9th International Rosaceae Genomics Conference

June 26-30, 2018
Hanyuan Mansion, Nanjing, China

Program and Abstracts

Organized by

College of Horticulture, Nanjing Agricultural University (NAU)
Jiangsu Academy of Agricultural Sciences (JAAS)
Nanjing Botanical Garden, Institute of Botany Jiangsu Province and Chinese Academy of Sciences (NBG，JIB)

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9th International Rosaceae Genomics Conference

Dear Rosaceae Genomics Community,

On behalf the Local Organizing Committee, we welcome you to attend the RGC9 from June 26-30, 2018, in the International Conference Center at NAU, Nanjing, China. The RGC9 is organized by Nanjing Agricultural University (NAU), Jiangsu Academy of Agricultural Sciences (JAAS), and Nanjing Botanical Garden,Institute of Botany Jiangsu Province and Chinese Academy of Sciences (NBG, JIB). This biennial conference will continue the tradition of past RGC conferences, serving as a platform for the international Rosaceae community to share updates on ongoing research projects in the fields of genomics and genetics, fostering collaboration and cooperation among the international community to solve the scientific problems related to Rosaceae crop pre- and post-production and gathering with the international colleagues and friends.

We are honored to be hosting RGC9 at NAU. Nanjing is rich in both history and culture, and conference attendees will have opportunities to enjoy many different fruits, visit the botanical garden, participate in a field tour of peach and pear breeding research sites and much more.

Please visit www.rgc9.org for more details.

Conveners

Zongming (Max) Cheng, Nanjing Agricultural University/University of Tennessee

Shaoling Zhang, Nanjing Agricultural University

October, 2017

Conference Program

June 26th, 2018 (Tuesday)		
14:00-20:00	Conference Registration First Floor Lobby, Hanyuan Mansion, Nanjing Agricultural University, Nanjing, China	
14:00-17:15 18:45-22:00	Workshop on Genomics and Molecular Breeding of Rosaceae, College of Horticulture, B4011 Instructed by Dr. Cameron Peace, Washington State University, USA	
17:00-19:00	Dinner: Zhongshan Hall of Hanyuan Mansion, third floor	
June 27th, 2018 (Wednesday)		
Conference Room of Hanyuan Hotel, sixth floor		
7:00-8:30	Breakfast, first floor dining room	Moderator
8:30-9:00	Opening Ceremony Welcome remark by Vice President Fadi Chen, Nanjing Agricultural University Welcome remark by Dean Juyou Wu, College of Horticulture, NAU	Max Cheng/Shaoling Zhang
9:00-9:30	Group Photo, first floor of Hanyuan Mansion	
Session 1: Genome Evolution and Epigenomes		
9:30-10:20	**Plenary Lecture:** The Evolutionary Significance of Polyploidy Yves Van De Peer, VIB/Ghent University, Belgium	Yi Li
10:20-11:10	**Plenary Lecture:** Update on Pear Genome and Pear Domestication Jun Wu, Nanjing Agricultural University, China	
11:10-11:30	Tea and coffee break	
11:30-11:50	Diversity and Evolution of Plant Epigenomes Chad E. Niederhuth, Michigan State University, USA	Francois Laurens
11:50-12:10	High Throughput Chromatin Interaction (Hi-C) of Fruit Tree Genomes David Chagné, The New Zealand Institute for Plant & Food Research Limited, New Zealand	
12:10-12:30	Origin and Evolution of the Armeniaca Species Shuo Liu, INRA, France/ Liaoning Institute of Pomology, China	
12:30-13:30	Buffet Lunch: Zhongshan Hall of Hanyuan Mansion, third floor	
Session 2: Genome and Genome Selection		
13:30-14:20	**Plenary Lecture:** Genomic Selection in Fruit Trees: Potential Evaluation in Japanese Pear and Apple Iwata Hiroyoshi, Tokyo University, Japan	Cameron Peace

14:20-14:40	Harnessing the High-Quality Apple Genome to Distinguish Genetic and Epigenetic Mutants Francois Laurens, INRA, France	Cameron Peace
14:40-15:00	The Improved Assembly of the European Pear Michela Troggio/Gareth Linsmith, Fondazione Edmund Mach, Italy/Ghent University, Belgium	
15:00-15:20	Tea and coffee break	
Session 3: Genetics and Molecular Biology		
15:20-16:10	**Plenary Lecture:** Is Tomato the Right Model for Fruit Ripening? Lesson from the FruitENCODE project Silin Zhong, The Chinese University of Hongkong, China	Pere Arús
16:10-16:30	Multiple Copies of a MYB-binding Site Confer Trans-regulation by Flavonoid-related R2R3 MYBs Richard V. Espley, The New Zealand Institute for Plant & Food Research Limited, New Zealand	
16:30-16:50	Identification of a Negative MYB Regulator of Anthocyanin Biosynthesis in Peach Fruit Yuepeng Han, Wuhan Botanical Garden, the Chinese Academy of Sciences, China	
16:50-17:10	Transcription Factor MYBX Is Involved in Anthocyanion Accumulation by Activating SUMO E3 Ligase MdSIZ1 in Apple Yuanyuan Li, Shandong Agricultural University, China	David Chagné
17:10-17:30	Lignification, a Firmness Research at Zhejiang University Kunsong Chen/Xueren Yin, Zhejiang University, China	
17:30-18:00	Poster view, Conference Room 1+2, sixth floor	
18:00-19:30	Dinner: Zhongshan Hall of Hanyuan Mansion, third floor	
19:30-20:30	Poster view, Conference Room 1+2, sixth floor	
June 28th, 2018 (Thursday)		
Conference Room of Hanyuan Mansion, sixth floor		
7:00-8:30	Breakfast, first floor dining room	Moderator
Session 4: Evolution and Genetics of Traits		
8:30-9:20	**Plenary Lecture:** Nuclear Phylogenomics and Evolution of Fruit Types in Rosaceae Hong Ma, Pennsylvania State University, USA/Fudan University, China	Jun Wu
9:20-9:40	New Marker-Based Breeding Strategies for Peach and Other Perennial Crops Pere Arús, IRTA, Spain	

9:40-10:00	Expression of MdCCD7 in the Scion Determines the Extent of Sylleptic Branching and Primary Shoot Growth Rate of Apple Trees Toshi Foster, The New Zealand Institute for Plant & Food Research Limited, New Zealand	Charles-Eric Durel
10:00-10:20	The Control of Apple Fruit Shape by Malus Domestica PISTILLATA (MdPI) Jialong Yao, The New Zealand Institute for Plant & Food Research Limited, New Zealand	
10:20-10:40	Tea and coffee break	
Session 5: Genome Editing		
10:40-11:30	**Plenary Lecture:** Development of CRISPR Gene Editing Technology for Plants Yunde Zhao, University of California San Diego, USA	Hong Ma
11:30-11:50	Efficient Targeted Mutagenesis in Apple Using the CRISPR-Cas System Elisabeth Cheverau/Emilie Vergne, IRHS, INRA, France	
11:50-12:10	Knock-out of the PDS Gene in Pear Using the CRISPR/Cas9 System Kui Lin-Wang, The New Zealand Institute for Plant & Food Research Limited, New Zealand	
12:10-13:30	Buffet Lunch: Zhongshan Hall of Hanyuan Mansion, third floor	
Session 6: Genome-enabled Selection and Breeding		
13:30-13:50	Breeding Apple and Pear Cultivars Using Genomic Selection Satish Kumar, The New Zealand Institute for Plant & Food Research Limited, New Zealand	Toshi Foster
13:50-14:10	New Insights into Assorted Markers and Genotypes in Peach Chunxian Chen, USDA-ARS, USA	
14:10-14:30	The Role of PmRGL2 in GA4 Signal Transduction during Floral Bud Dormancy Release in Japanese Apricot Zhihong Gao, Nanjing Agricultural University, China	
14:30-14:50	Understanding Molecular Regulators of Chilling-Mediated Bud Dormancy Release in Apple through Genomics Approaches Anil Kumar Singh, Indian Institute of Agricultural Biotechnology, India	Zhongshan Gao
14:50-15:10	New Insights into the Evolution and Mechanisms of the S-RNase-based Self-incompatibility in Prunus Obtained Based on the Genome-wide DNA Sequencing Analysis Ryutaro Tao, Kyoto University, Japan	
15:10-15:30	Phosphatidic Acid Counteracts S-RNase Signaling in Pollen by Stabilizing the Actin Cytoskeleton Juyou Wu, Nanjing Agricultural University, China	
15:30-15:50	Tea and coffee break	

Session 7: Genome Database and Tools		
15:50-16:10	GDR (Genome Database for Rosaceae): Resource for Genomic, Genetic and Breeding Research Doreen Main/Sook Jung, Washington State University, USA	Satish Kumar
16:10-16:30	Reconstruction of Multi-Generation Pedigrees Involving Numerous Old Apple Cultivars Thanks to Whole-Genome SNP Data Durel Charles-Eric, INRA, France	
16:30-16:50	Sweet cherry: new genomic tools for the creation of varieties adapted to future conditions Elisabeth Dirlewanger, INRA, France	
16:50-17:10	Visualizing the Genetics of Elite Genomes Cameron Peace, Washington State University, USA	Ryutaro Tao
17:10-17:30	Genomic Tools of Sweet Cherry for The Breeding Programs Kenta Shirasawa, Kazusa DNA Reserch Institute, Japan	
17:30-17:50	T-Lidar as A New High-Throughput Methodology for Studying the Genetic Determinisms of Apple Tree Architecture Evelyne Costes, INRA, France	
17:50-18:10	Bioinformatics Analysis for Genome Sequencing and Population Genetic Study Gang Zhou, Biomarker Technologies Corporation, China	
18:10-19:30	Dinner: Zhongshan Hall of Hanyuan Mansion, third floor	
19:30-20:30	Poster view, Conference Room 1+2, sixth floor	
19:30-22:00	Social Time, Zijin Hall of Hanyuan Mansion, third floor	
June 29th, 2018 (Friday)		
Concurrent Session One: Conference Room of Hanyuan Mansion, sixth floor		
7:00-8:00	Breakfast, first floor dining room	Moderator
Session 8: Genomics, Genetics and Breeding for Biotic and Abiotic Stresses		
8:00-8:20	Genetic Mapping of Novel Loci for Resistance to European Canker and Apple Scab Sue Gardiner, The New Zealand Institute for Plant & Food Research Limited, New Zealand	Fengwang Ma
8:20-8:40	Importance of Bacterial Strain-Specificity to Improve Fire Blight Resistance and Management in Apples Awais Khan, Cornell University, USA	
8:40-9:00	Exploring the Genetic Variability of Peach Skin Phenolics and Triterpenoids as Natural Defenses against Brown Rot Bénédicte Quilot-Turion, INRA, France	Awais Khan

9:00-9:20	Resistance to Sharka in Stone Fruit Trees: Genetic and Genomic Technologies for New Breeding Strategies Véronique Decroocq, INRA, France	Awais Khan
9:20-9:40	Advances in Peach Breeding: from Discovery to Application Ksenija Gasic, Clemson University, USA	
9:40-10:00	Development of a 70K SNP Array for Pear and its Efficiency in Characterizing the Genetic Diversity of *Pyrus* Sara Montanari, UC Davis, USA	
10:00-10:20	Tea and coffee break	
Session 9: Genetics and Breeding		
10:20-10:40	RNA Binding Protein MhYTP2 Enhances Drought Resistance and Water Use Efficiency by Activating ABA and Ethylene Signaling in Apple Fengwang Ma, Northwest University of Agriculture and Forestry, China	Ksenija Gasic
10:40-11:00	PbWoxT1 mRNA from Pear (*Pyrus betulaefolia*) Undergoes Long-distance Transport Assisted by a Polypyrimidine Tract Binding Protein Tianzhong Li, China Agricultural University, China	
11:00-11:20	Exploration and Innovation of Apple Germplasm Resources Xuesen Chen, Shandong Agricultural University, China	Iwata Hiroyoshi
11:20-11:40	Genetic Breeding of Apple Rootstocks Zhenhai Han/Ting Wu, China Agricultural University, China	
12:00-13:30	Buffet Lunch: Zhongshan Hall of Hanyuan Mansion, third floor	
Session 10: Rosaceae Crop Breeding and Genetics in China		
13:30-13:50	Peach Breeding and Innovation in JAAS Ruijuan Ma, JAAS, China	Véronique Decroocq
13:50-14:10	Research Progresses in Cherry Group of Beijing Academy of Forestry and Pomology Sciences Kaichun Zhang, Beijing Academy of Forestry and Pomology Sciences, China	
Session 11: Selected Presentations in All Areas		
14:10-14:20	Leveraging Historical Performance Data from Multiple Environments through International Collaboration to Predict Germplasm Performance on a Global Scale Craig Hardner, University of Queensland, Australia	Yuepeng Han
14:20-14:30	Collaborative Project to Identify Direct and Distant Pedigree Relationships in Apple Nicholas Howard, Carl von Ossietzky-University, Germany	
14:30-14:40	Transcriptomic Profiling of Endodormancy to Ecodormancy Transition in Apricot (P. armeniaca). Jiali Yu, University of Tennessee, USA	

14:40-14:50	Elucidating the Molecular Mechanisms Underpinning a Novel Acyanic Trait in Apple Khethani Mhelembe, Agrcultural Research Council, South Africa	Sara Montanari
14:50-15:00	QTL Analysis of Flowering Time in Sweet Cherry Alejandro Calle, CITA-IA2, Spain	
15:00-15:10	Methylation Analysis of Dormancy Breaking in Almond Flower Buds [*Prunus* dulcis (Mill.) D.A. Webb] Pedro Martinez-Gomez, CEBAS-CSIC, Spain	
15:10-15:30	Tea and coffee break	
Session 12: Selected Presentation in All Areas		
15:30-15:40	Heritability of Epigenetic Marks and its Impact on Phenotypic Variability in Apple Perrin Adrien, IRHS-INRA, France	Anze Svara
15:40-15:50	Variation of Allergenic Lipid Transfer Protein in Diverse Chinese Peach Cultivars Zhongshan Gao, Zhejiang University, China	
15:50-16:00	Developing Genomic and Epigenomic Resources for Almond [Prunus dulcis (Mill.) D.A.Webb] Jonathan Fresnedo-Ramirez, The Ohio State University, USA	
16:00-16:10	Control of Bud Dormancy Process in Apple: A Genetic-Molecular Study Fernando Andrés, INRA, France	Valerio Pompili
16:10-16:20	Toward the Molecular Cloning of Two Genes Conferring Susceptibility to Apple Chlorotic Leaf Spot Virus Derived from Wild Malus Accessions Shigeki Moriya, Institute of Fruit Tree and Tea Science, NARO, Japan	
16:20-16:30	Allopolyploid Origin in Rubus (*Rosaceae*) Inferred from Nuclear Granule-bound Starch Synthase I (*GBSSI*) Sequences Yan Wang, Sichuan Agricultural University, China	
16:30-16:40	Genomic Selection - which Prospects in Prunus armeniaca? Preliminary Results Issued from Fruit Quality Traits and Phenology Patrick Lambert, INRA, France	Daniel Edge-Garza
16:40-16:50	Polyploidy Iinfluences Resistance to Venturia Inaequalis in *Malus x domestica* Anze Svara, KU Leuven, Belgium	
16:50-17:00	Analyses of 127 Chloroplast Genomes Provide New Insights into the Phylogenetic Relationships among Cherry Species and Taxonomic Status of Cerasus (Rosaceae) Xiaorong Wang/Jing Zhang, Sichuan Agricultural University, China	
17:00-17:10	Introgressing Blue Mold Resistance into Elite Apple Cultivars with DNA Tests, a High-Density SNP Array, and Rapid Cycle Breeding Feixiong Luo, Washington State University, USA	Tingting Gu

17:10-17:20	Genome Edited and T-DNA-free Apple Plants Resistant to Fire Blight Valerio Pompili, Fondazione Edmund Mach, Italy	Tingting Gu
17:20-17:30	Investigating Global Changes in Gene Expression during Bud Development in Different Sweet Cherry Cultivars Noémie Vimont, INRA, France	
17:30-17:40	Poster Award Announcement	
17:40 - 17:50	Welcome Remark for the RGC10, Pere Arús	
17:50- 18:00	Closing Ceremony	
18:00-19:30	Dinner: Zhongshan Hall of Hanyuan Mansion, third floor	
June 29th, 2018 (Friday)		
Concurrent Session Two: Conference Room of Hanyuan Mansion, eleventh floor		
7:00-8:00	Breakfast, first floor dining room	Moderator
Session 13: Genomics of Rosoideae		
8:00-8:50	**Plenary Lecture:** The Rose Genome and Beyond: Understanding Rose Domestication and the Mechanisms Underlying Major Traits Mohammed Bendahmane, INRA-Ecole Normale Superieure, Lyon, France	Wensuo Jia
8:50-9:10	Multi-allelic QTL Analysis in Tetraploid Rose Using a Dense Linkage Map Rene Smulders, Wageningen University & Research, Netherlands	
9:10-9:30	A High-quality Sequence of *Rosa Chinensis* to Elucidate Genome Structure and Ornamental Traits Fabrice Foucher, INRA, France	
9:30-9:40	Characterizing Black Spot Resistance Genes in Polyploid Roses Nahla Bassil, USDA-ARS Corvalis, USA	Rene Smulders
9:40-9:50	Development of a Complete Process of in Vitro Culture and Agrobacterium Tumefaciens-Mediated Genetic Transformation of Rose Cultivars Hibrand-Saint Oyant, INRA, France	
9:50-10:00	Coexpression Network Comparison of *Rubus Idaeus* and *Fragaria Vesca* in Early Fruit Development Haley Wight, University of Maryland, USA	
10:00-10:10	MIKCC-type MADS-box Genes in Rosa Chinensis: the Remarkable Expansion of ABCDE Model Genes and Their Roles in Floral Organogenesis Jinyi Liu, Nanjing Agricultural University, China	
10:10-10:30	Tea and coffee break	
Session 14: Genomics, Breeding & Molecular Biology of Strawberry I		

10:30-10:50	Gene Discovery for Flavor and Disease Resistance in Cultivated Strawberry Chris Barbey, University of Florida, USA	Kenta Shirasawa
10:50-11:10	Genomic Prediction in Strawberry Breeding Vance Whitaker/Luis F. Osorio, University of Florida, USA	
11:10-11:30	Micro-dissected Single Chromosome and its Sequence Analysis in Cultivated Strawberry (Fragaria × ananassa Duch.) Tomohiro Yanagi, Kagawa University, Japan	
11:30-11:50	Optimization of Transient Gene Manipulation in Strawberry Fruit: Adoption of Percentage Difference of Phenotype (PDP) to Evaluate Gene Function in Fruit Ripening Wensuo Jia, China AgricultureUniversity, China	
11:50-13:30	Buffet Lunch: Zhongshan Hall of Hanyuan Mansion, third floor	
Session 15: Genomics, Breeding & Molecular Biology of strawberry II		
13:30-13:50	Genomic and Genetic Approaches to Identify Key Genes That Regulate Strawberry Reproductive Development Zhongchi Liu, University of Maryland, USA	Mizhen Zhao
13:50-14:10	Plant Hormones Coordinate Receptacle Fruit Development in Fragaria Vesca Yamamuro Chizuko, Fujian Agriculture and Forestry University, China	
14:10-14:30	Regulation of Phosphate Uptake and Allocation in Strawberry by PHR1-miR399-PHO2 Module Zhihong Zhang/Junxiang Zhang, Shenyang Agricultural University, China	Chunying Kang
14:30-14:50	'Who' and 'How' to Regulate the Ripening in Strawberry: a Model for Non-climacteric Fruit Yuanyue Shen, Beijing University of Agriculture, China	
14:50-15:10	Progress Report on Interactions of Strawberry with Fungus Pathology in SAAS-FFRI Qinghua Gao, Shanghai Academy of Agricultural Sciences, China	
15:10-15:30	Tea and coffee break	
Session 16: Genomics, Breeding & Molecular Biology of Strawberry III		
15:30-15:50	RAP Codes for a GST Anthocyanin Transporter That Is Essential for the Foliage and Fruit Coloration in Strawberry Chunying Kang, Huazhong Agricultural University, China	Zhongchi Liu
15:50-16:10	Advances of Strawberry Germplasm Collection and Breeding in Jiangsu Mizhen Zhao, Jiangsu Academy of Agricultural Sciences, China	
16:10-16:30	Lineage-specific Duplications of NBS-LRR Genes Occurring Before Divergence of Six Fragaria Species Yan Zhong, Nanjing Agricultural University, China	

16:30-16:40	Transcriptome Network-guided Identification of Transcription Factors Regulating Fruit Flesh Softening in Wild Strawberry Matthew Fischer, University of Maryland, USA	Yan Zhong
16:40-17:00	Transcriptome and Hormone Analyses Provide Insights into Hormonal Regulation in Strawberry Ripening Tingting Gu, Nanjing Agricultural University, China	
17:30- 17:40	Poster Award Announcement, Conference Room, Six floor	
17:40- 17:50	Welcome Remark for the RGC10, Pere Arús, Conference Room, Six floor	
17:50- 18:50	Closing Ceremony, Conference Room, Six floor	
18:00-19:30	Dinner: Zhongshan Hall of Hanyuan Mansion, third floor	

June 30th, 2018 (Saturday) Field Trip

7:00-8:30	Breakfast, first floor dining room	Coordinator
8:30-12:00	Field Trip 1: Pear Breeding & Cultivation	Shaoling Zhang/Jun Wu
8:30-12:00	Field Trip 2: Peach Germplasm Collection & Breeding & Cultivation	Ruijuan Ma

Field trip 1: The trip will take you to the Hushu & Baima Research Station of Nanjing Agricultural University and Jiangsu Academy of Agricultural Sciences, where intensive Asian pear breeding and cultivation are conducted. Baima station is about 45 minutes near the airport.
Route: Hanyuan Mansion - Hushu Research Station - Baima Research Station - Hanyuan Mansion

Field trip 2: The trip will take you to the Jiangsu Academy of Agricultural Sciences where a large peach germplasm is collected and an intensive breeding program has been carried out for decades.
Route: Hanyuan Mansion - Jiangsu Academy of Agricultural Sciences - Hanyuan Mansion

Abstracts

INDEX

The evolutionary significance of polyploidy

NOTE

VAN DE PEER, Yves[1,2,3,*]

([1] *Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium*; [2] *Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium*; [3] *Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0028, South Africa)*

Abstract: Thousands of species are currently polyploid, and contain multiple copies of their genome. On the other hand, the long-term establishment of organisms that have undergone ancient whole genome duplications (WGDs) has been exceedingly rare. The apparent paucity of ancient genome duplications and the existence of so many species that are currently polyploid provides a fascinating paradox. Interestingly, many ancient WGDs seem to have been established at very specific times in evolution, for instance during major ecological upheavals and periods of extinction. Our work has shown that WGDs observed for many different plant lineages seem to have coincided with the most recent major mass extinction, i.e. the K/Pg extinction, 66 million years ago. I will put forward different hypotheses of why polyploids, compared to their diploid progenitors, might have had some selective advantage that might explain their survival at times of extinction. Also, I will discuss how WGD events might lead to an increase in biological complexity. WGDs copy entire pathways or networks, and as such create the unique situation in which such duplicated pathways or networks could evolve novel functionality through the coordinated sub- or neofunctionalization of its constituent genes. I will describe a remarkable case of coordinated gene expression divergence following WGDs in Arabidopsis thaliana. We identified a set of 92 homoeologous gene pairs that all show a similar pattern of tissue-specific gene expression divergence following WGD, with one homoeolog showing predominant expression in aerial tissues and the other homoeolog showing biased expression in tip-growth tissues (root-tip and pollen-tube).

Key words: Whole genome duplication; polyploidy; K/Pg extinction; adaptation

Update on pear genome and pear domestications

NOTE

Wu Jun, Tao Shu-tian, Yao Gai-fang, Zhang Ming-yue, Li Xiao-long, Yin Hao, Qi Kai-jie, Wu Ju-you, Xie Zhi-hua, Huang Xiao-san, Wang Run-ze, Li Jia-ming and Zhang Shao-ling*

(*Centre of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, 210095, China*)
*Correspondence: nnzsl@njau.edu.cn

Abstract: The first draft genome of the Asian pear 'Dangshansuli' (Pyrus bretschneideri) using a combination of BAC-by-BAC and next-generation sequencing was reported. A 512.0-Mb sequence corresponding to 97.1% of the estimated genome size of this highly heterozygous species is assembled with194 x coverage. A total of 42,812 protein-coding genes were annotated. Repetitive sequences of 271.9 Mb in length, accounting for 53.1% of the pear genome, are identified. Simulation of eudicots to the ancestor of Rosaceae has reconstructed nine ancestral chromosomes, pear and apple diverged from each other ~5.4–21.5 million years ago, and a recent whole-genome duplication(WGD) event must have occurred 30–45MYA prior to their divergence, but following divergence from strawberry. In order to reveal the genetic variation pattern of different germplasm, we report the genome resequencing of 113 pear accessions from worldwide collections, representing both cultivated and wild pear species. Based on 18,302,883 identified SNPs, we conduct phylogenetics, population structure, gene flow, and selective sweep analyses. Furthermore, we propose a model for the divergence, dissemination, and independent domestication of Asian and European pears in which pear, after originating in southwest China and then being disseminated throughout central Asia, has eventually spread to western Asia, and then on to Europe. We find evidence for rapid evolution and balancing selection for S-RNase genes that have contributed to the maintenance of self-incompatibility, thus promoting outcrossing and accounting for pear genome diversity across the Eurasian continent. In addition, separate selective sweep signatures between Asian pears and European pears, combined with co-localized QTLs and differentially expressed genes, underline distinct phenotypic fruit traits, including flesh texture, sugar, acidity, aroma, and stone cells. Furthermore, it provides substantive and valuable genomic resources that will significantly advance pear improvement and molecular breeding efforts. Additionally, combine with the high-density genetic map, QTL localization and corresponding chromosomal physical map, a candidate R2R3 MYB TF, PyMYB114, was identified regulating the red skin color of pear. Furthermore, the interaction network bHLHS-PyMYB114-ERF3 provides insight into the coloration of fruits and the

interaction of different TFs to regulate anthocyanin biosynthesis. Additionally, we recently reassembled the Dangshansuli' genome by integrating different technologies, including PacBio, 10× Genomic, BioNano and Hi-C. The scaffold N50 increased to ~ 3 Mb, and the length of anchored assembly increased to ~500 Mb, which will provide the better reference genome for further genetics and breeding related study.

