6-2.9 g kg<sup>-1</sup> FW), indoxyl (0.3-1.9 g kg<sup>-1</sup> FW) and residual indigo (0.3-0.5 g kg<sup>-1</sup> FW).

That information can be used together with genetic and environmental data to assist local farmers to re-introduce *Isatis* species in the European agricultural system, not only indicating the higher indigo yielding genotypes, but also the most suitable harvest time and extraction procedure.

This study was supported by FCT project POCTI/AGR/56087/2004.



### PM11 - Studying genetic variability of Colombian yellow pitahaya *Selenicereus megalanthus* (Cactaceae) by different tools

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Although Colombia is the most important country in the production of yellow pitahaya, there are no selected genetic materials and established practices for cultivation. The fruit is stationary, which implies that in two seasons are given high production peaks. There is no marketing system in an organized and streamlined way to attach this volume of fruit in the domestic and international markets. This results in difficulties to producers, as prices placed by intermediaries are so low that they can recover the costs of production. In order to find some material with interesting traits for supporting a breeding program, genetic variability was evaluated by using morphological, molecular (RAMs), physicochemical, nutritional, cytogenetic and phytochemical tools. In addition, presence of a phytoalexin able to control the major disease of yellow pitahaya, the fruit basal rot, and propagation systems were investigated. Therefore, wild and cultivated introductions were collected in the departments of Bolívar, Boyacá, Cauca, Cesar, Cundinamarca, Huila, Risaralda, Santander, Tolima and Valle del Cauca. Preliminary study of botany resulted in the description of *Selenicereus megalanthus* for Colombia. A list of morphological descriptors for the species and their relatives was established. It was confirmed the chromosome number  $2n = 4x = 44$  for yellow pitahaya. All analysis showed a very low genetic variability, with only a basic chemotype, which has implications for crop health and planting material selection. Ten genotypes were selected (pre-breeding). A field collection with 300 introductions was constituted at Palmira municipality, Valle del Cauca, being the first and unique of the country. This project was financed by the Colombian Agriculture and Rural Development Ministry MADR and ASOHOFrucol.

### PM12 - Genetic diversity within and among European accessions of woad (*Isatis tinctoria* L.) detected by ISSR marker

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Woad (*Isatis tinctoria* L.) was introduced into Europe in ancient times to produce indigo, a natural blue pigment used principally for dyestuff. The cultivation of woad was abandoned in the 19th century when synthetic dyes were developed. The recent interest in alternative crops and the increasing demand for natural products has prompted reconsideration of woad as a dye crop for the production of natural indigo. The aim of this work was to evaluate the genetic diversity in an attempt to determine the genetic relatedness and identify genetic markers specific to ten European woad accessions. DNA from young leaves of nine plants from each accession was extracted using the DNeasy Plant Mini-kit. A set of nine inter-simple sequence repeat (ISSR) markers were used to analyse, generating 177 reproducible fragments, being 171 polymorphic. The mean number of fragments per accessions was 110, with a range between 100 (Coimbra) and 124 (Poland). The total polymorphism ( $P_T$ ) observed was 0.3272, the accessions polymorphism ( $P_S$ ) 0.1784, and the gene differentiation between accessions ( $G_{ST}$ ) 0.4546. Polymorphism accessions ranged between 32.2% (Ostrich) to 49.2% (Belgium). The genetic relationship among woad accessions was obtained with UPGMA dendrograms based on molecular marker, clearly clustering the European woad accessions, according to their geographic origin. This study could contribute to the molecular knowledge of this species and to answer to the increasing demand for natural dyes in Europe, within the framework of the crop diversification policy of the EU, suggesting that woad could become an economically viable new crop in the coming years.

This study was supported by FCT project POCTI/AGR/56087/2004.



PM13 - Validation of two breeding schemes and molecular analysis in three runner beans local landraces (*P. coccineus*) grown under organic farming conditions

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Runner bean (*Phaseolus coccineus*) is one of the most popular grain legumes in the Mediterranean countries. The objective of this study was the evaluation of two breeding schemes (mass selection and a modified plant (pod) to row method) and their effectiveness after 3 years of selection. Three landraces cv. Distrato, cv. Zagora and cv. Prespes, entered field evaluation at the experimental organic field in the University of Thessaly (Greece). Sixty individual fully competitive plants from each landrace were characterized according to UPOV, and evaluated using molecular analysis with RAPDs in a grid mass selection arrangement (1 plant *per m*<sup>2</sup>). Dry bean yield estimated on per plant basis along with number of pods and 100-seed weight. Selection for high yield was practiced with a selection pressure of 10%. Equal number of seeds of each selected plant was mixed to form C<sub>1HY</sub>, C<sub>2HY</sub> and C<sub>3HY</sub> populations during the period 2005 to 2006. From the C<sub>1HY</sub> population negative selection was applied to form C<sub>2LY</sub> and C<sub>3LY</sub> populations. Furthermore five plants from each Co were self-pollinated to create S<sub>1</sub> and S<sub>2</sub> families. Two cycles of selection were followed to form three families of high yield HY<sub>1 CYCLE</sub> and three families of low yield LY<sub>1 CYCLE</sub>. The same procedure was followed in the next year to develop three HY<sub>2 CYCLE</sub> and LY<sub>2 CYCLE</sub> populations for each cultivar. Simultaneous field evaluation of the Co populations along with the S<sub>1</sub>, S<sub>2</sub>, C<sub>1HY</sub>, C<sub>2HY</sub>, C<sub>3HY</sub>, C<sub>2LY</sub>, C<sub>3LY</sub>, HY<sub>1 CYCLE</sub>, LY<sub>1 CYCLE</sub>, HY<sub>2 CYCLE</sub> and LY<sub>2 CYCLE</sub>, was carried out in two locations (Velestino, Monastery), in 2007. The results of this study, shown that for *mass positive selection*, the yield gain cycle<sup>-1</sup> was +9.6-, +8.2-, and +2.3% for Distrato, Prespes and Zagora landraces, respectively. Furthermore, the application of *negative mass selection* into the same bean populations has shown yield loss (-2.1, -4.2, and -9.5%) per cycle. Data from the *plant to row selection scheme* for positive selection indicated that the gain per cycle was much higher (+15.5, +25.9, and +21.2%) and the loss was significant lower (-3.7, -1.8, and -6.6%) for Distrato, Prespes and Zagora cultivars, respectively. The data from shelling shown that inbreeding depression exists, expressed as low number of pods plant<sup>-1</sup> and low vigor.

## PM14 - Integration of approaches from agronomy, genetics, and biotechnology to use the *Helianthus tuberosus* genetic resources for the sustainable production of liquid biofuels

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Jerusalem artichoke, *Helianthus tuberosus* (*Ht*) (2n=102; 1C DNA content=12.5pg; total chromosome length=156.3 mm), has the full set of attributes that make it an important dedicated bioenergy crop. *Ht* plant contains inulin as carbohydrate reserve and is a valuable raw material for inulin, fructose, or liquid biofuels (i.e. ethanol) from either the fructo-cellulosic stem biomass or the fructo-oligosaccharides rich tuber biomass. *Ht* crop offers several management advantages (low agrochemical inputs, minimal cultivation, and harvesting options) and environmental sustainability services (soil and water conservation, wildlife habitat, CO<sub>2</sub> sequestration). Enterprise structures used so far for bioethanol production from grape and sugar beet pomaces, can fit the needs and values of *Ht* processing to get liquid biofuels.

Handling *Ht* genetic resources may help overcoming some technical and biological limitations. *Ht* floret fertility is low and transplantation of tubers is required for propagation. Because in Europe, it is a nonnative perennial with broad environmental adaptation, *Ht* may pose risk of becoming an invasive weed, if not managed appropriately. It is resistant to most of the pathogens that cause diseases in sunflower, but it may show susceptibility to polyphagous fungi such as *Sclerotinia sclerotiorum* (*Ss*). To become economically viable, the total amount of biomass produced per hectare per year must be maximized, as does the amount of fuel produced per unit of biomass. Integrating agronomic, genetics and biotechnological approaches, many of the mentioned limitations have been rapidly overcome. In pilot experiments, it has been ascertained that adaptation of tuber-harvesting and transplanting machinery for potato, ease tuber biomass recovery and planting; mutants for plant architecture and phenology increased the biomass yield per hectare; multiyear crop rotation avoided *Ht* spreading as a weed; matching rainfall regime with plant phenology maximized biomass output and restricted the use of irrigation water to emergency situations (i.e. extreme dry summer periods); micromethods to screen for *Ss* resistance have been effective. Currently, the available genetic resources are used to (a) enlarge genetic diversity for carbohydrate content and *Ss* resistance through somaclonal variation, (b) devise rapid screening methods based on *in vitro* microtuber formation, and (c) select microbial strain with improved saccharification and sugar fermentation efficiency for converting *Ht* biomass to liquid biofuels.

## PM15 - A focus on strawberry tree (*Arbutus unedo* L.) genetic resources of Turkey

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From two significant species of *Arbutus* genus (*Arbutus unedo* L. and *Arbutus andrachne* L.), *Arbutus unedo* L. are commonly found in the flora of Turkey. The strawberry tree (*Arbutus unedo* L.) which belongs to the genus *Arbutus* of Ericaceae family within Ericales is a typical evergreen plant of Mediterranean climate. It is native to Greece, Lebanon, Ireland, Southern Europe and Anatolia. Kocayemis is a common Turkish name for strawberry tree fruit (*Arbutus unedo* L.). In Turkey, strawberry trees grow in marquis areas of the Mediterranean, Aegean, Marmara and Black Sea Regions. The strawberry tree population is generally located along the coasts in these different regions. This short review offers distribution of *A. unedo* in Turkey, soil and climatic characteristics of its growing areas, usages of it in Turkey and finally studies on *A. unedo* in Turkey.

## PM16 - Improvement of grain nutrient content in red rice local variety through mutagenesis

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Indonesia has a wide diversity of local rice genetic resources. One of the potential genetic resources is red rice variety. Red rice represents the local genetic resource that needs to be conserved from totally disappeared and lost, cause its rich source in protein, minerals, vitamins B1, and fiber that are an important source of nutrition especially for children and pregnant women. The improvement in local red rice with mutation induction technique is very useful to generate mutant plants with agronomic traits and nutritional seed better. Therefore this research was aimed to improve the genetic variability and nutrition content in red rice through seed irradiated by gamma rays. Some mutant lines have been developed from a red rice variety Solegreng, through gamma rays treatment with different doses (15, 30 and 45 kRad of Cobalt-60). Seeds of these mutant lines from M2 generation were collected along with their parents and have been observed for morphology and nutrition content. The results showed that the seeds are irradiated with a dose of 45 kRad have the highest protein content (10.81%), compared to the other dose and parent. Amylose analysis showed that mutant lines have the same amylose content with a range 18.05-18.2%, while the parent 18.86%. For mineral content analysis showed that the Fe and Mn content is highest (1.6-1.65 mg/100g and 2.46-2.57 mg/100g), for irradiated dose of 15 and 30 kRad but low Mg content (143.81-146.09 mg/100g). Instead seeds irradiated with a dose of 45 kRad have an Mg content is higher than Fe and Mn content. Morphological observations showed that the treatment of radiation dose 15 and 30 kRad give better results for plant height, tillering number, and the amount of grain fill, compared to dose 45 kRad and parent.

