RGC8

8th International
Rosaceae Genomics Conference

PROGRAM / ABSTRACTS

JUNE 21-24/2016
ANGERS - FRANCE

www.rgc8angers2016.com

IRHS
Institut de Recherche en Horticulture et Semences

INRA
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FOREWORD

It is a great pleasure to welcome you to Angers for the 8th Rosaceae Genome Conference (RGC8).

More than 160 people from 24 countries are registered.

We organized this event following the spirit of the past RGCs with the dual goal of providing international researchers with the opportunity to interact, and to share cutting edge research in genomics and genetics of Rosaceae species.

The scientific program of RGC8 is organized around eight scientific sessions showing cross-disciplinary thematic research on flower initiation and plant development, fruit development, fruit quality, biotic and abiotic stresses, but also transversal approaches: domestication and diversity, genome and markers and methodology of selection. A total of 45 oral presentations and 70 posters of excellent quality will be presented.

RGC8 will not only be a great scientific event, the whole conference has been scheduled and organized to give more time for informal discussions and to provide opportunities for young scientists and new comers to be integrated in the community: for this reason, we favoured shorter and punchier oral presentations to maintain time for breaks; poster presenters will be given a short time to present their work. Don't miss the Gala Diner: it will be held in a very nice place; you'll enjoy Anjou wine tasting, and nice music, which hopefully will encourage you to dance…

I would like to thank the Scientific Committee for their help in the review of the papers and in the setting up of the scientific program; thanks also to the chairpersons who will manage the different sessions.

RGC8 would never have happened without the help of Valérie, Hélène and Elisabeth from the BDCE of Angers and Heidi, Patricia, Fabienne and Nathalie at INRA-IRHS who did a great job in the preparation, registration and practical organization of RGC8. Flower arrangements in the conference room and the gala dinner have been managed by Sandrine from INRA-IRHS: thanks Sandrine.

Many thanks to all the people who will give a hand during the conference and to our generous sponsors.

Thanks to all of you for being here. Together we will have a great RGC8!

Francois
RGC8 convenor

GENERAL PROGRAM

Tuesday, June 21

7h-9h	Welcome Hours Registration and poster putting up
9h	Welcoming address
9h05	**Session 1: Domestication, evolutionary genetics and diversity** Chairpersons: Véronique DECROOCQ - Jose Antonio CAMPOY
10h30	Coffee break
10h50	**Session 2: Genome and markers** Chairpersons: Nahla BASSIL - Etienne BUCHER
12h10	GDR Whorkshop Chairpersons: Satish KUMAR - Stijn VANDERZANDE
12h55	Lunch
14h10	**Session 3: Cross-disciplinary research on flower initiation and plant development** Chairpersons: Kate EVANS - Baptiste GUITTON
16h20	Coffee break
16h50	Poster Sessions 1 to 5 Session 1, chairpersons: Riccardo VELASCO - Jérémy CLOTAULT Session 2, chairpersons: David CHAGNÉ - Luca BIANCO Sessions 3 & 4, chairpersons: Amy IEZZONI - Herman SILVA Session 5, chairpersons: Lamia KRICHEN - James LUBY
18h	**Session 4: Cross-disciplinary research on fruit development** Chairpersons: Bénedicte QUILOT - XiongWei LI
19h30	Official Reception at Museum of Fine Arts located in the downtown of Angers. *10' walk from the Congress Center.*
	Free dinner

Wednesday, June 22

09h	**Session 5: Cross-disciplinary research on abiotic stresses** Chairpersons: Cecilia DENG - Jun ZHU
10h40	Coffee break
11h10	**Session 6: Methodology of selection** Chairpersons: Anne Marie AUWERKERKEN - Cameron PEACE
12h35	Lunch
13h45	**Session 6: Methodology of selection** Chairpersons: Sue BROWN - Daniel EDGE GARCIA
14h30	**Session 7: Cross-disciplinary research on fruit quality** Chairpersons: Beatrice DENOYES - Mario di GUARDO
15h50	Coffee break

16h15	Poster Sessions 6 to 8 Session 6, chairpersons: Anna PIKUNOVA - Craig HARDNER Session 7, chairpersons: Hilde NYBOM - Shigeki MORIYA Session 8, chairpersons: Larissa GUSTAVSSON - David NEALE
17h30	**Session 7: Cross-disciplinary research on fruit quality** Chairpersons: Mathilde ORSEL-BALDWIN - Pere ARUS
20h00	Departure from hotels and Congress Center by bus
20h30	Gala Dinner in the heart of the vineyard of Anjou
0h00/01h	Return shuttles to hotels and Congress Center

Thursday, June 23

09h	**Session 8: Cross-disciplinary research on biotic stresses** Chairpersons: Sara MONTANARI - Bert ABBOTT
10h55	Coffee break
11h25	General matters and RGC9
12h20	Lunch
14h	Departure to professional tour by bus
18h20	Return to Angers City by bus

Friday, June 24

08h	Departure by private coach from Congress Center Visit of INRA and VEGEPOLYS
12h45	Lunch Château d'Artigny
14h30	Visit of the regional fruit experimental station of La Morinière (Ste Maure de Touraine, near Tours)
17h30	Return to Angers City by bus

ORAL PRESENTATIONS: POSTERS:

DETAILED PROGRAM

Tuesday, June 21

7h-9h		Welcome Hours Registration and poster putting up	
9h		Welcoming address	
9h05		**SESSION 1: DOMESTICATION, EVOLUTIONARY GENETICS AND DIVERSITY**	P. 18
		ORAL PRESENTATIONS: Chairpersons: Véronique DECROOCQ - Jose Antonio CAMPOY	
9h05-9h30	S101	**Genetics and genomics of perennial domestication: what can we learn from apples?** Keynote Speaker: Amandine CORNILLÉ	P. 18
9h30-9h45	S102	**The evolutionary history of apricot (*Prunus armeniaca*) might have contributed to a loss of resistance to sharka disease** Véronique DECROOCQ	P. 19
9h45-10h	S103	**Population genomics of European woodland strawberry** Tuomas A TOIVAINEN	P. 20
10h-10h15	S104	**Nineteenth century French rose (*Rosa* sp.) germplasm shows a continuous shift over time from a European to an Asian genetic background** Jeremy CLOTAULT	P. 21
10h15-10h30	S105	**Genetic diversity and genome-wide association studies in sweet cherry** Jose Antonio CAMPOY	P. 22
		POSTERS: Chairpersons: Riccardo VELASCO - Jérémy CLOTAULT	
	S1P1	**Domestication of the cultivated apple tree, *Malus domestica*, and its hybridizations with the European crab apple tree, *Malus sylvestris*** Alice FEURTREY	P. 23
	S1P2	**On *Rosa arvensis* genetics and intra-specific biodiversity** Pascal HEITZLER	P. 24
	S1P3	**Characterization of the genetic diversity among cultivated pears using SSR markers** Arnaud REMAY	P. 25
	S1P4	**Evaluating the influence of the microsatellite marker set used on the genetic structure inferred in *Pyrus communis* L** Jorge URRESTARAZU	P. 26
	S1P5	**Tools for increasing the loquat diversity** Mar NAVAL	P. 27
10h30-10h50		Coffee break	
10h50		**SESSION 2: GENOME AND MARKERS**	P. 28
		ORAL PRESENTATIONS: Chairpersons: Nahla BASSIL - Etienne BUCHER	
10h50-11h15		Genomics Assisted Breeding to Improve genetic gain in Rosaceous crops using Illumina's discovery, development and deployment tools Venkatramana PEGADARAJU (Ag Consortium Manager, Illumina) - Iain Mac LAREN LEE (Ag Specialist EMEA, Illumina)	
11h15-11h40	S201	**Genetic mapping in polyploid crops: SNP development and use for integrated linkage map construction in rose and potato using a custom pipeline for tetraploid data** Keynote Speaker: Rene SMULDERS	P. 28
11h40-11h55	S202	**The Golden Delicious doubled haploid: A toolbox for genomic and epigenomic studies** Etienne BUCHER	P. 29
11h55-12h10	S203	**Resequence analysis of interspecific and intraspecific recombination in an [(almond × peach) × peach] backcross progeny** Octavio SERRA	P. 30

DETAILED PROGRAM

Tuesday, June 21

		POSTERS: Chairpersons: David CHAGNÉ - Luca BIANCO	
	S2P1	**Edmund Mach Foundation's Genomics Platform** Massimo PINDO	P. 31
	S2P2	**De novo sequencing of the almond genome** Pere ARÚS	P. 32
	S2P3	**Rose Genome Sequence Initiative: project and recent advances** Fabrice FOUCHER	P. 33
	S2P4	**Rose genome sequencing initiative: challenges and benefits** Jérémy JUST	P. 34
	S2P5	**SNP discovery and development of a high density array for large-scale genotyping in pear** Sara MONTANARI	P. 35
	S2P6	**Development of the Axiom® Apple480K SNP genotyping array and its application for genome wide association study in apple** Michela TROGGIO	P. 36
	S2P7	**A pipeline for the curation of genetic data for pedigree-based QTL studies, demonstrated on RosBREED's apple data set** Stijn VANDERZANDE	P. 37
	S2P8	**CrossLink: Genetic Mapping Software for Allopolyploid Outbreeding Species** Robert VICKERSTAFF	P. 38
	S2P9	**High-density genetic maps and comparative genomics using restriction site associated DNA sequencing technology in sweet cherry** Kenta SHIRASAWA	P. 39
	S2P10	**High density, multi-population consensus genetic linkage map for peach (*Prunus persica* L. Batsch)** Ksenija GASIC	P. 40
	S2P11	**Identification of haplotypes in octoploid strawberry cultivars using 90K IStraw** Amparo MONFORT	P. 41
	S2P12	**Haploblock structure in sweet cherry using a pedigree-based approach** Amy IEZZONI	P. 42
	S2P13	**A Blackberry DNA test to verify parentage in RosBREED** Nahla V. BASSIL	P. 43
	S2P14	**An improved *Pyrus* SSR fingerprinting DNA test to confirm parentage in RosBREED** Nahla V. BASSIL	P. 44
12h10		GDR Whorkshop	
		ORAL PRESENTATIONS: Chairpersons: Satish KUMAR - Stijn VANDERZANDE	
12h10-12h25		**GDR: More Data and Functionality** Dorrie MAIN	P. 45
12h25-12h40		**TripalBMS: Toward a Comprehensive Breeding Information Management System** Dorrie MAIN	P. 46
12h40-12h55		**Evaluation of Field Book: An open-source Android app for collecting phenotypic data in a peach breeding program** Ksenija GASIC	P. 47
12h55-14h10		Lunch	