Key words: Pear, genome assembly, population structure, domestication, genetic map, physical map

NOTE

Diversity and evolution of plant epigenomes

NOTE

Chad E. Niederhuth[1]

(Department of Plant Biology, Michigan State University, East Lansing Michigan)

Abstract: The epigenome consists of those factors that regulate and define the genome into regions of open and expressed euchromatin and regions of closed and silenced heterochromatin. DNA methylation is a major component of plant epigenomes and is a reversible modification made to DNA through the addition of methyl groups to cytosine and adenine bases. It is involved in the regulation of gene expression and the silencing of transposons. We are trying to understand the evolution of DNA methylation in plants, how it shapes genome evolution, and its functional consequences. Using a comparative epigenomics approach, we find widespread variation in DNA methylation between plant species and genetic variation underlying these differences.

Keywords: Epigenomics, Evolution, DNA methylation

High throughput chromatin interaction (Hi-C) of fruit tree genomes

NOTE

David Chagné[1], Gareth Linsmith[2], Cecilia Deng[3], Amali Thrimawithana[3], Jibran Tahir[1], Rubina Jibran[1], Toshi Foster[1], Ivan Liachko[4], Shawn Sullivan[4]

1 Plant & Food Research, Palmerston North, New Zealand;2 VIB Department of Plant Systems Biology, University of Gent, Belgium;3 Plant & Food Research, Auckland, New Zealand;4 Phase Genomics, Seattle, USA

Abstract: The Hi-C method combines capture of chromatin interaction within the nucleus, next-generation sequencing (NGS) and new bioinformatics tools for developing near-complete pseudo-chromosome genome assemblies. The method relies on the fact that chromosomes are folded inside the nucleus and that fragments of the same chromosome are in close 3D proximity to other fragments of the same chromosome, but fewer of other chromosomes. It is possible to crosslink and capture chromosome fragments that are in close proximity, ligate them and then sequence these using NGS. The probability of finding fragments that are in close proximity compared with distant fragments within paired-end NGS reads decreases with physical distance within chromosomes, and decreases even more drastically between chromosomes. We used the Hi-C method to assemble the genomes of Rosaceae species, including European pear, apple and black raspberry as well as other species, such as New Zealand mānuka, myrtle rust, trevally and kiwifruit. We demonstrated that Hi-C is very efficient for scaffolding genomes into very large fragments.

Keywords: genome assembly, scaffolding, Rubus, primocane, dwarfism

Origin and Evolution of the Armeniaca species

NOTE

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Abstract: During the protracted process of plant domestication and subsequent variety breeding, the traits of the wild ancestors of our modern crop species have been considerably modified. In consequence, many important traits, such as resistance to major crop pest or diseases, may be lacking in the cultivated germplasm while being present in wild relatives. Investigating the genetic and phenotypic diversities of wild relatives of crop species allows identifying interesting traits and understanding domestication, and more generally the processes of adaptation and diversification. Apricot, *Prunus armeniaca* L., is an emblematic fruit tree species corresponding to both the domesticated form, cultivated worldwide, and the wild form. Our recent genetic data combined with an approximate Bayesian approach supported the hypothesis that the center of origin of European cultivated apricots is Central Asia (Decroocq et al, 2016). This previous study also showed a significant genetic differentiation between Chinese apricot landraces and both European cultivars and wild Central Asian apricot trees, with however limited sampling in China (Decroocq et al, 2016). *Prunus armeniaca* has been cultivated for over 3,000 years in China, where shares its distribution with three other *Armeniaca* wild species, *i.e. P. sibirica, P. manshurica* and *P. mume.* The geographical origin and genetic structure of the wild Armeniaca species across Asia remain unclear. Furthermore, the identity of the wild species having contributed to European and Chinese cultivated apricots and the number of independent domestication events have been poorly studied so far.
We therefore studied the domestication history of *Armeniaca* species, with an emphasis on the cultivated and wild forms endemic to China. With a large-scale dataset of cultivated and wild Armeniaca collected over Eurasia, we addressed the following questions: (1) what is the genetic subdivision in the Chinese apricots and what is the history of lineage divergence in Eurasia? (2) How many independent domestication events occurred in Eurasia and from which wild species?

Keywords: *Prunus*, apricot, evolution, domestication, China

Genomic selection in fruit trees: potential evaluation in Japanese pear and apple

NOTE

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Abstract: Genomic selection (GS) has a great potential to accelerate fruit tree breeding via shortened breeding cycles and increased selection intensity, because of its ability to select seedlings during their juvenile phase and to filter out substantial proportion of progeny that will proceed to evaluation in field trials. GS can also be used for selecting an optimal parental combination that has high probability of generating offspring with desirable characteristics. Although GS is expected to streamline and accelerate fruit tree breeding, it is definitely important to evaluate the potential of GS with real breeding materials in a target species. In this lecture, I will introduce our studies for evaluating potential of GS in Japanese pear and apple. Japanese pear (*Pyrus pyrifolia* Nakai) is an important fruit tree, whose production is the third largest in Japan. ‘Kosui’ is the top cultivar which accounts for 34% of production, and a new cultivar superior to ‘Kosui’ has been long awaited. We developed a new method for predicting the segregation of target traits in a progeny population. The method provides the Bayesian posterior probability of getting offspring that fulfills selection criteria. Verification using a breeding population suggests that segregation in a progeny population can be predicted with reasonable accuracy in advance of crossing. We also evaluated the potential of GS with a parental population of 86 varieties and breeding populations of 765 trees from 16 full-sib families, which were phenotyped for 18 traits and genotyped for 1,506 single nucleotide polymorphisms (SNPs). The accuracy of genomic prediction was improved when we combined data from the breeding populations and the parental population. The power of GWAS was also improved with the combined datasets. The result suggests the necessity of a system for routine collection of the phenotype and marker genotype data for breeding populations. Apple (*Malus* x *domestica* Borkh.) is an important worldwide fruit, whose production is the second largest in Japan. The most of Japanese varieties are descendants of a small number of (mainly seven) founders. For example, the world-famous variety ‘Fuji’ was derived from the cross of two founders, ‘Ralls Janet’ and ‘Delicious’. Thus, the genetic composition of the varieties can be modelled with ancestral haplotypes of founders. We developed a new method for assigning founder haplotypes to descendants and applied it to the dataset of 185 varieties and 659 trees from 16 full-sib families, which were genotyped with 11,796 SNPs. The method attained reasonable accuracy in comparison to careful manual assignment of founder haplotypes. We can reduce the number of markers required for GS based on the

assigned haplotypes. GS has a good potential in fruit tree breeding, and development of new methods and systems will enhance the potential further.

Key words: genomic selection, genomic prediction, segregation pattern, *Pyrus pyrifolia*, *Malus* x *domestica*

NOTE

Harnessing the high-quality apple genome to distinguish genetic and epigenetic mutants

NOTE

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Abstract: Using the latest sequencing and optical mapping technologies we have produced a high-quality *de novo* assembly of the apple genome. Repeat sequences, representing over half of the assembly, provided an unprecedented opportunity to investigate the 'dark matter' of a tree genome: we identified a new hyper-repetitive retrotransposon sequence which is over-represented in heterochromatic regions. It is arranged in tandem repeat arrays that were detectable thanks to the optical maps. We estimate that a major transposable element (TE) burst occurred 21 My ago which seems to coincide with the uplift of the Tian Shan mountains, the center of origin of apple, suggesting that TEs may have contributed to its divergence from pear. We also report on epigenetic phenomena that contribute to apple fruit development. This high-quality genome now unlocks the possibility to carry out detailed epigenetic studies. We will present perspectives to decipher the mechanisms controlling apple skin colour and develop molecular markers that will allow genetic and epigenetic characterization of apple mutants.

Key words: *apple genome, epigenetics, fruit development, external fruit color*

The improved assembly of the European Pear

NOTE

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Abstract: Apple and Pear diverged from each other between 5.4 and 21.5 MYA and are believed to share a common whole genome duplication event between 35 and 50 MYA (Velasco et al. 2010, Wu et al. 2012). Size differences have been observed between the Apple and Pear genomes which are estimated at 527Mb (Pyrus x Bretschneideri Rehd) and 700Mb (Malus x Domestica Borkh) respectively (Wu et al. 2013, Li et al. 2016). The difference in genome size has been accounted for primarily by the proliferation of transposable elements, with the gene space thought to be fairly similar between the two species (Wu et al 2012). Comparative genomics of the lineage has however, been hampered by the fragmented nature of the reference assemblies. A new chromosome scale apple assembly was recently produced (Daccord et al. 2017) and now also a chromosome scale assembly of the European Pear (this study), which shows strong collinearity with Apple, greatly facilitating the comparative study of these genomes.

Key words: *Pyrus communis;* pear; *Malus domestica*; apple; genome assembly; comparative genomics; Hi-C; Bionano; Pacbio

Is tomato the right model for fruit ripening? Lesson from the fruitENCODE project

NOTE

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Abstract: Fleshy fruit has evolved many times throughout the angiosperm history, and constitute an important part of human and animal diet. For plants, ripening is an irreversible developmental process that the seed-bearing organ is transformed from repelling to attracting frugivore to aid seed dispersal, and a tight control could be of significant evolutionary advantage, as accidental activation would cause unnecessary seed loss. Much of what we know about this process came form the study of ripening mutants in tomato, a climacteric fruit that ripening requires autocatalytic ethylene production, MADS-box transcription factors and whole-genome demethylation. But the precise molecular mechanism and whether it is conserved in other species remain largely unknown.

The fruitENCODE project has systematically profiled gene expression, accessible chromatin, histone modifications and DNA methylation changes in 11 fleshy fruit species to investigate the molecular circuits controlling ripening. We found most climacteric fruits utilize a common angiosperm senescence-related NAC transcription factor to create a positive feedback loop to synthesize the autocatalytic ethylene. For plants undergone recent whole-genome duplication like tomato, apple and pear, their loop depends on neofunctionalization of the duplicated MADS-box transcription factors. Banana, a monocot climacteric species that diverged from eudicot over 100 myr and undergone three recent WGD, uses leaf senescence-related NAC transcription factor to generate a positive feedback loop and an additional loop with three MADS-box transcription factors that makes its ripening ethylene independent once initiated. It turns out that DNA methylation changes associate with ripening genes could be unique for tomato, while other species utilize H3K27me3 to regulate their genes involved in ripening and autocatalytic ethylene production.

The fruitENCODE study has raised important questions regarding how we should interpret complex developmental processes while the data is often derived from a single model species like tomato.

Key words: fruit ripening, DNA methylation, histone modification, accessible chromatin

Multiple copies of a MYB-binding site confer trans-regulation by flavonoid-related R2R3 MYBs

NOTE

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Abstract: There is increasing interest in anthocyanins and their contribution to a healthy diet. In apple, the R2R3 MYB transcription factor, MYB10, controls the accumulation of anthocyanin. MYB10 is also able to activate its own expression by binding its own promoter at a specific motif; the R1 motif. In red-fleshed apple germplasm this motif is mutated and comprises a further five tandem repeats of R1 to form a minisatellite repeat unit, R6. This modification results in a gain of function, producing an increase in anthocyanins throughout the plant and a strikingly pigmented phenotype. We have used this mutation to show that other anthocyanin-related R2R3 MYBs from pear, strawberry, petunia, and Arabidopsis are able to up-regulate promoters containing R6. Insertion of the R6 motif into orthologous promoters of pear and *Arabidopsis* resulted in auto-regulation of the MYB and plants containing this construct showed elevated anthocyanin. Introduction of the R6 motif into the promoter region of an anthocyanin biosynthetic gene, the cytochrome P450 enzyme, F3’5’H, altered the resulting anthocyanic profile, producing elevated levels of delphinidin in both tobacco and kiwifruit. This naturally occurring motif provides a versatile tool to re-engineer novel MYB-regulated responses across a range of plant species. Now we are using apple transformed with the native R6:MYB10 to understand the relationship between environmental factors and MYB-driven anthocyanin production.

Key words: Apple, anthocyanin, MYB transcription factor, pigments, promoter engineering

Identification of a negative MYB regulator of anthocyanin biosynthesis in peach fruit

NOTE

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Abstract: Anthocyanin accumulation in fruit is beneficial for human health due to the anti-oxidation activity. Here, we report the feedback regulation mechanism underlying anthocyanin accumulation in peach mesocarp. In blood-flesh peach cultivar 'Dahongpao', the expression level of the positive regulator *PpMYB10.1* increased during fruit developmental stages, and reached a peach at the ripening stage, which accorded with the content change of anthocyanins. Interestingly, one MYB gene, designated *PpMYB6*, also showed high expression level at ripening stages. Functional analysis showed that *PpMYB6* could negatively regulate two anthocyanin structural genes *PpDFR* and *PpUFGT* in dual luciferase assay and inhibited anthocyanin coloration when it was co-infiltrated *PpMYB10.1* in transient color assay. These results suggest that anthocyanin accumulation in blood-peach fruit may be regulated by a feedback loop comprising *PpMYB10.1*, *PpMYB6* and anthocyanin structural genes.

Keywords: *Prunus persica*, anthocyanin, repressor

NOTE

Transcription Factor MYBX Is Involved in Anthocyanion Accumulation by Activating SUMO E3 ligase MdSIZ1 in apple

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Abstract: Small ubiquitin-like modifier (SUMO) conjugated to target proteins is an important post-translational modification. MdSIZ1 is a SUMO E3 ligase in apple, which mediates its target MdMYB1 sumoylation to promote anthocyanion accumulation and apple fruit coloration under low temperature. However, how the MdSIZ1 senses low temperature signal and if the sumoylation is regulated by transcriptional level is unclear. In this study, by analyzing the promoter of *MdSIZ1* gene, we found a MBS (MYB Binding Site) motif in the promoter sequence of *MdSIZ1*, which was essential for the response of *MdSIZ1* to moderate low temperature conditions. Subsequently, a MYB transcription factor *MdMYBX* was found to directly bind to the MBS motif of *MdSIZ1* promoter with a yeast one-hybridization screening approach. MdMYBX was induced by moderate low temperature conditions and activated *MdSIZ1* expression. Finally, the *MdMYBX* promoted the anthocyanin accumulation in both apple fruits and calli in an *MdSIZ1* dependent manner. Taken together, our findings reveal an important role for transcriptional regulation of sumoylation and bring a new insight into the mechanism in the regulation of anthocyanin biosynthesis in plants.

Key words: anthocyanin biosynthesis; MYB transcription factors; MdSIZ1; transcriptional regulation

Lignification, a firmness research at Zhejiang University

NOTE

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Abstract: Loquat fruit lignification is a typical case of flesh lignification, where increase in fruit firmness is highly correlated with increase in lignin content. Lignification in plant cell is radical coupling reaction of lignin monomer, where the biosynthesis of lignin monomer is the base of lignification.
A brief introduction of our research in the past decade in fruit lignification research includes the cultivar comparison, biochemical and molecular biological mechanisms of the lignin biosynthesis, and effects of temperature regulation of this event. Our results showed that different cultivars have different textural change patterns in response to different postharvest temperature stresses. Temperature affects lignification and treatments such as low temperature conditioning (LTC) effectively alleviated fruit lignification. Among the lignin biosynthesis related structural genes, expression of *EjCAD1*was highly correlated with flesh lignification, and it was induced by ethylene and inhibited by 1-MCP treatment. LTC and 1-MCP regulated gene expression of *EjEIL1* and *EjETR1*, respectively. Big data analysis revealed that plant lignin biosynthesis is regulated by multiple transcription factors, and such regulation has become a new focus of research. EjAP2-1 indirectly regulated lignin biosynthesis through interacting with MYB and the formation of transcription protein complex.

Keywords: Lignification; Loquat fruit;firmness; transcriptional regulation

Nuclear Phylogenomics and Evolution of Fruit Types in Rosaceae

NOTE

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Abstract: Rosaceae is a family with ~3000 species in approximately 100 genera, and has an unusually large number of different fruit types, including fleshy fruit types, such as drupes (peach, plum, cherry and apricot) and pomes (apple, pear, and hawthorns) and complex fruits (strawberry with fleshy receptacles, and blackberry with multiple drupelets), which are consumed by human and animals. In addition, several types of dry fruit types are found in Rosaceae, such as achenetum and follicetum with features facilitating seed dispersal. Therefore, Rosaceae provides an excellent system for studying fruit type evolution. However, a rigorous examination of the evolutionary history of fruit types in Rosaceae was hampered by the uncertainties in the relationships among different genera and tribes bearing distinct fruit types. To reconstruct a highly supported phylogeny of Rosaceae and to address fruit evolution and other questions, we generated 125 new transcriptome and genomic datasets and identified hundreds of nuclear genes, then used these genes to reconstruct a Rosaceae phylogeny with highly supported monophyly of all subfamilies and tribes and relationships among these groups. Ancestral character reconstruction for fruit types supports independent origins of fleshy fruits from dry-fruit ancestors, including the evolution of drupes (e.g., peach) and pomes (e.g., apple) from follicetum, and drupetum (raspberry and blackberry) from achenetum. In addition, molecular clock analysis revealed an estimated age of ~101.6 Mya for crown Rosaceae. Furthermore, phylogenomic analysis yielded strong evidence for several whole genome duplications (WGDs), including one shared by the apple tribe and another ancestral to the peach tribe. In particular, a WGD event shared by the lineages in the apple tribe with fleshy fruits was very strongly supported, with numerous paralogous gene pairs detected in multiple members of the apple tribe. We propose that WGDs and environmental factors, including animals, contributed to the evolution of the many fruits in Rosaceae, providing a foundation for further investigation of fruit evolution and possible contributions of WGDs.

Key words: Dry fruits, fleshy fruits, low-copy nuclear genes, phylogenetic analysis, molecular clock, multiple gene duplications

New Marker-Based Breeding Strategies for Peach and Other Perennial Crops

NOTE

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Abstract: Perennial crops occupy approximately one eighth of the world's cultivated surface. In addition of having long intergeneration periods, most of them have a functional sexual mating system and are clonally propagated commercially. The classical breeding scheme for these crops involves the cross between two partly heterozygous parents, and the selection of the best individuals in their F1 progeny that are subsequently multiplied vegetatively. This approach is efficient as it can be done in a single generation, but has several unwanted consequences: recombination can only be used to a very limited extent (only one meiosis per parent), and the use of exotic sources of variability is generally excluded. Here we propose the use of two marker-based approaches for peach: the first one, Marker-Assisted Introgression (MAI), integrates genes from exotic sources (i.e. almond or another *Prunus* relative) only after two backcross generations by introgressing single almond fragments into the peach background from the F1 between peach and the exotic donor, and the second, Resynthesis, generates individuals with a genetic composition very close to a top cultivar, which can be done in two selfing generations. Both approaches can be combined in another breeding scheme, VORI, (Varieties obtained by Resynthesis and Introgression) to obtain individuals very similar to a leading cultivar and with one exotic gene conferring a desired property.

Key words: Prunus persica, perennial crops, breeding methods, marker-assisted introgression, resynthesis

NOTE

Expression of *MdCCD7* in the scion determines the extent of sylleptic branching and primary shoot growth rate of apple trees

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Abstract: Branching has a major influence on the overall shape and productivity of a plant. Strigolactones (SLs) have been identified as plant hormones that have a key role in suppressing the outgrowth of axillary meristems. *CAROTENOID CLEAVAGE DIOXYGENASE* (*CCD*) genes are integral to the biosynthesis of SLs and are well characterised in annual plants, but their role in woody perennials is relatively unknown. We identified *CCD7* and *CCD8* orthologues from apple and demonstrated that *MdCCD7* and *MdCCD8* are able to complement the Arabidopsis branching mutants *max3* and *max4* respectively, indicating conserved function. RNAi lines of *MdCCD7* show reduced gene expression and increased branching in apple. We performed reciprocal grafting experiments with combinations of *MdCCD7* RNAi and wild-type 'Royal Gala' as rootstocks and scion. Unexpectedly, wild-type roots were unable to suppress branching in *MdCCD7* RNAi scions. Another key finding was that *MdCCD7* RNAi scions initiated phytomers at an increased rate relative to wild-type, resulting in a greater node number and primary shoot length. We suggest that localised SL biosynthesis in the shoot, rather than roots, controls axillary bud outgrowth and shoot growth rate in apple.

Key words: *CAROTENOID CLEAVAGE DIOXYGENASE* (*CCD*), *Malus* x *domestica*, shoot growth rate, Strigolactone, sylleptic branching

The control of apple fruit shape by *Malus domestica PISTILLATA* (*MdPI*)

NOTE

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Abstract: Fruit shape represents a key trait that consumers use to identify and select preferred cultivars, and although the manipulation of this trait is an opportunity to create novel, differentiated products, the molecular mechanisms regulating fruit shape are poorly understood in tree fruits. In this study, we have shown that ectopic expression of *Malus domestica PISTILLATA (MdPI),* the apple ortholog of the floral organ identity gene *PISTILLATA (PI),* regulates apple fruit tissue growth and shape. *MdPI* is a single-copy gene and its expression is high during flower development but barely detectable soon after pollination. Transgenic apple plants with ectopic expression of *MdPI* produced flowers with white sepals and a conversion of sepals to petals. Interestingly, these plants produced distinctly flattened fruit as a consequence of reduced cell growth at the basipetal position of the fruit. These altered sepal and fruit phenotypes have not been observed in studies using *Arabidopsis.* This study using apple has advanced our understanding of PI functions outside the control of petal and stamen identity and provided molecular genetic information useful for manipulating fruit tissue growth and fruit shape.

Keywords: floral organs, fruit development, *Malus* x *domestica*, MADS-box, *PISTILLATA*, transgenic plants

Development of CRISPR gene editing technology for plants

NOTE

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Abstract: CRISPR gene editing technology enables precise editing of DNA sequences in vivo and it has been widely used in modifying genes in many organisms including plants. Editing genes in plants has unique challenges. In this presentation, I will first describe our effort to develop gene editing technologies in rice that have enabled us to do 1) generation of targeted knockout mutants; 2) replacement of DNA fragments through homologous recombination; 3) introduction of targeted point mutations. More importantly, I will present technologies for transgene-free gene editing. The gene editing technologies we developed have allowed us to systematically analyze genes related to auxin biosynthesis, degradation, transport, and signaling in rice. I will use auxin as an example to demonstrate the power of CRISPR in crop improvement.

Efficient targeted mutagenesis in apple using the CRISPR/Cas system

NOTE

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**Equal participation to This work*
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Abstract: Targeted genome engineering has emerged as an alternative to classical plant breeding and transgenic methods to improve crop plants. Among others methods (zinc finger nucleases or TAL effector nucleases), the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas type II prokaryotic adaptative immune system proved to be the most effective, convenient and less expensive method.
In apple we used the CRISPR/Cas system with the Cas9 nuclease from *Streptococcus pyogenes* optimized for application in eukaryotic organisms. As a proof of concept, we chose to knock-out *Phytoene Desaturase* (*PDS*) and *Terminal Flower 1* (*TFL1*) genes. To improve the edition efficiency, two different single guide RNA (sgRNA) were associated to the Cas9 nuclease for each target gene. These sgRNAs were placed under the control of the U3 and U6 apple promoters.
A very high efficiency of edition was obtained among the 86 lines produced in total. Characteristic albino phenotype was obtained for 85 % of the apple transgenic lines targeted in *PDS* gene. Early flowering was observed in 93 % of the apple transgenic lines targeted in *TFL1* gene. Sequencing of the target zones in apple *PDS* transgenic lines showed that the two sgRNA induced mutations but not at the same frequency. Cas9 nuclease cut the DNA in the twenty targeted base pairs near the protospacer adjacent motif and insertions were more frequent than deletions or substitutions. The most frequent editing profile was chimeric biallelic. Mutations analyses of *TFL1* apple transgenic lines and the search for off-targets are underway.
We conclude that CRISPR/Cas 9 system is a powerful and precise method to induce targeted mutagenesis in the first generation of apple transgenic lines.

