PlantEd

Genome editing in plants

Cost Action CA18111

4th PlantEd Conference

18-20 September 2023

Porto, Portugal

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

4th PlantEd Conference

18-20 September 2023

Porto, Portugal

Book of Abstracts

Book of Abstracts of the 4th PlantEd Conference

EDITORS:

Dr. Isabel Mafra

Dr. Joana Costa

Dr. Caterina Villa

Dr. Carla Teixeira

Prof. Isabel Ferreira

EDITION:

COST Action CA18111, PlantEd – Genome editing in plants - a technology with transformative potential

Website: https://plantgenomeediting.eu/

DATE:

September 2023

Table of contents

Committees

Scientific Committee

Dennis Eriksson, Swedish University of Agricultural Sciences, Sweden

Isabel Mafra, REQUIMTE-LAQV/Faculty of Pharmacy, University of Porto, Portugal

Götz Hensel, Heinrich-Heine-University, Dusseldorf, Germany

Katrijn Van Laere, EV ILVO, Belgium

Dragana Miladinovic, Institute of Field and Vegetable Crops, Serbia

Jeremy Sweet, JT Environmental Consultants, UK

Jale Tosun, Heidelberg University, Germany

Patrick Rüdelsheim, Perseus bvba, Belgium

Tomasz Twardowski, Institute of Bioorganic Chemistry, Poland

Ewa Wozniak, Polish Academy of Sciences, Poland

Geraint Parry, Association of Applied Biologists, UK

Matina Tsalavouta, University of Liverpool, UK

Vladislava Galovic, Institute of Lowland Forestry and Environment- ILFE, Serbia

Anna Coll, National Institute of Biology, Slovenia

Ankica Kondic-Spika, Institute of Field and Vegetable Crops, Serbia

Sebastien Carpentier, KU Leuven , Belgium

Local Organising Committee

Isabel Mafra, REQUIMTE-LAQV/FFUP, Portugal

Joana Costa, REQUIMTE-LAQV/FFUP, Portugal

Caterina Villa, REQUIMTE-LAQV/FFUP, Portugal

Carla Teixeira, REQUIMTE-LAQV/FFUP, Portugal

Isabel Ferreira, REQUIMTE-LAQV/FFUP, Portugal

Scope

The 4th PlantEd Conference (COST Action 18111) will be held over three days, with open scientific sessions on genome editing technology in plants, followed by PlantEd Working Group (WG) sessions and a Management Committee (MC) meeting. The conference will be a hybrid event, with a limited number of participants physically present, combined with live streaming (Zoom). The PlantEd conference, a network for plant genome editing research across Europe and beyond, is an excellent platform for disseminating information, discussion, and connections and updating the latest research and innovation.

The PlantEd Conference, being a network for research on plant genome editing across Europe and beyond, is an excellent platform for dissemination, discussions and connections, and for updating on the latest research and innovation forefront.

Topics to be covered: The conference will host sessions on the application of genome editing in various types of economically important plants (cereals, oil crops, roots and tubers, legumes, fruits and vegetables, trees, algae), as well as the latest technological advancements for genome editing in plants.

The conference will take place towards the end of the action and final grant period, which marks the closing of PlantEd activities, identifying the main achieved outcomes, but most importantly, planning/on-going activities by the prospection of new resources.

Venue

Faculty of Pharmacy, University of Porto (FFUP)
Rua Jorge Viterbo Ferreira, 228
4050-313 Porto
DIRECTIONS

The conference will have the support of the Associated Laboratory REQUIMTE and Faculty of Pharmacy, University of Porto.

Supporters

PlantEd

PlantEd – COST Action CA18111 – Genome editing in plants

Financial supporters

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

Local supporters

LAQV requimte
LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

U.PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

Programme

4th PlantEd Conference

Porto, Portugal – September 18-20, 2023

Monday 18 Sept		**Session Chair: Dennis Eriksson**
	08:00-09:00	Registration
	09:00-09:30	Welcome **Local Organizer – Isabel Mafra**; REQUIMTE- LAQV, Faculty of Pharmacy, University of Porto/Portugal Welcome **Executive Board of Faculty – Marcela Segundo**; Faculty of Pharmacy, University of Porto/Portugal Welcome **COST Action Chair – Dennis Eriksson**; Swedish University of Agricultural Sciences/Sweden
Session I – GE applications and molecular mechanisms	09:30-10:00	**Keynote: Dirk Bosch and Katarina Cankar**; Wageningen University/The Netherlands *Genome editing to improve health benefits of root chicory*
	10:00-10:20	**Justyna Boniecka**, Department of Genetics, Nicolaus Copernicus University in Toruń/Poland *CRISPR/Cas9-directed editing of RelA/SpoT Homologs in tomato (Solanum lycopersicum L.)*
	10:20-10:40	**Zoe Hilioti**, Institute of Applied Biosciences/CERTH/Greece *Tomato breeding by design using non-transgenic genome editing*
	10:40-11:20	Coffee break and poster session
	11:20-11:40	**Daria Navrotska**, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine/Ukraine *Brachypodium distachyon DOF transcription factor gene analysis and genome editing*
	11:40-12:00	**Tjaša Lukan**, Department of Biotechnology and Systems Biology, National Institute of Biology/Slovenia *CRISPR/Cas9-mediated miRNA editing in tetraploid potato*
	12:00-12:20	**Yordan Dolaptchiev**, The Sainsbury Laboratory/United Kingdom *Efficient targeted gene insertions in diploid potatoes*
	12:20-12:40	**Katrijn Van Laere**, ILVO - Plant Sciences Unit/Belgium *CRISPR-based visualisation of centromere sequences in chicory*
	12:40-13:50	Lunch
Monday 18 Sept		**Session Chair: Isabel Mafra**
Session II – Improving resistance to abiotic stress	13:50-14:20	**Keynote: Sílvia Coimbra**; Faculty of Sciences, University of Porto/Portugal *CRISPit - Bridging fundamental knowledge and novel technology to increase rice heat tolerance*
	14:20-14:40	**Cecilia Sarmiento**, Tallinn University of Technology/Estonia *Optimized Lolium perenne L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications*
	14:40-15:00	**Muneeb Hassan Hashmi**, University of Siegen, Siegen/Germany *Establishment of highly efficient and reproducible Agrobacterium-mediated transformation system for tomato (Solanum lycopersicum L.)*
	15:00-15:20	**Luca Nerva**, CREA - Research Centre for Viticulture and Enology/Italy *Improving grape resilience to climate change exploiting the CRISPR/Cas technology: different approaches to face drought*
	15:20-16:00	Coffee break and poster session
	16:00-17:30	PlantEd Working Group meeting (WG1-WG5 together)

Tuesday 19 Sept		**Session Chair: Götz Hensel**
Session III – Improved technologies	09:30-09:50	**Hilal Betul Kaya**, Manisa Celal Bayar University/Turkey *Optimizing protoplast isolation and transformation efficiency for enhanced plant genome editing in grapevine*
	09:50-10:10	**William de Martines,** Wageningen University and Research/The Netherlands *Exploring alternative approaches for efficient gene targeting in plants: high fidelity nonhomologous end-joining with CRISPR-Cas12a in potato protoplasts*
	10:10-10:30	**Angelo Ciacciulli,** CREA OFA Acireale/Italy *New genomic techniques in citrus, step-by-step solutions for more efficient and successful procedures*
	10:30-11:10	Coffee break and poster session
Session IV – Nutritional improvement and characterisation	11:10-11:40	**Keynote: Nélida Leiva Eriksson**; University of Lund/Sweden *Nutritional enrichment of sweetpotato with highly bioavailable iron*
	11:40-12:00	**Ellen Slaman**, VIB-Ugent/Belgium *In-depth characterization of Cas9 specificity in tomato using high-throughput amplicon sequencing, GUIDE-seq and whole genome resequencing*
	12:00-12:20	**Concetta Licciardello**, CREA/Italy *A dual single-guide RNA approach used to edit the b-cyclase 2 gene in anthocyanin-rich sweet orange varieties*
	12:20-12:40	**Fabio D'Orso**, Research Centre for Genomics and Bioinformatics/Italy *HQT gene editing to study chlorogenic acid metabolism and its physiological role in tomato*
	12:40-13:50	Lunch
Tuesday 19 Sept		**Session Chair: Vladislava Galovic**
Session V – Improving resistance to biotic factors	13:50-14:20	**Keynote: Johan Hunziker**; INRAE/France *Gene editing in potato to enhance PVY resistance*
	14:20-14:40	**Éva Csaba**, ELKH Centre for Agricultural Research/Hungary *Studying potato resistance and susceptibility factors against pathogens with the use of genome editing*
	14:40-15:00	**Senne Van den Broeck,** KU Leuven/Belgium *Gene editing in triploid banana cultivars*
	15:00-15:20	**Kim Hebelstrup**, Department of Agroecology, Aarhus University/Denmark De novo domestication of wild tuber-bearing Solanum species
	15:20-16:00	Coffee break and poster session
	16:00-17:30	Management Committee meeting
	19:30	Social dinner

Wednesday 20 Sept		**Session Chair: Katrijn Van Laere**
	09:00-09:30	**Keynote: Elke Vereecke**; EV ILVO/Belgium *Increase the production of industrially valuable compounds in the microalgae Chlorella – the GeneBEcon approach*
Session VI – Regulation and public perception	09:30-09:50	**Juan Vives-Vallés**, University of the Balearic Islands - INAGEA/Spain *Preliminary analysis of the European Commission Proposal for a Regulation on the production and marketing of plant reproductive material*
	09:50-10:10	**Tomasz Zimny,** Institute of Law Studies, Polish Academy of Sciences/Poland *The new NGT legislation proposal of the European Union. Analysis of selected EU and national regulatory obstacles for the introduction and market viability of NGT plant products*
	10:10-10:30	**Anna Linkiewicz,** Cardinal Wyszyński University in Warsaw/Poland *The awareness of the Polish society on new genomic techniques*
	10:30-11:00	Coffee break and poster session

	11:00-11:15	**Agnés Ricroch;** AgroParisTech and University of Paris Saclay/France *"Roadmap for Plant Genome Editing" – a Springer book production from PlantEd*
Session VII - STSM	11:15-11:30	**Vladislava Galovic;** University of Novi Sad, Institute of Lowland Forestry and Environment/Serbia *Overview on the 4-year STSM activities*
	11:30-11:45	**Alvaro Valenzuela**, Fondazione Edmund Mach/Italy *Leveraging system biology and new breeding technologies for water stress tolerance in grapevines*
	11:45-12:00	**Karam Mostafa**, Ondukuz Mayis University/Turkey and Agriculture Research Center/Egypt *Application of multiplexed CRISPR-ACT3.0 gene activation system in tomato roots for enhancing resistance against plant-parasitic nematodes*
	12:00-12:15	**Kubilay Yıldırım**, Ondokuz May University, Department of Molecular Biology and Genetics, Samsun/Turkey *Development of resistant sunflower lines to broomrape using crispr-cas9*
	12:15-12:30	**Sara Yasemin**, Siirt University/Turkey *Exploring the role of snrk2 genes in salinity stress response of Petunia axillaris through CRISPR-based genome editing*
	12:30-12:45	Poster prizes
	12:45-13:00	Closing of conference

Useful information

Registration

Registration will take place at the main entrance on the ground floor (2nd floor).

Oral and poster presentations

All oral presentations will be held in the Salão Nobre (ICBAS/FFUP complex), 4th floor. Posters should be placed at the beginning of the conference and removed at the end of the conference in the placards at the Foyer.

Internet access

Wireless internet Eduroam is available everywhere in the complex ICBAS/FFUP, using self-login and password.

Lunch and coffee breaks

Lunch and coffee breaks will be served at the Foyer and Bar, which are next to the Salão Nobre.

Social dinner

19th September - The social dinner will take place at Torreão Restaurante (Rua das Virtudes 37, 4050-630 Porto (41.1432926702757, -8.618372119053081).

Oral Presentations

Session I – GE applications and molecular mechanisms

Keynote

Genome editing to improve health benefits of root chicory

Katarina Cankar[1], Dirk Bosch[1,*]

[1]Wageningen Plant Research, Wageningen University and Research, Wageningen, The Netherlands
*E-mail: dirk.bosch@wur.nl

Root chicory is a European crop from which the health promoting dietary fiber inulin is extracted at industrial scale. The roots also accumulate bitter sesquiterpene lactones (STLs), but these are currently discarded as waste. During the presentation, the multidisciplinary approach of the recently finished EU-CHIC project to implement genome editing to develop root chicory as a crop from which multiple health beneficial products can be extracted, will be illustrated.
Variants of CRISPR methods were evaluated and implemented to generate new chicory varieties: varieties in which the accumulation of bitter STLs was completely prevented, which greatly facilitates inulin extraction, as well as varieties that accumulate different specific STLs with potential for medicinal use. Additionally, chicory varieties with higher quality of inulin and varieties in which the self-incompatibility of chicory was disrupted, were generated. Through these experiments scientific knowledge on chicory biology was increased, particularly regarding its bioactive compounds, their biosynthesis and storage.
CHIC demonstrated that genome editing can be a powerful tool to help to stimulate agricultural biodiversity in Europe by improving niche crops. Within five years multiple improved varieties with potential benefits throughout the value chain, from farmer to consumer, as well as for the environment and economy, were generated.

Acknowledgements: This research has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 760891 [H2020-NMBP-BIOTEC-07-2017: New Plant Breeding Techniques (NPBT) in molecular farming: Multipurpose crops for industrial bioproducts].

Oral presentation

CRISPR/Cas9-directed editing of RelA/SpoT homologs in tomato (*Solanum lycopersicum* L.)

Justyna Boniecka[1,*], Eugenio Butelli[2], Jie Li[2], Matthew Downie[2], Vera Thole[2], Cathie Martin[2]

[1] Department of Genetics, Nicolaus Copernicus University in Toruń, 87-100, Toruń, Poland
[2] John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom
*E-mail: jboniecka@umk.pl

One of the solutions to withstand the effects of climate change is the development of economically and environmentally sustainable horticultural crops through the production of improved varieties. For this, an understanding of the mechanisms regulating plant growth and development and responses to stress is a prerequisite. One promising target for crop improvement is the stringent response (SR) – a procaryotic stress response that controls adaptation to nutrient deprivation, that is also active in chloroplasts of higher plants, where it regulates transcription, translation and production of many metabolites. The effectors of this response – guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) – are synthesized by nucleus-encoded RSH (RelA/SpoT Homologs) proteins. Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables worldwide due to the delicious taste and wide variety of forms and colors of the fruit, and thus is an economically important crop plant. To analyze the involvement of the tomato SR in plant growth and development and responses to stress, we started to generate *SlRSH* (*SlRSH1.1*, *SlRSH1.2*, *SlRSH2*, *SlRSH3* and *SlCRSH*) mutants. For that, sequence-specific sgRNAs were designed using web-based tools (CRISPR-P v2.0 and CCTop-CRISPR/Cas9 target online predictor), and three/four constructs per gene with two sgRNA per CRISPR/Cas9 constructs were assembled using the Golden Gate technology. To test the efficiency of different sgRNA combinations for their potential to generate edits in the loci of interest, the constructs were introduced into *Agrobacterium rhizogenes* via electroporation, and their presence was verified using colony PCR with Cas9-specific primers. Positive clones were amplified and resuspended in liquid MS to reach OD_{600} 0.2-0.4. Cotyledon explants were isolated from tomato seedlings (up to 20 per transformation) and co-cultivated with the bacteria. After 20 min, explants were transferred onto MS plates (3% sucrose, no antibiotics), and after three days, on the same media but with 200mg/L cefotaxime and 100 mg/L kanamycin. Once roots were at least one cm long, 12-14 individual roots, each from single explant, were excised and used for DNA isolation. Targeted regions were amplified, analyzed with gel electrophoresis and sent for sequencing (6-10 products per construct). In all instances both biallelic and monoallelic mutations were observed, and the efficiency of some sgRNAs was high, reaching even 100%. The vectors with the most effective/efficient sgRNA combinations are currently being used for stable plant transformation with *A. tumefaciens* to regenerate *SlRSH* mutants.

Acknowledgments: The research experiments conducted by JB, which results were an inspiration to conduct the work, were financed from the funds of the National Science Centre in Poland (2017/01/X/NZ1/01981 [JB]). The work is to high extent the result of scientific missions supported by funds from the NCU IDUN Mobility Vth edition and CA18210 STSM grants.

Oral presentation

Tomato breeding by design using non-transgenic genome editing

Nestor Petrou[1], Zoe Hilioti[1,*]

[1]Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thermi, Greece
*E-mail: zhilioti@gmail.com

Genome editing has become an important tool for plant geneticists to modify the genetic sequences of plants and induce novel gene expression-associated phenotypes. This technology has been used to target and modify the tomato (Solanum lycopersicum L. var. Heinz 1706) NF-YA transcription factor (TF), which encodes a subunit of the heterotrimeric nuclear transcription factor Y (NF-Y). The aim of this study was to generate local mutations within the coding region of NF-YA gene, study their function(s) and create novel tomato varieties. We used a non-transgenic approach based on the Zinc Finger Nuclease (ZFN) technology for this purpose. ZFNs are DNA-binding proteins engineered to target and cleave specific sequences of DNA, resulting in the creation of double-stranded breaks (DSBs). The delivery of the ZFN pair into seeds through electroporation along with their transient expression induced site-specific DSBs, which were repaired by the Non-Homologous End Joining (NHEJ) mechanism. Phenotypic alterations where evident since the early seedlings, and mutations were confirmed in the NF-YA coding region through molecular techniques and sequencing analyses. In mature plants changes emerged both in vegetative growth and reproductive development. Specifically, the results revealed that the TF is a powerful regulator of new branches formation, stem and leaf development, flowering time, inflorescence architecture and the size and shape of tomato fruit in tomato plants. Overall, the results demonstrated that this application of genome editing technology is a powerful tool to create novel tomato varieties with altered traits in a precise and efficient way.

Acknowledgments: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 3873).