## PM17 - Genetic diversity of sesame by principal components analysis of some common quantitative and physiological indices in drought condition of Caspian border region of Iran

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In order to determine the best quantitative index for assessment of sesame genotypes response to drought stress and determination of physiologic indices related to water stress condition this experiment conducted with 20 treatments in the northwest region of Iran during 2007 and 2008 for two years with three replications and experimental design was RCBD. Two separate experiments were done: first in normal irrigation conditions and second with dry conditions with only one time irrigation after planting. During growth season some morphological and phenological traits recorded, some physiologic traits as cell membrane stability, leaf water content leaf EC at flowering date were recorded too. To study of response of genotypes to drought resistance, five different indices were used, including: SSI, STI, TOL, MP and GMP. Results showed that: 1) Correlation coefficients, distribution diagram, and principle components Analysis (PCA) showed that, STI and GMP indices are suitable indices for evaluating of drought resistance. First and second principal components could explain, 99.5% of total changes between indices. First principal component with GMP, MP, TOL, SSI and YP indices showed positive correlation and negative correlation with STI. By consider that, each principal component introduce different aspects of variables; thus, second principal component showed positive correlation with STI and YS indices. 2) Drought conditions have very large effect on increasing of EC, through cell membrane damage and Ion leakage between cells spaces and large decreasing of cells water content. 3) More resistance genotypes using GMP and STI to drought stress during growth season had less EC, more leaf water content and more cell membrane stability, too. 4) Final results showed that Panama, Karaj and Yekta genotypes respectively had the highest yield grain and also less EC, more leaf water content and more cell membrane stability in stress condition and IS.CO-1, and Indian 14 genotypes showed lowest grain yield and most EC, least leaf water content and least cell membrane stability in such condition.

### PM18 - Diversity and variability for production abilities of some maize landraces (*Zea mays* L.) in rural areas of Kosovo

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In Kosovo with maize in the last century on average were sowed near 100,000 ha, with minimal oscillations from year to year. Recently with maize are sowing about 70,000-80,000 ha, according to the data from (MAFRD, 2009).

Landraces and varieties of local maize, before the 1955<sup>ths</sup> 100% of surfaces were sowed , but actually now only 8.42% are sowed with landraces.

The research expedition in 2009, for collection of landraces and old varieties, in different rural areas of municipalities: (Malishevë L-1, Drenas L-2, Prishtinë L-3, Kamenicë L-4 and Kaçanik L-5, located in central and eastern parts of Kosovo). Research design was: Municipality-M-5 x Accessions-Acc-2 x Parameters-P- 6 x Repetitions R- 5= 300 combinations.

Experimental model was "Split-plot", and achieved results are calculated with different mathematical and statistical models (MSM).

The research was focused in determination of diversity and variability of accessions from collecting activities-2009, in particular the production ability according to effects of locality and farmers traditions. Variability determined for production ability for quantitative parameters was; Ear length (EL 2.41%), Ear weight (EW 63.01%), Number of kernels per Row (NKR, 23.79%), Number of Kernels per Ear (NKE, 39.57%), Kernels Weight per Ear (KWE, 65.07%). Maximal effects of variability for landraces and localities were 46.41-95.43%, with highly significant differences.

The existing diversity and variability, represents a precious fund for production ability and gene resource that can be used in plant breeding and food production. The diversity of landraces includes different forms of *Z. mays* (*Z. identa*, *Z. indurata*, *Z. everta* and the cross combinations between them).

### PM19 - Generation and establishment of mutant lines of the H7996 tomato (*Solanum lycopersicum*)

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Tomato is one of most important vegetable crops grown worldwide for fresh vegetable market and food processing industry. With the completion of the genome-sequencing projects in various crops, the major challenge will be to determine the gene function. Mutational approaches are being carried out to analyze mutant phenotypes. The paper reports the generation of gamma-irradiated and EMS-treated mutant populations, identification and phenotypic characterization of dominant and visible mutations in tomato mutant lines. Mutant populations of tomato H7996 were established using physical (Cobalt 60 gamma ray) and chemical (ethylmethane sulfonate, EMS) mutagens. Generally, based on high-throughput phenotypic characterization, mutations were observed on the plant habit, size, morphology, leaf and flower color and morphology and fruit characteristics. Specifically, the most common dominant and visible mutations noted in the M<sub>1</sub> generation were monopodial, compact, short internodes, multi-branch plant type, light yellow and ghost leaf coloration, tiny and long pedicel leaf morphology and small or short plant size. In the M<sub>2</sub> generation, homogeneous and segregating M<sub>2</sub> families were selected to constitute the core set of visible tomato mutants. Initial bacterial wilt resistance (BWR) gene knockouts were also identified. The mutant lines will be used as a rich source of genetic materials for breeding and functional genomics of tomato.

## PM20 - Effect of genotypes and culture media on embryogenic callus induction and plantlet regeneration from immature embryos of durum wheat

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Seven genotypes of durum wheat (*Triticum turgidum* L. var. *durum*) were cultured to identify an efficient medium for induction of embryogenic callus formation from immature embryos for biolistic-mediated transformation. The immature embryos were placed in petri dishes containing different induction media (M1, M2, M3, M4 and M5). All the media induced embryogenic callus, except, the medium 2, which produced brown callus in all the varieties. M3 medium induced very low frequency of embryogenic callus. However, the embryogenic callus derived from these media gave low regeneration frequency, when cultured on the regeneration medium, except for the M3. The M3 media was further amended with by additions of vitamins, sucrose and 2,4, D with different combinations and generated five additional media (M6 to M10). Upon testing these new media for embryogenic callus induction and regeneration, M6 and M8 were found to be very efficient in inducing embryogenic callus. These two media are being used for induction of embryogenic callus for biolistic transformation of durum wheat.

## PM21 - An improved somatic embryogenesis in bread wheat using mature and immature embryos

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One of the major obstacles of *in vitro* culture in wheat is its low efficiency of plantlets regeneration. It is known that the yield of callus and somatic embryos depends on the composition of the medium and explants sources, namely immature embryos and mature embryos. The regeneration efficiency also depends on genotype and age of embryos. In this study, we used mature and immature embryos as explants from four bread wheat varieties, The embryos were placed in petri dishes containing different induction media (M1, M2, M3, M4 and M5) to define culture conditions suitable for obtaining high frequencies of somatic embryogenesis and plant regeneration *in vitro* and compare results between mature and immature embryos in order to use mature embryos as a source of explant because it is a best alternative to save time and costs. All the media induced embryogenic callus, except, the medium 2, which produced brown callus with mature and immature embryos in all varieties. The results showed a differential response of media and varieties in their ability to induce embryogenic callus. For higher frequency of embryogenic callus induction and plantlet regeneration, the medium 1 is favorable for bread wheat varieties 'Aguilal', 'Mehdia' and 'Achtar'; medium 3 is favorable for 'Aguilal', 'Arrehane', 'Mehdia' and 'Achtar'. The experiments on regeneration using immature embryos as explants are currently in progress and they are at callus stage. The variety specific media are being used for somatic embryogenesis, as an integral part of wheat transformation process to improve drought tolerance by incorporating a late embryogenesis abundant gene.

**PM22 - Genetic variability for yield, yield parameters and oil content in safflower germplasm collection**

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Safflower (*Carthamus tinctorius* L.) is one of the premier *rabii* oilseed crops grown primarily for its much-valued edible oil rich in polyunsaturated fatty acids (linoleic acid 78%). It is known to reduce blood cholesterol level and hence recommended for heart patients. The presence of genetic diversity or variability is a basic requirement for crop improvement by plant breeding approaches. The objective of this study is to characterize the safflower germplasm collection consisting of 223 lines for morphological and yield contributing characters, which is primary step in crop improvement. The safflower germplasm collection was evaluated for two successive seasons (2008-09 and 2009-10) at Agricultural Research Station, Annigeri, Karnataka, India. Among the 223 germplasm collections, 27 were non-spiny & 196 spiny types; 23 were basal branching, 7 appressed types, & 193 normal branching types; 12 were early in maturity, 18 late maturing types & 183 lines have normal maturity duration of 130 days; 9 were dwarf statured, 25 were tall & 189 lines have normal plant height. The range of variation or diversity in the germplasm collection for important yield and yield contributing traits is as follows: number of capitula/plant: 6-41; diameter of main head: 1.5-3.1 cm; number of seeds/capitula: 10.6-60.3; hundred seed mass: 3.1-8.64 g; seed yield: 66.2-1901 kg/ha. The range of variation for oil content in the germplasm collection was 20.1 to 32.8%. The range of variation observed for oil content can be exploited for further improvement of oil content in safflower by hybridization and/or induced mutations or both.

**PM23 - GIS utilization in phytogeography of *Aegilops cylindrica* Host in Slovakia**

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In the Slovakia a lot of threatened and rare plant species are occurring. In this part of plants is listed also *Aegilops cylindrica* Host. This plant's origin is in south-eastern Europe and Asia Minor areas. It is annual grass and in the present increasing winter wheat production areas influence his occurrence and to modify his epizoochory. Most commonly, *Aegilops cylindrica* Host is found by vineyard lines, roadsides, buildings and anthropogenic biotope. Within the scope of project "Characterization and evaluation of diversity of wheat and their wild relatives and utilizing in breeding" were carried out survey in 2008 – 2009 years on the six localities in southern areas of Slovakia (localities: Sere, Dunajská Streda, Chaba, Kamenica nad Hronom, ierna nad Tisou and Dobrá). To locate the occurrence of the species we used GPS system. From that compile passport data we utilized by graphic display of map layers with GIS application. This method will help to us in following study and chorology and reproduction biology analysis of this species. Additional attributes in GIS will be molecular biology analyses of this species, which we deal with other part of this work.

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## PM24 - Enhancement of callus induction and cucurbitacin production in *Citrullus colocynthis* L. (Schrad) using plant growth regulators

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*Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) is a medicinal plant traditionally used as an abortifacient and to treat constipation, oedema, bacterial infections, and diabetes. The aim of this study was to investigate the effect of growth regulators and different explant type on callus induction and to increase the yield of cucurbitacin, an anti-inflammatory compound through tissue culture techniques. Leaf, stem and root explants of *Citrullus colocynthis*, taken from 15-day-old plants were cultured on Murashige and Skoog (MS) medium. The medium supplemented with different concentrations and combinations of 2, 4- dichlorophenoxy acetic acid (2, 4-D), Kinetin (kin), Benzyl adenine (BA) and -naphthalene acetic acid (NAA). The different concentrations of 2, 4-D + kin and BA + NAA as well as different explant organ types increased the callus fresh weight and dry weight. The callus cultures derived from stem explants grown on BA + NAA were proved to be appropriate protocol for callus induction, while 2,4-D failed to stimulate callus growth in the same manner. Different callus explants and the *in vitro* raised seedling leaf, stem and root were harvested and subjected to extraction of active principle compounds. The results revealed that, stem derived callus cultured on 2,4-D (2 mg/L) + kin (4 mg/L) produced the highest total cucurbitacins content with values reached 10.89% compared to the control seedling stem, leaf and root which produced 4.95, 4.97 and 5.12%, respectively. The HPLC analysis of cucurbitacin-E showed distinct changes in the different cultures initiated from various explants. The present study highlights the importance of biotechnological interference in this plant in order to overcome plant-to-plant chemo- variability to be used for large-scale production of drug at cost affordable levels.