Key words: CRISPR/Cas, apple, Phytoene desaturase, Terminal flower 1, knock-out

Knock-out of the PDS gene in pear using the CRISPR/Cas9 system

NOTE

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Abstract: The CRISPR/Cas9 system is a recently developed tool used to modify genomes rapidly in a precise and predictable manner. This gene editing method can create specific mutations that knock-out or alter target gene function and has successfully applied to the annual crops. However, there are only a few reports of the genome editing of fruit tree crops. We demonstrate the use of RNA-guided Cas9 to generate mutations of the phytoene desaturase (PDS) gene in pear. Four guide RNAs were cloned into a single CRISPR/Cas9 vector and stably transformed into pear. Full and partial albino phenotypes were observed in regenerating plantlets. Plant tissue was sequenced at the DNA level to characterize the specific gene edits. One of the four guide RNAs had a base pair mismatch to its target region, which led to a relatively low editing efficiency. This is the first report of CRISPR/Cas9 genome editing in pear, which will provide useful knowledge for the future alteration of gene sequences to introduce desired pear traits.

Key words: *CRISPR/Cas9, pear, phytoene desaturase*

Breeding apple and pear cultivars using genomic selection

NOTE

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Abstract: Perennial fruit crops such as apple and pear are important among horticultural crops, providing livelihoods, health, and adding billions of dollars to the world economy. These fruit crops have a long juvenility period, which can last up to eight years, so that traditional fruit breeding often takes up to 20 years to develop commercial cultivars. The timeline could be even longer if novel attributes (e.g., disease resistance) are introgressed from wild germplasm to first develop parents for cultivar breeding. Genomics-assisted breeding provide more direct and quicker solutions to fast-forward the development of high-value cultivars. Sequencing of genomes of apple and pear has facilitated the development of high-throughput cost-effective genotyping platforms such as single nucleotide polymorphic (SNP) arrays and reduced-complexity genotyping-by-sequencing (GBS). Access to these genomic resources is providing opportunities for application of a novel tool called genomic selection (GS). The most attractive feature of GS is that it has the potential to dramatically shorten the breeding cycle length by obviating the need to phenotype selection populations. Fruit characters that constitute a superior cultivar are often polygenic in their inheritance, and GS is best suited for selection of cultivar candidates for such traits. Evaluation of GS in apple and pear have provided promising selection accuracies. As a result, GS is now being used in some commercial fruit breeding programmes. Results from empirical studies and future potentials of GS will be discussed.

Key words: Genomic selection; genetic architecture; complex traits; breeding value; fruit quality

New Insights into Assorted Markers and Genotypes in Peach

NOTE

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Abstract: DNA markers are useful resources and tools in genetics and breeding, facilitating cultivar true-to-type authentication, gene mapping, association study, phylogenetic analysis, marker-assisted selection, and other applications. The release of a high-quality peach reference genome has promoted fast, fruitful progress in various genomic researches. In this report, genome-wide microsatellites from nuclear expressed sequence tags and chloroplast genomes in peach were computationally characterized and categorized to ensure optimal selection of considerably downsized subsets of markers with known distribution, high reliability, and predicted polymorphism. Genotyping data of selected nuclear microsatellites markers showed great genotyping variability in the number of polymorphic genotypes, detected alleles, polymorphic information content, gene diversity, and heterozygosity among the categories. Based on the genotyping data of selected polymorphic chloroplast microsatellite markers, a large number of peach breeding materials were clustered into a few maternal lineage groups, reflecting the unique geographic or genetic origin of each group. Markers for several important peach fruit traits were also genotyped to determine the alleles and allelic association to the traits. This report provided new insights into development and utilization of assorted markers to decipher peach genotypes and traits in a large scale.

Key words: *Prunus persica*, simple sequence repeat, expressed amplicon size, genomic amplicon size, molecular breeding

The Role of *PmRGL2* in GA4 Signal Transduction during Floral Bud Dormancy Release in Japanese Apricot

NOTE

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Abstract: Bud dormancy release is regulated by gibberellic acids (GAs). DELLA proteins are highly conserved and act as negative regulators in gibberellins signaling pathway. In this study, we mainly report the identification and functional characterization of DELLA protein RGL2 gene from Japanese Apricot. qRT-PCR date showed the *PmRGL2* was “down-up-down” regulated under different periods, besides, the determination of GA4 content by LC-MS/MS (liquid chromatography-tandem mass spectrometry) method was gradually up-regulated in different dormancy periods. At the same time, the expression profiles of *PmGA20ox2*, *PmGA3ox1* and *PmGID1b* genes which showed the relationship between GA4 and *PmRGL2* in Japanese apricot. The transgenic populus leaves became yellow were later and its height decreased than wild-types. These results showed that *PmRGL2* acts as an integrating regulator of dormancy through GA signal channel and act as a negative regulator , In addition, the interaction links between RGL2 and SLY1 by the yeast hybrid suggested that SCF E3 ubiquitin ligases SLY1 might be a critical factor in regulating the DELLA protein RGL2 by an SCFSLY1-proteasome pathway in Japanese apricot.

Keywords: Gibberellins; dormancy; RGL2; SLY1; Japanese Apricot

NOTE

Understanding Molecular Regulators of Chilling-Mediated Bud Dormancy Release in Apple through Genomics Approaches

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Abstract: Chilling accumulation plays an important role in establishment and release of bud dormancy, flowering and fruit set in apple. Insufficient chilling leads to inevitable physiological symptoms which adversely affect the yield and quality of the fruit. During recent decades, decreasing trend in available chilling has been observed in apple growing regions of Himachal Pradesh, India, which has caused severe yield losses. Thus, to understand the molecular mechanism of chilling-mediated dormancy release in apple, we employed comparative transcriptomic and epigenomic approaches. Under differential chilling conditions differential expression of genes related to phytohormones metabolism, floral time regulation and DNA methylation was observed. The weighted gene co-expression network analysis (WGCNA) identified two high chill specific gene modules enriched with GO term “post-embryonic development” which might be involved in the regulation of meristematic activity during dormancy release. In addition, the differential expression of genes encoding DNA methyltransferases and DNA glycosylases was also observed. The genomic DNA methylation was also compared under differential chilling using methylation sensitive amplified polymorphism (MSAP). The high chilling availability during dormancy was found to be associated with less cytosine methylation along the dormancy release and fruit set. The MADS-box transcription factors are known to regulate various physiological processes including dormancy and flowering. The comparative phylogenetic and transcriptional analysis of whole MADS-box family in apple identified six dormancy-associated MADS-box (*DAM*) and four flowering locus C-like (*FLC*-like) genes. Their expression analysis with some other flowering related genes (*FRI*, *FT*, *LFY*, *SOC1* and *CO*) lead to hypothetical model of dormancy and flowering regulation in apple. Taken together, our study suggests the effect of chilling availability on various pathways, including phytohormone metabolism, flowering time regulation, post-embryonic development and epigenetic modifications during dormancy release in apple.

Key words: Bud dormancy; chilling, DNA methylation; flowering; methylation sensitive amplification polymorphism; network analysis; transcriptome

NOTE

New insights into the evolution and mechanisms of the S-RNase-based self-incompatibility in *Prunus* obtained based on the genome-wide DNA sequencing analysis

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Abstract: Most fruit tree species in *Prunus* in the Rosaceae show the S-RNase-based self-incompatibility system, which utilizes S-RNase and F-box proteins as the pistil *S* and the pollen *S*, respectively. Although similar molecules are involved in specificity determination of the SI recognition reaction across the three plant families, Rosaceae, Solanaceae, and Plantaginaceae, accumulating data suggest the presence of distinct SI recognition mechanisms in *Prunus*. The pistil *S* determinant S-RNase is considered to have a cytotoxic effect commonly in the three plant families. However, functions of the pollen *S* determinant F-box proteins are suggested to be different between the genus *Prunus* and the other taxa that show the S-RNase based SI. Pollen *S* in *Prunus* is assumed to release cytotoxicity of self S-RNase, while in the other taxa, pollen *S* is considered to be involved in S-RNase degradation and detoxification. Since whole genome sequence data of various plant taxa have been available, it is now possible to utilize new approaches such as evolutionary analysis and genome re-sequencing to uncover molecular mechanism of SI. This report presents new insights to SI mechanism in *Prunus* obtained from the evolutionary and genome-wide DNA sequence analyses. The novel gene for self-compatibility found recently in *Prunus* is also discussed.

Phosphatidic acid counteracts S-RNase signaling in pollen by stabilizing the actin cytoskeleton

NOTE

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Abstract: S-RNase is the female determinant of self-incompatibility (SI) in pear (*Pyrus bretschneideri*). After translocation to the pollen tube, S-RNase degrades rRNA and induces pollen tube death in an *S*-haplotype-specific manner. In this study, we found that the actin cytoskeleton is a target of *P. bretschneideri* S-RNase (PbrS-RNase) and uncovered a mechanism that protects the pollen tube from PbrS-RNase cytotoxicity involving phosphatidic acid (PA). PbrS-RNase interacts directly with the actin protein PbrActin1 in an *S*-haplotype-independent manner, causing the actin cytoskeleton to depolymerize and promoting programmed cell death in the self-incompatible pollen tube. P156 of PbrS-RNase is essential for the PbrS-RNase–PbrActin1 interaction, and the actin cytoskeleton-depolymerizing function of PbrS-RNase does not require its RNase activity. The expression of phospholipase D (PbrPLDδ1) is enhanced by PbrS-RNase cytotoxicity, resulting in increased PA levels in the incompatible pollen tube. PbrPLDδ1-derived PA initially prevents depolymerization of the actin cytoskeleton elicited by PbrS-RNase and delays the SI signaling that leads to pollen tube death. This work provides insights into the orchestration of the S-RNase-based SI response, in which increased PA levels initially play a protective role in incompatible pollen, until sustained PbrS-RNase activity reaches the point of no return and pollen tube growth ceases.

GDR (Genome Database for Rosaceae): resource for genomic, genetic and breeding research

NOTE

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Abstract: The Genome Database for Rosaceae (GDR, https://www.rosaceae.org) provides online resources to facilitate basic, translational and applied research for the many fruit, nut and ornamental crops belonging to the economically important Rosaceae family. Fully integrated data includes curated genome sequences, genes, transcripts, genetic maps, markers, QTL, traits, germplasm, and publications, made accessible to browse, query and download through easy-to-use web interfaces and tools. New features include MapViewer, Synteny Viewer and Breeding Information Management System (BIMS). MapViewer allows users to view and compare genetic maps with hyperlinks to map features, Synteny Viewer allows users to view the overall pattern of synteny and collinearity between genomes displayed in a circular layout. Homologous gene pairs in each block are also displayed both graphically and as tables with hyperlinks to the gene pages. BIMS has a secure and comprehensive management system for breeders to store, manage, archive, analyze and search their private breeding data which is integrated with the public genomic and genetic data in GDR. In this presentation we will highlight these new features and future development as well as provide an overview of existing resources in GDR.

Key words: Database, Genomic, Genetic, Breeding, Rosaceae

NOTE

Reconstruction of multi-generation pedigrees involving numerous old apple cultivars thanks to whole-genome SNP data

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Abstract: A number of European apple cultivars are old, some of them dating back to the Renaissance, Middle Ages or even earlier. Many other cultivars have been developed during subsequent times. In order to decipher the relationships that link some of these old cultivars, whole-genome SNP data (~250K) for over 1400 genotypes were analyzed to infer first-degree relationships and reconstruct pedigrees. We used simple exclusion tests based on a count of Mendelian error to identify up to a thousand potential parent-offspring duos, including 295 complete parent-offspring trios and a hundred duos that could be oriented. Grand-parents for some missing parents could also be inferred. Combining all this information allowed us to reconstruct multigeneration pedigrees (up to 6 generations) highlighting the central role of major founders such as 'Reinette Franche', 'Margil', and 'Alexander'. Haplotypes were deduced from genotypic data and pedigrees, and used to measure haplotype sharing between supposedly unrelated cultivars, allowing investigating further links between them. To our knowledge, such a large analysis to reconstruct multigeneration pedigrees involving (very) old cultivars selected over such time has never before been performed in perennial fruit species.

Key words: *Malus* x *domestica*; relationship inference; Mendelian error; Single Nucleotide Polymorphism; genealogy; founder; haplotype sharing

Sweet cherry: new genomic tools for the creation of varieties adapted to future conditions

NOTE

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Abstract: Sweet cherry (*Prunus avium* L.) is one of the most popular temperate fruit crops despite of its relatively high price. As sweet cherry ripens first among stone fruits, followed by apricot, peach and plum, the demand for this fruit is very high in fresh market during late spring and early summer. The annual global sweet cherry production is about 2.2 million t and shows a slightly increasing tendency. However, this species is seriously threatened by the impact of climate change. Warmer winters and higher risks of frosts in the early spring, lead to a wide range of problems related to flower and fruit set, pollen fertility, desynchronization of pollinators or novel host-pest interactions. Additionally, a higher frequency of rainfall events in spring is responsible of important damages due to fruit cracking, which is one the main agronomic problems for cherry growers. Hence, breeding programs should integrate new traits to create better adapted varieties in terms of bloom date, and its components chilling and heat requirements, in addition to classical traits related to fruit quality such as fruit weight, firmness and cracking tolerance. Given that sweet cherry has a long juvenility period and required large areas to evaluate thousands of new hybrids, Marker Assisted Breeding (MAB) will allow breeders to rationalize their programs and plant only hybrids with favorable allelic combinations for the most critical agronomic traits. With the advent of new-generation sequencing technologies, a great number of genomic data are now available in sweet cherry. High saturated linkage maps have been built using SNPs identified using the RosBreed SNP arrays (6K array and more recently the 15K array) or Genotyping By Sequencing technology, allowing the detection of numerous and accurate Quantitative Trait Loci. Using the new 'Regina' genome sequence obtained with a combination of sequencing strategies (PacBio RSII sequencing and BioNano optical mapping), we are able to identify numerous molecular markers in the QTL arears allowing a more precise MAB. Moreover, the genomic selection methodology to select hybrids for traits difficult to phenotype on many hybrids and in a short period of time is in progress. All these genomics tools will considerably increase the efficiency of sweet cherry breeding programs.

Key words*: Prunus avium L., phenology, fruit quality, climate change, adaptation, marker assisted breeding*

Visualizing the Genetics of Elite Genomes

NOTE

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Abstract: The power of SNP array data is the information contained on the genetics of genome-scanned individuals. But, for breeders, such data are usually an inaccessible deluge. The common way to deal with large datasets is a quantitative summary across germplasm, but this approach easily overlooks each germplasm individual. We propose that sensory experience of DNA information on elite individuals (parents, selections, and cultivars), just as for phenotypic information, would help breeders understand better what they have and then target development of what they want. While DNA-based genetic information cannot yet be heard or tasted, one way for breeders to "experience the genotype" of an elite individual is by seeing it. Visual aids are recognized as vital tools for transmitting information for long-term memory retrieval and empowering users to leverage their creativity and understand large data patterns. With quality-controlled results from SNP arrays, the holistic "genotype" of an elite individual can be described by three genetic vectors of breeding relevance: allelic variation (patterns of similarity among alleles within each locus), linkage (patterns of recombination among loci within each individual), and relatedness (patterns of shared ancestry among individuals within a population). Together, the patterns of alleles over loci over generations can provide comprehensive genetic knowledge on elite germplasm individuals to improve the precision with which individuals are used in breeding. For apple and sweet cherry, we have visualized the genetics of elite genomes to reveal pedigree relationships among cultivars, trace origins and distributions of valuable trait locus alleles in ancestors and parents, and observed outcomes of introgression. The practical implications of these new insights are enormous. Routine use of genome-wide genetic information in breeding decisions will benefit from improvements in graphical genotyping tools.

Key words: ancestry, fruit breeding, haplotype mosaics, graphical genotyping, IBD, recombination, SNP arrays, trait locus alleles, translational genetics

Genomic tools of sweet cherry for the breeding programs

NOTE

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Abstract: Sweet cherry (*Prunus avium*) is one of the most popular fruit crops over the world. In Japan, fruits of sweet cherry are sometimes compared to jewelries because of the extremely good quality. The breeding programs would contribute for developing new sweet cherry cultivars with higher fruit quality. However, the sweet cherry breeding is time- and cost consuming because large fields are required for a long period. To overcome this situation, we prepared genetic resources for sweet cherry, which can improve the breeding efficiency, such as whole-genome sequence data, high-density genetic maps with single nucleotide polymorphisms (SNPs) through restriction-site associated DNA sequencing technology, microsatellite, indel, and SNP markers based on whole-genome resequencing analysis of modern varieties, and a user-friendly genome database, i.e., DBcherry (http://cherry.kazusa.or.jp). The information was indeed helpful for quantitative trait locus analysis to identify candidate genes for the harvesting date and the fruit flesh colors. The genomic information obtained from this study would accelerate the breeding programs in sweet cherry and its relatives like sour cherry (*P. cerasus*) and Chinese sour cherry (*P. pseudo-cerasus*).

Key words: draft genome; genetic maps; single nucleotide polymorphisms; sweet cherry

T-Lidar as a new high-throughput methodology for studying the genetic determinisms of apple tree architecture

NOTE

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Abstract: Growth, branching and reproduction processes are highly organized in space and time in plants. In many Rosaceae fruit tree species, this organization has been described by architectural analyses to evaluate tree plasticity in response to environmental conditions and genetically determined traits. However the study of genetic determinism of architectural traits requires the observation of large populations of individuals over several years which is hardly compatible with manual or classical digitizing techniques. We thus investigated new solutions based on T-Lidar technology that is currently developed mainly to characterize forest stands.
First, we explored the possibility to extract from T-Lidar scans features of tree form on a limited set of genotypes with contrasted architectures. Different precisions and distances of laser scans were tested to assess sensitivity of branches detection and determine an optimal combination between acquisition times and scan resolution. The scan analysis required the use of different algorithms to segment and extract the point clouds corresponding to each individual tree and to rebuild their 3D architectures. This allowed us to extract several descriptors of tree height and shape, total leaf area, leaf area distribution and density and light interception efficiency. Tree reconstructions gave promising results with R^2 ranging from 0.91 to 0.78 for the total number of axes and leaf area when T-Lidar data were compared to digitizing or manual measures. Second, scans were collected on a large range of individuals (around 1000) corresponding to the French core collection of apple varieties. All variables showed a high variation and heritability and architectural morphotypes were distinguished within the population by a clustering method. GWAS analyses are currently performed. The next steps of our research will be the creation of a dedicated workflow for scan analysis allowing faster computations and the improvement of the set of descriptors related to tree topology that could be used for genetic studies.

Keywords: scans, growth, branching, leaf area, tree shape, morphotypes

Bioinformatics Analysis for Genome Sequencing and Population Genetic Study

NOTE

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Abstract: In recent years, a number of *de novo* genome assemblies have been published. Complete genomes could greatly improve our understanding of the species. However, producing high-quality reference genomes especially for complex plants is challenging because of the short-read lengths of second-generation technology. Third-generation sequencing technologies, which can generate multi-kilobase sequences with the potential to greatly improve genome assembly. Here we provide several assembly strategies using Third-generation sequencing technologies and Hi-C-based proximity-guided assembly. We also introduce some comparative genomic approaches, such as evolutionary processes and adaptation to environment. In addition, we highlight bioinformatics strategies used in population genomics: i) Genome-wide association study (GWAS) identifies candidate functional genes; ii) Identification of selective sweeps in domesticated populations.

Genetic mapping of novel loci for resistance to European canker and apple scab

NOTE

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Abstract: Although marker assisted selection (MAS) is now accepted technology for improving efficiencies in apple breeding programmes internationally, there are two major reasons why identification of new genetic markers must remain as high priority for researchers. Firstly, some significant diseases lack any markers for resistance genes used in breeding. An example is the intractable disease European canker (*Neonectria ditissima*), for which resistance is difficult and laborious to phenotype in breeding populations. We report the identification of a novel large-effect quantitative locus for resistance to European canker (*Rnd1*) on Linkage Group (LG) 14 of *Malus* 'Robusta 5' (R5) and the design of a Taqman® marker to screen breeding populations derived from R5.

The second reason for continuing with marker discovery is to enable pyramiding of resistances to a single pest or disease from the onset of breeding: this increases durability of resistance by preventing the selection of new races of the pathogen. Breeders who are introgressing resistances into new selections require a toolbox of robust high-throughput genetic markers for different resistances to each pathogen. We report the mapping of the apple scab (*Venturia inaequalis*) resistance *Rvi19*/*Vr* derived from Russian apple R12470-7A, located distally on LG2, distinct from *Rvi2*/*Vh2*, which is approximately 7 Mb distant. Work is in progress to develop a high-throughput MAS marker for *Rvi19*/*Vr*.

Key words: *Neonectria ditissima, Rnd1, Venturia inaequalis, Rvi19*/*Vr*, high-throughput, MAS

NOTE

Importance of bacterial strain-specificity to improve fire blight resistance and management in apple

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Abstract: Fire blight susceptibility of commercial apple cultivars is a great risk to orchard profitability, requiring intensive disease management. Although expensive, chemical use can prevent fire blight infection, but not once the pathogen is in plant tissue, and could lead to antibiotic resistant strains. The majority of fire blight genetic loci identified so far in apple provide resistance against single or few strains of the fire blight bacterium *Erwinia amylovora*. Populations of *E. amylovora* are mixtures of multiple strains, characterized by genomic and pathogenicity variability. Infection severity is largely due to interaction between a specific bacterial strain and specific apple cultivar, influenced by the environment. Knowing the response of a cultivar to the strains it might encounter will benefit fire blight management and resistance breeding, including more accurate disease forecasting, spray and pruning scheduling, seedling and elite breeding material screening, identification of new resistance sources, and development of improved resistance cultivars. We have identified a total of 13 novel marker-trait associations linked to fire blight resistance from a *Malus sieversii* × *Malus* × *domestica* mapping population through interval mapping at 95% confidence and Kruskal-Wallis analysis at *P*-value= 0.005. Interaction between experimental conditions in the greenhouse and field, and differences in virulence levels of strains might be responsible for strain- and year-specific QTLs. Stability of identified loci must be tested over multiple years, and may need to be validated in different genetic backgrounds before application. Our results also show significant differences in cultivar response to specific strains, indicating the importance of cultivar x strain interactions for fire blight management and breeding. Profiling and pathogenicity testing of fire blight bacterial strains present across orchards will make the results relevant for fire blight management and breeding scion and rootstocks. Durable resistance barriers can be created by combining multiple monogenic and polygenic resistances with complementary action toward different strains, through marker-assisted backcrossing, cis-genically or *via* genome-editing.

Key words: *Erwinia amylovora*, genetic resistance, QTL mapping

Exploring the genetic variability of peach skin phenolics and triterpenoids as natural defenses against brown rot

NOTE

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Abstract: Brown rot caused by *Monilinia* spp. provokes dramatic losses of peach in all production regions. In this context, the general objective of our work is to develop sustainable ways to fight against this damaging disease.
This study explored phenolic and triterpenoid diversity of fruit skin together with brown rot resistance. A Brazilian peach collection and an interspecific cross between *P. davidiana* and *P. persica*, each of 120 genotyped individuals, have been screened for 3 years, for susceptibility to *M. fructicola* and to *M. laxa* respectively. Some genotypes of the two populations showed low or null infection the 3 years.
Secondary compounds of fruit skin of all genotypes were analyzed two years by HPLC-DAD. Depending on the genotypes, 30 to 40 compounds including triterpenoids, hydroxycinnamic derivatives and flavonoids, were quantified and around 20 were identified by mass spectrometry. Among these, phenolic esters of triterpenoids were identified for the first time in peach fruit.
The correlation of quantitative data between years showed their robustness. A large diversity in contents of the compounds was observed.
The association and QTL analyses proved to be complementary and led to assumptions about the co-locations of loci controlling different traits, especially for compounds with differential levels in the skin of peaches and nectarines. These analyses will help deciphering the genetic control of fruit skin phenolics and triterpenoids and pave the way to the identification of the underlying genes. Detection of loci related to resistance will provide tools for the implementation of marker assisted selection.
Correlations between compounds and infection traits led us to hypothesize that some compounds from peach skin could play a role in the brown rot control. The fungicide activity of these compounds is being tested *in vitro*.

Key words: Disease resistance, QTL, GWAS, *Monilinia spp.*, *Prunus persica*

This project is supported by Agropolis Fondation reference ID 1503-003 _ ANR-10-LABX-0001-01

Resistance to sharka in Stone Fruit trees : Genetic and genomic technologies for new breeding strategies

NOTE

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Abstract: Sharka is the most detrimental disease for stone fruit trees. It is due to the infection, through aphids or grafting, by the *Plum Pox Virus* (PPV). Few sources of resistance have been described in *Prunus* sp. and it is seriously lacking in crop species, threatening fruit production.

Depending on the *Prunus* species, different approaches have been set up. In *Armeniaca*, we searched for the geographical origin and genetic diversity of resistance (Decroocq et al., 2016). We identified PPV resistant apricot natural populations among the Central Asian mountain forests. They are currently being used for mapping the resistance determinants through GWAS which will be compared to the ones identified in the cultivated germplasm (Mariette et al., 2016).

In *Amygdalus*, resistance to sharka was documented in two peach related species, i.e. *P. davidiana* and *P. dulcis* –almond- (Pascal et al. 1998, 2002). While in *P. davidiana* the number of resistance sources is limited to one clone, previous studies described almond as non-host, resistant or susceptible to PPV. Such discrepancies reflect a wide variability of responses depending on the almond genotype or on the viral isolate. In the frame of the FP7 STONE project, *P. dulcis* and related species sampled in their area of origin were assessed experimentally. The most promising are being used to map the genetic determinants for resistance and to implement genomic selection to speed up the introgression of resistance in peach.