Oral presentation

Brachypodium distachyon DOF transcription factor gene analysis and genome editing

Daria Navrotska[1,2,3,*], Thomas Greb[2]

[1]Center for Plant Molecular Biology (ZMBP), Tübingen, Germany
[2]Centre for Organismal Studies (COS), Heidelberg, Germany
[3]Institute of Molecular Biology and Genetics (IMBiG) of the National Academy of Sciences of Ukraine, Kyiv, Ukraine; *E-mail: navrotska.daria@gmail.com

The plant body is pervaded by a network of vascular tissues that provide long-distance transport of water, energy metabolites, and signaling molecules. Despite similar functions, the vasculature of monocotyledonous and dicotyledonous plant species is organized differently. Underscoring its functional importance, dicotyledonous species have the capacity to establish vascular tissue on demand throughout their life cycle, resulting in radial growth of shoots and roots and contributing significantly to their plastic growth mode. In contrast, most monocotyledonous species do not maintain vascular stem cells in differentiated organs and have therefore lost their ability to grow radially. Remarkably, the molecular mechanisms underlying the differences in vascular development in these clades remain unexplored.

Vascular development is a complex process that integrates intercellular signaling events and gene regulation at the transcriptional level. Among the players controlling the fate of vascular stem cells is a group of plant-specific DOF (DNA-binding with one finger) transcription factors. They have been described in maize, rice, barley, tomato, poplar, and the dicotyledonous model Arabidopsis thaliana, where the vast majority of them are expressed during vascularization. Recently, DOFs have been found in the monocot grass Brachypodium distachyon.

Among the previously reported BdDOFs (27), we identified six (BdDOF1, BdDOF6, BdDOF12, BdDOF21, BdDOF22, and BdDOF23) that showed clear homology to AtDOFs and significant expression levels at sites of vascularization. Analysis of the BdDOF12 overexpression line revealed differences in stem diameter and vascular bundle area compared to WT plants. It was also confirmed by qRT-PCR. Single mutant alleles of BdDOF1, BdDOF6, both homologs of the well-characterized PEAR2 transcription factor in Arabidopsis, and BdDOF23 were generated using CRISPR-Cas9 targeted genome editing. We are currently analyzing their expression levels and testing for phenotypic differences.

Taken together, we provide data that BdDOFs genes are promising candidates for understanding the mechanisms of vascularization in monocots and that their further investigation will help to elucidate other factors within the regulatory network.

Oral presentation

CRISPR/Cas9-mediated miRNA editing in tetraploid potato

Tjaša Lukan[1,*], Florian Veillet[2], Maja Križnik[1], Anna Coll1, Tjaša Mahkovec Povalej[1], Karmen Pogačar[1], Katja Stare[1], Laura Chauvin[2], Jean-Eric Chauvin[2], Kristina Gruden[1]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Večna pot 111, Ljubljana, 1000 Slovenia

[2]IGEPP, INRAE, Institut Agro, Universite´ de Rennes, Ploudaniel 29260, France

*E-mail: tjasa.lukan@nib.si

MicroRNAs (miRNAs) are small noncoding RNAs, which modulate the abundance and spatiotemporal accumulation of target mRNAs at transcriptional and post-transcriptional levels and through that play important roles in several biological processes in plants. Here we show that in polyploid species, CRISPR/Cas9 system can be used for fine-tuning of miRNA expression, which can have broader range of applications compared to knock-out mutants. We established the complete pipeline for CRISPR-Cas9-mediated modulation of miRNA expression in potato. It consists of (1) design and assembly of dual sgRNA CRISPR/Cas9 constructs, (2) transient transfection of protoplasts following fast and efficient screening by high resolution melting analysis to select functional sgRNAs, and (3) stable transformation of potato explants with functional sgRNAs and selection of regenerated transgenic lines with desired mutations and desired miRNA abundance based on sequencing and RT-qPCR. We show that miRNA-editing using dual sgRNA approach results in different types of mutations among transgenic lines but also in different alleles of the same plant, which are target site-dependent. The most frequent were short deletions, but we also detected 1-nt insertions (T or G), deletions between two sgRNAs and larger deletions. miRNA abundance correlates with the frequency and type of introduced mutations, as more extensive mutations in more alleles result in lower miRNA abundance. Interestingly, some mutated loci can generate alternative miRNAs, now novel targets were however predicted for those. In all transgenic lines with Cas9 expression, we detected mutations, suggesting high efficiency of Cas9-editing. We confirmed the miRNA-editing efficiency of our optimised approach in two different potato genotypes and three different loci.

Oral presentation

Efficient targeted gene insertions in diploid potatoes

Yordan Dolaptchiev[1,*], Lila Grandgeorge[1], Matthew Smoker[1], Mark Youles[1], Sara Perkins[1], Azka Noureen[2], Aga Alexander[3], Jonathan D G Jones[1]

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK.
[2]John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK.
[3]Crop Science Centre, NIAB, Cambridge, CB3 0LE, UK.
*E-mail: yordan.dolaptchiev@tsl.ac.uk

Potatoes are a crucial subsistence crop but are vulnerable to various pathogens, most notably *Phytophthora infectans* which causes Late Blight. To keep this at bay, farmers rely on heavy pesticide use, as traditional breeding methods have been limited by tetraploid potatoes' difficult genetics. Novel diploid potato breeding provides a promising alternative, but adoption requires rapid germplasm improvement. Several strong resistance genes against *P. infectans* have been isolated from wild potato relatives, yet these lie behind breeding barriers and cannot be introgressed. One solution is "knock-in breeding," whereby resistance genes encoding NLR receptors are inserted into the genome at a predetermined location through homologous recombination. To establish and optimize this technique, an anthocyanin-based visual reporter system was employed to assay the efficiency of knock-in constructs with varying architectures and optimize best practices. Anthocyanin-rich plants were recovered at a moderate efficiency in several experiments. PCR screening identified multiple plants containing the expected targeted insertion. Sequencing confirmed the insertion of the knock-in cassette in a targeted and clean manner. The insertions were heritable, and the anthocyanin phenotype was visible in the seed. This work constitutes a proof of concept for a new technology which could be used to deploy R-genes in crops with precise control over the insertion locus.

Oral presentation

CRISPR-based visualisation of centromere sequences in chicory

Evelien Waegneer[1,2], Tom Ruttink[1,3], Ilya Kirov[4], Katrijn Van Laere[1,*]

[1]ILVO, Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food, Caritasstraat 39, 9090 Melle, Belgium
[2]Laboratory for Plant Genetics and Crop Improvement (PGCI), Division of Crop Biotechnics, Department of Biosystems, KU Leuven, Willem de Croylaan 42, 3001, Heverlee, Leuven, Belgium
[3]Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium
[4]Russian Research Institute of Agricultural Biotechnology, Moscow, Russia
*E-mail: katrijn.vanlaere@ilvo.vlaanderen.be

Cytogenetics refer to the study of chromosome structures and functions and allow for visualizing and tracking specific sequences on the chromosomes, like centromere and telomere repeats. Besides Fluorescence In Situ Hybridization (FISH), more recently CRISPR-based visualization techniques have been developed, with the advantages to be faster, to enable visualisation of sequences in different plant tissues and to be compatible with immunohistochemistry (Ishii et al., 2019). Here we optimized a CRISPR-based visualization technique called RNA-guided endonuclease in situ localisation (RGEN-ISL) to track centromere and telomere sequences in chicory.

First, we identified potential centromeric and telomeric tandem repeats (TRs) by identifying highly abundant repeat sequences in raw sequence data of Cichorium intybus, using TAREAN and NanoTRF (Kirov et al., 2022, Novak et al., 2017). Based on these results, we identified five TRs; here named CL1, CL2, CL85, CL90 and CL133. As a first confirmation the location of the TRs were determined by a BLASTn search in two different C. intybus reference genome assemblies; the L8001_v1 reference and the Puna reference (Fan et al., 2022, Waegneer et al., 2023). The results were different depending on the genome used, most likely due to different assembly strategies; while the Puna reference had few loci with highly abundant TRs, in the L8001_v1 reference sequence TRs were scattered throughout the genome. Second, we verified physical localization of these TRs using FISH. CL1 was located in the centromeric region of six out of nine chromosomes of the karyotype of C. intybus. All other repeats were located in telomeric regions, with CL2, CL90 and CL133 showing signals on 12 out of 18 telomeres, and CL85 showing signal in 10 out of 18 telomeres. Third, using centromeric CL1 and telomeric CL2, we optimized the RGEN-ISL procedure on chromosome metaphase spreads in chicory. We used three different hybridization times: 1 hour, 4 hours and overnight, and three different hybridization temperatures; 26°C, 37°C and 42°C. CL1 showed strong centromeric signals in six out of nine chromosomes, consistent with the FISH results, in all conditions, although strongest signals were observed using 4 hour incubation at 37°C. CL2 had weaker signals in 12 telomeres, again consistent with FISH, and the best results were obtained using 4 hour incubation at 37°C, similar to CL1 results.

In conclusion, we were able to identify and visualize both centromeric and telomeric TRs in chicory, and successfully optimized the RGEN-ISL procedure for visualization of these TRs. In next steps, we want to optimize RGEN-ISL for visualization of TRs in different plant tissues, which can have applications for studying processes such as chromosome elimination during haploid induction in chicory.

Session II - Improving resistance to abiotic stress

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

Keynote

CRISPit - Bridging fundamental knowledge and novel technology to increase rice heat tolerance

Sílvia Coimbra

Faculty of Sciences, University of Porto, Porto, Portugal
E-mail: scoimbra@fc.up.pt

Seeds are the link between the end of the reproductive cycle of adult plants and the establishment of their next generation. Seeds are the means through which plants can adapt to climatic changes. Feeding the ever-growing population is a major challenge, especially in light of rapidly changing climate conditions. Genome editing is set to transform plant breeding and help secure the global food supply. Advances in genome editing technologies provide new opportunities for crop improvement by employing precision genome engineering for targeted crop traits.

This proposal, which addresses critical stages of the reproductive development, integrates several approaches to comprehensively investigate sexual reproduction in Rice under heat stress (HS) conditions with the aim of using the novel knowledge generated to improve rice production. The model plant system *Arabidopsis thaliana* will be used as proof of concept, to generate quick knowledge that can be readily translated into concrete outcomes in rice, species of high importance to the European agricultural sector.

CRISPit main goal is to understand the mechanisms that regulate heat stress tolerance during the reproductive process in rice. CRISPit proposes to obtain and fully characterize mutants from chosen genes in order to deliver rice HS lines produced by CRISPR technology.

Oral presentation

Optimized *Lolium perenne* L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications

Ferenz Sustek-Sánchez[1], Anete Borodušķe[2], Madara Balode-Sausiņa[2], Erki Eelmets[1], Sanda Astra Bērziņa[2], Olav Kasterpalu[1], Merike Sõmera[1], Kristina Jaškūnė[3], Odd Arne Rognli[4], Nils Rostoks[2], Cecilia Sarmiento[1,*]

[1]Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia
[2]Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Riga, Latvia
[3] Laboratory of Genetics and Physiology, Lithuanian Research Centre for Agriculture and Forestry, Stoties 2, Akademija, 58343 Kedainiai distr., Lithuania
[4] Faculty of Biosciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
*E-mail: cecilia.sarmiento@taltech.ee

The project "Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology – EditGrass4Food" aims to improve adaptability and resilience of perennial ryegrass (*Lolium perenne L.*) for safe and sustainable food systems through gene editing. The three Baltic countries and Norway are members of the consortium that is coordinated by the University of Latvia. In Europe*, Lolium perenne* L. is the most widely distributed and grown forage grass used to feed livestock. However, this species does not grow well under freezing or drought conditions, which implies a problem for expanding its cultivation towards northern-eastern European regions. Genome editing with CRISPR-Cas9 can help obtain resilient genotypes and identify mechanisms underlying stress tolerance. Since perennial ryegrass is not a model organism, there is a lack of available experimental data regarding standardized transformation and regeneration procedures. Moreover, *Lolium perenne* L. is a highly heterogenous species, which creates an additional challenge when performing genetic studies.

We aimed to create a platform for screening the efficiency of different guide RNAs (gRNAs) knocking out genes potentially involved in drought and frost stress tolerances. Therefore, we started by establishing protocols to generate, in an asexual manner, plant material that could be used for gene editing. For this, we set up the *in vitro* culture of tillers. Tillers grown in solid media supplemented with growth promoters were processed to generate protoplasts. We tested different protocols for both the isolation and transformation of protoplasts. For the isolation of protoplasts, we optimized the following variables: enzymes' concentrations, mannitol pretreatment, enzymatic treatment duration, and vacuum infiltration. The optimized protocol yields, on average, 1.5 x 10^6 protoplasts per ml of enzyme solution, which is sufficient for downstream applications such as transformation assays. Later, we compared two different protoplasts transformation methods: PEG and electroporation.

The gRNAs tested in protoplasts can be used for agrobacterium-mediated transformation. In our case, we focus on the co-cultivation of either meristematic calli or meristems with agrobacterium strains carrying the previously tested gRNAs.

Acknowledgments: "EditGrass4Food" (ID No. EEA-RESEARCH-64, Contract No. EEZ/BPP/VIAA/2021/4) is financially supported by the European Economic Area (EEA) grants.

Oral presentation

Establishment of highly efficient and reproducible Agrobacterium-mediated transformation system for tomato (*Solanum lycopersicum* L.)

Muneeb Hassan Hashmi[1,2,*], Faisal Saeed[2], Ufuk Demirel[2], Allah Bakhsh[3]

[1]Department Chemistry-Biology, University of Siegen, 57068, Siegen, Germany
[2]Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, 51240 Nigde, Turkey
[3]Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
*E-mail: muneebshah9@gmail.com

A simple and improved *Agrobacterium*-mediated transformation protocol of tomato (*Solanum lycopersicum*) cultivar Rio Grande was developed to inspect the potential of producing transgenic tomatoes. In this study, regeneration and transformation efficiency as assessed in response to the seedling age, explant type, co-infection, co-cultivation duration, selection pressure (kanamycin), and the optimal concentration of plant growth regulators (PGR) 6-benzylaminopurine (BAP), gibberellic acid (GA3), and indolebutyric acid (IBA) in a Murashige and Skoog (MS) basal medium. To accomplish this goal, 2- to 4-wk-old tomato explant cotyledons, hypocotyl, and cotyledonary nodes were excised and transformed with the EHA105 *Agrobacterium* tumefaciens strain harboring pBIN19 binary vector containing uidA reporter gene and nptII as a selectable marker. Results revealed that 14-d-old cotyledonary nodes and leaves inoculated for 15 min with A. tumefaciens strain EHA105 following 48-h co-cultivation were optimal for the highest percent transformation efficiency 27.31. Antibiotic kanamycin (Kan) at 25 mg L^{-1} in the regeneration selection medium was found to be effective. MS medium containing optimal concentrations of 1.5 mg L^{-1} of BAP, 0.2 mg L−1 GA3, and IBA 1.5 mg L^{-1} showed a significant level of percent regeneration, shoot elongation, and rooting efficiency of transformed plantlets. Molecular analysis of T0 transgenic tomato plants showed integration and a higher relative expression level of the uidA gene. The optimized A. tumefaciens–mediated transformation method for tomato cultivar Rio Grande showed the highest percent transformation efficiency (TE) and regeneration efficiency (RE) and is likely to give consistent results with different tomato cultivars.

Oral presentation

Improving grape resilience to climate change exploiting the CRISPR/Cas technology: different approaches to face drought

Loredana Moffa[1], Manuela Campa[2], Ivan Bevilacqua[1-3], Chiara Pagliarani[4], Giorgio Gambino[4], Irene Perrone[4], Riccardo Velasco[1], Walter Chitarra[1,4], Luca Nerva[1,4,*]

[1]Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano, Italy
[2]Department of Genetics, Stellenbosch University, Matieland, South Africa
[3]University of Padua, Department of Agronomy, Food, Natural Resources, Animals and Environment, Agripolis, Viale dell'Università 16 - 35020 Legnaro (Pd), Italy
[4]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino, Italy
*E-mail: luca.nerva@crea.gov.it

Climate change has been significantly impacting the food chain production, resulting in decreased quality and yield. In response, the international scientific community has made substantial efforts to enhance resilience and sustainability in agriculture. Despite their importance, fruit crops face challenges with conventional breeding approaches, particularly regarding financial commitments, limited land resources, and lengthy generation times. In this context, the utilization of 'New Genomic Techniques' (NGTs) has emerged as a promising avenue for expediting the development of genetically improved cultivars, with precise targeting of specific DNA sequences. We capitalized on these technologies to enhance grape resilience against drought stress, employing three distinct genome editing approaches. Initially, we functionally characterized a grape glutathione-S-transferase gene, *VvGST40*, using spray-induced gene silencing (SIGS), which effectively downregulated the gene expression. By employing this approach, we examined the effects of transient downregulation during a drought stress event, ultimately identifying enhanced resilience in the treated plants. Subsequently, we utilized a self-designed cisgenic-like construct to transform embryogenic calli of Chardonnay and 110 Richter genotypes. Currently, multiple independent lines have been regenerated and are now undergoing thorough examination. Concurrently, we targeted two genes belonging to the pectin methyl esterase gene family (PME), previously associated with cavitation in poplar. In that case, utilizing an RNAi construct, it was observed a reduced susceptibility to cavitation, resulting in enhanced resilience to drought stress. Here we targeted two homologues of *Vitis vinifera* to then evaluate the effects. Plants of genotypes Chardonnay and 100 Richter have already been obtained. Lastly, we employed gene editing to modulate *VvMYB60* expression through targeted modifications of its promoter, specifically focusing on the DOF regions. Regenerated plants from all approaches are now undergoing rigorous evaluation to identify the most drought-tolerant specimens.

Session III – Improved technologies

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

Keynote

Increase the production of industrially valuable compounds in the microalgae *Chlorella* – the GeneBEcon approach

Elke Vereecke[1,2,*], Tom Ruttink[1,3], Sofie Van Nerom[1], Evelyne Delezie[1], Johan Robbens[1], Sil Allaert[1], Carla Mingolla[1], Marc De Loose[1], Johan Robbens[1], Katrijn Van Laere[1]

[1]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium
[2]VIB Center for Plant Systems Biology, Belgium
[3]Department of Plant Biotechnology and Bioinformatics, Ghent University, Belgium
*E-mail: elke.vereecke@ilvo.vlaanderen.be

Microalgae in general are promising for biobased applications as they yield high-value biomass that can be processed in different industries e.g. as food/feed supplements, photoprotective agents and/or biodiesel feedstocks. An interesting microalgae genus is *Chlorella* producing, besides a high-nutritional biomass, several specific high-value compounds, including mycosporine-like amino acids (MAAs). *Chlorella* would be a promising production platform for these high-value compounds if the production of MAAs can be increased. Metabolic engineering via gene-editing in *Chlorella* could be one of the strategies to improve the production of high-value compounds in general and MAAs in particular.
In the GeneBEcon project, the innovation potential of gene-editing in enabling a sustainable bioeconomy will be explored through the application of CRISPR/Cas in *Chlorella* and studying the associated economic and societal issues. First, a systems thinking approach was followed to map the value chain of microalgae production allowing the identification of potential benefits, and risks of gene editing in *Chlorella*. Second, a gene-editing toolbox is being developed for *Chlorella* to enhance the production of MAAs containing selection markers, transformation methods, and molecular screening methods. The gene editing toolbox will not only result in *Chlorella* strains with enhanced production of MAAs but will also provide scientific data to feed into discussions related to associated economic, societal, and regulatory factors of gene-edited products. Finally, after extraction of MAAs we will explore if the residual biomass can be used as a feed additive in poultry feed by performing chicken trials.
Successful increased production of MAAs, as case study for high-value compounds in general, and valorisation of the residual biomass would enable a zero-waste production chain in *Chlorella* for a circular bioeconomy.

