June 24-26, 2014

Seattle, Washington, USA

Program and Abstracts

WASHINGTON STATE UNIVERSITY

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Welcome to the 7th International Rosaceae Genomics Conference

RGC7 is an opportunity for Rosaceae researchers from around the globe to meet and share their research and knowledge. This year's conference has over 135 attendees from 19 countries. The variety of rosaceous species covered is diverse and the discoveries and applications stimulating.

This conference has been organized by Washington State University (WSU). The primary campus of WSU is in Pullman which is located 300 miles (480 km) east of Seattle, close to the border with the state of Idaho. WSU research and extension faculty working on rosaceous crops are also located at four centers throughout the state. Tree fruit research occurs at the research stations in Prosser (south central Washington) and Wenatchee (north central Washington). Significant research programs within the USDA Agricultural Research Service are located in Prosser, Wapato, and Wenatchee. Berry research occurs at the research stations in Puyallup (south western Washington) and Mount Vernon (north western Washington).

Rosaceous crops are grown throughout Washington State; we are the largest producers of apple, cherry, pear, and fresh market raspberry in the US. Tree fruits are primarily grown in central Washington while the majority of the berries are grown in western Washington.

Rosaceae research in the Pacific Northwest has strong links with its vibrant and forward-thinking industries. Several of the major crop-producing groups self-tax to fund research to the tune of $4 million US annually. Tree fruit industry relationships with WSU are particularly strong and recently resulted in Washington growers voting to impose an additional self-tax to create a $32 million US endowment to help expand the research and extension activities at WSU.

Washington has a very diverse climate. It is known as the "Evergreen State" for the dense forests and green flora of western Washington. Seattle gets an average of 36 inches (910 mm) of precipitation a year. The state is divided by the Cascade Mountains, creating a rain shadow. As a result, central Washington gets very little precipitation, around 9 inches (230 mm), and is classified as a shrub steppe. Irrigation is a must for the agriculture in this area. The average amount of precipitation increases from the central part of the state to the eastern edge. Pullman receives 17 inches (430 mm) of precipitation per year and this allows for the dry-land cultivation of wheat, barley, lentils, canola, and garbanzos (chickpeas).

We have a busy program for the conference, but we hope that you also have time to enjoy Seattle. Pike's Place Market is just four blocks away from the hotel. It is a very good place to see and enjoy the agricultural products of Washington. In fact, it is the state's original farmers market, first opened in 1907. Seafood and crafts are also available for purchase. The hotel is also just a block away from the monorail station that goes to the Space Needle, Pacific Science Center, and Experience Music Project Museum. Downtown Seattle also has many things to explore. Thank you for attending the conference and we hope you have a great time not only at the conference, but in Seattle as well.

Conference Schedule

Tuesday, June 24 – Seattle Ballroom

7:00 AM – 9:00 AM	Registration and poster set-up	
9:00 AM – 9:15 AM	Opening remarks	Jim McFerson
9:15 AM – 9:30 AM	Welcome from Washington State University	Ronald Mittelhammer
9:30 AM – 10:20 AM	*Plenary address:* RGC: Then and now	Pere Arús Albert Abbott
10:20 AM – 10:30 AM	Announcements	
10:30 AM – 11:00 AM	Break *Sponsored by Affymetrix, Inc.*	
Topic Session – Genomics, Part I		Moderator: Dan Sargent
11:00 AM – 11:15 AM	Simultaneous sequencing of hundreds of nuclear loci for phylogenomic analyses across Rosaceae: A next generation sequencing approach	Aaron Liston
11:15 AM – 11:30 AM	GenSAS v2.0: an easy-to-use, web-based DNA annotation platform	Jodi Humann
11:30 AM – 11:45 AM	New insights in apple genomics	Riccardo Velasco
11:45 AM – 12:00 PM	Deciphering gene networks involved in fruit texture evolution during cold storage in 16 apple varieties	Mathilde Orsel
12:00 PM – 1:00 PM	Lunch, Emerald Ballroom	
Topic Session – Genomics, Part II		Moderator: Chris Dardick
1:00 PM – 1:15 PM	Transcriptome profiling on apple root defense responses to infection by *Pythium ultimum*	Yanmin Zhu
1:15 PM – 1:30 PM	Sequencing the *Potentilla micrantha* genome and transcriptome	Dan Sargent
1:30 PM – 1:45 PM	GDR: Current functionality and future direction	Dorrie Main
1:45 PM – 2:30 PM	*Genomics, 5 minute poster presentations*	
	Development of genomic resources in black raspberry	Douglas Bryant
	Activation of anthocyanin-related biosynthetic genes by bHLH transcription factors in *Rubus idaeus* and *Fragaria vesca*	Andrea Lorena Herrera Valderrama

	Softening of *F. chiloensis* fruit. An effort to understand its hormonal regulation using transcriptomic and genomic analyses	Maria A. Moya-León
	Global transcriptome analysis reveals the implication of one PME gene in apple mealiness development	Sandrine Mikol
	A population genomics approach for unraveling the genetic bases of differentiation between dessert and cider apples	Diane Leforestier
	Genome-wide copy number variation (CNV) detection in *Malus* x *domestica*	David Chagne
	Addressing fruit quality issues in *Prunus persica* varieties using a deep transcriptomic approach.	Ariel Orellana
	Transcriptome sequencing of *Prunus* sp. rootstocks roots to identify candidate genes involved in the response to root asphyxia	Rubén Almada
2:30 PM – 3:00 PM	Break	
Topic Session – Genomics to Genetics, Part I		Moderator: Lee Meisel
3:00 PM – 3:15 PM	The molecular basis for tree growth habit in *Prunus persica* (peach)	Chris Dardick
3:15 PM – 3:30 PM	The peach volatilome modularity is reflected at the genetic and environmental response levels	Maria Luisa Badenes
3:30 PM – 3:45 PM	First evaluation of the IStraw90 Axiom® Array in the cultivated ctrawberry (*Fragaria* × *ananassa*)	Nahla Bassil
3:45 PM – 4:00 PM	A dense genetic map of tetraploid rose on the basis of the WagRhSNP AXIOM SNP array and build using a pipeline for genetic analyses in polyploids	Marinus Smulders
4:00 PM – 5:00 PM	*Keynote address:* Why and how genome wide selection may work (or not work at all) in rosaceous crops	Rex Bernardo
5:00 PM – 7:00 PM	Poster reception, Emerald Ballroom *Sponsored by Affymetrix, Inc.*	

Wednesday, June 25 – Seattle Ballroom

Topic Session – Genomics to Genetics, Part II		Moderator: Ryutaro Tao
8:00 AM – 8:15 AM	Molecular characterization of cytokinin responsive genes during stone-fruit development in *Prunus persica*	Lee Meisel
8:15 AM – 8:30 AM	Functional characterization of a SAP protein expressed in dormant buds of peach	Maria Luisa Badenes
8:30 AM – 8:45 AM	Characterisation of the QTL associated with chill requirement during endodormancy in *Malus* x *Domestica* Borkh	Stephanie Cornelissen
8:45 AM – 9:00 AM	Characterization of unique plastid-targeted genes in apple (*Malus* x *domestica*)	Ryan Christian
9:00 AM – 9:15 AM	Fruit quality in sweet cherry: from QTLs to candidate genes	Elisabeth Dirlewanger
9:15 AM – 9:30 AM	Validation of a sweet cherry transcriptome for gene expression in fruit development	Paul Wiersma
9:30 AM – 10:15 AM	*Genomics to Genetics, 5 minute poster presentations*	
	A functional genomics approach to understand cracking in sweet cherries	Herman Silva
	A transcriptomic approach to understanding pedicel-fruit abscission in sweet cherry	Benjamin Kilian
	Sweet cherry cultivar fingerprinting using single nucleotide polymorphisms detected by high resolution melt analysis	Nadia Sokal
	Genome wide scan with the IPSC peach SNP array for the identification of QTLs controlling fruit quality, phenological and tree architecture traits.	Ignazio Verde
	Unique small RNA (sRNA)-based gene regulatory networks and their potential function in fruit crops	Zongrang Liu
	Phylogeny of species within the genus *Fragaria* revealed by next generation sequencing of multiple low copy nuclear markers	Yilong Yang
	A high density linkage map for the ancestral diploid strawberry *Fragaria iinumae* using markers from GBS and the ISTRAW90 Axiom® SNP Array	Lise Mahoney

	Carotenoids in reproductive organs of the diploid strawberry, *Fragaria vesca*	Janet Slovin
10:15 AM – 10:45 AM	Break	
Topic Session – Genetics		Moderator: Maria Jose Aranzana
10:45 AM – 11:00 AM	TxE revisited. Use of a *Prunus* reference mapping population in the NGS era	Pere Arús
11:00 AM – 11:15 AM	Identification of genomic regions associated with harvesting date and mealiness susceptibility in peach using QTL analysis	Claudio Meneses
11:15 AM – 11:30 AM	Comprehensive genotyping of the peach collection at the National Clonal Germplasm Repository in Davis	Ksenija Gasic
11:30 AM – 11:45 AM	Application of genetic and spatial analyses to identify collection priorities for wild *Malus* species	Gayle Volk
11:45 AM – 12:00 PM	Genetic diversity, structure and parentage analysis within several European apple germplasm collections assessed by microsatellite markers	Charles-Eric Durel
12:00 PM – 12:15 PM	CBFs, DAM, and DELLA Genes: The coordinated regulation of cold hardiness, dormancy, and growth in apple	Michael Wisniewski
12:15 PM – 1:15 PM	Lunch, Emerald Ballroom	
1:15 PM – 1:45 PM	*Genetics, 5 minute poster presentations*	
	Mapping and identification of disease resistance candidate genes in three *Malus* populations	John Baison
	A linkage map for black raspberry (*Rubus occidentalis*)	Jill Bushakra
	Roles of auxin biosynthesis by the flavin monooxygenase genes in developmental control and environmental responses in strawberry	Ke Duan
	An improved version of cultivated strawberry linkage map using the IStraw90 Axiom® Array for QTL analysis	Amparo Monfort
	Axiom® Genotyping Arrays: Automated analysis of complex plant genomes	Bridget Moore

Topic Session – Genetics to Breeding, Part I		Moderator: Jay Norelli
1:45 PM – 2:00 PM	Mapping loci for pest and disease resistance and hybrid necrosis in pear	Sara Montanari
2:00 PM – 2:15 PM	Genetic determinants of vigour control and precocity by pear rootstocks	Mareike Knaebel
2:15 PM – 2:30 PM	Genetic characterization of Japanese plum (*Prunus salicina* L.) cultivars and segregant populations through genotyping-by-sequencing (GBS) and simple sequence repeat (SSR) technologies	Basilio Carrasco
2:30 PM – 2:45 PM	QTL discovery and validation for soluble solids content, titratable acidity and remontancy within RosBREED strawberry germplasm	Sujeet Verma
2:45 PM – 3:15 PM	*Genetics to Breeding, 5 minute poster presentations*	
	Identification of QTL underlying soluble solids content and titratable acidity in sweet cherry (*Prunus avium* L.)	Yunyang Zhao
	Development of DNA markers for sucrose content in pear fruit	Akihiro Itai
	Fine mapping and candidate gene analysis to find the bitterness gene in almond	Raquel Sánchez Pérez
	Identification of QTL for volatile organic compounds in apple (*Malus* x *domestica*)	Carolyn Watkins
	Cross Assist: Online software to identify efficient cross combinations, integrating rosaceous crop genomics, genetics, and breeding	Cameron Peace
3:15 PM – 4:00 PM	Break	
4:00 PM – 5:00 PM	RosIGI meeting, First Hill conference room	
5:00 PM – 6:00 PM	US RosExec meeting, First Hill conference room	
6:00 PM – 10:00 PM	Conference dinner at Space Needle	

Thursday, June 26 – Seattle Ballroom

Topic Session – Genetics to Breeding, Part II		Moderator: Sue Gardiner
8:30 AM – 8:45 AM	Progress and challenges in pedigree-based QTL analysis utilizing high density marker data on related full sib families: A case study on fruit firmness in apple	Marco Bink
8:45 AM – 9:00 AM	Genetic determinism of budbreak timing in apple, a pedigree based analysis approach	Alix Allard
9:00 AM – 9:15 AM	Exploiting the genetics of the top of apple's chromosome 16 for breeding	Cameron Peace
9:15 AM – 9:30 AM	Genome-wide selection in apple: A pilot study in European breeding programs	Hélène Muranty
Topic Session – Breeding and Breeding Tools		Moderator: François Laurens
9:30 AM – 9:45 AM	Apple Breeding Populations as Research Resources	Susan Brown
9:45 AM – 10:00 AM	Field Trial Optimization for the Washington State University Apple Breeding Program	Julia Harshman
10:00 AM – 10:15 AM	Optimal Application of New Genotyping Technologies to Accelerate Tree Fruit Breeding Programs	Farhad Ghavami
10:15 AM – 10:45 AM	Break	
10:45 AM – 12:30 PM	Concluding Remarks and voting on next RGC location	
12:30 PM – 1:30 PM	Lunch, Emerald Ballroom	

Fifteen Minute Oral Presentation Abstracts

Genomics, Part I

Simultaneous sequencing of hundreds of nuclear loci for phylogenomic analyses across Rosaceae: A next generation sequencing approach

Aaron Liston[1], Richard Cronn[2]

[1]Oregon State University, Corvallis, OR, USA; [2]Pacific Northwest Research Station, USDA Forest Service, Corvallis, OR, USA

Abstract:
Phylogenetic reconstruction using organelles or small numbers of nuclear loci often fails to accurately reconstruct organismal relationships, especially in groups with high rates of speciation and/or reticulate evolution, such as Rosaceae. The apple, peach and strawberry genomes have been sequenced, and these genera represent 3 of the 4 lineages of the family. To simultaneously obtain sequence data from hundreds of nuclear loci, probes were designed for solution hybridization-based target enrichment followed by sequencing on the Illumina HiSeq platform (Hyb-Seq). A total of 1419 conserved orthologous exons (<15% sequence divergence in pairwise comparisons across the three genomes and >10% divergence among loci in a genome) were targeted from 257 single-copy nuclear loci. The analysis included 24 species representing the 12 recognized Rosaceae tribes and 4 unplaced genera. A mean of 1342 exons and 392,000 base pairs were obtained per species. In addition, nearly complete plastomes were assembled via genome skimming. Maximum likelihood phylogenetic analysis was conducted using nucleotide and amino acid sequences with concatenation and gene tree approaches. The robust phylogenetic resolution of all inter-tribal relationships provides a strong foundation for studies of gene family evolution, biogeography and character evolution in Rosaceae. The probes are available for phylogenetic analysis within genera of Rosaceae, and are currently being used in *Fragaria*, *Rubus*, *Crataegus* and others. These studies highlight their utility for the resolution of species delimitation, interspecific hybridization and polyploid origins.

Keywords: Rosaceae, phylogenomics, strawberry, hyb-seq

GenSAS v2.0: an easy-to-use, web-based DNA annotation platform

Taein Lee[1], Stephen Ficklin[1], **Jodi Humann**[1], Dorrie Main[1]

[1]Washington State University, Pullman, WA, USA

Abstract:
Genome and transcript sequencing are common tools in molecular biology and genetics research, but most researchers in these fields do not have the computer science knowledge or server access to use the common bioinformatics tools to analyze these data. Bioinformatics tools that are user-friendly and allow access to server resources via the web are becoming more common. The goal of this project was to design and implement a web-based DNA annotation pipeline that allows researchers to analyze their DNA sequences with command line based annotation tools though a graphical user interface. The Genome Sequence Annotation Server (GenSAS) allows users to upload DNA sequences, create a task of user selected annotation tools, and submit the task to the server for execution. Users return to the GenSAS web page to view and work with the results when the task is complete. Results from tasks are visualized in the GenSAS Browser and users can create custom curation tracks by selecting features from the results of the annotation tools. Users export their results in the GFF3 file format which is compatible with many downstream bioinformatics tools and genome browsers. We have tested GenSAS with DNA from a variety of organisms and of different lengths and GenSAS has been further tested in undergraduate and graduate classes. Based on feedback from users, GenSAS is an easy to use customizable online DNA annotation pipeline tool that allows users with little computer science knowledge to create a custom DNA annotation for their sequence(s) of interest.

Keywords: annotation, genome analysis

New insights in apple genomics

Michela Troggio[1], Luca Bianco[1], Elisa Banchi[1], Alessandro Cestaro[1], Diego Micheletti[1], Massimo Pindo[1], Daniel J Sargent[1], Mario Di Guardo[1], Fabrizio Costa[1], Eric van de Weg[2], **Riccardo Velasco**[1]

[1]Fondazione Edmund Mach, San Michele all'Adige, San Michele all'Adige, Trento, Italy; [2]Wageningen University and Research Centre, Wageningen, Netherlands

Abstract:
Next generation sequencing has significantly reduced sequencing costs and has permitted de-novo assembly of complex genomes, and re-sequencing of multiple genotypes within a species. The availability of plant reference genomes has accelerated genetics and genomics research, providing tools to identify functional elements, leading to the efficient development of improved varieties. Understanding the links between phenotypic variation and DNA variation the major challenge for plant geneticists. High-density SNP arrays for genome-wide assessment of allelic variation, have made high resolution genetic characterization of crop germplasm feasible. Such arrays are now commercially available for plant species with complex polyploid genomes. Here we present an Illumina Infinium 20K array in apple that will be implemented in Genome Wide Association Studies as well as Pedigree Based Analyses. In both cases it will be employed to dissect the genetic mechanism controlling important fruit quality traits, towards the identification of valuable markers suitable for the assisted selection activity in apple breeding programs. SNPs contained on the array were predicted from re-sequencing data derived from 14 apple genotypes and two doubled-haploids of Golden Delicious. A customised pipeline for SNP selection was devised avoiding the pitfalls of paralogous sequence variants. The performance of the array was assessed using data from mapping 23 populations from controlled crosses and germplasm collections. Marker positions based on the 23 genetic maps provided new insights on the apple genome, which will be presented and which were used to improve the scaffolding and anchoring of the apple reference genome, now available in the 3.0 new assembly.

Keywords: apple genome, genomics, genetics, molecular breeding, genotyping

Deciphering gene networks involved in fruit texture evolution during cold storage in 16 apple varieties

Jean-Marc Celton[1], **Mathilde Orsel**[1], Maryline Bruneau[1], Robert Scheaffer[2], Jean-Pierre Renou[1], François Laurens[1]

[1]INRA, Beaucouzé, France; [2]Plant & Food Research, Auckland, New Zealand

Abstract:
Fruit crop species, and particularly apple (*Malus* x *domestica*), are an important component of human diet. Most apples can usually be stored for several months before consumption in controlled environments (cold temperature, controlled atmosphere), thus slowing down the developmental processes associated with maturation. During this storage period, one of the most significant modification occurs to the quality of the fruit texture. Indeed, some varieties are known to develop undesirable characteristics such as softness, loss of crispiness, or mealiness. Physiological and biochemical studies are currently being performed in several research groups to understand the basis of these changes. However, it is only recently that genomic approaches have been used to investigate fruit maturation. To understand the molecular bases of fruit maturation, we developed a transcriptomic approach using a 120k AryANE microarray (1). Within the frame of the EU-FP7-Fruitbreedomics project, 16 varieties were characterised with sensory, instrumental and transcriptomic analyses over four months of cold storage, during two consecutive years. The objective is to identify differentially expressed genes and gene networks associated (i) with fruit maturation, (ii) fruit texture changes. Results from this study will provide us with an overall picture of the gene networks involved in fruit maturity development, and may allow us to identify early markers associated with fruit texture deterioration. (1)Celton et al., New Phytol, 2014. In Press.

Keywords: apple, fruit quality, texture, transcriptome, gene network

Fifteen Minute Oral Presentation Abstracts

Genomics, Part II

Transcriptome profiling on apple root defense responses to infection by *Pythium ultimum*

Yanmin Zhu[1], Ping Zheng[2], Sungbong Shin[3], Gennaro Fazio[4], Mark Mazzola[3], Dorrie Main[2]

[1]USDA-ARS Tree Fruit Research Lab, Wenatchee, WA, USA; [2]Washington State University, Pullman, WA, USA; [3]USDA-ARS, Wenatchee, WA, USA; [4]USDA-ARS, Geneva, NY, USA

Abstract:
The defense response of apple rootstock to necrotrophic soilborne pathogens inciting replant disease is poorly defined. In this study, apple rootstock seedlings were inoculated with *Pythium ultimum*, a primary member in replant disease pathogen complex. Root tissues including mock inoculated were sampled at 0, 1, 4, 8, 24, 48, 72 and 96 hour post inoculation (hpi). RNA-Seq based transcriptome profiling was performed with at least 20 million reads per sample. The large gap mapper plugin and the transcript discovery plugin in combination with the existing RNA-Seq tool in the CLC Genomics Workbench were used to map the reads and identify the differentially expressed genes (DEGs); gene ontology analysis and KEGG pathway analysis were applied to annotate DEGs. The peak response is at 48 hpi based on the number of the identified DEGs (1061). Members from gene families functioning in hormone signaling such as ET, JA, GA, CK and auxin, encoding NAC, WRKY, MYB and ERF transcription factors; encoding enzymes in several biosynthesis pathways of potential antimicrobial secondary metabolites and cell wall modification are among the identified DEGs. The results from this study represent a crucial step in dissecting the resistant mechanisms in perennial root system to soilborne pathogens. It seems that the molecular framework of defense responses in perennial root system is largely conserved with that characterized using foliar pathosystem. Further investigation on the defense responses specific to various pathogens and rootstock genotypes (resistant and susceptible) should reveal valuable information for future genomics-assisted breeding of resistant apple rootstock.

Keywords: apple rootstocks, resistance, replant disease, defense responses, molecular regulation

Sequencing the *Potentilla micrantha* genome and transcriptome

Marco Moretto[1], Matteo Buti[1], Paolo Sonego[1], Kristof Engelen[1], Nada Surbanovski[1], Lara Giongo[1], Duccio Cavalieri[1], Riccardo Velasco[1], Judson Ward[2], **Daniel Sargent**[1]

[1]FEM, San Michele all'Adige, TN, Italy; [2]Driscoll's, Watsonville, CA, USA

Abstract:
The genus *Potentilla* (Rosaceae) is large and diverse and is closely-related to the economically important *Rubus* and *Fragaria*. *Potentilla* species exhibit many morphological similarities to *Fragaria*, but the majority do not form fleshy accessory 'berries' characteristic of all members of the *Fragaria*. The close relatedness, morphological similarities, small genome size and high homozygosity of a number of *Potentilla* species make them an ideal comparative organism to study characteristic features of *Fragaria* such as 'berry' formation. To develop an experimental system to study such traits, the genome of *P. micrantha* was sequenced using the Illumina HiSeq and MiSeq and the PacBio RS sequencing platforms. Data were generated from overlapping paired end libraries, large insert mate-pair libraries and PacBio SMRT-bell libraries. The sequence data was assembled and a genome with an approximate size of 315 Mb was recovered, and confirmed with flow-cytometry against a *F. vesca* 'Hawaii 4' standard. RNA-seq was performed on reproductive tissue samples from five stages of fruit development in *P. micrantha*, corresponding to the stages of development defined for *F. vesca*. Additionally RNA-seq was performed on young leaf samples. Comparisons of the *P. micrantha* genome and transcriptome sequence to that of *F. vesca* 'Hawaii 4' revealed a high degree of expected synteny, but also clear patterns of global and localized divergence. The details and implications of these differences with respect to fruit development will be discussed.