In plum species, which are more amenable to genetic transformation, PPV susceptibility factors have been targeted through gene silencing. PPV resistant lines of diploid plums (*P. salicina*) in which one susceptibility host factor has been impaired are by now being used for introgression in other diploid *Prunus* sp. as well as for genome editing in the frame of the H2020 TESS project (2018-2021).

Keywords: sharka, *Prunus*, GWAS, genomic selection, genome editing

Advances in peach breeding: from discovery to application

NOTE

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Abstract: Environmental challenges, changes in production systems, and human preferences are driving the need for delivery of new peach scion and rootstock cultivars for sustainable production. The latest innovations in science and technology are integrated in plant breeding to provide sustained solutions to production challenges and market demands. Understanding the genetics of the key traits of importance for peach tree production and sustainability is the main generator of advances in breeding of new cultivars that can address changing needs of the industry. Characterization and utilization of genetic diversity, and application of genomic technologies are needed to improve breeding efficiency in both scion and rootstock cultivar development. Functional and comparative genomics studies utilizing synteny and collinearity among species and genera are often applied in development of new cultivars with desirable traits via marker-assisted selection in breeding programs. Routine DNA-informed breeding has become reality via translation of the new discoveries into practical breeding-friendly tools and knowledge. A multidisciplinary approach that incorporates the application of modern technological tools in peach scion and rootstock cultivar development in the Clemson University peach breeding program will be presented.

Key words: marker-assisted breeding, fruit quality, disease resistance, germplasm introgression

Development of a 70K SNP array for pear and its efficiency in characterizing the genetic diversity of *Pyrus*

NOTE

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Abstract: The development of genetic tools for high-density and large-scale genotyping have enabled the dissection of important traits and the evaluation of genetic diversity in several plant species. With the final objective of enhancing the implementation of marker assisted breeding in pear, we designed a highly efficient Affymetrix Axiom Pear 70K Genotyping Array and we used it to screen the entire *Pyrus* collection held at USDA National Clonal Germplasm Repository (NCGR) in Corvallis, OR. This large collection includes more than 2,000 clonal pear accessions, encompassing nearly every known *Pyrus* species and providing a valuable source of diversity to be exploited in pear breeding programs. From the whole-genome re-sequencing of 55 diverse pear accessions we developed 700,000 SNP markers that we tested on a subset of the collection. We then selected the most robust and informative 70,000 SNPs to build the commercial Axiom Pear 70K Genotyping Array, currently the densest SNP array available for pear. We further validated this array by genotyping the remaining NCGR pear accessions and more than 90% of the SNPs were classified as high quality and polymorphic (PolyHighResolution). We are currently using this large dataset to evaluate the genetic diversity of this pear germplasm collection, which will provide useful information for pear breeding.

Key words: Marker assisted breeding; germplasm collection; large-scale genotyping; high-density SNP array

NOTE

RNA binding protein MhYTP2 enhances drought resistance and water use efficiency by activating ABA and ethylene signaling in apple

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Abstract: Identification and characterization of genes involved in improving water use efficiency (WUE) are crucial for crop breeding. In this study, we characterized a gene encoding an RNA-binding protein cloned from *Malus hupehensis*, *MhYTP2*, for its function in relation to the WUE. *MhYTP2* overexpression improved apple WUE based on the discovery that, transgenic plants had more biomass accumulation than wide type plants under both well-watered and drought conditions. Further analysis indicated that MhYTP2 improved drought resistance and WUE probably by decreasing the stomata aperture via increasing ABA level and activating ethylene signaling. MhYTP2 may activate ethylene signaling by up-regulating the gene transcription of its protein target, an acireductone dioxygenase, MhARD4, which participates in recycling of the ethylene precursor S-adenosylmethionine. *MhYTP2* predominantly expresses in apple roots. Interestingly, higher P_n, instantaneous WUE and root water potential were observed when *35S:MhYTP2* plants were used as rootstocks than being used as scions, indicating MhYTP2 plays crucial roles in roots than in aerial parts, implying it can be used for improving drought resistance and WUE of apple rootstocks, and potentially also of other crops suitable for grafting.

Keywords: apple, abscisic acid; ethylene, graft; RNA-binding protein; drought stress; water use efficiency

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NOTE

PbWoxT1 mRNA from pear (*Pyrus betulaefolia*) undergoes long-distance transport assisted by a polypyrimidine tract binding protein

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Abstract: In woody plants, some noncell-autonomous mRNAs can move through the phloem to regulate development. Recent researches have identified some phloem-mobile mRNAs, but little is known about the mechanisms of the long-distance transport of mRNAs in the phloem. Here, we reported that *PbWoxT1* mRNA, containing a WUSCHEL-RELATED HOMEOBOX (WOX) domain, was showed to be phloem-mobile in pear(*Pyrus betulaefolia* cv.Du Li). Grafting experiments indicated that a 548-bp fragment is critical in the long-distance movement of *PbWoxT1* transcripts. In addition, electrophoretic mobility-shift assays and further analyses demonstrated that PbPTB3, a polypyrimidine tract binding protein, could bind to the CUCU motifs present in the *PbWoxT1* transcripts, to regulate the trafficking of *PbWoxT1* mRNA. Taken together, our results suggested the noncell-autonomous manner of WOX gene in pear and enriched the mechanism of long-distance transport of WOX gene.

Key word: mRNA transport; PbPTB3; *PbWoxT1*; *pyrus betulaefolia*; ribonucleoprotein(RNP) complex

Exploration and Innovation of Apple Germplasm Resources

NOTE

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Abstract: Apple (*Malus domestica* Borkh.), one of the most widely produced and economically important fruit crops in temperate regions, is a significant source of flavonoids in the human diet and is among the top nutritionally rated and most widely consumed fruits worldwide. However, apple production is challenged by serious inbreeding problems. The narrowing of the hereditary base has resulted in apples with poor nutritional quality and low flavonoid contents. Thus, the whole genome re-sequencing and population genetics analysis of 117 germplasm resources of apple were performed. The origin and domestication of apple were studied and the original characters and breeding value of *M. sieversii* were demonstrated. Besides, using *Malus sieversii* f. *niedzwetzkyana*, the hybrid isolated populations were constructed and its trait heredity and flavonoid metabolism mechanism were explored. Transcription factors involved in flavonoid metabolism of red-fleshed apple, such as *MYB12*, *MYB22*, *MYB16* and *bHLH33* were isolated and identified. And eventually, a scientific apple breeding system including quality formation, quality breeding, quality varieties, and quality maintenance were summarized. These processes, combined with good horticultural practices, are essential for the development of high-flavonoid apple lines and for broadening the genetic basis of cultivated varieties.

Key words: *Malus sieversii f. niedzwetzkyana*; origin; flavonoids; breeding

Genetic breeding of apple rootstocks

NOTE

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Abstract: Rootstock is increasingly a key factor for plant growth and development, fruit yields and quality of tree fruits. China has plenty of germplasm resources of Malus, considerable efforts have been made for the apple rootstock breeding over the last 60 years in China. In this presentation, overview for breeding challenge of apple rootstocks in China was firstly introduced. Thenafter description for our research of apple rootstocks genetic breeding was given and lastly, future outlook on apple genetic breeding was discussed on basis of the current advances in the rootstock, particularly dwarfing rootstock breeding.

Key words: Apple rootstocks，genetic breeding，gene markers

Peach breeding and innovation in JAAS

NOTE

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Abstract: Peach breeding program was started in the 1950's in JAAS, with the first cross was made in 1956. Early ripening and for fresh market are the main objectives in 1960s, and canning peach is another objective in 1970s breeding program. Flat peach and nectarine breeding program were started in 1980s. Good fruit quality and keeping quality, diversity are the main objective in nowadays. 49 varieties have been released total, including 35 peach, 4 nectarine, 6 flat peach, 2 flat-nectarine and 2 ornamental peach. Among the 20 varieties released since 2001, 18 varieties are for the fresh market, 2 for ornamental use. Most of the varieties are pollen fertility, do not need artificial pollination in production. The flesh texture are getting firmer, with 2 varieties soft-melting, 2 varieties crisp, the others are hard-melting. Some of the varieties are planted wildly in China. Germplasm improvement and innovation mainly work on the red flesh, narrow leaf, pillow tree and low chill. Flat peach with red flesh, or narrow leaf, and narrow leaf peach and nectarine have been selected after generations, though the fruit size, quality need to improve. Low chill peach selections show good quality and adaptability in tested area in south China, imply that these area can harvest peach in early May.

Key words: peach; breeding; genetic improvement

Research Progresses in Cherry group of Beijing Academy of Forestry and Pomology Sciences

NOTE

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Abstract: Three main research contents have been investigated in our group.
The first one is germplasm collection. Till now, germplasm resources including sweet cherries, sour cherries and rootstocks were collected, conserved and evaluated. An in vitro conservation method was created, which could conserve these germplasm to 18 months. The guideline for the conduct of tests for distinctness, uniformity and stability-cherry was established by us and germplasm evaluation was conducted based on the guideline.
The second one is sweet cherry breeding. The breeding aims are self fertile, large size, hard flesh, multiple resistances, and elongated mature window and so on. Molecular markers, such as self fertile marker, fruit skin color marker and leaf spot marker were developed and used in sweet cherry breeding. Furthermore, a high density genetic linkage map of sweet cherry was built and QTLs of important fruit characters, such as fruit size, sweetness and acid were mapped on it. Up to now, 5 cultivars have been released, which are ‘Caihong’, ‘Caixia’, ‘Xiangquan 1’, ‘Xiangquan 2’, ‘Zaodan’. Besides, 9 superior lines and 15 superior plants of sweet cherry were under investigation. In addition, molecular fingerprint of 50 sweet cherry varieties were built by 18 SSR makers.
The third main job is rootstock breeding. The breeding objectives are easily asexual reproduction, highly grafting compatibility, early fruiting, and multiple resistances, and so on. By using distant hybridization and embryo rescue technique, serials of filial generation were obtained. Through molecular identification and multiple resistance screening, several superior lines were gained, which were propagated by cuttage. After 15 years grafting evaluation, ‘Landing’ series and ‘Jingchun’ series root stocks were released and applied to Plant Variety Protection.
These achievements will provide technical support for the sweet cherry industry.

Key words: Germplasm collection; Sweet cherry; Root stock; Molecular marker; Breeding;

Leveraging historical performance data from multiple environments through international collaboration to predict germplasm performance on a global scale

NOTE

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Abstract: Knowledge of environmental stability of predicted performance of selection candidates can improve confidence in deploying locally adapted germplasm in new environments as well as introducing potentially elite exotic germplasm into domestic environments. However, despite the global scale of rosaceous tree crop production, there is limited information to study genotype-by-environment interaction in these crops. Most breeding programs only trial their material in local environments and there is limited documented international replication of germplasm among field evaluation trials. Here we describe initiatives supported by RosBREED and the Genome Database for Rosaceae to develop methods and infrastructure to predict phenotypic performance on a global scale for rosaceous crops. Given that historical phenotypic data is the response of an individual's cumulative alleles to the specific environment (climatic, edaphic, management, etc.), and chromosome segments shared among individuals can be characterised using genome-wide SNPs, our hypothesis is that a multivariate genomic prediction model can be used to predict the genetic potential of all germplasm for all environments included in the analysis. This approach does not require clonal replication of individuals among locations and produces predictions of germplasm in environments in which they have not been specifically tested. Our vision is to develop a database to manage anonymous-contributed data with which users can interact to input genotype and phenotypic data and output performance predictions of their specific germplasm across the range of global environments.

NOTE

Collaborative project to identify direct and distant pedigree relationships in apple

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Abstract: Pedigree information is fundamentally important in breeding programs, enabling breeders to know the source of valuable attributes and underlying alleles and to enlarge genetic diversity in a directed way. Many apple cultivars are related to each other through both recent and distant common ancestors. As apple trees are clonally propagated, long-lived, and widely adapted, many of the ancestors of modern cultivars are still present in global germplasm collections. Use of apple SNP arrays enables identification of direct and distant pedigree relationships with precision. An example is the elucidation of the 'Honeycrisp' pedigree using the 8K SNP array, which enabled further findings regarding the inheritance of important alleles for traits including scab resistance and soft scald susceptibility.

To facilitate more discoveries across apple germplasm, a large-scale collaborative apple pedigree reconstruction project has been initiated. This project utilizes output from the Illumina Infinium 20K and Affymetrix Axiom 480K apple SNP arrays, a high quality genetic linkage map for the 20K array SNPs, and a data curation pipeline developed through the FruitBreedomics and RosBREED projects. Techniques using shared haplotype length statistics will be used alongside historical information to deduce distant pedigree relationships. The project involves various experts, germplasm collections, and academic institutions around the world and is open for further extension. It will provide findings useful for breeding programs, germplasm collections, geneticists, and historians.

Key words: haplotype, *Malus* × *domestica*, pedigree, SNP array

Transcriptomic profiling of endodormancy to ecodormancy transition in apricot (*P. armeniaca*).

NOTE

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Abstract: To overcome harsh environmental conditions in winter, perennial trees protect themselves and arrest vegetative growth by entering dormancy. Endo-dormancy is maintained until a genotypic-specific period of chilling has been fulfilled. The fulfillment of chill requirement leads the trees to eco-dormancy, in which the buds can sense optimal temperature for development, resulting in bud break. The genetic mechanism underlying the transition of endo-dormancy to eco-dormancy is not fully understood. To characterize the genetic controls of bud development and maintenance of dormancy, we examined gene expression profiles in bud tissue over time and across cultivars with different chill requirements. In this study, we utilized apricot (*Prunus armeniaca*) varieties in the range of ~500 to 1000 hours of chill requirements. Floral buds from four apricot cultivars were collected at 0 chill hours, 100 chill hours, 400 chill hours, 800 chill hours, sepals visible stage, and petals visible stage, then investigated by RNAseq. Over 90% of the reads were mapped to the peach (*Prunus persica*) reference genome. As trees with different chill hours were moving through stages of dormancy and tissue development at different rates, the samples were analyzed based on physiological stage instead of time point. Prior work points to the importance of the DAM gene family, which is a group of transcription factors that regulated peach bud dormancy. This works confirms their importance, with 3 of 6 genes being differentially expressed over time. Overall, 26,873 genes in the genome were analyzed by DEseq2 to identify differential expression genes. By comparing the expression between endo-dormancy and eco-dormancy, 1,367 genes were significantly differentially expressed, with 1,203 upregulated and 164 downregulated at the eco-dormancy stage. Examining genotype-specific differences, which may illuminate pathways responsible for chill requirement, 5,923 significant genes were differentially expressed between two genotypes. Gene orthology analysis identified genes involved in oxidoreductase activity which may be involved in stress response or regulating sugar transportation during bud development, and genes which can sense photoperiod,

resulting the alteration of dormancy phases. Additionally, differential expressed genes are also involved in plant hormones such as gibberellin and abscisic acid biosynthesis associated with dormancy regulation. Further investigation is needed to integrate the gene expression profiles with molecular pathways associated with chill requirement and dormancy.

Key words: dormancy; floral bud; gene expression

NOTE

Elucidating the Molecular Mechanisms Underpinning a Novel Acyanic Trait in Apple

NOTE

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Abstract: Apple (*Malus pumila* Mill.) is an important deciduous fruit crop in South Africa, with approximately 43% exported overseas or to African markets. Although traditional "green" fruited cultivars such as 'Granny Smith' and 'Golden Delicious' are valuable export cultivars, they are prone to development of blush (red pigmentation of the skin) under certain environmental conditions, which may in turn lead to downgrading or rejection of shipments. Nearly all apple cultivars have red tinged shoots and pink flowers and fruitlets, which either stay red or turn green during development. However, representatives of a rare acyanic (absence of anthocyanins) phenotype, has been observed in Meopham, England and in Drostersnes, South Africa. The purpose of the current study is to elucidate the genetic and molecular mechanisms underlying this phenotype. Through genetic mapping, the chromosomal location of the gene has been established (to a region <3.5MB) and two strong candidate genes have been identified. Functional analysis of the coding sequences and surrounding sequences for these two genes is currently being performed to determine which gene is likely responsible for the observed phenotype. In addition, metabolomic and transcriptomic analyses are currently underway to elucidate the failure of anthocyanin accumulation in the acyanic apple phenotypes. Knowledge of the gene/s responsible will provide fundamental knowledge regarding anthocyanin metabolism, and will guide design of markers that could be used to select acyanic carriers to reliably breed "green" cultivars.

Key words: acyanic; Malus, metabolomics, transcriptomics

QTL analysis of flowering time in sweet cherry

NOTE

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Abstract: Flowering time is a relevant trait in sweet cherry (*Prunus avium* L.) production. Flowering time depends on climatic conditions and varies for each genotype. Sweet cherry cultivars must be suited to climatic conditions in growing areas to prevent crop loss associated with floral freeze injury and irregular floral development. Cultivars with low chilling requirements often show early flowering and may be relevant for growing at low chilling regions. In this work, 411 individuals from six sweet cherry families (4 cross-pollinations and 2 self-pollinations) were used to investigate the genetics of flowering time. These families derive from the landraces 'Cristobalina' and 'Ambrunés' and breeding cultivars including 'Brooks', 'Lambert' and 'Vic', and show from extra-early to late flowering dates. The families were phenotyped during four years (2015 to 2018) and genotyped with RosBREED Cherry 6K Illumina Infinium SNP array. Quantitative trait loci (QTL) analysis was carried out in a combined way for the six populations using the Bayesian approach implemented in FlexQTL™ software. Various QTLs showing strong to decisive evidence were identified in various linkage groups. Major QTLs controlling this trait in this plant material as well as QTLs alleles putatively associated to extra-early flowering time in the low-chilling cultivar 'Cristobalina' will be discussed.

Key words: 'Cristobalina'; flowering date; early flowering; QTL; marker-assisted selection.

NOTE

Gene expression changes in relation to the phytohormonal signaling of the resistance to *Plum pox virus* (PPV, sharka) induced in peach by almond grafting

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Abstract: *Plum pox virus* (PPV) is a limiting factor for peach production in those areas that are affected. Although no natural sources of resistance have been described for peach, recent studies have demonstrated that grafting almond cultivar Garrigues onto GF305 peach seedlings heavily infected with PPV progressively reduces disease symptoms and virus accumulation. This response appears to be specific between almond and peach. Furthermore, grafting Garrigues onto GF305 before PPV inoculation completely prevented virus infection, showing that resistance is constitutive and not induced by the virus. Previous results also showed the role that SA and the cytokinin tZ play in virus infection and in the induced resistance in peach grafted onto almond. To unravel the phytohormonal signaling of this mechanism, we analyzed the gene expression of 14 different genes involved in pathways related to the growth-related and stress-related phytohormones involved in this induced resistance. First results showed that expression of *PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN*, *CHORISMATE MUTASE 2* and *ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR 4* genes seem to be related to the signaling of this induced resistance.

Key words: PPV, sharka, *Prunus*, phytohormones; breeding; qPCR

Heritability of epigenetic marks and its impact on phenotypic variability in apple

NOTE

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Abstract: Because of its auto-incompatibility, apple is an obligate outcrosser and highly heterozygous. To maintain traits, apple trees are grafted thereby ensuring the stability of these varieties. The Golden Delicious variety was initially identified over 120 years ago and has been grafted since. Due to this clonal propagation, the Golden Delicious genome today is mostly identical to the initial pippin initially obtained. Yet, phenotypic variability in clonally propagated apple is apparent, suggesting that epigenetic mechanisms may contribute to that variability. However, epigenetic transmission mechanisms are not yet well known in perennial plants. Moreover, the plasticity of the epigenome over time may allow adaptation to environmental conditions and pathogen attacks. Therefore, we can expect that the current apple epigenome differs from the original progenitor. To explore this question, we defined two main lines of research:(i) Because DNA methylation is the hallmark of epigenetic marks, we want to better understand the importance of this mark in apple development using two approaches based on the CRISPR-Cas9 technology: a) Global reduction of DNA methylation by targeted knockout of the apple homologues of the DNA methylation maintenance enzyme MET1 of Arabidopsis. b) By targeting DNA methylation at specific loci in the genome and assessing its effect on gene expression. (ii) In order to assess the heritability of epigenetic marks during sexual and asexual reproduction in apple, we compare the epigenomes of donor-trees, grafts (asexual) and seedlings obtained from self-fertilization (sexual). Here, as a model variety we use our Golden Delicious double haploid line, which greatly simplifies downstream bioinformatic analyses. First results suggest numerous differentially methylated regions (DMRs) between each of the analyzed samples. Interestingly, there are more DMRs between seed and graft or tree compared to tree and graft. To understand the impact of these DMRs on gene expression we will carry out transcriptomic analyses. This work will improve our understanding of the contribution of epigenetics to the phenotypic diversity that is observed within an apple genotype.

Key words: DNA methylation, DMR, CRISPR-Cas9

NOTE

Variation of allergenic lipid transfer protein in diverse Chinese peach cultivars

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Abstract：Background: Fruits are a major cause of food allergy in adults. Peach is the most frequently reported allergenic fruits in China, the main allergen is Pru p 3 (lipid transfer proteins) that can cause severe allergic reactions to fruits, but little is known about Pru p 3 content in a large set of Chinese different cultivars.**Method:** This study was to determination of the levels of Pru p 3 LTP in about 100 of core peach cultivars and accessions selected by genetic diversity evaluation. Pru p 3 content was measured by a reliable and sensitive double monoclonal antibodies sandwich ELISA. Different ripening stages and parts of fruit were also assessed.**Results:** The content of Pru p 3 was increased with the progress of ripening for most peach cultivars, decreased for a few cultivars. The Pru p 3 content of mesocarp was much lower than that in epicarp. We divided the tested peach accessions into three groups according to their potential allergencity, the first is below 10μg/g fresh weight, including some early ripe nectarine and hard-melting peach (landraces) and wild peaches, the second is the medium group with Pru p 3 content between 10-40μg/g, including most peach cultivars from north China. The high Pru p 3 content is above 40ug/g, majority is in the cluster of Shanghai-cling juicy-honey cultivars, such as Yulu flat peach, Hujingmilu in the southeastern China. Since the nature of pathogenesis related protein for Pru p 3, its content was variable under different cultivation practices and among different years. It seems that the Pru p 3 content is related to peach aroma and flavor quality, the most delicious peach usually have higher Pru p 3 content, those of lower quality with no aroma have lower Pru p 3. A few very low Pru p 3 content cultivars have been selected out.

Key Words: Pru p 3, Allergen quantification, Cultivar, Lipid transfer protein.

Developing genomic and epigenomic resources for almond [*Prunus dulcis* (Mill.) D.A.Webb]

NOTE

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Abstract: Almond, a relevant nut crop whose production in California has been valued at more than $21.5 billion, exhibits an age-related disorder known as non-infectious bud failure (BF) that affects vegetative bud development, indirectly affecting kernel-yield. Despite the relevance of this major crop and threat of an epigenetic-related disorder, the genomic resources and application of epigenomic approaches for almond are lacking. As a multi-institutional interdisciplinary effort, genomic resources are being developed and deployed to address relevant questions related to the genetics, epigenetics and productive performance of almond. Additionally, BF represents an opportunity to address aging in a commercially-relevant and vegetatively-propagated, perennial crop. By generating whole-genome sequences, gene predictions and annotations, as well as resequencing select almond genotypes via bisulfite sequencing, the interrogation of aging in almond will provide a novel perspective to the study of aging in perennial productive plants. Application of these methods will allow for the identification of biomarkers of age and predictors of BF potential in almond accessions. Deployment of such genomic resources will enable researchers to ask and address relevant questions considering almond as a unique biological system and will no longer be solely reliant on the resources available from other species (i.e. peach). These resources will also allow for more comprehensive comparative genomics within *Prunus*. The outcomes from this research will have an impact on almond breeding, commercial nursery propagation, almond crop management and can also be translated to other important perennials within Rosaceae species.

Key words: *Prunus*; epigenetics; methylome; genome assembly; comparative genomics

Control of bud dormancy process in apple: a genetic-molecular study.

NOTE

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Abstract: Dormancy is an adaptive mechanism that enables plants to survive unfavorable climatic conditions and allows flowering to occur only when the conditions are more permissive. The production of temperate fruits, such as apple (*Malus x domestica* Borkh.), is closely related to bud dormancy, given that a well-adjusted dormancy cycle is crucial for the achievement of their full genetic potential. Dormancy in apple is triggered by exposure to low temperatures and therefore, the predicted impact of the ongoing climate change will result in difficulties for apple production. The mechanisms that regulate dormancy are highly heritable, suggesting a strong genetic control of this trait. However, the genetic networks controlling dormancy process in apple are still unknown. In this communication, we will present our last results on the identification and characterization of candidate genes in the control of dormancy process in apple. At the genetic level, we have explored an apple core collection established in France to identify allelic variation present in genes involved in bud dormancy. For this end, we have developed a target-enrichment approach of candidate genes (CGs) coupled to next generation sequencing. We have made use of the sequencing data to perform a GWAS that allowed us to narrow down the zone of interest and precise the list of CGs associated to bud break and dormancy. Notably, the allelic variation found in some of the identified CGs could have an effect on their biological function. At the molecular level, we have carried out protein-protein interaction studies in order to define molecular complexes formed by Dormancy-Associated MADS-box (DAM) and other MADS-box proteins related to the dormancy process regulation. This study revealed that an intricate transcriptional regulatory network formed by MADS-box protein complexes might operate at different phases of the dormancy process.
Together, these studies contribute to a better characterization of key processes in dormancy molecular control, as well as to identify possible biotechnological resources for application in breeding programs.