Oral presentation

Optimizing protoplast isolation and transformation efficiency for enhanced plant genome editing in grapevine

Hilal Betul Kaya[1,*], Gulsen Kolasinliler[1], Cengiz Akkale[2]

[1]Department of Bioengineering, Manisa Celal Bayar University, Manisa, 45140, Turkey
[2]Department of Bioengineering, Adana Alparslan Türkeş Science and Technology University, Adana, 01250, Turkey
*E-mail: hbkaya@gmail.com

Protoplasts provide a useful alternative strategy in plant genome editing instead of testing genome editing reagents directly in plants, requiring a genotype-dependent, time-consuming, and labor-intensive tissue culture process. There are many studies on protoplast isolation from various crops and model plants, but studies on protoplast isolation from grapevine are limited. Although enzymatic removal of cell walls has been proven to be the most successful method in protoplast isolation, various parameters such as plant species and even cultivar, digestion duration, enzyme concentration, type and age of explants, and type of mechanical disruption methods can directly affect the efficiency of protoplast isolation. In testing genome editing reagents, it is critical not only to obtain high amounts of protoplasts but also to keep them healthy to achieve high transformation efficiency, which is also affected by incubation time on PEG, protoplast concentration, and plasmid DNA concentration. In this study, these parameters were tested in coordination to improve protoplast yield and increase transformation efficiency. Protoplast isolation experiments yielded a maximum of 9 million protoplasts per gram of fresh leaves where enzymatic digestion duration was eight h using 0.75% Macerozyme, 1.5% cellulase, and 0.6 M D-mannitol.
On the other hand, a 5-minute PEG incubation time for transforming 500.000 protoplasts with ten µg of plasmid DNA resulted in 90.3% transformation efficiency when using 35S:GFP construct. It was observed that increasing the number of protoplasts did not improve efficiency. This optimized and improved method could be used in future grapevine genome editing research to streamline the discovery of novel strategies. Overall, this optimized and improved method could contribute to ongoing efforts to enhance plant genetic manipulation in the grapevine.

Oral presentation

Exploring alternative approaches for efficient gene targeting in plants: high fidelity nonhomologous end-joining with CRISPR-Cas12a in potato protoplasts

William de Martines[1,*], Ellen Slaman[2], Richard Visser[1], Jan Schaart[1]

[1]Plant Breeding, Wageningen University & Research, PO Box 386, 6700 AJ Wageningen, The Netherlands

[2]Laboratory of Molecular Biology, Wageningen University & Research, PO Box 633, 6700 AP Wageningen, The Netherlands

*E-mail: william.demartines@wur.nl

Gene targeting in plants has predominantly relied on homology-directed repair, but despite substantial investments, its efficiency remains limited. Thus, investigating alternative approaches for gene targeting in plants is imperative. This study demonstrates the successful replacement of 3.3 kb genomic DNA fragments in potato protoplasts using CRISPR-Cas12a, coupled with directional insertion of 58 bp replacement sequences delivered as double-stranded oligodeoxynucleotides (dsODNs) via high fidelity nonhomologous end-joining (NHEJ). Our investigation focuses on the impact of end modifications in dsODNs on the efficiency of seamless integrations, aiming to unravel the intricacies of our high fidelity NHEJ pathway. In the most efficient cases, we observe seamless integrations in up to 50% of total excision events. Notably, we identify the use of dsODNs with 5-nucleotide overhangs, exhibiting left and right complementarity to the expected Cas12a DNA cleavage sites, as the most efficient approach. Any further alteration in the overhang design results in significantly lower efficiencies. Based on our observations, we propose that the repair mechanism likely relies on a process involving simple base pairing followed by ligation for DNA repair. However, the complete classification of this pathway within the existing nonhomologous end-joining pathways described in the literature remains uncertain. Further investigation into these mechanisms holds promise for advancing this type of repair in diverse genome editing applications.

Oral presentation

New Genomic Techniques in citrus, step-by-step solutions for more efficient and successful procedures

Angelo Ciacciulli[1,*], Helena Domenica Pappalardo[1], Mickael Malnoy[2], Umberto Salvagnin[2], Marco Caruso[1], Concetta Licciardello[1]

[1]Council for Agricultural Research and Economics (CREA) - Research Centre for Olive, Fruit and Citrus Crops, Acireale, Italy
[2]Research and Innovation Centre, Trento with S. Michele all' Adige, Trento, Italy
*E-mail: angelo.ciacciulli@crea.gov.it

In recent years, the development of New Genomic Techniques (NGTs, cisgenesis and genome editing) is promoting the biotechnological approach to gene studies and to the varietal innovation in fruit tree crops. In particular, cisgenesis allows the gene transfer between sex-compatible species without crossing; genome editing simplifies the induction of target mutations, not only for knock-down but also for specific replacements. There are still several bottlenecks in the application of both techniques, especially for tree fruit species: the availability of the optimal in vitro media and transformation and regeneration protocols; the long juvenility that limits the time to see the induced phenotype and the reduced possibility to segregate out the transgenes necessary for the editing machinery. Our lab tried to overcome most of these limitations in *Citrus* with the aim of studying the target genes and obtaining transgenes-free plants. Here we list the limiting steps for the application of NGTs in citrus and the solutions we adopted. **The explants transformation**: after comparing different sources of explants, we opted for the seeds that are slightly less efficient than the epicotyls, but easier and faster to process and regenerate. **The selection of regenerants**: a variable number of shoots escape the selection process; therefore, transformation must be validated through molecular assays. To simplify this step, a visual marker was added to the plasmid: *VVmybA1* from grape, *Ruby* (myb-like) from citrus. *VVmybA1* resulted in the most efficient, in terms of easy detection, with the strongest and the most stable pigmentation of the shoots. **The substrates**: Despite several efforts being performed to optimize the substrates for faster growth of the transformants, the most efficient practices for growth are represented by in-vivo minigrafting and micrografting of transformant onto young rootstocks. **The production of transgene-free plants**: We equipped our plasmids with the FLP/FRT system, allowing the excision of the cassette enclosing the Cas and the selection marker, after optimized heat-shock conditions. **The detection of transgene-free plants**: The heat-shock works with a certain efficiency, and sometimes more than one treatment is necessary, requiring several molecular analyses to evaluate the effect. Again, the use of VVmybA1 facilitated the detection of transgene-free plants, because only the plants that have lost the anthocyanins pigmentation are tested for the excision of the transgenes. **The overcoming of juvenility**: The transgene-free plants obtained from juvenile tissues need five to ten years to pass the juvenile phase and bear the first fruits. To overcome this limitation, our plasmids were equipped with Cas9 and a single-guide RNA able to edit two genes reported as inhibitors of flowering, TFL1 and CEN. Despite some steps that need to be optimized, in our laboratory we improved several steps for the generation of cisgenic and edited plants, making the transformation system for citrus cost-effective, labour-saving, faster, and more efficient. The developed plasmids and procedures could be applied to other fruit tree species using citrus as a model.

Session IV - Nutritional improvement and characterisation

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

Keynote

Developing gene editing in sweetpotato to increase its nutritional status

Nélida Leiva Eriksson*

Lund University, Department of Biotechnology, Lund, Sweden
*E-mail: nelida.leiva_eriksson@biotek.lu.se

Classical plant breeding together with modern methods of plant biotechnology, such as integrated omics analysis, can contribute to the efficient development of improved crops. Central America is a region that is seeing its levels of malnutrition increase, putting at risk the fulfilment of the Second Sustainable Development Goal: Zero Hunger, by 2030. In response to this situation, the Sweden-Central America network was established to develop functional crops with high nutritional value and resistant to the effects of climate change. Within this collaboration, field tests are being carried out with sweetpotato crops in different locations in Guatemala and Honduras, to determine their productivity as well as to analyse the iron content in leaves and roots and see what the effect of the environment on its absorption and accumulation is. At the same time, during field tests, samples are being taken for integrated omic analysis that will allow us to understand the molecular basis of iron accumulation in sweetpotato. In this presentation, we will report our first results from field trials in both countries and preliminary results on protoplast isolation as a first step to develop gene editing in sweetpotato. The omics results together with the gene editing protocol will be used to increase bioavailable iron levels in leaves and roots.

Oral presentation

In-depth characterization of Cas9 specificity in tomato using high-throughput amplicon sequencing, GUIDE-seq and whole genome resequencing

Ellen Slaman[1,2,*], Sven Warris[3], Jacqueline Busscher[2], Rick Dekker[2], Michiel Lammers[2], Gerco C. Angenent[1,2], Ruud A. de Maagd[2]

[1]Laboratory of Molecular Biology, Wageningen University & Research, Wageningen, The Netherlands
[2]Plant Developmental Systems, Bioscience, Wageningen University & Research, Wageningen, The Netherlands
[3]Applied Bioinformatics, Bioscience, Wageningen University & Research, Wageningen, The Netherlands
*E-mail: ellenslaman@gmail.com

CRISPR-Cas9 technology has revolutionized genome editing and offers great potential to accelerate plant breeding. However, the specificity of this technology in plants remains insufficiently investigated, particularly regarding unbiased off-target detection methods. Here, we studied off-target mutations using biased and unbiased approaches. Initially, we screened 224 predicted off-target sites belonging to 89 sgRNAs with up to 4 mismatches to their respective target site using high-throughput amplicon sequencing of tomato protoplasts.

Mutations were found at only 17 predicted off-target sites. No mutations were observed at sites containing more than 2 mismatches. Notably, most mutated sites (14 out of 17) had only a single mismatch to the target site. To complement our biased screening, we applied the unbiased off-target detection method GUIDE-seq to a subset of identified promiscuous sgRNAs. This method is based on the integration of short dsDNA fragments in DSBs. Using this technique, we could detect off-target mutations with a minimum frequency of 0.7% in protoplast pools. Importantly, no new off-target sites were identified compared to the biased screen. Additionally, whole genome resequencing of 38 mutant T1 tomato lines at ~34x coverage revealed no significant increase of SNVs, indels, or structural variation in CRISPR-Cas9 edited lines as compared to control plants. Moreover, no evidence of off-target mutations was found among 29,903 predicted off-target sites with up to six mismatches. Overall, our study comprehensively assessed the specificity of CRISPR-Cas9-mediated genome editing in tomato.

We successfully applied the unbiased off-target detection method GUIDE-seq in plants for the first time. By combining biased and unbiased approaches, we conclude that CRISPR-Cas9 mediated genome editing is highly specific. Our results show that off-target mutations are predictable and can thus effectively be avoided in products for farmers and consumers.

Oral presentation

A dual single-guide RNA approach used to edit the *β-cyclase 2* gene in anthocyanin-rich sweet orange varieties

Fabrizio Salonia[1,2], AngeloCiacciulli[1], Helena Domenica Pappalardo[1], Lara Poles[2], Massimo Pindo[3], Simone Larger [3], Paola Caruso [1], Marco Caruso [1], Concetta Licciardello[1]*

[1]Council for Agricultural Research and Economics (CREA) - Research Centre for Olive, Fruit and Citrus Crops, Acireale, Italy,
[2]Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy,
[3]Research and Innovation Centre, Trento with S. Michele all' Adige, Trento, Italy
*E-mail: concetta.licciardello@crea.gov.it

Citrus represent one of the most important fruit tree species in the world, whose fruits are used for fresh consumption and industrial purposes. They are also crucial for the human diet, highly appreciated by consumers for taste, aroma, and juiciness, and also for their high content of antioxidant compounds, such as vitamin C, polyphenols, flavonoids and carotenoids. One of the aims of breeding programs in citrus is the production of varieties with an increased amount of bioactive compounds, such as anthocyanins and lycopene. The most common genotypes characterized by the presence of anthocyanins are blood oranges (i.e. Moro, Tarocco, Sanguigno and Sanguinello varieties). On the other hand, the presence of pink colour in fruit tissues, typical of varieties enriched in lycopene, is diffused among sweet orange, grapefruit, pummelo, and lemon varieties.
Traditional breeding approaches to conjugate both pigments are hampered by several factors, such as chimerism, the high level of heterozygosity, the polyembryony, the partial or complete sterility, and the long juvenile phase. For this reason, we decided to apply genome editing on *b-cyclase 2* (the gene converting lycopene into b-carotene) in anthocyanin-rich sweet orange varieties. In particular, we used a dual single guide RNA (sgRNAs) approach based on GoldenBraid technology to produce a large deletion of 250 bp (the region between the cutting sites of both sgRNAs), in addition to inducing point mutations in one or both sgRNAs. Five anthocyanin-rich sweet oranges varieties belonging to the Tarocco and Sanguigno varietal groups, were transformed using the EHA105 *Agrobacterium tumefaciens* strain. Among the tested varieties, 'Doppio Sanguigno' showed the best transformation efficiency, reaching around the 20%; the combination of appropriate transformation substrates and procedures made 'Doppio sanguigno' a highly efficient transformation system for citrus. The 86% of the 58 plantlets sequenced in the target region resulted successfully edited. The most frequent mutations were deletions (from -1 to -74 nucleotides) and insertions (+1 nucleotide). Moreover, the inversion of the region between the cutting sites of the sgRNAs was also observed in six plantlets. We excluded chimeric events for 20 plantlets in which a single mutation occurred.
After three years since the transformation, no visible changes were detected in the vegetative tissues. Based on our knowledge, this work represents the first example of using genome editing to improve citrus fruit quality.

Oral presentation

HQT gene editing to study chlorogenic acid metabolism and its physiological role in tomato

Fabio D'Orso[1,*], Lionell Hill[2], Ingo Appelhagen[2], Tom Lawrenson[2], Marco Possenti[1], Jie Li[2], Wendy Harwood[2], Giorgio Morelli[1] and Cathie Martin[2]

[1]CREA Research Centre for Genomics and Bioinformatics, Via Ardeatina 546, Rome, Italy
[2]John Innes Centre, Norwich NR4 7UH, United Kingdom
*E-mail: fabio.dorso@crea.gov.it

Phenolic compounds play crucial roles in plant development and physiology, and they are part of defence strategies against biotic and abiotic agents especially when plants have to face extreme environmental events. Understanding metabolic pathway regulation is a key step towards a more sustainable agriculture as it will help plant breeding programs to generate resilient plants adapted to survive and keep high growth performance and yield in adverse conditions.
In Solanaceous plants, chlorogenic acid (CGA) is the most abundant phenolic compound which has several protective properties including antimicrobial and antioxidant activities, but also UV-B absorbing capacity. Its biosynthesis has been subject of several studies in the past and it has been shown that in tomato and potato the most important metabolic way to produce CGA is mediated by the enzyme hydroxycinnamoyl-CoA:quinate hydroxycinnamoyl transferase (HQT). Nevertheless, so far the absence of natural or induced mutants of this gene has prevented to understand if the HQT-dependent way is the only one active or if other ways can give a significant contribution in CGA production and accumulation.
Here, through CRISPR technology, we generated several tomato lines by inducing knock-out mutations in HQT gene. The resultant *slhqt* plants did not accumulate relevant amounts of CGA nor other caffeoyl-quinic acids (CQAs) in several parts of the plant. This demonstrated that in tomato, and possibly in other Solanaceaes, CQA biosynthesis almost completely depends on HQT-mediated pathway.
As in WT plants CGA is the most accumulated phenolic compound, its depletion can cause a significant metabolic change, therefore we investigated how the phenylpropanoid metabolome was remodelled in *slhqt* leaf in normal and stress conditions. We also explored the expression change of genes coding enzymes and transcription factors involved in phenylpropanoid biosynthesis and regulation. Also, in this work we showed that in WT leaves CGA is accumulated in the upper epidermis of leaf and we explored the physiological effect of UV-B light exposure in *slhqt* leaves.

Session V – Improving resistance to biotic factors

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

Keynote

Gene editing in potato to enhance PVY resistance

Johan Hunziker

INRAE, France
E-mail: johan.hunziker@inrae.fr

Plant scientists have rapidly adapted New Genome editing Tools for crop improvement, to complement traditional breeding approaches. The current technical challenge is to efficiently induce precise and predictable targeted point mutations valuable for crop breeding purposes. Several base editing tools such as the CRISPR/Cas9 system and its derivate Base Editors, have demonstrated their utility in plant breeding. However, such technologies proved their limits with non-predictable mutations introduced, resulting in difficulty to transfer known mutations between cultivars or species. New technologies of precise base editing currently in development such as the Prime Editing, support the possibility to obtain expected DNA substitution in different species, without having to characterize large populations of mutants. In combination, new varieties can be created without requesting bacterial DNA insertion, with established protoplast transfection and regeneration protocols. In this lecture, the objective will be to explain the application of genome editing tools to transfer well-characterized mutations involved in PVY resistance, isolated from diploid Solanaceae such as pepper and tomato, to the tetraploid potato. A presentation of the application of the different generations of genome editing tools and their results on plants will be introduced and the advantages and disadvantages of each of them will be pointed out.