DETAILED PROGRAM

Tuesday, June 21

14h10		**SESSION 3: CROSS-DISCIPLINARY RESEARCH ON FLOWER INITIATION AND PLANT DEVELOPMENT**	P. 48
		ORAL PRESENTATIONS: Chairpersons: Kate EVANS - Baptiste GUITTON	
14h10-14h35	S301	**Deciphering the molecular and genetic control of tree architecture** Keynote Speaker: Chris DARDICK	P. 48
14h35-14h50	S302	**Identification of the dwarfing gene Dw1** Toshi FOSTER	P. 49
14h50-15h05	S303	**Transcriptomic comparison between deflowered and overloaded apple trees: toward the understanding of biennial bearing in apple** Baptiste GUITTON	P. 50
15h05-15h20	S304	**Unravelling the balance between sexual and asexual plant reproduction in an herbaceous perennial, the strawberry** Béatrice DENOYES	P. 51
15h20-15h35	S305	**Control and selection of blooming seasonality in rose** Fabrice FOUCHER	P. 52
15h35-15h50	S306	**How to breed novel flowering traits in strawberry?** Timo HYTÖNEN	P. 53
15h50-16h05	S307	**Next Generation Sequencing data as a useful tool to decipher self-incompatibility mechanism in *Prunus*** Elena ZURIAGA	P. 54
16h05-16h20	S308	**Next generation sequencing analysis to identify modifier gene candidates conferring pollen-part self-compatibility in sweet cherry 'Cristobalina'** Takuya MORIMOTO	P. 55
		POSTERS: Chairpersons: Amy IEZZONI - Herman SILVA	
	S3P1	**Tree architecture of apple genotypes with columnar growth** Radek VÁVRA	P. 56
	S3P2	**Auxin Transporter PIN1 in rootstock regulates the growth of scions in *Pyrus*** Ran WANG	P. 57
	S3P3	**The players of the «collaborative non-self recognition» *Malus* self-incompatibility system** Cristina P. VIEIRA	P. 58
	S3P4	**Phenotyping of inflorescence morphology for flowering-cherry 'Sakura' genetic resource** Tomoya ESUMI	P. 59
	S3P5	**Mapping flowering time QTLs in a diploid strawberry** Samia Samad	P. 60
16h20-16h50		Coffee break	
16h50-18h00		Poster Session	

DETAILED PROGRAM

Tuesday, June 21

18h		**SESSION 4: CROSS-DISCIPLINARY RESEARCH ON FRUIT DEVELOPMENT**	P. 61
		ORAL PRESENTATIONS: Chairpersons: Bénedicte QUILOT - XiongWei LI	
18h-18h15	S401	**Apple cell division and elongation during fruit development is associated with marked changes in hemicelluloses composition, structure and related gene expression** Marc LAHAYE	P. 61
18h15-18h30	S402	**Genomic approach in identifying genes and gene networks that regulate strawberry fruit development** Zhongchi LIU	P. 62
18h30-18h45	S403	**From gene to phenotype: genetic control and modeling of sugar metabolism during peach fruit development** Bénédicte QUILOT-TURION	P. 63
18h45-19h	S404	**Effects of exogenous application of GA4+7 and 1-Naphthaleneacetic acid on Sugar Accumulation and Related Gene Expression in Peach Fruits during ripening stages** Xiong-wei LI	P. 64
19h-19h15	S405	**Understanding development, ripening and postharvest performance of peach fruit using a system biology approach** Athanassios MOLASSIOTIS	P. 65
		POSTERS: Chairpersons: Amy IEZZONI - Herman SILVA	
	S4P1	**Study of the regulatory mechanism controlling the climacteric ripening physiology in apple (*Malus x domestica* Borkh.) through an integrative approach combining transcriptomic assay with metabolite and physical analysis** Alice TADIELLO	P. 66
	S4P2	**Transcriptomic analysis and comparison of early and late harvest peaches and nectarines during ripening** Claudio MENESES	P. 67
19h30		Official Reception at Museum of Fine Arts located in the downtown of Angers. *It is a 10 minute walk from the Congress Center.*	
		Free dinner	

DETAILED PROGRAM

Wednesday, June 22

09h		**SESSION 5: CROSS-DISCIPLINARY RESEARCH ON ABIOTIC STRESSES**	P. 68
		ORAL PRESENTATIONS: Chairpersons: Cecilia DENG - Jun ZHU	
9h-9h25	S501	**Stress responses in perennials; or how do trees "chill out"?** Keynote Speaker: Bert ABBOTT	P. 68
9h25-9h40	S502	**Effect of drought stress on gene expression, metabolites and phenotype in diploid and tetraploid apple (*Malus x domestica*)** Wannes KEULEMANS	P. 69
9h40-9h55	S503	**Transcriptional analyses of root responses under soil water stress of one-year old apple cultivars** Lamia KRICHEN	P. 70
9h55-10h10	S504	**Flowering time response to temperature in sweet cherry across Europe: multi-cultivar and multi-environment analysis** Béatrice WENDEN	P. 71
10h10-10h25	S505	**Identification of a new pear QTL associated with spring vegetative budbreak** Gilad GABAY	P. 72
10h25-10h40	S506	**Small RNA sequencing and DNA methylation analysis in floral bud reveal that RNA directed DNA Methylation (RdDM) participates during cold accumulation and dormancy release in sweet cherry (Prunus avium L.)** Karin ROTHKEGEL	P. 73
		POSTERS: Chairpersons: Lamia KRICHEN - James LUBY	
	S5P1	**Inheritance of chilling and heat requirements for flowering in an inter-specific almond x peach progeny** Célia CANTIN	P. 74
	S5P2	**Genotype x watering interaction of architectural and physiological characteristics in rose bush** Laurent CRESPEL	P. 75
	S5P3	**Study on Abiotic Stress of MdSBP20 Gene from *Malus*** Jun ZHU	P. 76
	S5P4	**A unique haplotype on chromosome 9 is associated with the ability to thrive under warm winter conditions, as revealed by characterization of old local apple accessions and their hybrids** Tal ISAACSON	P. 77
	S5P5	**Transcriptome analysis of differentially expressed genes upon treatment with hydrogen cyanamide in peach** Mercy OLMSTEAD	P. 78
	S5P6	**Metabolites analysis of flower buds during dormancy in sweet cherry** Rémy BEAUVIEUX	P. 79
	S5P7	**Molecular and epigenetic mechanisms during dormancy in sweet cherry flower buds** Mathieu FOUCHÉ	P. 80
10h40-11h10		Coffee break	
11h10		**SESSION 6: METHODOLOGY OF SELECTION**	P. 81
		ORAL PRESENTATIONS: Chairpersons: Anne Marie AUWERKERKEN - Cameron PEACE	
11h10-11h35	S601	**Connection Research-industry in Fruit breeding** Keynote Speaker: Jim Mac FERSON	P. 81
11h35-11h50	S602	**Towards gene pyramiding for apple scab resistance following the working results at VNIISPK** Anna PIKUNOVA	P. 82

DETAILED PROGRAM

Wednesday, June 22

11h50-12h05	S603	**Validation of genetic markers for fruit quality and disease resistance in apple breeding germplasm using the openArray® technique** David CHAGNE	P. 83
12h05-12h20	S604	**From QTLs to routine DNA-informed breeding: prospects, advances, and needs** Cameron PEACE	P. 84
12h20-12h35	S605	**Using SNP arrays to leverage historic data sets for improved prediction accuracy and estimation of GxE of fruit maturity in sweet cherry** Craig HARDNER	P. 85
12h35-13h45		Lunch	

ORAL PRESENTATIONS:
Chairpersons: Sue BROWN - Daniel EDGE GARCIA

13h45-14h	S606	**Character integration, breeding goals compatibility and selection indexes using genome wide breeding values: a study case** Alix ALLARD	P. 86
14h-14h15	S607	**Integration of Genomic Selection into the University of Florida strawberry breeding program** Luis F. OSORIO	P. 87
14h15-14h30	S608	**Prediction of apple fruit phenotypes using genome-wide markers: progress and challenges** Satish KUMAR	P. 88

POSTERS:
Chairpersons: Anna PIKUNOVA - Craig HARDNER

	S6P1	**Main achievements of COST Action FA1104 'Sustainable production of high-quality cherries for the European market'** José QUERO GARCIA	P. 89
	S6P2	**Improving the stability of high-quality traits of berry in different environments and cultivation systems for the benefit of European farmers and consumers** Guillaume VALLIN & Aurélie PETIT	P. 90
	S6P3	**RosBREED: Combining disease resistance and horticultural quality in new rosaceous cultivars** Amy LEZZONI	P. 91
	S6P4	**Cornell Apple Breeding and Genetic Diversity Studies** Susan BROWN	P. 92
	S6P5	**The development of scab immune (Vf), triploid (3x) and columnar (CO) apple cultivars** Anna PIKUNOVA	P. 93
	S6P6	**Development and evaluation of a temporary immersion system for mass propagation of sweet cherry cultivars and cherry rootstocks** Humberto PRIETO	P. 94
	S6P7	**Conversion of DNA tests to high-throughput technologies supporting apple breeding decisions** Daniel EDGE-GARZA	P. 95
	S6P8	**Marker Assisted Selection (MAS) in apple: case studies for red skin coloration and Rvi12 (Vb) scab resistance** Lara POLES	P. 96
	S6P9	**Integration of a molecular marker for the highlighter (red skin color suppression) trait in a peach breeding program** Iban EDUARDO	P. 97
	S6P10	**Identification of almond genomic regions in four 3-way interspecific hybrid progenies** Beatriz BIELSA	P. 98
	S6P11	**An apple amiRNA efficiently silences the phytoene desaturase gene in apple** Aurélie CHARRIER	P. 99

DETAILED PROGRAM

Wednesday, June 22

14h30		**SESSION 7: CROSS-DISCIPLINARY RESEARCH ON FRUIT QUALITY**	P. 100
		ORAL PRESENTATIONS: Chairpersons: Beatrice DENOYES - Mario di GUARDO	
14h30-14h45	S701	**An unexpected candidate gene may be involved in the control of fruit acidity in peach** Elisabeth DIRLEWANGER	P. 100
14h45-15h	S702	**Transcriptional, genetic and chemical approaches to understand tolerance to cracking in sweet cherry fruits** Herman SILVA	P. 101
15h-15h15	S703	**Analysis of genetic control of fruit size in apple using both multiple, pedigree-related and single full-sib families** Hélène MURANTY	P. 102
15h15-15h30	S704	**Unraveling the dynamics of QTLs associated to fruit firmness in apple over postharvest storage using a multi-family Pedigree Based Analysis (PBA) approach** Mario Di GUARDO	P. 103
15h30-15h50	S705	**Deciphering the genetic determinism of flowering/harvest period and several fruit sensory quality traits in apple by a Genome-Wide Association approach** Jorge URRESTARAZU - Shigeki MORIYA	P. 104
15h50-16h15		Coffee break	
16h15-17h30		Poster Session	
		ORAL PRESENTATIONS: Chairpersons: Mathilde ORSEL-BALDWIN - Pere ARUS	
17h30-17h45	S706	**Mealiness in apple is associated with changes in specific ACC synthases genes** Haya FRIEDMAN	P. 105
17h45-18h	S707	**Identification of a candidate gene for fruit flat shape in peach** Maria Jose ARANZANA	P. 106
18h-18h15	S708	**Genetic and biochemical characterization of fruit from different apricot accessions highlights apricot as a rich source of phytoene and phytoflouene, and indicates Carotenoid cleavage deoxygenase4 (Ccd4) gene as a potential regulator of fruit color** Tal ISAACSON	P. 107
18h15-18h30	S709	**Mapping the distinctive aroma of «wild strawberry» using a NIL collection** Amparo MONFORT	P. 108