Keywords: bud dormancy, *Malus x domestica*, *DAM* genes.

NOTE

Toward the Molecular Cloning of Two Genes Conferring Susceptibility to Apple Chlorotic Leaf Spot Virus Derived from Wild *Malus* Accessions

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Abstract: Apple chlorotic leaf spot virus (ACLSV) causes top working disease on some apple accessions, particularly the Asian ones, thereby significantly reducing fruit production. Some susceptible accessions, such as *Malus sieboldii* and *M. prunifolia*, are used by East Asian apple growers as rootstock and as breeding material for rootstock. Thus, the control of top working disease is crucial to achieve sustainable crop production. Although a solution is to develop ACLSV resistant rootstock, inoculation tests may require up to 3 years to obtain promising results. Therefore, molecular markers that can distinguish resistant plants would dramatically save time and associated labor. In the present study, we focused on two susceptible accessions *M. sieboldii* 'Sanashi 63' and *M. prunifolia* 'Seishi' because they are used in our rootstock breeding program and show distinct disease symptoms and severity. Both accessions demonstrated that they have one dominant gene conferring susceptibility to ACLSV and resulting in top working disease. These genes are designated as *Cv1* and *Cv2* and thus 'Sanashi63' and 'Seishi' are designated as *Cv1*/*cv1* and *Cv2*/*cv2*, respectively. We performed linkage analyses using two different segregating populations and mapped *Cv1* in the upper distal end of chromosome (chr) 10 and *Cv2* in the lower distal end of chr 4. Their estimated candidate regions spanned 4.7 Mb and 0.56 Mb in the GDDH13 genome, respectively. Furthermore, to identify *Cv1* and *Cv2* themselves, we obtained approximately 2000 additional F1 individuals for the fine mapping of *Cv1* and *Cv2*. Currently, the screening of recombinant plants, their detailed genotyping and phenotyping are being simultaneously performed. The molecular cloning of responsible genes will help elucidate the virus–plant interaction.

Key words: *Cv*; molecular marker; rootstock breeding; top working disease

NOTE

Allopolyploid origin in *Rubus* (Rosaceae) inferred from nuclear granule-bound starch synthase I (*GBSS*I) sequences

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This work was supported by the National Natural Science Foundation of China (31272134).

Abstract: *Rubus* L. is a large and taxonomically challenging genus which exhibits apomixis, polyploidy and frequent hybridization ubiquitously. Most of Chinese *Rubus* are mainly concentrated into two major sections, diploid *Idaeobatus* and polyploid *Malachobatus*. It has long been controversial to discuss the evolutionary direction of *Rubus*, main focusing on woody or herbaceous plants are primitive or advanced. To illustrate this disputation, we investigated the copy number and subparalog structure of *GBSS*I-1 (granule-bound starch synthase I) gene in 140 *Rubus* samples representing 102 species from 17 subsections in 7 sections with various ploidy levels. Based on gene structure and sequence divergence comparisons, we defined three subparalogs, *GBSS*I-1a, *GBSS*I-1b, and *GBSS*I-1c. *GBSS*I-1a or *GBSS*I-1b was detected in 56 diploid species including 82 samples of sect. *Idaeobatus* except for *R. pentagonus* and *R. peltatus* with both two subparalogs. Both *GBSS*I-1a and *GBSS*I-1b were identified in 39 taxa (48 samples) of *Malachobatus* polyploids, as well as in two sects. *Dalibardastrum*, one *Chamaebatus* and three *Cylactis* species. In addition, *GBSS*I-1a, *GBSS*I-1b and *GBSS*I-1c were observed simultaneously in three tetraploid species. By comparison, *GBSS*I-1b had a shorter fourth intron than that of *GBSS*I-1a, while *GBSS*I-1c had a short fourth intron and a missing fifth intron. We further reconstructed the phylogenetic tree and network of *Rubus* based on multiple *GBSS*I-1 copies. These results revealed that the two kinds of *GBSS*I-1 copies in polyploids were inherited from different diploid progenitors, indicating most sect. *Malachobatus* species were allopolyploids. Thus, we supported Yü's taxonomic view that evolution in *Rubus* probably proceeded from diploid to polyploid, and from woody to herbaceous plants. This study firstly provided powerful evidence for the evolutionary direction and allopolyploid origin within the genus.

Key words: *Rubus*, *GBSS*I-1, section *Malachobatus*, allopolyploid origin, hybridization, evolutionary direction

Genomic selection - which prospects in *Prunus armeniaca*? Preliminary results issued from fruit quality traits and phenology

NOTE

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Abstract: Genomic selection (GS) refers to a selection method which intends to assess individuals according to their genomic values using genome-wide dense markers. Particularly efficient in animal improvement strategies, opening interesting perspective in crops, it is already engaged in perennial species such as apple where, genetic gain was maximized for fruit quality traits quantitatively inherited by comparison with conventional breeding strategy.

In the present work, GS was applied on a biparental apricot population (184 individuals) characterized over two consecutive years for phenology (blooming and maturity dates) and fruit quality traits (fruit weight, color, sugar and acidity content, ethylene production). A ridge regression (RR-BLUP) modelling has been used. The performance of GS was assessed by cross validation using the accuracy defined as the correlation between true phenotypes values and the estimated ones.

The effects of markers density and number, training set size and composition as well as the heritability of the investigated traits were evaluated on the accuracy of the model. The main results issued from the two years multi-annual modelling approach are showing that (i) genomic predictions are accurate even with a little number of markers (>100 markers), (ii) the accuracy increases with the training population's size and with high heritability traits, (iii) and the optimization of the training set improves the performance of genomic selection model.

To conclude, a clear interest exists in continuing the evaluation of the genomic selection in biparental apricot populations even for traits that are difficult to measure. The robustness of the approach needs to be tested both on other progenies and on a large set of genetic resources.

Key words: Genomic selection, Apricot, RR-BLUP, Cross validation, Accuracy, Bi-parental population

NOTE

Polyploidy influences resistance to *Venturia inaequalis* in *Malus* x *domestica*

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Abstract: Apple scab caused by the fungus *Venturia inaequalis* is yearly threatening apple yields. Despite extensive control by fungicides, the primary inoculum of *V. inaequalis* is increasing. Therefore alternative control measures to improve natural resistance and its durability are necessary. A promising approach could be the implementation of polyploid cultivars in combination with current control practices. This study aims to unravel the effect of polyploidy on resistance to *V. inaequalis* in *Malus* x *domestica*.

In a greenhouse inoculation trial, macroscopic scab symptoms were monitored in diploid and tetraploid isoforms of scab-susceptible 'Gala' and monogenic-resistant G58 genotypes. This phenotypic evaluation indicated different degrees of susceptibility with the highest degree of symptoms in diploid 'Gala' plants, and the lowest in G58 tetraploid plants. Over all conditions polyploidy significantly reduced sporulation symptoms when comparing it to the diploid genotype. The highest reduction in tetraploid plants in comparison to diploid ones was detected in 'Gala' by 38.3 %. Moreover, the sporulation symptoms were almost absent in the tetraploid G58 genotype. These phenotypic observations correlated with qPCR quantification of *V. inaequalis* DNA. Next, RNA-sequencing was performed to detect differences in gene expression between tetraploids and diploids. In the transcriptomic analysis, about 7-13 million 100 base pairs reads were obtained per cDNA library. Enrichment analysis of differential genes between diploid and tetraploid 'Gala' showed enriched bins belonging to defense response to fungus, response to salicylic acid stimulus and secondary metabolic process. Among various differentially expressed genes, significant changes have been detected in expression of genes involved in the phenylpropanoid metabolic pathway. The differences in transcriptomic data have been confirmed via RT-qPCR in an independent experiment. Finally, total and targeted phenolic compounds have been identified and quantified via LC-MS and HPLC. Changes in accumulation of total as well as specific polyphenolic compounds have been detected.

Key words: Polyploidy, Apple scab, Biotic stress, Transcriptomics, Phenolic compounds

NOTE

Phylogeoeraphic analysis of genus *Cerasus* elucidates the domestication origin of Chinese cherry (*Cerasus pseudocerasus* Lindl.)

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This work was supported by the National Natural Science Foundation of China (31672114).

Abstract: Cherry is a typical perennial fruit crop belonging to *Cerasus*. However, the origin of the cultivated cherry species remains unclear. Domestication pattern of cultivated Chinese cherry (*Cerasus pseudocerasus*) and its phylogenetic history is also largely absent. In this study, we analyzed a dataset of 910 cherry accessions, genotyped by three chloroplast DNA sequences and ITS fragment. Phylogeographic approaches were applied to investigate the origin of cultivated cherry species. Haplotype genealogies were also analyzed to reveal the domestication pattern and history of cultivated Chinese cherry. Genetic data and geographic ranges consistently identified two major phylogeographic clades. One, European clade, comprises European cheery species. Another, Chinese clade, comprise Chinese cherry landraces (LC), wild populations (WC), wild relatives (RC) as well as *Microcerasus* taxon (MC). Admixture shaped haplotype lineage of LC, WC and RC taxa suggests the common genetic ancestry for accessions from this lineage. WC taxon is an independent clade from RC taxon and the accessions from Longmenshan Fault Zones (LFZ, China) may represent the origin center of Chinese cherry. Overall, our results offered evidence for the existence of two independent origin centers for *Cerasus* accessions, China and Europe. Chinese cherry landraces were proved domesticated from WC populations in Longmenshan Fault Zones (LFZ, China).

Keywords: Cherry; Domestication pattern; Genetic divergence; Origin center; Phylogeography

NOTE

Introgressing Blue Mold Resistance into Elite Apple Cultivars with DNA Tests, a High-Density SNP Array, and Rapid Cycle Breeding

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Abstract: Apple blue mold is a postharvest disease resulting in significant economic losses worldwide. A source of resistance has been described in *Malus sieversii* PI 613981 and its responsible locus qM-*Pe*3.1 has been mapped on chromosome 3. However, PI 613981 has unfavorable fruit quality. Blue mold resistance and the effect of PI 613981 genome on fruit quality cannot be phenotypically evaluated until fruiting that takes approximately five years. To improve introgression efficiency, a strategy was devised that uses DNA markers to keep resistance alleles (foreground selection) and discard the remaining PI 613981 DNA (background selection), and utilizes rapid cycle breeding to hasten fruiting. This study was to validate a DNA test targeting the qM-*Pe*3.1 locus and determine an efficient mean of background selection, which can be affected by the overall amount, location, and size of PI 613981 DNA segments, and by favorable recombination on chromosome 3. Of 141 second-generation ([‘Gala’× PI 613981] × T1190 [carries *BpMADS4*]) individuals, 75 carried the transgenic *BpMADS4* early flowering gene. Of those, 43 carrying the qM-*Pe*3.1 resistance allele were identified by the DNA test Md-Pe3s-SSR. To validate the latter DNA test, blue mold susceptibility of the 75 *BpMADS4* carriers is being phenotypically determined. Using apple 20K Illumina SNP array data, the genome of PI 613981 and other parents used were compared with genomes of the 43 individuals to trace the inheritance of PI 613981 DNA segments. Three individuals were identified with favorable recombinations flanking the qM-*Pe*3.1, which have 18.6%, 21.8%, and 24.9% of PI 613981 genome, respectively. By adopting similar strategies for introgression, other tree fruit breeding programs could improve the efficiency and creativity of disease resistance breeding.

Key words: background selection; foreground selection; *Malus sieversii; Penicillium expansum*

Genome edited and T-DNA-free apple plants resistant to fire blight

NOTE

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Abstract: Fire blight, caused by the bacterium *Erwinia amylovora* (*E.a.*), is one of the most devastating diseases affecting members of the Rosaceae family, in particular apple (*Malus x domestica*) whose worldwide production is extremely hit by this disease. For this reason, many efforts have been made to understand the molecular mechanisms underlying both plant host resistance and *E.a.* pathogenesis, especially those induced by effector proteins during bacterial-host interaction. In this regard, the DspA/E effector, codified by a disease specific gene localized in the pathogenicity island of *E.a.*, is absolutely required for the pathogenesis and interacts specifically with four Disease Interacting Proteins of *Malus* (DIPM1-4). This interaction suggests that *DIPM* genes may act as susceptible genes during the infection, therefore their silencing could lead to the reduction of plant susceptibility. In this study, conducted on two *Malus x domestica* susceptible varieties, 'Royal Gala' and 'Golden Delicious', a genome editing approach based on CRISPR/Cas9 via *Agrobacterium tumefaciens* (*A.t.*) was applied to mutate and silence *DIPM4*. The binary vector used contained FRT sites next to the *A.t.* left and right borders and the *Flp* gene under the control of an inducible promoter in order to remove the T-DNA cassette in those lines selected for the desired mutation. About sixty putative edited lines were analyzed using an high throughput screening approach by Next Generation Sequencing in order to verify the CRISPR/Cas9-induced mutations. Seventy percent of the plants was completely mutated and showed different types of mutations, especially deletions producing premature stop codons. Some of these lines were treated and the removal of the T-DNA cassette was proved. The selected lines are under *ex vivo* investigation to test their resistance to *Erwinia amylovora*.

Key words: *Malus x domestica*; fire blight; DIPM; CRISPR/Cas9; NGS; marker-free plants

NOTE

Investigating global changes in gene expression during bud development in different sweet cherry cultivars

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Abstract: Plants are sessile organisms and must adapt their physiology to seasonal changes to survive over time. Their ability to perceive and respond to changes of temperature is determinant for their survival. For perennial plants, this is especially important because flowering occurs during the subsequent season exposing reproductive structures to high variation of temperature, in particular cold winter temperatures. One of the strategies to survive under low temperatures is a period of dormancy. In sweet cherry (*Prunus avium* L.), which is a perennial fruit tree belonging to the *Rosaceae* family, dormancy is mainly controlled by temperature. However, the mean surface temperature of the earth is increasing and this climatic change may have serious negative consequences on the dormancy release, potentially resulting in lower cherry production. Despite this strong effect of temperature on dormancy, the transcriptional events regulating dormancy and the effect of temperature on dormancy are still not very well understood. We performed a transcriptome analysis on flower buds of different cherry cultivars displaying contrasted flowering time to identify genes required for the control of dormancy. A time course spanning the entire bud development to flowering was carried out to explore specific biological processes. A list of genes involved in dormancy progression was identified and can be used as a decision-making tool to estimate the dormancy status. This is particularly relevant for growers who need to know the specific timing to apply dormancy release products. In addition, we will present information that will be used to create predictive models, which will be powerful tools to assist the breeding strategies.

Key words: Transcriptomic; bud development; RNA-seq; dormancy; *Prunus avium* L.

The rose genome and beyond; Understanding rose domestication and the mechanisms underlying major traits

NOTE

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Abstract: Roses hold high cultural and economic importance as ornamental plants in many societies worldwide. The rose is well suited to be an original model organism for woody ornamental species as it has a relatively small genome size (560Mbp) and it has a short life cycle. During centuries, generations of breeders had fastidiously selected the showy and desirable traits (mainly floral) of *Rosa* species based on keen and meticulous observations. However, the molecular and genetic mechanisms controlling these traits remain poorly understood[1]. During the past years, we generated a number of biotechnology and molecular tools, such as reproducible genetic transformation and a database that provides useful information on *Rosa sp.* genome structure and expressed genes with thorough genes annotation and an overview of their expression patterns with good accuracy[1-3]. These tools allowed to discover the molecular mechanisms controlling the double-flower formation[4] and the biosynthesis of major scent molecules[5]. Recently, we used Single Molecule Real-Time sequencing and an original meta-assembly approach to obtain a very high-quality genome assembly for *Rosa chinensis*, known to have extensively participated in breeding and the creation of modern roses[6]. Resequencing of the genome of 14 major genotypes that contributed to rose domestication, along with genome diversity analyses, highlighted the mosaic origin of the genome of modern rose hybrids that combines European species traits and Chinese species traits [6]. Expert gene annotations along with gene expression data permitted the reconstruction of gene regulatory pathways associated with major rose traits, and allowed to describe epigenetic variation landscapes along the rose genome[6]. Reconstruction of regulatory and secondary metabolism pathways involved in scent and flower color, validated by biochemical and molecular analyses, allows to propose models of interconnected regulation of flower color and scent compounds[6]. The data also provide indication on why roses evolved alternative routes to produce scent compounds, such as terpenes, in the petals. Comparative genomic investigation permitted to assess rose paleohistory within the Rosaceae family. Together, these resources provide a solid foundation for understanding the mechanisms governing rose traits and their diversity and will accelerate improvement in roses, *Rosaceae* and ornamentals. Recent advances will be presented and discussed.

Multi-allelic QTL analysis in tetraploid rose using an ultra-dense linkage map

NOTE

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Abstract: In rose the development and use of genetic and molecular tools for breeding has been slow because most of the breeding is in tetraploids. In addition, novelty is a major driver, and this is judged visually, decreasing the need to develop and implement these tools. However, increased competition is a driver for faster and more predictable breeding programs. The sector is also facing new demands from society, among which a decrease in the use of pesticides, for which breeding has to identify and employ disease resistances.

Marker development has benefited from the developments in sequencing technology, while genotyping can now be one for 10,000's of SNP markers automated using SNP arrays. As a result, we can now develop the markers and genotype ornamental crops for which little prior information exists and generate dense genetic maps that are the basis for QTL mapping of relevant traits. For tetraploid rose we developed the 68k WagRhSNP array and used it to genotype the K5 population and develop a genetic map consisting of 25695 SNP markers across all four homologues of each of the seven rose chromosomes. We used the haplotype composition of the offspring to resolve differences in pairing behavior among chromosomes in the meiosis in the parents. Examples of QTL mapping of ornamental traits in rose will be shown where the effects of individual homologues are estimated, and linked markers identified that can be used to optimize the breeding process. As producing populations, markers, and software for QTL mapping are now in place, phenotyping may be the limiting factor, e.g., developing reliable phenotyping tests for diseases.

Key words: *Rosa*; genetic map; QTL mapping; software.

A high-quality sequence of *Rosa chinensis* to elucidate genome structure and ornamental traits

NOTE

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Abstract: Rose is the world's most important ornamental plant with economic, cultural and symbolic value. Roses, genus *Rosa,* are cultivated worldwide and sold as garden roses, cut flowers and potted plants. Roses are outbreeding with high heterozygosity and can have various ploidy levels. Our objectives were (i) to develop a high-quality reference genome sequence for the genus *Rosa* by sequencing a doubled haploid, combining long and short reads, and anchoring to a high-density genetic map and (ii) to study the genome structure and (iii) the molecular and genetic basis of major ornamental traits as double flower, continuous flowering, self-incompatibility and prickle density.

We produced a doubled haploid rose line, obtained from *R. chinensis* 'Old Blush', an old Chinese cultivated variety. Using a combination of long and short read sequencing and genetic map anchoring, we generated a rose genome assembly anchored to seven pseudo-chromosomes (512 Mbp with N50 of 3.4 Mbp and 564 contigs). The length of 512 Mbp represents 90.1-96.1% of the estimated haploid genome size of rose. Of the assembly, 95% is contained in only 196 contigs. The anchoring was validated using high-density diploid and tetraploid genetic maps. We delineated hallmark chromosomal features including the pericentromeric regions through annotation of transposable element families and positioned centromeric repeats using Fluorescent In Situ

Hybridisation. The rose genome displays extensive synteny with the *Fragaria vesca* genome, as we delineated only two major rearrangements, but also whithin the Rosaceae family. Genetic diversity was analysed using resequencing data of seven diploid and one tetraploid *Rosa* species, that represent the various sections of the genus. Combining genetic and genomic approaches, we identified potential genetic regulators of key ornamental traits, including prickle density, continuous flowering, self-incompatibility locus and number of flower petals. A rose *APETALA2/TOE* homologue is proposed to be the major regulator of petal number in rose. We proposed that a misregulation of the *APETALA2/TOE* homologue is responsible for the increased number of petals in double flower rose. W*e* also detected a new allele controlling continuous flowering. This new allele is due to a large genomic rearrangement at the *RECURRENT BLOOMING* locus, previously described.
This reference sequence is an important resource for studying polyploidisation, meiosis and developmental processes as we demonstrated for flower and prickle development. It will also accelerate breeding through the development of molecular markers linked to traits, the identification of the genes underlying them and the exploitation of synteny across Rosaceae.

Key words: *Rosa, double flower, prickle, FISH, synteny, haploid, flower development, genome annotation, centromere*

NOTE

Characterizing Black Spot Resistance Genes in Polyploid Roses

NOTE

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Abstract: The foliar fungal disease black spot, caused by *Diplocaron rosae*, is a constant problem for rose (*Rosa hybrida*) growers. While fungicides are effective in managing black spot, disease resistant roses could reduce the need for chemical inputs. Phenotyping was conducted with 12 *D. rosae* races to better characterize resistance in four popular polyploid rose cultivars (Brite Eyes™, High Voltage™, Lemon Fizz™, and Morden Blush). Subsequently, two populations ('Morden Blush' × Brite Eyes™ and High Voltage™ × Lemon Fizz™) were developed to study resistance segregation and map the genes mediating black spot resistance using the rose Axiom array. 'Morden Blush' was susceptible to all *D. rosae* races while the remaining three cultivars displayed differing disease responses. A 1:1 segregation ratio was observed for the two study populations where each progeny was either resistant or susceptible to all races tested to date, suggesting resistance is conferred by a single resistance gene in Brite Eyes™ (R to races 1-4, 6-11, & 13) and Lemon Fizz™ (R to all races but 7). The observed disease resistance in High Voltage™ (R to races 2, 6, 7, & 12) suggests a different resistance gene than both Brite Eyes™ and Lemon Fizz™. Linkage mapping in the 'Morden Blush' × Brite Eyes™ population identified a single resistance gene that mapped to a chromosome 5 homeolog (*Rdr4*). To date, three black spot resistance genes, *Rdr1, Rdr2*, and *Rdr3*, have been identified. *Rdr1* and *Rdr2* both map to chromosome 1 indicating they are not allelic to *Rdr4*, while *Rdr3* has not been mapped. *D. rosae* races 3 and 9 are virulent on *Rdr3* but avirulent on *Rdr4*, therefore, we cannot confirm if *Rdr4* is a unique gene or an allele of *Rdr3*. Future work will focus on developing tools for marker assisted breeding and pyramiding the identified resistance genes into new cultivars.

Key words: *Rosa*, *Diplocaron rosae*, *Rdr4*

NOTE

Development of a complete process of *in vitro* culture and *Agrobacterium tumefaciens*-mediated genetic transformation of Rose cultivars

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Abstract: Rose is one of the most important plant in the ornamental sector worldwide and is increasingly gaining importance as a major driver for research to study ornamental traits such flower initiation and development or scent emission. By combining genetic and genomic approaches, candidate-gene can be more and more easily isolated; however their functional validation is still a bottleneck. To develop functional validation protocol, it is essential to use biotechnology methods such as regeneration tissues combined with transgenic approaches. Here our objectives are to develop such a methods in different rose cultivars.
In rose, we developed a complete process of clonal cycle from meristem introduction including plant elongation, multiplication and rooting on several genotypes. Using these processes, we developed several lines of embryogenic callus in order to use them as target in *Agrobacterium tumefaciens*-mediated rose genetic transformation protocol. From this new developed protocol, we obtained efficiency rates that can reach 12.5% of transformation events. These low transformation rates are real problems for mastering the key factors controlling regeneration and transformation in rose.
Recently, we applied this protocol to study *RoFT* gene, a floral activator and a *FLOWERING LOCUS T (FT)* rose homologue. We obtained *RoFT* transformed roses for two genotypes *Rosa* Deltrimen 'Guy Savoy'® and *Rosa* deldog 'Pimprenelle'®. As expected, the *RoFT* ectopic expression led to early flowering, with plants that can flower even in *in vitro* condition. *RoFT* integration was validated with PCR technique. Nevertheless, we observed a difference in the rooting capacity between both genotypes: *Rosa* Deltrimen Guy Savoy® transgenic lines produce roots while *Rosa* deldogPimprenelle® lines are unable to produce them.
The key steps of our protocol, the strategy for practical application and the main results obtained will be presented.

Key words: *Rosa*, genetic transformation, *RoFT*

Coexpression Network Comparison of *Rubus idaeus* and *Fragaria vesca* in Early Fruit Development

NOTE

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Abstract: The molecular mechanisms underlying the immense diversity in fleshy fruits of *Rosaceae* are of great interest from a fundamental evolutionary development perspective. It is still a mystery how the same developmental signals from phytohormones auxin and gibberellic acid can induce formation of diverse types of fleshy fruit, even in closely related species. While the ovary wall becomes the fleshy fruit in the red raspberry (*Rubus idaeus)*, the stem tip develops into the fleshy fruit in strawberry. Therefore, the *Rosaceae* family serves as an ideal model to investigate the evolution and morphological diversity in fleshy fruit development. Here we generated a spatial and temporal transcriptome profile of both *Rubus idaeus* throughout stages of fruit development pre and post fertilization. We then constructed a coexpression network integrating a comparable spatiotemporal transcriptome of the woodland strawberry, *Fragaria vesca*. This cross-species coexpression network provides insight into the similar and disparate mechanisms of fleshy fruit development in closely related organisms with highly distinctive fruit morphology. Further, to make our data accessible we have constructed a comparative electronic fluorescent pictograph (eFP) web viewer that allows the user to view RNA expression patterns of orthologs across the two species. Therefore, providing a key resource for understanding early fruit development.