Keywords: Rosaceae, comparative genomics, evolution, sequencing, genomics

GDR: Current functionality and future direction

Dorrie Main[1], Sook Jung[1], Taein Lee[1], Stephen Ficklin[1], Chun-Huai Cheng[1], Anna Blenda[2], Jing Yu[1], Ping Zheng[1], Julia Piaskowski[1], Sushan Ru[1], Cameron Peace[1], Kate Evans[1], Nnadozie Oraguzie[1], Mercy Olmstead[3], Albert Abbott[2]

[1]Washington State University, Pullman, WA, USA; [2]Clemson University, Clemson, SC, USA; [3]University of Florida, Gainesville, FL, USA

Abstract:
GDR (www.rosaceae.org) is a curated and integrated web-based relational database providing access to publicly available genomic, genetic and breeding data for Rosaceous crops. GDR contains annotated whole genome sequences, transcripts, markers, trait loci, genetic maps, genes, taxonomy, germplasm, publications and communication resources for the Rosaceae community. In this update we report on new functionality and data including access the addition of RNASeq and GBS data viewable through implementation of the JBrowse genome viewer, as well as new genome, trait, map and marker data, and new or improved search tools. The accepted community database for Rosaceae research, GDR was accessed by 14,227 users from 127 countries in 2013, with over 170,000 pages accessed.

Keywords: Rosaceae, genomics, genetics, breeding, database

Fifteen Minute Oral Presentation Abstracts

Genomics to Genetics, Part I

The molecular basis for tree growth habit in *Prunus persica* (peach)

Chris Dardick[1], Courtney Hollender[1], Ralph Scorza[1], Chinnathambi Srinivasan[1], Ann Callahan[1], Tetyana Zhebentyayeva[2], Karina Ruiz[3], Michael Whitaker[1], Renate Horn[4], Albert Abbott[2], Tom Tworkoski[1]

[1]USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV, USA; [2]Clemson University, Clemson, SC, USA; [3]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Colina El Pino, La Serena, Chile; [4]University of Rostock, Rostack, Germany

Abstract:
The large size and spreading growth habit of trees requires excessive labor, land space, and pesticides. Genetically improving tree shapes so that they can be planted at higher density and/or more readily adapted to mechanization would increase productivity and be more environmentally friendly. Currently, very little is known about the genes and signaling pathways that regulate tree growth and development. Using peach as a model system, we have mapped and identified several genes that control branch growth and tree size via a new, Next-Gen sequencing enabled method. The peach pillar trait associated with fastigiated growth was found to be controlled by a gene called PpeTAC1 (Tiller Angle Control) homologues of which were previously described in rice and maize. Knock outs of PpeTAC1 in Arabidopsis showed a similar fastigiated growth habit as did transgenic plums which were silenced for PpeTAC1. PpeTAC1 was found to be a member of a broader gene family that includes the previously described LAZY1. Knock outs of LAZY1 in rice and *Arabidopsis* displayed prostrate growth as well as a loss of gravitropism associated with impaired polar auxin transport. RNAseq studies of apical shoots from TAC1 and LAZY1 mutants in peach and *Arabidopsis* did not show changes in auxin biosynthesis, transport, or signaling but instead revealed coordinated changes in stress, defense, and secondary metabolic pathways. The knowledge gained from this work has important implications for improving agricultural productivity and sustainability in not only fruit trees but potentially a wide variety of different crops.

Keywords: tree architecture, genomics, peach, branch angle, next generation sequencing

The peach volatilome modularity is reflected at the genetic and environmental response levels

Gerardo Sánchez[1], José Martinez[2], Jesús Garcia[3], Antonio Monforte[4], **Maria Badenes**[5], Antonio Granell[4]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), San Pedro, Argentina; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain; [3]Instituto Murciano de Investigación y Desarrollo Agrario (IMIDA), Murcia, Spain; [4]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain; [5]Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain

Abstract:
The improvement of fruit aroma is currently one of the most desirable objectives in peach breeding programs. To better characterize and asses the genetic potential for increasing the aroma by breeding, a quantitative trait locus (QTL) analysis approach was carried out in a F1 population segregating largely for fruit traits. Linkage maps were constructed using the IPSC peach 9 K Infinium ® II array, rendering dense genetic maps except of some chromosomes, probably due to identity-by-descent of those chromosomes in the parental genotypes. Aroma variability was analyzed by a metabolomic approach based on GC-MS to profile 81 volatile compounds across the population in two locations. Additionally, quality-related traits were also studied to assess possible pleiotropic effects. Correlation-based analysis of the volatile dataset revealed that the peach volatilome is organized in modules formed by compounds from the same biosynthetic origin or sharing similar chemical structures. QTL mapping showed clustering of volatile QTL included in the same volatile modules, indicating that some of them are subjected to joint genetic control. The monoterpene module is controlled by a unique locus at the top of LG4, a locus previously showed to affect the levels of two terpenoid compounds. At the bottom of LG4 a locus controlling several volatiles but also melting/nonmelting and maturity-related traits was found, suggesting putative pleiotropic effects. In addition, two novel locus controlling lactones and esters at linkage group 5 and 6 were discovered. The results presented here confirmed previously locus controlling the aroma of peach but also identified novel ones.

Keywords: peach, aroma, breeding, improvement, QTL

First Evaluation of the IStraw90 Axiom® Array in the Cultivated Strawberry (*Fragaria* × *ananassa*)

Nahla Bassil[1], Thomas Davis[2], Iraida Amaya[3], Laurent Bellon[4], Luca Bianco[5], Fiona Brew[6], Beatrice Denoyes[7], Thijs van Dijk[8], Stephen Ficklin[9], Amy Iezzoni[10], Sook Jung[9], Lise Mahoney[2], Dorrie Main[9], Michael Mittman[4], Amparo Monfort[11], Cameron Peace[9], Ali Pirani[4], Umesh Rosyara[10], Daniel Sargent[5], Sujeet Verma[12], Teresa Webster[4], David Wood[2], Yilong Yang[2], Hailong Zhang[2], Vance Whitaker[12], Eric Van De Weg[8]

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Abstract:
The RosBREED project aimed to enhance the infrastructure for marker-assisted breeding, including platform development for high throughput genome scans. For the allo-octoploid, cultivated strawberry, this goal was met by the public release of the International Strawberry 90K (IStraw90TM) Axiom® genotyping array developed in collaboration with Affymetrix. Array design mainly exploited polymorphisms identified in the cultivated strawberry. The challenges of allo-octoploidy were addressed by maximizing the number of SNP targets to compensate for potential low conversion of candidate to functional SNPs, and sub-genome-specific probe targeting. Additionally, some reduced ploidy genomic sites were targeted via fortuitous probe placement. The array was evaluated on four mapping populations, numerous pedigreed breeding populations, various cultivars and selections, and several genetically unusual strawberry genotypes. New filters were developed and octoploid-specific thresholds were used in the genotyping procedure to identify potentially well-resolved and more complex SNP markers. A total of 12,609 well-resolved SNPs were obtained and evaluated through the generation of a genetic linkage map for 'Holiday' × 'Korona' (HK), where 6,689 of the 8,084 polymorphic HK-SNPs were successfully mapped. These mapped SNPs were also checked for marker consistency in the pedigreed cultivars and breeding selections using FlexQTLTM. Results of the SNP development strategies, improved gridding algorithm and automated genotype scoring will be presented. This array is the first high throughput genotyping platform in an octoploid organism and we expect it to enable genome-wide scanning in the cultivated octoploid strawberry and facilitate QTL discovery for many traits of economic significance in this important Rosaceous fruit crop.

Keywords: single nucleotide polymorphism, high throughput genotyping, allo-octoploid

A dense genetic map of tetraploid rose on the basis of the WagRhSNP AXIOM SNP array and build using a pipeline for genetic analyses in polyploids

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Abstract:
Rose, as many other ornamental, vegetable and field crops, is tetraploid. This poses constraints in genetic analyses, due to the occurrence of multiple alleles at marker and trait loci and the existence of multiple allele dosages. Developments in marker discovery (next generation sequencing), genotyping (SNP arrays) and analysis (software for dosage scoring) now make it feasible to develop high-density molecular marker maps for the homologous chromosomes in tetraploids separately, and perform QTL analysis. We developed the WagRhSNP Axiom array based on sequences from tetraploid garden roses and cut roses, to be used for inheritance studies and genetic mapping. The array design can be used by all researchers working in rose. We have also developed a general strategy for mapping in polyploid crops including stringent error filtering, which we will also apply to other ornamentals, including tetraploid *Alstroemeria* and hexaploid *Chrysanthemum*. Using this strategy we have built genetic maps for cut and garden roses. They are based on the simplex:nulliplex, simplex:simplex and duplex:nulliplex segregating SNPs. The cut rose map contains a total of 2515 (parent P514) and 2762 (P867) SNP markers. Each linkage group has between 127 and 705 SNP markers. Twenty six of the 28 haplogroups (homologous chromosomes) are represented with 30-246 SNPs per haplogroup. Both maps enabled establishing that the predominant mode of inheritance is tetrasomic with a possible disomic inheritance in one chromosome. Furthermore, occurrence and distribution of double reduction is calculated, and a detailed overview of the synteny with the *Fragaria vesca* genome sequence is produced.

Keywords: linkage map, Rosa, tetrasomic inheritance, synteny, SNP arrray

Fifteen Minute Oral Presentation Abstracts

Genomics to Genetics, Part II

Molecular characterization of cytokinin responsive genes during stone-fruit development in *Prunus persica*

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Abstract:
The phytohormone cytokinin plays a major role in plant development. Recently we have reported the identification and comparative analyses of genes associated with cytokinin signaling and homeostasis pathways in two hardwood tree species: *Populus trichocarpa* and *Prunus persica* (Immanen et al., 2013). To better understand the role that cytokinin responsive genes play in stone fruit development, we have performed RNA-seq and qPCR analyses of peach fruits treated with exogenously applied t-zeatin at different developmental stages. These analyses reveal a developmental stage specific expression of the cytokinin signaling and homeostasis pathway genes in peach fruits. We have also identified cytokinin responsive genes during pre-lignification, lignification and post-lignification stages of stone-fruit development in *Prunus persica*. Furthermore, transient overexpression of a putative type-B response regulator (PpRR1) in peach fruits increased the expression of putative downstream genes, PpShy2 and PpRR6. These results suggest that the cytokinin response pathway that has been described in *Arabidopsis* is conserved in peach, and that this response pathway is differentially expressed during peach stone-fruit development. This work was funded by CONICYT Fondecyt /Regular N°1121021

Keywords: *Prunus persica*, cytokinin, stone fruit, development, lignification

Functional characterization of a SAP protein expressed in dormant buds of peach

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Abstract:
The meristems of many perennial plants in temperate and boreal climates remain in a cyclic quiescent state within buds during the cold period of autumn and winter, which ensures protection against the effects of cold and water stress. Photoperiod and temperature control growth cessation and bud dormancy induction, whereas dormancy release requires the quantitative perception of chilling. We performed two transcriptomic approaches for the identification of differently expressed transcripts in reproductive buds of peach (*Prunus persica* [L.] Batsch). Among different genes involved in transcriptional regulation of dormancy, pollen development and stress tolerance, we identified a gene coding for a protein similar to Stress Associated Proteins (SAP) containing two specific Zn-finger domains named A20 and AN1 (PpSAP). SAPs have been described as regulators of the abiotic stress response in plant species, emerging as potential candidates for improvement of stress tolerance in plants. We have studied the developmental and stress dependent expression of PpSAP in reproductive buds and vegetative tissues. PpSAP was highly expressed in leaves and dormant buds, being down-regulated after the release of bud dormancy and before bud break. PpSAP strongly interacted with ubiquitin proteins in the yeast two-hybrid system, in accordance with previous works supporting an E3 ubiquitin ligase activity for SAP proteins. PpSAP was constitutively expressed in transgenic plum plants under the control of CaMV 35S promoter. PpSAP over-expression led to alterations in leaf shape and an increased tolerance to leaf desiccation, which conferred to this gene a high interest for the manipulation of abiotic stress tolerance in plants.

Keywords: stress associated proteins, PpSAP overexpression, transgenic plums, abiotic stress, dormancy

Characterisation of the QTL associated with chill requirement during endodormancy in *Malus* x *domestica* Borkh

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Abstract:
Temperate sub-tropical countries, for instance South Africa, receive very little chill measured in Richardson Units (RU) during winter months, which causes great concern for farmers producing high chill requirement fruit crops like apples. Failure to fulfill chill requirement in apples causes prolonged dormancy symptoms for instance reduced branching and extended fruiting period in trees. The genetic mechanism of chill accumulation during endodormancy is not well understood and the aim of this study is to investigate this phenomenon. The two apple varieties, 'Anna' and 'Lady Williams' were used to compare the differentially expressed genes during chill accumulation. 'Anna' is a low chill apple variety that requires less than 300 hours of chill. 'Lady Williams' is a very high chill variety that requires more than 1500 hours of chill. QTL analysis was performed for a Lady Williams' x 'Anna' mapping population using the apple 8K Infinium SNP-array. One major QTL on linkage group 9 and several minor QTLs on linkage groups, 2, 8 and 15 were detected. RNA was extracted from Lady Williams and Anna at 100h time intervals and analysed by RNA-Seq. Transcripts were investigated between time intervals to identify genes that were differentially expressed and then mapped against the QTL's to locate the genes within the loci. A number of transcripts were highlighted as being differentially expressed between time intervals. The differentially expressed genes from Anna were compared to those from Lady Williams. Differences between the patterns of expression were identified for further analysis.

Keywords: dormancy, chill accumulation

Characterization of unique plastid-targeted genes in apple (*Malus* x *domestica*)

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Abstract:
Until recently, tomato (*Solanum lycopersicum*) was regarded as a model for plastid development for all fruits. However, recent studies of plastid morphology and development in apple (*Malus* x *domestica*) have revealed that not only are there extreme differences between apple and tomato, but there are also significant differences between apple cultivars as well throughout fruit development. The recent sequencing of the 'Golden Delicious' genome has provided a wealth of sequence information that has shed some light on why such a stark difference exists; the apple genome is predicted to contain nearly 5,000 unique plastid-targeted proteins which are not represented in the genomes of *Arabidopsis*, grape, peach, pear, poplar, or tomato. These unique genes represent over 40% of the total plastid proteome in apples, which is currently predicted to have 10,000 proteins. Because these proteins have not been documented in plastids before now, each may have functions that are entirely new to plant biology. Of the 5,000 unique genes, we have narrowed our focus to genes which may function in crosstalk between the plastid and nucleus or in modulation of photosynthesis. The gene set was enriched with GO terms for electron transfer, chromatin-binding, transcription, translation, and proteins containing transmembrane domains; members of these categories are undergoing expression analysis, localization, and photosynthesis phenotyping experiments. This work will shed light on the plastid morphodevelopment in non-model fruit systems such as apple, and may unveil proteins with novel functions that can be utilized in the future for development of improved varieties.

Keywords: protein transport, plastids, plant science, cell biology

Fruit quality in sweet cherry: from QTLs to candidate genes

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Abstract:
In sweet cherry, fruit weight, firmness, and organoleptic quality are very important traits for both producers and consumers. Their genetic determinism has been analysed in three F1 sweet cherry progenies. 'Regina' × 'Lapins' (RxL), 'Regina' × 'Garnet' (R×G) and 'Fercer' × 'Burlat' (F×B), were analysed for fruit weight and firmness, during four to seven years. R×L was also analysed for pH and titratable acidity for three years. Major soluble carbohydrates (glucose, fructose, sucrose and sorbitol) and major organic acids (citrate and malate) were quantified during one year on R×L and F×B progenies. Genotyping was performed with the RosBREED's cherry 6K SNP array v1 and linkage maps constructed with JoinMap 4.0. Many QTLs were detected for both fruit weight and firmness and in most cases in the same position, the most significant being detected on LG2, 5 and 6. Major QTLs for pH, titratable acidity and malic acid were identified on LG6, in the same region. On LG2 the QTL for fruit weight co-localised with a CELL NUMBER REGULATOR gene (CNR 12). For fruit weight and firmness, 14 candidate genes (CGs) were identified on the QTL on LG5 and 29 on the QTL on LG6. On LG5, three were selected for their potential involvement in fruit weight control: cytochrome p450 78A3-like, CNR1 and plac8 family protein. The 11 other CGs were selected for their potential involvement in fruit firmness. On LG6, three and 26 CGs were selected for their potential involvement in the control of fruit weight and firmness, respectively.

Keywords: sweet cherry, fruit quality, QTLs, candidate genes

Validation of a sweet cherry transcriptome for gene expression in fruit development

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Abstract:
The development of cherry fruit follows a well-defined pattern that ends in a desirable, healthy and valuable product. Associated with development is a large scale change in gene expression pattern with differential regulation. Next-generation sequencing provides the tools for examining this process at a level of detail never before possible. This study constitutes three parts: sequence discovery to provide a reference transcriptome; RNA-Seq analysis across fruit development; and validation of the RNA-Seq data as a measure of gene expression changes during development. A sweet cherry transcriptome was constructed from RNA libraries made from multiple stages of leaf, buds and fruit tissues. Assembly was done using SOAPdenovoTrans, producing more than 30k scaffolds over 500 bp and an N50 over 1500. Predicted orfs were compared to the peach model and over 12k cherry transcripts matched to peach in sequence and peptide size. RNA-Seq was conducted from 20 libraries spanning the entire fruit developmental timeframe. Alignment of these reads to the cherry transcriptome produced between five and seven million matches for each library. Read counts were roughly normalized by dividing by total counts and expression changes graphed for genes identified by homology to the peach model. Gene expression was further characterized by quantitative PCR to identify: “house-keeping” genes useful for normalizing expression levels across tissues; stage-specific changes flagging transitions in fruit development; and cell wall modifying gene products associated with fruit firmness. These quantitative PCR results confirmed the gene expression patterns observed by RNA-Seq.

Keywords: *Prunus avium*, RNA-Seq

Fifteen Minute Oral Presentation Abstracts

Genetics

TxE revisited. Use of a *Prunus* reference mapping population in the NGS era

José Manuel Donoso[1], Octavio Serra[1], Roger Picañol[1], Iban Eduardo[1], Konstantinos Alexiou[1], María José Aranzana[1], Werner Howad[1], **Pere Arús**[1]

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Abstract:
During the last decade, the high-density marker map based on the F2 progeny of the 'Texas' almond and 'Earlygold' peach (TxE) cross has been a resource for the *Prunus* scientific community. TxE has been used to identify markers for the construction of anchored linkage maps in crosses involving peach and other *Prunus* and mapping major genes and QTLs on them, for fast mapping of markers or candidate genes with the bin mapping approach, for the comparison of maps with other rosaceous crops, for physical map construction, and recently as one of the reference maps employed for the alignment of the peach genome assembly. Once the peach genome sequence has become available, a new and more powerful avenue for most of these applications is open. This communication will present some of the new ways in which this population, and others derived from the 'Texas' x 'Earlygold' hybrid, are currently being used for a better understanding of the *Prunus* genome and the genetics of characters of interest for plant breeding. This will include: a) the new maps of TxE and the first backcross progeny to peach (T1E population) based on the 9k IPSC peach SNP chip, b) the resequencing of individuals of T1E to find the precise positions of recombination breakpoints, and c) the use of these two populations to study the inheritance of a broad set of characters and to identify alleles of almond which may be valuable for peach improvement.

Keywords: SNP, resequencing, introgression, peach, almond

Identification of genomic regions associated with harvesting date and mealiness susceptibility in peach using QTL analysis

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Abstract:
Chile is the first peach exporter in the South Hemisphere and the fifth producer in the world. Chilean initiatives of peach breeding have been carried out, where the fruit quality and postharvest performance are the main goals for the new peach varieties. Breeding is a time consuming and costly process. For this reason, the development of genomic tools to support the early selection of genotypes is quite relevant to improve the efficiency of breeding programs. Thus, the aim of this work was to identify genome regions associated with fruit quality traits in peach using a QTL analysis. Harvesting date, soluble solids content (SSC), color, weight, firmness, titratable acidity and mealiness were evaluated. The 'Venus' x 'Venus' segregating population was genotyped with microsatellite markers and SNPs using 9K SNP array for peach. A linkage map was built with 1,820 markers, which were mapped in 8 linkage groups. The resulting map spanned a total distance of 382.9 cM with an average of 0.21 cM between adjacent markers. A QTL for titratable acidity was associated to chromosome 1, another QTL linked to cover skin color was associated to chromosome 3, and QTLs detected in chromosome 4 were related with weight, harvesting date, soluble solids contents and firmness. Finally, six QTLs were detected for mealiness, four of them located in chromosome 4 and two in chromosome 2. These results provide insights into the genetic determinants of quality traits in peach, but further work is required to identify and to validate candidate genes and polymorphisms. (This work was supported by Fondecyt 11121396, FONDAP CRG 15070009; Genoma G13I0005, Conicyt fellowship D-21120635 to ACE and Basal PFB-16).

Keywords: *Prunus persica*, QTL, harvesting date, mealiness, genetic linkage map

Comprehensive genotyping of the peach collection at the National Clonal Germplasm Repository in Davis

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Abstract:
We used genotyping by sequencing (GBS), a low-cost, high-throughput sequencing technology to genotype 333 *Prunus* accessions, preserved at the *Prunus* collection of the National Clonal Germplasm Repository (NCGR) in Davis, California. The *Prunus* collection is the second largest in this genebank with more than 90 taxa and in excess of 1600 accessions of *Prunus* spp. that includes almonds, apricots, cherries, peaches and plums. The accessions genotyped here consist of heirlooms (old cultivars never patented, or off patent), landraces, breeder's lines, and wild relatives of the peach from all over the world. Majority of accessions belonged to *Prunus persica* (84%), with 10% of them being wild relatives (*P. mira*, *P. davidiana*, and *P. ferganensis*) and 6% categorized as hybrids between peach and other related species and *Prunus* spp. The method produced on average 1 million sequence reads per accession, with majority of the accessions having more than 500,000 reads. We identified 18,008 single-nucleotide polymorphism (SNP) markers, present in at least ≥ 80% of analyzed accessions distributed across the entire genome. These genomic data will serve as a resource for breeders seeking to develop peach cultivars that will meet the challenge of changing climates, markets, and horticultural practices. The use of these SNP markers for conservation, management and utilization of the NCGR collection as well as for genome-wide association studies (GWAS) in combination with phenotypic data available through Germplasm Resources Information Network (GRIN) will be discussed.