DETAILED PROGRAM

Wednesday, June 22

POSTERS: Chairpersons: Hilde NYBOM - Shigeki MORIYA		
S7P1	**Apple breeding for the improvement of biochemical composition of fruit. the inheritance of sugar, ascorbic acid and phenolic compound contents** Anna PIKUNOVA	P. 109
S7P2	**Primary metabolite fruit profile is altered in response to source-sink imbalance and can be used as early quality predictors in nectarine** Andrea MIYASAKA ALMEIDA	P. 110
S7P3	**Impact of defoliation treatments on apple fruit quality before and after cold storage** Mickael DELAIRE	P. 111
S7P4	**Transcriptional regulation of carotenoid and vitamin C contents in apple fruits during postharvest and shelf-life storage** Eline LEMMENS	P. 112
S7P5	**Genetic structure based on EST-SSR: A promising tool for fruit color selection in Japanese plum (*Prunus salicina* L.) breeding programs** Basilio CARASCO	P. 113
S7P6	**Identification of QTLs for aesthetic properties in apple using image analysis** Marjin RYMENANTS	P. 114
S7P7	**QTL mapping for phytochemical compounds in peach [*Prunus persica* (L.) Batsch]** Ksenija GASIC	P. 115
S7P8	**Mapping the human taste experience on the apple genome** Béatrice AMYOTTE	P. 116
S7P9	**Identification of candidate genes associated with fruit softening rate in nectarine (*Prunus persica*) using QTLs and expression QTL** Claudio MENESES	P. 117

20h00	Departure from hotels and Congress Center by bus
20h30	Gala Dinner in the heart of the vineyard of Anjou
0h00/01h	Return shuttles to hotels and Congress Center

DETAILED PROGRAM

Thursday, June 23

9h		**SESSION 8: CROSS-DISCIPLINARY RESEARCH ON BIOTIC STRESSES**	P. 118
		ORAL PRESENTATIONS: Chairpersons: Sara MONTANARI - Bert ABBOTT	
9h-9h25	S801	**What do evolutionary histories of pathogens teach us about their various capacities to overcome plant resistances?** Keynote Speaker: Bruno LE CAM	P. 118
9h25-9h40	S802	**Using 'omics to understand the genetic mechanisms of the R-Avr model between *Maleae* hosts and *Venturia* species** Cécilia DENG	P. 119
9h40-9h55	S803	**Intergeneric transfer and functionality of a major apple scab resistance gene** Elisabeth CHEVREAU	P. 120
9h55-10h10	S804	**Targeted Mutagenesis of MLO-Homologous Genes in the Rose Genome** Juliane GEIKE	P. 121
10h10-10h25	S805	**Mapping black spot resistance in autotetraploid rose using genotyping-by-sequencing** Travis BANKS	P. 122
10h25-10h40	S806	**Exploring horticulturally important traits in an apple population using genome-wide association studies** Kendra A. Mc CLURE	P. 123
10h40-10h55	S807	**Comparison of the transcriptomes of a partially resistant and highly susceptible apple cultivars in response to Neonectria ditissima infection** Larisa GARKAVA-GUSTAVSSON	P. 124
		POSTERS: Chairpersons: Larissa GUSTAVSSON - David NEALE	
	S8P1	**NBS-LRR resistance genes polymorphism in genus *Malus* revealed by NBS profiling** Ekaterina SAVELYEVA	P. 125
	S8P2	**Effector-Mining in the genome of Diplocapon rosae** Enzo KLEIN	P. 126
	S8P3	**Exploring the genetic basis of host specificity in *Pseudomonas syringae* of *Prunus*** Richard HARRISON	P. 127
	S8P4	**Positive selection acting on cherry (*Prunus avium* L.) resistance gene analogs (RGAs)** Antonios ZAMBOUNIS	P. 128
	S8P5	**BAC library screening for the identification of Dp-fl resistance gene to *Dysaphis plantaginea* in the apple cultivar Florina** Michela DALL'ARGATA	P. 129
	S8P6	**Screening candidate genes for resistance to Sharka disease in *Prunus* species** David TRICON	P. 130
	S8P7	**De novo transcriptome sequence assembly in apricot using PPV infected and healthy plants** Elena ZURIAGA	P. 131
	S8P8	**Apple scab and Powdery Mildew: from Applied Genomics and NBTs the Ultimate Solution?** Riccardo VELASCO	P. 132
	S8P9	**The holy grail for plant geneticists: good phenotyping data!** Hilde NYBOM	P. 133
	S8P10	**Identification of genomic regions for virulence in the fruit canker fungus *Neonectria ditissima*** Kerstin DALMAN	P. 134
	S8P11	**Phenotyping pathogen resistance in cultivated strawberry roots using hyperspectral imaging** Helen COCKERTON	P. 135

DETAILED PROGRAM

Thursday, June 23

	S8P12	**Improving disease resistance in strawberry** Charlotte NELLIST	P. 136
	S8P13	**Identification of one major QTL associated with gummosis disease in peach (*Prunus persica*) using the 9K SNP array** Xiong-wei LI	P. 137
	S8P14	**Polygenic inheritance of resistance to *Cacopsylla pyri* in a *Pyrus communis* x P. ussuriensis population explained by four QTLs and an epistatic interaction** Laure PERCHEPIED	P. 138
	S8P15	**Two large effect QTL identified and characterized for soft scald incidence in apple** Nicholas HOWARD	P. 139
	S8P16	**Identification of new genomic regions for rose resistance to black spot** Brice MAROLLEAU	P. 140
	S8P17	**Update on molecular characterization of aphid resistance in black raspberry germplasm** Nahla V. BASSIL	P. 141

10h55-11h25	Coffee break
11h25	General Matters and RGC9
12h20-14h00	Lunch
14h00	Departure to professional tours by bus
18h20	Return to Angers City Bus

Friday, June 24

08h00	Departure by private coach from Congress Center Visite of **INRA** and **VEGEPOLYS**
12h45-14h30	Lunch Château d'Artigny
14h30	Visit of the regional fruit experimental station of La Morinière (Ste Maure de Touraine, near Tours)
17h30	Return to Angers City Bus

Committees

Convener:

Francois Laurens

Local Organizing Committee:

Valérie Fichot (Angers Convention Bureau)
Hélène Guillot (Angers Convention Bureau)
Elisabeth Fourmy (Angers Convention Bureau)
Nathalie Mansion (INRA Angers)
Patricia van Daele (INRA-IRHS Angers)
Heidi Eppe (INRA-IRHS Angers)
Fabienne Dupont (INRA-IRHS Angers)
Sandrine Pierre (INRA-IRHS Angers)

Scientific Committee:

Francois Laurens (INRA-IRHS, France)
Albert Abbott (Kentucky University, USA)
Suzan Brown (Cornell University, USA)
Etienne Bucher (IRHS, Angers University, France)
David Chagné (Plant &Food Research, New Zealand)
Max Cheng (University of Tennessee, USA)
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ABSTRACTS

Genetics and genomics of perennial domestication: what can we learn from apples?

S1O1

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Domestication has been used since Darwin as a model to understand adaptation and divergence. Among domesticated species, one major distinction is between annual and perennial life cycles. Here I will analyze domestication of perennials from a population genetics perspective, with a focus on apple domestication. The cultivated apple is a major fruit crop in temperate zones. Recent research has revealed a major role of hybridization in the domestication of the cultivated apple and has highlighted the value of apple as an outstanding biological model for unraveling adaptive divergence processes in perennial fruit crops. I will discuss the implications of this knowledge for apple breeding and for the conservation of wild apples. More generally, I will highlight motivations to study perennial plants, and new approaches that can lead to further progress in understanding the domestication of perennials.

Key words: grafting, local adaptation, introgression, clonal propagation, bottleneck, epigenomics.

The evolutionary history of apricot (*Prunus armeniaca*) might have contributed to a loss of resistance to sharka disease

S1O2

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Apricot (Prunus armeniaca L.) is an important fruit in the Northern hemisphere, where it is severely threatened by the Plum Pox Virus (PPV), causing the sharka disease. In the frame of the FP7 Marie Curie STONE project (#246795), we searched for the geographical origin and performed a world-wide genetic diversity analysis of resistance source(s) to sharka in apricot. Indeed, the apricot wild progenitor, P. armeniaca vulgaris, is still present as a forest tree in its native area, on the slopes of the Tien Shan ranges, in Central Asia. However, the history of apricot domestication and of landrace diversification is still poorly understood.

We used sixteen microsatellite markers amplified on a comprehensive dataset of 230 trees sampled in Central Asian natural populations (Kazakhstan, Uzbekistan, Western China and Kyrgyzstan) and on 142 cultivated apricots representatives of its ecogeographical groups. This revealed high levels of genetic diversity in Central Asian and Chinese germplasm, in agreement with an origin of this species in this region. Three differentiated genetic clusters with distinct geographic ranges were detected, encompassing respectively: i) cultivated apricots from Europe, North America and the Irano-Caucasian region, ii) Chinese cultivated apricots, and iii) wild apricots from Central Asia. Wild populations could be further differentiated into Southern and Northern Central Asian clusters. Scenarios of apricot evolution were tested and revealed two separate and consecutive events of domestication. Moreover, in its native Central Asian range, wild apricots exhibited a high frequency and variability in resistance to sharka. Altogether, our results contribute to the understanding of the domestication history of cultivated apricot. We also unraveled the origin of resistance to sharka and point to valuable genetic diversity in the extant gene-pool of wild apricot.

Population genomics of European woodland strawberry

S1O3

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Woodland strawberry (Fragaria vesca ssp. vesca, hereafter strawberry) is a perennial model species with a wide geographical distribution throughout Europe. Environmental conditions vary greatly between the margins of its geographical range; it can grow in various climates from the Mediterranean region with hot and dry summer (southern Spain, latitude 37°N) to the arctic climate in the most northern parts of Norway (latitude 70°N). As a perennial plant it must accurately time its life-history events according to length of the growing season. Adaptation to short growing season in the North has required directional selection for specific traits, such as timing of flowering and cessation of growth. In concordance with this, strawberries show phenotypic differentiation along a latitudinal cline in Europe. We are using population genomic methods to explore what are the genetic changes underlying latitudinal adaptation in strawberry. We sequenced the genomes of 110 accessions across Europe (from southern Spain to northern Norway), an average genome-wide coverage per sample being 18. Based on these data, we identified ca. 2 million SNPs which were first used to explore population structure and the amount of genetic diversity within the species. Moreover, we conducted genomic scans for recent directional selection (selective sweeps) in populations located in different latitudes and showed that selection has acted extensively on strawberry genome.

Nineteenth century French rose *(Rosa sp.)* germplasm shows a continuous shift over time from a European to an Asian genetic background

S1O4

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Modern cultivated roses would be the result of hybridizations between 7 to 10 species from the genus *Rosa*, coming from Asia, Middle East or Europe. Hybridizations between Asian and European roses which occurred in Europe, especially in France, during the 19th century have contributed for a large number of varieties and important trait introgressions in European varieties like recurrent blooming or tea perfume. In this study, we aimed at understanding the variations of genetic structure of French rose hybrids along the 19th century and the contribution of exotic introductions to these variations. A large sample of 1,228 garden roses, including 991 European garden roses from the 18th and 19th centuries, was constituted from prospecting in ten French rose gardens. As a comparison, Asian, botanical and modern roses were included in the study. Ploidy levels of the studied genotypes ranged from 2× to 6×. The individuals were genotyped with 32 single sequence repeat (SSR) primer pairs and the genetic diversity and structure of the sample were assessed. A large genetic diversity was found in the sample, in accordance with the interspecific origin of the material. SSR markers support the finding of sixteen genetic groups, which were then interpreted according to botanical sections or horticultural groups, breeding years, geographic origins and ploidy levels. A genetic differentiation was detected between ancient European and Asian accessions and a continuous temporal shift was observed in cultivated hybrids from a European to an Asian genetic background during the 19th century. Frequent crosses with Asian roses along the 19th century and/or selection for Asiatic traits may have induced this shift. Implications for rose breeding and germplasm conservation will be discussed.