Key words: fruit development, transcriptomics, coexpression network, raspberry, strawberry

NOTE

MIKC^C-type MADS-box genes in rose: insights into the floral organogenesis and flowering

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Abstract: MIKC^C-type MADS-box (MIKC^C) genes encode transcription factors that play crucial roles in controlling floral organogenesis and flowering time in plants. Although they have been well characterized in many plant species, evolutionary and comprehensive functional analysis of this gene family in rose is lacking. In this study, 58 non-redundant MIKC^C uni-transcripts were extensively identified from rose transcriptomes. Phylogenetic analysis then placed these genes into 12 clades with their Arabidopsis and strawberry counterparts, and revealed that ABCDE model (including AP1/FUL, AP3/PI, AG and SEP clades), SOC1, and AGL6 clade genes are remarkably expanded in *Rosa chinensis*, while the genes from FLC and AGL17 clades were undetectable. Sequence alignments suggest that the AP3/PI clade may contribute to more specific functions in rose due to high variation of amino-acid residues within their MADS-box domains. A comparative analysis of gene expression in specific floral organ differentiation stages and floral organs between *Rosa chinensis* cv. Old Blush and the closely related mutant genotype *Rosa chinensis* cv. Viridiflora (floral organs mutated into leaf-like structures) further revealed the roles of ABCDE model genes during floral organogenesis in rose. Analysis of co-expression networks gave an overview of the regulatory mechanisms of rose MIKC^C genes and shed light on both the prominent roles of AP3/PI clade genes in floral organogenesis and the roles of *RcAGL19*, *RcAGL24* and *RcSOC1* in regulating the floral transition in rose. Our analyses provide an overall insight of MIKC^C genes in rose and their potential roles in floral organogenesis and flowering.

Key words: rose, MADS-box, floral organogenesis, RNA-seq, co-expression network

Genomic Prediction in Strawberry Breeding at the University of Florida

NOTE

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Abstract: The potential value of genomic selection (GS) for parent selection in the University of Florida Strawberry Breeding Program was determined in a series of annual trials conducted in successive years at the Gulf Coast Research and Education Center in Balm, Florida. This first series of trials comprised two clonally replicated tests of seedlings and two advanced selections tests, with 1628 field-tested phenotypes, established in the 2013-2014 and 2014-2015 seasons. Genotyping was performed with the Affymetrix Axiom®IStraw90®SNP array (Bassil *et al*., 2015) and approximately 17,500 markers were used in the analyses after quality control. In all trials, we evaluated five yield and quality fruit traits and tested several genomic selection methods.

We found small differences among the methods and on average the Prediction Ability (PA) of Genomic Breeding Values (GBV), across methods and across years, was moderate to high ranging from 0.31 for Total Marketable Yield to 0.49 for Average Fruit Weight. The efficiency of GS for four out of the five traits indicated that 40-70% of the gains obtained using marker and phenotypic information could be obtained by selection using markers only (Gezan *et al*., 2017). We implemented GS into the breeding program in the 2016-2017 season by selecting a subset of parents one year before phenotyping had occurred.

A second series of clonally replicated trials were established in consecutive years during the 2015-2016, 2016-2017 and 2017-2018 seasons. We used these advanced selection trials to determine 1) how aggregating data from multiple breeding cycles in the training population affects Predictive Ability (PA) 2) the potential of reducing the number of markers by different methods, i.e., random selection, MAF selection, etc. and 3) the potential of within-family selection for seedling selection (clones). In general, these analyses were performed either with Bayes B or RKHS and used 1,000 genotyped individuals with 8,000 to 10,000 markers after quality control.

In the first case, the multi-cycle analysis indicated that the PA for a test population increased steadily, for all traits, as data were aggregated across cycles in the training population. Our results from the second objective showed that by randomly selecting between 500 and 1,000 markers we can reach, for all traits, from 88% to 97% of the PA

obtained with all 10,000 markers. In our third objective, the results suggest that by using within-family selection in elite crosses, additional gains 4.4% to 15.6% above the family mean could be realized by selecting the top 10% of predicted individuals within families. In total, these research efforts in genomic selection have allowed us to integrate this tool into our breeding program for parental selection, reducing the length of the breeding cycle, with strong potential for additional gains via seedling selection if cost-effective, low-density genotyping can be developed.

Key words: *Fragaria x ananassa*, Strawberry Breeding, Genomic Selection.

NOTE

NOTE

Micro-dissected single chromosome and its sequence analysis in cultivated strawberry (*Fragaria* × *ananassa* Duch.)

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Abstract: Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is an allooctoploid. Thus, when conducting ordinary de novo assembly of DNA sequences, it is difficult to make clear in the detail of the genome sequence, because of the existence in homoeologous chromosomes. Hence, the sequence analysis using microdissected single somatic chromosomes of cultivated strawberry was conducted. Approximately three hundred chromosomes of the Japanese octoploid strawberry 'Reiko' were individually taken under a light microscope with a microdissection system. The 288 dissected chromosomes were successfully amplified DNA by the amplification kit. We decoded the base sequences of the DNA segments by a next-generation sequencer. As the results of the mapping with the reference of the octoploid strawberry, *F*. × *ananassa*, and the ancestry diploid *F*. *vesca*, the 144 samples could be confirmed that the those plants. In our presentation, the methods of the chromosome microdissection and sequence analysis by the single chromosome will be exhibited.

Key words: *Fragaria* × *ananassa* Duch.; allopolyploid; chromosome microdissection; sequence analysis

NOTE

Optimization of transient gene manipulation in strawberry fruit: adoption of percentage difference of phenotype (PDP) to evaluate gene function in fruit ripening.

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Abstract: Strawberry has been increasingly become a model plant for researches on fruit growth and development. Transient Gene Manipulation (TGM) is a powerful tool for gene function identification, but its application for a precise identification of gene function in strawberry fruit is still difficult. In this study, we optimized the condition for the TGM in strawberry fruits and developed a novel method, by which the gene function can be more precisely and efficiently identified. The results showed that successful TGM must be based on a conditional optimization and the type of vectors, temperature and fruit developmental stage are three major factors determining whether a TGM was successful. Notably, TGM was found to be only applied to large green stage, and hence especially suitable for the researches on gene function in fruit ripening. Based on a conditional optimization, we established a novel method designated as 'Percentage Difference of Phenotype (PDP)', in which the functional capability of a gene can be estimated by a PDP values from 0 to 100%, and hence it can be adopted to compare a set of genes for their relative capabilities to regulate fruit ripening. This study is of great significance for accelerating the researches on molecular basis of strawberry fruit ripening.

Key words: Strawberry; Transient gene manipulation; Percentage Difference of Phenotype (PDP), FaMYB10

Regulation of phosphate uptake and allocation in strawberry by PHR1-miR399-PHO2 module

NOTE

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Abstract: Although phosphorus (P) nutrition has long been one of the hot topics in plant nutrition research, the relationship between P content and fruit quality has been studied very little. We found the positive correlation relationship between soluble solids content (SSC) and P content in strawberry fruits with the correlation coefficient r=0.95 and the P content and SSC in strawberry fruits could be significantly increased by applying 6 mM phosphoric acid. To improve the ability of strawberry plants to uptake P from soil, the PHR1-miR399-PHO2 module in strawberry was studied. *FvPHR1*, a member of MYB-CC gene family in woodland strawberry, was characterized. FvPHR1 was also demonstrated to directly bind to and activate the miR399 promoter via the recognition of the P1BS element. Overexpression of miR399 in woodland strawberry plants could significantly increase P content in leaves and fruits, and the contents of sugars and Vc in transgenic fruits were significantly higher than those in non-transgenic controls. miR399 could direct the cleavage of target mRNA of *FvPHO2* gene, so overexpression of miR399 in woodland strawberry plants significantly down-regulated expressions of *FvPHO2* gene. Expressions of Pi starvation-induced gene *FvPHO1*, *FvPHT1;1* and *FvSPX1* were up-regulated in woodland strawberry plants overexpressing miR399. The full-length CDS regions of three *PHO1* genes (*FaPHO1*, *FaPHO1;H1*, *FaPHO1;H9*) in cultivated strawberry were cloned, and overexpression of these genes in Arabidopsis could significantly increase P content in transgenic plants, while silencing *FvPHO1;H9* gene in woodland strawberry significantly reduced P content in plants. Our results provide theoretical guidance for developing strawberry cultivar efficiently utilizing P fertilizer by molecular breeding technique.

Genomic and Genetic Approaches to Identify Key Genes That Regulate Strawberry Reproductive Development

NOTE

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Abstract: Strawberry exhibits two alternative modes of reproduction. First, sexual reproduction leads to the formation of abundant seeds which then produce auxin and GA to initiate fruit development. The resulting fruit not only ensures seed disposal but, for human, serves as an important source of nutrient and fiber. However, strawberry also reproduces asexually through stolon, a stem that grows horizontally above the ground and is composed of successive units of young daughter plants. The ability to form stolon is the basis of commercial propagation of strawberry cultivars. We are interested in understanding the molecular mechanisms governing both fruit initiation and stolon development and how these two modes of reproduction are mutually exclusive. *Fragaria vesca,* the diploid strawberry, is emerging as a better model to investigate gene function than the octoploid garden strawberry due to its diploidy, simpler genome, and the availability of molecular genetic tools. Further, abundant natural variations exist, providing rich genetic materials for novel allele identification. Therefore, we have developed genomic resources for this emerging model as well as use this model to identify key genes in reproductive development. Through transcriptome profiling, network data analysis, CRISPR/Cas9, forward genetic screens, and mapping-by-sequencing, we identified a number of key regulatory genes for strawberry reproduction. Our study provides examples of how genome-wide analyses and network building nicely complement forward genetic screens to effectively link genes to specific traits of economic importance.

Plant hormones coordinate receptacle fruit development in *Fragaria vesca*

NOTE

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Abstract: Fruit growth and ripening are coordinated to determine the final fruit size and are modulated by multiple phytohormones. How these hormones coordinate and interact with each other to control these processes at the molecular level is not clear. In the early stages of *Fragaria vesca* fruit development, auxin increases both widths and lengths of receptacle fruits, while gibberellin (GA) mainly promotes their longitudinal elongation. We showed that auxin promoted GA biosynthesis and signaling by activating GA biosynthetic and signaling genes, suggesting auxin function is partially dependent of GA function. At the onset of fruit ripening, both auxin and GA levels decreased, leading to a steep increase in the endogenous level of ABA that drives receptacle fruit ripening. ABA repressed the expression of *FDR1* gene that inhibit fruits ripening but promoted the expression of *FveNCED, a rate limiting step in* ABA biosynthesis. I will discuss how we use strawberry receptacle fruits as a model to understand cross-talk among plant hormones.

Key words: *Fragara vesca*; plant hormone interaction; fruits development

Gene Discovery for Flavor and Disease Resistance in Cultivated Strawberry

NOTE

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Abstract: Associating crop traits with their underlying genetics is crucial for rapid agricultural improvement in auto-allooctoploid strawberry. Flavor is frequently cited as the most important consumer trait, yet progress in this area has been slow due to the immense genetic and chemical complexity of flavor. To identify candidate genes controllin0g these traits, high-resolution genomics assays were applied to detect sequence variations at the subgenomic level. For associating flavor phenotypes, fruit volatile metabolomes were derived from 263 individuals using a series of statistical alignment techniques on non-targeted GC/MS data. Over 25,000 subgenomic sequence variants were tracked through multiple related populations. These inherited sequence variants were correlated to the production of various strawberry flavor and aroma volatile compounds. In a similar approach, high-variance transcripts from 65 fruit transcriptomes were robustly correlated to their genotypes, indicating differential expression due to cis and trans genetic factors (eQTL). In a separate study, targeted genomic capture and Pacbio sequencing of strawberry R-genes (SRMT-Renseq) was deployed to finely resolve the physical sequences related to previously-detected disease resistance QTL. This approach allows for facile sequence comparison of disease resistance loci. For this study, a 50,000 capture probe panel was designed against strawberry R-gene sequences mined from public databases. These capture probes were used to enrich R-gene sequences in gDNA and mRNA prior to third-gen sequencing via Pacbio-SMRT. Sixteen important strawberry lines were selected, representing the full diversity of the University of Florida's disease resistant germplasm. Genomic regions containing canonical R-gene-like sequences were assembled to reveal sequence and expression-level differences at the loci correlated with the hypersensitive response.

Key words: Strawberry, flavor, aroma, disease, R-genes, genomics, QTL.

NOTE

‘Who’ and ‘How’ to regulate the ripening in strawberry: a model for non-climacteric fruit

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Abstract: Fruits are an essential part of the human diet, nutrition and health. Based on fruiting structures of angiosperms, fruits are ranged from completely dry to highly fleshy organs, among of which flowering plants utilize different floral structures to develop various flesh tissues; seeds are a common organ and are responsible for fleshy fruit ripening, thus the conservation and diversification mechanisms exist in fleshy and dry fruits. In the model plant Arabidopsis (*Arabidopsis thaliana*), which has dry fruit, a high-level regulatory network for controlling fruit development has been defined. Studies on various unripening mutations in tomato (*Solanum lycopersicum*), a model for fleshy fruit, have provided new insights into the networks responsible for the control of ripening.

Based on the presence or absence of a transient rise in respiration rate and the production of autocatalytic ethylene, fleshy fruit ripening is divided into climacteric and non-climacteric types. In climacteric fruits, typically as tomato, the ripening process is marked by increased respiration and is induced and co-ordinated by ethylene, while in non-climacteric fruits, typically as strawberry *(Fragaria ananassa)*, it is controlled by an ethylene-independent process with little change in respiration rate, and now not only emphasizing roles of abscisic acid in non-climacteric fruit ripening, but also which are indeed involved in several other plant hormones, including ethylene, brassinosteroid, jasmonate, axin, and polyamines.

Generally, color break is the visual manifestation of the developmentally regulated transition of chloroplasts to chromoplasts in fleshy fruit ripening, which are coupled with sugariness, softening, colorfulness, and flavor. A combination of both mutant and inhibitor applications as well as a set of comparative transcriptomic, proteomic and metabolic throughput techniques, the molecular mechanisms of climacteric tomato fruit ripening are absolutely defined by ethylene perception and signaling transduction; in contrast, the molecular mechanisms of non-climacteric strawberry fruit ripening are not fully understood. Here, great effort has been made to outline the molecular mechanisms of strawberry fruit ripening regulated by plant hormones, now highlighting on an ABA-dominated and many other hormone-participating process.

Key word: Ripening; non-climacteric fruit; strawberry; plant hormones; ABA

This work was supported the National Natural Science Foundation of China (Project 31672125), the Natural Science Foundation of Beijing Municipality (6171001), and Beijing Training Project for the Leading Talents in S & T (grant no. LJ201612).

Progress report on interactions of strawberry with fungus pathology in SAAS-FFRI

NOTE

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Abstract: The investigation will deliver molecular markers for fungus disease such as anthracnose resistance genes and improve the precision of breeding. It will allow us to incorporate resistance genes from more than one source into the same variety, thus improving both the strength and durability of the resistance. It is likely that soil fumigation will not be a sustainable form of wilt control, except in the short term, and varieties with strong resistance will become essential for production in the soil. *Colletotrichum fructiola*, a hemibiotrophic fungus within the C. gloeosporioides species complex, could cause diseases in strawberry worldwide. So far, molecular interaction between *C. fructiola* and strawberry is largely unknown.
A deep RNA-sequencing approach was applied to gain insight into the pathogenicity mechanisms of *C. fructiola* and the defense response of strawberry at different stages of infection including the appressoria, biotrophic and necrotrophic stages.
In our recently study, the transcriptome data showed stage-specific transcription accompanied by a step-by-step strawberry defense responses and the evasion of these defense systems by fungus. Fungal genes involved in plant cell wall degradation, secondary metabolism and detoxification were up-regulated at different infected stage. Host cells first expressed 4-coumarate-CoA ligase and dirigent-like proteins to stabilize cell wall. While *C. fructiola* utilized pectin-degrading enzymes and glucanase inhibitor proteins to destroy host cell wall and then invade into host cells. Most importantly, *C. fructiola* infection was accompanied by a large number of highly expressed effectors. However, the most important receptors recognizing fungal chitin were not significantly up-regulated. The activation of ETI was characterized by the up-regulation of several R genes. The necrotrophic stage displayed a dramatic up-regulation of genes involved in reactive oxygen species activation. The early activation of ABA and auxin signaling, the delayed activation of SA pathway, as well as the continuous suppression of JA and ET pathways all contributed to the deployed basal defense and the insufficient induced defense system in strawberry.
Collectively, the transcriptome information of both *C. fructiola* and strawberry gives us a useful clue that even strawberry builds the multilayered defense against infection, *C.*

fructiola employs a series of escape or antagonizing mechanisms to complete its infection process.

Key words: Fungus disease, Strawberry, Effector, Pathogenicity, Resistance

NOTE

NOTE

RAP codes for a GST anthocyanin transporter that is essential for the foliage and fruit coloration in strawberry

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Abstract: The red color of the foliage and fruit in strawberry comes from anthocyanins stored in the vacuole. However, how anthocyanin accumulation is regulated in strawberry is still unclear. A *reduced anthocyanin in petioles* (*rap*) mutant was identified in an ENU mutagenized population of YW5AF7, a white-fruited variety of the wild strawberry *Fragaria vesca*. The causative mutation was identified to be a premature stop codon in a *glutathione S-transferase* (GST) gene. In addition to the foliage coloration, *RAP* also mediates fruit pigmentation and acts downstream of the fruit-specific transcription factor *FveMYB10*. Among all eight GST genes in the same subfamily, *RAP* is most abundantly expressed in the ripening fruit. Expression analysis and transient expression assay demonstrated that RAP is the principal transporter of anthocyanins among the paralogs. Domain swap experiments showed that both N- and C-termini of RAP are essential for the binding capability of anthocyanins. Moreover, stable over-expression of *RAP* driven by the 35S constitutive promoter in *rap* not only restores anthocyanin accumulation in leaf petiole, but also results in strong coloration in fruit receptacle starting from early developmental stages. In addition, transient knock-down of *RAP* resulted in reduced fruit coloration in cultivated strawberry. Collectively, our results demonstrate that *RAP* encodes the principal GST transporter of anthocyanins in the strawberry foliage and fruit and could be modified to alter the fruit color in strawberry.

Key words: Anthocyanin transporter; foliage coloration; fruit coloration; glutathione S-transferase; mutant analysis; strawberry; *Fragaria vesca*

Advances of Strawberry Germplasm Collection and Breeding in Jiangsu

NOTE

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Abstract: China is the largest genetic information repository of strawberry in the world. Of about 24 recognized *Fragaria* species, 13 are native from China. Jiangsu Academy of Agricultural Sciences (JAAS) as one of the earliest strawberry germplasm collection center (1981-) in China, 520 accessions of the approximately 22 species and natural hybrids of *Fragaria*. have been conserved from all over the world. According to the yearly observation and investigation, we found some strawberry resources showed changed phenotype, which compared from their native area, during our conservation process in Nanjing. Such as *F*. *daltoniana*., *F*. *nubicola*., *F*. *pentaphylla*. and *F*. *tibetica*. couldn't flowering in Nanjing, and *F*. *iinumae*. couldn't fruiting even though flowering normally. The height of *F*. *tibetica*., *F. nilgerrensis*. and *F*. *moupinensis*. changed in to a higher level and their leaves also grown larger than their original plant, respectively. The potential characteristics of native resources in China could be used in strawberry breeding, for example *F*. *viridis*. has strong resistance and perfect aroma. *F*. *tibetica*. showed high-tolerance for hot weather in summer of Nanjing and growth well. *F*. *nilgerrensis*. has the white fruit with a kind of peach aroma. Cross breeding among wild species and cultivated strawberries has been carried out, and a number of intermediate materials have been obtained. In recent years, our strawberry breeding work focus on early-maturing, perfect-quality, high resistance to different pathogens and big-fruit. A series of varieties were selected and released in the strawberry production of China.

Keywords: Strawberry; Germplasm; Breeding

NOTE

Lineage-specific duplications of NBS-LRR genes occurring before divergence of six *Fragaria* species

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Abstract

Background: The plant disease resistance (*R*) genes are evolving extremely rapidly and play a critical role in the plant innate immune system. The nucleotide binding sites-leucine rich repeat (NBS-LRR) genes are one of the largest classes in plant *R* genes. Previous studies have focused on NBS-LRR genes from one or several species in different genus, the sequenced genomes in genus *Fragaria* offer the opportunity to study the evolutionary processes of these *R* genes among the closely related species.
Results: In this study, 325, 155, 190, 187, and 133 NBS-LRRs were discovered from *F. x ananassa*, *F. iinumae*, *F. nipponica*, *F. nubicola*, and *F. orientalis*, respectively. Together with the 144 NBS-LRR genes from *F. vesca*, a total of 1134 NBS-LRRs contained 866 multi-genes to compose 184 gene families across the six *Fragaria* genomes. Extremely short branch lengths and shallow nodes were widely present in the phylogenetic tree constructed with all NBS-LRR genes of the six strawberry species. The identities of orthologous genes were highly significantly greater than those of paralogous genes, while the *Ks* ratios of formers were very significantly lower than those of latters in all NBS-LRR gene families. In addition, the *Ks* and *Ka*/*Ks* of TIR-NBS-LRR genes (TNLs) were significantly greater than those of non-TIR-NBS-LRR genes (non-TNLs). Furthermore, the expression patterns of NBS-LRR genes demonstrated that the same gene differently expressed under different genetic backgrounds in response to pathogens.
Conclusions: These results, combined with the shared hotspot regions of duplicated NBS-LRRs on chromosomes, could indicate that lineage-specific duplications of NBS-LRR genes occurred before the divergence of the six *Fragaria* species. The *Ks* and *Ka*/*Ks* ratios suggested that the more early duplicated TNLs are rapidly evolving and driven by stronger selective pressures compared with non-TNLs.

Keywords: NBS-LRR genes, *Fragaria* species, Disease resistance genes, Lineage-specific duplication, Duplication time.

Transcriptome network-guided identification of transcription factors regulating fruit flesh softening in wild strawberry

NOTE

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Abstract: Fruits of temperate plants can be classified according to their softening properties throughout ripening, for some soften considerably, while others maintain crispness. Strawberry falls within the former category, undergoing rapid softening during the later-ripening stages of fruit development. Pectate lyases (PELs) are a class of calcium-dependent enzymes that catabolize pectin, a major component of the plant cell wall. High transcriptional expression of PEL genes can be observed in the strawberry receptacle during the ripening stage, and it is well known that these enzymes are responsible for the rapid softening of this tissue. However, the proteins that mediate the transcriptional regulation of the pectate lyase mRNA remain to be identified. Here we propose two candidate transcription factors, the R2R3 MYB protein MYB123a and HLH protein BQNa, as the key regulators of pectate lyase transcription during Fragaria vesca receptacle ripening. Using a novel computational approach to generate a consensus transcriptional network, we have found that both transcription factors are highly correlated with two of the ripening-specific pectate lyases throughout strawberry fruit development. MYB123a and BQNa appear to directly interact to form a transcriptional complex in yeast. Our results suggest for the first time how pectate lyases may be regulated in non-climacteric fruits such as *Fragaria vesca*, the wild strawberry. Additionally, we demonstrate how R2R3 MYB and HLH proteins mediate combinatorial regulation of ripening-specific processes, and the regulatory targets of these complexes may be identified via transcriptional network analysis.

Key words: *Fragaria vesca*, fruit texture, ripening quality, pectate lyase, pectin, transcriptome network, MYB, HLH, combinatorial regulation, non-climacteric

NOTE

Transcriptome and hormone analyses provide insights into hormonal regulation in strawberry ripening

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Abstract: Strawberry is emerging as a model for studies on non-climacteric fruit ripening. Regulation of fruit ripening involves a complex interplay among gene expression and signaling events. Plant hormones are important regulators of strawberry fruit ripening. However, the knowledge on whether and how these hormones are involved is still limited. To understand hormonal actions in the ripening process, we performed wet bench experiments and transcriptome analysis in achene and receptacle (flesh) at different ripening stages of the woodland strawberry *Fragaria vesca.* Our results demonstrate that the pre-turning stage is the transition stage from immature to ripe fruits. Auxin is synthesized predominantly in achenes, while ABA, bioactive free base cytokinins, gibberellins, and ethylene are mainly produced in receptacles. Combinatorial analyses of exogenous hormone treatments, hormone and transcriptome profiles show that gibberellin may delay ripening, while ethylene and cytokinin are likely involved at later stages of the ripening process. Our results also provide additional evidence that ABA promotes ripening while auxin delays it. This study provides a global picture for hormonal regulation of non-climacteric strawberry fruit ripening and also evidence for a possible mechanism of ABA and auxin interaction on the ripening process.