Keywords: *Prunus*, genotyping by sequencing, genetic resources, SNP, genetic diversity

Application of genetic and spatial analyses to identify collection priorities for wild *Malus* species

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Abstract:
The USDA-ARS National Plant Germplasm System has 33 species of wild *Malus*, many of which were acquired from plant explorations performed over the past 30 years. The phylogenetic relationships among these species were determined by chloroplast sequencing (1681 bp from four regions). Five primary clades of species were identified. *Malus* x *domestica* and the primary progenitor species, *M. sieversii*, *M. orientalis*, *M. sylvestris*, and *M. prunifolia* were localized within one of the two clades specific to species of Chinese origin. *Malus fusca*, native to the western US and Canada, also localized within this Chinese clade. The other three *Malus* clades originate from Taiwan (*M. doumeri*), southern Italy (*M. florentina*), and central/eastern North America (*M. ioensis*, *M. coronaria*, *M. angustifolia*). Genetic diversity and admixture of individuals within each clade and species was evaluated using nuclear microsatellite markers. Together these data provide the basis for integrating genetic structure information with species range distribution modeling approaches to prioritize novel collection sites.

Keywords: geography, genetic diversity, *Malus*, wild species, phylogeny

Genetic diversity, structure and parentage analysis within several European apple germplasm collections assessed by microsatellite markers

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Abstract:
Cultivated apple (*Malus* × *domestica*) is the most important fruit crop of the temperate regions worldwide. The accurate phenotypic and genetic characterization of apple genetic resources is essential for enlarging the genetic bases of breeding populations. Here we investigated the genetic diversity and structure of almost 2,700 European dessert apple accessions (old cultivars) originating from 12 germplasm collections located in 9 European countries, Russia, and Kyrgyzstan using 16 common SSR markers. Funding derived from both national and European (FruitBreedomics) projects. Priority was given to accessions chosen to be both diverse and representative of regional/national landraces. Genotyping made the identification and clarification of many redundancies, among accessions with different names but identical SSR profiles, possible both within and between collections. A total of 1,750 unique diploid genotypes (unique SSR profiles) were retained for further analyses. The average allelic diversity was very high (Na ~23), as were the observed and expected heterozygosities (0.81-0.83). The genetic structure of these accessions was studied by Factorial Component Analysis (FCA) and Structure software. A slight differentiation according to the geographic origin of the accessions (when known) was found (Fst = 0.021 +/-0.003), when subdividing the accessions into 3 large regions (West, South, North-East). Three groups (K=3) were also identified by Structure, but did not fully coincide with the pre-defined geographic regions. Parentage analyses made it possible to infer parents of several old cultivars. Overall, the genetic diversity was very high, but with a weak structure confirming large gene flow across Europe.

Keywords: *Malus* × *domestica*, SSR fingerprinting, genetic diversity, structure, gene flow

CBFs, DAM, and DELLA Genes: The coordinated regulation of cold hardiness, dormancy, and growth in apple

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Abstract:
CBFs are cold-induced transcription factors (TFs) that regulate the expression of many cold-acclimation-related genes. To better understand and improve cold hardiness in apple, transgenic lines of 'M26' apple have been created that overexpress a peach CBF gene or various native apple CBF genes. Alternatively, silencing constructs have been used to generally or specifically inhibit native CBF genes. These transgenic lines have been studied in both laboratory and field studies over the past several years. Overexpression of a peach CBF gene in apple improved cold hardiness and unexpectedly also made trees enter dormancy earlier in the fall, delayed budbreak in the spring, and significantly decreased growth. These effects have been verified in 3 years of field studies. Transcriptomic studies indicate that CBF genes regulate approximately 8.5% of the total gene set identified in apple during normal cold acclimation, including a number of other transcription factors, including dormancy-associated-MADs box (DAM) genes. A bioinformatic analysis has revealed that several apple DAM genes possess one to several C-repeats (CBF protein binding motif) in their upstream promoter sequences. CBFs have also been proposed to regulate growth by inducing the accumulation of growth repressing DELLA proteins in the nucleus. Thus it appears that, in addition to cold acclimation, CBF TFs have the potential to integrate the regulation of both dormancy and growth. A bioinformatic analysis of CBF and Inducer of CBF Expression (ICE) genes in several fruit (grape, apple, peach, citrus, and blueberry) and forest tree (poplar, eucalyptus) species has also been conducted and will be discussed.

Keywords: cold hardiness, dormancy, climate change, environmental stress, transgenic apple

Fifteen Minute Oral Presentation Abstracts

Genetics to Breeding, Part I

Mapping loci for pest and disease resistance and hybrid necrosis in pear

Sara Montanari[1], Laure Perchepied[2], Hélène Muranty[2], Philippe Robert[3], Vincent Bus[4], Lester Brewer[5], Mickael Malnoy[1], Sue Gardiner[6], Riccardo Velasco[1], David Chagné[6], Charles-Eric Durel[2]

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Abstract:
We present our findings on genetics of resistance to fire blight (caused by the bacterium *Erwinia amylovora*) and psylla (*Cacopsylla pyri*) in a pear inter-specific segregating population between P128R068T003 (*Pyrus* x *bretschneideri* X *P. communis*) and 'Moonglow' (*P. communis*). Asian pears are usually less susceptible to psylla than their European relatives and P128R068T003 was previously demonstrated to be a source of resistance to *C. pyri*. The other parent, 'Moonglow', originated from five *P. communis* cultivars, and we demonstrated 'Roi Charles Würtemberg' to be the source of fire blight resistance. We performed the phenotyping of P128R068T003 x 'Moonglow' population at INRA in Angers (France) and detected Quantitative Trait Loci (QTLs) for both fire blight and *C. pyri* resistance. Amongst the progenies of this cross, we also observed some instances of hybrid necrosis. This phenomenon is caused by epistatic interactions between genes that have deleterious effects in the hybrid, resulting in dwarfism, tissue necrosis and in some cases lethality. This type of genetic incompatibility has been observed in several plant species and has been known for a long time by plant breeders. We were able to collect leaf samples from the seedlings showing the hybrid necrosis phenotype, scan them with molecular markers using the High Resolution Melting (HRM) technique and identify, for the first time in pear, some of the genomic regions where the lethal genes are located.

Keywords: pear, resistance, fire blight, *Cacopsylla pyri*, hybrid necrosis

Genetic determinants of vigour control and precocity by pear rootstocks

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Abstract:
We examined the genetic determinants of vigour control and precocity conferred on a pear scion by *Pyrus* rootstocks, for the purpose of enabling breeders to develop new pear rootstocks with improved vigour and floral precocity. A segregating population of 450 F1 rootstocks from a *P. communis* cross between 'Old Home' and 'Louise Bonne de Jersey' (OHxLBJ), grafted with 'Doyenne du Comice' scions, was comprehensively phenotyped for traits related to tree architecture and flowering, and the data modelled in order to describe the growth of the scions. Genotyping was performed using the apple and pear Infinium® II 9K SNP array. A high density SNP-based genetic map was constructed using 546 polymorphic pear and 99 apple markers. QTLs relating to vigour control and precocity were detected on LG5 and LG6 of 'Old Home'. The LG5 QTL is orthologous to the Dw1 locus from 'Malling 9' apple rootstock (Rusholme Pilcher et al. 2008). Analysing the syntenic region linked to this QTL in the *P. communis* ('Bartlett'), *Malus* x *domestica* ('Golden Delicious') and *Prunus persica* reference genomes should lead to the identification of candidate genes for vigour control in both pear and apple.

Keywords: *Pyrus communis*, rootstock, vigour control, precocity, QTL

Genetic characterization of Japanese plum (*Prunus salicina* L.) cultivars and segregant populations through Genotyping-By-Sequencing (GBS) and Simple Sequence Repeat (SSR) technologies

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Abstract:
Next-generation sequencing technologies have dramatically accelerated the use of genetic information for genome diversity analysis and genetic mapping in plants. Genotyping-by-sequencing (GBS) is a next-generation DNA sequencing (NGS) technology that can analyzed hundreds of thousands of short reads from a genome, allowing a direct and inexpensive single nucleotide polymorphism (SNP) detection from germplasm collections. Our objective was to identify and characterize SNPs through commercial cultivars and segregant populations with known paternity relationships. We used the GBS technology to characterize a collection of 29 commercially important Japanese plum (*Prunus salicina* L.) cultivars plus 116 offspring derivated from controlled cross among Japanese plum cultivars. We analyzed 68 offspring from Angeleno (♀) by Aurora (♂) and 48 offspring from Flavor Rich (♀) by Larry Ann (♂). Previously, a paternity analysis was conducted in order to verify the filiations for individuals of each segregant population. We calculate the paternity index (PI), combinated paternity index (CPI) and probability of paternity (W) using 17 simple sequence repeat (SSR). The sequencing and filtering process gave 1,784,547 tags. The tags were aligned to the peach reference genome, where 50% aligned to unique position, 5% aligned to multiple position and 45% could not be aligned. Finally after filtering, 57,542 SNP were identified. We discussed the useful of high-throughput GBS to characterize the genetic diversity of commercial cultivars and segregant populations of Japanese plum. Acknowledgements: This research was supported by CONICYT, FONDECYT/Regular Nº1120261.

Keywords: GBS, SNPs, *Prunus salicina*, SSR, Genetic characterization

QTL discovery and validation for soluble solids content, titratable acidity and remontancy within RosBREED strawberry germplasm

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Abstract:
Strawberry is the most consumed Rosaceae small fruit in the world where flavor is a key for the success of a new cultivar. Overall flavor in strawberry is driven by a balance between sugars, acids, and several aroma compounds. Strawberry breeders are interested in using marker-assisted selection to combine better flavor with remontancy (day-neutrality). While quantitative trait loci (QTLs) have been reported for some of these traits, markers do not exist for routine application in breeding. The RosBREED project is focused on enabling marker-assisted breeding via identification and validation of traits important to strawberry breeders. The development of the first genome-wide scanning platform (Affymetrix Axiom IStraw90TW) for the cultivated octoploid strawberry enabled high-throughput genotyping of 384 pedigree-connected strawberry accessions. Phenotypic data for titratable acidity (TA), soluble solids content (SSC) and remontancy were collected in the field in 2011 and 2012 in Oregon and Michigan. FlexQTL™ software was used for a marker consistency check and genome-wide QTL analysis for these three traits using SNP markers that were already genetically positioned using a 'Holiday' × 'Korona' linkage map. The preliminary QTL analysis resulted in 2 QTLs for TA, 1 QTL for SSC and 7 QTLs for remontancy. These results validate some of the previously reported QTLs. Estimation of haplotype effects and phenotypic variation explained by these QTLs is in progress for RosBREED strawberry germplasm. Haplotypes associated with desirable QTL alleles should enable the development of DNA-based markers and improve efficiency in the development of remontant and flavorful strawberry cultivars.

Keywords: *Fragaria*, fruit quality, genetic mapping, haplotype, marker-assisted breeding

Fifteen Minute Oral Presentation Abstracts

Genetics to Breeding, Part II

Progress and challenges in pedigree-based QTL analysis utilizing high density marker data on related full sib families: A case study on fruit firmness in apple

Marco Bink[1], Johannes Kruisselbrink[1], Hans Jansen[1], Roeland Voorrips[2], Amy Iezzoni[3], Cameron Peace[4], Herma Koehorst van Putten[2], Sara Longhi[2], Mario di Guardo[2], Michela Troggio[5], Fabrizio Costa[5], Erica Di Pierro[6], Luca Gianfranceschi[6], Thomas Letschka[7], Lidia Lozano[7], Stefano Tartarini[8], Giulia Pagliarani[8], Larissa Garkava-Gustavsson[9], Wannes Keulemans[10], Evelyne Costes[11], Alix Allard[11], Ines Ben Sadok[12], François Laurens[12], Hélène Muranty[13], Eric van de Weg[2]

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Abstract:
The power to identify, map and quantify QTL underlying complex traits may substantially increase when considering multiple segregating progenies simultaneously. The relatedness among these progenies can be fully exploited when the pedigree is known and the ancestors are available for genotyping. Previously, we successfully used the FlexQTL software to map QTL in such a Pedigree Based Analysis (PBA) approach to a large pedigreed population in apple with only 87 SSR markers (Bink et al. 2014). The current availability of high-throughput SNP genotyping infrastructures allows marker genotyping at much higher densities, which will contribute to higher mapping resolution and more accurate QTL characterization but also poses challenges to the QTL mapping software. Within the framework of the projects SCRI-RosBREED (#2009-51181-05808) and EU-FP7 FruitBreedomics (#FP7- 265582), we have improved and extended functionality of the FlexQTL software to better handle large numbers of SNP markers with regard to computation time, the phasing of the lowly-informative SNPs in complex pedigrees (e.g., inbreeding loops). Furthermore visualization tools were added to inspect recombination events. Furthermore, highly informative multi-allelic haplotypes can now be built comprising consecutive low informative SNPs, which will reduce computational effort without losing information from the high density marker data. The progress and challenges on utilizing high density SNP data in Rosaceae crops will be highlighted via a case study, i.e., applying the new version of FlexQTL software to fruit firmness trait on a large 20K genotyped apple population as available from the FruitBreedomics project. Reference: Bink MCAM et al (2014). Theor Appl Genet: 1-18. (doi: 10.1007/s00122-014-2281-3).

Keywords: Bayesian analysis, VisualFlexQTL software, identity by descent (IBD), genomic breeding values, genotype probabilities

Genetic determinism of budbreak timing in apple, a pedigree based analysis approach

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Abstract:
Since the late 80's, significant increases of mean temperatures has been recorded worldwide. This change leads to earlier flowering dates in apple, possibly making frost damage and phenological disorders related to fruit setting more frequent. This situation urges studies on genetic determinism of chilling and heat requirements in apple tree leading to timing of bud phenology. The present study aimed at clarifying the genetic determinism of timing of budbreak for five full sib families related by their pedigrees. Two of these families are located in Montpellier, South-East of France, and three in Angers, North-West of France. Up to five development stages, green point, vegetative budbreak, floral budbreak, flowering and end of flowering, were observed for the five families, over two years. The climatic years and locations were characterized by the duration of chilling and heat requirement completion for a reference cultivar 'Golden Delicious'. On that basis, mixed models were used to estimate the genotype effects and the GxE interaction effects. The five populations were also genotyped with the Illumina 20k SNP array within the EU-FP7 project FruitBreedomics. These SNP data and the Best Linear Unbiased Prediction values on genotypes and GXE interaction for the different phenological stages are used for pedigree-based QTL mapping with the FlexQTLTM software. The outcomes of this study will provide new insights on the genetic determinism of timing of budbreak in apple, leading to new tools for apple breeders.

Keywords: budbreak timing, apple, genetic determinism, pedigree based analysis

Exploiting the genetics of the top of apple's chromosome 16 for breeding

Cameron Peace[1], Jim Luby[2], Amy Iezzoni[3], Sujeet Verma[1], Matthew Clark[2], Cari Schmitz[2], Paul Sandefur[1], Susan Brown[4], Eric van de Weg[5], Kate Evans[6]

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Abstract:
A fascinating region of the apple genome is the proximal third of chromosome 16. Features detected in breeding germplasm using resources generated by the RosBREED project include a hotspot for heterozygosity, a hotspot for recombination, an introgressed segment from an enigmatic crab apple source, and a hotspot for QTLs covering all major categories of fruit quality: flavor via acidity and sweetness, texture via firmness and crispness, appearance via fruit size and bitter pit incidence, and even nutritional composition via phenolics content! The region was genetically dissected into several haplotype blocks ("haploblocks"). Focusing on U.S. apple breeding germplasm, breeding utility was assigned to each haploblock. Breeding utility was primarily characterized by effects on trait levels and the relative economic value of trait levels differentiated and the allele frequencies and sources in breeding germplasm. Often, alleles associated with desirable levels of one trait are linked in repulsion phase with those of another trait (e.g., acidity and phenolics; crispness and bitter pit incidence). However, most such linkages can be broken. Ideally, breeding parents contain the most valuable haploblocks in coupling phase and in homozygosity for use in efficiently enriching subsequent breeding families with alleles for superior performance in multiple traits. The best haploblock combinations identified within rare individuals of RosBREED's reference U.S. germplasm represent valuable parents for imparting superior genetic potential. As collaborative research efforts rapidly accumulate DNA information across the genome for apple and related rosaceous crops, useful genetic stocks can be developed by targeted haploblock assembly into desired configurations.

Keywords: fruit quality, haploblocks, Ma locus, QTLs, RosBREED

Genome-wide selection in apple: A pilot study in European breeding programs

Hélène Muranty[1], Michela Troggio[2], Inès Ben Sadok[1], Mehdi Al Rifaï[1], Annemarie Auwerkerken[3], Elisa Banchi[2], Piergiorgio Stevanato[4], Eric Van de Weg[5], Mario Di Guardo[5], François Laurens[1], Marco C.A.M. Bink[6]

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Abstract:
The tremendous increase in throughput of genotyping techniques opened appealing perspectives for genome-wide selection (GWS), which could enhance breeding efficiency by decreasing generation interval and increasing selection intensity and/or accuracy of breeding values. In GWS a large training population with both phenotypic and genotypic data is used to construct a statistical prediction model which is then applied to estimate Genomic Breeding Values (GBV) of individuals that only have genotypic data. In the EU-FP7 project FruitBreedomics, we performed a pilot study of GWS in apple. The two main objectives were to provide proof of principle and to evaluate the accuracy of prediction with respect to the relatedness between the test and the training populations. Hereto the phenotypic means of the 50 best and 50 worst predicted individuals of a test progeny comprising 700 individuals were compared and correlations between predicted GBV and phenotypic data were compared for smaller full-sib families of different relatedness. The training population included 20 full-sib families comprising 992 individuals genotyped with an Illumina 20K SNP array and phenotyped for fruit quality traits. The test population comprised four progenies from commercial breeding programs totaling 1500 individuals that were genotyped with 512 SNP that had been selected among the 20K for heterozygosity in the parents of the test population and genome-wide coverage. SNP genotypes were completed through imputation and fed into the calibrated prediction model to obtain GBV on the 1500 offspring. We will present the results and discuss challenges remaining to bring GWS into practice in Rosaceae species.

Keywords: breeding strategy, 20K SNP array, fruit quality, accuracy of prediction, relatedness

Fifteen Minute Oral Presentation Abstracts

Breeding and Breeding Tools

Apple breeding populations as research resources

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Abstract:
Apple breeding populations are valuable resources in genomic, physiological and genetic research. They help to reveal unique traits, assess characteristics of distantly related *Malus* species and generate useful materials for elucidating components of fruit quality and plant morphology. The germplasm resources within breeding programs allow the comparison of traits across a host of harvest times and genetic backgrounds. Using cultivars as both a maternal and paternal parent often yield differences in trait expression in these reciprocal crosses. This presentation will provide a "behind the scenes" look at unexpected traits found within progenies. The purpose of the cross will be provided, including parental attributes, followed by images of traits, which deviate from expectations. Variants in tree architecture, fruit cuticle attributes, fruit size and shape and postharvest attributes will be explained. Different types of genetic dwarfs will be described, along with variants for leaf morphology. This presentation seeks to foster further discussions on such resources within breeding programs and how best to leverage them for studying traits of importance.

Keywords: apple, germplasm, progenies, *Malus*, plant morphology

Field trial optimization for the Washington State University Apple Breeding Program

Julia Harshman[1], Kate Evans[1], Cameron Peace[2], Craig Hardner[3]

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Abstract:
Plant breeding is undergoing a major change in methodology with the application of DNA-based markers for selection. Little has been done to quantify the efficiency of apple breeding, or indeed tree fruit breeding, with or without the new technology. This study aims to investigate the impact on different optimization criteria of alternative designs for an evaluation of selection candidates established in phase 2 field trials of the Washington State University Apple Breeding (WABP) program using two metrics. Acceptance probability, an extension of the least significance difference (LSD) common in statistics, has been used to evaluate alternative trial designs. Acceptance probability expresses the observed percentage difference a candidate must have when compared to a standard(s) to reject the hypothesis that the mean of the candidate is equal to the mean of the standard(s). Response to selection was also used to compare alternative designs which differ in trial size. This is because variation in numbers of candidates may affect both variation of predicted cultivar mean (similar to the account in LSD and acceptance probability) and the intensity of selection. Apple breeding is a costly and long-term investment (18 years minimum from cross to release), for which improvements in efficiency have huge positive repercussions. Opportunities for improvement identified in the WABP can then be adopted by other tree fruit breeding programs, resulting in faster development of new varieties.

Keywords: Breeding efficiency

Optimal application of new genotyping technologies to accelerate tree fruit breeding programs

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Abstract:
Rapid development of single nucleotide polymorphism (SNP) markers and their automation have resulted in SNPs becoming the molecular markers of choice in high throughput genotyping of a wide variety of plant species. Advantages of SNPs include prolificacy of polymorphism in any genome, ease of their development, low error rate of genotype calling, and the possibility of finding polymorphism within genes of interest. SNP genotyping platforms provide low cost and high throughput capabilities resulting in a dramatic reduction in cost efficiency. This cost reduction has made the application of marker-assisted breeding technology affordable for even the smallest plant breeding programs. We will describe how the combination of High Marker Throughput (HMT) and High Sample Throughput (HST) platforms implemented at BioDiagnostics, Inc. (BDI) has been used to assist breeding programs. This combination can be used to optimize the value of breeding projects' funds by increasing success and reducing cost and time to market of new varieties. We will explain how BDI can reduce the time between developing useful trait markers and implementing them in your breeding programs, using association mapping studies. Advantage of genomic selection in plant breeding with practical examples and also in tree fruit breeding will be discussed. We will also provide examples of projects that have been conducted at BDI to assist breeding programs in the new genomics era.

Keywords: biodiagnostics, SNP, genotyping, marker assisted selection, genome

Five Minute Poster Presentation Abstracts

Genomics

Development of genomic resources in black raspberry

Douglas Bryant[1], Jill Bushakra[2], Kelly Vining[3], Michael Dossett[4], Chad Finn[5], Sergei Filichkin[3], Jerry Weiland[5], Nahla Bassil[2], Todd Mockler[1]

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Abstract:
For over 75 years the United States black raspberry industry has been in steady decline due in large part to a lack of adapted and disease resistant cultivars. Recently, potential health benefits associated with black raspberry's high concentration of bioactive compounds have sharply increased interest in breeding and production of new cultivars. To facilitate and enhance breeding efforts, we have developed a wide set of genomic resources based on deep sequencing of the black raspberry genome and broad sampling of the transcriptome. Further, we have performed reduced representation genotyping-by-sequencing (GBS) for the ORUS 4305 mapping population that was propagated and planted in growers' and research fields across five US production areas. A high-quality genome assembly was generated through high-throughput genome sequencing of a highly homozygous accession (~2200 scaffolds, ~240 Mbp, ~353kb N50, 0.06% SNP). Over 50% of the genomic sequence was placed onto linkage groups based on our GBS analyses. RNA-seq data from seven replicated libraries were assembled by de novo and reference-guided approaches, forming the basis for our empirically-based structural annotation (~30,000 transcription units). Associated functional predictions are in progress. These broad genomic resources are poised to enable marker-assisted breeding efforts and lead to development of improved black raspberry cultivars.