Oral presentation

Studying potato resistance and susceptibility factors against pathogens with the use of genome editing

Éva Csaba*, Jeny Jose, Zoltán Bozsó, József Bakonyi, Tetiana Kyrpa, Kamirán Áron Hamow, Ervin Balázs, László Sági

ELKH Agricultural Institute, Centre for Agricultural Research, Martonvásár, Hungary
*E-mail: evacsaba@gmail.com

Potato is a staple crop worldwide, but its cultivation is threatened by diseases like bacterial wilt caused by Ralstonia solanacearum and late blight as result of *Phytophtora infestans* infection. The outcome of these infections may depend on the presence or absence of plant resistance factors (physical barriers, recognition receptors, stress hormones, production of antimicrobial compounds and pathogenesis related proteins, etc.) and susceptibility factors (interaction partners of plant-injected bacterial proteins, plant genes feeding the pathogen like sugar transporters, and plant negative immunoregulators). Here, we targeted various potato genes for CRISPR/Cas genome editing in order to improve the metabolic and immunological response of potato plants, thereby giving them an edge over these highly effective pathogens. The knockout of polyphenol oxidase genes caused widespread metabolic and hormonal changes (e.g., increased dihydrokaempferol, taxifolin, salicylic acid, and jasmonic acid production) but still compromised the resistance against Ralstonia solanacearum, while the late blight resistance did not change. A mutation of the coryne receptor, a possible interaction partner of *Ralstonia solanacearum* proteins, resulted in a slight resistance to the bacterium. We attempted to boost the immune system by knocking out the microRNA genes miR396 and miR159, but the results were mixed. The miR396 KO plants showed a delay in bacterial wilt symptoms, but their tubers were more sensitive to late blight. The miR159 mutants were slightly more susceptible to Ralstonia solanacearum. Our studies show successful application of the CRISPR/Cas system for gene function studies. Strong plant resistance may be achieved by the combination of successful single-gene strategies.

Acknowledgements: The project has been supported by grants NKFI K-132829; ATK-MATE no. 0205K0035P and National Laboratory project no. RRF-2.3.1-21-2022-00007

Oral presentation

Gene editing in triploid banana cultivars

Senne Van den Broeck[1,*], Bart Panis[1,2], Hervé Vanderschuren[1,3]

[1]Laboratory of Tropical Crop Improvement, KU Leuven, Willem de Croylaan 42, 3001 Heverlee, Belgium
[2]Alliance of Bioversity International and CIAT @ KU Leuven Willem de Croylaan 42, 3001 Heverlee, Belgium
[3]Plant Genetics Laboratory, TERRA Teaching and Research Center, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium. Av. de la Faculté d'Agronomie 41/13, Gembloux, Belgium
*E-mail: senne.vandenbroeck@kuleuven.be

Banana is the most important fruit crop globally, with an annual production of over 145 million tonnes. They are both a commercial export crop of important economic value as well as a staple food for over 500 million people in several regions with chronic food security issues. Their triploid nature and sterility limit their use in breeding programs, resulting in monoclonal cultivation and thus high susceptibility to pests and diseases. To introduce new characteristics in banana cultivars, Agrobacterium tumefaciens-mediated transformation is most often used for the introduction of full genes or gene edits, for which a protocol was recently established at our laboratory. However, given that outcrossing of the T-DNA cassette is impossible, gene-edited banana remains classified as GMO, even after further potential loosening of the regulatory framework in the European Union. Therefore, DNA-free genome editing techniques are required for these sterile and vegetatively propagating crops. One of the main challenges in banana horticulture is the presence of an endogenous virus in the B genome of banana, namely Banana Streak Virus (BSV), which limits all uses of the B genome for breeding. Therefore, we are developing DNA-free genome editing techniques, which could later be utilized for the removal of the endogenous BSV of the B genome of banana.

Oral presentation

De novo domestication of wild tuber-bearing *Solanum* species

Aristotelis Azariadis[1], Olga A. Andrzejczak[1], Hussam H. Nour-Eldin[2], Zeinu M. Belew[2], Harsh Yadav[2], Frida M. Carlsen[3], Ida Westberg[3], Henrik B. Pedersen[1], Bent L. Petersen[3], Kim H. Hebelstrup[1,*]

[1]Dept. of Agroecology, Crop Genetics and Biotechnology, Aarhus University, Denmark
[2]Dept. of Plant and Environmental Sciences, Molecular Plant Biology, Copenhagen University, Denmark
[3]Dept. of Plant and Environmental Sciences, Plant Glycobiology, Copenhagen University, Denmark
*E-mail: kim.hebelstrup@agro.au.dk

De novo domestication by genome editing is a technology whereby wild plants are taken into agriculture and cultivation by introducing domestication traits using new breeding technologies. We screened through 107 wild *Solanum* species, for relevant traits of high resilience against various biotic and abiotic stresses. Nine wild *solanum* species were selected. We developed transformation and genome editing protocols to target genes for introducing early tuberization and low glycoalkaloid content. We were able to *de novo* domesticate one of the wild species by using genome editing. It exhibited high resistance to *Phytophthora infestans* (Late blight), good tuber yield and acceptable content of glycoalkaloids, and so we have established this technology as a proof-of-concept for *de novo* domestication of wild tuberous plants for future agriculture.

Session VI – Regulation and public perception

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

Oral presentation

Preliminary analysis of the European Commission Proposal for a Regulation on the production and marketing of plant reproductive material

Juan Antonio Vives-Vallés

University of the Balearic Islands – INAGEA, Spain
E-mail: ja.vives.valles@gmail.com

Finally, on July 5, the European Commission published the Proposal for a Regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 (COM(2023) 411 final). On that date, the European Commission also published the Proposal for a Regulation of the European Parliament and of the Council on the production and marketing of plant reproductive material in the Union (COM(2023) 414 final). Both proposals, together with others published jointly proposals and documents, are set to have a major impact on agriculture and plant breeding in the EU (Henriksson, 2023; Stokstad, 2023). The academia's attention seems to be mainly focused on the NGTs' Proposal, however, the PRM Proposal brings many significant changes, with relevant implications, even on the biotech regulatory scheme in the EU, including the NGTs' Proposal. This paper focuses on the analysis of the PRM Proposal, with special attention to the synergistic and related aspects between such regulatory initiative, the EU biosafety scheme, and the NGTs' Proposal.

Funding: Research leading to this paper has been partially carried out within the framework of a funded research stay at the Oxford Intellectual Property Research Centre of the Faculty of Law of the University of Oxford, under the TED2021-129992A-I00 research project, funded by the Spanish Ministry of Science and Innovation.

References:

Henriksson, P. (2023). The Swedish Presidency – A Time of Anticipation Pending the New Regulatory Texts From COM. EuropeanSeed, May 31. https://european-seed.com/2023/05/the-swedish-presidency-a-time-of-anticipation-pending-the-new-regulatory-texts-from-com/

Stokstad, E. (2023). European Commission proposes loosening rules for gene-edited plants. Science Insider. https://www.science.org/content/article/european-commission-proposes-loosening-rules-gene-edited-plants

Oral presentation

The new NGT legislation proposal of the European Union. Analysis of selected EU and national regulatory obstacles for the introduction and market viability of NGT plant products

Tomasz Zimny

Institute of Law Studies, Polish Academy of Sciences, Poland
E-mail: tzimny@inp.pan.pl

The speech presents results of an analysis of regulatory thresholds not directly connected with the GMO or New Genomic Techniques (NGT) legislation that the newly defined NGT plant products may still face, even if the proposed revision of the EU GMO legislation enters into force.
The European Commission's proposal for a regulation on plants obtained by certain new genomic techniques and their food and feed (COM(2023) 411) introduces two new categories of regulated plants on the EU market. The NGT type 1 plants, which while mostly deregulated in comparison to classic GMOs, will still be excluded from the organic production chain and are going to be labelled to a limited extent. The NGT type 2 plants are largely going to be treated as classic GMOs, when it comes to authorization, labelling, market presence etc., yet with some of the authorization requirements relaxed and some incentives to develop plants with traits that the legislator deems as promoting sustainability in agriculture.
Newly developed NGT-1 products, even if mostly exempted from the legislation, may still face market entry thresholds connected with other pieces of legislation. These include the food law and in particular the 2015/2283 novel foods regulation, seed laws of the member states, implementing the 2002/53 Directive on the common catalogue of varieties of agricultural plant species, national laws on fair competition, non-GMO labeling, as well as some environmental law provisions on the absence of GMOs from certain protected areas. Depending on such provisions, the position on the common market of a given product may be different in comparison to conventional products, even if such a product will be largely exempted from the general GMO legislation. Such differences will impact its economic and legal viability on the market. Unlike some of its closest neighbors and trade partners (e.g. the UK, Argentina, Brazil, Canada, USA) the European Union seems to be against a general exemption or exclusion of certain NGT-1 products from the legislation. This hybrid approach gives rise to regulatory obstacles not necessarily connected with the GMO legislation itself.
In the speech I present an overview and analysis of the regulatory burdens not contained in the GMO or NGT legislation that the products of NGTs may still face on the common market, even if the proposed EU legislation enters into force. I present some of those less obvious regulatory limitations to marketing of NGT products, along with an analysis of their influence on the viability of NGT 1 and 2 products across the Union.

Oral presentation

The awareness of the Polish society on new genomic techniques

Anna M. Linkiewicz*, Wiktoria Mołodziejko and Justyna Nowakowska

Laboratory of Molecular Biology and Genetics, Institute of Biological Sciences, Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszynski University in Warsaw, Wóycickiego 1/3, 01-938 Warsaw, Poland
*E-mail: a.linkiewicz@uksw.edu.pl

Genome editing methods increase the precision of mutations compared to current breeding methods and can lead to the rapid production of desired plant and animal varieties. However, the future of the technology will depend in part on consumer acceptance. The first comprehensive survey of public opinion among Polish citizens on new genomic technologies (NGTs) is presented. The main purpose of the study was to obtain information on the reception of the latest biotechnological inventions that may affect people in Poland. The survey was conducted from August 2022 to February 2023 among a group of 194 respondents on the basis of an anonymous, self-developed questionnaire, designed as a 3-part study. The first part of the survey concerned the socio-demographic characteristics of the respondents. The second and third parts of the survey were designed to find out the knowledge and attitudes of Polish citizens who, due to their education or profession, have contact with biology or agriculture, towards the use of NGTs. We were interested in the extent to which Polish citizens are aware of the possibilities offered by NGT methods and whether they know if the law permits the use of these methods. A low level of awareness was found for terms such as genome editing, CRISPR/Cas9 or TALEN. The majority of respondents recognised the potential benefits of using the technology for medical purposes or in plant breeding, agriculture and the food industry, but with appropriate regulations guaranteed by the EU. Respondents were not convinced about the personal use of NGTs if they were available in Poland. For most of the questions asked, there were no statistically significant differences in the answers, regardless of gender, age, region of residence, occupation or education. Our findings can be used to assess the attitudes of EU citizens towards advances in biotechnology, including GMOs.

Session VII – Short Term Scientific Missions - STSM

Oral presentation

Leveraging system biology and new breeding technologies for water stress tolerance in grapevines

Álvaro Vidal Valenzuela

Fondazione Edmund Mach (San Michele, Italy), TOMS biolab I2SysBio (Valencia, Spain) and CII Viña Concha y Toro, Chile
E-mail: alvaroignaciovidalvalenzuela@gmail.com

Climate change poses a serious threat to the quality and quantity of fruits and vegetables, affecting food security and sovereignty. Moreover, legislation hinders the development of biotechnological solutions, creating new challenges. Therefore, it is necessary to find a way to generate stress-tolerant plants without exogenous DNA, that can be planted worldwide. To address this problem, I present the pipeline I developed for the identification of molecular hubs that can be silenced by CRISPR-CAS based methods to increase the tolerance of grapevine to water stress: starting with literature analysis, using public RNA-seq data to generate a hydric-stress heatmap app to compare gene expression at different drought stages, tissues and cultivars, using co-expression network and DAP-Seq results (Related to the STSM CA18111-bddbbd8d) to identify pathways related to water stress response, validating in silico results by Q-PCR analysis, comparing different grapevine genomes for key elements associated with tolerance (Related to the VM CA18111-bddbbd8d), designing a CRISPR-CAS9 FRT/FLP vector to transform grapevine calli and remove the exogenous DNA by a heat shock induced recombinant system, developing a new somatic embryogenesis process to reduce the time-consuming transformation of trees based on a stress dependent regeneration, analyzing the site of insertion and copy number of transformed plants, and outlining future perspectives:
- Presentation of RNA-SEQ heat map app for water stress
- Presentation of STSM and VM results
- Pipeline used to select target genes to silence
- New protocol for somatic embryogenesis of grapevine calli.

Oral presentation

Application of multiplexed CRISPR-ACT3.0 gene activation system in tomato roots for enhancing resistance against plant-parasitic nematodes

Karam Mostafa[1,2,*], Mohamed Farah Abdulla[1], Musa Kavas[1]

[1]Department of Agricultural Biotechnology, Faculty of Agriculture, Ondokuz Mayis University, Samsun 55270, Turkey.
[2]The Central Laboratory for Date Palm Research and Development, Agricultural Research Center (ARC), Giza 12619, Egypt
*E-mail: karamkrush1@gmail.com

Plant-parasitic nematodes (PPNs) pose a substantial economic burden on a wide range of plants and crops globally. To combat their detrimental effects, plants release a complex mixture of water-soluble and volatile organic compounds (VOCs) into the soil, collectively called plant root exudate. This exudate is critical in inhibiting nematode reproduction and minimizing the damage inflicted by tomato-specific Meloidogyne incognita. In our study, we focused on enhancing the expression of multiple endogenous genes (FATA, FATB1, FATB2, and FATB3) responsible for synthesizing palmitic acid and its derivatives in the roots of Tomato plants. To achieve this, we utilized the CRISPR-ACT3.0 technology under root-specific promoters. To ensure efficient gene activation, we employ two single guide RNAs (sgRNAs) to target the promoter region of each gene. Subsequently, we assemble the multi-sgRNA cassette into a single vector to achieve a comprehensive gene activation approach. For assessing the efficiency and rapid evaluation of CRISPR-ACT3.0 vectors, we optimized in vitro A. rhizogenes-mediated hairy root transformation in Tomato plants. We evaluated various parameters, including *A. rhizogenes* strain, co-cultivation period, tomato genotype, and explant type. Our experiments demonstrated that the highest transformation efficiency of our target genes occurred in 10-day-old hypocotyls and cotyledons of cv. Bobcat, inoculated for 3 to 4 days by *A. rhizogenes* (ATCC-15834). After two weeks, we observed root growth originating from the hypocotyl, whereas hairy roots emerged from cotyledons after more than 4 weeks. The CRISPR-ACT3.0 system exhibited remarkable effectiveness in activating the targeted genes, resulting in gene expression levels ranging from 2.5- to 23.3-fold compared to the wild type. These findings provide compelling evidence for the efficacy of CRISPR-ACT3.0 systems in achieving multiplex gene activation, thereby enhancing the feasibility of implementing transcriptional activation in this economically important plant species. Furthermore, by augmenting the concentration of palmitic acid and its derivatives in the root exudates, it is anticipated that the repulsion and subsequent reduction of M. incognita damage can be achieved.

Oral presentation

Development of resistant sunflower lines to broomrape using CRISPR-CAS9

Kubilay Yildirim[1,*], İlkay Sevgen Küçük[1], Musa Kavas[2], Dragana Miladinović[3]

[1]Department of Molecular biology and Genetics, Ondokuz Mayıs University, Samsun, Turkey
[2]Department of Agricultural Biotechnology, Ondokuz Mayıs University, Samsun, Turkey
[3]Institute of Field and Vegetable Crops, Novi Sad, Serbia
*E-mail: kubilay.yildirim@omu.edu.tr

Sunflower is one of the most important oil crops in the world that become a strategic plant due to the increased demand for its oil in recent years. Sunflower has low climate demand that enables it to grow in many regions of Europe. The biggest problem in sunflower cultivation is the presence of parasitic plants called broomrape (*O. cumana*). The seeds of these non-photosynthetic parasitic plants germinate with the secretion of Sesquiterpene Lactones (STL) from the roots of the sunflower. After attachment to the roots of the sunflower, it absorbs the water and all the nutrients from the host. Just one broomrape plant can produce millions of tiny seeds that can survive more than 15 years in the soil and can contaminate many fields in a region. Classical herbicides and mechanical techniques are not effective on these parasitic plants, since it already causes great damage to the plant when it rises above the ground. Many sunflower lines resistant to broomrape have been developed in last decades. However, the resistance of these lines was broken by emergence of new virulent broomrape strains. In recent years, secretion of Sesquiterpene Lactones (STLs) from sunflower roots has been found to trigger the germination of broomrape seeds. The genes encoding the enzymes (HaGAS, HaGAO, HaG8H, HaCOS) functional in STL biosynthesis in sunflower have been well characterized in recent years. In the light of all these information, genes of the enzymes that catalyze the production STLS was aimed to knockout with CRISPR/Cas9 technique in the project. It has been hypothesized that mutant sunflower lines developed in this way will have full resistance to broomrape. The sequences of four genes were retrieved from the database and processed with CRISPR-P 2.0 software to find out the best guide RNAs (gRNAs) that can target exon parts of the genes. By this way, four best gRNAs (one gRNA for each gene) were selected for simultaneous targeting of the first exon of the genes. All gRNAs were then transferred into a Cas9 containing agrobacterium plasmid (pHSE401) by using golden gate cloning. gRNA/Cas9 containing Agrobacterium (Gv3101) strains was treated to the seed, cotyledon and hypocotyl parts of the sunflower genotype. Tissue culture-based regeneration process has been established and first transgenic candidate seedlings were obtained in the current study. After obtaining fully regenerated mutants (T0) sunflower lines, transgenic ones will be selected with PCR and sequencing test. STL level in the roots of mutant line will be determined broomrape germination test will be applied to select the resistant genotypes. This is the first study developing broomrape resistant sunflower genotypes by using CRISPR genome editing system. Optimization of CRISPR mediated gene transfer and regeneration protocol will fasten and made important contribution to sunflower breeding. Genome editing-based strategies used to enhance crop resistance to parasitic weeds and its prospective applications will be discussed in the congress.

Funding: The project was supported financially by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with a project number; TOVAG-122O340 and the Cost Action-CA18111 with STSM.

Oral presentation

Exploring the role of *SnRK2* genes in salinity stress response of *Petunia axillaris* through CRISPR-based genome editing

Sara Yasemin[1,2,*], Katrijn Van Laere[2], Tom Ruttink[2], Tom Eeckhout[2]

[1]University of Siirt, Faculty of Agriculture, Department of Horticulture, Kezer, Siirt, Turkey
[2]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Melle, Belgium
*E-mail: sarayasemin1988@gmail.com

Salinity stress in plants results in cellular and genetic alterations, as well as physiological challenges, including diminished photosynthesis, membrane and enzyme damage, and metabolic disruptions. The salt stress also has a negative impact on both the growth and market value of Petunia plants. The plant response to salinity stress involves a complex cascade of reactions, with abscisic acid (ABA) playing a crucial role as a signaling molecule that assists in maintaining cellular regulation and dehydration tolerance, thereby balancing water levels. The ABA signalling pathway involves ABA receptors, such as SnRK2s and PP2Cs, where the activation of SnRK2 stimulates the expression of stress-tolerant genes through downstream signalling.