Key words: *Fragaria vesca*; fruit ripening; transcriptome; plant hormones

NOTE

An SNP marker for Resistance to *Colletotrichum gloeosporioides* in Apple (*Malus* × *domestica* Borkh.)

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Abstract: Apple anthracnose caused by *Colletotrichum gloeosporioides* is one of the highly devastating diseases. To develop molecular markers and to screen for candidate genes, 1401, 2637, 467 and 235hybrids derived from 'Jonathan' × 'Golden Delicious' (J×G), 'Zisai Pearl' × 'Red Fuji' (Z×F), 'Zisai Pearl' × 'Golden Deliciou' (Z×G) and 'Jonathan' × 'Tsugaru' (J×T), and also 61 *Malus* germplasm accessions were phenotyped in 2017. The response of the hybrids to natural pathogen segregated into two distinct phenotypes, incident and non-incident with 709:692, 0:2637, 235:232 and 117:118 ratios for J×G, Z×F, Z×G and J×T populations, respectively. The data indicated obviously that the inheritance of the resistance to *C. gloeosporioides* is qualitatively controlled by likely one locus. The incident: non-incident ratio was 1:1 in Malus accessions. Using high density genetic linkage maps of J×G, one major QTL for resistance to *C. gloeosporioides* were mapped to 3.8-4.8 Mb interval region on G15. and candidate genes were searched in the region. Within this region, five candidate genes were pre-selected including a LEUNIG gene. By using kompetitive allele-specific PCR test, a C/T SNP marker on CDS domain of LEUNIG gene was closely associated with the resistance to *C. gloeosporioides*, with a recombination frequency of 0.8% (1/119). The CT and TT genotypes were linked to incident phenotype and the CC genotype indicated non-incident phenotype. The genotype of 'Golden Delicious' and 'Tsugaru' is CT and the genotype of 'Jonathan' 'Zisai Pearl' and 'Red Fuji' is CC. Of the 60 Malus germplasm accessions, the genotype frequency of TT, CT and CC were 6.7%, 36.7% and 56.7%, respectively, while the gene frequency of T was 25%. The results confirmed the qualitative inheritance of the resistance and a C/T SNP can be used efficiently in marker assisted selection.

Key words：Apple anthracnose; QTL mapping; SNP; Marker assisted selection

NOTE

The effect of promoter methylation on *MdMYB1* expression determines the level of anthocyanin accumulation in skins of two non-red apple cultivars

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Abstract: Fruit color in apple (*Malus domestica* Borkh.) is ascribed mainly to the accumulation of anthocyanin pigments, and is an important trait for determining fruit market acceptance. Bagging is a commonly used treatment to enhance the red pigmentation in apple skin. The *MdMYB1* transcription factor gene plays an important role in the biosynthesis of anthocyanin in apple after bag removal, but little is known about how *MdMYB1* transcription is regulated. In this study, we investigated pigmentation in the non-red skinned cultivars 'Granny Smith' and 'Golden Delicious' after bag removal. The fruit skins of the two cultivars showed red/pink pigmentation after bag treatment. Transcript levels of *MdMYB1*, the master regulator of anthocyanin biosynthesis in apple, increased, and showed a correlation with anthocyanin content in both cultivars after bag removal. The *MdMYB1* genomic sequences were compared in the two cultivars, which showed that the green-fruited cultivar 'Granny Smith' harbors the *MdMYB1-1* and *MdMYB1-2* alleles, while the yellow-fruited cultivar 'Golden Delicious' harbors only *MdMYB1-2*. A comparison of methylation levels in the 2 kb region upstream of the *MdMYB1* ATG between the bag-treated fruits after removal from the bags and the unbagged fruits showed a correlation between hypomethylation and the red-skin phenotype in 'Granny Smith'. Moreover, 'Granny Smith' fruits responded to treatment with 5-aza-2'-deoxycytidine, an inducer of DNA demethylation. An investigation of the *MdMYB1* promoter in 'Granny Smith' showed reduced methylation in the regions -2026 to -1870 bp, -1898 to -1633 bp, and -541 to -435 bp after bag removal and 5-aza-2'-deoxycytidine treatments. Differences in anthocyanin levels between 'Granny Smith' and 'Golden Delicious' can be explained by differential accumulation of *MdMYB1*-specific mRNA. Different levels of *MdMYB1* transcripts in the two cultivars are associated with methylation levels in the promoter region. Hypomethylation of the *MdMYB1* promoter is correlated with the formation of red pigmentation in 'Granny Smith' fruit skins. As a result, red pigmentation in Granny Smith' was more intense than in 'Golden Delicious' fruits after bag removal.

Keywords: Apple, Pigmentation, Anthocyanin, *MdMYB1* promoter, Methylation

NOTE

QTL Mapping Suggests the Regulation Downstream of Ethylene in Room Environment Shelf-life in Apple (*Malus* × *domestica* Borkh.)

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Abstract: Room environment shelf-life (RESL) is an important trait for fresh market apple quality maintenance and commercial benefits. The climacteric during ripening and senescence of apples usually results in RESL collapse. To understand the genetic and molecular mechanism of apple RESL, candidate genes were screened based on Bulk Segregation Analysis (BSA) mapping, the functional annotation and the sequence variations. Prior to and during six weeks room environment storage, ethylene emission, flesh firmness and crispness of 1623 hybrids derived from 'Zisai Pearl' × 'Golden Delicious' were phenotyped in 2015~2017. Obvious segregation in flesh firmness, crispness and ethylene emission was detected in the population. The genotypes of previously reported *MdACS1* were *MdACS1*-1/1 and *MdACS1*-1/2 in 'Zisai Pearl' and 'Golden Delicious', respectively, indicating the segregating ratio should be 1:1 in the hybrid population. The long RESL bulk was constructed of 30 hybrids with extremity phenotype, the flesh firmness and crispness of apples were > 7.0 kg/cm^2 and > 0.7 kg/cm^2, respectively, after six weeks postharvest storage at room environment. Whilst the short RESL bulk consisted of 30 hybrids which fruit flesh firmness and crispness were < 7.0 kg/cm^2 and < 0.7 kg/cm^2 immediately after harvest. Three major QTLs, G03.1, H08.2 and Z16.1, were identified using BSA-seq strategy. *MdPAE*, *MdPL*, *MdHAT* and *MdWRKY* were screened as candidate genes from the QTL intervals. The data implied that RESL is likely controlled by regulatory and functional genes downstream of ethylene.

Key words: Apple; Shelf-life; QTL; BSA-seq

BSA-seq Identifies Key Loci Linked to Spur Type Tree Architecture in Apple (Malus × domestica Borkh.)

NOTE

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Abstract: Spur type tree architecture mutants in apple have been frequently reported and commercially used worldwide, but their molecular signature is not fully understood. Totally 4041 12-year-old hybrids derived from the reciprocal crosses between two spur type cultivars, 'Starkrimson' and 'Miyazaki Spur Fuji', were phenotyped in 2017. The hybrids segregated into three tree architecture types, spur, semi-spur, and standard, with a 1:2:1 ratio. We supposed that the spur tree architecture should be controlled by at least two independent non-complete dominance genes with unequal effects. Based on the phenotype data, 30 spur and 30 standard type trees were randomly chosen for bulked segregant analysis via Illumina re-sequencing (BSA-seq). Prior to BSA-seq, the chosen individuals were genotyped for 8 structure variation markers and were ensured as true diploid hybrids. By using BSA-seq data, 16 significant QTLs were identified across seven chromosomes. Of these QTLs, the G' values of H03.1 and S15.2 were 7.81 and 6.08, respectively, and were thus considered as major QTLs. Between the intervals of H03.1 and S15.2, 65 and 8 genes contained nonsynonymous variation in their coding region domains (CDS) or cis-element altering variations at their 2 kb upstream sequences, and of these genes, *MdMBD4* and *MdARF19* were predicted as candidate genes, respectively. The single nucleotide polymorphic variations at CDS of *MdMBD4* and *MdARF19* were then validated via Sanger sequencing. The data implied that the spur tree architecture should be qualitatively controlled by the variations on at least two major genes.

Key words: Apple; Spur-type; BSA mapping; QTL

NOTE

Comparative Transcriptomic Analysis Reveals That Ethylene/H_2O_2-Mediated Hypersensitive Response and Program Cell Death Determine the Compatible Interaction of Sand Pear and *Alternaria alternata*

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Abstract: A major production restriction on sand pear (*Pyrus pyrifolia*) is black spot disease caused by the necrotrophic fungus *Alternaria alternata*. However, pear response mechanism to *A. alternata* is unknown. Here, host responses of a resistant cultivar Cuiguan (CG) and a susceptible cultivar Sucui1 (SC1) to *A. alternata* infection were investigated. We found that the primary necrotic lesion formed at 1 dpi and the expansion of lesions was aggressive in SC1. Data from transcriptomic profiles using RNA-Seq technology identified a large number of differentially expressed genes (DEGs) between CG and SC1 in the early phase of *A. alternata* infection. K-mean cluster and Mapman analysis revealed that genes involved in ethylene (ET) biosynthesis and ET signaling pathway such as ACS, ACOs, ERFs, and in hypersensitive response (HR) and programmed cell death (PCD) were significantly enriched and up-regulated in the susceptible cultivar SC1. Conversely, genes involved in response to hydrogen peroxide and superoxide were differentially up-regulated in the resistant cultivar CG after inoculation. Furthermore, ET levels were highly accumulated in SC1, but not in CG. Higher activities of detoxifying enzymes such as catalases were detected in CG. Our results demonstrate that the ET-/H_2O_2-mediated PCD and detoxifying processes play a vital role in the interaction of pear and *A. alternata.*

Keywords: Sand pear, *Alternaria alternata* (Fr) Keissler, hypersensitive response, programmed cell death, ethylene, hydrogen peroxide, antioxidant enzyme

A Chilean germplasm core collection for sweet cherry (*Prunus avium* L.) breeding by the means of SSR markers

NOTE

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Abstract: During the last decade Chile is increasing its Sweet cherry (*P. avium* L.) production and new varieties with improved fruit quality are very important to ensure its competitiveness in the global market. Supported by the state, the Chilean industry has implemented its own breeding programs for sweet cherries such as the INIA-SCBP (INIA-Sweet Cherry Breeding Program). The obtaining a germplasm collection has been one of the most important goals and therefore, several genotypes from Europe and North America have been introduced. Nowadays, more than 60 genotypes constitute our collection. The large size of germplasm collections often complicates their characterization, evaluation, utilization and maintenance; this situation is particularly important in sweet cherry since trees require extensive field surface and significant maintenance labor. Therefore, a core germplasm collection with a minimal set of progenitors representing the genetic diversity but carrying the best alleles to improve the fruit of sweet cherry is desirable. Using a set of 18 SSR, including 5 markers associated to color, fruit size, maturity time and cross compatibility, with criteria of maximum genetic diversity, best allele combinations for desirable quality of cherry fruits and cross compatibility, a minimal group of genotypes as core collection for the INIA-SCBP is proposed.

Key words: Prunus avium, core collection, SSR

Acknowledgments: Fondecyt Regular 1161377; INNOVA-CORFO: 09PMG-7243.

A global view of transcriptome dynamics during floral transition in sweet cherry (*Prunus avium* L.)

NOTE

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Abstract: The floral transition is an important developmental event, but little is known about the underlying regulatory networks in sweet cherry trees. To obtain a comprehensive overview of the dynamic transcriptome during the floral bud development in *P. avium*, high-throughput RNA-seq was conducted during four flowering related stages (S) (S1: rounded meristem; S2: flower primordia; S3: early differentiation of the flower whorls; S4: differentiation of the pistils). Among the 113,878 de novo assembled unigenes, 5,265 were differentially expressed during the four stages analyzed. Gene co-expression network and Gene Ontology (ClueGO) enrichment analysis revealed that the larger cluster is enriched in several biological processes, from which the most representative are “multicellular organism development (reproductive structures)”, “response to abscisic acid,” “phosphorelay transduction system”, “response to acid chemicals”, “cell wall macromolecule metabolic process” and “organophosphate metabolic process.” This enrichment analysis suggests that the highly connected genes from the largest cluster in the network are involved in the regulation of complementary processes triggered by floral transition. Furthermore, many gene families of transcription factor (such as bHLH, ERF, WRKY, MADS, B-box) were found to be differentially expressed genes in the S1-4. Several genes and gene families were analyzed in depth including MADS-box TFs, CONSTANS, as well as LEAFY and FLOWERING LOCUS T. Our analysis of the genes underlying the floral transition in sweet cherry provides a basis a basis for further functional analysis. This work was funded by FONDECYT Project 1160706; CONICYT Regional/CEAF/R08I1001; CONICYT R16F200006.

Key words: *Prunus avium*; bud development; flowering induction; gene expression.

Phylogenetic relationship between Western China Pear accessions and abroad pear accessions based on SSR markers

NOTE

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Abstract: Pear is one of the oldest fruits of family *Rosaceae* with a cultivation history of about 3000 years ago. The current study was carried out to detect and understand the genetic diversity and phylogenetic relationship between western China pear accessions and abroad pear accessions using SSR markers. Abroad pears were originated from ten different countries/locations. However, origin of some accessions was unknown. Chinese pears were originated from two different locations, Qinghai and Xinjiang. A total of 17 Simple sequence repeats were capable to elucidate the genetic variation/diversity and association between accessions. In the current investigation, a total of 324 alleles were observed/found in the population ranged from 11 to 29 alleles with average of 19.05 alleles per locus. The heterozygosity observed among accessions ranged from 0.52 to 0.89 with mean of 0.29 per locus respectively. The dendrogram were gained based on the simple sequence repeat (SSR) genotype, the Western and Chinese accessions clustered in two groups based on their geographical area, all abroad pears were in group one and western China pears were in group two. Luosha from unknown and J. No.1 from Czech Republic were held same genotype. Population structure analysis with K value of 4 reflected a clear genetic composition within different genotypes, Group I was composed of Chinese accessions, Group II and Group III were composed of occidental accessions, Xingyeli and Bajiaoli were clustered together with occidental pear in the Group IV.

Key words: Phylogenetic relationship; SSR; Pears

BPPCT034: An Efficient DNA-Based Genetic Assay for Avoiding Small-Fruited Cherry Trees

NOTE

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Abstract: New sweet cherry cultivars must meet a minimum fruit size threshold to succeed commercially. Cherry fruit size is a highly heritable trait with QTLs on chromosome 2 putatively contributing to fruit size by regulating mesocarp cell number. Despite extensive knowledge about these QTLs, predictiveness of nearby markers has not been quantified. We report on the estimated allele effects and predictive accuracy of a DNA test targeting this locus, BPPCT034, and SNP-based haploblocks spanning the genomic region. BPPCT034 is an SSR marker with seven alleles located 10 cM from a candidate gene. The SSR and candidate gene are embedded in and flanked by four haploblocks totaling 76 SNPs across 17 cM, with 14-20 haplotypes per haploblock. Allele effects were estimated using a mixed model approach where the DNA test was treated as a fixed effect and the background genomic effects accounted for as random effects using a genomic relationship matrix. BPPCT034 explained 13% of the total phenotypic variance and 27% of the genotypic variance for fruit diameter and similarly for other measures of fruit size. The coefficient of determination between the genetic values and BPPCT034 predictions was 0.14. The most predictive haploblocks explained 28% and 41% of the phenotypic and genotypic variance, respectively, with a coefficient of determination of 0.60. Because sweet cherry breeding is slowed by its long juvenility, using BPPCT034 to choose parents with ideal allelic combinations and cull a seedling population of inferior small-fruited individuals would enhance breeding efficiency. A new DNA test capturing the haploblock information of this fruit size genomic region could double the efficiency.

Key words: DNA test, fruit size, haploblock, marker-assisted selection, sweet cherry

Characterization of a *Hexokinase-like* gene induced by root hypoxia in *Prunus* rootstocks

NOTE

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Abstract: Root hypoxia in fruit trees affects growth, vegetative and reproductive development, which is reflected in low productivity, poor fruit quality, and other negative effects in trees. Most stone fruit trees (*Prunus* genus) are hypoxia-sensitive and for this reason they are grafted on rootstocks. We performed a large-scale transcriptome sequencing of roots from two different stone fruit rootstocks with contrasting responses to hypoxia, Mariana 2624 and Mazzard F12/1, which are tolerant and sensitive to this stress, respectively. Among flooding-responsive genes, we discovered one gene encoding a hexokinase (HXK3-like, ppa004715m) that was highly induced in the tolerant genotype. Hexose sugars, such as glucose and fructose are present in all plants and are the origin for most organic matter found in nature, but these hexose compounds must first be phosphorylated to be used. In plants have been identified only two families of enzymes capable of phosphorylate glucose and fructose: hexokinases (HXKs) and fructokinases (FRKs). To determine the role of *Prunus HXK3-like* in the context of the mechanisms of hypoxia tolerance, its function and subcellular localization was characterized. The *HXK3-like gene* was isolated and sequenced, and the structure of the codified protein was modeled. Also, we analyzed the subcellular localization of HXK3–GFP by transient expression in tobacco and the protein was localized or associated with the chloroplast. To analyze its role under hypoxia or other abiotic stresses, we overexpressed *HXK3-like* gene under 35S promoter in *Arabidopsis thaliana*. The overexpression of *HXK3-like* resulted in improved drought and salt tolerance in Arabidopsis. Additionally, we performed an *in silico* analysis of the promoter region of these two different rootstocks. Ongoing transcriptomic analyses of Arabidopsis overexpressing lines will provide further understanding of its role in abiotic stress tolerance. This work was funded by FONDECYT-CONICYT 3160292 and 1161377; CONICYT-R16F200006.

Key words: *Prunus*; hexokinase; rootstocks; hypoxia.

Coordinated Functional Divergence of Genes after Genome Duplication

NOTE

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Abstract: Gene and genome duplications have been rampant during the evolution of flowering plants. Unlike small-scale gene duplications, whole-genome duplications (WGDs) copy entire pathways or networks, and as such create the unique situation in which such duplicated pathways or networks could evolve novel functionality through the coordinated sub- or neofunctionalization of its constituent genes. Here, we describe a remarkable case of coordinated gene expression divergence following WGDs in *Arabidopsis thaliana*. We identified a set of 92 homoeologous gene pairs that all show a similar pattern of tissue-specific gene expression divergence following WGD, with one homoeolog showing predominant expression in aerial tissues and the other homoeolog showing biased expression in tip-growth tissues. We provide evidence that this pattern of gene expression divergence seems to involve genes with a role in cell polarity and that likely function in the maintenance of cell wall integrity. Following WGD, many of these duplicated genes evolved separate functions through subfunctionalization in growth/development and stress response. Uncoupling these processes through genome duplications likely provided important adaptations with respect to growth and morphogenesis and defense against biotic and abiotic stress.

Key words: whole-genome duplication; subfunctionalization; gene expression

NOTE

Single-Base Methylome Analysis Reveals Dynamic Epigenomic Differences Associated with Water Deficit in Apple

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Abstract: Cytosine methylation is an essential feature of epigenetic regulation and is involved in various biological processes. Although cytosine methylation has been analysed at the genomic scale for several plant species, there is a general lack of understanding of the dynamics of global and genic DNA methylation in plants growing in environments challenged with biotic and abiotic stresses. In this study, we mapped cytosine methylation at single-base resolution in the genome of commercial apple (Malus x domestica), and analysed changes in methylation patterns associated with water deficit in representative drought-sensitive and drought-tolerant cultivars. We found that the apple genome exhibits ~54%, ~38% and ~8.5% methylation at CG, CHG and CHH sequence contexts, respectively. We additionally documented changes in gene expression associated with water deficit in an attempt to link methylation and gene expression changes. Global methylation and transcription analysis revealed that promoter-unmethylated genes showed higher expression levels than promoter-methylated genes. Gene body methylation appears to be positively correlated with gene expression. Water deficit stress was associated with changes in methylation at a multitude of genes, including those encoding transcription factors (TFs) and transposable elements (TEs). These results present a methylome map of the apple genome and reveal widespread DNA methylation alterations in response to water deficit stress. These data will be helpful for understanding potential linkages between DNA methylation and gene expression in plants growing in natural environments and challenged with abiotic and biotic stresses.

Key words: Apple, Methylomes, Epigenetics, Gene expression, Water deficit, Transcriptome.

Insights into the effect of human civilization on *Malus* evolution and domestication

NOTE

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Abstract: The evolutionary history of the *Malus* genus has not been well studied. Cultivated apples are generally accepted to domesticate from the wild species *M. sieversii*, which originates from Central Asia, and cultivated apples also exhibit strong introgressions from the European *M. sylvestris*. However, the details of these two wild species' contributions are unclear. Here we present genetic evidence on the origin of the *Malus* genus based on genome sequencing of 297 *Malus* accessions, revealing contributions from East Asia, North America, and Central Asia. Introgressions from *M. sylvestris* in cultivated apples appear to be more extensive than those from *M. sieversii*, whose genetic background flowed westward across Eurasia and eastward to wild species including *M. prunifolia*, *M.* × *asiatica*, *M.* × *micromalus*, and *M* × *robust*. Our results suggested that the loss of ancestral gene flow from *M. sieversii* in cultivated apples accompanied the movement of European traders around the world since the Age of Discovery. We identified several genes that have undergone a selective sweep and/or are related to nine agronomic traits of the domesticated apple. Using a genome-wide association approach, we identified several loci associated with these traits. An *NB-ARC* domain-containing gene was found to strongly affect anthocyanin accumulation, and an apple *ERECTA*-like gene that underwent selection during domestication was identified as a likely major determinant of fruit length and diameter. Our results provide new insights into the origin and domestication of apples, and will be useful in new breeding programs and efforts to increase fruit crop productivity.

Key words: *Malus*, Apple, sequencing, Evolution, Domestication

A Genomewide Association Study for Brown Rot Tolerance in Peach

NOTE

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Abstract: Brown rot, caused by *Monilinia* spp., is one of the most devastating diseases of stone fruit worldwide. Severe yield loss can be caused by pre- and post-harvest fruit decay. Although some degree of tolerance has been reported in peach and almond, the genetic resistance in peach cultivars is still lacking. To date, few genomic regions in peach associated with brown rot response in fruit skin and flesh have been detected. Limited knowledge suggests brown rot tolerance in peach is a quantitative trait controlled by multiple genes with small effect. To further understand the genetics behind brown rot tolerance in peach, we phenotyped 26 cultivars and 138 progeny from 9 crosses across two seasons (2015 and 2016) for skin and flesh response to brown rot infection, and genotyped using newly developed 16K peach SNP array. Association mapping revealed total of 32 SNPs ($p<0.0000001$) significantly associated with brown rot response in peach fruit skin (20), flesh (11) and in both fruit skin and flesh (1) across whole genome. Candidate gene analysis within the haplotype regions of the detected markers identified 23 predicted genes associated with pathogen infection response/resistance. Two candidate genes, Prupe.7G072600 and Prupe.7G072700, sharing high identity with polygalacturonase-inhibiting protein (PGIP) genes were identified. Detailed analysis of the trait values associated with the haplotypes (H) in haploblock 7_2, suggested absence of H5 significantly reduced brown rot disease severity index in skin and flesh. The information presented here provides an important foundation for further dissection of the genetics behind brown rot tolerance in peach.

Key words: *Prunus persica*, disease resistance, candidate gene, haploblock

NOTE

Comparative transcriptomes analysis of Chinese crispy pear and melting pear after harvest to identify genes associated with texture formation

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Abstract: Pears are rich in the type of fruit texture. Based on the changes in fruit texture during the ripening process, the pear varieties can be divided into melting and crisp types. In this study, we integrated phenotypic, microscopic, transcriptomic and biochemical analyses to gain insights into the molecular basis of the formation of different textures. Four oriental pear varieties used as experimental materials, including two crispy varieties and two melting varieties. The physiological characteristics of melting type varieties were active after harvest, which were significantly correlated with ethylene production and respiration rate. The physiological performance of crisp pear were not active, and ethylene production was significantly different between the two varieties, so the crisp type pear showed the ethylene insensitive characteristic. According to transmission electron microscopy, the middle lamella of melting type pear were all degraded, plasmolysis occurred, the cell microfibrils degraded and lighter in color, and organelles also occurred obvious degradation and destruction as the fruits matured .On the contrary, the middle lamella of crisp-pear only become blurred slightly, the cell wall structure and organelles did not materially change. RNA-seq was employed to survey the differentially expressed genes (DEGs) during the ripening process of these two types of pears, and we focused on the genes about regulation of ethylene biosynthesis and putative ethylene signaling transduction pathway. The ethylene synthesis gene ACS 2 is highly expressed during the ripening of melting pears, this result consisted with previous findings of pear fruit wound response. High expression of genes ethylene insensitive 3, ethylene receptor 2 and five ethylene response factors (AP2-ERF 1, AP2-ERF 2, AP2-ERF4, AP2-ERF 53, AP2-ERF 113) in the transition of melting pears suggests those genes may be involved as signals triggering the transition to responsiveness to ethylene in pear fruit. The genes PG, PME, PL, EXP, CES, and GAL, which are involved in cell wall degradation, are responsible for the different textures of pear fruits. The expression of these genes was highly consistent with the physiological basis. This study provides us with a systematic biochemical basis for the texture change of pears.