Keywords: genomics, transcriptomics, annotation, GBS, bioinformatics

Poster number: 1

Activation of anthocyanin-related biosynthetic genes by bHLH transcription factors in *Rubus idaeus* and *Fragaria vesca*

Andrea Lorena Herrera Valderrama[1], Antje Feller[1], Stefan Martens[1], Andrew Allan[2]

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Abstract:
Anthocyanin biosynthesis is regulated through the interaction of the MYB-bHLH-WD40 complex, which has already been characterized in several crops from diverse plant families, including some Rosaceae species. Using genome wide phylogenetic analysis, we identified three possible bHLH candidates from *F. vesca* and two from *R. idaeus* based on sequence homology with reported bHLH proteins from *Malus*, *Arabidopsis* and *Fragaria*. To date it is not clear which of these bHLH proteins are regulators of anthocyanin biosynthesis, and how this regulation is occurring in strawberries and raspberries. Here we show the results of gene expression studies of these bHLH genes as well as of putative anthocyanin biosynthetic genes at several fruit developmental stages. In addition, we show the results of promoter activation studies of "key" genes of the anthocyanin pathway like CHS, DFR, UFGT. Gene activation was determined by co-transformation of the promoter regions of biosynthetic genes fused to the luciferase gene and bHLH genes under 35S promoter expression in *Nicotiana benthamiana*, followed by luciferase transient assay; we determined that some of the bHLH candidate proteins are essential components for activation of key genes inolved in the anthocyanin pathway.

Keywords: *Fragaria vesca*, *Rubus idaeus*, anthocyanins, bHLH transcription factors

Poster number: 2

Softening of *F. chiloensis* fruit. An effort to understand its hormonal regulation using transcriptomic and genomic analyses

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Abstract:
The Chilean strawberry (*Fragaria chiloensis*) has emerged as a new berry with excellent organoleptic characteristics, however its fast softening is a limiting step for commercialization. *F. chiloensis* is a non-climacteric fruit and its ripening seems to be coordinated by hormones like auxins (Aux) and abscisic acid (ABA). During ripening Aux levels decrease while that of ABA increases. Softening is related to cell wall dissembling and several related enzymes have been described to take part during softening of *F. chiloensis* fruit, including polygalacturonase (PG), xyloglucan endotransglycosylase/hydrolase (XTH1) and expansin 2 (Exp2). The level of their transcripts increases during fruit softening. When the fruit is subjected to several hormonal treatments (Aux, ABA, GA3, ethylene, 1-MCP) in order to establish their effects on the expression of these genes, significant variations are recorded. An activator effect of auxin on the expression of XTH1 and a repressive effect on PG is found. ABA induces the expression of XTH1 and PG, GA3 displays an activator effect on XTH1, Exp2 and PG, and finally 1-MCP represses the expression of PG and Exp2 while induces that of XTH1. To explain this regulatory mechanism, the promoter regions of these genes were obtained by Genome walker, cloned and *in silico* analyzed to reveal putative cis elements using PlantCARE. The promoters contained several regulatory elements responding to hormones which could explain their differential responsiveness. This provides evidences to clarify the hormonal regulation of key genes during ripening of *F. chiloensis* fruit. Acknowledgments to Anillo ACT-1110 and Fondecyt 1110792 Projects.

Keywords: *Fragaria chiloensis*, fruit softening, cell wall dissembling, hormonal effect, cis regulatory elements

Poster number: 3

Global transcriptome analysis reveals the implication of one PME gene in apple mealiness development

Sandrine Mikol[1], Maryline Bruneau[2], Jean-Marc Celton[2], Sophie Le Gall[3], Axelle Bouder[3], Mathilde Francin-Allami[3], Marjorie Juchaux[4], Mathilde Orsel[2], François Laurens[2], Jean-Pierre Renou[2]

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Abstract:
Fruit development and maturation involve many physiological and biochemical changes. Some apple cultivars show undesirable ripening process leading to mealiness, which is characterized by texture deterioration resulting in soft, grainy and dry fruit. To understand the molecular bases of mealiness development, we used the 120k AryANE microarray (1). Based on sensorial analysis over 6 years, six genotypes with contrasted fruit texture (mealy or not) were selected among a progeny. A global transcriptome analysis was performed over four time points during fruit development and cold storage. Interestingly, this analysis revealed one transcript coding a pectin methyl esterase (PME) which displayed an important modification in its expression level in accordance with the development of mealiness in fruits. Protein fusion experiment showed that this specific MdPME is secreted to the apoplast. Microscopic analysis revealed a progressive loss of cell to cell adhesion in mealy fruits in accordance with specific cell wall disassembly. Biochemical analysis pointed out specific alteration of pectin during fruit ripening. However, no global significant changes in pectin structural properties could be ascribed to mealiness. Consistent with the observations of Ng et al (2), we hypothesize that mealiness may be partially due to qualitative and spatial variations of pectin microarchitecture rather than quantitative pectin differences, and that these changes may occur early in fruit development. Overall, these data support the role of PME in cell wall remodeling during fruit maturation and suggest that PME act locally. This MdPME could be an early marker of texture disorder in apple.
(1) Celton et al., New Phytol, 2014. In Press. (2) Ng et al., BMC Plant Biol. 2013.

Keywords: apple, fruit quality, transcriptomics, cell wall

Poster number: 5

A population genomics approach for unraveling the genetic bases of differentiation between dessert and cider apples

Diane Leforestier[1], Elisa Ravon[2], Hélène Muranty[2], Aurélie Bérard[3], Dominique Brunel[3], Marie-Christine Le Paslier[3], Christophe Lemaire[1], Antoine Branca[4], Tatiana Giraud[4], Charles-Eric Durel[2]

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Abstract:
Understanding the processes that occurred during domestication remains one of the central questions in genetics. In addition to fundamental importance, it can indeed provide knowledge on the genetic bases for further crop improvement. As an example, apple is one of the most important fruit crops in temperate regions, having both an economic and a cultural value. Dessert apples are used for consumption as fruits while cider apples are used to produce cider. Yet few traits are known to be different, despite overlapping between dessert and cider apples, namely fruit size and bitterness. A population genomics approach was used for detecting loci involved in the differentiation between cider and dessert apples, on two core collections of old cultivars, one for dessert apples and one for cider apples. A set of 96 gene fragments, localized in 6 areas of the apple genome bearing QTLs for traits of agronomic interest, was re-sequenced and the Illumina 8K SNP chip was used to genotype these two collections. The low estimates of per locus differentiation (ie Fst) between dessert and cider apples indicated that these two pools recently derive from common ancestors, except for 2 genomic regions exhibiting higher Fst values and potentially involved in selective processes. Departure from neutrality was also tested for each "population" using Tajima's D and Fu and Li's D and several areas of the genome were found to have significant decrease of both. Such knowledge could be helpful in new selection programs, bringing their efficiency to a higher level.

Keywords: *Malus* x *domestica*, genetic structure, signatures of selection

Poster number: 6

Genome-wide copy number variation (CNV) detection in *Malus* x *domestica*

David Chagne[1], James Boocock[2], Tony Merriman[2], Mik Black[2]

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Abstract:
Genetic variation takes many forms, from large chromosomal anomalies to individual base pair changes. Copy number variants (CNVs) are generally considered to be gains or losses of DNA segments larger than 1 kb. In humans CNVs are a factor in determining phenotype, as they provide rapid genetic response to a changing environment and have been associated with risk of disease. Preliminary analyses of plant genomes have indicated that CNVs are important to the evolution of plant genomes which have been under strong selection. Surprisingly, CNVs have been an untapped source of genomic variation, compared with SNPs, for understanding the genetic control of key traits in plants and as tools to improve selection efficiency of new varieties. Domesticated apples first appeared in the Near East around 4,000 years ago and it seems pertinent to investigate the CNV of apple varieties, as many CNVs may have emerged since its domestication. We have performed a genome-wide analysis of CNV using low coverage genome re-sequencing of 34 apple varieties using the R package CNVrd2. CNVs were detected across the apple genome. CNVs associated with candidate genes for aroma, and pest and disease resistance were identified.

Keywords: apple, structural variants, bioinformatics

Poster number: 7

Addressing fruit quality issues in *Prunus persica* varieties using a deep transcriptomic approach

Ariel Orellana[1], Dayan Sanhueza[1], Paula Vizoso[1], Claudio Meneses[1], Reinaldo Campos-Vargas[1]

[1]Universidad Andres Bello, Santiago, Chile

Abstract:
Peach/nectarine varieties exhibit unique features that are the basis for distinctive traits involved in fruit quality. The repertoire of genes that each variety expresses at a given time, is a key issue in acquiring the characteristics that distinguish them. In order to get further insights in the molecular aspects that are important for fruit quality and postharvest life, we carried out a deep transcriptomic analysis of four *Prunus persica* varieties. Two early-season and two late-season varieties were analyzed for different traits such as firmness, color, solid soluble, acidity as well as their susceptibility to mealiness and response to postharvest treatments aimed to control the postharvest disorders. The late season varieties resulted to be more susceptible to chilling injury than the early season varieties. In addition, the comparison between the late-season varieties showed a differential response to treatments such as controlled atmosphere and conditioning. The transcriptomic analysis during ripening, cold storage and postharvest treatments using an Illumina-based RNA-Seq approach, showed clear differences in the repertoire of genes that are expressed among varieties during ripening, cold storage and postharvest treatments. The expressed genes were identified using the *Prunus persica* reference genome and those differentially expressed were mapped to metabolic networks. Our results indicate that there are set of genes that are differentially expressed in each of the condition analyzed. Their relationship to the traits analyzed in the present study will be discussed. Supported by: Fondap-CRG 15090007, P10-062-F, PB-16, DS Conicyt D-21090737, UNAB DI-64-12/1 and Fondecyt 3140294

Keywords: *Prunus persica*, transcriptomics, fruit quality, postharvest

Poster number: 11

Transcriptome sequencing of *Prunus* sp. rootstocks roots to identify candidate genes involved in the response to root asphyxia

Rubén Almada[1], María José Arismendi[1], Paula Pimentel[1], Patricio Hinrichsen[2], Manuel Pinto[3], Alex Di Genova[4], Dante Travisany[4], Adriana Bastias[5], Alejandro Mass[4], Ariel Salvatierra[1], Boris Sagredo[5]

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Abstract:
Root hypoxia limits stone fruit tree (SFTs) development. To overcome this problem, SFTs are grafted on rootstocks with different degrees of tolerance to root hypoxia. However, the molecular base of such variability is largely unknown. Here we performed a transcriptome analysis of roots from rootstocks with contrasting responses to hypoxia using Illumina deep sequencing. Plants of the genotypes 'Mariana 2624' and 'Mazzard F12/1', tolerant and sensitive respectively, were subjected to hypoxic conditions by waterlogging. Transcriptomes from root samples collected from control (without waterlogging) and hypoxia stressed plants (6, 24 and 72h) were compared. The 81% of generated sequences were mapped to the reference genome of *P. persica* (L.). Analysis of the differentially expressed genes (DEG) revealed that most of the genes were down-regulated in the waterlogging stages. Regarding their identities and mode of regulation (up or down regulated) among DEGs, there was a high number of common DEG between the two genotypes for each sampling time, but also a high number of exclusive DEG was detected per genotype at each time. A few DEG presented opposite modes of regulations between the two genotypes throughout the hypoxia treatment. The most differentially affected functional categories are "oxidation reduction", "carbohydrate metabolic process" and "oxidoreductase activity" at both genotypes, but only the susceptible genotype showed significant alteration of genes involved in responses to "oxidative stress", "oxidoreductase activity", "peroxidase activity" and "antioxidant activity". This research represents a valuable source of information for further studies to identify the mechanism and genes that define tolerance to hypoxia in *Prunus*. Acknowledgment: CEAF_R08I1001 – CONICYT, FONDECYT 1121117, FONDECYT11110079 (RA).

Keywords: root hypoxia, *Prunus*, rootstock, transcriptome sequencing, qRT-PCR

Poster number: 12

Five Minute Poster Presentation Abstracts

Genomics to Genetics

A functional genomics approach to understand cracking in sweet cherries

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Abstract:
Chile is one of the main exporters of fresh fruits from the south hemisphere. Among them, sweet cherries are very important from a commercial point of view. Unfortunately cherries suffer a series of problems such as cracking, which is one of the major reasons for losses in its production. In sweet cherry the damage occurs when the cherries are wetted by rain water. It is hypothesized that the susceptibility to cracking in cherry fruits could be related to structural components of the exocarp and differential gene expression associated with this physiopathology. In collaboration with Washington State University the sweet cherry genome (Stella; 83.5 x coverage) was sequenced. In addition the fruit transcriptomes of three sweet cherry varieties with different susceptibility to fruit cracking (Bing, Lapins and Rainier) was sequenced. 5,124 contigs were assembled from fruit reads and 65% of the contigs were annotated. A transcriptome of the Bing variety under water stress was also generated. A differential expression analysis, based on Digital Northern technique and qPCR, identifing genes that are differentially expressed between the three varieties in fruit was performed. We are trying to verify a potential correlation between differences in amounts of alkanes in fruit wax and gene expression. This research was supported by CONICYT, FONDECYT/Regular Nº1120261.

Keywords: sweet cherry, cracking, functional genomics

Poster number: 19

A transcriptomic approach to understanding pedicel-fruit abscission in sweet cherry

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Abstract:
In some genotypes of non-climacteric sweet cherry, ethylene induces the development of a clearly defined abscission zone between the fruit and pedicel. The aim of this study is to identify and analyze the genetic components of sweet cherry abscission in response to exogenous ethylene application. We performed a time course transcriptome analysis of the fruit-pedicel abscission zone treated with exogenously applied ethylene. Three unique genotypes were used, representing three distinct classes of phenotypes of fruit-pedicel abscission zone formation in sweet cherry. RNA-seq captured a global snapshot of the transcriptome at each time point sampled. The transcriptome data was assembled and relative expression values for each gene (RPKM) were calculated. Genes with at least a five-fold difference in expression between treatment and control were selected for annotation using Blast2GO and polymorphism analysis. Gene ontology and pathway information identified both known and cherry specific gene networks involved in the abscission process. In the future, the polymorphisms embedded in differentially expressed abscission related genes will be validated in populations segregating for ethylene-induced abscission. Additionally, they are expected to be used as gene-based markers for the development of varieties that exhibit desired traits for new and developing harvest technologies.

Keywords: abscission zone, sweet cherry, ethylene, gene expression

Poster number: 20

Sweet cherry cultivar fingerprinting using single nucleotide polymorphisms detected by high resolution melt analysis

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Abstract:
The Summerland breeding program has a long history of producing unique sweet cherry cultivars with a variety of desirable characteristics. Recent successes include the licensing of Sentennial, Sovereign and Staccato, which are progeny of Sweetheart, an earlier Summerland cultivar. Plant Breeders Rights for these cultivars are currently held by the Okanagan Plant Improvement Corporation (PICO) with detailed descriptors of the cultivars as the defining characteristics. We are currently developing molecular techniques to discriminate between these very closely related cultivars. Previously, AFLP and SSR analysis have successfully differentiated most cherry cultivars. However, only one SSR and one AFLP marker distinguished Sweetheart from Staccato. The requirement for more unique markers led to development of our current SNP fingerprinting strategy. New single nucleotide polymorphisms (SNPs) were identified from analysis of Illumina RNA-Seq libraries generated for Sweetheart and its three progeny. High coverage Sweetheart SNPs were examined for segregation in the progeny and confirmed by High Resolution Melt (HRM) assays. A set of 16 SNPs was chosen for independent segregation across the genome that provided a unique fingerprint of Sweetheart and each of its progeny. The straightforward SNP HRM technique was further tested on a broader set of 35 cultivars of interest to PICO, providing unique fingerprints for each. Within this group of 35 cultivars, 16 were selected for genotyping-by-sequencing to identify additional SNP markers. The choice of multiple unlinked SNPs increases the probability that each pattern of inheritance will be unique and can be used to verify the identity of unknown cultivars.

Keywords: plant breeders rights, RNA-Seq libraries, genotyping-by-sequencing, *Prunus avium*

Poster number: 21

Genome wide scan with the IPSC peach SNP array for the identification of QTLs controlling fruit quality, phenological and tree architecture traits

Maria Teresa Dettori[1], Sabrina Micali[1], Laura Gazza[1], Valeria Aramini[1], Elisa Banchi[2], Elisa Vendramin[1], Michela Troggio[2], **Ignazio Verde**[1]

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Abstract:
A BC1 progeny (PxF) obtained crossing two peach accessions, the selection IF7310828 used as female recurrent parent and the oriental accession Ferganensis (formerly *P. ferganensis*) used as male donor parent was genotyped with the IPSC 9K SNP array. Out of 8144 SNPs available on the chip 3326 (40.8%) resulted polymorphic. We mapped 1660 SNPs and 15 SSR loci, all informative for the F1 parent and segregating in a 1:1 ratio in the PxF population, using JoinMap 4.1 and the Maximum Likelihood mapping algorithm. Grouping was performed at a LOD higher than 10. After grouping, identical loci were eliminated, with the exception of SSRs that were always retained. The final map consisted of 242 loci distributed in 8 groups, covering 607 Haldane cM. This map was used to scan the genome for QTLs using MapQTL6. Interval mapping and MQM mapping were used, both with the mixture Model Algorithm. Traits related to plant structure (internode length, plant height, trunk perimeter), plant physiology (blooming and maturity date),fruit quality (fruit weight, fruit juice pH, titratable acidity, soluble solid content , fruit red overcolor) were analyzed for at least two years. Several QTLs were identified, the major ones for blooming and maturity date, soluble solid content, fruit red overcolor.

Keywords: *Prunus persica*, SNPs, QTLs

Poster number: 23

Unique small RNA (sRNA)-based gene regulatory networks and their potential function in fruit crops

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Abstract:
MicroRNAs (miRNAs), interfering RNAs (siRNA), phase-based siRNAs (phasiRNA) and trans-acting siRNAs (tasiRNAs) have recently emerged as powerful regulators in plants. However, how they operate and function, in context of fruit productivity, in fruit crops remain largely unknown. Recently, we took advantage of a deep sequencing approach, in combination with computation and molecular analyses, to thoroughly characterize the profile of miRNASs, tasiRNAs, phasiRNAs and other types of siRNAs as well as their interaction in apple and peach, respectively. Our work showed that both apple and peach shared the conserved miRNAs with model species as well as evolved a large number of unique ones. We also demonstrated that a multitude of apple and peach miRNAs were capable of triggering phasiRNA production in a few gene families, and many of the produced phasiRNAs were able to target genes inside and outside the families. These miRNA-triggered, phasiRNA-cascaded robust networks represent novel regulatory mechanisms that have not been elucidated in other species. The potential function of the sRNA-based regulatory networks in regulation of plant growth, development, productivity and defense will be discussed.

Keywords: miRNAs, phasiRNAs, tasiRNAs, apple, peach

Poster number: 24

Phylogeny of species within the genus *Fragaria* revealed by next generation sequencing of multiple low copy nuclear markers

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Abstract:
While the utilization of next generation sequencing technologies is rapidly growing, the application of this approach to phylogenetic analysis has been fairly limited to date. We are conducting large scale phylogenetic analyses of *Fragaria* (strawberry) species using the Fluidigm Access Array system and 454 sequencing platform. Our aim is to clarify phylogenetic relationships among *Fragaria* species and to elucidate the sub-genome composition of the polyploid species. Twenty-four single-copy or low-copy nuclear genes distributed across the genome were selected, and amplicons were sequenced from ninety six genomic DNA samples representing species from diploid to decaploid. Individual gene trees and species trees were reconstructed by different tree-building methods. Our results support the monophyly of *Fragaria*, and illuminate phylogenetic relationships among diploid species. Our findings also suggest the presence of three types of sub-genomes within the octoploid strawberry genome, and support the prior implications of ancestry for three diploid species: *F. vesca* and/or *F. mandshurica*, and *F. iinumae*. We developed a bioinformatics pipeline that is useful for large scale phylogenetic analysis of other polyploid species, and demonstrate the power of high throughput sequencing technology to enable robust phylogeny estimation from multiple nuclear genes.

Keywords: *Fragaria*, polyploidy, low-copy nuclear genes, phylogeny, next generation sequencing

Poster number: 25

A high density linkage map for the ancestral diploid strawberry *Fragaria iinumae* using markers from GBS and the ISTRAW90 Axiom® SNP array

Lise Mahoney[1], Daniel Sargent[2], Judson Ward[3], James Hancock[4], Kevin Folta[5], Dave Wood[1], Hailong Zhang[1], Michael Mittmann[6], Nahla Bassil[7], Thomas Davis[1]

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Abstract:
In recent years, phylogenetic, and other evidence has accumulated implicating diploid (2n=2x=14) *F. iinumae* as a second subgenome contributor to the octoploid (2n=8x=56) strawberries, including the cultivated strawberry, *Fragaria* × *ananassa* and its ancestors *F. chiloensis* and *F. virginiana*. Previously, diploid *F. vesca* had been identified as an ancestral subgenome donor, and the representative 'Hawaii4' genome was published in 2011 as the first *Fragaria* reference genome. We have developed germplasm and genomic resources for *F. iinumae*, including high throughput genomic sequence data and a linkage mapping population. We now report construction of two high density linkage maps, constructed using GBS markers and SNPs from the IStraw90 Axiom ® Strawberry array. The maps, each of seven linkage groups, are based on segregation data from an F2 population (F2Ds) derived from a cross between two *F. iinumae* accessions (CFRA1955 and CFRA1849) collected in Hokkaido, Japan by Tom Davis and Kim Hummer in 2004. The maps differ with regard to the numbers of genotyped individuals and the markers employed. A GBS-only map based on 85 F2 plants defines 220 loci comprising 972 markers, with a map density of 2.1 cM and a map length 457.4 cM. A combined GBS-SNP map of only 21 individuals defines 158 loci comprising 3,181 SNP and 994 GBS markers. We plan to expand the SNP genotyping to include all 85 F2 individuals. The *F. iinumae* linkage maps will be used to anchor an *F. iinumae* genome assembly, thereby providing an important new genomic resource for *Fragaria*.

Keywords: *Fragaria*, SNP, linkage map, IStraw90, GBS

Poster number: 26

Carotenoids in reproductive organs of the diploid strawberry, *Fragaria vesca*

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Abstract:
Carotenoids are tetraterpenoid lipophilic pigments found in most plant organs. In leaves, carotenoids serve as light harvesting complexes, and they are responsible for protection against reactive oxygen species generated in the chloroplast. Carotenoids are also precursors for the production of signaling compounds such as abscisic acid and strigalactone. In flowers and fruits, the colors of carotenoids play roles as visual attractants for pollinators and seed dispersers, and roles as antioxidants in these organs are also probable. Strawberry production depends on successful development of reproductive structures and fertilization, processes highly susceptible to abiotic stresses. Insight into the functions of carotenoids in reproductive organs could provide the groundwork for breeding or genetically engineering more stress tolerant strawberries and other horticultural crops. Flowers of the diploid woodland strawberry, *F. vesca*, have yellow-orange anthers, yellow carpels, and yellow pollen due to the presence of carotenoids, but the precise role(s) of these pigments have not been defined. We are assessing the potential for these compounds to protect reproductive organs against elevated temperature stress using biochemical analysis, genomics, and genetics. Unlike in leaves, HPLC analysis showed that developing *F. vesca* carpels and stamens have no detectable beta-carotene. Candidate genes involved in carotenoid biosynthesis were identified, revealing the existence of small gene families. Gene expression patterns throughout the plant, and expression in response to heat stress in the reproductive structures were analyzed by QRTPCR. Mass spectrometric analyses of carotenoids in control and heat stressed tissues, and in a male sterile mutant with pale anthers are underway.