## PM25 - Estimation of genetic variability for agro-morphological traits among red rice (*Oryza sativa* L.) genotypes

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Germplasm accessions of 110 red rice genotypes including landraces, elite lines and improved cultivars from ZARS, Mudigere, University of Agricultural Sciences, and Bengaluru, were evaluated for 14 Agro-morphological traits in three replications. Significant variability was observed for all the 14 Agro-morphological traits. All the genetic variability parameters like GCV, PCV, heritability and genetic advance as per cent mean were high for spikelets per panicle, fertile spikelets, panicle exersion and straw yield per lot. Positive significant correlation was observed for grain yield with days to 50% flowering (0.57), plant height (0.62), straw yield (0.90), days to maturity (0.56) and panicle length (0.50). Plant height showed positive and significant correlation with panicle length (0.67) indicating importance of plant height in improving panicle length. In genotypic path coefficient straw yield had higher magnitude of positive direct effect on grain yield (0.847). Accessions with best individual character performance were identified. To exploit their genetic potential and their beneficial use in the breeding programme.



## PM26 - Agro-morphological characterization of the cowpea (*Vigna unguiculata* L.) landraces in Turkey

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Turkey is one of the significant countries for plant genetic diversity. Although cowpea (*Vigna unguiculata* L.) originated in Africa, there are high variations of Turkish landraces since natural selection during the adaptation to different ecological regions and farmer's selection for their preference and different use of landraces. Due to diversity of cowpea landraces of Turkey and tendency of drought tolerance of this legume, 102 landrace accessions from Aegean and Mediterranean Regions of Turkey were collected and used to evaluate for 47 qualitative and quantitative agro-morphological characters in field conditions of AARI experimental area in 2009. The observed characters were analyzed by using the principle component analysis (PCS). The first three principle components (PCs) were accounted for 36.2% of total variation. The characters of days to flowering, days to first mature pods, terminal leaflet shape, pod curvature, and matured pod length were affected to compose of the PCs. In first two PCs, one distinct group and two sub-groups were formed for two third the accessions. The rest of landrace accessions were scattered out of this distinct group. Because of the significant variation of landraces in observed characters, there was no distinct grouping on other PCs. Eye pattern was significantly correlated with seed width, seed thickness, and 100-seed weight as 0.566, 0.652, 0.636 ( $P < 0.01$ ), respectively. Terminal leaflet shape and pod length were also significantly correlated with 0.539 ( $P < 0.01$ ). As a result, significant variation exists within and between cowpea landrace populations of Turkey.

## PM27 - Genetic variation and character interrelationship studies for identification of selection parameters in tomato genetic resources under subtropical conditions of Jammu, India

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Forty-nine genotypes of tomato collected from different sources and maintained in the Division of Vegetable Science and Floriculture, FOA, Chatha, SKUAST-Jammu, India, were evaluated for various quantitative and quality traits. The material showed wide range of variability for gross yield (8.64-379.04 q/ha), marketable yield (3.09-325.0q/ha), average fruit weight (12.23-82.21 g), marketable number of fruits per plant (1.67-177.00), pericarp thickness (0.15-1.05 cm) and total soluble solids (3.40-6.05%). The character association analysis indicated that total numbers of fruits per plant were significantly and positively correlated with gross yield (q/ha), marketable yield (q/ha), marketable number of fruits per plant, plant height (cm) and total soluble solids (%), where as plant height was negatively and significantly correlated with fruit shape index . Fruit shape index was negatively and significantly correlated with number of locules per fruit. Gross yield (q/ha) was significantly and positively correlated with total number of fruits per plant, marketable number of fruits per plant and marketable yield (q/ha). The estimates of direct and indirect effects showed that marketable yield (q/ha) gave highest positive direct effect on gross yield (0.904), followed by marketable number of fruits / plant (0.845) and total number of fruits plant (0.796). Maximum indirect contribution towards gross yield (q/ha) was through marketable yield (0.819) via total number of fruits. The present studies revealed considerable scope for improvement in tomato as total number of fruits per plant, average fruit weight (g), marketable number of fruits and plant height (cm) showed a wide range of variability. The phenotypic coefficients of variability (PCV) were higher with smaller magnitude than genotypic coefficients of variability (GCV), indicating little influence of environmental factors. The characters like total number of fruits, gross yield (q/ha) and marketable number of fruits showed high genetic advance coupled with high heritability and genotypic coefficient of variability, indicating thereby that selections based on phenotypic performance could be effective for improvement of these characters.

## PM28 - Genotype x tillage interaction in wheat improvement for conservation agriculture

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Increasing interest in zero tillage has resulted in cultivation of thousands of hectares under conservation agriculture worldwide. However, the breeders' ability to breed cultivars better adapted to these systems still need to be assessed. The presence of genotype x tillage (G x T) interaction across target environments, and assimilation of desired genome wide variation into the improved genotypes is crucial in increasing the genetic adaptation under zero tillage. Earlier studies showed a minimal G x T interaction in most of the cases but this should not limit further quest in this regard. Studies conducted in north-western NSW at Plant Breeding Institute University of Sydney on Berkut-Krichauff mapping population comprising of 160 wheat genotypes, showed significant differences among genotypes but non-significant G x T interaction across a two years study. However a changed depiction emerged when a subset comprising of two tails of the ZT-CT (ZT- zero tillage, CT-conventional tillage) distribution was separately analysed for analysis of variance. The revised analysis showed significant genotypic differences, along with significant G x T interaction among the genotypes. The studies were extended to a third year on a Sokol-Krichauff mapping population comprising of 150 wheat genotypes. The extreme genotypes subset (ZT- CT) verified the results of previous two years with significant genotypic and G x T differences in the subset. The results of this study will add up to the existing knowledge about G x T interaction in wheat and may have implications on future work to breed cultivars better adapted to conservation agriculture.

## PM29 - Isolation and characterization of new microsatellite marker in *Taxus baccata* L.

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*Taxus baccata* L. (English yew) is one of the most important medicinal tree species globally. It is well-known for its Taxol content. Here, we report the isolation and characterisation of 31 new polymorphic microsatellite loci from a repeat-enriched genomic library of *T. baccata* L. The genetic diversity of these loci was assessed in 48 individual samples of *T. baccata* L. All loci were variable: the number of polymorphic alleles per locus ranged from 2 to 9 (average 4.45). The observed and expected heterozygosities ranged from 0.15 to 1 and from 0.14 to 0.83, respectively. The loci were informative with polymorphic information content values that ranged from 0.21 to 0.82 (average 0.55). Nineteen of the 31 loci conformed to Hardy-Weinberg expectations. The loci identified in this study should provide useful tools to study the population structure and genetic diversity of *T. baccata* L. and promote its management and conservation.

### PM30 - Determination of criteria of selection for the tolerance of durum wheat to salinity at germination and the three sheets stage

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This study was carried out with five varieties of durum wheat cultivated in Tunisia: two old accessions (Chili and Roussia) and three relatively new (Maghrebi, Om Rabii and Khiar). These varieties were treated with six concentrations: 0, 1, 3, 5, 7 and 9 g/l of NaCl. During germination, the discriminative character for the tolerance with salt was the emergence of the coleoptile. At the stage 3 sheets, the weight growth of the seedling and especially of the stems constitutes the best parameter of appreciation of the tolerance to salinity. The degree of tolerance to the salt of the plants also seems to be correlated with the  $K^+/Na^+$  coefficient. According to these criteria, Roussia and Chile behave like tolerant varieties, Om Rabii, Maghrebi and Khiar like fairly tolerant varieties, the last being most sensitive. The results obtained by one or the other of the tests are completely concordant. Indeed, the classification obtained at the stage seedling is in conformity with that of germination. Thus the percentage of germination at the stage emergence of the coleoptile can constitute a reliable criterion for the evaluation of the tolerance to the salinity of durum wheat and this until the stage seedling.

### PM31 - Studying of the adaptive reaction of *Zea mays* L. accessions in drought and salinity conditions

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The adaptation of plants to the unfavorable environmental condition consists of in quality different stages - stress reaction and specialized adaptation (Kusnetsov, 2001). During the effect of adverse factors, stress reaction provides the temporary protection of plants from death and promotes the forming of the specialized long-term resistance.

It is known that, the plants are more sensitive to the stress factors during the seed germination. The decreasing of the germinating ability of seeds of different varieties differs in the same level of drought and salt and this fact reflects their distinctive stress tolerance potential (Udovenko, 1988). Therefore, the assessment of stress reaction of the different *Zea mays* L. accessions were carried out based on the seed germination technique in sucrose and salt medium. Consequently, the KF-38, KF-64, KF-58 and KF-63 genotypes of maize were characterized with more tolerance to drought, KF-38, KF-55, KF-57, KF-58, KF-39, KF-60, KF-63 and KF-67 with more tolerance to salt stress. The KF-38, KF-58, KF-63 and KF-64 were both drought and salt tolerant accessions among the studied genotypes.

**PM32 - Estimation of resistance of varieties of a sugar beet to *Erysiphe communis* Grev. and *Cercospora beticola* Sacc.**

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Researches of immunity and also selection of a sugar beet is conducted concerning the most harmful diseases. Such as *Pythium Pringsh.*, *Cercospora beticola* Sacc., *Erysiphe communis* Grev., *Peronospora schachtii* Fuckel f. *betae* Jacz., *Botrytis cinerea*.

Studying photopathological resistans of samples of a sugar beet to *E. communis* Grev. has shown that six varieties were immune against this disease, it has made 12.5%, 29.2% were highly resistant, 20.8% were resistant, 35.4% were tolerant. Only one variety appeared to be susceptible, that is 2.1%, and there were no highly susceptible varieties to the disease. The phytopathologic estimation of resistans of varieties of a sugar beet has established that one variety only was immune to *C. beticola* Sacc., that is 2.1% 4.2% were highly resistant, 10.4 % were resistant, 52.1% were tolerant, 29.2% were susceptible, and highly susceptible varieties were 2.0%.

The comparative estimation of phytopathologic resistance to *E. communis* Grev. and *C. beticola* Sacc. has shown that varieties of a sugar beet are resistant against *E. communis* Grev. than to *C. beticola* Sacc., since percentage of immune, highly resistant and resistant varieties to *E. communis* Grev. in the sum have made 62.5%, and against to *C. beticola* Sacc. - 16,7%. Percentage of susceptible and highly susceptible varieties in the some made 2.1% (*E. communis* Grev.) and 31.2% (*C. beticola* Sacc.) respectively. Thus, resistance to *E. communis* Grev. and *C. beticola* Sacc. varieties of a sugar beet are a valuable material and can be used in selection as donors of resistance to these diseases.