Key words: Pear texture , Comparative transcriptomes, Ethylene synthesis, Signal transduction

NOTE

Construction of Genetic Linkage Map and Comparison with Different Population Using Simple Sequence Repeats in Pear (*Pyrus* spp.)

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Abstract: This study was conducted to construct a pear (*Pyrus* spp.) genetic linkage map using co-dominant markers developed by next generation sequencing technologies. On this basis, other map constructed from different pear population was compared to our map. A total of 4,801 single nucleotide polymorphisms (SNPs) were developed using previous genotyping by sequencing (GBS) raw data of F_1 derived from 'Whangkeumbae' × 'Minibae' based on pseudo-chromosome level of reference genome of 'Dangshansuli'. In addition, 13 insertion/deletions (InDels) exploited in resequencing data from 'Whangkeumbae' and 'Minibae' and 42 simple sequence repeats (SSRs) developed in preceding research in pear and apple were used to construct F_1 linkage map of 'Whangkeumbae' × 'Minibae' (WM map). The most SSRs were derived from the Chinese pear 'Dangshansuli'. The map of 'Bayuehong' × 'Dangshansuli' (BD map) developed by Chen et al. in 2015 was used as comparative map. The 758 markers including 721 SNPs, 6 InDels, and 31 SSRs were positioned in 17 linkage groups (LGs) on the WM map. Twenty-four SSRs out of the 31 SSRs were observed in 14 LGs of both maps in common and order of the 24 SSRs was highly conserved. The LG1 and 13 of WM map, which correspond to LG8 and 16 of BD map respectively, had the largest number of SSRs. Integration of transferable SSRs into our SNP-based map enabled to compare with other maps and improved the reliability. Our genetic map could be used as a basic frame map for comparative analysis of genomic structure between different research groups in pears.

Key words: comparative mapping; genetic linkage map; *Pyrus* spp.; simple sequence repeat; single nucleotide polymorphism

Genetic Linkage Map of Apple Using SSRs and SNPs Detected by GBS

NOTE

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Abstract: Genetic linkage map is valuable tool for genetic, genomic, and crop breeding studies. In the present study, we describe an apple (*Malus* × *domestica*) genetic linkage map constructed using single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. The F_1-mapping population was produced from a cross between 'Gala' and 'Jonathan' and consisted of 94 individuals. A new set of 27,151 polymorphic genotyping-by-sequencing (GBS)-based SNPs was detected from the two parents with their F_1 population. Additionally, polymorphism of 160 SSR markers developed in apple were checked in the two parents and 94 F_1 individuals. After that, 53 polymorphic SSR markers were selected to construct a linkage map. The apple linkage map was successfully constructed with 1,016 SNP markers and the 37 SSR markers. This map covered 17 linkage groups (LG) over a total length of 1,594.7 cM with an average distance of 1.5 cM between markers. LG 15 spanned the longest genetic distance with 151.5 cM, while LG 4 spanned the shortest with 73.1 cM. The SSR markers located in each chromosome covered most of LGs with 1 to 5 SSR markers. The present apple genetic linkage map has higher marker density by saturating GBS-based SNPs. Moreover, the apple SSR markers well-scattered on the LGs and this allowed of construction of reliable genetic map. This map could be used to identify fruit-related quantitative trait loci (QTL) as well as apple anthracnose resistance related QTLs.

Key words: Gala; Genetic linkage map; Jonathan; *Malus* × *domestica*; SNP; SSR

Development of a Cleaved Amplified Polymorphic Sequence Marker Associated with Presence or Absence of Pollen in Pear (*Pyrus* spp.)

NOTE

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Abstract: Because commercial pear (*Pyrus* spp.) orchards that grow pollenless cultivar require time-consuming and labor-intensive artificial pollination to produce marketable fruit with stable production, presence and absence of pollen is an agriculturally trait of interest. In our previous study, a candidate region associated with presence and absence of pollen have been identified based on high-density of single nucleotide polymorphism (SNP) linkage map in the mapping population from a cross of pollenless 'Whangkeumbae' (*P. pyrifolia*) and normal 'Minibae' (P. hybrid) using genotyping-by-sequencing (GBS) technology. Fourteen candidate SNPs associated with presence and absence of pollen were identified. Among them, 300-bp of flanking sequences of a SNP (s162_40781) were used to design a pair of primers (BfuCI-1) based on reference genome of 'Dangshansuli' (*P. bretschneideri*). While pollenless 'Whangkeumbae' showed two bands, 'Minibae' exhibited three bands. Most of pollenless F_1 individuals had two bands as with 'Whangkeumbae', whereas normal F_1 individuals showed three bands as with 'Minibae'. However, genotypic value of F_1 individuals by cleaved amplified polymorphic sequence (CAPS) marker were not fully consistent with that by GBS, indicating the probability of false positive SNP calling via GBS featuring reduced representative library. Out of 70 F_1 individuals, 55 individuals were successfully predicted by the combination of BfuCI-1 with *Bfu*CI. The CAPS marker developed in this study would provide an opportunity to validate the transferability in other mapping populations as well as pear breeding program.

Key words: CAPS; GBS; 'Minibae'; pollen; 'Whangkeumbae'

Selection of Suitable Restriction Enzyme Combination to Increase Efficiency of GBS in Korean Pear (*Pyrus* spp.)

NOTE

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Abstract: Restriction enzyme (RE) is an important factor in genotyping-by-sequencing (GBS) library construction because it influences size and the number of DNA fragments. The present study was carried out to improve GBS efficiency in pears (*Pyrus* spp.) by selecting optimized RE combination. To prove efficiency of selected RE combination, classification results of Korean native pears were compared in different GBS libraries. After *in silico* digestion, *Ape*KI, *Ape*KI/*Tfi*I, and *Ape*KI/*Mse*I were selected to construct GBS libraries and the number of single nucleotide polymorphisms (SNPs) obtained from *Ape*KI/*Tfi*I library were about six times more than *Ape*KI library. In addition, the SNPs of *Ape*KI/*Tfi*I library showed high accuracy in classification of Korean native pear accessions. Both genetic diversity and population structure analysis indicated that the SNPs generated from *Ape*KI/*Tfi*I library distinguished most pear accessions unlike *Ape*KI. Thus, *Ape*KI/*Tfi*I combination is suitable for construction of GBS library in pears and this RE combination could provide higher SNP density for pear genomic studies.

Key words: genotyping-by-sequencing; *Pyrus*; restriction enzyme; single nucleotide polymorphism

NOTE

The verification of function of Sucrose-phosphate synthase (*SPS2*) in the metabolism of sucrose biosynthesis in peach fruit

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Abstract: Sugar is one of the most important traits to determine the fruit organoleptic quality. The improvement of fruit sweetness is currently one of the most sought-after objectives in peach breeding and cultivation technique research programs. Sugar in peach mainly includes sucrose, glucose, fructose and sorbitol, in which sucrose accounts for above 70% of the total soluble sugar content. Our previous studies showed a positive correlation between the transcript level of the candidate gene (*SPS2*, ppa000636m, 566bp) in sucrose phosphate synthase family cloned from the fruit of cv. 'Jin Xiu ' and the content of sucrose. In order to verify the gene function, Virus-induced gene silencing (VIGS) have been widely used for gene function analysis in plants, especially for those species which are difficult to create transgenic plants, such as peach. And it is proved to be an efficient way for functional studies of the genes related to fruit traits. In this study, VIGS was implemented to construct the interference vector TRV2-SPS2, and to be transformed by Agrobacterium successfully. The results showed that the sucrose content in the fruit of 'Jin Xiu' showed a rapid increasing trend during the peach ripening stage, the sucrose content in the peach fruit treated with TRV2-SPS2 was lower than empty carrier, while the difference is not significant. The expression level of *SPS2* in the interfered fruits with TRV2-SPS2 was significantly decreased compared with that of empty carrier, which means that the recombinant interfering vector was successfully transferred into the peach fruit and the target gene SPS2 was silenced successfully. For sucrose biosynthesis, *SPS2* might not be the key factor to catalyze the formation of sucrose, Other genes' function must be verified in the future study.

Key words: peach, sucrose, virus-induced gene silencing (VIGS), Sucrose Phosphate Synthase

NOTE

Integrated high-density genetic maps improve *Pyrus bretschneideri* 'DangshanSuli' v1.0 genome to chromosome level

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Abstract: Chromosomal level reference genome provides crucial foundation for genomics researches such as genome-wide association studies (GWAS) and whole genome selection (WGS). Both the European (*Pyrus communis*) and Chinese (*P. bretschneideri*) pear genomes published so far are in scaffolds level. To help anchoring *P. bretschneideri* 'DangshanSuli' (DS) v1.0 genome of 2103 scaffolds into pseudo-chromosomes, two genetic maps (MH and YM maps) were constructed using half sibling populations of Chinese pear crosses, 'Mantianhong' (MTH) × 'Hongxiangsu' (HXS) and 'Yuluxiang' (YLX) × MTH, where 345 and 162 samples were prepared for SNP discovery using genotyping-by-sequencing (GBS) technology. Two sets of maps, MH and YM, each with 17 linkage groups (LGs), were constructed from 2606 and 2489 SNP markers and spannign 1847 and 1668 cM, respectively, with average marker intervals of 0.7. The two maps were further merged with a previously published genetic map (BD) based on the cross 'Bayuehong' (BYH) × 'Dangshansuli' (DS) to build the new integrative MH-YM-BD map. A total of 670, 671 and 679 scaffolds (covering 69.1%, 69.5% and 66.1% of the assembled genome length) were anchored to the MH, YM and BD maps, in which 97.91%, 95.23% and 68.78% of scaffolds were oriented with two or more markers, respectively. By using 7757 markers located on the integrated MH-YM-BD map, 898 scaffolds (400.57 Mb) of the DS v1.0 assembly were successfully anchored into 17 pseudo-chromosomes, accounting for 78.8% of the total genome size. About 88.31% of them (793 scaffolds) were directionally anchored with two or more markers on the pseudo-chromosomes. Furthermore, pseudo-chromosomes 1 and 7 were extended by adding nineteen and fourteen scaffolds respectively, while seven scaffolds from pseudo-chromosome 1 were transferred into pseudo-chromosome 7 in the newly constructed DS v1.1 genome. Synteny analyses revealed that the DS v1.1 genome had high collinearity with the apple genome, and the homologous fragments between pseudo-chromosomes were similar to those found in previous studies. Moreover, the red-skin trait of Asian pear was mapped

to identical locus as identified previously. All these findings effectively confirmed the accuracy of DS v1.1. With more than 400 MB anchored to 17 pseudo-chromosomes, the new DS v1.1 genome provides a critical tool that is essential for studies of pear genetics, genomics and molecular breeding.

Key words: pear; GBS; genetic map; genome assembly; SNP; QTL-seq

NOTE

Genetic identification of cv ‘Susina di Dro’ (*Prunus domestica L.*) ecotype using microsatellites

NOTE

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Abstract: The widest distributed plum in Europe is 'German prune', during the years many cultivars were described and have showed different synonyms in each region. ‘Susina di Dro’ is a plum of *Prunus domestica* species grown traditionally in Trentino region (north Italy) since Middle Ages. In this work, Short Sequence Repeat (SSR) markers were used to characterize the profiles of 9 ancient clones of cv ‘Susina di Dro’ and compare them with other German selections. The set of primers was chosen from previous work on *P. domestica* and international reference cultivars were added to the study to facilitate alleles scoring.
SSR profiles were compared and most of the 9 ancient cv 'Susina di Dro' shared the same alleles profile for 7-8 markers but showed tiny differences in just one; this could be due to clonal polymorphism. In fact, *P. domestica* is hexaploid,that high number of alleles could result in a higher mutation rate, so these samples could be considered as clonal variants. To check this hypothesis we also characterized the S-locus (SSRs markers for S-RNase intron) and we found no differences between these profiles, confirming the homology.

Key words: European plum, ‘Susina di Dro’, *P. domestica*, SSR, fingerprint

Development of Molecular Markers for Fruit Skin Color in Japanese Plum (*Prunus Salicina* Lindl.)

NOTE

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Abstract: Fruit skin and flesh color is one of the most considered traits in Japanese plum breeding programs. Commercial Japanese plum varieties show a broad variation in fruit skin coloration, going from black to green and yellow through a range of purple and red hues. In Japanese plum, red fruit coloration is due to the accumulation of anthocyanin compounds. MYB transcription factor have been shown to be the major determinant of variation in anthocyanin pigmentation in the Rosaceae family. With the aim of finding molecular markers useful for marker assisted selection (MAS) for Japanese plum fruit skin color we explored the variability within the MYB10 gene group in Japanese plum and its association with fruit skin color in a panel of varieties. The association analysis and a subsequent fragment cloning allowed for phylogeny and putative prediction of the partial proteins and for the development of a dominant marker highly linked with the absence of anthocyanin accumulation in the fruit skin. The validation of this marker in progenies and additional germplasm population will validate its use in MAS.

Key words: Japanese Plum; fruit color; Marker Assisted Selection.

QTL detection for key aroma compounds in cultivated strawberry.

NOTE

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Abstract: The cultivated strawberry, *Fragaria x ananassa*, is the most important berry specie of the Rosaceae family. Its octoploid nature (2n = 8x = 56) presents a challenge to the development of molecular breeding tools, although it's closely related to diploid Fragaria species (2n = 2x = 14) which provides great knowledge and advanced genomic tools. Strawberry has interesting characters to be studied for breeding such as color, sugars content, acidity and volatile compounds. For this study, we have two segregating populations, F1 population (67 individuals) and F2 (117 individuals), all 2 have been phenotyped for organoleptic traits and for volatile composition. These population's phenotypes were performed during 2 years for weight, penetrance, shape, fruit color, puree color, brix, pH and titration. Volatile profiles using GC-MS were taken place for two populations during the same period paying more attention in the 20 more relevant key compounds for the strawberry flavor. 2018 data collection is underway. Genotyping was done by IStraw35K or IStraw90K hybridization on populations to generate a high resolution maps. QTLs for agronomic traits and volatile compound accumulation in linkage maps for these populations has been detected in LG 1, 3, 6 and 7 for key volatile compounds, and in all LG for different agronomic traits. QTLs are stable in different years and populations.

Key words: *Fragaria x ananassa*; aroma, agronomic traits, QTL, high resolution map.

Towards the development of an interespecific collection of NILs between peach and almond

NOTE

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Abstract: One of the main limitations in peach breeding is the low level of variability found in the commercial gene pool. Introgression of genetic variability from wild or cultivated *Prunus* species may provide new alleles useful to improve disease resistance or fruit quality. For that, we developed an approach, marker-assisted introgression (MAI), allowing the integration of a DNA fragment coming from a donor species (almond) in the background of a peach cultivar only two backcross generations after the hybrid. Additionally, a first survey of the genetics and map position of interesting major genes from the donor species can be done using a small subset of BC1 plants with a low number of introgressions (2-4). We have applied this method in a cross between peach ('Earlygold') and almond ('Texas'), where some interesting almond alleles providing powdery mildew resistance and red flesh color have already been identified. As a side project, and starting from this small set of BC1 plants with few introgressions, we are developing a introgression line collection of almond genomic fragments in the peach genome background. Some problems encountered were the identification of a cytoplasmic male sterility, that forced us to change the direction of the crosses including an additional generation, and the use of an early ripening peach cultivar that requires embryo rescue, increasing the resources needed to obtain the collection. We already obtained a collection of lines with 2 or 3 introgressions covering the whole almond genome, and two sets of lines with one introgression in heterozygosis and homozygosis covering the 81% and 45% of the almond genome respectively. This collection will be a very useful tool for quantitative variation studies and can be considered as pre-breeding material to be used in peach breeding programs.

Key words: *Prunus*; introgression; marker-assisted selection; pre-breeding

Identification and validation of a QTL for fruit firmness on linkage group 4 in three sweet cherry populations

NOTE

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Abstract: Fruit firmness is an important market driven trait in sweet cherry (*Prunus avium* L.) as firmness dictates the harvesting, handing and marketing practices. In addition, consumers prefer firm sweet cherries. To investigate the genetic basis of fruit firmness, three different mapping populations were used: an INRA bi-parental F_1 population, an INRA sweet cherry diversity population, and the RosBREED pedigreed population. For all three populations, the effects of different genotypes for fruit firmness were highly significant, and the broad-sense heritabilities were high ranging from 0.73 to 0.97. The INRA F_1 sweet cherry population exhibited a bimodal distribution for fruit firmness which suggested that this population was segregating for a major locus controlling this trait. A major QTL for fruit firmness, named *qP-FF4.1*, that has not previously been reported, was identified in all three sweet cherry populations. Thirteen haplotypes (alleles) associated with either soft or firm fruit were identified for *qP-FF4.1* in the sweet cherry germplasm. The soft fruit alleles for *qP-FF4.1* contributed by the wild 'mazzard' sweet cherries were dominant over the firm fruit alleles. The identification of candidate genes for this fruit firmness QTL is underway. These results advance our understanding of the genetic basis of fruit firmness and will help to enable the use of DNA informed breeding for this trait in sweet cherry breeding programs.

Key words: Fruit firmness; QTL; Sweet cherry; Haplotype; DNA informed breeding

Transcriptome and metabolome profiling of *Botrytis cinerea* inoculated strawberry fruit during ripening

NOTE

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Abstract: Strawberry is one of the world's most popular fruit bearing crops. However, disease management is a never-ending challenge in strawberry cultivation. One of the most severe diseases is gray mold caused by the necrotrophic fungus *Botrytis cinerea*, leading to substantial economic losses in strawberry production worldwide. Currently, *Botrytis cinerea* fruit rot (BFR) is mainly controlled via fungicides. However, the adverse effects of fungicide use on human health and environment and the development of fungicide-resistant *B. cinerea* strains urge the development of alternative methods to limit BFR of strawberry. Infection generally occurs via the strawberry flower after which the fungus grows into the developing fruit without causing any disease symptoms. During ripening, fruit rot symptoms will appear and there are strong indications that both structural and biochemical changes during fruit ripening lead to optimal conditions for development of gray mold including shifts in phenolic compounds, modifications in cell wall composition and the influence of volatiles. However, more knowledge is required to elucidate which mechanisms specifically trigger BFR development. The aim of this work is to unravel the mechanisms that induce gray mold development during strawberry fruit ripening. To this end, we will analyze the transcriptome and metabolome of *B. cinerea* inoculated strawberry fruits during different ripening stages and in a *B. cinerea* resistant and susceptible cultivar. In this project we focus on *Fragaria vesca,* the woodland diploid strawberry. At first, we determined the different ripening stages of the fruit based on instrumental color, brix and firmness measurements. Secondly, we performed qRT-PCR to analyze the expression of various defense response marker genes at these at different ripening stages. Finally, these analyses will be performed on *F. vesca* cultivars with different levels of BRF susceptibility after which we will perform a transcriptome and metabolome profiling of a selected BFR susceptible and resistant cultivar. Results obtained within this project will be a first step in unraveling the mechanisms that induce gray mold development during strawberry fruit ripening and it will help to enhance the prospects for improving the plant immunity or to guide resistance breeding.

Key words: *Botrytis cinerea, Fragaria vesca*, strawberry, transcriptome, metabolome, fruit ripening

Genome-wide association study for detection of Apple Marssonina Blotch(AMB) disease resistance genes in Apple.

NOTE

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Abstract: Apples are one of the important agricultural crops in worldwide. Apple Marssonina Blotch disease, caused by *Diplocarpon mali*, is one of the most serious diseases known in Korea. Apple Marssonina blotch occur on leaves and fruits, which reduces the fruit quality and hinders nutrient accumulation leads to lower tree growth and lowers the fruit quality. 'Fuji' and 'Hongro', main cultivars in Korea, are known as susceptible to apple Marssonina blotch. But relative genome study has been rare. GBS-GWAS is useful for searching for genes that are related to the target trait. This study was carry out to detect candidate genes affection resistance to apple Marssonina blotch establish basic data for genomic study in future. From May, large quantity of conidia was produced, we monitored severity of infection on leaf of 730 apple germplasm(RDA korea) until October scored on a six scale. Results of pathogenicity showed 1.9% Immune(I) and 4.2% resistant(R), 23.0% Moderately resistant(MR), 28.1% Moderately susceptible(MS), 26.0% Susceptible(S), 16.7% Highly susceptible(HS), respecively. Carry out pretreatments for Genotyping-by-Sequencing (GBS), and then SNPs are called and filtered thru TASSEL-GBS pipeline with apple reference genome with 187 core collection. We performed GWAS analysis with GBS data of apple germplasm.

Key words : Apple Massonina blotch, resistance, *Malus x domestica*

NOTE

Development of a SCAR Marker for Discrimination of Bud Sports from *Malus* × *domestica* cv. Hongro through S-SAP Analysis

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Abstract: Retrotransposons (RTs) insert large number of copies into the plant genome, some of which occasionally causes mutation. Thus, RT-based molecular markers could be useful tool for genetic diversity analysis. Two large retrotransposon derivative (LARD)-RTs identified in our preceding study were used to develop the sequence-specific amplification polymorphism (S-SAP) markers for identification of ‘Hongro’ (*Malus* × *domestica*) bud sports from progenitor varieties. Two ‘Hongro’ bud sports including ‘Gumi Hongro’ and ‘Cheongsong Hongro’ were identified from progenitor variety and ‘Jahong’ by polymorphic peaks using primer combination LTR2+A3. Because ‘Gumi Hongro’ and ‘Cheongsong Hongro’ were not identified from each other in S-SAP analysis, the polymorphic band was excised with a cutter and purified to be sequenced. A pair of primers was designed based on 5′ upstream sequence and LTR2 primer, respectively. ‘Cheongsong Hongro’ was finally discriminated from ‘Gumi Hongro’ using cultivar-specific sequence characterized amplified region (SCAR) marker. Our strategy that features S-SAP analysis followed by SCAR marker might be effective to discriminate bud sports in fruit trees.

Key words: bud sport; ‘Hongro’; retrotransposon; SCAR; S-SAP

Characterisation, identification and mapping of genes controlling dwarf trait in apple (*Malus pumila* Mill.)

NOTE

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Abstract: Apple (*Malus pumila* Mill.) is one of the most widespread and commercially important fruit crops worldwide. Various dwarf genes in apple have been described in the literature but few, if any, have been mapped and characterised. An apple progeny derived from a cross of 'McIntosh', segregating for dwarf habit usually associated with crinkled leaves is growing at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij's Bien Donné Research Farm. Preliminary phenotypic scoring indicates a segregation ratio of approximately 9:7 for normal *versus* dwarf seedlings, which is consistent with control by two complementary genes. Mapping of two epistatic genes segregating simultaneously may not be straightforward. Therefore, a supplementary apple progeny derived from another cross of 'McIntosh' which segregates in a 3:1 ratio, presumably at just one of the two loci, has been chosen initially for mapping. This progeny has been verified for trueness to parentage with a set of 12 microsatellite markers. Rather than use microsatellites, in-depth-genotyping is being performed using the apple Illumina Infinium® 20K SNP (single nucleotide polymorphism) array in association with an Italian group. A high density SNP-based genetic map will be generated to map the dwarf trait and identify potential candidate genes. Concurrently, a transcriptomics approach using RNA-seq is being pursued with extracts from apical buds collected at several developmental growth stages to identify genes differentially expressed between the contrasting phenotypes. The results gained from this study should help elucidate the underlying molecular genetics and biological pathways that regulate growth in apple.

Key words: dwarf; growth habit; microsatellite; single nucleotide polymorphism

New Genomic Resources for Blackberry (*Rubus subgenus Rubus*) Breeding

NOTE

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Abstract: Blackberries (*Rubus* subgenus *Rubus*) are one of the most important small fruit crops in the US, with a production value of $38 M in Oregon and a growing fresh-market industry in other regions. Domestication and conventional breeding of blackberry started about 150 years ago. Cultivated blackberries are bred at the tetraploid level for the fresh-market industry and higher order polyploid levels for the processing industry concentrated in the Pacific Northwest. Historically, blackberry breeders have focused on improving crop yield, fruit size, firmness and postharvest quality, extended harvest season, disease resistance, primocane fruiting, thornlessness, and flavor. Molecular tools can help expedite the conventional breeding process. However, the molecular resources in blackberry are limited and consist of a few expressed sequence tags, a limited number of simple sequence repeat (SSR) markers, and a sparse linkage map. Here, we report high-quality draft genomes for two diploid blackberry accessions, Burbank Thornless (*R. ulmifolius*, PI 554060) and Hillquist (*R. argutus*, PI 553951). These two accessions were chosen as they represent important sources of thornlessness, and primocane fruiting, respectively, in the fresh-market blackberry breeding programs. The genome size for each cultivar was estimated to be 405 MB in Burbank and 376 MB in Hillquist using nuclear flow cytometry. Single-Molecule Real-Time (SMRT) sequencing technology using PacBio generated ~80X genome coverage for each of the two genomes. The reads were assembled using Falcon and Falcon-unzip software packages. The Burbank Thornless reads were assembled into 425MB genome with contig N50 of 915.3KB while Hillquist reads produced 374MB genome with ontig N50 of 486.5KB. We are in the process of using 10X genomic and HiC scaffolding tools to obtain chromosome-level assemblies. These genomes will be valuable resources for blackberry breeding programs and for comparative genomics in *Rubus* and the Rosaceae.

Key words: Thornless Burbank, Hillquist, genome sequence