Keywords: carotenoids, gene expression, reproductive organs, heat stress, mass spectrometry

Poster number: 27

Five Minute Poster Presentation Abstracts

Genetics

Mapping and identification of disease resistance candidate genes in three *Malus* populations

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Abstract:
Apple scab and powdery mildew are a major concern for apple breeders and producers. Control of these apple diseases by the development of varieties with durable disease resistance is the most efficient and environmentally friendly way. Therefore, the gene-pyramiding route for the development of apple varieties with durable disease resistances requires a supply of a diverse resistance gene pool. The identification and mapping of these genes is therefore of paramount importance. In this study genetic mapping of Quantitative Trait Loci (QTLs) for apple scab and powdery mildew was performed on progeny of three mapping populations ('Mildew Resistant' x 'Golden Delicious', 'Russian Seedling' x 'Golden Delicious' and *Malus platycarpa* x 'Mildew Resistant'). Three marker systems were used and these were Simple Sequence Repeats (SSRs), Diversity Arrays Technology (DArTs) and Single Nucleotide Polymorphisms (SNPs) markers. A total of 17 putative QTLs were detected for the 'Mildew Resistant' x 'Golden Delicious' population, 10 for the *Malus platycarpa* x 'Mildew Resistant' population and nine for the 'Russian Seedling' x 'Golden Delicious' populations for the three diseases. Two putative QTLs for apple scab detected on LG 02 of the 'Russian Seedling' x 'Golden Delicious' coincided with loci previously identified as encoding two apple scab resistance genes Vh2 and Vh4 on 'Russian apple'. SNP markers R_8936738_Lg2 and R_32981524_Lg2 were strongly linked to the Vh4 and Vh2 QTL regions respectively. The analysis of chromosome two led to the identification of two candidate genes for the Vh4 locus and a cluster of ABC (PDR) for Vh2.

Keywords: disease resistance, quantitative trait loci, genetic mapping, marker systems, candidate genes

Poster number: 30

A linkage map for black raspberry (*Rubus occidentalis*)

Jill Bushakra[1], Douglas Bryant[2], Kelly Vining[3], Michael Dossett[4], Todd Mockler[2], Jungmin Lee[5], Chad Finn[6], Nahla Bassil[1]

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Abstract:
The genus *Rubus* (subfamily Rosoideae) contains an estimated 600-800 species distributed world-wide. Several of these, including black raspberry (*Rubus occidentalis* L.) are grown as crops. Since the early 1900s, the black raspberry industry in the USA has steadily declined due to lack of adapted, disease resistant cultivars. Renewed interest in production and breeding new cultivars has been fueled by news regarding potential health benefits of black raspberry bioactive compounds. We present a genetic linkage map comprised of simple sequence repeat (SSR) markers derived from both genomic and expressed sequence tag libraries, and single nucleotide polymorphic (SNP) markers derived from genotyping by sequencing (GBS). The map was constructed using 93 progeny of the full-sibling population ORUS 4305 (ORUS 3021-2 x ORUS 4153-1). The map consists of seven linkage groups representing the seven haploid chromosomes of this diploid species. The consensus map covers 613.1 cM with the longest linkage group spanning 101.7 cM with 103 markers (G1) and the shortest spanning 77.6 cM with 61 markers (G7). The construction of a densely populated genetic linkage map will be used for quantitative trait locus (QTL) mapping of economically important traits and for comparative genomic studies with other members of the Rosaceae.

Keywords: Rosaceae, simple sequence repeat markers, marker-assisted breeding

Poster number: 35

Roles of auxin biosynthesis by the flavin monooxygenase genes in developmental control and environmental responses in strawberry

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Abstract:
Auxin has been regarded as the main signal molecule coordinating the growth and ripening of fruits in strawberry, the reference genomic system for Rosaceae. Functional information regarding the key regulators of auxin biosynthesis during both vegetative and reproductive development and environmental responses is scarce in strawberry. We have isolated and characterized whole YUCCA family genes encoding the key enzyme for auxin biosynthesis in both diploid strawberry (*Fragaria vesca*) and octoploid strawberries (*F.* × *ananassa* Duch.). Transcriptional profiling was performed in different tissues, fruits of different developmental stages, and leaves under different abiotic stresses. Functionality of YUCs was studied in transgenic *F. vesca* by over-expression or RNA interference, also in *F.* × *ananassa* by tobacco rattle virus (TRV)-induced gene silencing (VIGS) technique. The genetic study showed that FvYUC6 has important role in vegetative and reproductive development in woodland strawberry. VIGS experiment provided evidence that the gene FaYUC11, most homologous to AtYUC11, is crucial for receptacle enlargement in octoploid strawberry. Also, our work supports the interpretation that auxin biosynthesis system in strawberry holds flexible responses to developmental and environmental signals. These results will provide useful information for strawberry production to improve plant growth and fruit yield.

Keywords: strawberry, auxin biosynthesis, fruit development, plant growth, abiotic stress

Poster number: 37

An improved version of cultivated strawberry linkage map using the IStraw90 Axiom® Array for QTL analysis

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Abstract:
Strawberries are economically important fruits around the world. *Fragaria* x ananassa, the cultivated strawberry, is an allo-octoploid species (2n=8x=56), their ploidy level difficult the application of marker assisted breeding. *F. vesca* has been considered as a diploid model organism. Berries, including strawberries, are known for their rich nutritional profile. Understanding the genetic bases controlling the production of phenolic and volatile compounds is extremely important for the selection of new varieties of cultivated strawberry with greater consumer acceptation. The genetic map of a F2 population (Camarosa x Dover) posses 192 loci distributed along the 28 expected linkage groups, representing each homeologous group with a high coverage (>70%) when compared to *F. vesca* genome. LC-MS analysis for polyphenol metabolites in full ripen fruits allowed the quantification of 22 metabolites and mapping 146 metabolic QTLs. The IStraw90™ (International Strawberry 90K Axiom®) Affymetrix array developed by Rosbreed based on diploid and octoploid sequences was hybridized with 120 F2 individuals and parentals. The analysis of the results using GenotypeConsole and SNPolisher softwares shows a high quality of hybridization, with a >97% probe call rate for all individuals. For the 93937 valid SNPs: 75% were classified as MonoHighResolution, 9356 as NoMinorHomozygous, and 4939 as PolyHighResolution segregating in our population. The SNPs position in *F.vesca* and the SSR map allow to construct 4x7 homeologous groups with an increased resolution and higher coverage. Distribution of SNPs in homeologous groups will help the assembly of contigs and scaffolds of the genome draft sequence covering 154x of one population individual.

Keywords: strawberry, SNPs, map, QTLs

Poster number: 38

Axiom® Genotyping Arrays: Automated analysis of complex plant genomes

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Abstract:
High-throughput automated genotyping of plants requires a technology that is robust in the face of complex plant genomes, and cost-effective for processing large numbers of samples. Plant genomes may be polyploid, comprised of high copy number sequences, and/or sub-population structural diversity. The Axiom® Genotyping Solution has three components that address these complexities. One, it uses the Axiom GT1 algorithm which dynamically adapts to cluster variation produced by plant genomes. Two, novel genotyping algorithms are used for markers in regions of high genomic complexity. Three, markers are sorted into relevant genomic classes, and the potentially most accurate and polymorphic markers are sorted into the PolyHighResolution class. The analysis workflow, algorithms and results are presented for allo-octoploid, cultivated strawberry samples that were genotyped with the IStraw90TM Axiom® Genotyping Array (in a 96-array layout) designed by the RosBREED project. Genotypes for SNPs in the PolyHighResolution class have greater than 99.8% reproducibility, and low Mendelian inheritance error rates. Cost effective genotyping is enabled by the Axiom® 384-array layout, where 384 samples are simultaneously processed in a 384 microplate for analyzing up to 35,000 polyploid or 50,0000 diploid SNPs per sample. Markers that have been identified as highly performing, such as those in the PolyHighResolution class as identified by the Axiom® Genotyping Analysis on Axiom 96-array layout can be transferred to Axiom 384-array layout with 100% fidelity. The multi-species capability of the Axiom 384-array layout enables the design of a single array for markers from all rosaceous species that can be used in marker-assisted breeding.

Keywords: Axiom, strawberry, octoploid, genotyping, SNP

Poster number: 39

Five Minute Poster Presentation Abstracts

Genetics to Breeding

Identification of QTL underlying soluble solids content and titratable acidity in sweet cherry (*Prunus avium* L.)

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Abstract:
Sweetness and acidity are important fruit quality traits that drive consumer acceptability of sweet cherry. Developing new sweet cherry cultivars with superior taste attributes is a major breeding objective in fruit breeding programs. This study was designed to identify quantitative trait loci (QTL) associated with soluble solids content (SSC) and titratable acidity (TA) in sweet cherry to facilitate development of new cultivars with exceptional taste characteristics. A total of 601 pedigree-linked individuals were used in this study. Five largest fruits from each individual were selected at maturity for SSC measurement. Juice from a bulked sample of 25 fruit was used for TA measurement. One thousand and ninety one (1091) single nucleotide polymorphism (SNP) and four simple sequence repeat (SSR) markers were used to provide genome-wide markers for determining marker-locus-trait associations. QTL analyses were performed in FLexQTL™ for SSC and TA using phenotypic data collected in 2010, 2011 and 2012. Three QTL for SSC were mapped on the 'Texas' x 'Earlygold' *Prunus* reference map on linkage group (LG) 2 (in 2011 and 2012), on LG 4 (in 2012) and on LG 7 (a minor one in 2011). Three QTL were identified for TA; one mapped on LG 2 in 2010, the other on LG 4 in 2011 and 2012 and the third on LG 6 in 2012. The haplotypes for these QTL are discussed in relation to breeding for SSC and TA in sweet cherry.

Keywords: quantitative trait loci, sweetness, acidity, haplotype, marker-assisted breeding

Poster number: 45

Development of DNA markers for sucrose content in pear fruit

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Abstract:
Fruit taste depends upon sugars, organic acids, firmness, amino acids, aromatic compounds and so on. Of these components, sugars are one of the most important components of fruit taste. The amount of sucrose, glucose, fructose and sorbitol plays a key role in sweetness of pear fruits. The differences in sugar composition within cultivars exist in Japanese pear. There is much difference in sucrose content and commercially important cultivars accumulate much sucrose. Cultivars with high sucrose accumulation exhibit a rapid increase in sucrose at later stage, while those with low sucrose accumulation exhibit no increase even at later stage. To study whether sucrose content is related to changes in the gene expression of sucrose metabolizing enzymes (sucrose synthase: SUS, soluble acid invertase: AIV and sucrose phosphate synthase: SPS), we examined the expression of five genes such as SUS (PpSUS1), AIV (PpAIV1 and PpAIV2) and SPS (PpSPS1) during development. PpAIV2 is specifically expressed in fruit at later stage by cultivars with low sucrose content. Moreover, we used RFLP analysis to identify PpAIV2 gene linked to sucrose content in ripening fruits. When total DNA was probed with PpAIV2, we identified two bands of 4.4kb and 2.8kb, which were specific to cultivars with low sucrose content. The absence of these bands enabled the identification of high or moderate sucrose content. We have converted these markers in to PCR-based markers. The markers identified here, which are linked to low sucrose content, should be useful for the selection of seedlings with good taste.

Keywords: *Pyrus*, sucrose, acid invertase, marker assisted selection

Poster number: 60

Fine mapping and candidate gene analysis to find the bitterness gene in almond

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Abstract:
The original taste of almond kernel is bitter. Due to human domestication, nowadays the majority of cultivated almond varieties are sweet. Since most of the cultivated almonds are heterozygous for bitterness, new bitter almond seedlings are usually obtained in the cross breeding programs. Therefore, it would be interesting to develop a molecular marker that enables the breeder to eliminate early in the nursery the bitter seedlings. Although its biochemical function remains unknown, the Sweet kernel (Sk) locus, was localized in linkage group five (G5) in an almond genetic linkage map. Fine mapping between the molecular markers flanking the Sk/sk gene, has been performed to obtain a shorter interval in which Sk locus should be localized. We will show new single nucleotide polymorphisms found in a 3 Mb region in which Sk locus is included within G5. In bitter almonds, hydrogen cyanide is produced upon hydrolysis of amygdalin following tissue disruption. Amygdalin and its precursor prunasin are cyanogenic glucosides. Amygdalin is mainly present in the kernels, whereas prunasin can be detected in the vegetative parts as well as in the fruit mother tissues. Parallel studies with candidate genes analysis based on the amygdalin pathway are also under study. The elucidation of the polymorphisms defining bitterness will be decisive to identify the Sk locus and the development of a molecular marker for bitterness in almond.

Keywords: almond, fine mapping, bitterness, cyanogenic glucosides, SNPs

Poster number: 59

Identification of QTL for volatile organic compounds in apple (*Malus* x *domestica*)

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Abstract:
Plant metabolites including volatile organic compounds (VOCs) play a major role in apple flavor notes. Breeding for improved flavor is a major objective in many breeding programs although there is no systematic selection for specific VOCs. Knowledge of the genetic systems controlling these compounds will facilitate breeding for enhanced concentration and combinations of these compounds in apple. This study profiled individual VOCs in ~200 individuals including commercial cultivars and their progenies from the Washington State University's (WSU) apple breeding program, maintained at the WSU Sunrise orchard, Wenatchee. Fruit was collected, juiced and frozen during the harvest season of 2012, then assayed for VOCs via GC-MS. The accessions were genotyped using ~1000 polymorphic SNPs of a total of 9K SNPs on the Infinium® II array developed in the RosBREED (www.rosbreed.org) project. The integration of phenotypic (VOCs) and genotypic data (SNPs) to identify quantitative trait loci (QTL) linked to these traits was done using FlexQTL™ software. In all accessions, pentyl acetate showed the least concentration (0.1-102.1 ng/ml) while hexanal had the highest concentration (55.8-3390.1 ng/ml). QTL were identified for precursors such as 1-butanol on LG 2 and LG 16, and for 2-hexenal on LG 4 (2 QTL) and LG 7.Methyl-butanol, 1-pentanol and hexanal each had one QTL on LG 13, LG 10 and LG 13, respectively. Among the esters assayed, QTL were identified for butyl-acetate on LG 5 and LG 8, on LG 4 for hexyl-acetate, on LG 2, LG 4, LG 10 and LG 16 for methylbutylacetate and on LG 5 for pentylacetate. The implications of these results are discussed in the context of breeding for enhanced flavors in apple.

Keywords: QTL (quantitative trait loci), VOCs (volatile organic compounds), heritability, SNPs, facilitated breeding

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Cross Assist: Online software to identify efficient cross combinations, integrating rosaceous crop genomics, genetics, and breeding

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Abstract:
Cross Assist (www.rosaceae.org/breeders_toolbox/cross_assist) is a decision-support tool for breeders to plan crosses. This software was developed as a component of the RosBREED project's Breeding Information Management System to support U.S. breeders of crops in the Rosaceae family, and the underlying database is housed on the Genome Database for Rosaceae. Cross Assist identifies efficient pairwise parental combinations from a breeder's available parent pool. This efficiency is determined by the number of seedlings needed to result in a target number predicted to each perform within specified trait thresholds. Efficient crosses achieve all trait thresholds with relatively few seedlings. The software uses available information on the breeding value of each candidate parent to predict how well resulting seedlings would perform. Calculations can be made on three increasing levels of breeding value information. The first method, "Phenotype", uses only phenotypic information in the database. "+Pedigree" adds information provided by pedigree – estimated breeding value. "+Ped+DNA" further adds information provided by functional genotypes for Mendelian trait loci and QTLs. Where such additional information is not available, calculations behind the latter two methods revert to use of phenotypes only. Users supply two specifications as input: target number of seedlings and target trait thresholds. Cross Assist's output provides a list of crosses, sorted initially by the estimated number of seedlings required. Further functionalities are being added, such as incorporation of linkage information, output addressing the trait loci involved and their available DNA tests, and integration with our sister software, Seedling Select.

Keywords: breeding value, BIM System, decision support, GDR, RosBREED

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Poster Abstracts

Genomics

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Inference of the *Fragaria vesca*'s transcriptome through the re-annotation of its genome

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Abstract:
The diploid *Fragaria vesca*, is recognized as model for more complex octoploid species such as *F.* × *ananassa* (the commercial strawberry) and *F. chiloensis* (the native Chilean strawberry). The genome of *F. vesca* has been recently sequenced nevertheless the annotation in its 1.1 version presented some inconsistencies. Therefore, in order to improve the annotation of its transcriptome the following steps were followed: 1) Prediction of open reading frames from scaffolds; 2) Assembling of ORFs in a contig or coding sequences (CDS); 3) Annotation of genes; 4) Functional classification of the genes through KOG, followed by homology with reference genomes using Blast (*Arabidopsis*, *Populus* and *Prunus*). From the 256 scaffolds available a total of 217,766 ORFs, longer than 150 bp, were predicted using Glimmer. A total of 17,818 ORFs were annotated: 6451 sequences were classified within the main KOG categories (737 in Information, storage and processing category; 4858 in Cellular processing and signaling; and 856 in Metabolism), 5409 sequences were classified as poorly characterized and 5958 as unknown. A total of 53,586 ORFs with a biological description were annotated by homology. The entire data has been deposited initially in a local database. The search of genes related to important quality traits of strawberry fruit was performed particularly on fruit softening. The search provided an entire list of genes including those previously described as main players in the softening of strawberry fruit. Research supported by Anillo ACT-1110 project.

Keywords: strawberry, fruit softening, *Fragaria vesca*, gene annotation, functional classification

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Poster number: 8

Impact of carbon deprivation on apple fruit quality during cold storage

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Abstract:
Fruit quality is built on complex traits such as taste, texture, colours and aromas, developed during fruit maturation and cold storage. The genetic, molecular and ecophysiological bases of these traits are still mainly unknown. Apple fruit development is characterized by a complex network of sink-source interactions due to concomitant vegetative and bourse shoots development during the growing season. Several studies are pointing out a possible direct relationship between dry mater accumulation and high quality texture (1, 2). In order to study the impact of trophic competitions on fruit quality, the sink-source equilibrium was modulated at the level of the fruit bearing branch or the inflorescence by defoliation treatments. The experiments were carried out on four apple varieties with contrasting sensory phenotypes. Apple quality was investigated with sensory, morphometric, mechanical, biochemical and transcriptomic analyses. The impact on fruit development and quality is depending on the timing of the defoliation treatment and on the variety. If late defoliation did not impact much fruit quality, data suggest an important role of bourse leaves in early stages of fruit development. Fruits transcriptome analyses also revealed a differential stress response to cold storage, probably due to increased sunlight exposition which depend on tree architecture. A drastic effect was observed on developing buds with a total fall during the next winter. (1) Palmer et al., 2010 Journal of the Science of Food and Agriculture 90(15). (2) Saei et al., 2011 Scientia Horticulturae 130(1).

Keywords: apple, fruit quality, dry matter, cold storage, transcriptome

Poster number: 9

Isolation and characterization of apple *Pythium ultimum* response genes and their ethylene and jasmonate mediated transcriptional regulation

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Abstract:
Apple Replant Disease (ARD) causes a serious economic loss for the apple industry. Although it has been known that a complex of necrotrophic fungi and oomycetes are the primary causal agents of ARD, the molecular response in apple to infection by these pathogens has not previously been examined. In this study, we identified apple genes that are activated by *Pythium ultimum*, an oomycete that is a significant component of the ARD pathogen complex. The apple genes examined in this study include those involved in ethylene (ET) biosynthesis and jasmonic acid (JA) biosynthesis, a transcription factor gene that functions as an ET/JA signal integrator (MdERF: ethylene response factor) and a pathogenesis-related (PR) gene that is a target of ERF (MdCHIB: CHITINASEB or beta-chitinase). Using real-time quantitative reverse transcription PCR, target gene expression was shown to a 10-60 fold up-regulation in apple root stock seedlings 1-2 days post *P. ultimum* inoculation. Transcriptional regulation of these target genes by exogenous application of ethylene and jasmonate was also examined. MdERF was not only up-regulated by either ethylene or JA, but the enhanced expression of MdERF was significantly greater when exposed to the combination of these hormones. For MdACS and MdAOS, ethylene and JA serve as a positive or negative regulator depending upon gene isoform and the plant tissues in which expression is monitored. Our data demonstrate that ET/JA cross-talk plays an important role in the apple rootstock response to infection by *P. ultimum*.

Keywords: apple replant disease, plant hormones, fungi, oomycetes, ERF

Poster number: 10

Application of the 8K Illumina Infinium apple SNP-chip to estimate linkage disequilibrium and population structure in an apple germplasm collection

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Abstract:
Discovering genetic variations that cause the observed variations for important plant traits, is one of the key challenges for plant researchers. For apple, the International RosBREED SNP Consortium developed an 8K SNP-chip on the Illumina Infinium platform that can be used for such association and genotyping studies. This SNP-chip was used to genotype our *Malus* x *domestica* germplasm collection consisting of both commercial and old cultivars as well as some genotypes from a Telamon x Braeburn progeny. We used the SNP data obtained from this SNP-array to estimate the linkage disequilibrium (LD) between adjacent SNPs as well as the LD decay across larger regions because this determines the number of markers necessary for association studies. In addition, association analyses can be biased by the population structure present in the collection used for these studies. Therefore we determined the population structure of our germplasm collection using the software STRUCTURE. Evanno's criterium indicated that the most likely numbers of subpopulations present in our collection were two and three subpopulations with two being the most likely. The first subpopulation was a group with the Telamon x Braeburn progeny and other Braeburn-related cultivars. The other subpopulation contained all other commercial and old cultivars. This latter group split into a group of commercial cultivars and a group of old cultivars when increasing the number of subpopulations to three while the first group remained unchanged. The estimated LD between adjacent SNPs using the r2 parameter was 0.23 and decayed quickly below 0.18 after about35kb.