Genetic diversity and genome-wide association studies in sweet cherr

S1O5

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Description of the genetic diversity, linkage disequilibrium and population structure is necessary for the efficient management of genetic resources and for genome-wide association studies (GWAS). In this study we present the evaluation of the genetic diversity, the detection of linkage disequilibrium (LD) patterns, the estimation of the levels of population structure, the identification of a first "core collection" and preliminary data on GWAS in sweet cherry.

A total of 210 genotypes including breeds and landraces from 16 countries were genotyped using the RosBREED cherry 6K SNP array v1. Structure analysis using STRUCTURE software detected two ancestral populations. Principal Coordinate Analysis confirmed these results. Further analyses identified nine subgroups using STRUCTURE and Discriminant Analysis of Principal Components. These sub-groups matched to different eco-geographic regions of landraces distribution. LD was evaluated showing lower values than in peach, the reference Prunus species. A core collection containing 156 accessions was constructed using the maximum length sub tree method. Preliminary analysis allowed the identification of significant SNPs controlling important agronomical traits.

We present the first population genetics analysis in cultivated sweet cherry using a medium-density SNP marker array. We provide data on LD, genetic structure and GWAS, and we propose the definition of a first INRA's sweet cherry core collection useful for breeding programs, germplasm management and association genetics studies.

Domestication of the cultivated apple tree, *Malus domestica*, and its hybridizations with the European crab apple tree, *Malus sylvestris*

S1P1

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Apple is the main fruit crop in temperate regions with, in addition, a high symbolic and cultural value. Elucidating its origin and the processes involved in the domestication of the cultivated apple tree, Malus domestica, is thus of great interest. It has long been considered that M. sieversii, a wild apple tree growing in Central Asia, was the ancestor of our cultivated apple tree. However, it has recently been shown that inter-specific gene flow played an important part in its domestication, with several other wild species contributing to the genetic makeup of M. domestica. In particular, M. sylvestris, a crab apple tree growing in European forests, seems to have hybridized frequently with M. domestica. However, several questions remain, such as the extent to which M. sylvestris contributed to the genome of Malus domestica. Indeed, different markers give different answers: microsatellite and chloroplastic markers gave M. domestica closer to M. sylvestris than to M. sieversii, while the re-sequencing of 23 genes across the Malus genus indicated the opposite. It is also unknown if the hybridizations between M. domestica and M. sylvestris had any functional impact on the cultivated apple tree. We addressed these questions here by comparing genomes of M. domestica, M. sieversii and M. sylvestris.

On *Rosa arvensis* genetics and intra-specific biodiversity

S1P2

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R. arvensis was first used as a model to explore physiological aspects of achene germination and hip development at the University College of North Wales, Bangor, UK. These studies have been published in the journal 'Nature' in 1959 (Prosser & Jackson) and 1965 (Jackson & Blundell). In the wild, *R. arvensis* has distinctive traits, with no or little variability at first glance. However, many natural variants, mutants and hybrids, that deviate from standard descriptions, were described by past French and Belgian taxonomists. Unfortunately, today, many competences and plants disappear with the past naturalists. I nevertheless decide to found an experimental and conservatory garden to restore and maintain *R. arvensis* biodiversity and to investigate the species genetically. I sampled from several regions of France, natural occurring mutants or variants affecting growth habit, leaf serration or the hip shape. Some variants fit with earlier descriptions, among others, 'biserrata', 'elipsoidea', 'gallicoides' or 'majus', a lost giant form, whereas some others, including several dwarf 'pumila' forms, are unrecorded from both science and horticulture. I found also hybrid taxons that clearly indicate that *R. arvensis* is involved in gene reticulation within the canina and gallica species. Altogether, I could progressively reconstitute most of the lost intra-specific biodiversity that was recorded from the 1800-1940 period.

R. arvensis was never explored at the level of formal genetics, despite the long-range breeding tradition with roses in Europe. In particular, a genetic model is missing that uses the advantages of reliable advanced wild type pedigree and natural variants from European species. I first made consanguineous controlled retro-crosses using standard wild-type clones of *R. arvensis*, in order to establish inbred semi-compatible lines. Second, I have begun to verify the nature of the inheritance of the wild-collected variants. Third, I cross *R. arvensis* with historic rose cultivars, bringing new horticultural traits in that common wild pedigree. Altogether, I will summarize my 20-years investigations and preliminary results and discuss whether this diploid species would be suitable as a genetic model.

Characterization of the genetic diversity among cultivated pears using SSR markers

S1P3

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GEVES – BioGEVES

Pear species (Pyrus spp.) is one of the most commercially important fruit tree species in the subfamily Maloideae of the Rosaceae. P. communis (European pear) is commonly cultivated in Europe and temperate regions, while P. pyrifolia is the main cultivated pear species in Asia. Registration of new cultivars either for Plant Breeders Rights or National Listing purposes requires Distinctiveness Uniformity and Stability test to be completed. DUS tests are based on the comparison between candidate cultivars and accessions of the reference collection. Developing new efficient tools to help to manage reference collections is a great concern.

The mains aims of the present study were to characterize the reference collection of pear using SSR markers, and to define a highly discriminating marker set.

Study has been carried out on leaves of 142 pear accessions (P. communis, P. pyrifolia, and P. calleyryana) collected from INRA ex situ collection, including 7 references genotypes recommended by ECPGR. Samples were genotyped with 37 SSR markers publicly available and evenly distributed throughout the genome.

On the 37 SSR markers, 6 were discarded due to weak amplification. The 31 remaining SSR markers were efficient to separate European and Asian pears and identify uniquely 111 accessions. Among the varieties with similar molecular profile, 19 were cultivars and its mutants. Nevertheless for 6 pairs of varieties with a genetic distance equal to 0 (concerning 12 accessions) further analysis are needed. A reduced set of markers was defined with an equivalent discriminatory power compared to the complete set of markers.

In conclusion, the set of 31 SSR markers and the reduced set could be used to establish strategies to manage the reference collection and provide true-to-type analysis.

Evaluating the influence of the microsatellite marker set used on the genetic structure inferred in Pyrus communis L.

S1P4

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Bayesian model-based approaches are nowadays majorly preferred to infer genetic structure, but it is still largely unresolved how marker sets should be built in order to obtain a robust inference. The objective of this study was to evaluate, in Pyrus communis L. germplasm, the influence of the SSR marker set size on the genetic structure inferred, also evaluating the influence of the criterion used to select those markers. Inferences were performed considering an increasing number of SSR markers that ranged from just two up to 25, incorporated one at a time into the analysis. The influence of the number of SSR markers used was evaluated comparing the number of populations and the strength of the signal detected, and also the similarity of the genotype assignments to populations between analyses. Our dataset allowed to evaluate three different situations: i) a very robust structuring reflecting major divisions in the germplasm, ii) a strong structure with moderate differentiation and iii) a weaker structure with little, but significant differentiation. In order to test if those results were influenced by the criterion used to select the SSR markers, several choosing scenarios based on the discrimination power or the fixation index values of the SSRs were tested. Our results indicate that population structure could be inferred accurately once a certain SSR number threshold was reached, which depended on the underlying structure within the genotypes, but the method used to select the markers included on each set appeared not to be very relevant. The minimum number of SSRs required to provide robust structure inferences and adequate measurements of the differentiation, even when low differentiation levels exist within populations, was proved similar to that of the complete list of recommended markers for fingerprinting. When a SSR set size similar to the minimum marker sets recommended for fingerprinting it is used, only major divisions are detected.

Tools for increasing the loquat diversity

S1P5

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Loquat (*Eriobotrya japonica* Lindl., Maloideae, Rosaceae) is a subtropical, evergreen fruit tree indigenous of China, where is located the center of origin of the species. Loquat is grown in all subtropical areas and was introduced in the Mediterranean basin in late 18th century. In Europe, the largest germplasm bank is located at Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia, Spain).

Genetic resources are the main breeding tool. In this project the genetic diversity of a germplasm collection has been studied using microsatellites which completed the phenotypic characterization made. The information gathered resulted in a key tool for planning crosses in the breeding program aimed at increasing the diversity.

Another biotechnology tool developed in the context of the breeding program was to set up techniques aimed at obtaining genotypes with different ploidy levels. Chemical mutagenesis followed by *in vitro* and *in vivo* selection resulted in polyploids with different ploidy level, which are of high interest in loquat, due to its potential for producing varieties with bigger fruits (tetraploids) o seedless fruits (triploids). We obtained stable tetraploids (4x) and triploids (3x). The ploidy level was determined by flow citometry and confirmed by chromosome counting in leaves and roots. On the other hand, experiments aimed at obtaining haploids and doubled haploids (DH) were carried by gametophytic embryogenesis in male gametes by isolated microspore culture and anthers. Haploids and double haploids are very useful for sequencing projects and allow homozygous lines in a unique generation, which is very useful in long juvenile period species as loquat. The anther culture resulted in a triploid plant (3x) probably explained by a natural chromosome duplication during the regeneration process. Nowadays, new experiments enlarging the genotypes used and experimental conditions are in progress.

Genetic mapping in polyploid crops: SNP development and use for integrated linkage map construction in rose and potato using a custom pipeline for tetraploid data

S2O1

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The construction of genetic linkage maps in polyploids has lagged behind diploids for several reasons:

1. SNP marker development may be more complicated
2. The mode of inheritance for many polyploid species is not clear: disomic, polysomic or a mixture.
3. To generate linkage maps for polyploids dosage scoring is needed, which is technically more difficult than in diploids.
4. Integration of the multiple homologs needs special attention.

The presentation will address these complexities, and subsequently illustrate how we solved them by means of a SNP marker data pipeline to construct a linkage map for polyploid mapping populations.

The pipeline consists of 3 steps:

- fitTetra (Voorrips et al. 2011) or fitPoly generates the dosage values of all individuals for the SNP markers
- various quality checks of the data, including checkF1
- TetraMappeR (in the future polyMappeR) that contains the R scripts to generate both linkage maps of the homologs as well as an integrated genetic linkage map.

Importantly, we build integrated maps that are based on maps for the separate homologs, so phasing of the markers is incorporated in the procedure.

The Golden Delicious doubled haploid: A toolbox for genomic and epigenomic studies

S2O2

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The main aim of this project is to better understand the contribution of epigenetic regulation of gene expression to the development of important traits in apple. Apple is an ideal subject to study epigenetic effects, because clones of the same individual have been grafted over long periods of time. In these presumably isogenic populations, phenotypic variations have been observed (e.g. fruit color). Using such material and genome-wide approaches, we want to identify epigenetic alleles (epialleles) in apple. Importantly, in the majority of the cases heritable epigenetic control of gene expression is caused by the presence of transposable elements (TEs) and other repetitive sequences. In order to be able to study epigenetics, however, a high quality reference genome with a good TE annotation is crucial. For that purpose we have decided to re-sequence the apple genome using a doubled haploid (DH) derivative of Golden Delicious. The advantage of using a DH is that the plant is completely homozygous which greatly reduces the complexity of the genome to be assembled and analyzed. Here we will report on the latest updates concerning the creation of a new reference genome for apple that will be very helpful for the community. Furthermore we'll present our latest results on the apple epigenome and how certain epigenetic marks may influence important apple traits.