**PM33 - Growth, yield and radiation use efficiency of cotton as affected by different irrigation scheduling and integrated plant nutrition**

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Two field experiments to evaluate the effect of different levels of integrated plant nutrition and irrigation scheduling on the growth, radiation use efficiency and yield of cotton during 2003 and 2004 growing seasons. The experiments were conducted on the Agronomic Research Area, Postgraduate Agricultural Research Station (PARS), University of Agriculture, Faisalabad, (31.25° N, 73.09° E, 184.0 m) Pakistan. Irrigation schedules I<sub>1</sub> (six irrigations), I<sub>3</sub> (irrigation at 25 mm potential soil moisture deficit) and I<sub>4</sub> (irrigation at 50 mm potential soil moisture deficit) increased seed cotton yield by 79.23, 80.26 and 81.70%, respectively over I<sub>2</sub> (three irrigations) during 2003 and 79.19, 80.20 and 81.65%, respectively during 2004. It was mainly due to the increase in total dry matter production (TDM) in former than the later. Increasing rate of integrated plant nutrition levels significantly enhanced seed cotton yield and TDM over control and lower rates of integrated plant nutrition. Integrated plant nutrition level N<sub>5</sub> (150-75-75 kg NPK ha<sup>-1</sup> + FYM @ 20 t ha<sup>-1</sup>) increased seed cotton yield by 53.63% in 2003 and 53.69% in 2004 over control that was followed by N<sub>6</sub> (150-75-75 kg NPK ha<sup>-1</sup> + Wheat straw @ 5 t ha<sup>-1</sup>) which gave higher seed cotton yield (35.49% in 2003 and 35.50% in 2004 over control) in comparison to the rest of integrated plant nutrition levels. The seed cotton yield was strongly dependent and related to the total dry matter production, as there was a positive and linear relationship between them. Higher TDM production in I<sub>4</sub> (irrigation at 50 mm potential soil moisture deficit) or in other higher integrated plant nutrition levels was due to higher crop growth rate in these treatments. Analyzing crop growth and yield in terms of leaf area duration (LAD) and yields, a strong and positive linear relationship was found. Maximum radiation use efficiency of 1.86, 1.86 and 1.88 gMJ<sup>-1</sup>, respectively in 2003 and 1.82, 1.82 and 1.84 in 2004. The minimum values of 1.21 gMJ<sup>-1</sup> and 1.19 gMJ<sup>-1</sup> were recorded in 2003 and 2004, respectively with I<sub>2</sub> (three irrigations). Maximum values of radiation use efficiency (1.99 in 2003 and 1.95 in 2004) were recorded with the treatment N<sub>5</sub> (150-75-75 kg NPKha<sup>-1</sup> + FYM @ 20 t ha<sup>-1</sup>). The minimum values (1.52 in 2003 and 1.49 in 2004) were observed with N<sub>0</sub> (control).

**PM34 - Integration of biotechnology for the studies of the grape fanleaf virus (GFLV) in Moroccan varieties diagnosis and control methods**AM Mdarhri <sup>1</sup>, S Tarek <sup>2</sup>, S Raji <sup>2</sup>, F Gaboune <sup>1</sup>, L. Belamri <sup>3</sup>, L El Alami <sup>1</sup><sup>1</sup> UR biotechnology National Institute of Agronomic Research CRRRA Rabat, Morocco<sup>2</sup> DPVCTRF Department of the plant protection and the Suppression of Fraud Rabat, Morocco<sup>3</sup> Hassan II, Institute of Veterinary and Agronomy (IAV), Rabat, Morocco

In Morocco, grape vines (*Vitis vinifera* L.) occupy fourth place in importance among the fruit trees after olive trees, almonds and citrus. The profile of Moroccan grape vine is composed of several varieties, some are introduced and others are local. This is an important channel for Moroccan agriculture and plays a significant socioeconomic role. The Moroccan vineyards are particularly susceptible to many biotic stresses namely viral diseases among them the Grapevine fanleaf virus (GFLV) is exclusively transmitted to its natural host in the vineyards by nematodes (*Xiphinema*), threat which could be dangerous to our national output. The establishment of a research program to study these diseases through biotechnology methods constitutes a track for the sound management of plant germplasm of this species, a way for the creation and production of healthy and / or resistant vines. The control strategies used nowadays are preventive measures which are based on sanitary selection and chemical control of the known vectors, sanitary selection calls for detection techniques the most used being biological indexing and immunochemical techniques. Actually molecular tools are being developed for the detection of viral RNAs in grapevines. Research programs are running which aim to develop new control strategies namely pathogen-derived resistance. This area is the main goal of our research program.

**PM35 - Study quantitative and qualitative characteristics of irrigated and drying farming of durum wheat**Mirakhorli N, Shojaei ZA

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This study was aimed to determine the effect of drought on characteristics of Iranian durum wheat germplasm to find out desirable traits of them. Two experiments were conducted under non stress and water stress conditions using an augment design. The results showed that the nutritional quality of grain increased under water stress condition. However other traits such as yield, plant height and 1,000-kernel weight were negatively affected by water stress. Additionally, the results of correlation, stepwise regression and path analysis showed under water stress condition, traits namely number of spike in plant, 1,000-kernel weight, spike yield, peduncle length, spike weight, number of kernel in spike and plant weight had significant effects on yield as well as spike yield, number of spike in plant, peduncle length under non stress conditions. Therefore, they can be used as criteria for yield improvement. Moreover, principal component analysis showed that six component accounted for most of the variations among traits in stress and non stress condition. The values of STI, GMP, MP were the best index because of their positive significant correlation with yield in both conditions. Consequently seed yield data had low repeatability thus they cannot be suggested as selection criteria. Nevertheless, selection is based on traits such as day to heading and plant height with more repeatability could be very useful.

### PM36 - Path analysis of yield and yield components for safflower (*Carthamus tinctorius*) genotypes

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An experiment was conducted with four genotypes of safflower in the research station of Faculty of Agriculture, Islamic Azad University, Kaleybar Branch, Iran. In this study, a randomized block design with three replications was carried out. After ripening, seed yield and its components were measured. Then, the relationship of seed yield and yield components was investigated by calculating Pearson's correlation and carrying out forward ridge regression and subsequently, phenotypic path analysis. After forward ridge regression, three characters remained in the model. In the path analysis only two characters (pod number and seed number in pod) had significant and positive direct effect on seed yield.

### PM37 - Influence of genotype, foliar disease pressure and date of sowing on oil quality in groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) is one of the major and important oilseed crops of the world and the fatty acid composition of oil determine the quality of groundnuts. Quality of groundnut oil depends upon the proportion of oleic and linoleic acid. Various factors, viz., genotype, seasonal variation and foliar diseases affect the fatty acid make up of groundnut oil. A study involving foliar disease resistant (28-2, D 39d and B 37c, ICGV 87165, and ICGV 86590) with ruling but susceptible Spanish bunch cultivars (JL 24, TMV 2, Dh 8, R 8808 and TAG 24) was conducted in two rainy seasons under unprotected (UP) protected (P) conditions. Analysis of variance for fatty acid composition (palmitic, stearic, oleic, linoleic, arachidic, eicosenic, behenic and lignoceric acids) and oil quality (O/L and P/S ratios) parameters indicated significant genotypic effects for all the parameters. Date of sowing had significant effect on linoleic acid, arachidic acid content and O/L ratio. While, disease control treatment significantly affected all the parameters except linoleic acid and arachidic acid content. However, the margin of change was less as a result of date of sowing and disease control on fatty acid composition and oil quality as compared to genotypes. All the interaction effects involving genotype were highly significant for all the parameters indicating the importance of genotypic effects in determining fatty acid composition and oil quality. Among the genotypes, foliar disease resistant inter-specific derivatives D 39d (Spanish bunch) and ICGV 87165 (Virginia bunch) had high oleic acid (46-48%) and O/L ratio (1.5-1.7) across dates of sowing and disease pressure revealing the improved shelf life and nutritional quality in these cultivars. D 39d also had higher unsaturated fatty acid content (78.8 %) next to Dh 8 (80.6%). Genotypic and interaction effects involving genotype were highly significant for fatty acid composition and oil quality parameters and margin of change was also more due to genotypes than date of sowing and disease control treatment indicating the importance of genotypic effects in determining fatty acid composition and oil quality. However, date of sowing had significant effect on linoleic acid, arachidic acid content and O/L ratio, while disease control treatment affected all the parameters except linoleic and arachidic acid content. This implies that genotype and its interaction effect with production practices have to be taken into account in improving the oil quality in groundnut.



**PM38 - Genetic analysis of oil quality traits in sunflower (*Helianthus annuus* L.)**

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Sunflower crop known for its excellent nutritional properties has gained rapid area expansion in India with its versatile nature of wide adaptability. However, the efforts on genetic improvement for oil quality traits are very much limited. In the present study, the new cytoplasmic male sterile (CMS) lines (3) developed with high oleate trait and two normal oleate CMS lines were crossed with seven different restorers and the hybrid combinations were evaluated for oil quality. CMS lines had significant variation for stearic, oleic & linoleic acid but restorers had significant variability for palmitic acid. However, hybrids had significant variation for all the above four major fatty acids studied. The proportion of sca variance was much higher (5 to 7 times) compared to gca variance for two major fatty acids (oleic & linoleic acid) indicating the role epistasis in determining the major oil quality trait of O/L ratio. Two new CMS lines and one mutant restorer line were identified as good general combiners for oil quality traits. The heterosis for two major fatty acids (oleic & linoleic acid) was significant in both the directions. Four hybrid combinations recorded significant positive heterosis (13.60-18.11%) over better parent with per se performance of around 90% oleic acid. This study indicates the importance of epistatic gene interactions in determining oil quality traits which could be exploited through heterosis breeding.

**PM39 - Genetic diversity in yield components and determination of selection indices in various cultivars of cotton**Nazemi Ghasemali<sup>1</sup>, Jafari Aliashraf<sup>2</sup>, Dehghani Gita<sup>3</sup><sup>1</sup> Islamic Azad University- Bandar abbas Branch, Iran.<sup>2</sup> Research Institute of Forests and Rangelands-Tehran, Iran<sup>3</sup> Education Office-Estahban, Iran

To produce a plant breeding variety, various traits are considered. Most of these traits have a high inter-relationship and a high correlation with yield, so that improved varieties are product of simultaneous or non-simultaneous selection of these traits. In order to the evaluation of genetic diversity in yield components and determination of selection indices in nine foreign varieties cotton and one domestic varieties were sown using a randomized complete block design with four replications in agricultural research stations Darab and Fars. Data were collected and analyzed for plant number, vegetative branch length, vegetative branch number, sympodial branch length, sympodial branch number, boll number, boll weight, earliness and total yield. Results of analysis of variance showed significant differences ( $P < 0.01$ ) among varieties for all of traits except the vegetative branch length. Path analysis was done in order to investigate the inter relationship between the traits and yield. This analysis showed that boll number had the most effect on yield production and direct selection based on this trait would be beneficial. Using broad sense heritability as the relative economic value, more gain than direct selection only based on the yield. The highest selection indices were obtained for both Bakhtegan (the commercial control) and 43259. This variety were suggested to cultivation selection indices were estimated. The results shown that selection based on the all of traits would make in Darab area in Fars province, as an alternative variety.