In this study, a CRISPR/Cas9 genome editing workflow was employed for *Petunia axillaris* to investigate the effects of SnRK2 genes on stress tolerance. Protoplast transfection mediated by polyethylene glycol (PEG) was optimized for the delivery of CRISPR/Cas9 elements, resulting in a transfection efficiency between 27.4% and 35.5%. To design targets, first a phylogenetic tree was constructed for SnRK2 genes from *P. axillaris* and Arabidopsis thaliana using the orthologous gene family information retrieved from the PLAZA Comparative Genomics platform, followed by manual curation of structural gene models using RNA-Seq data. Based on this, three SnRK2-related genes were selected as target genes for CRISPR/Cas, namely Peaxi162Scf00016g01538, Peaxi162Scf00684g00579, and Peaxi162Scf00993g00343. For these three genes, 45 guide RNAs (gRNAs), both in the gene promotors and in the CDS gene sequences, were designed with the SMAP design module of the SMAP package. The optimal gRNAs were selected and efficient amplification was confirmed through amplicon sequencing of 28 amplicons. Selected gRNAs were cloned into the Cas9 vector and delivered into protoplast cells using PEG aiming to create knock out mutations enabling to assess the involvement of the selected genes in the pathway and evaluate the efficiency of the gRNAs.

In our further research, overexpression of the targeted genes is aimed by using the gRNAs targeting the promoter regions introduced in a vector containing dCas9 fused to a promotor activator and deliver this in Petunia cells through Agrobacterium-mediated transformation. By this salinity stress tolerance in Petunia could be increased.

Poster presentations

Poster presentations in-person (PP) and online (PO)

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

PP01

Optimization of gene editing in *Lactuca sativa*

Ainhoa Morillas*, Pilar Cubas

Plant Molecular Genetics Department, National Centre of Biotechnology, Madrid, Spain
*E-mail: amorillas@cnb.csic.es

New genomic techniques (NGTs) provide new opportunities to alter in a specific manner the genetic material of plants allowing the rapid development of crop varieties with improved characteristics. In particular, targeted mutagenesis mediated by CRIPSR/Cas9 is increasingly popular due to its precision, effectiveness, programmability, and relative ease of use.
Lettuce is an important cultivar in Spain. Due to the climate change and the rising temperatures the adaptation of some of its cultivars to the changing environmental conditions can be compromised thus reducing yield. In close collaboration with a Spanish seed company we are optimizing transgenic mediated and DNA-free (which does not require insertion of exogenous DNA) CRISPR-Cas9 gene editing in two lettuce ecotypes for which no protocol has been published yet. For this we are developing protocols to generate transgenic lettuce plants of these varieties and we are also generating lettuce protoplasts, and performing protoplast lipofection of Cas9-gRNAs RNP complexes. The optimization steps of these protocols will be presented.

PP02

Improvement of drought stress tolerance in poplars (*Populus*) by modification of candidate genes

A. Fendel*, M. Fladung, T. Bruegmann

Thünen Institute of Forest Genetics, Genetic Technologies, Grosshansdorf, Germany
*E-mail: alexander.fendel@thuenen.de

Climate change and associated drought scenarios threaten the tree production of European forest trees. The adaptability of plant species to such environments is strongly dependent on the genetic information and genetic regulations, controlling morphological, physiological and biochemical adaptive mechanisms. However, the knowledge of underlying genes in forest tree species is still scarce. To estimate the impacts of single genes on the drought stress tolerance in trees, we selected several candidate genes in the poplar hybrid *Populus × canescens* (grey poplar) and genetically modified the genes by CRISPR/Cas generated loss-of-function mutations in the model clone INRA 717-1B4. The set of candidate genes comprises genes potentially involved in abscisic acid metabolism or stomatal development. The analysis of genome-edited poplars revealed several editing patterns and allow conclusions on the gRNA efficiencies, probably based on differences in the secondary structure. Edited lines were *in vitro*-propagated and transferred to drought stress experiments in the greenhouse, where several morphological, physiological and biochemical analyses aim to reveal differences in the drought stress tolerance between edited and wildtype lines. Based on preliminary results from the experiments, the impact of the respective edited candidate gene on drought stress tolerance is to be determined. The approach of this study contributes to the understanding of the natural genetic potential in poplar regarding their drought stress tolerance and will allow us to reveal genetic mechanisms important for the consideration of further forest tree species and breeding purposes. Accordingly, gained insights are planned to be transferred to the European beech (*Fagus sylvatica*) as it is prone to drought scenarios but indispensable for the ecosystem of Central European forests.

PP03

CRISPR/Cas-mediated efficient knock down of vacuolar invertase gene to address cold sweetening in potatoes

Aneela Yasmeen, Allah Bakhsh*, Abdul Qayyum Rao

Centre of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Bank Road Lahore-53700, Pakistan

*E-mail: abthebest@gmail.com

We have attempted to knock down vacuolar invertase (*VInv)* gene in local cultivar of potato plants using CRISPR-Cas9 genome editing approach. The primary transformants were screened through PCR, Sanger sequencing, digital PCR, and ELISA. The amplicon sequencing data showed maximum indel frequency for potato plant T12 (14.3%) resulting in gene knock out and 6% frame shift. While for plant B4 the maximum indel frequency of 2.0% was found which resulted in 4.4% knock out and 4% frameshift. The qRT-PCR data revealed that mRNA expression of *VInv* gene was reduced up to 90 % in edited potato plants when compared to the non-edited control potato. Following cold storage, chips analysis of potatoes proved B4 and T12 as best lines. Reducing sugars analysis by titration method determined fivefold reduction in reducing sugars in tubers of B4 lines compared to the control. Physiologically genome edited potatoes behaved like their conventional counterpart.

PP04

Use of prime editing to mutate the thiol reductase (TR) activity of AtYUCCA 6 in *Arabidopsis Thaliana*

Amna Faryad*, Christa Kanstrup, Elizabeth Heather Jakobsen Neilson, Meike Burow

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
*E-mail: af@plen.ku.dk

The important YUCCA gene family encodes flavin-containing monooxygenases (FMO) that catalyze the rate-limiting and irreversible oxidative decarboxylation of indole-3-pyruvic acid (IPA), to form indole-3-acetic acid (IAA), also known as auxin. Auxin is an essential hormone that orchestrates virtually every aspect of plant development. Furthermore, YUCCAs are involved in many biotic and abiotic stress responses, including herbivory, drought, and metal toxicity. Taken together, YUCCAs are critically important for plant growth, performance, and resistance under a wide range of environmental conditions. A keynote study in 2015 made the novel discovery that AtYUCCA6 also possesses additional thiol (TR) activity, independent of IAA functionality. This TR activity conferred with holdase chaperone properties, enhanced peroxidase activity, and improved ROS scavenging; ultimately increasing drought tolerance independent of IAA biosynthesis. Moreover, they have found a “CELP” Motif which is conserved in all 11 Arabidopsis YUCCAs. Specifically, it has been investigated that this Cysteine (C) amino acid in the CELP region is responsible for TR activity. However, the potential of this moonlighting function of AtYUCCA6 has not been researched for other abiotic stresses including heat, cold, and salinity. This study will focus on the role of TR activity for other stresses as well.
To create the AtYUCCA6 mutants with altered TR region, we have used Prime editing. Introduced in 2019 by Anzalone et al, this search and replace genome editing technique has the potential of mediating all possible base conversions and combinations. The most interesting part is that it does not need double-stranded breaks (DSBs). However, it requires a longer guide RNA (compared to the CRISPR/Cas9 approach) known as prime editing gRNA (PegRNA) which carries, primer binding sites, RT region, edit site, and RNA scaffold. In this study, we have used nickase Cas9 which has one mutation for nicking enabling it to do one-strand nicking, fused with an engineered reverse transcriptase enzyme. We have created two prime editing constructs based on two experimental approaches (one with only one PegRNA while the strategy involved one PegRNA and an extra gRNA to do the nicking of the WT strand) to compare the efficiency of both in Arabidopsis. These constructs were transformed in Arabidopsis plants. Following the transformation, the transformed seeds were screened based on the expression of red fluorescence protein in the seed coat.

PP05

Multiplex genome editing and trait improvement for complex multicrop systems

Ana Marjanović Jeromela*, Ankica Kondić Špika, Dragana Rajković, Dragana Miladinović

Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia
*E-mail: ana.jeromela@ifvcns.ns.ac.rs

Breeding for diverse crop rotations involves selecting objectives that often overlap with current breeding programs. However, in the context of diverse crop rotations, a new target population of environments emerges, necessitating selection strategies to maximize genetic gain. The primary breeding goal remains productivity, but additional factors specific to diverse crop rotations, such as yield stability, postharvest quality, and traits associated with disease and insect pests, need to be considered. Occasionally, crops are bred for unexplored regions or novel crops are developed, such as perennial grain crops like intermediate wheatgrass, winter oilseeds like pennycress, and cover crops like hairy vetch. In these cases, breeding efforts may focus on crucial domestication traits such as seed shattering and harvestability.

Improving performance in a multicrop system can enhance breeding efficiency by identifying traits that can be observed in monoculture. Breeders can then select for these traits in multicrop systems without establishing a dedicated multicrop nursery, known as a "trait-informed approach." This approach proves particularly useful when heritability is low due to environmental heterogeneity. However, in the absence of highly correlated and observable traits, direct selection in multicrop systems becomes necessary to achieve optimal genetic gain. As high-throughput phenotyping technology advances, breeding programs can improve efficiency even when additional nurseries and trials are required. Collaboration with engineers to develop high-throughput phenotyping platforms suitable for complex multicrop systems presents further opportunities.

Complex traits like yield and disease tolerance often require edits in multiple independent loci. Multiplex genome editing enables the simultaneous modification of multiple, related or unrelated, loci within a single cell and subsequently within a single regenerated plant. Some of these loci may have negative impact on other important traits and it make this technology difficult for application. For example, reducing seed glucosinolates in Brassica plants can be achieved through mutation of glucosinolate transporter genes (GTRs). Previously created low seed glucosinolate germplasms in B. napus using gene-editing cannot be utilized for breeding due to their negative effects on other traits, possibly caused by the involvement of edited genes in other trait formation. However, in 2018 researchers demonstrated that CRISPR/Cas9-mediated mutations of three homologous BnWRKY70 genes in rapeseed increased resistance against S. sclerotiorum, offering theoretical guidance and germplasm resources for developing rapeseed varieties with high resistance to S. sclerotinia and proving the concept that multiplex genome editing is useful not only for scientific reasons but also for application in breeding.

Acknowledgements: This work is part of the project supported by Ministry of Education, Science and Technological Development of Republic of Serbia, grant number 451-03-47/2023-01/200032**,** and COST Action CA18111.

PP06

Genome editing technologies for engineering the phytonutrient content in tomato

Aurelia Scarano, Angelo Santino

Institute of Science of Food Production, C.N.R. Unit of Lecce, 73100 Lecce, Italy
E-mail: aurelia.scarano@ispa.cnr.it; angelo.santino@ispa.cnr.it

The application of precise genome editing represents an important step-forward in plant functional genomics research and crop improvement by generating tailored modifications within a target genome sequence. Among the genome editing technologies, the CRISPR/Cas9 system has been the most largely one applied in many crop species, thanks to its high customizable specificity. In recent years, the number of studies on the genome editing application in tomato has increasingly grown, particularly for the improvement of fruit quality and nutritional value. Tomato fruit is a good source of lycopene, β-carotene, phenolic compounds, and micronutrients, with most of these phytonutrients playing an important role in human nutrition. Therefore, several structural genes or transcription factors regulating different metabolic pathways can be interesting targets for genome editing.
For instance, several genes within the carotenoid pathway, such as SGR1, LCY-E, Blc, LCY-B1 and LCY-B2 genes (stay-green 1, lycopene δ-cyclase, β-lycopene cyclase, lycopene β-cyclase 1 and 2, respectively), implicated in lycopene content improvement have been taken into consideration.
A similar approach has also been applied to the polyphenol biosynthetic pathway, to edit transcription factors (SlAN2, SlMYB12) related to polyphenols biosynthesis and accumulation in vegetative tissues and fruits.
Highly efficient CRISPR/Cas9 systems have been also employed for metabolic engineering of other important micronutrients in tomato, such as provitamin D3 in tomato by targeting the 7-dehydrocholesterol reductase gene (7DR2).
The use of genome editing technologies offers an effective strategy to ameliorate agronomic and quality fruit traits bypassing the conventional breeding programs and transgenic approaches which pose some ethical problems in the public acceptance. Transgene-free genome editing provide a promising alternative to rapidly innovate germplasm and possibly introduce new traits for agrobiodiversity. Even though many progresses have been achieved in comparison to other crops (for example those recalcitrant to plant transformation), more efforts are still required to efficiently target other genes involved in tomato fruit quality and nutritional value, and to overcome the barrier of gene overexpression, system deliveries, and improve methods for a more rapid screening of mutant lines.

PP07

Identification of the Pectate Lyase gene family in *Vitis vinifera* and its role in the berry development

Bárbara Rojas[1,2,*], Jorge Lagrèze[1,2], Mickael Malnoy[2], Claudio Moser[2], Lorenza Dalla Costa[2], Giulia Malacarne[2]

[1]Center Agriculture Food Environment (C3A), University of Trento/Fondazione Edmund Mach, San Michele all'Adige (Trento), Italy
[2]Research and Innovation Center, Edmund Mach Foundation, San Michele all'Adige (Trento), Italy
*E-mail: barbara.rojassanmartin@fmach.it

Grapevine (*Vitis vinifera* L.) is one of the most commercially valuable fruit trees worldwide. Table grapes represent an important economic sector, where consumers highly appreciate the berry firmness trait. Although several studies have addressed the key role of the cell wall in fruit firmness, the main players among cell wall degrading enzymes during fruit ripening are still unclear. This work characterizes the grapevine Pectate Lyase (VvPL) gene family which catalyses the eliminative cleavage of de-esterified pectin during the berry development. Using the latest grapevine genome assembly and annotation, 17 members of the family containing the PL domain were identified. To identify the VvPL members most involved in pectin degradation during fruit softening, an in-silico analysis in Expression Atlas and in public RNA-Seq repositories was performed. Additionally, gene expression of the VvPL genes was evaluated in table grape varieties showing contrasting texture profiles. Our results demonstrated that specific VvPL genes were up-regulated in the softer variety compared to the firmer one, suggesting their active role in the softening process during berry development. Furthermore, two of the up-regulated VvPL genes were selected for functional characterization via genome editing with CRISPR/Cas9 technology in the table grape variety 'Sugraone'

PP08

CRISPR/Cas9 mediated resistance to Becurtovirus in sugar beet

Kubilay Yıldırım[1], Musa Kavas[2], Ilkay Sevgen Küçük[1], Zafer Seçgin[2], Çiğdem Gökçek Saraç[3]

[1]Ondokuz Mayıs University, Dep.of Molecular Biology and Genetics, Samsun, Turkey
[2]Ondokuz Mayıs University, Dep.of Agricultural Biotechnology, Samsun, Turkey
[3]Akdeniz University Faculty of Engineering Dep. of Biomedical Engineering, Antalya, Turkey
*E-mail: kubilay.yildirim@omu.edu.tr

Beet Curly Top Iran Virus (BCTIV, Becurtovirus) is a dominant and widespread pathogen responsible for great damage and yield reduction in sugar beet production in the Mediterranean and Middle East. CRISPR-based gene editing is a versatile tool that has been successfully used in plants to improve resistance against many viral pathogens. In this study, the efficiency of gRNA/Cas9 constructs targeting the expressed genes of BCTIV was assessed in sugar beet leaves by their transient expression. Almost all positive control sugar beets revealed systemic infection and severe disease symptoms (90%), with a great biomass reduction (68%) after BCTIV agroinoculation. On the other hand, sugar beets co-agronioculated with BCTIV and gRNA/Cas9 indicated much lower systemic infection (10–55%), disease symptoms and biomass reduction (13–45%). Viral inactivation was also verified by RCA and qPCR assays for gRNA/Cas9 treated sugar beets. PCR-RE digestion and sequencing assays confirmed the gRNA/Cas9-mediated INDEL mutations at the target sites of the BCTIV genome and represented high efficiencies (53–88%), especially for those targeting BCTIV's movement gene and its overlapping region between capsid and ssDNA regulator genes. A multiplex CRISPR approach was also tested. The most effective four gRNAs targeting all the genes of BCTIV were cloned into a Cas9-containing vector and agroinoculated into virus-infected sugar beet leaves. The results of this multiplex CRISPR system revealed almost complete viral resistance with inhibition of systemic infection and mutant escape. This is the first report of CRSIPR-mediated broad-spectrum resistance against Becurtovirus in sugar beet.

PP09

Generation of the barley why2 knock-out mutant

Entela Malkaj[1,*], Karin Krupinska[2], Götz Hensel[1,3]

[1]Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich Heine University, Dusseldorf, Germany

[2] Institute of Botany, Christian Albrecht University of Kiel, Kiel, Germany

[3]Division of Molecular Biology, Centre of the Region Hana for Biotechnological and Agriculture Research, Faculty of Science, Palacký University, Olomouc, Czech Republic

*E-mail: malkaj@uni-duesseldorf.de

WHIRLY2 belongs to a small family of single-stranded DNA-binding proteins together with WHIRLY1. Both these proteins are dual targeted. WHIRLY1 to the nucleus and chloroplasts, and WHIRLY2 to the nucleus and usually mitochondria. Using RNAi WHIRLY1 barley mutants, it has been found that WHIRLY1 was involved in stress response and chloroplast development. A barley WHIRLY1 knock-out mutant (why1) was generated using the CRISPR-Cas gene-editing technology to investigate its role further. The primary foliage leaves of the mutant seedlings were initially white/pale green but eventually became green foliage leaves and grains as they developed. Immunological analysis showed that WHIRLY2 accumulated in the chloroplast with increasing greenness of the why1 mutant leaves. and was present in its green leaves. These results lead to the hypothesis that WHIRLY2 of barley compensates for the absence of WHIRLY1. The barley why1 mutants complemented with the WHIRLY2 sequence driven by the promotor of WHIRLY1 either died or did not produce any grains. To further investigate the role of WHIRLY2, a barley WHIRLY2 knock-out mutant (why2) was also generated using the CRISPR-Cas gene-editing technology and Agrobacterium-mediated transformation. Four single guide RNA sequences were designed to target four different exons of the WHIRLY2 gene. The guides and the Cas9 cassette, were assembled into a construct that was used to transform immature barley embryos via Agrobacterium-mediated transformation. Out of 51 regenerants, 31 were transgenic. Eight of the regenerants had indels in one or two exons, whereas four regenerants had mutations in the gene's introns. The plants had no obvious phenotypes and will soon be harvested. Afterward, Cas-free homozygous M1 plants will be selected and molecularly and phenotypically characterised.