Resequence analysis of interspecific and intraspecific recombination in an [(almond × peach) × peach] backcross progeny

S2O3

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The use of next generation sequencing (NGS) technologies is becoming routine, allowing the study of entire genomes in short periods of time. Crosses between Prunus species are often possible and a very important source of useful variability for breeding. However very little is known about interactions between the genomes of different species in interspecific hybrids and their offspring. In the past, the F2 progeny of the 'Texas' almond and 'Earlygold' peach (T×E) provided with maps homogeneously populated with markers useful for the location of major genes and QTLs. More recently, a first backcross population of the T×E hybrid with 'Earlygold' peach (T1E population) was produced that represents an opportunity to further study the interplay of Prunus genomes. In this work we present the analysis of the resequencing data of 125 individuals of T1E, where it is possible to study independently the recombinations occurring in the meiosis of the hybrid (interspecific) and of 'Earlygold' (intraspecific). In addition, the parents used in this progeny had a very diverse level of genetic variability (2 SNPs per polymorphic kb in 'Earlygold' vs 7 SNP/kb in the T×E hybrid). After developing a strategy to genotype in silico the T1E individuals, based on SNP markers called from resequencing data, we precisely mapped the recombination breakpoints. Results were compared with the genetic map previously developed for this population using SNP data from the peach IPSC 9k array and a perfect collinearity was found. Our results suggest that: (1) the rate of recombination within peach is 50% higher than in almond x peach hybrids; (2) recombination tends to occur closer to chromosome ends at both inter- and intraspecific levels; (3) there are "hotspots" of recombination, both inside species and between species and (4) DNA motifs poly-A and (CT)n are significantly associated with the occurrence of recombinations (the latter also associated with recombination "hotspots»).

Edmund Mach Foundation's Genomics Platform

S2P1

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The Edmund Mach Foundation's Genomics Platform takes part in the research activities using innovative methods of experimental investigation relating to sequencing and genotyping. The Platfrom provides comprehensive genomic services that include genome sequencing, transcriptome analysis, gene expression, genome-wide SNP genotyping, molecular assisted breeding and de-novo DNA marker discovery. Our application fields ranges from agriculture to environmental studies, passing through the metagenomic studies of the microbial communities in their environment (human and animal gut, plants, environmental, food).

The availability of three robotic workstations can automate a wide range of applications including primary and secondary screening, DNA extraction, amplification set-up, sample dilution, normalization and assay development. Thanks to the 96-capillary 3730xl DNA analyzer and the 16-capillary 3130xl DNA analyzer a wide variety of sequencing and fragment analysis applications including re-sequencing, microsatellite analysis, AFLP, LOH, SSCP, SNP screening and SNP validation are available to allowing researchers to save time, reduce costs and increase productivity. The 454 and Illumina platforms combined with the Illumina HiScanSQ system integrates the power and resolution of Next generation sequencing with the high-throughput capacity of genotyping and gene expression arrays, delivering unprecedented flexibility for experimental design.

The Genomics Platform explores new investigative methods, with the acquisition of new knowledge in the green biotechnology field to make them widely available.

De novo sequencing of the almond genome

S2P2

José L. Garcia, Amit Dhingra, Henry Duval, Ángel Fernández i Martí, Michelle Wirthensohn, Pere Arús

IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB and many others, *Campus UAB, Edifici CRAG, Cerdanyola del Vallès (Bellaterra), 08193 Barcelona, Spain*

Almond is one of the oldest cultivated nut crops with its origin in central and western Asia. The selection of the sweet type (Prunus dulcis) distinguishes the domesticated almond from its bitter wild relatives. It is economically important, especially in California with the highest worldwide production, followed by Spain. The almond belongs to the same subgenus as the peach, for which there already exists a reference genome. However, to fully understand the genetic underpinnings marking the key phenotypic differences between almond and peach, we have sequenced the genome of the 'Texas' almond, one of the traditional cultivars producing a sweet nut. Whole-genome shotgun sequencing of Illumina paired-end libraries gave an initial low-contiguity assembly of 512 Mbp, nearly double the estimated genome size. Counting of k-mers indicates a 275 Mbp genome with a substantial level of heterozygosity as well as repetitive sequence. In order to tackle both problems, we constructed a fosmid library and sequenced 68 pools of ~500 clones per pool. We then assembled the pools, merged them and finished the assembly by scaffolding with paired end and mate pair libraries, which resulted in a 240 Mbp assembly with a scaffold N50 of 500 kbp, a contig N50 of 33.5 kbp and CEGMA completeness of 99%. Two thirds of the assembly was anchored to the peach-almond genetic map, and using re-sequencing data of peach-almond hybrids and their parents we inferred the two haplotypes of the sequenced almond tree. We performed additional validation of the assembly using Oxford Nanopore MinION sequencing.

Rose Genome Sequence Initiative: project and recent advances

S2P3

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Rose, genus *Rosa*, is the largest ornamental plant cultivated worldwide, in the form of garden roses, cut roses and flowerpots. Rose is a woody plant for studying different aspects of plant development, such as blooming seasonality, flower development and scent emission. To decipher these developmental processes and also to understand rose domestication and selection, genomic approaches have to be developed. One important issue in rose is the high level of heterozygosity, which will complicate assembling the rose genome sequence.

In the framework of an international initiative (Rose Genome Sequencing Initiative), coordinated by M. Bendahmane (RDP, Lyon) and F. Foucher (IRHS, Angers), we have decided to produce a high quality genome sequence of rose, based on the old Chinese variety R. *chinensis* 'Old Blush'. This genotype is highly heterozygous. In order to tackle the heterozygosity problem, we have implemented two additional strategies: development of a high-density genetic map to anchor the genome and development and sequencing of a haploid of 'Old Blush'.

Using an F1 progeny (151 individuals) from a cross between 'Old Blush' and R. x *wichurana*, a genetic map based on SNP markers has been developed (using the 68k rose Axiom Array). The female map (from 'Old Blush') contains 5635 SNP markers on 7 linkage groups with a size of 482 cM (1.15 SNP marker/cM). In parallel, Genoscope (Evry) sequenced and assembled 'Old Blush'. The first version of the rose genome consists of 16000 scaffolds, with a N50 of 226kb. This version is under annotation. To reduce the number and increase the size of the scaffolds, a haploid of 'Old Blush' has been obtained. Sequencing, assembling and annotation of this haploid is in progress. The recent advances of this project will be presented.

Rose genome sequencing initiative; challenges and benefits

S2P4

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Roses are of high symbolic value and have great cultural importance in many societies world¬wide. The rose is well suited to be an original model organism for woody ornamental species as it has a relatively small genome size (560 Mbp) and it has a short life cycle for a perennial woody plant. Several characters, such as recurrent blooming, flower morphogenesis, scent[1,2,3]… are of high economic importance. During the past years, we generated a number of molecular, genomic and biotechnology tools[4] such as an efficient genetic transformation protocol5 and a database that provides useful information on *Rosa* expressed genes with thorough annotation and an overview of gene expression patterns with good accuracy6; the latest represented a valuable prerequisite to the the rose genome sequencing. We have under¬taken the genome sequencing of the diploid *Rosa chinensis* cv. 'Old Blush', an ancestor of modern roses that contributed several important characters. We generated and assembled a first draft genome sequence for this cultivar, however, its relatively high heterozygosity hampered high quality genome assembly with about 16,000 scaffolds and a N50 of about 230 kb. To overcome this difficulty, we have generated and sequenced a *R. chinensis* homo¬zygous tissue (line *HzRc-RDP12*) using Old Blush as starting material. The availability of this homozygous material yielded high quality genome assembly with reduced number of scaffolds and a N50 of about 1 Mb. The availability of the rose genome sequence will be of great help for discovering the molecular and genetic mechanisms controlling many traits in *Rosa sp.* and likely in other rosaceae species. Latest developments will be presented and discussed.

[1]Magnard et al., 2015 Science 349:81-3. [2]Iwata et al., 2012 Plant J 69:116-25. [3]Randoux et al., *New Phyt* 202(1):161-73. [4]Bendahmane et al., 2014 *J Exp Bot* 64:847-57. [5]Vergne et al, 2010 *PCTOC* 100:73-81. [6]Dubois et al., 2012 *BMC Genomics* 13:638. [7]Koning-Boucoiran et al., 2015 *Front Plant Sci* 6:248.

SNP discovery and development of a high density array for large-scale genotyping in pear

S2P5

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Pear production can be increased by developing new varieties with improved agronomic characteristics, such as size-control and precocity in rootstocks, and resistance to biotic and abiotic stresses. Marker-assisted selection (MAS) technologies, which are currently routinely and successfully applied for several plant crops, can potentially increase pear breeding efficacy. Currently, a Single Nucleotide Polymorphism (SNP) array including about 1000 European pear SNPs is available (Montanari et al., 2013), and it has been proved useful for the construction of dense genetic maps and application in quantitative trait locus (QTL) mapping projects. However, sequencing technologies have progressed at a very fast pace in the last few years, and it is now possible to design arrays with a much greater number of SNPs at a relatively small cost. In this project, we have sequenced at a low-coverage 55 pear accessions, representing founding cultivars and 29 species and hybrids, selected within the collections held at the National Clonal Germplasm Repository (NGCR) in Corvallis (OR) and the Appalachian Fruit Research Station (AFRL) in Kearneysville (WV). The analysis of the sequences generated from this diverse panel enabled us to discover large numbers of SNPs, which will be included in a high density array and used to genotype the entire NCGR pear collection, consisting of about 2000 accessions belonging to different species. This large set of genotypic data will be useful to study the genetic diversity within the genus Pyrus, and to find marker-trait associations to be applied for MAS in pear.

Development of the Axiom® Apple480K SNP genotyping array and its application for genome wide association study in apple

S2P6

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During the last decade, high-throughput genotyping has facilitated the dissection of complex traits in species with large/complex genomes and high level of genetic diversity. Array-based marker systems have been increasingly adopted for high-throughput genotyping, not only in model organisms, but also in many non-model plant species for which genomic resources are now available. A new high-density Affymetrix Axiom® SNP array has been built for the domesticated apple (Malus x domestica). It gathers more than 487K SNPs that are evenly distributed over the 17 chromosomes. The array has been built from the high-depth resequencing (~10-20x) of 63 different varieties covering most of the genetic diversity in cultivated apple. SNPs have been chosen by applying a focal points approach to enrich genic regions, but also to reach a uniform coverage of non-genic regions as to support SNP haplotype approaches. A total of 1324 apple accessions, including the 92 progenies of two mapping populations, have been genotyped with the Axiom®Apple480K to assess the effectiveness of the array. The majority of SNPs (359,994; 74%) fell in the most interesting class of Poly High Resolution polymorphisms. A novel filtering procedure was also devised to identify a subset of 275K robust markers that can be safely used for germplasm surveys in apple. A first application to genome wide association (GWA) study of two phenology traits (flowering time and maturity date) in six European germplasm collections is also presented. The Axiom®Apple480K has been publicly released and will likely be a reference tool for GWA studies in apple.