## PM40 - Evaluation of Barnyardmillet (*Echinochloa frumentaceae* Rox.) germplasm

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Barnyardmillet is native of Eurasia and is an important crop of dry land agriculture. The success of Barnyardmillet is attributed to prolific seeding, rapid growth and flowering in a range of photo and thermo periods and seed dormancy. It is grown over a wide array of environments and poor to varied soil health conditions. It is equally important as a grain and fodder crop. Its cultivation is mainly confined to tribal belts of the states of Orissa, Maharashtra, Gujarat, Madhya Pradesh, Tamil Nadu and Bihar besides hilly tracks of Uttaranchal state of India. It is generally cultivated in hill slopes and undulating fields where a few options exist for crop diversification. Germplasm is the best raw material in any crop improvement programme. The progress so far achieved in plant breeding is directly related to the available diversity in the crop. Recognizing the fact, Department of Millets, Centre for Plant Breeding and Genetics, TNAU, Coimbatore assembled accessions of Barnyardmillet. The characterization and evaluation are the important pre requisites for effective utilization of germplasm and also to identify sources of useful genes. So, an effort was made for detailed evaluation of 268 accessions for 27 descriptors as per IBPGR (1983) guidelines at Millet Breeding Station during 2008-2009. The experiment was laid out in randomized complete block design with three replications. The data were analyzed by following standard statistical procedure for arriving range of variability, frequency, distribution, co-efficient of correlation matrices depicting character associations and querying data base for desired traits. The study showed sufficient variability for screening the germplasm materials for important qualitative and quantitative characters. The ultimate goal of evaluation is to serve as a useful document to breeders in selecting most useful germplasm for their research activities.

## PM41 - The genetic resources of the largest Russian grapevine collection

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The largest Russian grape germplasm collection belongs to North Caucasian Regional Research Institute of Horticulture and Viticulture of the Russian Academy of Agricultural Sciences and is located on Black Sea coastal area of Krasnodar region in Anapa. Collection contains over 3,000 accessions. 80% of the samples were obtained from diverse regions of ex-Soviet Union, 20% from different areas of Europe, Asia and America. Almost half of the collection is represented by table cultivars (48.1%), whereas 40.3% are wine cultivars, and 11.6% table-wine. Rootstock cultivars are widely presented. There are 110 seedless cultivars. According to the terms of ripening, samples of collection could be divided into seven groups. *V. vinifera* L. accessions prevail in the collection. Among those more than 70% are local cultivars from different region of viticulture, the rest are intravarietal hybrids. Cultivars of another species of genus *Vitis* (Tournef.) L. belongs to *V. labrusca* L. (less than 2% of the collection).

Interspecific cultivars compose 23.4% of the collection. Mostly these are hybrids between *V. Vinifera* L. and *V. amurensis* Rupr., which have high level of tolerance to low temperature. There are series of genotypes, combining high fruit quality with high tolerance to pests, diseases and freezing, which were bred using interspecific hybrids Seyve Villard. Replenishment of gene pool with new samples takes place routinely. Study of cultivars and search for donors of valuable traits are conducted. Breeding for high content of biologically active substance in berries, resistance to fungal diseases and phylloxera, drought and cold tolerance is carried out on the collection. Recourses of the collection were used to breed high quality cultivars for production (17 wine-, 14 table- and 4 rootstock). Currently electronic data bank of ampelografic collection is being formed. It contains more than 50 characteristics of each cultivar. From the date of establishment in 1995 plantations of the collection were exposed to different abiotic and biotic stresses. It gave the opportunity to determine groups of cultivars with high adaptive potential for further use in agricultural production and breeding.

#### PM42 - Effect of drought stress in the field and the relationship between an in vitro method (Polyethylene glycol 6000) for screening sugar beet genetic resources

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This study is done in order to determine the relation of some laboratory traits with farming traits for finding the appropriate traits for screening sugar beet genotypes in the condition of laboratory stress instead of fulfilling the stress in the farm. Of 15 pollinate groups (O-Type) which were tolerant of drought, in five genotypes (control) three were tolerant, one semi-tolerant and one sensitive were evaluated in farm and laboratory conditions. Drought stress in permanent and severe drought conditions were fulfilled the experimental design was randomized complete block with six replications in researching farm located in Karaj in 2008. Permanent and severe drought stress started by stop of irrigation after plant establishment continued to the end of growth season. Pollinates of G4 (O-Type 9590), G8 (O-Type 7617) and G7 (O-Type 8090) were distinguished as hopeful genotypes concerning drought toleration in the farm. In the laboratory, Polyethylene glycol 6000 was used in solid medium for creating various levels of water stress. Four water stress level with osmosis potential of 0, -0.6, -0.7 and -0.8 MPa and 20 sugar beet genotypes were arranged in factorial experiment on CRD with three replications under control experiment. The genotypes G7 (O-type 8090), G10 (O-type 463) and G5 (O-type 1609) should superiority among pollinates under different drought stress levels for most tested traits than other genotypes. The correlation of these laboratory and farm traits showed that in stress of -0.6 MPa, the length and dry weight of root has negative and significant correlation with sugar percentage at farm in 5% of P value. The correlation of laboratory attribute of wet weight of root had positive and significant correlation in p value level of one percent with farm attributes of root yield, sugar and white sugar, dry weight of root and proportion of dry weight of root to shoot. Under stress condition of -0.8 MPa in laboratory, wet weight of root showed positive and significant correlation with attributes of root yield, sugar and white sugar and total dry weight of root and shoot ( $P < 0.05$ ). In general, according to existence of positive and significant correlation between root's wet weight of sugar beet plantlet in laboratory condition with farm traits we can take actions for implying water stress of about -0.6 MPa in laboratory instead of water stress at farm for genotypes tolerant to drought and from root's wet weight of genotypes having high yield of root, they can be sifted under intense water stress at farm.

#### PM43 - Effect of continuous and sever water stress on the morphologic, quantitative and qualitative characteristics of 20 sugar beet genotype

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The current research was done in order to evaluate genetic diversity, and screening the tolerant genotypes of sugar beet subjected to continuous and severe water stress in the experimental farm of Sugar Beet Seed Institute in Karaj in 2008. The experimental design was randomized complete block with six replications. Fifteen O-Types which were tolerable to drought plus five (check) genotypes of which three of them were tolerable, one semi-tolerable were evaluated. Permanent and severe drought stress started by stop of irrigation after plant establishment continued to the end of growth season. The soil water suction reached to about 4 MPa in the horizon of 0-30 cm at the end of growth period. During the growth season, the attributes of morphology characters, and the end of growth season, yield and quality of genotypes were determined. The results of ANOVA showed that the effect of genotypes on the root yield, shoots dry weight, total dry weight, roots dry weight, root dry weight/ shoot dry weight ratio, sugar yield, achievable sugar yield, sugar percentage, harmful nitrogen and sugar percentage were significant ( $P < 0.01$ ). Genetic diversity among the new surveyed genotypes concerned drought tolerance of sugar beet genotypes. Thus, there is chance to identify tolerant genotype for drought stress. Existence of meaningful difference and genetic diversity among different species of sugar beet has been confirmed in other studies too. The results confirmed that under the situation of permanent and severe water stress the genotypes of G8 (O-Type 7617) and G4 (O-Type 9590) were distinguished as tolerant O-Types and may be used in the breeding program for provision of tolerable to drought stress species.

#### PM44 - Enhancement nutrient content and yield in mungbean using mutation induction

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The genetic variability is the basic requirement for making progress in crop breeding. Thus, mutation induction has become an establishment tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Especially in mungbean (*Vigna radiate* (L.) Wilczek) that has been favoured by children and elders due to its easy digestibility, rich source of protein, vitamin B1 and low flatulence problem. Therefore this research was aimed to improve the genetic variability and nutrition content in mungbean through seed irradiated by Gamma ray treatment with doses 200 Gy. Three mutants and parent have been observed on yield potential and nutrition content in M<sub>7</sub> generation. They were Ps-30, Ps-31, Ps-15, and Gelatik. The result showed that Ps-31 (24.55%) has the highest protein content. The other mutant lines (Ps-30, Ps-15, and Gelatik) have ranges of protein percentage were 21.84–24.40%. Subsequently, oil, vitamin B1, P and Fe content of the mutants were achieved 1.20% (Ps-31), 0.90 mg/100g (Ps-31), 580.25 mg/100 g (Ps-15) and 7.11 mg/100 g (Gelatik) respectively which superior among the mutants and parent. The multilocation trial test has been performed on the mutant lines and control in four marginal land locations in Indonesia. The mutant line has the highest yield potential is Ps-31 (3.00 ton/ha) among mutants and parent (2.36 ton/ha) significantly.

#### PM45 - Evaluation of promising barley lines for terminal drought tolerance

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In order to evaluate of 15 promising barley lines for terminal drought stress tolerance, a research as two experiments was conducted under drought stress and non stress condition at Research Center of Jihad -Agriculture of Eastern Azerbaijan in 2007. In both experiment were used randomize complete block design. The results showed that were significant difference between studied lines for all traits except filling period. Interaction of line × experimental conditions was not significant for any traits. So comparison of means with average data based on combined analyses showed that, line 1 having the highest value for all traits. Path analysis for grain yield showed that plant height and number of grain per spike were important and effectual traits for grain yield of studied barley lines.

#### PM46 - Diversity and characterization of Jerusalem artichoke using agronomic and morphological characters

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A better understanding on genetic diversity and characterization of germplasm are priority in any breeding program. Jerusalem artichoke (*Helianthus tuberosus* L.) is a promising candidate for inulin production in the tropical climates, and genetic improvement should increase crop productivity and product quality. The objectives of this study were to investigate genetic diversity in 87 accessions of Jerusalem artichoke germplasm from different sources in Europe and America and to characterize these accessions using agro-morphological characters. The accessions were evaluated in a randomized complete block design with two replications for two seasons in the late rainy season 2008 and early rainy season 2009. Days to harvest, fresh tuber yield, biomass and inulin content were recorded at harvest. Significant variations were found in all characters. Days to harvest ranging from 92 to 104 days were observed. Fresh tuber yields from 2.3 to 25.1 t ha<sup>-1</sup> were recorded. Variation in biomass was observed, ranging from 1.4 and 11.9 t ha<sup>-1</sup>. Inulin contents were found between 53.9 and 75.4% of dry weight. Days to harvest was not associated with fresh tuber yield ( $r = 0.19^{ns}$ ) but was significantly associated with biomass ( $r = 0.49^{**}$ ). The relationship between days to harvest and inulin content was negative ( $r = -0.36^{**}$ ). Biomass and fresh tuber yield were well correlated ( $r = 0.78^{**}$ ), but they were not associated with inulin content. Euclidean coefficients of genetic dissimilarity ranged from 0.50 to 13.53. Cluster analysis based on dissimilarity matrix revealed six distinct groups that should be useful to breeder. These data would enable breeders to make informed decisions about suitable parents for their breeding programs.