PP10

The "SMART-BREED" project: innovative molecular technologies for the adaptation of horticultural species to climate change through precision breeding

Fabio D'Orso[1,*], Giovanna Frugis[2], Floriana Augelletti[1], Simona Baima[1], Ilaria Brozzi[3], Monica Carabelli[3], Chiara D'Attilia[2], Alessandro Del Bufalo[2], Vanessa De Luca[2], Alberico Di Pinto[3], Valeria Chiatti[2], Marco Fabriani[2], Barbara Felici[1], Valentina Forte[1], Davide Gentile[2], Maria Adelaide Iannelli[2], Valentina Iori[2], Giorgio Morelli[1], Daniela Palma[1], Marco Possenti[1], Simone Samperna[2], Giovanna Sessa[3]

[1]CREA, Centre for Genomics and Bioinformatics, Unit of Rome, Italy
[2]CNR, Institute of Agricultural Biology and Biotechnology (IBBA), Unit of Rome, Italy
[3]CNR, Institute of Molecular Biology and Pathology (IBPM), Rome, Italy
*E-mail: fabio.dorso@crea.gov.it

Climate change poses an alarming threat to the agricultural system affecting the ecological balance of the planet, food security and human nutrition. Its impact on agriculture risks to neutralize all the domestication efforts exerted by the breeders to adapt the genetics of plant species to the cultivation environment, compromising the agricultural production. The SMART-BREED project aims to develop innovative molecular technologies to study the effects of genetic variability and use this information to accelerate the breeding of new resilient varieties that maintain their productive and typical characteristics in more variable and extreme environmental conditions.

Genetic diversity, in natural or domesticated populations, constitutes a source of allelic variants "tested" naturally in the field of evolution, and are therefore an important resource for genetic improvement. More than 60% of adaptive mutations are associated with genes encoding transcription factors (TF), which constitute the main target genes on which the process of adaptation acts in wild and domesticated plant populations.

As a "proof of concept", the project focuses on target transcription factors with a proven role in two agronomic traits that are important for the production and adaptation of horticultural species: flowering time and shade avoidance. Knowledge from Arabidopsis is being translated in *Lactuca sativa* as a model for Asteraceae and *Solanum lycopersicum* as a model for Solanaceae.

Three technological platforms (genetic diversity, genome editing and gene co-expression networks) are being integrated to design new molecular strategies of genetic improvement based on a candidate gene approach, that could potentially extend to any candidate TF and agronomical trait.

In this view, genome editing is strategic to develop new crop varieties adapted to the new climatic conditions. In the framework of SMART-BREED project, this platform is being applied to edit Arabidopsis, tomato and lettuce candidate genes but also to expand the CRISPR toolbox in terms of number Cas nucleases and precise gene editing approaches (geminivirus mediated gene-targeting and Prime Editing).

Acknowledgements: SMART-BREED project, POR FESR LAZIO 2014 – 2020, Gruppi di ricerca 2020" Det. n. G04052 del 04/04/2019, Codice Progetto A0375E0166, prot. n. A0375-2020-36510.

PP11

The application of CRISPR-Cas9 for the creation of multiple mutants in the genes coding for the nascent polypeptide associated complex

Jan Fíla[1,*], Božena Klodová[1,2], Petr Šesták[1,2], David Honys[1,2]

[1]Institute of Experimental Botany of the Czech Academy of Sciences, Laboratory of Pollen Biology, Rozvojová 263, Praha 6 – Lysolaje, 165 02, Czech Republic
[2]Charles University, Faculty of Science, Department of Experimental Plant Biology, Viničná 5, Praha 2, 128 00, Czech Republic. *E-mail: fila@ueb.cas.cz

Flower and fruit developments represent important processes of plant reproduction. Conventional Arabidopsis thaliana double homozygous mutant in the genes encoding for the β-subunit of the nascent polypeptide associated complex (that were acquired by the cross of the available T-DNA insertion lines) showed notable phenotypic defects in the flowers and siliques. Moreover, seeds of the double homozygous mutant showed a reduced germination efficiency under the salt and osmotic stress. The aim of this study was to employ modern genome editing techniques to acquire the multiple mutants in the genes coding for the subunits of the nascent polypeptide-associated complex (a quintuple mutant of genes encoding its α-subunit and a double mutant in genes encoding the β-subunit). The acquired mutants were characterized in more detail and highlighted the role of nascent polypeptide-associated complex during flower and silique developments of Arabidopsis thaliana. Then, both mutants were notably delayed in their development, and the nacα mutants showed also less chlorophyll in their leaves but the other photosynthetic parameters were not influenced.

Acknowledgments: The authors gratefully acknowledge the financial support from Czech Science Foundation (22-29717S, 23-07000S), and Mobility Plus Program with DAAD (DAAD-23-06).

PP12

Employing protein-interaction techniques to identify pathogen effectors to better devise targeted genome editing in plants

Jeny Jose[1,2]*, László Sági[1], Lauren Crean[3], Florence Danila[3], John Rathjen[3]

[1]Centre for Agricultural Research, Martonvásár, H-2462 Hungary
[2]Doctoral School of Plant Science, Hungarian University of Agriculture and Life Sciences, Gödöllő, H-2100 Hungary
[3]Research school of Biology, Australian National University, Canberra, 2601 Australia
*E-mail: jeny.jose@atk.hu

Plants resist pathogen attacks yet often succumb to diseases owing to the pathogen's ability to suppress immune responses. Pathogens secrete virulence effector molecules into the plant cell to aid their infection. Molecular genetic strategies aiming to counteract plant diseases must therefore include a thorough understanding of plant immunity proteins and their interactions with effectors. The host plasma membrane is such an interacting interface where numerous plant protein components contact effector molecules. Hence, characterizing this interface is of fundamental importance.
Plasmodesmata (PD) are nanopores in plant cell walls that mediate the exchange of metabolites and signaling molecules. They are targeted, exploited, and modified by pathogens like *Phytopthora* sp. for host invasion. Plasma membrane-localized effectors of *Pseudomonas syringae pv. tabaci* (*Pst*) interact with tobacco protein complexes to manipulate plant responses. This interaction has been studied at protein level with BioID techniques, where the target protein (bait) is fused to a mutant biotin ligase that labels interacting proteins (prey) with biotin. This *in-planta* technique is particularly effective in identifying new components in large complexes and cellular structures such as the PD.
I will use the model plant *Nicotiana benthamiana,* which allows transient transformation, infection with *Pst,* and facile extraction of leaf proteins, to identify host and pathogen proteins associated with the PD. As a bait, I will use a unique plasmodesmata-located protein 1 (PDLP1) fused to an engineered biotin ligase variant, TurboID. By comparing protein-protein interactions in infected and control tobacco cells, we expect to identify essential effectors in the tobacco-*Pst* interaction.
Progress in this work will pave the way for future applications in *Solanum tuberosum* against its important pathogens and validate effector interactions for the identification of new targets of gene editing.

Acknowledgments: This project has been fully financed by the PlantEd COST Action CA 18111 (STSM Grant No. c4837117)

PP13

Changes in the public perception of genetically modified organisms (GMOs) in Poland

Justyna Nowakowska*, Anna Linkiewicz

Institute of Biological Sciences, Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszynski University in Warsaw, Wóycickiego 1/3 Street, 01-938 Warsaw, Poland
*E-mail: j.nowakowska@uksw.edu.pl

We present a multi-year study and targeted survey of attitudes towards different uses of GMOs, level of knowledge and trust of the population in the authorities responsible for GMO safety, conducted among 1135 Polish citizens in 2017-2019. It has been shown that the knowledge of inhabitants about GMOs is growing, but the reported concern about genetically modified organisms is still present in more than half of the society. Most women are more afraid of GMO products, pay more attention to their labelling, but would not pass them on to their children. People under 20 have a positive attitude towards GMOs, while people over 35 show negative effects of consuming GMO products. The general awareness of GMOs increased with the education of the respondents. In a group of 75 selected respondents, who were mainly plant breeders and scientists, opinions in favour of GMOs were generally similar. The biggest disagreements within this group were on the environmental impact of GMOs, the nutritional value of food GM and food labelling requirements. This suggests that Poles are less concerned about GMOs compared to the earlier surveys by Nowakowska et al. (2021). Most people living in rural areas are still afraid of GMO products, and the opinion of urban dwellers is divided into two almost equal groups, both supporters and opponents of GMOs. The observed trend in the acceptance of GM in Polish society is in line with the results from Sweden (e.g. Spendrup et al., 2021) and the European Union (EFSA, 2019).

PP14

A rocky road to CRISPR - Dealing with in vitro recalcitrance in European beech (*Fagus sylvatica* L.)

Virginia Zahn*, Alice-Jeannine Sievers, Matthias Fladung, Tobias Brügmann

Thünen Institute of Forest Genetics, Grosshansdorf, Germany
*E-mail: virginia.zahn@thuenen.de

As the most common deciduous tree species in Central Europe, European beech (*Fagus sylvatica*) is an important component of European forest ecosystems and an important economic factor. Due to increasing drought, especially since 2018, *F. sylvatica* is suffering in European forests. As a result, numerous beech stands are threatened ("beech dieback"). Modern biotechnological methods such as genome editing with CRISPR/Cas can be used to investigate the genetic basis of tolerances to abiotic stress and thus support the selection of drought-tolerant beech.
To date, however, neither genome editing nor the previously required genetic transformation of F. sylvatica has been reported. This is mainly because F. sylvatica has been known to be recalcitrant to in vitro propagation and regeneration since the 1980s. Consequently, the road to CRISPR (in vitro regeneration, transformation, CRISPR/Cas components) must be followed from the very beginning. To obtain a stable in vitro line for propagation, regeneration, and transformation, seedlings of *F. sylvatica* were transferred to in vitro culture using shoots and shoot tips. Protocols were adapted for an in planta transformation approach to circumvent in vitro regeneration. For this purpose, in vitro shoot tip transfer was combined with needle perforation and vacuum infiltration with Agrobacterium tumefaciens GV30101-pMP90RK and EHA105 transferring a RUBY marker. Seedling leaves were used for protoplast isolation. Initial experiments on PEG-mediated transformation of protoplasts with plasmid-DNA were performed using a 35S::mEGFP reporter construct. Both transformation systems will be used for proof-of-principle CRISPR/Cas9 and ttCas12a gene editing tests in *F. sylvatica*.

PP15

Generation of a brc1 knock-out mutant using CRISPR-Cas9

Liping Tang*, Michael Nicolas, Pilar Cubas

Plant Molecular Genetics Department, National Centre of Biotechnology, Madrid, Spain
*E-mail: Harlequinnv@gmail.com

The intricate coordination of gene expression during animal and plant development relies on gene regulatory networks (GRNs), controlled by transcription factors (TFs) and other regulatory proteins. TFs acting as the master regulators of particular GRNs, bind to specific DNA sequences to either activate or repress gene expression in a cell-specific manner. In higher plants, the formation of branches, lateral shoots derived from axillary buds, is a key developmental program with significant impact on aboveground architecture. In Arabidopsis thaliana, BRANCHED1 (BRC1) that encodes a TEOSINTE BRANCHED1, CYCLOIDEA, PCF (TCP) [1] TF, acts as a suppressor of shoot branching and a master regulator of bud dormancy. Although the gene targets and GRNs controlled directly by BRC1 begin to be elucidated [2], many questions regarding BRC1 regulation and function are still unknown. For instance, very few interactors of the BRC1 protein have been characterized, and the mechanisms of how they could affect BRC1 activity are unclear. The TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN1 (TIE1) directly interacts and antagonizes BRC1 and thus regulates shoot branching [3]. In addition, FT-like proteins have been reported to display a functional in planta interaction with BRC1-like proteins in Arabidopsis [4], poplar [5] and potato [6]. The advent of biotin ligase-based proximity labeling (PL) technology has provided sensitivity and specificity to identify protein complexes and (local) proteomes on a cell-type-specific level. In PL, a genetically encoded biotin ligase (i.e. TurboID, [7]) is used to covalently attach biotin to nearby proteins (in a radius of less than 10 nm). In this study, our goal is to screen for the interactors of the BRC1 protein in axillary bud tissue, where BRC1 is expressed. However, to avoid competition with the endogenous BRC1 protein, it is desirable to use a total loss-of-function for BRC1 as a background to transform the pBRC1: BRC1: TurboID construct. However, so far only T-DNA insertional mutants and TILLING point mutants are available for this gene. We will describe our strategy to generate full knock-out mutants using CRISPR/Cas9 for BRC1 and our current results.

References:

1. Cubas P, et al. An epigenetic mutation responsible for natural variation in floral symmetry[J]. Nature, 1999, 401(6749): 157-161.
2. van Es S W, et al. A gene regulatory network critical for axillary bud dormancy directly controlled by Arabidopsis BRANCHED1[J]. Biorxiv, 2020: 2020.12. 14.394403.
3. Yang Y, et al. The TIE1 transcriptional repressor controls shoot branching by directly repressing BRANCHED1 in Arabidopsis[J]. PLoS Genetics, 2018, 14(3): e1007296.
4. Niwa M, , et al. BRANCHED1 interacts with FLOWERING LOCUS T to repress the floral transition of the axillary meristems in Arabidopsis[J]. The Plant Cell, 2013, 25(4): 1228-1242.
5. Maurya J P, et al. Branching regulator BRC1 mediates photoperiodic control of seasonal growth in hybrid aspen[J]. Current Biology, 2020, 30(1): 122-126. e2.
6. Nicolas M, et al. Spatial control of potato tuberization by the TCP transcription factor BRANCHED1b[J]. Nature Plants, 2022, 8(3): 281-294.
7. Branon T C, et al. Efficient proximity labeling in living cells and organisms with TurboID[J]. Nature biotechnology, 2018, 36(9): 880-887

PP16

New Plant Breeding Techniques to mitigate biotic stresses in grapevine

Loredana Moffa[1,*], Luca Nerva[1,2], Irene Perrone[2], Chiara Pagliarani[2], Riccardo Velasco[1], Giorgio Gambino[2], Walter Chitarra[1,2]

[1]Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano, Italy
[2]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino, Italy
*E-mail: loredana.moffa@crea.gov.it

In the current global context characterized by environmental change, New Plant Breeding Techniques (NPBTs) have emerged as a means to overcome the limitations associated with conventional breeding methods in enhancing plants' resistance to biotic and abiotic stresses. These techniques align with European Policies that promote chemical inputs reduction and a more sustainable approaches in agriculture. In our research, we implemented genome editing utilizing the CRISPR/Cas9 system in grapevine, focusing on specific disease susceptibility genes. To counteract powdery mildew, we targeted two genes, VvMLO7 and VvMLO6, belonging to the Mildew Locus O (MLO) family. Additionally, we directed our attention towards the Non-Expressed Protein 3 (NPR3), which plays a crucial role in defense responses against pathogenic biotrophic fungi, negatively impacting the accumulation of salicylic acid. Alongside genome editing, we employed cisgenesis to introduce the resistance locus RPV3-1 (Resistance to Plasmopara viticola) into economically significant grape cultivars, such as Chardonnay. This locus comprises two distinct genes, TNL2A and TNL2B, which were inserted together using their native promoters and terminators. To address a limitation associated with conventional Agrobacterium tumefaciens-mediated transformation, namely the incorporation of unrelated transgenes, we employed an inducible excision system based on Cre-Lox recombinase technology. This system is controlled by a heat-shock inducible promoter that will be activated upon confirmation of the transformation and/or editing event(s), facilitating the removal of CRISPR/Cas components and selection markers in both the genome editing and cisgenic approaches. Currently, the regenerated plants are undergoing scrutiny to assess editing efficiencies and the copy number inserted in the genome. The edited plants have entered the acclimatization phase and will be studied under laboratory conditions while awaiting the possibility of field evaluation.

PP17

CRISPR/Cas9 technology to reduce the levels of allergenic molecules in tomato fruit

M. Dellino[1,*], S. Presa[3], D. Orzaez [3], C. Montemurro[1], C. De Giovanni[1], M. M. Miazzi[1], A. Fiore[2], G. Diretto[2], F. Sevi[2], A. Granell[3].

[1]Department of Soil, Plant and Food Science (DiSSPA), University of Bari "Aldo Moro," 70125, Bari, Italy
[2]Italian National Agency for New Technologies, Energy, and Sustainable Development, Casaccia Research Centre, 00123, Rome, Italy
[3]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas, Universidad Politécnica de Valencia, Valencia, Spain
*E-mail: maria.dellino@uniba.it

Tomato is one of the most cultivated fruit crops worldwide and is considered an important part of the Mediterranean diet. Tomato fruit is rich in nutrients and contains various health-related compounds such as iron (Fe), calcium (Ca), vitamins, antioxidants, polyphenols, and carotenoids. However, while regular consumption of tomatoes may have health benefits, the fruit can also trigger the onset of allergies in some consumers. In fact, different studies indicated that the prevalence of tomato allergy is approximately 1.7% to 9.3% in different populations of Europe with an average of 4.9%. In this work, a genome editing technology known as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR- associated protein 9) was used to improve the qualitative aspect of tomato by reducing the level of allergenic proteins. In particular, the CRISPR/Cas9 system was used to induce mutations in the Solyc08g080640 and Solyc10g080210 genes, which encode respectively for the NP-24 protein, belonging to the Thaumatin-like protein (TLP) family and the Polygalacturonase 2a (PG2a) enzyme, two allergens present in tomato fruit.
Gene-specific sgRNAs were designed and constructed with low off-target scores. The DNA constructs were assembled using Goldenbraid, and components of the CRISPR-Cas9 system were delivered into plant cells via Agrobacterium-mediated transformation. The transformation was performed on cv Moneymaker tomato plants previously edited by the CRISPR/Cas9 system, which led to the silencing of two genes: the GAME4 gene, involved in the biosynthetic pathway of glycoalkaloids, and Sola l 4, one of the major tomato allergens. Regeneration of plant tissues rapidly occurred on selective media containing the antibiotic kanamycin, and Cas9 and sgRNA expression cassettes were stably integrated into the plant genome.
Finally, transformed or regenerated plants with the desired modifications were identified by polymerase chain reaction (PCR) genotyping and confirmed by sequencing. Eight edited plants for the TLP and PG2a genes were obtained by the CRISPR/Cas9 system and transferred to the greenhouse. Lines derived from those initial edited plants will be obtained and characterized by sequencing around the edited locus, off target and by detailed phenotypic evaluation (absence of the targeted gene expression) and susceptibility to biotic and abiotic stresses.
Eventually, our approach will allow us to obtain tomato cultivars with reduced levels of allergenic and anti-nutrient proteins, thus contributing to higher fruit quality. In addition, our results will show that it is possible to target and stack different genes using CRISPR/Cas9 genome editing.