A pipeline for the curation of genetic data for pedigree-based QTL studies, demonstrated on RosBREED's apple data set

S2P7

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Obtaining high quality genetic data is a requirement for many genetic analyses. For any crop, errors in SNP genotype calls, pedigree records, or linkage maps can lead to erroneous missing marker data imputations, haploblock and haplotype determination, origin assignment of favorable alleles, and marker-locus-trait associations. To mitigate such data problems, a common data curation pipeline is followed for all crops within the RosBREED project. This pipeline used and extended a preceding pipeline from the European FruitBreedomics project. Here, we demonstrate this pipeline on RosBREED's multi-generation germplasm set of apple. A subset of SNPs with good intensity data and clustering performance was composed, using ASSIsT software, from genome scans performed with the 8K apple SNP array. First, SNP data were used to remove polyploid and aneuploid individuals. Individuals with low-quality SNP data were also removed. Second, SNP data were used to confirm and deduce pedigrees with an R-script. Third, SNPs with more than 1% of segregation errors were removed. In the fourth step, FlexQTLTM software identified any remaining genotype calls that caused segregation errors and those calls were manually corrected or removed. Fifth, a consensus genetic map from five 'Honeycrisp' full-sib families was used as a scaffold to determine genetic positions for all mapped SNPs. Genetic locations of unmapped SNPs were estimated based on their physical positions in the apple genome v3.0.a1 relative to those of the genetically anchored SNPs. Sixth, the resulting map was examined for cases of double recombination using FlexQTLTM, and SNP orders were adjusted or SNPs removed where necessary. The outcome was a high quality genetic data set for apple, free of segregation errors and with few double recombinations, that can be used for downstream genetic analyses targeting DNA-informed breeding applications.

CrossLink: Genetic Mapping Software for Allopolyploid Outbreeding Species

S2P8

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Genetic mapping plays a central role in the study and breeding of plants, facilitating scaffolding of full chromosome-length genome sequences, interspecies synteny comparisons and quantitative trait loci identification.

Map construction is relatively straightforward where a traditional backcross or F2 population from inbred lines can be used. Some species, including many crops, suffer inbreeding depression from the recessive deleterious alleles present in outbreeding populations. Maps are therefore produced from outcrosses between highly heterozygous parents, making more specialized mapping approaches necessary. For polyploid outbreeders, such as cultivated strawberry, Fragaria x ananassa, additional difficulties can result from markers segregating on more than one homeologous chromosome.

We present CrossLink, software for the rapid and automated construction of genetic maps for outbreeding species, using biallelic markers such as can be obtained from SNP arrays, genotyping-by-sequencing or whole genome sequencing, also incorporating additional features for polyploid genomes.

Incorporating the best methods available from existing mapping programs, our software maps markers using algorithms designed to scale efficiently to dense maps. Initial ordering uses the fast minimum spanning tree method of the (outcross-incompatible) MSTmap program. Maximum likelihood map ordering and multipoint distance estimation employ a genetic algorithm and Gibbs sampler respectively, similar to the methods employed by JoinMap(R). An alternative map ordering method uses a global measure of map quality for cases where local maximum likelihood fails to produce a good order. Two visualisation tools facilitate final map verification. Additional error correction procedures deal specifically with genotyping and grouping errors found to result from the allopolyploidy of strawberry, whereby the heterozygous and homozygous genotypes can become confused and where homeologous

High-density genetic maps and comparative genomics using restriction site associated DNA sequencing technology in sweet cherry

S2P9

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To improve efficiency of breeding programs in sweet cherry, high-density genetic maps were developed using restriction site associated DNA sequencing (RAD-Seq) technology and comparative analysis between the maps and the peach genome was performed. Three F1 mapping populations were generated from crosses between Beniyutaka and Benikirari, between Yamaen C12 and Benikirari, and between Nanyo and Benisayaka, and employed for RAD-Seq analysis. Totals of 1384, 1475, and 1157 polymorphic loci were identified in the three populations. Using a pseudo-test cross mapping strategy, six parent-specific genetic maps were obtained. The maps consisted of eight linkage groups except for one map with six groups, and total map lengths and numbers of mapped loci were 661 – 1224 cM and 422 – 782 loci, respectively. The maps were compared with the structure of the peach genome, indicating that high synteny relationships were conserved in the genomes of sweet cherry and peach. The genetic maps developed in this study would be useful tools for not only accelerating the sweet cherry breeding programs but also identification of genes for agronomically important traits.

High density, multi-population consensus genetic linkage map for peach (*Prunus persica* L. Batsch)

S2P10

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Highly saturated genetic linkage maps are extremely helpful to breeders and are essential prerequisite for a number of biological applications, such as the identification of marker-trait associations, mapping quantitative trait loci (QTL), candidate gene identification, the development of molecular markers for marker-assisted selection (MAS), and association and comparative genetic studies. Several high density inter- and intraspecific genetic maps, constructed using 9K SNP peach array, are available for peach. However, these genetic maps are based on single mapping population and have limited use for QTL discovery and comparative studies. A consensus genetic linkage maps developed from multiple different populations provide not only a higher marker density and a greater genome coverage when compared to the individual maps, but also serve as valuable tools for estimating genetic positions of unmapped markers. In this study, four high density intraspecific *Prunus persica* genetic maps, 'O'Henry' × 'Clayton' (OC^2), PI91459 ('NJ Weeping') × 'Bounty' (WB), 'Venus' × 'Venus' (VV), and updated 'Zin Dai' × 'Crimson Lady' (ZC^2), and interspecific 'Texas' × 'Earlygold' (T × E) map were used for the development of a consensus genetic linkage map for peach. The consensus linkage map contains a total of 4,015 molecular markers, consisting of 9 SSRs, 4,005 SNPs and 1 morphological marker associated with slow ripening in peach. The map spans a genetic distance of 844.8 cM with an average marker density of 0.7 cM/marker. This consensus genetic linkage map represents the most comprehensive peach map available to date and will serve as a new reference map for the *Prunus* genus. It will support QTL identification and molecular marker development for peach and other genetic studies within the *Rosaceae*.

Identification of haplotypes in octoploid strawberry cultivars using 90K IStraw

S2P11

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Cultivated strawberry (Fragaria x ananassa) is a hybrid octoploid species whose fruit is highly appreciated due to its organoleptic properties and health benefits. Despite its economic importance, the complex octoploid genome represents a great challenge to study the genome structure and molecular mechanisms related to fruit quality and agronomic traits. Recently, a strawberry SNP genotyping array (IStraw 90K) was developed providing thus a powerful tool for genome-wide scanning. The present study centered on utilizing SNP data from 37 cultivars, bred by the company PLANASA, to determine trait-relevant haplotypes in the cultivars and to improve a strawberry genetic linkage map in development, generated from a F2 population derived from a “Camarosa” x “Dover” cross. We developed a strategy to extend the genetic linkage map by association of high quality SNPs to the previously mapped ones based on genotype similarity with consistent results. The resultant 33 maps, composed of 2,106 markers, were derived from joining 71 fragments in function of their annotated homeolog group; the highlight is on the 633 markers that were newly incorporated. As genetic distance among cultivars responded to both phenotype and genotype properties, a thorough analysis on those linked by a common feature may shed light on the markers involved with it. Functional annotations of SNPs consisted of prediction of effects on genes, with focus on those variants that disrupted gene transcription; a list of 280 markers with predicted high impact on genes is provided. This work may be continued by performing association analysis when cultivars are fully phenotyped in order to define candidate genes related with traits of economic significance and will generate a small chip 60 SNPs (Open Array) for Varietal Identification.

Haploblock structure in sweet cherry using a pedigree-based approach

S2P12

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Sweet cherry (Prunus avium) is a clonally propagated diploid out-crossing crop where cultivar development is done using a pedigree-based breeding approach. The best parents and offspring are selected based on segregating F1 families. Recently available genome-wide single nucleotide polymorphism (SNP) arrays enables tracing the inheritance of chromosome segments in multiple generations of cherry breeding pedigrees. Visualizing the inheritance of hundreds of bi-allelic SNPs is facilitated when the data are presented as multi-allele intervals consisting of non-randomly associated SNPs that are in linkage disequilibrium called "haploblocks". Our objectives were to define statistically supported haploblocks for sweet cherry using a pedigree-based approach and explore the utility of this approach for DNA-informed breeding with a focus on the fruit size QTL region on linkage group 2. Genome-wide SNP data were used from a cherry 6K SNP array for a pedigree-linked set of 515 individuals of the Washington State University sweet cherry breeding program comprising the RosBREED sweet cherry Crop Reference Set and Breeding Pedigree Set. Pedigree-based haploblocks were estimated in FlexQTL™ and haplotypes for each haploblock were performed using PediHaplotyper. A total of 146 haploblocks were identified across the 8 Prunus chromosomes, with a mean length of 1.25 Mb based using the peach genome sequence as reference. Five haploblocks spanned the 29.4 cM, 6.14 Mb QTL region of linkage group 2, with 6–11 haplotypes per haploblock. The number of annotated peach genes per haploblock were also calculated. Inheritance of identical-by-descent SNPs in a four-generation pedigree illustrated the prevalence of effective recombination events in this five-haploblock region in the pedigree of modern U.S. sweet cherry cultivars. Furthermore, the observed effective recombination events within this pedigree illustrated how pedigree-based haploblocks can help reduce the width

A Blackberry DNA test to verify parentage in RosBREED

S2P13

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USDA-ARS National Clonal Germplasm Repository

A 6-SSR (simple sequence repeat) DNA test was developed to verify parentage of seedling populations representing important blackberry (Rubus sp.) parents two breeding programs (University of Arkansas and USDA-ARS, Oregon) participating in the SCRI-funded RosBREED project. This fingerprinting set consisted of six trinucleotide-containing SSRs and was used to genotype six seedling populations from UA and 12 seedling populations from USDA-ARS to ensure identity by descent, a necessary criterion for subsequent pedigree-based analyses. Presence of alleles not found in either parent but in a seedling was used to identify individuals in a population with incorrect parentage. The initial analysis of the 6-SSR test revealed 19 seedlings out of the 264 from UA and 24 out of the 202 seedlings representing 11 USDA-ARS populations with incorrect parentage. In the 12th population, ORUS 4647, all 23 seedlings contained alleles found in one of the parents, 'Obsidian', but also contained an allele not found in either 'Obsidian' or ORUS 2532-1, suggesting that the wrong parent plant was sampled for ORUS 2532-1. This hypothesis will be confirmed by genotyping each of the three available ORUS 2532-1 plants. Eventhough the 6-SSR test was able to identify some individuals with incorrect parentage, it could not differentiate 23 groups of individuals representing 55 seedlings from the UA populations and seven pairs of individuals from the USDA-ARS populations. To achieve better resolution, marker RH_MEa0016bC11 was removed and replaced with RH_MEa0008cF01, and two additional markers were added, resulting in a more polymorphic 8-SSR DNA test. With this revised set of primers, undifferentiated samples were reduced to eight in UA material. Genotyping the 14 undifferentiated USDA-ARS samples with 13 additional Rubus SSRs generated similar results for 12 of the samples, indicating good distinguishing ability of this 8-SSR test.