#### PM47 - Morpho-agronomic characterization of woad (*Isatis tinctoria* L.) accessions

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In the 18th/19th century dye plants were very important in European agriculture. After beginning of 20<sup>th</sup> century the increase use of synthetic dyes by the industry leads to a decreasing interest of natural dyes and to the abandonment of dye crops.

Woad (*Isatis tinctoria* L.) was the most important source of natural blue indigo, a pigment used mainly for dyestuff.

The control of toxic effluents and the classification of many synthetic colorants as toxic, when in contact with the skin, were responsible for an increasing interest of natural dyes by the dyestuff industry.

The aim of this study was to assess similarities/differences regarding morphological and agronomical traits of woad accessions from different European countries.

Ten accessions from nine countries were characterized by five qualitative traits and fifteen quantitative traits. From each accession were characterized 10 plants. The plants were grown at Vila Real (41°17'N; 7°44'W; 460 m above sea level) – Portugal.

Qualitative traits, such as rosette vigour, colour, pubescence and consistency of leaf and growth habit, revealed a low variability. A great variation for agronomic traits like leaf size, plant height, number of secondary stems, days of vegetative and reproductive growth, size and number of siliques and 1000 siliques weight was observed. PCA diagrams separated the accessions in four groups primarily according with their geographic origin. The first three components of the PCA accounted 74% of the total variation.

The wide diversity observed in this woad collection shows that it can be used for selection and for hybridization in order to obtain improved varieties.

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## PM48 - Evaluation of Iranian bread wheat landraces under cold and drought conditions in the drylands area

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Wheat is one of the major crops grown in the Islamic Republic of Iran. The total area covered by wheat in dryland is about 3.8 to 4.2 million hectares. Drought stress is one of the most important threatening factors for the production of crop in the arid and semi-arid regions of the country. Studies to identify the important traits affecting drought and cold tolerance on grain yield stability are a very important aspect in wheat breeding programs in DARI. Average of grain yield in the rainfed areas was low, due to drought, excessive cold in mountains, high temperature during late spring, diseases and insect pests. A total of 3,500 Iranian gene bank bread wheat landraces were evaluated from 1997-08 in Maragheh, Qamlo, Ardebil, Zanjan, Shirvan and Sararood Research Stations. The experiments were arranged as observational nursery and RCBD with four replications. Analysis of data on 16 evaluated characteristics, showed, there was a lot of variability for all traits, especially for grain yield. The difference of heading time between earliest and latest lines was 25 and for maturity time was 15 days. Factor analysis with extraction of Eigen values by Principal Component Analysis (PCA) revealed five factors explaining 67.57% of total variation. The two important factors were 2<sup>nd</sup> factor including grain-filling period, heading time, 1,000-kernel weight and 3<sup>th</sup> factor including grain yield, plant height, cold tolerance.

Overall, results of 9 years of research showed that, line No. 14 Gene Bank was found among the suitable line with 2230 and 4494 kg ha<sup>-1</sup> of grain yield, under rainfed and supplementary irrigation conditions respectively, and produced the highest yield. Result of stability analysis on grain yield using parametric method of C.V.% and Lin and Binns, and non-parametric method of rank, showed that No. 14 Gene Bank, was the most stable genotypes. Finally results based on the means of grain yield, stability parameters, bread quality, and diseases results, indicated that genotype No. 14 Gene Bank was found as the highest in yielding, stable and tolerance to drought and cold therefore was selected as a desirable genotype for releasing in cold and moderate-cold dryland areas of Iran in 2009-10 cropping season.

## PM49 - Estimate biological nitrogen fixation in horse bean

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Research projects as split plot experiments in a randomized complete block design with four replications in field research in Islamic Azad University of Ahvaz 3 consecutive years (2006,2007,2008) implementation was the main plot assembly, four cultivar horse bean (*Vicia faba* L.) plant: BARAKAT, ZOHRE, SHAMI and JAZAYERI, damascene the number of islands in the province have grown and sub-plots in 2006 and 2007 three levels of nitrogen fertilizer (N1, N2 and N3 treatments, respectively 20 and 40 and 80 kg fertilizer N ha simultaneously planting) and the third year, 2008 values were doubled care. After the propagation earth, using cultivar with Rizobium bean plant (*Rh. Leguminosarum*) inoculation and immediately cultured. Survey cultivar, BARAKAT highest percentage of mean total nitrogen plant 1.97 percent won. In sub-plots, with increasing amounts of nitrogen, accumulation of this element bean plants increased. Percent nitrogen treatments nodes N2 and N3 showed a significant difference, but the highest accumulation of nitrogen treatments N1 nodes with 1.67 percent won, thus whatever amount of fertilizer increased, the amount of biological nitrogen fixation nodes decreased. N3 treatment reduced accumulation of 40 to 50 percent nitrogen found in to other treatments. With increasing N rate, weight, number and size of the plant nodes decreased blessing average number of nodes 1250 nodes per plant among the highest number of cultivars grown offered. Number of nodes equal treatment and 1450 to increase the amount of fertilizer treatments 80 kg 998 nodes per plant decreased in all fertilizers in small amounts or how large gland enlargement process was observed. The mean largest tumor diameters in the treatment 1.98 cm were measured. Green and white non-effectiveness of enzyme Nitrogen's stated that usually the primary growth was achieved in pink and red and efficient biological nitrogen fixation, approximately 35 days after planting continued until after flowering and 10 days after flowering, gland Posts brown and black, showed the node representing aging and lack of nitrogen is established.



### PM50 - Assessment of genetic diversity between local grapevine accessions and wild relatives belonging to Near-Caspian zone of Azerbaijan by SSR markers

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In this research, microsatellite markers were used to estimate genotypic similarities, genetic diversity, and clustering of 65 grapes samples, including 31 cultivated and 34 wild accessions belonging to the regions near Caspian Sea in Azerbaijan Republic. A total of 184 alleles were identified giving in average 12.26 alleles and 5.7 effective number of alleles for 15 SSR markers. Among the primers under study, VVMD24 primer had the lowest diversity, VVMD28, VVMD36 and VVS2 primers had the highest diversity regarding expected heterozygosity and PIC. VVMD28 and VVMD36 primers, more than just showing the highest diversity among studied samples, had lowest values of polymorphic identities (PI), which makes them the most appropriate markers for the identification of accessions and determination of genetic diversity among cultivated and wild grapes. Among the investigated grape populations, wild types sampled in Davachi region had the highest diversity, regarding the average number of alleles, the effective number of alleles and expected heterozygosity. Since wild types are very rich sources of diversity at different useful genes, especially the genes of adaptation to biotic and abiotic stresses, we can take advantage of the existing diversity in these samples, in order to use them in collection management and breeding studies. Clustering analysis based on SSR markers led to a fine separation between cultivated and wild samples of different regions. Accordingly, it can be concluded that the observed genetic diversity in studied samples based on genetic markers under study corresponded to the geographic diversity.

### PM51 - Evaluation of response of wheat genotypes to a humic fertilizer against terminal drought by use of stress tolerance indices

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Breeding for drought tolerant of wheat is an important task and objective in arid and semiarid regions with Mediterranean climate during the grain filling period. Humic substances (HS) are major components of the natural organic matter in soil and water as well as in geological organic deposits such as lake sediments, peats, brown coals and shales. Mitigating activity of HS is observed under biotic and abiotic stress conditions. An experiment was conducted for assessing tolerance of six bread wheat genotypes to terminal drought at presence of a liquid humic fertilizer (HF) based on peat. Experimental conditions were well watered; well watered + HF; terminal drought; and terminal drought + HF. Based on grain yield of genotypes in these conditions, stress susceptibility index (SSI), tolerance index (TOL), mean productivity (MP), stress tolerance index (STI), and geometric mean productivity (GMP) were calculated. There were significant correlations (at  $P < 0.01$ ) for MP, GMP and STI with potential yield ( $Y_{pi}$ ); and MSTI with stress yield ( $Y_{si}$ ) in the condition of unused HF. But there weren't such relations with application of HF. There was significant correlation (at  $prob < 0.05$ ) between TOL and SSI with  $Y_{si}$  at presence of HF. These correlations weren't observed for unused HF condition. Without application of this fertilizer, genotype 4057 had the highest grain yield in the both stressed and non-stressed environments. HF reduced average grain yield differences between stressed and non stressed conditions from 1.0 to 0.1 ton/ha. This natural fertilizer can ensure production levels in the both environmental conditions. Genotype 4057 selected as a tolerant to terminal drought of Ardabil region, with or without HF. This genotype had the highest MP, GMP and STI. Also, it had the lowest susceptibility relative to stress. Humate use, in addition to 4057, caused to select Gascogen as a tolerant genotype. Numerical values of indices for Gascogen were similar to 4057. But increasing of its grain yield from 3.73 ton/ha ( $Y_{pi}$ ) to 4.28 ton/ha ( $Y_{si}$ ) was unbelievable. It seems this is the miraculous effect of applied humic substance on this genotype.

### PM52 - Harvest index associated characters in winter wheat genotypes against terminal drought at presence of a humic fertilizer

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Wheat is the world's most important crop and drought is a worldwide problem. Wheat production is subjected to water deficit after anthesis in Ardabil. Humic preparations are increasingly applied as natural stimulators in plant breeding. This investigation was done for evaluation response of six bread wheat genotypes to a liquid humic fertilizer based on peat against terminal drought stress. Humic fertilizer decreased drought stress intensity by 20%. Experimental conditions (well-watered; well-watered + humic fertilizer; terminal drought and terminal drought + humic fertilizer) had significant differences for economic yield and biological yield. But wasn't significant for harvest index. Humic fertilizer increased economic and biological yield respectively by 0.74 and 1.58 ton/ha in drought condition. Genotypes had significant differences for economic yield and harvest index. But interactions between conditions and genotypes were not significant for measured traits. Genotypes Gascogen, Sabalan and 4,057 had the highest harvest indices and economic yield. The lowest amounts of economic and biological yield were belonged to drought stress condition. But other three conditions were placed in a same situation for these three traits. Correlation between economic and biological yield was positively significant for all of four experimental conditions. Also, there were positively significant linear relationship between economic yield and harvest index for well-watered and drought stress + humic fertilizer conditions. But they hadn't linear relations in the other two conditions. Correlation of biological yield and harvest index was negatively significant for well-watered + humic fertilizer. But relationships between these characters weren't a linear correlation for other conditions.