Keywords: linkage disequilibrium, population structure, *Malus* x *domestica*, SNP-chip

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Determination of DNA methylation and histone modification kinetic in the promoter region of dormancy associated MADS-box gene (DAM6) in sweet cherry (*Prunus avium*) during bud dormancy release

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Abstract:
Bud dormancy release in Rosaceae depends on a minimum accumulation of chilling hours. DORMANCY ASSOCIATED MADS-BOX (DAM) genes are negative flowering regulators and strong candidates for the regulation of bud growth cessation and dormancy maintenance. Epigenetic mechanisms appear to control the silencing of DAM genes in peach in a similar way to its ortologous in *Arabidopsis* during vernalization. To better understand the molecular mechanism of dormancy release in sweet cherry (*Prunus avium* var. Bing); we isolated the promoter and 5'UTR regions of DAM6. Histone modifications and DNA methylation pattern kinetic of this region in response of chilling hours (CH) accumulation was studied in floral buds sampled weekly during the dormancy period. Using chromatin immunoprecipitation (ChIP) assay, we observed that trimethylation of histone H3 at K27 increased reaching a maximum at 800 CH accumulated. This histone modification is related to silencing of gene expression. On the other hand it was observed that methylation of histone H3 at K4, a modification related to gene activation decreases with CH accumulation. In parallel, the results obtained from bisulfite sequencing suggest a dynamic change of methylated cytosines as the chilling requirement is being fulfilled indicating silencing of DAM6. It was also observed an increase in FLOWERING LOCUS T (FT) gene at 700 CH with a peak at 1200 CH. FT is a transcription factor known as the universal signal inducing flowering. In *Arabidopsis*, FT expression is repressed by the DAM ortologous gene. Our results strength the role of epigenetic mechanisms in chilling requirement for dormancy release and blooming in Rosaceous species as sweet cherry. This work is supported by FONDEF G09I1008; FONDAP CRG 15070009 and Basal PFB-16.

Keywords: bud dormancy, histone modification, DNA methylation, DAM6, chilling hours

Poster number: 14

Resistance and susceptibility of *Prunus persica* to *Xanthomonas arboricola* pv. *pruni*: molecular signatures in response to early infection

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Abstract:
Bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (Xap), is the most dangerous bacterial disease for *Prunus*. 'Redkist' peach variety is spot resistant whereas 'J.H. Hale' is susceptible. In order to characterize the molecular mechanisms underlying the incompatible and the compatible interaction between peach and Xap, we performed RNA sequencing for differential gene expression profiling on both varieties during very-early phases of interaction: 30 min, 1 h, 3h post-infection (hpi). Over 550 million reads provided an extensive view on the defense responses in both genotypes. RNA-Seq analysis detected 20,837 expressed genes in leaf, representing 75% of peach predicted genes. A total of 803 and 838 differentially expressed genes (DEGs) were identified as spot responsive genes in 'Redkist' and 'J.H. Hale' respectively during all the time points. The former regulated 88.9% of DEGs at 3 hpi while the latter only 53.4%. Both varieties induced genes involved in signal transduction, hormone biosynthesis, secondary metabolism, ROS burst, defense proteins, transcription factors. Also, GO term enrichment analysis resulted in terms including ‘immune system process’, ‘defense response’, ‘cell death’. Finally, the validation by Q-PCR confirmed the relative gene expression to be consistent with RNA-Seq data analysis. This study revealed very similar molecular responses to Xap in 'Redkist' and 'J.H. Hale' after bacterial infection and are consistent with previous studies. Between the two, crucial differences were detected in the expression of transcription factors, salicylic acid biosynthetic genes, and ROS burst, shedding light on the effective disease response exerted by a resistant host relative to a susceptible

Keywords: peach, bacterial spot, RNA seq

Poster number: 15

Development of ALSV-mediated VIGS in *Prunus* fruit trees

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Abstract:
Apple latent spherical virus (ALSV) vectors have been shown to effectively induce stable virus-induced gene silencing (VIGS) in a wide range of plant species including Rosaceous fruit tree species, such as apple and pear. In this study, we attempted to develop a VIGS-based gene evaluation system for fruit tree species in *Prunus*, using the ALSV viral vector system. Partial sequence of PHYTOENE DESATURASE (PDS) of apricot (*Prunus armeniaca*) was cloned and ligated into the T-DNA region of a binary vector pBICAL2. The T-DNA region of pBICAL2, designed based on the RNA2 of ALSV, contains a single ORF for the ALSV polyprotein under the control of CaMV35S promoter sequence. The partial ParPDS sequence was ligated in frame with the coding sequences for the movement protein and the capsid protein Vp25 flanking the cloning site. The resultant pBICAL2-ParPDS was introduced into a disarmed *Agrobacterium* strain EHA105. The pBICAL1, a binary plasmid for the expression of RNA1 of ALSV in plants, was also introduced into EHA105. To amplify and produce recombinant ALSV particles, leaves of *Nicotiana benthamiana* were infected with pBICAL1/EHA105 and pBICAL2-ParPDS/EHA105 at the same time. The amplified ParPDS-ALSV in *N. benthamiana* was isolated and infected to the cotyledons of *Prunus* seeds right after germination by a particle bombardment. Uniform discoloration of the upper leaves, a typical knock-down phenotype of PDS, was observed several weeks after inoculation in a certain range of fruit tree species in *Prunus*.

Keywords: gene evaluation system, photo-bleached leaf, phytoene desaturase, post-transcriptional gene silencing

Poster number: 16

Genetic structure of sweet cherry with 6K SNP array v1

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Abstract:
A set of 45 sweet cherry (*Prunus avium*) varieties which includes ancestors of modern varieties, modern breeding cultivars and local germplasm was genotyped and evaluated with the 6K SNP chip array of Illumina by an Infinium® assay. Genotyping filtering revealed that from 5696 SNPs available in the 6K array, 431 (7.5%) were not scored, 3368 (59%) were monomorphic and 1897 (33%) were polymorphic and informative (MAF>0,1). From these up to 1.5% failed Hardy-Weinberg equilibrium ($p<0.001$). The SNP dataset was used to study the genetic structure of the sample. Cluster analysis identified clear substructure within the dataset by differencing 3 major groups. Modern cultivars and breeding founders grouped together in a single cluster suggesting a common Western European Origin (WEO). Local varieties from Spain were separated in two clusters, one from Western Spain (WS) and another from Eastern Spain (ES). Preliminary studies pinpoint to the existence of a unique genomic pool in the Spanish sweet cherry germplasm, evidencing its potential for breeding programs.

Keywords: *Prunus avium*

Poster number: 17

Unique evolutionary pattern of NBS-LRR genes among five Rosaceae species

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Abstract:
Disease resistance (R) genes from different Rosaceae species have been identified by map-based cloning for resistance breeding, and most disease resistance genes encode NBS-LRR protein. However, reports describing evolutionary pattern of R-genes in Rosacese species are rare because several Rosaceae genome sequences have only become available in last several years. We performed a systematic genome-wide survey of NBS-LRR genes among five Rosaceae plants, including *Fragaria vesca* (woodland strawberry), *Malus* × *domestica* (apple), *Pyrus bretschneideri* (Asian pear), *Prunus persica* (peach) and *Prunus mume* (mei, or Japanese apricot) with 144, 748, 469, 354 and 352 NBS-LRR genes. The high proportions of multi-genes and similar Ks peaks (Ks = 0.1- 0.2) of gene families in the four woody genomes indicate that recent duplications played an important role in the four woody perennial Rosaceae species. Subsequently, 385 species-specific duplicate clades were dominant in the phylogenetic tree constructed by all 2067 NBS-LRR genes. High percentages of NBS-LRR genes involved in species-specific duplication were found among the five genomes (54.86% in woodland strawberry, 68.05% in apple, 57.56% in pear, 44.07% in peach and 45.74% in mei). It might be inferred that species-specific duplication mainly contributes to the expansion of NBS-LRR genes in the five Rosacese species. In addition, the Ks and Ka/Ks values of TIR-NBS-LRR (TNL) were significantly greater than those of non-TIR-NBS-LRR (non-TNL), suggesting that rapidly evolved TNLs have different evolutionary patterns to adapt to different pathogens compared with non-TNL genes. In addition, some of the RPW8 domain-containing NBS-LRRs had Ka/Ks ratios less than 1, suggesting that they were driven by positive selection.

Keywords: NBS-LRR genes, Rosaceae species, disease resistance genes, species-specific duplication

Poster number: 18

A proposed naming convention for genes in Rosaceae species

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Abstract:
In the last few years a considerable effort has yielded the complete genome sequence of four key Rosaceae species: apple, strawberry, peach, and pear with more to come. With this genomic information it is now possible to identify gene family members rapidly and through other genomic technologies such as mRNA-seq and test gene function. This has greatly accelerated the pace at which research is done. As currently only a few of the 30 - 60,000 predicted genes in the genome have been described in each of these species, the Rosaceae community has a unique opportunity to standardise gene names within and across the Rosaceae species. The Rosaceae Gene Name Standardization Subcommittee has been formed to propose a naming guideline around naming genes in Rosaceae. This poster details the issues around naming genes and proposes a common nomenclature standard for species gene, and splice variation. Curated gene database in GDR (Genome Database for Rosaceae) will contain user-submitted gene names in addition to those curated from NCBI nr database. GDR will also host gene family submission site, as well as gene name submission site, to maintain a user-curated list of gene families. To avoid confusion in future publications, the subcommittee recommends researchers to register their gene names and gene family data to GDR prior to publication to facilitate the effort on the gene naming standardization.

Keywords: gene name standardization, GDR

Poster Abstracts

Genomics to Genetics

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Analysis of candidate genes involved in flowering date in sweet cherry (*Prunus avium*)

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Abstract:
In sweet cherry, flowering process is controlled by specific chill and heat requirements. In the context of global climate change, the knowledge of the genetic determinism of flowering is necessary to develop new cultivars that are able to face the challenges associated with an increase of temperature. Two intraspecific F1 mapping progenies, 'Regina' × 'Lapins' (R×L, 124 individuals) and 'Regina' × 'Garnet' (R×G, 117 individuals), were genotyped and used in the detection of quantitative trait locus (QTL) involved in flowering process. Using a candidate gene approach, a list of one hundred functional candidate genes was established according to those found in other plant species. Cherry gene homologues were identified using the sweet cherry 'Regina' transcriptome database developed in our lab. Polymorphism between parents was analysed and candidate genes were mapped in the two progenies. The expression of the most promising candidate genes, located in QTL regions for flowering date, were analysed using qRT-PCR on reproductive buds at different stages during dormancy progression.

Keywords: sweet cherry, flowering date, candidate genes, QTL mapping, expression analysis

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Profiling the transcriptomic and metabolomic changes associated with apple fruit controlled-atmosphere storage related peel disorder

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Abstract:
'Golden Delicious' (*Malus* x *domestica* Borkh.) (GD) is one of the most widely available pome fruit. External CO_2-injury, a postharvest controlled-atmosphere storage related peel disorder, significantly impacts long-term storability and fruit quality for fresh apple and pear fruit cultivars, although annual incidence varies greatly. The experiment included 5% CO_2-controlled atmosphere (CA), 5% CO_2-CA plus 1-methylcyclopropene (CA+1-MCP), and regular air (RA) treatments and peel tissues were sampled at 14, 28, 56, 84 days storage over a three year period. RNA-Seq based transcriptome profiling (2011) and Lipdome profiling by LCMS (2011-2013) were carried out for sampled apple peel tissues. KEGG pathway analysis indicated oxidation/reduction, glycolysis, sodium transporters and glutamate biosynthesis enriched in CA but not RA and CA+1-MCP. Accordingly, gene ontology for CA emphasized the importance of the endoplasmic reticulum (ER), ER stress and the Unfolded Protein Response while CA+1-MCP down regulated these ER stress response and protein folding genes. Multivariate and time course analysis of RNA-seq and LCMS/APCI data provided genes and lipids of interest. SKP2A, an F-box protein known to be involved in severe ER stress, was detected only in CA stored fruit after 28 days storage. Results for the lipidome show (C18:3/18:2) diglyceride, β-SitoGluclinolenate, two monogalactose-diacylglycerol and two triacylglycerols species having a significant differences between treatments. Highly relevant network gene models selected by sparse Partial Least Squares is being validated by RTqPCR using all three years GD peel tissue. These results will determine the key molecular responses of apple peel tissue to high-CO2 controlled-atmosphere storage.

Keywords: controlled-atmosphere, physiology disorder, CO_2 injury, abiotic fruit, endoplasmic unfolded protein response

Poster number: 29

Applying physiogenomics-based solutions to address ethylene and ripening challenges in European pear

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Abstract:
Development of genomics-guided physiological solutions in biological systems lacking significant genomic resources is challenging. Utilization of controlled physiological models with time course gene expression analysis can address these challenges. Harboring unique ripening biology and responses to the ethylene signaling inhibitor 1-MCP, pear is an ideal model for investigation of the molecular underpinnings of System 2 ethylene induction. Induction of autocatalytic ethylene biosynthesis in climacteric fruits acts as a powerful indicator of – and trigger of –ripening. We identified differential expression of genes in a ripening-related pathway during postharvest conditioning in peel tissue of two pear varieties: Anjou and Bartlett. Anjou requires 60-day cold at 0-5°C to gain competency for ripening and System 2 ethylene biosynthesis, while Bartlett requires 15 days. Further, pear exhibits variable recovery from 1-MCP treatments, with some fruit 'locked' permanently in an unripe state. We developed ripening-stimulating compounds (RCs) thought to increase the activity of the pathway we previously identified in comparison of Anjou and Bartlett tissue. Initial tests of mature unconditioned, 1-MCP treated fruit have shown accelerated ethylene biosynthesis, respiration rate and development of fruit quality traits from RC exposure. Future work will seek to characterize global transcriptomic responses to exposure of these RCs in 1-MCP treated and control Anjou and Bartlett fruit, as well as identify means to enhance RC penetration into fruit interior. This work demonstrates a unique means of accelerating pear ripening, and can help facilitate the use of 1-MCP in the pear postharvest management chain.

Keywords: pear, ripening, ethylene, 1-MCP, postharvest

Poster Abstracts

Genetics

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Identification of two QTLs for flesh mealiness of apple (*Malus* x *domestica* Borkh.) fruit on linkage group 10

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Abstract:
Flesh softening, particularly mealiness, which occurs in apples during storage, is an unfavorable characteristic as it decreases the commercial value of the produce. Although many studies have been conducted on firmness and texture at harvest as well as changes in them during storage, the degree of mealiness (i.e. loss of cellular adhesion) during storage has rarely been investigated. In the present study, we quantified flesh mealiness under 20°C with 85% relative humidity by measuring the degree of cell separation in flesh discs after 7 hours shaking in a sucrose solution. Samples were taken over a period of 2 years from 137 F1 individuals from a cross between 'Orin' x 'Akane.' QTL analysis revealed two QTLs for flesh mealiness on linkage group (LG) 10, and the genetic distance of these QTLs was approximately 35 cM. Md-PG1SSR10kd marker was the nearest to one of the QTLs with a LOD score of 5.13. This marker is located on 10 kbp downstream of the stop codon of MdPG1, known to be involved in firmness and texture of flesh. AF057134-SSR marker was the nearest to the other QTL with a LOD score of 5.30. The location of this marker is almost same as a QTL reported for flesh firmness at harvest and after cold storage for 2 months in 'Mondial Gala' apples. These results indicate that LG 10 has the significant importance in controlling not only fruit texture at harvest but also loss of texture during post-harvest storage.

Keywords: mealiness, genetic linkage map, quantitative trait locus

Poster number: 32

Mapping of dwarf growth habit traits in apple (*Malus pumila* Mill.)

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Abstract:
Apple (*Malus pumila* Mill.) is one of the most widespread and commercially important fruit crops worldwide. There is increasing interest in the genetics of tree architecture. Various dwarf genes have been described in the literature but few if any have been mapped and characterised. At the ARC-Bien Donné Experimental Farm are two young progenies, both derived from 'McIntosh', segregating for two novel dwarf traits, one monogenic and the other apparently digenic. These progenies are being scored with markers to map the traits and to identify potential candidate genes. And a transcriptomic approach will also be pursued to identify candidate genes. Combining the results will help clarify the molecular genetics and mechanisms behind these dwarf traits in apple. Tracing the genes in relevant pedigrees will help avoid raising progenies segregating for these traits in future.

Keywords: *Malus*, dwarf, mapping, trait

Poster number: 33

Characterization of resistance to fire blight (*Erwinia amylovora*) and blue mold (*Penicillium expansum*) in exotic and domesticated *Malus* (apple) germplasm

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Abstract:
Genetic improvement of host resistance has been identified by the U.S. apple industry as a major way to mitigate production risks associated with economically important diseases such as fire blight and blue mold. Several fire blight resistance QTLs have been described in *Malus*, however most come from exotic germplasm of poor fruit quality. Two bi-parental populations derived from 'Splendour' apple, which has excellent flavor and is resistant to fire blight, were found to segregate for resistance to fire blight (1R:3S) when challenged with *Erwinia amylovora*. These populations are being genotyped to identify the genetic basis of this resistance. Another approach to identify marker trait associations for fire blight resistance within domesticated *Malus* cultivars is to utilize the pedigree-linked germplasm of the RosBREED project. The *Malus* Crop Reference and Washington State University (WSU) Breeder Pedigree Sets were propagated and a replicated research orchard will be established at WSU, Wenatchee, WA in spring 2015 and used to determine the resistance of this germplasm to fire blight. Although there is no known resistance to blue mold in *M.* × *domestica*, *M. sieversii* PI613981 collected from the wild in Kazakhstan is resistant. Fruit collected from a 'Royal Gala' X PI613981 mapping population (GMAL4593) were inoculated with *P. expansum* and evaluated for decay over a three year period. A genetic framework map has been developed for the GMAL4593 population and was used to identify QTLs for blue mold resistance on linkage groups 4 and 10.

Keywords: Splendour, PI613981, GMAL4593, *Malus* crop reference set

Poster number: 34

A global conservation strategy for apple

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Abstract:
Apple (*Malus* x *domestica*) production is #17 for agricultural products both in the U.S. and world with a value of more than $31 billion worldwide. This important perennial crop is expensive to produce, with high costs for land, labor and inputs. The industry is dominated by a relatively few number of scion cultivars and rootstocks, which increases its susceptibility to threats of new diseases, pests, and changing climate conditions. There are approximately 38 wild *Malus* species which are native to Asia, Europe and North America. Some of these wild species exhibit desirable resistance to biotic and abiotic stress, unique fruit quality, and useful rootstock traits. Apple is an Annex 1 crop and is covered under the multilateral system of The International Treaty (IT) on Plant Genetic Resources for Food and Agriculture. Countries that ratify the IT agree to make genetic resources and related information about these crops stored in their gene banks available to facilitate research and information exchange. Global Conservation Strategies have for many of the Annex 1 crops summarized the information available regarding gene bank collections, methods for conservation, and safe germplasm movement. This discussion will define the process of developing a global strategy for apple conservation. An expert committee will be chosen. A survey of global genebanks will be conducted. Global collections will be summarized and gaps will be discussed. The results will be summarized in recommendations for genebank standards for the management of apple genetic resources. Through the development of a Global Conservation Strategy for Apple, we aim to facilitate the flow of information and apple genetic resources internationally.

Keywords: *Malus*, genetic resources, genebank, conservation, diversity

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Poster number: 36

Genetic relationships and ploidy levels of *Rubus* spp. of the tropical Americas

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Abstract:
The demand for blackberries (*Rubus* spp.) has increased in Costa Rica, which represents an important income for small farmers in the country. The material grown in Costa Rica consists of four species and nine genotypes, mostly landraces of *R. adenotrichos* and is grown from southern Mexico, to northern South America. Costa Rica possesses diversity of *Rubus* with desirable economic traits, such as high ORAC values, but little is known about their genetics and genomics. In this study, genotypes were analyzed using 9 RAPD, and 13 SSR markers as well as using preliminary flow cytometry work. Nei's genetic distances were calculated, and the UPGMA procedure was used. 'Qualicum', 'Tulameen' and 'Navaho' were comparative controls. Both RAPD and SSR analyses concurred with morphological data except for *R. urticifolius* ld. 'Caballo', which showed a closer relationship to *R. adenotrichos* landraces than *R. urticifolius* ld. 'Ratón'. Although, *R. urticifolius* was reported as a diploid, allelic patterns were similar to that of a tetraploid. *R. glaucus* was reported as a blackberry-raspberry hybrid; both RAPD and SSR analyses support this as the genotype showed the closest relationship to *R. idaeus*. Flow cytometry analyses showed that *R. adenotrichos* landraces were diploid. Ploidy measurements for all other genotypes are underway to determine whether different ploidy levels can be found in *R. urticifolius*, or that 'Caballo' is a different species from 'Ratón'. Genetic relationships and ploidy information is crucial to breeders for determining crossing compatibly among the genotypes, and initializing a competent breeding program in the future.

Keywords: Flow cytometry, tropical blackberries, SSR, RAPD

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Poster number: 39, see page 62

Poster number: 40

Formation of the SCF complex with the F-box proteins encoded by genes linked to the S locus in *Prunus*

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Abstract:
Many species in the Rosaceae, Solanaceae and Plantaginaceae exhibit the S-RNase-based self-incompatibility (SI). Their S loci contain the genes encoding S-ribonuclease (S-RNase) and F-box protein(s) for the pistil and the pollen specificities, respectively. The pollen S specificity in the Solanaceae is determined by multiple S locus F-box proteins (SLFs), while that in *Prunus* (Rosaceae) is by a single S haplotype-specific F-box protein (SFB). In addition to SFB, three other pollen-expressed F-box genes called S locus F-box protein like 1-3 (SLFL1-3) are located in the flanking regions of the *Prunus* S locus, although it is not clear whether they are involved in SI. An F-box protein generally forms the SCF complex with Skp1, Cul1 and Rbx1. The SCF complex is known to polyubiquitinate substrate proteins to be degraded by the ubiquitin proteasome system. Although solanaceous SLFs have been shown to form the SCF complex and to participate in the SI system, it remained to be elucidated whether *Prunus* SFB and SLFLs would form the SCF complex. We previously identified SFB-interacting Skp1-like protein 1 (SSK1), which showed the binary protein interactions with SFB, SLFLs and the pollen-expressed Cul1 homologs (Cul1A and Cul1B). Here, we report the ternary protein interactions of GST-fused SFB/SLFLs, SSK1 and Cul1A/Cul1B, in pull-down assay, indicating that SFB and SLFLs could form the SCF complex with either of the Cul1 homologs and polyubiquitinate unidentified substrate proteins in pollen tube.

Keywords: SCF complex, self-incompatibility, SFB, SLFL, protein-protein interaction

Poster number: 41

New advances on the flesh's red pigmentation in peach

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Abstract:
Anthocyanin-rich fruit, because of their antioxidant properties and strong attractiveness to consumers, generates great interest in varietal innovation programs that increasingly integrate genomics and plant breeding. This is the case in peach in which the anthocyanin concentration in the mesocarp of the fruit is controlled by three different loci: i) the dominant Cs locus, mapped to LG3 and probably associated with MYB10, determines a red pigmentation of the mesocarp around the stone; ii) the dominant locus DBF, for Dominant Blood-Flesh, that we recently mapped to LG5, determines a fully red mesocarp appearing at the later stages of the fruit development. Using the peach reference sequence, we identified as good candidates a cluster of three predicted members of the dihydroflavonol-4-reductase gene family in the 505-kbp region containing DBF; iii) similarly, we narrowed the region (<80-kpb) of the recessive locus bf, for blood-flesh, that had been previously mapped to LG4. It determines a more or less substantial accumulation of anthocyanin in both immature and mature fruits, associated with the red pigmentation of midrib (rpm) on the underside of leaves and a reduced tree height (rth). However for bf, uncertainty still exists over whether these simply inherited traits are controlled by three linked loci (bf, rpm, and rth) or one pleiotropic locus. From biochemical, molecular and genomics analyses of one segregating population of peach (850 progenies), we bring new elements to answer this question and propose candidate genes involved in anthocyanin expression and the growth of plants.