An improved *Pyrus* SSR fingerprinting DNA test to confirm parentage in RosBREED S2P14

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USDA-ARS National Clonal Germplasm Repository

A primary objective of the USDA-SCRI funded RosBREED project in pear is to develop DNA tools to combine fire blight disease resistance with excellent fruit quality in new cultivars. Pedigree-based analyses will be used for QTL discovery in the USDA-ARS breeding program from which 288 individuals were selected to represent important breeding parents. A DNA test was needed to confirm parentage and identity by descent of these 288 pears. The European Cooperative Program for Plant Genetic Resources (ECPGR) fingerprinting set is made up of 12 di-nucleotide-containing simple sequence repeats (SSRs) that are amplified in two PCR reactions and contains SSRs that exhibit a number of artifacts such as stutter, split peaks and binning errors. Our objective was to develop a DNA test containing easy to score higher core repeats of 3-6 bp motifs to use in parentage verification. Of 23 higher core-containing SSRs evaluated in a test panel of 13 accessions representing 5 Pyrus species, nine were easy to score and generated three to nine products. These nine SSRs were combined into a 6-SSR multiplex that contains also the dinucleotide-containing SSR, CH01d08, and a 5-SSR multiplex that contains the di-SSR CH04e03. Neither DNA tests could distinguish the full-sibs from the 11 biparental populations tested. Using all 11 SSRs distinguished all but five pairs of full-sibs, indicating insufficient polymorphism to distinguish each genetic variant. Seven more high core repeat SSRs were then identified and evaluated to either be added to the 6-SSR set or replace the least polymorphic SSR, resulting in a 9-SSR fingerprinting set that contained five high-core SSRs and four di-SSRs that are easiest to score among the 12 SSRs in the ECPGR set. This 9-SSR DNA test distinguished all unique accessions evaluated and is a useful tool for quick parentage verification in pear.

GDR: More Data and Functionality

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The Genome Database for Rosaceae (GDR, www.rosaceae.org) is the community database for basic, translational and applied research in almond, apple, apricot, blackberry, cherry, peach, pear, raspberry, rose and strawberry. Built using the Tripal platform, GDR provides an online portal of up to date, curated and integrated genomics, genetics and breeding data, combined with a suite of tools facilitating intuitive data mining and analysis. With a new theme included, GDR has been redesigned to provide more efficient access to the data, tools and resources for crops of interest. New and updated data and functionality in GDR include reference transcriptomes using published RNASeq data, new PlantCyc metabolic pathway databases, synteny data, the most current genome data, genetic maps, molecular markers, and QTL data. We will also discuss plans for further development through 2019 for this USDA and NSF funded resource.

TripalBMS: Toward a Comprehensive Breeding Information Management System

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Breeding programs produce large amounts of data that require efficient management systems to keep track of performance, pedigree, geographical and image-based data. With the development of DNA-based screening technologies, more breeding programs perform genotyping in addition to phenotyping for performance evaluation. The integration of breeding data with other genomic and genetic data is instrumental in the refinement of marker-assisted breeding tools, enhances genetic understanding of important crop traits and maximizes access and utility by crop breeders and allied scientists. We have previously developed a breeding database in the Genome Database for Rosaceae (GDR) and integrated with other genomic and genetic data. While it is developed using Chado and Drupal, it is not developed as a Tripal module that can be shared by any other databases that uses the Tripal platform. We report the progress on TripalBMS, a comprehensive Breeding Management System in GDR. The key improvement is a web interface where breeders can directly import various types of data including the data collected using the app 'Field Book'. We highlight future plans for the development of this comprehensive breeding management system.

Evaluation of Field Book: An open-source Android app for collecting phenotypic data in a peach breeding program

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Concomitant advances in high-throughput sequencing, phenotyping, and computational technologies, are generating petabytes of genomic, genetic, and breeding data for Rosaceae crops. Efficient utilization of this data by the research community requires analysis, integration, and visualization. The Genome Database for Rosaceae (GDR) is working towards enabling high-resolution dissection of traits and relating molecular diversity to functional variation for efficient development of new cultivars through marker-assisted breeding. Plant breeding programs generate and search through thousands of plants to find the best plant types which will become new improved cultivars. A typical breeding program produces hundreds of thousands of phenotypic data points in a given year. Inefficient and poor handling of this data decreases the genetic gain of the breeding program, consequently reducing its efficiency. While data handling has been a limiting area for breeding programs in the past, advances in computing have provided simple solutions that can address the needs of plant breeding programs. Using open-source software and relatively inexpensive phone and tablet hardware, GDR is supporting development of a platform that will allow researchers to replace hard-copy field books, thus alleviating the possibility of transcription errors while providing faster access to the collected data. The peach breeding program at Clemson University is testing the Field Book app for collecting data on field research plots. Field Book data input is customized for a large number of data types including numeric, percentage, categorical, multicategorical, date, Boolean, text, counter, rust score, photo, and audio. These data types allow the user to create custom traits to suit their own personal needs. We report our experience of Field Book app in a peach breeding program and further development plans.

Deciphering the molecular and genetic control of tree architecture

S3O1

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Genetic manipulation of plant architecture has been at the heart of modern advances in agricultural productivity. Grain crops such as barley, wheat, rice, maize, and sorghum, have been bred with shorter, thicker stalks and an overall upright shape to enable high density production. Comparable gains from architectural optimization have not been sufficiently realized for many crop species such as fruit/nut trees that have long generation times and rely on expensive horticultural manipulation to manage plant size and shape. A major obstacle is a lack of knowledge about the genetic basis for key architectural traits such as the number and orientation of lateral organs (branches, leaves, flowers, and roots). Recent advances in the field of plant development are now beginning to uncover some of the underlying molecular and genetic pathways and, more importantly, have demonstrated a great degree of untapped architectural plasticity. Our group is currently studying a number of novel genes identified in peach that specifically control the orientation of lateral shoots and roots. This information is being used to both investigate the underlying molecular mechanisms as well as to deliver trees with a range of desirable sizes and/or shapes via breeding and biotechnology. Such technologies have the potential to increase productivity, limit management costs, minimize environmental impacts, and improve water/nutrient use in Rosaceous crops.

Identification of the dwarfing gene *Dw1* S3O2

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The 'Malling 9' ('M9') dwarfing rootstock is used widely in apple breeding and commercial cultivation to shorten the juvenile period, reduce vegetative growth and increase scion flowering. We identified two genetic loci from 'M9' that confer rootstock-induced dwarfing. Phenotypic analysis indicates that the combination of *Dw1* and *Dw2* has the strongest influence on rootstock-induced dwarfing, and that *Dw1* has a stronger effect than *Dw2*. Genetic markers linked to *Dw1* and *Dw2* were used to screen over 41 rootstock accessions that confer a range of effects on scion growth. The majority of the dwarfing and semi-dwarfing rootstock accessions screened amplified marker alleles linked to *Dw1* and *Dw2*. This suggests that most apple dwarfing rootstocks have been derived from the same genetic source. Using recombinants from our mapping population, we narrowed the interval containing *Dw1* to 1.1 Mb on LG5. Several candidate genes were identified that show altered expression in 'M9' and other dwarfing rootstocks. Genomic sequencing of 'M9' revealed that it is heterozygous for a non-synonymous SNP in a conserved region of the top candidate gene. Tobacco transformed with the mutant 'M9' allele of this gene were short, with thick stems, reduced apical dominance, abnormal vascular patterning and floral patterning defects. Non-transformed tobacco grafted onto 'M9' transgenic roots had shorter shoots, reduced leaf area and altered flowering time relative to wild type/wild type homografts. Transgenic apple plants expressing the *Dw1* candidate gene and *Dw2* will be grafted with wild-type scions and phenotyped. Identification of the *Dw1* gene would enable the development of dwarfing rootstocks in crops where there are no dwarfing rootstocks.

Transcriptomic comparison between deflowered and overloaded apple trees: toward the understanding of biennial bearing in apple

S3O3

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Under the assumption that flower induction is inhibited in the trees carrying heavy fruit load or induced by the absence of fruit, we artificially placed in situation of alternation adult apple trees of the Gala variety to study the differentially expressed genes in their apical meristems, from the period of floral induction to floral differentiation. Analysis of these genes by qRT-PCR and microarray enabled to identify the key biological processes involved. Indeed, classes of differentially expressed genes suggest that the meristems are in contrasting physiological states resulting from various metabolic, hormonal and redox states. In addition, several genes known to be involved in the control of floral induction, such as *TEMPRANILLO (TEM1), FLORAL TRANSITION AT MERISTEM (FTM1) and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* were differentially expressed.

These results represent a foundation toward the understanding of molecular processes leading to the inhibition vs. promotion of floral induction in fruiting trees.

Unravelling the balance between sexual and asexual plant reproduction in an herbaceous perennial, the strawberry

S3O4

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To ensure the sustainability of species, plants have developed different reproduction modes: sexual and asexual reproduction. Most often, in polycarpic perennial plants, floral transition happens once a year ('once flowering', OF) but some perennials have the ability to flower more than once during the favourable season, therefore offering a lengthened period of fruit production ('perpetual flowering', PF). In addition, in many polycarpic perennial plants, sexual reproduction is often combined with asexual reproduction. In perennial crop species, both PF and asexual reproduction constitute highly desirable traits, though they may antagonize each other. Strawberry stands as an interesting model in perennial plants for studying the balance between these two processes since the length of the flowering period and the strength of production of ramets, the so-called runnering process, can show wide variation.

We investigated the genetic and molecular control of this balance in diploid and cultivated octoploid strawberry and by studying segregating populations. In the diploid F. vesca, the characterization of the epistatic interaction between FvTFL1, which controls the PF behaviour, and the RU locus, which controls the runnering process, suggested a balance between flowering and runnering.

In the octoploid cultivated F. x ananassa, a single locus named FaPFRU controls both the PF and the RU traits, with opposite effects. Recently, we succeed on the development of a strategy based on selective mapping using a reduced sample of individuals to fine map target homoeologous loci in a complex polyploid species. Using this strategy, we were able to drastically reduce the locus FaPFRU, which now comprises 234 genes, including 15 flowering-associated genes. Despite non-orthology between key genes and locus controlling perpetual flowering and runnering between both species, physiological results suggested common bases for the control of the balance in Fragaria.

Control and selection of blooming seasonality in rose

S3O5

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Rose is the largest economical plant in the ornamental sector for gardens, cutting flowers and flowerpots. This tremendous success can be explained by the ability of roses to flower continuously. Rose, genus *Rosa*, is a good model to study seasonality of blooming, a developmental process which is poorly understood. We have previously demonstrated that continuous flowering in rose is due to a mutation of a gene encoded a floral repressor, *RoKSN*, a *TFL1 (TERMINAL FLOWER 1)* homologue. In continuous-flowering roses, a transposon (with a *copia* element) is inserted into the gene leading to non-accumulation of the floral repressor and so to continuous flowering.

Our objectives were to understand the mode of action of this floral repressor and its selection during the process of human breeding.

To analyse the mode of action of *RoKSN*, we ectopically expressed the gene in *tfl1* mutants of *Arabidopsis thaliana* and continuous-flowering roses. We also studied the proteins that interact with RoKSN. We clearly demonstrated that RoKSN is a floral repressor, as *RoKSN* could complement *tfl1* mutant of *A. thaliana* and its ectopic expression led to an absence of blooming in the transgenic roses. To inhibit blooming, RoKSN is interacting with a transcription factor, RoFD. There is a competition between a floral activator, RoFT, a FLOWERING LOCUS T homologue, and RoKSN for the regulation of RoFD

In collaboration with historians, we studied the process of rose selection during the 18th and 19th centuries in France. By genotyping and sequencing *RoKSN* on a large collection of roses from this period, we showed a progressive selection of the *copia* allele (bringing continuous flowering). Furthermore, we detected a new allele that can be responsible for intermediate phenotype (occasionally-reblooming). This new allele at the *RoKSN* locus encodes a functional RoKSN but the transcript accumulation is weaker.