### PM53 - Effect of reduction of drought stress using supplementary irrigation of dryfarming chickpea (*Cicer arietinum* L.) varieties in Kermanshah climate

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An experiment was carried out in 2007 to investigate the effects of different irrigation regimes, and chickpea cultivars on chickpea production in the Agricultural Research Station, College of Agriculture, Islamic Azad University, Kermanshah Branch. The experiment was split-plot in a randomized complete block design with three replications. Supplementary irrigation at three levels: control treatment (without irrigation)(I<sub>0</sub>), one-time irrigation at 50%-flowering stage(I<sub>1</sub>) and one-time irrigation at pod-filling stage(I<sub>2</sub>), was allocated to main plots and the varieties(ILC-482 (V<sub>1</sub>), Hashem(V<sub>2</sub>) and Arman(V<sub>3</sub>)) was allotted to subplots. A significant difference was observed between irrigation treatments in terms of plant height, number of axillary branches, distance to the first pod from soil surface, number of grains per plant, number of pods per plant, grain yield, biological yield, harvest index and 100-grain weight; such a difference was observed between test varieties in terms of all trials rather than 100-grain weight. The grain yield mean was significantly higher for Arman than that of Hashem and for Hashem was significantly higher than that of ILC-482. Of course, there was no significant difference between Hashem and ILC-482 in terms of grain yield. The highest values of the number of grains per plant relate to Arman and pods per plant pertained to Arman and Hashem, respectively. High rate of grain yield in irrigation treatment at pod-filling stage was associated with yield components, especially with the number of pods per plant and 100-grain weight. The grain yield was positively correlated with number of pods per plant ( $r = 0.654^{**}$ ), number of grains per plant ( $r = 0.902^{**}$ ) and 100-grain weight ( $r = 0.707^{**}$ ). This research showed that grain formation and pod-filling stages are the most sensitive to water-deficit, and under water limitation conditions, we can considerably increase grain yield at this stage by irrigation, especially for Arman.



**PM54 - Microorganisms caused rotting of grape root infected by phylloxera**

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Root samples from infected by phylloxera grape varieties Tebrizi and Xindogni collected from farms of Fuzuli region were analyzed and species composition of microorganisms caused to the second pathologic process-rotting were determined. The amount of microorganisms obtained from roots, of Tebrizi grape variety was 78%. The phytopathogens belonged to *Cylindrocarpon* genus were 18%, whereas phytopathogenes of *Fusarium* genus were 12%. At the same time 30% of phytopathogenes were detected to be belonged to bacteria of *Pseudomonas* genus. Also among phytopathogenes of this grape variety 6% were saprophytic fungi from *Penicillium* genus, 4% were fungi from *Mucor* genus, 3% were from *Molissia* genus and 5% were from *Rhacodiella* genus.

Spreading rates of phytopathogens from *Cylindrocarpon* (18%) and saprophytic fungi from *Penicillium* (6%) genera were more wider. Pathogens obtained from roots, of Xindogni grape variety infected by pests were 100%. These were fungi from *Gliocladium* genus - 24%, fungi from *Cylindrocarpon* genus-16% and from *Fusarium* genus - 17%. At the same time 13% of bacteria were from *Pseudomonas* and 18% bacteria were from *Bacillus* genera. There were 2% saprophytic fungi from *Penicillium* genus, 7% fungi from *Mucor* genus and 3% fungi from *Absidia* genus on roots of grape variety Xindogni. As it seen spreading rates of phytopathogens from *Gliocladium* genus (24%) and saprophytic fungi from *Mucor* genus (7%) was higher. Spreading rate of bacteria from *Bacillus* genus (18%) was also higher.

**PM55 - Investigate quantitative and qualitative traits and their relation in durum wheat**Shojaei ZA, Mirakhorli N

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It is of importance to find out characterizations of desirable traits in collections so as to increase utilization of germplasm resources in breeding programs. In relation to factors such as grain yield and protein quantity, it is important to measure simply quantitative traits which can be used to identify worthy genotypes for further evaluation. An agument design was used to study quantitative and qualitative traits of 516 Iranian durum wheat (*Triticum turgidum* L. var durum) morphotyps. The results showed that the mean of 1,000-kernel weight of all wheats was 40.93 g and the mean of kernel protein was 14.02 which were acceptable values. Additionally, the results of correlation, stepwise, and path analysis revealed that the traits such as spike seed yield, number of leaf per plant, number of sterile spiklet per spike, awn length and number of spiklet per spike, can be used as criteria for yield improvement. Furthermore, principle component analysis showed that seven components accounted for most of the variations among traits. The components included traits relating to duration of plant survival, yield components and plant morphology.

### PM56 - Diversity for oleic, linoleic acids, O/L ratio and agronomic traits of peanut (*Arachis hypogaea* L.)

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Peanut is an important oilseed crop. Oleic (O) and linoleic (L) acids are a major fatty acid in peanut seed. They are related with seed quality. Linoleic acid is more susceptible to oxidation than oleic acid. A better understanding on diversity and characterization of germplasm are priority in breeding program. The objectives of this study were to investigate genetic diversity of 22 peanut genotypes for oleic, linoleic acids, O/L ratio and agronomic traits and their correlations. The experiments were conducted at the agronomy farm of Khon Kaen University. The peanut genotypes were planted in the dry season 2006/07 and the rainy season 2007. A randomized complete block design with four replications was used. Agronomic traits were recorded at harvest including pod yield, biomass, harvest index (HI). Mature kernels sample for each plot were analyzed for oleic and linoleic acid content by gas liquid chromatography (GLC) method, and then O/L ratio was determined. Highly significant variations in the oil compositions and agronomic traits were found in these genotypes ( $P \leq 0.01$ ). The average oleic and linoleic acids content have ranging from 41.96 to 80.11 and 3.55 to 33.66% of total fatty acid, respectively. It is interesting to note that all released cultivars and breeding lines in Thailand had very low O/L ratios compared to the elite germplasm lines. The variation in pod yield and biomass varied from 1213 to 2758 and 5785 to 10467 kg ha<sup>-1</sup>, respectively, and HI varied from 0.18 to 0.44. Oleic acid was strongly negatively correlated to linoleic acid ( $r = -0.99$ ,  $P \leq 0.01$ ) and positively correlated to O/L ratio ( $r = 0.79$ ,  $P \leq 0.01$ ). Correlations between pod yield and oleic acid and O/L ratio were not significant ( $r = 0.05$  and  $r = -0.14$ , respectively) indicating independent segregation of these traits. The variations in the oleic linoleic O/L ratio and agronomic traits observed in these germplasm might indicate the possibility to incorporate high O/L ratio into commercial cultivars with good agronomic backgrounds.

### PM57 - Evaluation of germplasm accessions in pearl millet (*Pennisetum glaucum* (L.) R. Br.) genotypes

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Pearl millet (*Pennisetum glaucum*) is an important food and forage crop in Africa and Asia, and forage in Americas. It is probably the world's hardiest crop and has great potential because of its suitability to the extreme limits of agriculture. This crop is extensively grown in dry areas of the arid and semi arid topics with 200-800 mm annual rainfall where no other cereal crop can be grown successfully. During the course of its cultivation in differing farming systems, pearl millet diversified into numerous ecotypes. The spread of high-yielding cultivars, crop substitution by other more remunerative crops, recurrent drought, and urbanization are gradually eroding land races. Over grazing and destruction of habitats are threatening the wild and weedy forms. The success in crop improvement programs depends largely on the extent of genetic variability available to the researchers. Pearl millet is endowed with enormous genetic variability for various morphological traits, yield components, adaptation and quality traits. In ensuring that the plant breeders will have genetic resources for use in plant breeding programs, collection, conservation, characterization, evaluation, documentation and distribution of plant genetic resources is very important. Large efforts to be made to collect and conserve the pearl millet diversity before it are lost forever. Considering the importance of assessing the utility of conserved germplasm, an effort was taken to characterize and evaluate 500 germplasm accessions of pearl millet which is being maintained at Department of Millets, Centre for Plant Breeding and Genetics, TNAU, Coimbatore during 2008-2009. The crop was raised in randomized block design with two replications. The data were collected for 26 standard descriptors followed at national and international level (NBPGR and ICRISAT) and were analysed statistically to depict the variability present in the accessions. Large phenotypic diversity in accessions has been observed for almost all the characters. There are accessions in the collection, which can flower as early as in 35 days and as late as in 130 days. Similarly, the variation was observed for plant height from very small to very height plants (more than 250 cm). The data were subjected to standard statistical procedure to analyse the available variability within the germplasm. This study also revealed the presence of maximum variability of different characters in pearl millet germplasm. Hence, this study will be helpful for the breeders for selecting the good materials based on their objective and to utilize it for future pearl millet improvement programme.

**PM58 - Study of genetic diversity of common chickpea landraces of College of Agriculture and Natural Resources Gene bank collection using multivariate statistical method**

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This study was conducted to investigate the genetic diversity and relationship between yield and its components in 633 chickpea landraces of College of Agriculture and Natural Resources Gene bank collection. The entries evaluated in Agricultural research station in 2005-6. The entries were planted in rows with 5 meter length and 0.5 m between rows. The varieties Jam and Kourosh were planted after every 15 rows as a check. The scored traits were day to germination, flowering time, biological yield, number of primary branches, number of secondary branches, day to 50% flowering, seed filling duration, plant canopy surface, plant height, number of nodes, node length, number of pods, number of filling pods, pod weight, pod length, pod width, number of seeds per pod, seed length, seed width, number of seed per plant, seed weight, 100 seed weight, and harvest index. One way analysis of variance was applied to determine the soil uniformity. The traits such as number of secondary branches, number of pods, number of filling pods, biological yield, and pod weight had a sufficient variability. The result of simple correlation and stepwise regression analysis showed that pod weight, biological yield, number of filling pods, 100 seed weight, number of seeds per pod, and number of seed per plant had the greatest effect on seed yield. The result of principal component analysis showed six principal components (having Eigen value more than 1) comprised 70.40% of total variation. Cluster analysis for geographical locations grouped samples in 13 clusters. No relationship was found between genetic diversity and geographical classification.

**PM59 - Variability and diversity in Ethiopian durum wheat land races, revealed on morphological markers**Tesema AG<sup>1</sup>, E Porceddu<sup>2,3</sup><sup>1</sup> Institute of Biodiversity Conservation and Research Addis Ababa, Ethiopia<sup>2</sup> International Doctoral Programme in Agrobiodiversity, Scuola Superiore Sant'Anna, Pisa, Italy<sup>3</sup> Dipartimento di Agrobiologia e Agrochimica, Università degli studi della Tuscia, Viterbo, Italy

In order to estimate the level of genetic variation among populations and within populations, Ninety seven tetraploid ( $2n = 4x = 28$ ) wheat originating from the Central Highlands, north, east, west and southern parts of Ethiopia, from nine regions, were investigated for morphological markers. 2910 plant individuals (30 plants per landrace) were examined. The characters recorded were glum colour, glum hairiness, spike density, seed colour, degree of shrivillage and seed vitreous. Mean diversity index, ranged from zero (monomorphic) to 0.98 (highly polymorphic). Bale and Gonder materials were entirely monomorphic for the glum hairiness. Whereas in Kefa observed highest diversity (94%). Analysis on the overall material indicated the existence of 54 variants, out of which 24 variants were > 1% frequency, whereas, 30 variants were < 1%. Three variants were the most frequent being present above 10%. White and glabrous glumes were more frequent in Arsi, Bale, Keffa and Hararghe, whereas red to brown and glabrous glumes were significantly present in Gonder, Gojam and Wello. The three glum hairiness classes were found in a similar proportion in Keffa. But, in Bale, Gonder and Gojam the highest percentage of glabrous glum hairiness (8%, 100%) and the lowest percentage of high glum hair (0%) were found. The dense of spike density type was more frequent in Gonder with a maximum frequency of 79%, while in Arusi its frequency was 22%. Dense spikes were frequent in about 50% of the overall material and prevalent in the Shewa, Tigray and Hararghe material, whereas, the intermediate spike density prevalent in Arsi, Bale, Gonder, Gojam and Keffa. Lax spikes were rather rare in all regions except Bale and Keffa after many centuries of exploitation, but still conserving very high levels of genetic variability.