Keywords: *Prunus persica*, anthocyanin, blood-flesh, fine mapping, pleiotropy

Poster number: 42

What determines apricot fruit color? Characterization of carotenoids profile in different apricot cultivars

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Abstract:
Carotenoids are 40 carbon isoprenoids pigment molecules, indispensable for plant life. They play essential roles in photosynthesis, and they serve as precursors to the plant hormones abscisic acid (ABA) and strigolactones. In addition, these compounds furnish many flowers and fruit with attractive yellow, orange and red colors. Carotenoids are important in human health since they serve as pro-vitamin A and are antioxidants with protective activity against various illnesses. Vegetables and fruits are a natural source of carotenoids, and apricot (*Prunus armeniaca* L.) has the potential to serve as a particularly rich source. Carotenoid biosynthesis in plants is well characterized thanks to extensive research in model plants such as tomato and *Arabidopsis*. However, little is known in regards to apricot. To better understand the factors that determine carotenoid profile in apricot fruit, we characterized the carotenoids composition and content of fruits from more than one hundred apricot cultivars and lines. We present the result of a HPLC analysis, showing a great variability within the different cultivars, in both carotenoid content and composition. Based on the carotenoid phenotype, we suggest candidate genes to explain the phenotypic diversity. The data obtained will be used to facilitate research on the genetic factors controlling carotenoid biosynthesis in apricot.

Keywords: carotenoid biosynthesis, apricot, fruit development, color

Poster number: 43

Development and flowering-related gene expressions in inflorescence and peduncles in Japanese flowering-cherries

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Abstract:
Japanese flowering-cherries called “Sakura” show various floral morphogenesis in such as the number of flowers in a cluster, flower size, shapes of petals, the number of petals, flower color, and so on. In this study, we are focusing on the variation of inflorescence structure which is possibly one of the important traits affecting ornamental quality for flowering-cherries. We investigated the lengths of inflorescence axis and peduncles in a number of selected cultivars of flowering cherries at the time of anthesis. The inflorescence types diverged widely from corymb to umbel. The expressions of genes expected to be involved in the inflorescence development were analyzed by RT-PCR. The expression of TFL1/CEN/FT homologous genes (PruTFL1, PruCEN, and PruFT), which are related to the flowering and inflorescence structure, were investigated in inflorescence axis and peduncles. Expression of PruTFL1 was hardly detected in selected cultivars. PruCEN expression was observed only in several cultivars. PruFT was highly expressed in the inflorescence axis and peduncles and the expression level was relatively higher in inflorescence. However, these expression levels were different in cultivars, and the relationship to morphological variation couldn’t be found. The expression of genes related to the biosynthesis of gibberellic acids, which are often involved in organ elongation, were also analyzed, but the expression pattern was ambiguous like the result of TFL1/CEN/FT homologous genes. We are planning transcriptome approach to found the other genes involved in the development of various inflorescences in flowering-cherries, and testing the effects of phytohormones to the inflorescence development.

Keywords: *Prunus*, morphology, inflorescence, flower

Poster number: 44

Metabolomic analyses in sweet cherry

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Abstract:
In sweet cherry (*Prunus avium* L.) organoleptic quality is part of the main fruit traits for breeders and consumers. Two F1 progenies, 'Regina' × 'Lapins' (R×L) and 'Fercer' × 'Burlat' (F×B), were used to analyse the genetic determinism of traits related to organoleptic quality: titratable acidity, pH and refractometric index were analysed on R×L during three years: 2006, 2008 and 2013 on 83, 106 and 114 individuals, respectively. Major soluble carbohydrates (glucose, fructose, sucrose and sorbitol) and major organic acids (citrate and malate) were analysed in 2012 on R×L and F×B on 106 and 111 individuals, respectively. Metabolite quantification was performed using robotised microplate-based assays in three replicates per tree, consisting of a mixture of fruit skin and pulp obtained by pooling five fruits. Genotyping was performed with the RosBREED's cherry 6K SNP array v1 and linkage maps for the two progenies were constructed with JoinMap 4.0 software. QTL mapping was carried out using MultiQTL V2.6 software. Titratable acidity and pH were negatively correlated, and major QTLs for both traits were detected on LG6. For sugars and organic acids a QTL with major effect was only detected for malic acid on LG6, co-localizing with pH and titratable acidity QTLs. QTLs for malic acid were also detected on LG1 and LG5, but having lower effect. QTLs for sorbitol were detected on LG2 and LG3. QTLs with very low effect were detected for glucose and sucrose on LG4 and LG3, respectively.

Keywords: sweet cherry, fruit quality, QTLs

Poster Abstracts

Genetics to Breeding

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Poster number: 46

Molecular characterization of sweet cherry germplasm collections in Chile

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Abstract:
Within the past decade, sweet cherry has become an important Chilean fresh fruit export crop. The cherry fruit industry has the potential to expand and increase the production, quality and volume of exported cherries. A large diversity of commercial sweet cherry cultivars are grown in Chile. In order to determine the genetic diversity and similarity of these germplasms for Sweet Cherry Marker-Assisted Breeding programs, we have created a collection of genomic DNA from 93 commercial sweet cherry cultivars. Sweet cherry (*Prunus avium* L., 2n=16), is a member of the Prunoideae genus, one of the four Rosaceae subfamilies. Since there is a high degree of synteny between different *Prunus* species, we have molecularly analyzed this collection of 93 commercial sweet cherry cultivars using 16 SSRs that have been described previously in other *Prunus* species. The molecular characterization and genetic diversity/similarities among these cultivars will be presented. This work was funded by FONDEF-Chile grant D04I-1060; 07CN13PBT-167 INNOVA Chile CORFO project; CONICYT Fondecyt 1121021; CONICYT-Regional/GORE O´Higgins/CEAF/ R08I1001; CONICYT Fellowships (21120115 and 24121484 for CK, and 24121618 for VG); Universidad Andrés Bello project DI-78-12/I for KC; and INIA.

Keywords: *Prunus avium*, sweet cherry, SSR, germplasm collection, Chile

Poster number: 47

Identification of QTL underlying powdery mildew and bacterial canker infection in sweet cherry (*Prunus avium* L.)

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Abstract:
Powdery mildew (PM), caused by *Podosphaera clandestina*, and bacterial canker (BC), caused by *Pseudomonas syringae* pv. *syringae*, are the major diseases of sweet cherry in the USA. Incorporation of natural resistance into elite cultivars would be an effective way to reduce reliance on fungicide and pesticide use and facilitate the transition to sustainable production systems. This study was designed to identify quantitative trait loci (QTL) underlying PM and BC infection to facilitate development of new resistant cultivars. Six hundred pedigree-linked germplasm were used in this study. PM was scored in the field on a 0-5 scale (0 = no visible symptoms and 5 = very severe infection on leaves) from 2011 to 2013. BC phenotyping was performed by inoculating five healthy and newly emerging leaves with 10 µl of 100,000,000 CFU/ml bacteria suspension mixed with 0.5% surfactant by mid-rib wound method in a detached leaf assay. Disease was scored in the lab on a 0-4 scale (0 = no necrosis and 4 = total necrosis). Approximately 1100 single nucleotide polymorphism (SNP) and four simple sequence repeat (SSR) markers were used for determining genome-wide marker-locus-trait associations. One PM QTL mapped on top of linkage group (LG) 5 in the three years while others mapped on LGs 1, 3 and 6 in a single year. For BC, one major QTL was identified on top of LG 5. The co-location of QTL for both diseases on LG 5 will be explored further to develop a breeding strategy for the two diseases combined.

Keywords: sweet cherry, powdery mildew, bacterial canker, quantitative trait loci, marker-assisted breeding

Poster number: 48

Phenotyping protocol for sweet cherry (*Prunus avium* L.) to enable an understanding of trait inheritance

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Abstract:
The USDA Specialty Crop Research Initiative-funded RosBREED project has the objective of enabling marker-assisted breeding (MAB) in the economically important agricultural family of the Rosaceae. To standardize and increase the accuracy of MAB in Rosaceae, it is necessary to characterize many horticultural and fruit quality traits in representative germplasm. A well-developed comprehensive phenotyping protocol for productivity traits, fruit quality traits and horticulturally objectionable traits, developed for use in the Washington State University (WSU) Sweet Cherry Breeding Program, is described and selected correlations among traits observed and quantified. The protocol facilitates standardization of data among researchers working with sweet cherry across various environments and institutions. Data collected from sweet cherry 'Crop Reference Set' and 'Breeding Pedigree Set' between 2010 and 2012 was evaluated for correlation among sweet cherry phenotypic traits, and the results are presented. Selected results indicate significant moderate correlations for harvest date with fruit weight and fruit firmness ($r=0.26$, $p<0.0001$ and $r=0.39$, $p<0.0001$, respectively), with later- maturing varieties tending towards larger and firmer cherries, in general. Also, fruit weight had a positive significant association with pedicel-fruit retention force with $r=0.43$ ($p<0.0001$). However, soluble solid content showed a negative relationship with fruit weight and firmness ($r=-0.34$ and $r=-0.20$, respectively). Progress in breeding for multiple traits simultaneously will be faster with the significant positive correlations obtained in this study.

Keywords: phenotyping protocol, fruit quality, MAB (marker assisted breeding), sweet cherry breeding, standardization of data

Poster number: 49

Analysis of trends in sweet cherry flowering data across Europe

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Abstract:
In temperate fruit trees, most key phenological stages are highly dependent on environmental conditions. In particular, correct timing for dormancy and flowering is essential to ensure good fruit production and quality. As a result, in a swiftly-changing environment, temperate fruit crop adaptation in many areas will be at risk in the coming decades. Global changes in environmental conditions include warmer winters and higher risks of frosts in the early spring, leading to a wide range of problems: flower and fruit set, sun-scald, cross-pollination or novel host-pest interaction. With the final aim of better understanding the response of flowering to climatic conditions, we present a large-scale analysis of flowering data for various sweet cherry cultivars from numerous sites in Europe, characterized by a wide range of climatic conditions. These phenology data were provided through a national network of experimentation (Ctifl) and a European COST action on sweet cherry that INRA-Bordeaux is leading. This approach allowed extracting the main trends in flowering behaviour under different temperature conditions and selecting the best phenology models. These results represent the first step towards developing a predictive model for flowering in sweet cherry based on both genomic and phenology data.

Keywords: sweet cherry, statistical analysis, phenology, flowering, environmental conditions

Poster number: 50

Differential expression of cherry MYB10 in white and red varieties is responsible for anthocyanin levels

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Abstract:
Fruit colour it is a key trait in sweet cherry as it is a main determinant of fruit quality. Fruit and flower color in other rosaceous species is caused by anthocyanin accumulation, which is regulated by transcriptional factors of the anthocyanin biosynthetic pathway. In sweet cherry a transcription factor, MYB10, has been cloned. This transcription factor correlates with anthocyanin production during sweet cherry development and co-localizes with a major QTL for cherry fruit colour. In this work, we studied MYB10 transcription, structure and function in a sweet cherry cultivar that produces white fruits, and in three cultivars with different red fruits. Results revealed a lack of MYB10 transcription in white sweet cherries and a large insertion in one allele of the MYB10 genomic sequence. We postulate that this mutation and slight differences in promoter sequence of MYB10 result in low expression of this gene in the white cultivar, confirming that MYB10 is a major determinant of fruit color in sweet cherry.

Keywords: *Prunus avium*, colour

Poster number: 51

Elucidation of the molecular mechanisms behind breaking flower bud dormancy in cherry trees with special focus on the role of hydrogen cyanide

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Abstract:
Dormancy is the ability of some species, i.e. perennial woody plants in the *Prunus* genus, to suspend and resume growth in response to seasonal changes. In order to re-start growth flower buds need a certain amount of chill units in winter and subsequent heat hours in spring. Especially in warmer production areas and now increasingly due to global warming, the chilling requirements cannot be completely fulfilled. Therefore, different cyanide-based chemicals have been used to fully achieve the required chill units, and also to synchronize flowering time, which increases the fruit yield and facilitates fruit harvesting. The best chemical used to date is Dormex® (AlzChem), which can bring forward flowering time around one week compared to untreated controls. However, the EU has banned this chemical in 2008 due to concerns towards environmental effects and operator exposure. The molecular mechanisms by which cyanamide, the principal component of Dormex®, brings forward flowering time is still unknown. Some studies suggest that cyanamide acts through the release of hydrogen cyanide. Hydrogen cyanide can also be produced by the hydrolysis of cyanogenic glucosides present in *Prunus*. Furthermore, it is known to inhibit the antioxidant enzymes catalase and superoxide dismutase. We propose that the consequently increased ROS levels activate certain 'breaking dormancy' genes. To prove this, we have treated dormant cherry flower buds with Dormex® to analyze the effects on the transcript, protein and metabolite level. Finally, we will also detect the cyanogenic glucosides in cherry flower buds and in different parts of the fully developed flower.

Keywords: sweet cherry, HCN, ROS, dormancy, cyanamide

Poster number: 52

Development and utilization of a DNA diagnostic test to predict flesh color in tetraploid sour cherry (*Prunus cerasus*)

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Abstract:
Fruit color in sour cherry (*Prunus cerasus*) is an important market-driven trait in the U.S. where the dominate cultivar 'Montmorency' has a brilliant red color. This unique red color allows for a distinction between sour cherries grown in the U.S. and those in Europe which have predominantly dark-purple flesh. The anthocyanin transcription factor, MYB10, has previously been shown to control flesh color in cherry and other rosaceous species. Thirteen allelic variants for the sour cherry MYB10 region were distinguished based on the linkage phase of 47 polymorphic SNPs determined using the 6K Infinium® II SNP array developed by the RosBREED project. Of these 13 haplotypes, four behaved as dominant alleles conferring dark-flesh color. No SNPs, however, were found in this region which would distinguish haplotypes conferring dark-flesh. Marker-assisted seedling selection (MASS) is a goal of the Michigan State University cherry breeding program to cull undesired seedlings before they are planted in the field. Those found to have dark-fleshed alleles could be culled if a simple DNA diagnostic tool were available. Due to the high synteny in *Prunus*, the peach genome sequence was used to design 36 SSRs that were screened for their ability to uniquely identify dark-fleshed haplotypes. One SSR primer pair was found to amplify fragments that successfully differentiated the two darkest-flesh haplotypes. This marker can now be used for MASS in any crosses with either of these two haplotypes to cull those individuals which are predicted to have dark-purple flesh.

Keywords: flesh color, *Prunus*, marker-assisted seedling selection

Poster number: 53

Expression of related genes to the response of drought in *Prunus* rootstocks

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Abstract:
Climate change conditions accentuate drought since the reduction of 10% of the precipitation is translated in a reduction of 25% of the soil water. Therefore, it is indispensable the use of adapted rootstocks to water stress conditions. In order to understand their response to drought, physiological and molecular parameters of three *Prunus* hybrid rootstocks, the almond x peach hybrid (*P. amygdalus* x *P. persica*) 'Garnem', their progenies 'P.2175' x 'Garnem'-3 trihybrid (*P. cerasifera* x [*P. amygdalus* x *P. persica*]) and 'P.2175' x 'Garnem'-9 trihybrid were investigated. Plants in pots were subjected to drought conditions (35% water soil content) during one month. Subsequently, plants were submitted to re-watering period. Physiological responses were monitored through transpiration and leaf water potential showing significant differences along the experiment and among the genotypes. Gene expression analysis of four genes coding for proteins related to ABA pathway and abiotic stress were analyzed by RT-qPCR. A dehydrin (ppa005514m), a LEA protein, (ppa008651m), an A20/AN1 zinc finger (ppa012373m), and a bZIP transcription factor (ppa013046m). The profile expression of the four genes showed a correlation with physiological parameters of drought response, being higher in root than in phloem tissue. The zinc finger and bZIP transcription factors showed differences in expression relative to the target genes LEA protein and dehydrin under drought and recovery treatment, showing a regulatory response to water stress in *Prunus* genotypes.

Keywords: ABA, LEA protein, real-time PCR, transcription factors, water stress

Poster number: 54

Phylogenetic analysis of F-box genes homologous to the pollen S determinant F-box genes in the S-RNase-based self-incompatibility system

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Abstract:
Self-incompatibility (SI) is one of the most important genetic systems maintaining genetic diversity in flowering plants. The gametophytic self-incompatibility (GSI) system of the Solanaceae, Rosaceae, and Plantaginaceae are called the S-RNase-based GSI system. In this GSI system, the self and cross incompatibility reaction specificities of pistil and pollen are determined by ribonuclease (RNase) and F-box protein, respectively. Although the three plant families use the same molecule as the pistil S and pollen S determinants, molecular and genetic analyses of *Prunus* SC S haplotypes and polyploid sour cherry reveal the possible existence of a distinct self and cross recognition mechanism in the S-RNase-based GSI system of *Prunus*. In *Prunus*, the specificity determinants of pistil and pollen are called S-RNase and S haplotype-specific F-box protein (SFB), respectively. We previously proposed a working hypothesis in which *Prunus* SFB is supposed to have a distinct function from the pollen S determinant F-box proteins (SLF) of Solanaceae and Plantaginaceae. Here, we performed a detailed phylogenetic analysis of *Prunus* SFB and its orthologs in the *Prunus* genome and other angiosperm genomes. Our results indicated that *Prunus* SFB was generated from recent *Prunus*-specific gene duplications. *Prunus* F-box protein genes that are located flanking regions the S locus (SLFLs) are classified to the same clade as the pollen S F-box protein genes of Solanaceae, Plantaginaceae, and the subtribe Pyrinae (Rosaceae), indicating that duplication and sub-functionalization of the original pollen S genes generated SFB. We discuss the possible mechanisms of (in)compatibility reaction in *Prunus*.

Keywords: SFB, S locus, SLFL, S-RNase

Poster number: 55

Identification and co-localization of MYB transcription factors with known and new red coloration traits mapped in the *Prunus* reference map

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Abstract:
Red coloration has become an important commercial trait in *Prunus* by improving consumer acceptance and health properties as well as ornamental aspects. This pigmentation is caused by the accumulation of anthocyanins in the vacuole of the cells that form the colored tissue. The biosynthetic pathway of the anthocyanins is being activated by transcription factors of the MYB family. With the *Prunus* genome sequence being available and red coloration traits mapped in the *Prunus* reference map or elsewhere candidate genes can be found. This communication will present the identification of new candidates of the MYB transcription factor family in the peach genome and their possible implication in the expression of red coloration traits. For the candidate gene-trait co-localization we will also include our latest results on newly mapped red pigmentation traits. Finally marker development for marker assisted breeding will be discussed.

Keywords: anthocyanin, MYB transcription factor, candidate gene, *Prunus* reference map, marker assisted breeding

Poster number: 56

Identification and characterization of QTLs for peach fruit quality by pedigree-based analyses (PBA) in the FruitBreedomics project

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Abstract:
The European project FruitBreedomics was conceived with the aim of improving the efficiency of peach and apple breeding. One of the strategies consists of enhancing the use of molecular markers in progeny selection (Marker Assisted Selection, MAS). In peach, many QTLs linked to more than one hundred agronomic traits have been identified and published so far, in all instances detected after analyzing single mapping populations. Despite the great amount of information, the number of molecular markers available to be used in MAS is still reduced. One of the reasons is the too large QTL intervals, which are due to low marker density and/or limited progeny size. With the final goal of reducing the confidence intervals of some relevant agronomic QTLs, in FruitBreedomics we have genotyped with the 9K peach SNP chip about 1,500 progeny from 18 peach and other *Prunus* crosses with phenotypic data from several years. Hereby, we present first results on fruit quality QTL mapping using pedigree-based analysis (PBA) methodology. PBA has its power in the joint analysis of progenies from different crosses at a time, enlarging the population size and consequently narrowing the candidate genomic regions. Moreover, as multiple parents with different genetic backgrounds are included in the analysis, this method may detect multiple favorable alleles. Additionally, the subsequent analyses of identity by descent (IBD) give insight into the peach founders that brought some of the favorable QTL alleles into these breeding populations.

Keywords: Peach quality traits, QTL mapping, Pedigree-based Analysis (PBA), FruitBreedomics

Poster number: 57

RosBREED peach mini SNP arrays v1 design, analysis and results

Terrence Frett[1], John Clark[1], Cameron Peace[2], **Ksenija Gasic**[3], Nahla Bassil[4], Jonathan Fresnedo-Ramírez[5], David Byrne[6], Tom Gradziel[5], Amy Iezzoni[7]

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Abstract:
The RosBREED project (http://www.rosbreed.org/) has successfully identified functional alleles associated with nine peach fruit quality traits: Fruit resistance to bacterial spot (Xap1 and Xap6), maturity date (G4mat), fruit type (G), blush, flesh color (Y), texture type (F-M), acidity (D), acidity and soluble solid content (G7Flav) and mealiness. Forty-eight single nucleotide polymorphism (SNP) markers, significantly associated with the nine phenotypic traits and capable of distinguishing all functional alleles were selected and divided into two 24-SNP mini arrays. Two DNA service providers [University of Arizona Genetics Core (UA) and BioDiagnostics (BDI)] were charged with the design and testing of the mini SNP arrays. Plant material from the four RosBREED peach demonstration breeding programs (University of Arkansas, Clemson University, Texas A&M University and University of California, Davis) representing advanced breeding material as well as several control samples were genotyped. Genotyping data were successfully translated into trait predictions and results provided to each breeding program. Depending on the material genotyped, genotyping data acquired accurately predicted phenotypic performance in 80-90% of cases across material from all breeding programs. These results are due to the vast haplotypic diversity in U.S. peach breeding germplasm. The additional 20-10% of material required specific attention to assigning the correct haplotypes to the correct phenotypes. Feasibility of using mini SNP arrays in breeding programs for cross planning and seedling selection as well as the level of service required from providers will be discussed.

Keywords: single nucleotide polymorphism (SNP), mini SNP arrays, functional alleles, haplotypes, DNA service providers

Poster number: 58

Harnessing the power of RosBREED: Development, validation, and application of DNA tests for predicting peach flavor and other valuable rosaceous tree fruit traits

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Abstract:
DNA tests that predict valuable trait levels are essential for widespread adoption of marker-assisted breeding (MAB) of rosaceous tree fruits. The RosBREED project has facilitated development of DNA tests for important traits in peach, apple, and cherry, including peach sweetness and acidity. Based on a quantitative trait locus (QTL), discovered on peach chromosome 7 by RosBREED collaborators, explaining ~20% of phenotypic variation for titratable acidity in normal acid peaches and ~10% of variation in soluble solids content, a DNA test ("G7Flav-SSR") was developed at Washington State University. Standard cultivars and University of Arkansas (UA) and Clemson University (CU) breeding germplasm were used to confirm predictiveness of the DNA test, where G7Flav-SSR clearly differentiated low:low, low:high, and high:high allelic combinations. Validation of this new DNA test was conducted on unselected families of CU and AR germplasm. G7Flav-SSR results were used to guide 2014 crossing decisions in the UA peach breeding program. This advance in DNA-informed breeding represents an example of successful collaboration among institutions and across disciplines. Other DNA tests emerging from RosBREED include those for the prediction of peach maturity time, bacterial spot resistance, firmness, and blush, apple sweetness, acidity, and firmness, and sweet cherry maturity time and firmness. By harnessing the power of collaboration, specifically the integration of pedigree, phenotypic, and genotypic data generated by RosBREED team members for QTL discovery, the development of these DNA tests was possible. Tools such as G7Flav-SSR are now available to make DNA-based predictions a routine part of tree fruit breeding.