PP18

Field trials in genome edited plants: a bibliometric approach

Melekşen Akın[1]*, Ankica Kondić-Špika[2], Dragana Miladinović[2], Agnès E. Ricroch[3], Marcel Kuntz[4]

[1]Department of Horticulture, Iğdır University, Iğdır 76 000, Turkey
[2]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia
[3]IDEST, Paris-Sud University, Sceaux, France
[4]Laboratory of Plant and Cell Physiology, University of Grenoble Alpes, CNRS, CEA, INRA, Grenoble, France
*E-mail: akinmeleksen@gmail.com

We have performed bibliometric analysis on field trials of genome edited plants utilizing Scopus and Web of Science core collections. All documents published until May, 2023 were extracted. The final bibliographic data consisted of 39 publications after merging both databases and removing duplicates. Bibliometrix package and Biblioshiny interface available in R studio were utilized to perform the bibliometric analysis. The documents were published in various sources (38) with diverse scopes. Frontiers in Plant Science and Plant Biotechnology Journal were the most active followed by Research Journal of Biotechnology. India and the USA were the leading countries on the topic. The Sankey plot demonstrated that besides publishing on genome editing and field trials, the USA was also publishing in CRISPR/Cas9, acrylamide and biosafety as sub-topics. India was also associated with acrylamide. Acrylamide as a keyword and sub-topic was published in Plant Biotechnology Journal based on our data collection. The most frequent keywords displayed with the word cloud following gene/genome editing and field trials were CRISPR, CRISPR/Cas9, genetic engineering, acrylamide, agronomic traits, biosafety, biotechnology, and crop productivity. Other notable keywords were gmo, hybrid poplar, lignin, metabolic engineering, regulation, abiotic stress, climate change, etc. Collaboration network analysis was run to demonstrate collaboration groups on the topic between countries, institutions and authors which resulted in three countries, 14 institutions and 12 author collaboration sub-networks with at least one collaborative paper. This research displays the progress of the field over time while projecting hot research topics and gaps in the literature, as well as hidden collaboration patterns among the scientific actors.

Acknowledgements: This article/publication is based upon work from COST Action "CA18111 PlantEd" (www.plantgenomeediting.eu), supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation (http://www.cost.eu).

PP19

CRISPR/Cas targeted inactivation of guaianolide oxalate formation in chicory

Milica Bogdanović*[1], Slađana Todorović[1], Katarina Ćuković[1], Jeroen van Arkel[2], Dirk Bosch[2], Katarina Cankar[2]

[1]Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia
[2]Wageningen Plant Research, Wageningen University and Research, 6708PB Wageningen, The Netherlands
*E-mail. milica.bogdanovic@ibiss.bg.ac.rs

Root chicory (Cichorium intybus var. sativum), an industrial crop species used for the production of a fructose polymer inulin, has been shown to contain a mixture of bitter tasting sesquiterpene lactones (STLs), that are currently discarded as waste. For several STLs found in plants of the Asteraceae family including chicory, interesting bioactivities have been demonstrated, including potent anti-cancer, anti-malarial, anti-inflammatory, anti-fungal and anti-bacterial activity. This activity is mainly attributed to guaianolide STLs; in chicory the most abundant STLs are lactucin, lactucopicrin, and 8-deoxylactucin, found predominantly in their oxalated forms in the latex of the plant. Several steps in the biosynthetic pathway of these compounds have been unraveled recently. However, the enzymes involved in the formation of STL oxalates, the most abundant form of STLs in chicory, have not yet been identified. Candidate genes for the chicory oxalate-CoA ligase (CiOxL) and chicory STL oxalyl transferases (CiOxT) putatively involved in the STL-oxalate formation were identified. Next, introduction of CRISPR/Cas reagents into chicory by Agrobacterium tumefaciens-mediated stable transformation was used to inactivate gene candidates putatively involved in STL-oxalate formation, and several chicory lines with edited genes were successfully regenerated. Detailed genotyping of mutant lines revealed the presence of indels leading to frame-shift predominantly, varying from 1 to 44 base pairs in length. Detailed genotyping also confirmed previous observations that plants transformed via Agrobacterium often showed chimerism, and a mixture of different on-target edits in one plant was observed. Leaves of plants carrying mutations in CiOxL or CiOxT were characterized by LC-MS to determine changes in terpene profile. The analysis showed that the production of STLs was reduced or eliminated in leaves of several CiOxL and CiOxT4 plants. Surprisingly, not only the oxalated terpenes were reduced but also the non-oxalated STLs, perhaps due to feedback regulation or toxicity of non-oxalated forms. These results contribute to further elucidation of the STL pathway in chicory and show that Agrobacterium-mediated plant transformation with CRISPR/Cas reagents requires detailed genotyping for characterization of genome edited plants.

Acknowledgements: This research has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 760891 [H2020-NMBP-BIOTEC-07-2017: New Plant Breeding Techniques (NPBT) in molecular farming: Multipurpose crops for industrial bioproducts]. Ministry of Science, Technological Development and Innovations of the Republic of Serbia, grant number 451-03-47/2023-01/ 200007 is also acknowledged.

PP20

Efficient gene editing in tomato facilitated by RNA virus vector systems

Mireia Uranga[1,*], Verónica Aragonés[1], Fabio Pasin[1], Arcadio García[1], Sophie Mirabel[1], Silvia Gianoglio[1], Silvia Presa[1], Antonio Granell[1], José-Antonio Daròs[1]

[1]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas - Universitat Politècnica de València, Avenida de los Naranjos s/n, 46022 Valencia, Spain
*E-mail: miurrui@alumni.upv.es

Precise genome editing based on CRISPR-Cas systems provides excellent opportunities in plant functional genomics and crop improvement. Conventional approaches for delivering CRISPR-Cas reagents in plants rely on genetic transformation or transient delivery to protoplasts, both of which are time-consuming, laborious, and challenging in many crop species showing recalcitrant regeneration. Plant RNA virus-derived vectors are an attractive alternative for the transient delivery of guide RNAs into adult plants because the virus capacity for genome amplification and systemic movement results in fast and efficient genome editing [1]. We previously engineered potato virus X (PVX) and tobacco rattle virus (TRV) to express guide RNA arrays that resulted in efficient multiplex, heritable editing in Nicotiana benthamiana [2-4]. PVX and TRV infect a wide range of host species, including important crops in the family Solanaceae. This work demonstrates that the PVX-gRNA system can be used for genome editing in Cas9-expressing tomato. The PVX-based vector efficiently delivered gRNAs targeting the two endogenous genes phytoene desaturase (SlPDS) and stay-green (SlSGR), producing ~30% indels in the corresponding genes in cells of systemic leaves. Moreover, plants showing a high rate of heritable biallelic edits were regenerated from infected tissues. Our preliminary data show that use of a TRV-based vector for SlPDS mutagenesis resulted in plant photobleaching and ~80% indel production in systemic leaves. In conclusion, our results highlight the utility of our viral vector systems for functional genomics studies and precision breeding in tomato, and eventually in all main Solanaceous crops.

References:

1. Uranga, M.; Daròs, J.-A. Plant Genome 2022, e20220. https://doi.org/10.1002/tpg2.20220.
2. Uranga, M.; Aragonés, V.; Selma, S.; Vázquez-Vilar, M.; Orzáez, D.; Daròs, J.-A. Plant J. 2021, 106 (2), 555–565. https://doi.org/10.1111/tpj.15164.
3. Aragonés, V.; Aliaga, F.; Pasin, F.; Daròs, J.-A. Biotechnol. J. 2022, 17 (7), e2100504. https://doi.org/10.1002/biot.202100504.
4. Uranga, M.; Aragonés, V.; Daròs, J.-A.; Pasin, F. STAR Protoc 2023, 4 (1), 102091. https://doi.org/10.1016/j.xpro.2023.102091.

PP21

CRISPR/Cas9-mediated genome editing in petunia plants: enhanced plant architecture with compactness and flower abundance

Mohamed Farah Abdulla[1], Karam Mostafa[1,2], Musa Kavas[1]*

[1]Ondokuz Mayis University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55200, Samsun, Turkey

[2]The Central Laboratory for Date Palm Research and Development, Agricultural Research Center (ARC), Giza 12619, Egypt

*E-mail: musa.kavas@omu.edu.tr

Petunia plants are renowned ornamental species widely cultivated as pot plants for their aesthetic appeal indoors and outdoors. The preference for pot plants depends on their compact growth habit and abundant flowering. While genome editing has gained significant popularity in plants for addressing abiotic and biotic stress factors, relatively less emphasis has been placed on its application in ornamental plant species. In this groundbreaking study, we aimed to optimize the procedure for genome editing in petunia plants using the highly efficient Multiplexed CRISPR/Cas9 system. Specifically, we targeted two genes associated with plant architecture traits: internode length, early flowering, number of flowers, and plant height. We successfully induced substitution, insertion, and deletions in the targeted genes through precise genome editing, resulting in significant phenotypic alterations in petunia plants. Notably, the plants with the edited SHORT PEDICLE gene exhibited a visibly early flowering time compared to the wild-type counterparts. Furthermore, mutants with alterations in the SELF-PRUNING 5g gene demonstrated shorter internodes than wild-type ones, creating a more compact and aesthetically appealing phenotype. This study represents the first successful endeavor to produce compact petunia plants with increased flower abundance through genome editing techniques. Our approach holds immense promise to improve economically significant petunia plants and serve as a potential foundation for further investigations into similar ornamental plant species.

PP22

Development of drought-resistant tomato plants by knocking out the AHP genes

Abdullah Aydın, Seher Yerlikaya, Bayram Ali Yerlikaya, Nisanur Yılmaz, Musa Kavas*

Ondokuz Mayıs University, Department of Agricultural Biotechnology, Atakum, Samsun, 55200, Turkey
*E-mail: musa.kavas@omu.edu.tr

Tomato (*Solanum lycopersicum*) is an important nutrient for human health. There are important biotic and abiotic stress factors for tomato, which is widely produced worldwide. These stress factors affect the yield and, in some cases, even lead to the death of the plant. In this study, it was aimed to develop tomato plants resistant to drought stress that are expected to increase as a result of climate change. For this purpose, Solc01g080540 (AHP1) and Solyc06g084410 (AHP2) genes, which are homologues of AHP genes previously known to provide drought tolerance by silencing in Arabidopsis thaliana, were selected and these genes were silenced in tomato plants using CRISPR/Cas9 genome editing technique. A total of 4 gRNAs were used to silence these genes, and a total of 20 candidate genome-edited tomato plants were obtained as a result of gene transfer. Genome-edited plants were determined by the evaluation of Sanger sequencing results with the Synthego ICE program. Using the seeds from seven genome-edited lines, drought and germination treatments were performed and the results were compared with those of plants without genome editing. As a result, it was observed that AHP plants were better able to sustain their development under drought conditions compared to plants without genome editing, and even survived in conditions where plants without genome editing could not survive. Additionally, It has been observed that genome-edited plants have a more developed root system while having fewer stomata.

PP23

Improving resistance to pod shattering in canola

Nay Chi Khin[1,2,*], Barry Pogson[1], Harsh Raman[2]

[1]Research School of Biology, The Australian National University, Canberra, Australia
[2]NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, Australia
E-mail: naychi.khin@anu.edu.au

Canola is a significant global oilseed crop and faces challenges that lead to seed loss and diminished overall yield. One significant challenge is premature pod shattering. Extensive efforts have been undertaken to understand the genetic basis of pod shatter resistance in canola. Previous studies, including genome-wide association studies involving crossbred populations of canola species, have provided valuable insights into the natural genetic variations that underlie resistance to pod shattering. These studies have identified specific loci associated with pod shatter resistance, highlighting their crucial role as key genetic factors in conferring resistance.

The current focus is on targeting these identified loci and introducing them into susceptible canola varieties to validate their significance and enhance the resistance of vulnerable lines. To introduce the candidate loci associated with pod shatter resistance, agrobacterium-mediated transformation via hypocotyl segments will be employed to insert the desired genetic elements into the canola genome. Additionally, multiple candidate loci will be assessed for their individual additive and digenic effects, providing a deeper understanding of the molecular mechanisms that control the resistance phenotype and shedding light on the complex interactions between different loci.

In addition to the targeted introduction of candidate resistance genes, haploid induction techniques will be utilised to expedite the development of homozygous pod shatter-resistant canola varieties. Haploid induction involves generating haploid lines, which can then be converted into fertile doubled haploid lines by doubling the chromosome number through natural or artificial means, such as the use of anti-microtubule drugs like colchicine.

The comprehensive exploration of different loci and their interactions, along with the targeted introduction of resistance alleles and the application of haploid induction techniques, presents a valuable toolkit for the development of pod shatter-resistant canola varieties. This research not only deepens our understanding of the genetic basis of pod shatter resistance but also holds the potential to revolutionize canola cultivation by enabling the implementation of more efficient and sustainable crop improvement methods.

PP24

Genome editing of VvPDS gene by using biolistics on grapevine (*Vitis vinifera* L.) somatic embryos

T. Mamić, F. Španić, D. Leljak-Levanić, N. Malenica*

Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Croatia
*E-mail: nenad.malenica@gmail.com

Grapevine cultivation is a worldwide industry which is known for its high pesticides inputs. Due to various climatic and economic pressures, conventional breeding for obtaining new cultivars is not cost-effective nor time-efficient. Therefore, there is a need to improve existing varieties by genetic engineering in terms of better biotic/abiotic stress tolerance and a decrease in pesticide application. Genetic manipulations are difficult in woody plants like grapevine due to its high heterozygosity and a long generation time. In this study, we used biolistic-mediated targeted mutagenesis of the reporter gene VvPDS in grapevine somatic embryos using a single CRISPR-Cas9 vector. We demonstrate the necessity of the initial genotyping of the targeted genomic region which may hinder biallelic targeting due to its heterozygosity. Chimeric plants were regenerated from grapevine somatic embryos after particle bombardment. We detected sequence alterations in the targeted region, although not in a homozygous state which would result in visible white sectors. Also, we tested vacuum agroinfiltration on young grapevine leaves to preliminary validate the constructed binary vectors.

PP25

Genome editing in perennial ryegrass: towards improving abiotic stress tolerance for safe and sustainable food systems (EditGrass4food)

Nils Rostoks[1,*], Anete Borodušķe[1], Ferenz Sustek Sánchez[2], Mallikarjuna Rao Kovi[3], Odd Arne Rognli[3], Cecilia Sarmiento[2], Kristina Jaškūnė[4]

[1]Faculty of Biology, University of Latvia, Riga, LV-1004, Latvia
[2]Department of Chemistry and Biotechnology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia
[3]Faculty of Biosciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[4]Laboratory of Genetics and Physiology, Lithuanian Research Centre for Agriculture and Forestry, Stoties 2, Akademija, 58343 Kedainiai distr., Lithuania
*E-mail: nrostoks@lu.lv

Due to increasing consumption of food, feed, and fuel, and to meet global food security needs for rapidly growing populations, there is a need to grow high-yielding crops that can adapt to future climate changes. Perennial ryegrass (*Lolium perenne* L.) is the predominant forage grass species in Europe because it grows back quickly, establishes rapidly, tolerates frequent mowing and grazing, and has high nutritional value for ruminants. However, perennial ryegrass performs poorly under unfavorable environmental conditions compared to other cool-season forage grasses, making climate change a major challenge for the cultivation of perennial ryegrass in the Baltic-Nordic region. The EditGras4Food project aims to improve perennial ryegrass in terms of winter hardiness, persistence, and biomass production under water limiting conditions. By improving forage production, the dairy and meat industries will directly benefit, so this project contributes to safe and sustainable food systems. The EditGrass4Food project uses unique pre-breeding material developed under the Nordic-Baltic public-private partnership project along with ecotypes of European origin and CRISPR/Cas9 gene editing to validate candidate genes involved in abiotic stress tolerance. Here, we focus on the two-candidate gene, LpCPB20 and LpCBP60g, which are associated with drought tolerance and improved plant immunity under elevated growth temperature, respectively. We will outline candidate gene identification in the perennial ryegrass genome sequences, development of CRISPR/Cas9 – sgRNA constructs, as well as validation of genome-editing efficiency in perennial ryegrass protoplast cultures.

Acknowledgements: This work was supported by the project "Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology – EditGrass4Food", ID No. EEA-RESEARCH-64, Contract No. EEZ/BPP/VIAA/2021/4 is financially supported by the European Economic Area (EEA) grants.