### PM60 - Estimation of genetic variability in Foxtailmillet (*Setaria italica* (CL.) Beauv)

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Foxtailmillet is one of the oldest crops cultivated for hay, pasture and food grain. It is an important crop in China besides India, Japan and some other countries in Asia and Europe. It is grown in North and South America, Australia and Africa as a minor cereal crop. Foxtailmillet is rich in nutrients and the best choice of feed for pet birds, apart from staple food for tribals and small and marginal farmers. It is a drought hardy plant and grows nicely in rainfed condition. The movement of germplasm that took place between Europe and Asia since time immemorial might have marked the evolutionary history of the crop. The long history of domestication and extensive cultivation has resulted in generation of large variability within the cultivated species of Foxtailmillet. Collection and conservation of plant genetic resources of crop plants have been receiving a lot of attention during the last two decades. The main reason for poor utilization of rich collection is lack of adequate information on the genetic worth of the conserved material. So, it is very important that the conserved germplasm need to be properly evaluated, described and analysed to aid in the selection of promising germplasm and promoting its use in crop improvement. Considering the importance of assessing the utility of conserved germplasm, a modest effort was made for a comprehensive characterization and evaluation of 715 accessions of Foxtailmillet at Department of Millets, Centre for Breeding and Genetics, TNAU, Coimbatore during 2009-2010. The crop was raised in randomized complete block design with three replications. The data collected for 27 descriptors (IBPGR, 1983) were analysed statistically to depict the variability present in accessions in the form of labels and graphs. The querying of database for important agronomic characters singly and in combination was made to identify promising germplasm accessions. Tremendous variability was estimated for most of the characters. The evaluation was intended to serve as a useful databank to breeders in selecting proper materials for crop improvement and varietal evaluation.

### PM61 - Comparison of bio-ethanol efficiency of several wheat varieties

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During this project, we studied the production of bioethanol in batch from different wheat varieties using the yeast *Saccharomyces cerevisiae*. The study was conducted on 32 wheat varieties whose names were coded. Each variety is grown in three different places with two different nitrogen fertilization rate: R1 (3 nitrate fertilizer) and R2 (2 nitrate fertilizer). First, we validated a new method for the determination of ethanol. Then, a comparative study between normal wheat (not waxy) and genetically modified wheat (waxy) was performed. The non-waxy wheats showed a little increase in the total starch content and ethanol yield for normal wheat compared to waxy wheats. Three methods for determining potential ethanol were used: the determination of starch seems to be only an estimating tool to design the fermentability of wheat. The average ethanol content for all varieties is around 225, 339, 450 and 464 L ethanol / t DM respectively after the 16, 22, 46 and 68 hours. Performance of bio-ethanol produced from different wheat varieties based on two factors: the place of culture and nitrogen fertilisation allowed us to propose a classification of different varieties of wheat. The multiple comparison test allowed us to distinguish seven groups significantly different. We can say that varieties of group A (AND, GAR, SOI, VOL, MAX, CF01085, GLA, CRO, DIN, TIM, BAG, SAN and ARA) are more productive while the varieties of group G (SOG, 101,013 2 4, PAL, CAL, RYT, MEN, AST, and THAT FAR) are the least productive.

## PM62 - Future developments for non-destructive 3D plant and root imaging

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High-throughput screening and high-throughput phenotyping have become key technologies for research in and development of active ingredients for pharmacology, new plant protection compounds and breeding for new traits in agricultural products. These technologies are fundamentally important for many fields of applied and basic research, enabling the examination and understanding of different plant gene functions and the overall effects of chemicals on various organisms. Most of these screening methods are measuring visible parameters of the plants such as color, shape, size, area, architecture, growth rate, performance or movement. Therefore, digital imaging of plants has become a very important tool in plant research, since modern image processing software algorithms are much better and more reproducible in quantifying these visual parameters than the human eye. Moreover, the spectrum of modern CCD cameras can be extended to lower or higher wavelengths far beyond the visual range of the human eye such as Near Infrared (NIR) for measuring the water distribution and dynamics in plants during drought stress experiments. However, all these reflective measurements are just able to target the visible part of the plant, the shoot, while the root keeps to be hidden in the soil or substrate. The goal of this joint study is, to explore whether Nuclear Magnetic Resonance Imaging (NMRI) or (Sub) Terahertz Imaging (THz) might be used for obtaining non-invasive and valuable information about plant roots in soil or substrate.

## PM63 - Studies on production potential of patchouli (*Pogostemon cablin* Pellet) as an under storey crop, nutrient management and processing

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A study on production potential of patchouli (*Pogostemon cablin* Pellet) as an understory crop, integrated nutrient management and processing was carried out during 2002-2005 at Division of Horticulture, UAS, Bangalore, Karnataka, India. Three year pooled data of the results revealed that, under the study on production potential of patchouli based cropping systems with other shade loving medicinal and aromatic crops, patchouli as a base crop in patchouli + kapur kachri (*Kaemferia galanga*) system exhibited significant influence on cumulative dry herb yield (3.73 t/ha) and essential oil yield (90.46 kg/ha) over other patchouli based cropping systems. Further, patchouli alcohol content was significantly high i.e. 48.77% in patchouli as a base crop in patchouli + kapur kachri system. The indices for evaluating cropping systems viz., Land Equivalent Ratio, Relative Crowding Coefficient, Aggressivity Index and Area Time Equivalent Ratio were found to be favoring the patchouli + kapur kachri cropping system. This system also recorded the maximum gross and net returns with higher B: C ratio of 1:9.45. Whereas, under the experiment on integrated nutrient management, the three year pooled data indicated higher cumulative dry herb yield of 3.60 t/ha with an application of 75% rec. dose of NPK in integration with Azotobacter + PSB + VAM biofertilizer and were 22% and 57% higher than the yield of patchouli compared to control [i.e., only rec. dose of inorganic fertilizers (2.82 t/ha) and only biofertilizer application (1.52 t/ha)]. The studies on post harvest processing in patchouli revealed that shade drying for 6 days was found to be ideal method for drying of patchouli herbage and was on par with mechanical drying. Storage period studies revealed that patchouli dry herbage could be stored up to a period of two months either in gunnysack or nylon net without significant reduction in oil content.



#### PM64 - Investigation of the possibility of using the natural biodiversity as ornamental plants in alata first natural site area dunes

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Because of its natural and cultural characteristics, Alata Horticultural Research Institute is taken under protection as a first grade natural site area and is being under dense pressure of surrounding inhabiting housing. Sand dunes have rich genetic resources. In this study, the natural biological diversity of the dunes use of as the ornamental plants possibilities were investigated. Natural plants which can be use as ornamental plants were selected with selection and taken culture. Suitable species as *Pancratium maritimum*, *Paronychia argentea*, *Ipomea stolonifera*, *Prasium majus* are selected. The whole species showed the best development similar to the their natural environment type of sand media. These plants, which are successfully taken culture, can be used for landscape of coastal areas.

#### PM65 - Improvement of grain nutrient quality and yield potential of soybean in marginal land through mutation induction

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The research was aimed to improve the genetic variability and nutrition content in soybean through seed irradiated by Gamma ray treatment with dose 200 Gy. The genetic variability is the basic requirement for making progress in crop breeding. Some mutant lines have been developed from soybean variety Muria. Soybean [*Glycine max* (L.) Merr.] has been recognize as valuable source of high quality protein, oil, vitamin and nutrition. In Indonesia, consumption of soybean has been increased, but the production has been decreased. Therefore, to supply the domestic demand, it is important to increased domestic production. Expansion growth areas and used of adapted varieties and high grain nutrient might overcome these problems among others. Yield test observation of four soybean mutant lines and parent as control (Muria variety) have been conducted at Mataram Central Indonesia in marginal land. Seeds of these mutant lines from M3 generation were collected along with their parents and have been observed for nutrition content. They were G1M, G2M, G3M, G4M and Muria. Kjeldhal method was used to analyze protein content, AAS for nutrition content and HPLC for vitamin content. The result showed that G3M (41.25%) has the highest protein content. The other mutant lines (G2M, G4M, G1M and Muria) have ranges of protein percentage were 40.57, 39.63, 38.95 and 38.50%, respectively. For mineral content, analysis showed that the P content Muria is highest 583.19 mg/100g, but P low in mutant line G4M, G1M, G2M and G3 M (580.02, 574.66, 568.72 and 557.42 mg/100g). G4M is highest Ca content (224.10 mg/100g). Subsequently, Fe and vitamin B1 content of the mutants were achieved 8 mg/100g (G4M), 7.40 (G1M), 6.45 mg/100 g G3M, 6.09 mg/100g (G2M) and 5.82 mg/100g (Muria). Vitamin B1 1.20 mg/100 g (G1M), 1.05 mg/100g Muria, 0.94 mg/100g (G4M), 0.82 mg/100 g (G3M) and 0.74 mg/100g (G2), respectively, which was superior among the mutants and parent. The yield trial test has been performed on the mutant lines and control in marginal land locations in Mataram, Central Indonesia. The mutant line has the highest yield potential is G2M (1.75 ton/ha) among mutants and parent (1.32 ton/ha) significantly.

## PM66 - Phenotyping agronomic traits and characterization of sweet sorghum as feedstock for bioethanol production

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Today, interest in developing sweet sorghum as feedstock for bioethanol production is increasing as it has the great potential to become a versatile feedstock for large-scale bioenergy production given the high content of sugars from stalk juice, large amount of cellulosic biomass from the entire plant, and rich starch from its grain, which fit for several ways of bioethanol production. However, sweet sorghum hybrids, cultivars, and varieties suitable for bioethanol production are essentially lacking at the present time. As high-performance energy feedstock, sweet sorghum crops require higher sugar yield and sustainable production. Like other energy crops, sweet sorghum has not been bred for biofuel. However, interest in growing sweet sorghum for fermentable sugars is increasing worldwide; thus there is strong demand for elite varieties and hybrids offering high sugar yield and sustainable production. Recently we initiated a research project focus on sweet sorghum germplasm enhancement and genetic improvement through selective germplasm evaluation, gene discovery and molecular breeding. A preliminary evaluation was conducted with a set of 687 germplasm accessions during the past two years. Phenotypic characterization focused on those traits that are important for high sugar content, high biomass yield, low lignin content, early maturity, resistance to diseases and insects, and wide adaptability. Based on the phenotypic data, substantially genetic variations were observed among the germplasm originating from a wide range of geographic locations worldwide. These untapped genetic resources harbor an excellent gene pool for crop improvement. Furthermore, a group selected germplasm lines will be valuable materials for breeders to do further manipulation toward the production of new cultivars or hybrids.



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