Keywords: marker-assisted breeding, soluble solids content, titratable acidity, simple sequence repeat marker

Poster number: 59, see page 65

Poster number: 60, see page 64

Poster number: 61

Analysis and Visualization of Genetic Diversity in Pear (*Pyrus* spp.)

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Abstract:
Pears (*Pyrus* spp.) are one of the most important tree fruit crops. They are an excellent source of fiber and vitamin C. The genus *Pyrus* is native to the Northern Hemisphere and consists of over 20 species. Little work has been done to analyze the genetic relationships among pears. It is important for breeders to understand pedigree relationships among pear germplasm in order to make informed crosses. To assess the genetic diversity and relationships among ~200 advanced pear genotypes, target region amplification polymorphism (TRAP) markers were used. Six primer combinations (three sets of polymerase chain reaction) produced a total of 86 polymorphic loci. Scoring information was subsequently analyzed with the software programs STRUCTURE, CLUMPP, and DISTRUCT in order to assign individuals into populations and determine admixture within individuals. STRUCTURE output indicates that these particular individuals fall into three populations and that there is admixture among the individuals. In addition to analyzing the genetic diversity and relationships among these individuals, a virtual representation of the data was created using the custom software seeDNA™. The software generates a unique genetic identity number based upon the loci amplified, this format is converted to a two dimensional barcode. seeDNA™ is compatible with all types of marker output including SNPs generated via next generation sequencing platforms.

Keywords: pear, TRAP, diversity

Poster number: 62

Differential expression of copper/zinc superoxide dismutase in pear/quince combinations during early stages of development

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Abstract:
Superoxide dismutase (SOD, EC 1.15.1.1) is one of the most important antioxidant enzymes and constitutes the first level of defence against superoxide radicals in plants. SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2. SOD enzymes fall into three distinct families that are classified according to metal cofactors. The most abundant SODs in plants are the CuZnSODs which are found mainly in the cytosol and in the chloroplasts providing enhanced tolerance to oxidative stress. In grafted plants, it is reported that oxidative stress could trigger cell and tissue degradation processes in incompatible grafts. The aim of this study was to evaluate the gene expression of different genes encoding CuZnSOD (CSD1, CSD2, CSD3) and cytosolic SOD activity in callus unions with different graft response from pear cvs. 'Conference' (Co) and 'William' (Wi) and the quince rootstock clone 'Ba29' during three weeks after union. CuZnSOD mRNA transcripts were detected in all combinations throughout the graft union development However, the mRNA expression patterns varied with the kind of combination examined. CSD1 and CSD2 transcript levels showed significant differences between 'Co/Co' and 'Co/BA29' at 10 and 21 DAG, respectively. Likewise, it was observed a 4-fold increase in CSD3 gene expression in the homograft 'Wi/Wi' at 21 DAG compared to the heterograft 'Wi/Ba29'. Furthermore, there was a higher SOD activity in the compatible cultivar (Co) than in the incompatible cultivar (Wi) from 1 day after wounding onwards. These results will be discussed in terms of wound/healing repair mechanisms and oxidative stress associated to grafted plants during graft union development.

Keywords: cytosolic SOD, *in vitro* callus unions, oxidative stress, real-time PCR, wounding mechanisms

Poster number: 63

QTL identification and phenotyping of fruit quality and disease resistance traits in octoploid strawberry (*Fragaria* × *ananassa*)

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Abstract:
Strawberry breeding for disease resistance and fruit quality traits is a challenging process due to its complex octoploid genetics. The use of marker-assisted selection (MAS) in plant breeding programs can significantly improve the selection of genotypes through linkage mapping and the identification of quantitative trait loci (QTL) approaches. The success rate of marker development for MAS depends on the complexity, stability and the nature of the QTL, and marker-trait combinations being utilized. In this study, work will be carried out to understand how different complex traits are correlated in the octoploid strawberry at the phenotypic and genotypic level and to investigate how this affects the deployment of markers in breeding. The discovery of the best and easiest traits to phenotype and developing simple ways of capturing phenotypic data in an automated fashion will speed up the development of novel breeding approaches, first through MAS and then genomic selection (GS). A particular focus of current research is on two suites of traits, disease resistance and fruit quality. Work is underway to characterize the nature of Verticillium wilt disease resistance and to uncover and map molecular markers linked to key fruit quality traits such as firmness, sugar levels, fruit size and shape in cultivated strawberry using QTL mapping. An octoploid strawberry mapping population ('Redgauntlet' × 'Hapil') containing a total of 122 seedlings was used for phenotyping flower-related traits, root architecture and plant characteristics. The existing SSR-based linkage map ('Redgauntlet' × 'Hapil') will be used for locating genes of interest for MAS and to identify QTLs associated with phenotyped traits.

Keywords: strawberry, MAS, QTL, phenotyping

Poster number: 64, see page 66

Poster number: 65

QTL mapping of soft scald in the RosBREED apple germplasm set

Nicholas Howard[1], Matthew Clark[1], Cari Schmitz[1], James Luby[1], James Bradeen[1], Yingzhu Guan[2], Benjamin Orcheski[3], Sujeet Verma[4], Susan Brown[3], Katherine Evans[2], Cameron Peace[4]

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Abstract:
Soft scald is a postharvest disorder of apple (*Malus domestica*) characterized by distinct brown and often longitudinal lesions on the fruit that often extend into the flesh and can lead to secondary infections in the fruit. Fruit that develop soft scald are unmarketable for fresh eating. The disorder is economically important because it usually develops in storage multiple weeks after producers invest considerable resources into harvest and cold storage. Incidence of soft scald varies among cultivars and can vary depending on orchard and storage conditions. Many cultivars are susceptible to the development of soft scald, including cultivars commonly used extensively as parents in US breeding programs. Observations by breeders suggest the trait is heritable. The development of new cultivars with reduced potential for soft scald development would benefit the apple industry and this could be made more efficient through the use of marker assisted breeding. A quantitative trait locus (QTL) detection study was conducted using FlexQTL software with data collected on soft scald incidence in 2011 and 2012 from the RosBREED apple germplasm set and additional data collected in 2013 at the University of Minnesota comprised of 4 half-sib families with 'Honeycrisp' as a common parent. Putative QTL were detected, though they were not consistently detected in all years and locations.

Keywords: apple, soft scald, RosBREED

Poster number: 66

Dissecting the QTL dynamics of harvest date in apple (*Malus* x *domestica*)

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Abstract:
Harvest date in apple (*Malus* x *domestica*) is an important trait for breeders making crossing and selection decisions. Early harvest date has been associated with lower hedonic ratings for fruit quality traits and decreased storability due to softening compared to apples that mature later in the season. An objective for breeders and producers is to have genotypes with early season ripening and improved texture and storage traits that could replace less desirable cultivars. Conversely, in production regions with short growing seasons, late season cultivars may never ripen fully before freezing and cannot be harvested. The development of molecular markers that inform plant breeders on harvest date would be useful in selecting parents for cultivar development and for screening seedlings as part of marker assisted breeding (MAB). Selected seedlings and advanced breeding lines could also be targeted to testing locations with appropriate season lengths. A pedigree-linked population was investigated for harvest date quantitative trait loci (QTL) for 2011 and 2012. Growing degree day accumulation (base 4.4 °C) at harvest was used as a proxy for calendar date in order to compare years and to normalize data for ancestors grown at other locations as part of the RosBREED project. The analyses evidenced QTL on linkage groups 3, 4, 7, 8, 11, 14, and 15. The QTL on LG 3 co-localized with a QTL for sensory firmness and harvest date from previous reports in apple. This study will provide marker-trait-loci associations through the identification of functional SNP haplotypes which span the QTLs and can be utilized in MAB.

Keywords: apple, QTL, harvest date, marker assisted breeding, SNP genotyping

Poster number: 67

Mapping and genetic dissection of QTL influencing bitter pit symptoms in apple (*Malus* × *domestica*)

Matteo Buti[1], Daniele Caset[1], Lara Poles[1], Pierluigi Magnago[1], David Chagne[2], Satish Kumar[3], Riccardo Velasco[1], **Daniel Sargent**[1]

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Abstract:
Bitter pit is the most economically important physiological disorder affecting apple fruit production. Brown pits develop in the cortical apple flesh – due to the breakdown of the cell plasma membranes – especially during storage, rendering the fruit unmarketable. Environmental conditions and cultural practices play a role in symptom expression, and there is a link between the severity of symptoms and relative concentrations of calcium in the fruit, leading to the application of calcium sprays and dips; however, these are not completely effective. Cultivars vary in susceptibility and thus there is scope for breeding for resistance. In this investigation, we have identified two major QTL controlling bitter pit symptom expression in four large apple mapping populations. The QTL intervals were defined using SSR and SNP markers, and candidate genes from both intervals were characterised. The markers identified will be useful for marker assisted selection in programmes for the genetic improvement of cultivated apple.

Keywords: physiological disorder, bitter pit, calcium, genomics, breeding

Poster number: 68

Fine mapping of the scab resistance locus Rvi12 derived from *Malus baccata* 'Hansen's baccata #2'

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Abstract:
Apple scab is a disease caused by the fungus *Venturia inequalis* which leads to significant economic losses in apple production especially in temperate regions. Breeding programs are attempting to introgress scab resistance genes from wild apple varieties into commercial cultivars for the control of the disease. Most of the commercially available scab resistance varieties to date rely on the Rvi6 (Vf) resistance gene from *Malus floribunda* 821. The evolution of new pathotypes of *V. inequalis*, which have caused the breakdown of Rvi6 based resistance at least in northern Europe highlights the need for the characterisation and pyramiding of scab resistance genes from different sources for durable disease resistance. In this study, the scab resistance gene 'Rvi12' from *Malus baccata* 'Hansen's baccata #2' was mapped to apple linkage group 12 in the cross 'Gala' × 'Hansen's baccata #2' in an interval between two SSR markers Hi02d05 and Hi07f01. Using the 'Golden Delicious' genome sequence, novel SSR markers and SNPs were identified in the Rvi12 mapping interval, and mapped in an extended mapping population of 635 plants. Rvi12 was fine-mapped to an interval of 0.79 cM, spanning 882 kb of the 'Golden Delicious' genome sequence. The 23 heterozygous SNPs fine mapped to the Rvi12 mapping interval were screened in eight apple breeding founder lines and for 21 of the 23 SNPs, the allele linked in coupling to the Rvi12 resistance locus were found only in 'Hansen's baccata #2'. The SNPs identified will therefore be useful for the efficient identification of apple genotypes carrying the Rvi12 resistance locus.

Keywords: apple scab, durable scab resistance, mapping, marker assisted selection, molecular markers

Poster number: 69

Genetic diversity of the Spanish apple genetic resources using SSRs

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Abstract:
The Spanish Program of Plant Genetic Resources integrates, among others, the collections located at Public University of Navarre, Centro de Investigaciones Agrarias de Mabegondo, Cabildos (Tenerife, La Palma and Gran Canaria), University of Lleida, Estación Experimental de Aula Dei-CSIC and CITA of Aragon. Those collections include mainly local cultivars from their respective regions, covering most of the Spanish apple-growing areas. Though some previous studies about the genetic variability of apple genetics resources from Spain were already performed, a complete analysis is needed in order to evaluate the complete diversity of *Malus* spp. in Spain. For doing that, the Spanish Government funded the project "Harmonization of the methodology of characterization, assessment of genetic diversity and definition of the core collection of the apple germplasm conserved in Spanish genebanks". In total, we have evaluated 1206 accessions using standardized methodologies, with SSR markers and morphological descriptors. SSR fingerprinting was performed with 13 SSR markers. SSR profiles were obtained independently and allele sizes were compared using a common set of cultivars selected as references. Results showed 601 genotypes for 1206 accessions. Most of the genotypes (438) were identified only in one accession. The other 163 genotypes were repeated in two to 81 accessions (involving 767 accessions in total). The harmonization of morphological descriptors will allow us to determine if the accessions with the same genotype are synonymies or closely related individuals. Results of this study highlight the interest of coordinated actions in order to optimize the management of germplasm collections and to evaluate the complete genetic diversity of *Malus* spp. in Spain.

Keywords: apple germplasm, biodiversity, identification, *Malus* x *domestica* Borkh, SSR markers

Poster number: 70

RNA-sequencing analysis to identify candidate genes associated with responses to fruit tree canker in apple

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Abstract:
Neonectria ditissima (formerly *Neonectria galligena*, anamorph *Cylindrocarpon heteronema*) is the causal agent of fruit tree canker, which is regarded as a serious economic problem in horticulture. This fungus occurs in a wide range of temperatures but is closely associated with wet weather, and geographic distribution is therefore strongly associated with local climate. Notable damage to apple trees is especially common in some regions like North-Western Europe. Significant efforts Fungicide and culture measures to control the disease usually are not very successful. An alternative approach could be the culture of more resistant cultivars. However, breeding for resistance is hard, resistance tests being time-lasting and labor intensive and resistance being quantitative in nature. Availability of molecular markers for resistance could greatly enhance the prospects of breeding. Currently, nothing is known on the genetic bases of resistance as present in some cultivars. Therefore a focused genomics approach was initiated in order to facilitate the identification of resistance genes. In this study, the transcriptomes of a partially resistant and highly susceptible cultivar are compared for healthy and infected wood. Samples were taken at three different time points, i.e., 5, 15, and 30 days after inoculation. This comparison enabled us to identify a list of candidate genes in response to fungus attack in apple trees. This information will be used in the study of signaling pathways are possibly involved in resistance to fruit tree canker.

Keywords: fruit tree canker, *Neonectria ditissima*, apple, partial resistance

Poster number: 71

Genetics of scab resistance in 'Geneva' apple

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Abstract:
Over 60 years ago, the existence of different races of *Venturia inaequalis* was demonstrated using inoculation experiments with monospore isolates of the pathogen on the red-fleshed scab-resistant apple cultivar 'Geneva'. The findings suggested the presence of at least three scab resistance genes in this host. More recently, our genetic studies with five monospore isolates of *V. inaequalis* have indicated the presence of at least four genes. Three of these, two dominant genes and one recessive gene, have been mapped to a 5 cM region on linkage group 4, which corresponds to a 2 Mbp region containing nine candidate NBS-LRR resistance genes on the physical map of 'Golden Delicious'. Primers specific to the candidate genes were designed for the fine-mapping of the resistance genes and used to construct a detailed genetic map defining the loci. The consequences of the complexity of the 'Geneva' scab resistance for breeding and the selection of a differential host (3) for *V. inaequalis* pathotype monitoring (www.vinquest.ch) will be discussed.

Keywords: genetic map, molecular markers, marker assisted selection, durable resistance, differential host

Poster number: 72

Are differentially expressed genes associated with contents of some chemical compounds in apple fruit challenged with *Penicillium expansum*?

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Abstract:
Fruit decay occurring during cold storage of apple fruit, e.g., blue mold caused by *Penicillium expansum*, is a major problem in apple production, especially in organic cultivation and other production systems that do not allow post-harvest anti-fungal treatments. Fruits of susceptible and resistant apple cultivars were challenged with *Penicillium expansum* spores, and samples of inoculated and control fruits were taken in time series. Differentially expressed genes were identified using an AryANE chip containing 60K apple transcripts. Differentially expressed genes were then analysed for blast similarity, gene ontology and pathway analysis. In another study we quantified some selected chemicals in both inoculated and control fruits, e.g. total polyphenols, individual polyphenolic compounds, total organic acids as well as individual acids to study the possible association of these chemical compounds with the level of fungal resistance. The results of the gene expression study together with the results of the chemical evaluation will enable us to find candidate genes that can be used in breeding programs for development of apple cultivars with improved resistance to storage diseases.

Keywords: transcriptomics, postharvest disease, chemical contents, fungal resistance

Poster number: 73

The power of two: Maximizing predictive strength in breeding for apple acidity by combining DNA tests

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Abstract:
The acidity content of an apple contributes to its delicious taste and overall enjoyment. Two major loci are known to contribute to the bulk of the heritability of acidity levels in apple. The Ma locus on chromosome 16, with its underlying malic acid transporter gene, accounts for ~30% of the phenotypic variance in Washington apple breeding program (WABP) germplasm. The recently discovered "A" locus on chromosome 8, hypothesized to be responsible for malic acid production, explains another ~20% of observed variation. A DNA test is available and in use for the Ma locus ("Ma-indel") but not for the A locus. At Washington State University, a new DNA test ("LG8A-SSR") was developed to help differentiate between apple fruit acidity levels. Primers were developed for microsatellites near the "A" QTL peak. From ten sets of primers, several were chosen that matched functional haplotype patterns obtained from RosBREED's high-resolution SNP data. By pairing outcomes of LG8A-SSR and Ma-indel, differentiation of five levels of acidity could be made, allowing for a more accurate prediction. In combination, these two tests explain > 50% of phenotypic variation within WABP germplasm and were used in the 2014 marker-assisted seedling selection. The predictive power of these two tests should be verified on breeding germplasm of other regions. The double DNA testing strategy creates a powerful predictive tool helping breeders select for the desired acidity levels.

Keywords: marker-assisted breeding, QTL, A locus, MA locus, malic acid

Poster number: 74, see page 67

Poster number: 75

Routine marker-assisted seedling selection focused on fruit quality improves breeding efficiency in three tree fruit programs

Daniel Edge-Garza[1], Terrence Rowland[1], Paul Sandefur[1], Bonnie Konishi[2], Lisa Brutcher[2], Kate Evans[2], Sue Watkins[3], Nnadozie Oraguzie[3], Matthew Clark[4], John Tillman[4], David Bedford[4], James Luby[4], Cameron Peace[1]

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Abstract:
Marker-assisted seedling selection in practice saves precious breeding resources by identifying and discarding genetically inferior seedlings early. Resources can then be reallocated to those seedlings more likely to become viable new cultivars or parents with higher genetic value. DNA-based seedling selection also helps free up breeders' time, opening opportunities for enhanced creativity. Washington State University's (WSU's) Washington apple breeding program (WABP) and Pacific Northwest sweet cherry breeding program (PNWSCBP) have now used this new technology routinely for five years. Since RGC6, these programs completed two more successful years of cost-efficient marker-assisted seedling selection. Over 2013 and 2014, the WABP improved efficiency by $82K by screening 15,938 seedlings and culling 66% that are unlikely to meet various fruit texture, flavor, and appearance targets. The PNWSCBP avoided wasting $83K by culling 85% of 3399 seedlings predicted to lack alleles for self-fertility and large fruit. Through RosBREED, WSU's Tree Fruit Genotyping Laboratory expanded operations in both years to include more than 6000 seedlings from the University of Minnesota's apple breeding program. Using DNA tests for several fruit quality traits and scab resistance, half of the seedlings were culled, for an estimated savings of almost $40K. RosBREED is changing the definition of "conventional" seedling selection for tree fruit breeding.

Keywords: apple, genotyping, MASS, RosBREED, sweet cherry

Poster number: 76

Seedling Select: A web-based software tool to facilitate cost modeling of marker-assisted seedling selection (MASS) in Rosaceae tree fruit

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Abstract:
The Rosaceae family includes most of the temperate tree fruit crops such as apple, apricot, peach, pear, and cherry. Seedling selection is the process of selecting superior seedlings generated from crosses for cultivar development. Traditional seedling selection (TSS) is expensive for most rosaceous tree fruit because of long juvenility and large plant sizes. Marker-assisted seedling selection (MASS) is a promising approach to improve the efficiency of TSS with the assistance of DNA markers. A lack of decision-support tools is a major challenge in identifying cost-efficient MASS schemes. Here we report on progress made in the development of a web-based software tool Seedling Select to enable accurate and flexible cost modeling for MASS in rosaceous tree fruit. Seedling Select consist of four modules: TSS Cost Structure (mod. 1), Seedling Family Structure (mod. 2), and DNA Test Cost Structure (mod. 3) lay the foundation of cost estimation by modeling costs in each TSS stage, seedling family, and DNA test, respectively, while MASS Cost Structure (mod. 4) calculates and compares total costs of TSS and MASS by integrating outputs from the previous modules with a user-defined MASS scheme. By comparing costs of all possible MASS schemes, this software tool will suggest cost-efficient MASS schemes for defined sets of breeding families. Seedling Select will be publicly available on the Genome Database for Rosaceae (www.rosaceae.org). Once available, Seedling Select will assist breeders to design efficient MASS schemes with a rational allocation of resources.

Keywords: marker-assisted seedling selection, marker-assisted selection, cost modeling, software, seedling select

Poster Abstracts

Breeding

Poster number: 77

Verticillium wilt resistance varies within ploidy levels in strawberry (*Fragaria* spp.)

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Abstract:
Verticillium wilt is a major obstacle to U.S. strawberry production. Our current and ongoing investigations include defining new sources of resistance in cultivated and wild strawberry germplasm, and advancing genetic studies on the basis of resistance/susceptibility. We screened 26 octoploid, 1 decaploid, and 23 diploid strawberry genotypes for response to root-dip inoculation with *Verticillium dahliae* isolate V1. Inoculated plants were individually rated at eight weeks post-inoculation using a categorical scale: 1=healthy; 1.5=slightly symptomatic; 2=moderately symptomatic; 2.5=very symptomatic; 3=dead. Qualitative classifications were assigned to genotypes on the basis of their respective mean disease resistance ratings. The rating ranges and corresponding classifications (in parentheses) were: 1.0-1.3 (very resistant = VR), 1.4-1.7 (moderately resistant = MR), 1.8-2.2 (intermediate = I), 2.3-2.6 (moderately susceptible = MS), and 2.7-3.0 (very susceptible = VS). Considerable variability in inoculation response existed within and between species at both the diploid and octoploid levels. VR or MR genotypes were found within each of the following species: diploids *F. vesca*, *F. iinumae*, and *F. nipponica*; and octoploids *F. chiloensis*, *F. virginiana*, and *F.* × *ananassa*. MS and VS genotypes were documented within *F. vesca*, within each octoploid species, and in a genotype of decaploid *F. cascadensis*. We compared our screening results to those of previous studies and constructed a pedigree of the evaluated and related octoploid cultigens for visualization. We also made resistant × susceptible crosses at both the diploid and octoploid levels as a step toward genetic analysis of wilt resistance/susceptibility and resistance gene identification in strawberry.

Keywords: strawberry, breeding, disease resistance

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