PP26

Targeted knockout of barley endosperm-specific storage proteins as a prerequisite for molecular farming purposes

Pouneh Pouramini[1,*], Robert Koller[2], Gregor Huber[2], Andreas Fischbach[2], Hildur Björg Birnisdóttir[3], Jón Már Björnsson[3], Philipp Westhoff[4], Goetz Hensel[5,6]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology, Seeland OT Gatersleben, Germany
[2]Forschungszentrum Jülich, IBG-2: Plant Sciences, Jülich, Germany
[3]ORF Genetics, Reykjavik, Iceland
[4]CEPLAS Metabolomics and Metabolism Laboratory, Institute of Plant Biochemistry, Heinrich-Heine-University, Dusseldorf, Germany
[5]Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich-Heine-University, Dusseldorf, Germany
[6]Division of Molecular Biology, Centre of the Region Hana for Biotechnological and Agriculture Research, Faculty of Science, Palacký University, Olomouc, Czech Republic
*E-mail: pouneh.pouramini@hhu.de

The concept for producing valuable proteins in plants is known as molecular farming. Besides transient expression using viral vector systems in tobacco leaves, the cereal grain is a natural bioreactor ideal for storing proteins under ambient conditions. Therefore, the cereal grain provides a cost-effective, easily scalable expression system for producing high-value proteins in the starchy endosperm.
To increase protein yield and overcome the competition between endogenous storage protein accumulation and the high-value protein, a targeted knockout of the Hordein B1 family members using Cas9 technology was performed. Targeted knockout of barley Hordein B1 family members was achieved, and Sanger and deep amplicon sequencing genotyped mutant plants. ELISA and Western blotting performed the abundance and quantification of the recombinant protein overexpressed in wildtype and mutant background. An automated phenotyping device evaluated the morphological changes of the mutant grains. Grains of altered morphology were used to determine if an altered amino acid composition accompanies the reduced protein content.
The recombinant human Epidermal Groth Factor (EGF) was overexpressed in wildtype and horb1 mutant background as a proof-of-concept. Grains of the resulting horb1 mutants showed altered biometric traits, reduced total protein and hordein content, and delayed germination behaviour compared to wild-type segregants. Western blotting and ELISA results confirmed a high EGF accumulation in the horb1 mutant background.
In conclusion, reducing endogenous storage protein accumulation successfully demonstrated the strategy of a higher abundance of a recombinant protein in barley grains. Further fine-tuning is required to minimize the germination disadvantage.

PP27

Using wheat transformation and gene editing strategies for wheat improvement

Sadiye Hayta

John Innes Centre, Department of Crop Genetics, Norwich Research Park, Norwich, Norfolk, NR4 7UH
E-mail: sadiye.hayta@jic.ac.uk

Despite wheat's global importance, it has trailed behind the other major cereals regarding genomic tools and resources as well as gene transformation. As a major staple crop, unrivalled in its geographic range of cultivation, wheat accounts for more than 20% of the protein and caloric intake of human diet worldwide. Plant breeders and scientists use crop diversity to develop new wheat germplasm. Recent major advances in genome editing (GE) and a step-change in wheat transformation efficiency, as well as cultivar flexibility, means that scientists are well placed to rise to these future challenges facing global wheat production. New plant breeding technologies (NPBTs) could contribute to increase crop yields and improve nutrition, pest management, resilience to climate change, and reduce postharvest losses. The timely arrival of UK legislation reducing the stringency of GE trial regulations places the UK agricultural research and development sector in a position to fully exploit these highly complementary breakthroughs. In this meeting I will discuss the transformation and gene editing strategies that are presently being applied for wheat improvement at JIC.

PP28

Mitochondrial genome editing in potato by mitoTALEN and mitoTALECD: induced mutations and phenotype of edited plants and vegetative progenies

Alessandro Nicolia[1], Nunzio D'Agostino[2], Rachele Tamburino[3], Giovanna Festa[1], Lorenza Sannino[3], Gaetano Aufiero[2], Rosa Paparo[3], Shin-Ichi Arimura[4], Nunzia Scotti[3], Teodoro Cardi[1,3,*]

[1]CREA, Research Centre for Vegetable and Ornamental Crops, Pontecagnano (SA), Italy
[2]Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy
[3]CNR-IBBR, Institute of Biosciences and Bioresources, Portici (NA), Italy
[4]Laboratory of Plant Molecular Genetics, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
*E-mail: teodoro.cardi@ibbr.cnr.it

Few and inefficient tools are available to study and exploit the molecular basis of nuclear-mitochondrial interactions sometimes affecting the development and performance of interspecific hybrids in crops. Hence, novel editing methods to induce mutations in higher plant chondriomes are highly desirable. The mitochondrial genomes of male sterile and male fertile *Solanum commersonii* (+) *S. tuberosum* interspecific somatic hybrids were fully sequenced and few candidate genes putatively involved in male sterility were identified. Due to the difficulties of importing gRNAs into cytoplasmic organelles, we used two TALE-based approaches (mitoTALEN and mitoTALECD) with the aim of mutating the region of the mitochondrial genome containing *orf125*, one of such candidate genes. A male sterile hybrid was stably transformed by *Agrobacterium*-mediated transformation to constitutively express two constructs with the *Fok*I nuclease (mitoTALEN), targeting two regions of *orf125,* and two with the DddA cytidine deaminase (mitoTALECD), inducing base editing in the same target regions. The N-terminal presequence of *Arabidopsis* mitochondrial ATPase delta-prime subunit was used to guide the expressed nuclear constructs into the mitochondria.
Short deletions (236 – 1066 bp) were induced in the target region by mitoTALEN, due to the repair of the induced double strand break (DSB) through recombination of short direct repeats (11 – 12 bp). In one case, induced DSB and subsequent repair resulted in the amplification of a substoichiometric molecule showing a 4288 bp deletion spanning the target sequence. With the mitoTALECD approach, base substitutions induced both missense and nonsense mutations, the latter leading to premature stop codons. Deletions and single nucleotide mutations were either homoplasmic or heteroplasmic, but only the former was generally retained in vegetative offspring. Plants with physical or functional knock-out of *orf125* showed no obvious alteration in plant growth and other vegetative traits. On the other hand, they displayed reversion to male fertility, strongly suggesting a role of *orf125* in nuclear-cytoplasmic interactions leading to male sterility in some potato interspecific hybrids. The fertility phenotype was stably maintained through vegetative tuber propagation. The involvement of *orf125* in the induction of male sterility was also confirmed by over-expression studies in which it was stably inserted into transgenic plants of a male fertile hybrid and its product expressed in the mitochondria. The possibility of inducing targeted mutations in plant chondriomes and plastomes by genome editing opens up exciting possibilities to study novel sources of variation and exploit them in plant genetics and breeding.

PP29

Genetic editing of CML genes in potato Solanum tuberosum

Tetiana Kyrpa[1,2]*, Csaba Éva[1], László Sági[1]

[1] Centre for Agricultural Research, Martonvásár, Hungary
[2] Institute of Cell Biology and Genetic Engineering of NASU, Kyiv, Ukraine
*E-mail: t-kirpa@ukr.net

The vast array of tools to generate or stimulate plant resistance to biotic stresses, such as bacteria, fungi, and viruses, is an important means for genetic improvement. However, using these tools in an open biogeocoenosis may break the natural interconnections between consumer, producer and reducer organisms. Therefore, it is preferable to induce or enhance the defence mechanisms in the crop plants. Such measures may include the (increased) expression or knockout of certain plant genes. We selected as candidates members of the CML (calmodulin-like) gene family. These gene may act both as positive and negative regulators of plant defence. Plant-specific CML genes play an important role in the perception and transduction of several types of environmental signals through the binding of the second messenger calcium ion. Knockouts of CML genes are predicted to reveal their specific physiological roles and may also lead to agronomically advantageous mutant phenotypes.
A literature review and bioinformatics analysis were performed to find target genes in potato for knockout by the CRISPR/Cas system. Two genes were found, the sequence of which was identified as potential targets. Two plasmid vectors were then constructed and transferred into the potato varieties 'Désirée'. At the present stage, mutant lines of potatoes were obtained, in which the knockout of the CML30 gene was proven. Currently, research is being conducted on the resistance of these plants to pathogen *Phytophthora infectans*.

Acknowledgements: The work was carried out with the support of a grant STSM of PlantEd Cost (CA18111).

PP30

Freedom to use and share regulatory data under the proposal for the EU revision of the New Genomic Techniques (NGT) legislation

Tomasz Zimny

Institute of Law Studies, Polish Academy of Sciences, Poland
E-mail: tzimny@inp.pan.pl

The poster presents a structured, systemic view of data protection instruments for products with different regulatory statuses (non-GMO, GMO, NGT 1-2, novel food etc.), as well as the consequences of the use of such instruments by the applicant, for other applicants and authorities, including control laboratories. Regulatory data is data submitted for different types of authorization processes. It comprises *inter alia* sequence information, information relevant for the risk assessment, information regarding detection or monitoring. Depending on the procedure and type, it enjoys protection, mostly through data exclusivity or confidentiality. If not for these, such data, obtained at the cost of one applicant, could be freely used by other parties in order to support their applications for the authorization or status confirmation of same or similar products. Regulatory data is also important for the development and use of efficient detection, identification and monitoring methods for regulated products.

The new EU proposal for the reform of the GMO legislation (COM(2023) 411 final) introduces new categories of GMOs (NGT type 1 and 2 plants). They shall be subjected to different regulatory conditions and procedures. Hence, the use and re-use of regulatory data will remain an important element of the notification and authorization processes. The current and proposed EU legislation on the placing of GMOs on the market contains provisions guaranteeing applicants protection of regulatory data (e.g. art. 31 of the Resolution 1829/2003/EC) and confidentiality of parts of the information submitted to the authorities (art. 30 of the Regulation 1829/2003/EC, art. 25 of the 2001/18/EC Directive, art. 11 of the proposal). For instance, the data protection provisions prevent (for 10 years) other applicants from reusing protected data in their own applications without the consent of the original applicant, unless the product falls under the novel food regulation, where the protection period is 5 years. The confidentiality provisions prevent authorities from revealing parts of the submitted dossiers. Similar provisions are also contained in the EU food law, as well as in the aforementioned EU proposal. The regulatory status of a given plant product ("classic" GMO, NGT-1 or 2) will influence the forms of protection available for regulatory data, hence also the freedom to re-use it by other parties.

The poster presents possible interruptions in data sharing and re-use, caused by regulatory data protection and confidentiality provisions for different types of NGT plant products under the proposed legislation.

PP31

CRISPR/Cas goes viral – viral replicons for gene targeting in poplar (*Populus* spp.)

Virginia Zahn*, Matthias Fladung, Tobias Brügmann

Thünen Institute of Forest Genetics, Grosshansdorf, Germany
*E-mail: virginia.zahn@thuenen.de

The genetic diversity of tree species offers the possibility of adaptation to changing environmental conditions as well as comprehensive insights in genetic interactions by comparing different genotypes. For genetic analyses, CRISPR/Cas-mediated gene knock-outs are well established in Populus, the model plant genus for tree genetics. CRISPR/Cas-based gene targeting (GT) can exploit more advanced applications, e.g., insertion, replacement or deletion of desired sequences. This editing technique is based on homology-directed repair (HDR), a rare DNA double-strand break (DSB) repair pathway. Therefore, GT mostly results in efficiencies below 1% to 5% while genome editing using non-homologous end joining (NHEJ) mostly results in efficiencies between 30 and 70%.
HDR efficiency is highly dependent on the accessibility of the DNA repair template, bearing the desired mutation (Donor-DNA). To enhance its accessibility, the Donor-DNA is placed within a viral replicon based on the African Cassava Mosaic Virus (ACMV). This enables the Donor-DNA to be proliferated extrachromosomal, when the viral replicase is expressed at the same time. The functionality of the ACMV replicon was tested in Nicotiana benthamiana as well as in *Populus × canescens*. For timed replicon proliferation, a GmHSP promoter was tested for its compatibility with a post-transformation heat treatment, which is expected to increase Cas activity and thus HDR efficiency simultaneously. To test GT in poplars, a transgenic GUS-expressing *Populus tremula* × *Populus tremuloides* was selected for insertion of a restriction enzyme site. To optimize GT in poplars, transgenic *P. × canescens* lines expressing both monomeric enhanced GFP (mEGFP) and temperature-tolerant (tt)LbCas12a or the D10A mutant nickase nCas9 were generated. Both Cas nucleases are compared in terms of their HDR efficiency with mEGFP as a target. For the conversion of mEGFP to mEYFP, a small but targeted amino acid exchange is sufficient so that the editing results can be easily validated. The gRNAs for both nucleases and targets were designed in silico. An out-of-frame (oof)GFP system for in vitro validation of gRNAs was tested in poplar protoplasts.

PO01

Protoplast applications in CRISPR/Cas9 genome editing to accelerate cabbage breeding

Ester Stajič

University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia
E-mail: ester.stajic@bf.uni-lj.si

Cabbage (*Brassica oleracea* var. capitata L.) is an important horticultural crop with many health benefits. Traditional breeding of cabbage is a time-consuming process because of its self-incompatibility and biennial growth cycle. Therefore, modern biotechnological methods are essential to overcome these limitations. Protoplasts are widely used in various fields of plant research. In genome editing, not only can they be used for rapid screening of genome editing reagents, but they can also be regenerated into plants. The aim of our study was to use cabbage protoplasts to investigate the effects of various parameters on genome editing efficiency and to improve the transient expression of CRISPR/Cas9 vectors. By optimising the protoplast regeneration protocol, we have explored the possibility of obtaining gene-edited cabbage plants. Our results will contribute to the development of new breeding strategies for cabbage and other brassicas.

Acknowledgements: This work was supported by the Slovenian Research Agency (postdoctoral research project Z4-3215 and research programme P4-0077).

PO02

Maximising pineapple production by controlling flowering time using CRISPR/Cas9

Ming Cheng[1,*], Yuri Trusov[1], Liu Guoquan[2], Yanfei Mao[3], Jimmy Botella[1]

[1]Plant Genetic Engineering Laboratory, School of Agriculture and Food Science, The University of Queensland, St Lucia, QLD, Australia
[2]Centre for Crop Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia
[3]Shanghai Institutes for Biological Sciences, Shanghai, China
*E-mail: ming.cheng1@uq.net.au

The pineapple (*Ananas comosus* (L.) Merr.) is a highly valued tropical fruit that ranks second in terms of harvesting and third in terms of consumption and international export. MD-2, the most successful hybrid from conventional pineapple breeding, was produced in 1973 and has since grown in popularity across the world, surpassing the previously dominant cultivar Smooth Cayenne. Precocious flowering is a big problem for modern day pineapple farming. It disturbs harvesting schedules, impacts all-year supply, and drives up market prices. It is established that flowering in pineapples can be triggered by a variety of environmental stimulants, including cold temperatures, shorter day, and mechanical disturbance. However, the mechanism of early flowering induction remains unclear. Stress-related hormone: ethylene, was found to be the most important regulator for pineapple flowering induction. From Yang's cycle, enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) controls the rate-limiting step in ethylene biosynthesis. It is demonstrated that silencing of the AcACS2 gene delays - pineapple flowering. We analysed several available pineapple genomes including those of MD-2 and identified seven AcACS genes grouped in four distinct clusters. To further understand the roles of the AcACS genes, we applied the CRISPR/Cas9 system to MD-2 pineapple. We optimized the protocol for pineapple transformation based on the existing literatures. We will establish a CRIPSR based gene knock out system using visual marker gene such as phytoene desaturase. We want to have a better understanding of the roles of AcACS genes at the end of this project. In addition, an enhanced pineapple line with controlled flowering can be developed.

PO03

Heavy metal (Cu, Zn) induced gene expression in *Brassica juncea*

Abdul Razaque Memon*, Nuriye Merakli

Department of Molecular Biology and Genetics, Faculty of Arts and Science, Usak University, Turkey
*E-mail: armemon@usak.edu.tr

Many plant species are well-documented as metal hyperaccumulators, most of which are restricted to metalliferous soils and are known as obligate hyperaccumulators. However, some other plant species are widely grown in metalliferous and non-metalliferous soils and accumulate metals when occurring in metalliferous habitats. These plant species are reported as facultative hyperaccumulators. This phenomenon of metal hyperaccumulation has profound implications in the field of phytoremediation. *Brassica juncea* is reported to accumulate several heavy metals, including Cu, Z, and Cd, in their shoots and is considered a hyperaccumulator plant. It has been a subject of extensive molecular biology and environmental science research. *B. juncea*, commonly known as Indian mustard or brown mustard, is a member of the Brassicaceae family and is considered a hyperaccumulator plant. Hyperaccumulators can accumulate unusually high levels of heavy metals like copper (Cu) and zinc (Zn) in their tissues without showing significant signs of toxicity. This remarkable ability has made *B. juncea* an important model organism for studying the molecular mechanisms underlying heavy metal tolerance and plant accumulation.

B. juncea has developed several regulatory mechanisms, including heavy metal absorption, transportation, chelation, and detoxification, to survive in the metal-contaminated environment. Several metalloproteins or metallochaperone-like proteins containing conserved heavy metal-associated (HMA) domains are involved in metal binding and transport. P1B-metal transporting ATPases are particularly interesting for their role in metal transport at the cellular and subcellular levels in accumulator plants. In the present communication, we report the gene expression pattern of heavy metal ATPases in *B. juncea* grown at different levels of Cu and Zn.

In this research, *B. nigra* and *B. juncea* plants were grown in the soil at different Cu and Zn concentrations (0 to 1000 µM). Plants showed no toxicity symptoms and accumulated a significant amount of metal in their leaves. In the leaves of *B. juncea*, Cu content increased significantly with an increase in Cu level in the media. HMA1 (Heavy Metal ATPase 1) in the leaves gradually increased with increased Cu levels until 50 µM. Then, its expression slowly decreased at the increase in Cu levels. The expression of HMA3 is also increased with an increase in Cu in the leaves. However, its expression pattern differed from that of HMA1. Our data showed that an increase in Cu levels in the leaves triggers the expression of both genes, suggesting that they play an active role in Cu detoxification.

Gene expression analysis showed that increased Zn levels in the leaves trigger the expression of the HMA2 and HMA4 genes, suggesting that they play an essential role in Zn detoxification and sequestration at the subcellular level. Furthermore, the bioinformatics analysis of these transporters in *B. juncea* showed a significant similarity at gene and protein levels to that of HMA2 and HMA4 transporters in other members of the Brassicaceae family. The data indicates the importance of these transporters in Zn sequestration and detoxification in accumulator plants in Brassicaceae.

Recently, CRISPR- Cas 9 technology (clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9) has been successfully used for genome editing in bacteria, plants, mammalian, and human systems. This new technology has great potential to help molecular biologists

and crop breeders edit or modify the genome or genes of crop plants that can produce high yields under biotic/abiotic stress conditions. CRISPR/Cas9 technique could introduce point mutations in the sequences of the metal transporter genes to enhance their activity or modify their selectivity toward the metal of interest. The increase in transporter activities (overexpression) will enhance the ability of the plant to uptake and translocate the metals in shoots efficiently, which could be of paramount importance for phytoremediation. Currently, we are trying to use CRISPR/Cas9 methods to understand the role of HMAs in metal transport and accumulation in *B. juncea*. In the present communication, we have preset the role of HMAs in metal transport in *B. juncea* and highlighted the role of transporter proteins in metal homeostasis in hyperaccumulator plants. We propose that this plant species could decontaminate Cu and Zn from polluted soils.