How to breed novel flowering traits in strawberry?

S3O6

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Homologs of TERMINAL FLOWER 1 (TFL1) are major floral repressors in the Rosaceae family. Recent studies in the Rosaceae model species Fragaria vesca, the woodland strawberry, have demonstrated the role of FvTFL1 as an integrator of light and temperature signals in the genetic flowering pathway and revealed a null mutation of FvTFL1 as a genetic basis of perpetual flowering in this species. FvFT1-FvSOC1 module controls the expression of FvTFL1 according to light signals, while the temperature regulation of FvTFL1 occurs through an unknown mechanism. To test this genetic model in cultivated strawberries, we silenced FaTFL1 in Fragaria x ananssa cv. Elsanta and analyzed mRNA levels of key flowering time genes in several cultivars with distinct environmental flowering responses. The silencing of FaTFL1 caused perpetual flowering under non-inductive long days without direct effect on runner formation. Gene expression studies showed that FaTFL1 mRNA levels correlated with flower induction in different cultivars in different conditions. However, no clear correlations were found between the expression of FaTFL1 and its putative upstream regulators FaFT1 and FaSOC1, indicating that additional mechanisms are involved in the transcriptional regulation of FaTFL1. We suggest that changing FaTFL1 expression patterns through different breeding approaches could result in new cultivars with desirable flowering patterns including novel perpetual flowering cultivars. Results will be discussed in the light of recent molecular and genetic studies in cultivated strawberry and other Rosaceae.

Next Generation Sequencing data as a useful tool to decipher self-incompatibility mechanism in *Prunus*

S3O7

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Prunus species exhibit a Gametophytic Self-incompatibility mechanism where specific recognition is determined by S-RNases (pistil S-determinant) and S-haplotype-specific F-box proteins (SFB; pollen S-determinant). However, non-S-factors (modifier factors) are also necessary for the mechanism to function properly. Pollen Part Mutations (PPMs) affecting modifier factors and conferring self-compatibility have been reported in apricot cultivars 'Canino' and 'Katy'. Interestingly, both PPMs mapped in an overlapping region at the distal end of chromosome 3 named as *M*-locus. This work is focused on facilitating the identification of the PPM by using NGS data. For this purpose, a supercontig was obtained after *de novo* assembly of BAC clones covering *M*-locus from the SI apricot cv. 'Goldrich'. To narrow down the *M*-locus map, new recombinant hybrids and molecular markers, some of them identified by Illumina Whole Genome Sequencing data, were used. A high resolution map for 'Canino'/'Katy' *M*-locus region of approximately 134 Kb in size was successfully defined improving previous genetic maps. Putative role of candidate genes comprised in the *M*-locus region is discussed. This work highlights how NGS data can be helpful for gene identification and future cloning.

Next generation sequencing analysis to identify modifier gene candidates conferring pollen-part self-compatibility in sweet cherry 'Cristobalina'

S3O8

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The Rosaceae, the Solanaceae and the Plantaginaceae have the S-RNase-based gametophytic self-incomaptibility (SI) system, which uses S-RNase and F-box proteins as the pistil *S* and the pollen *S* determinants, respectively. Despite the commonality of the specificity determinants, SI recognition mechanism in *Prunus* in the Rosaceae is supposed to be distinct from those in the other taxa, and various *Prunus*-specific self-compatible (SC) mutants have been reported. Sweet cherry (*Prunus avium*) 'Cristobalina' is such a Prunus-specific SC mutant of which the pollen-part modifier gene, unlinked to the *S* locus, confers SC phenotype. Here, we performed subsequence cataloging from Illumina HiSeq genomic reads (paired-end 150-bp) in 44 F_1 progeny of 'Cristobalina' segregating for SI and SC phenotypes. The reads including the SC progeny-specific subsequences were assembled into polymorphic contigs covering the candidates of the pollen modifier locus. Most of these contigs showed significant homology to the bottom edge of the peach chromosome 3, to which the syntenic region in the cherry genome has been reported to contain the pollen modifier locus of 'Cristobalina'. Next, we further filtered the polymorphisms specific to 'Cristobalina' by mapping of Illumina HiSeq reads from various sweet cherry cultivars to the candidate contigs. This second screening based on association analysis confined the 66 SC-specific contigs with the average length of *ca.*400-bp. Considering the results from pollen mRNA-Seq analysis together, we could identify the candidates of the modifier genes expressed in the SC-specific genomic contigs.

Tree architecture of apple genotypes with columnar growth

S3P1

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High density planting needs small or dwarf trees with modified canopies for better light interception and distribution, and to ease mechanization of field operations. Very low tree growth is achieved by choosing suitable low growth cultivars and dwarf rootstocks. A new alternative for this system is growing columnar apples that are characteristic by central tree axis with short fruiting spurs. Columnar tree growth habit of apples was discovered in the 1960s and was described as the sport 'McIntosh Wijcik'. Columnar materials differ in tree vigour, spurring density and length of fruiting spurs. The objective of this study was the orchard evaluation of 80 select apple columnar genotypes on M9 rootstock. Genotypes were evaluated in their 3th, 4th, 5th and 6th growing year after planting. Differences among genotypes in total tree growth, length of annual central axis growth, length of spurs and their density in the central tree axis was assessed. Selecting the ideal phenotype focused on an individual with optimum length of annual central axis growth with sufficient density of fruiting spurs on central axis of the tree. The length of annual central axis varied from 0.14 m to 0.35 m. There was a positive correlation between length of annual central axis growth and fruit size ($R^2 = 0.24$) and fruit number at harvest ($R^2 = 0.44$). There was a negative correlation between number of fruiting spurs and fruit size ($R^2 = 0.19$).

Auxin Transporter *PIN1* in rootstock regulates the growth of scions in *Pyrus*

S3P2

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Dwarfing and high density planting cultivation is an inevitable trend in the pear cultivation production worldwide, which needs dwarfing rootstocks. A series of dwarfing rootstocks were got through resource collection and the traditional crossbreeding techniques. The dwarfing rootstocks were grafted with the same scions in order to determine the mechanism of dwarfing effects. It was found that the auxin content in the bark of the grafting combination with dwarfing rootstocks is much lower than that with standard rootstocks, and the IAA or TIBA treatment *in vivo* did promote or suppress the growth of scions. So we characterized an auxin transporter gene *PIN1*, from *Pyrus*, and found it was associated with dwarfing ability. The transgenic *Pyrus* lines with *PIN1* over-expressed showed that more thick and dwarf stems than that of wild-type plants. Through cross techniques with DR5::GUS and 35S::PIN1 transgenic *Arabidopsis*, we got the DR5::GUS X 35S::*PIN1* plants. The DR5::GUS X WT plants were used as control. It was found that the *PIN1* enhanced the GUS activity in leaves, stems, and siliques. It maybe affect through accelerating the transport of auxin. It was postulated that *PIN1* in the rootstock played a role in controlling elongation of shoots of scion, through affecting the transport of auxin, which will be verified through micro grafting in the next step test program.

The players of the «collaborative non-self recognition» *Malus* self-incompatibility system

S3P3

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In flowering plants, a widespread mechanism to prevent inbreeding is gametophytic self-incompatibility (GSI), where the self-incompatible phenotype of the pollen is determined by its haploid genome. In Solanaceae, Plantaginaceae, Rubiaceae, and Rosaceae species pistil specificity is determined by an extracellular ribonuclease, called S-RNase. The male determinant, always a F-box gene, can be determined by one gene, as in the self recognition Prunus (Rosaceae) system, or multiple genes, as in the non-self recognition Pyreae (Rosaceae; called SFBBs) and Solanaceae (called SLFs) systems. In the collaborative non-self recognition system, each of the multiple S-pollen genes interacts with only a subset of its non-self S-RNases, and none of them can interact with their respective self-S-RNase. Therefore, in such systems, a large number of S-pollen genes must exist in a single S-haplotype. In M. domestica we have identified18 SFBB like genes using a de novo RNA-seq approach to analyze the pollen transcriptomes of 10 S-haplotypes. As expected, these genes are not present in the leaf, ovary, stigma, style, sepal, and petal transcriptomes of Golden delicious (S2S3). Furthermore, these genes are in linkage with the S-RNase gene, as expected for S-pollen genes. High diversity and/or deletion of SFBB genes as predicting targets of non self-S-RNases are here addressed as the mechanism of GSI recognition in M. domestica.

Phenotyping of inflorescence morphology for flowering-cherry ‘Sakura’ genetic resource

S3P4

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Phenotyping is an important step to characterize and evaluate individuals. However, phenotyping is a laborious process and data are often dependent on environmental conditions and tree developmental stages, especially for perennial horticultural plants. The morphological characteristics in flower and inflorescence are generally quantified by measuring the size, counting the numbers, or comparison to the color standards. The sensory evaluations can be also adopted to quantify the ornamental value. Human errors and measurement skill’s variation influence on the quantification. We are assessing the ornamental value of Japanese flowering-cherry ‘Sakura’ tree by measuring the number of flowers per cluster and the length of inflorescence axis and peduncles in this study. We repeated the measurement of inflorescence architecture of the flowering-cherry over four years. In the first two years, manual measurements were performed by using caliper tool, but over the following years, image capturing by commercial document scanner and image analysis using ImageJ software was employed. The image analysis method improved the measurement efficiency and the accuracy. Based on the coefficient of variation from each data, morphologically stable individuals (cultivars) that are not affected by environmental conditions could be found. Moreover, the inflorescence architecture models for each individual were obtained by multi-year measurements. We discuss about these phenotypic data of inflorescence from 130 cultivars of flowering-cherries with consideration for using in genomic analyses such as GWAS.

Mapping flowering time QTLs in a diploid strawberry

S3P5

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Flowering time is an important event in plants' life cycle, and therefore, many studies are focusing on the molecular and environmental conditions which favor flowering. This project illustrates QTL mapping conducted in the field as well as in the greenhouse using the diploid woodland strawberry (Fragaria vesca) F2 population of 335 lines. The study shows three additive QTLs on linkage groups LG4, LG6 and LG7 that explain about half of the flowering time variance in the population. The LG4 QTL was found both in the field and greenhouse, but the location varied slightly. QTL regions on LG4 contain several homologs of flowering time genes that can be candidate genes for the QTL such as FLOWERING PROMOTING FACTOR1 (FPF1) and SQUAMOSA PROMOTER BINDING LIKE (SPL) in the greenhouse experiment and two MADS box genes, SHORT VEGETATIVE PHASE (SVP) and DORMANCY ASSOCIATED MADS BOX (DAM), in the field. Furthermore, the QTL in LG7 was found close to homologs of EARLY FLOWERING 6 (ELF6) and FLOWERING LOCUS D (FLD). In the LG6, QTL peak co-localized with the previously identified floral repressor TERMINAL FLOWER1. Our results show that a few QTLs have a significant effect on flowering time in diploid strawberry. We suggest that these results may aid to understand the regulation of flowering time also in cultivated octoploid strawberry which has a high commercial value.

Apple cell division and elongation during fruit development is associated with marked changes in hemicelluloses composition, structure and related gene expression

S4O1

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Fleshy fruit growth and mechanical properties impacting texture quality depend on the close interplay between cellular water partition and cell wall mechanical properties regulating turgor pressure all along fruit development and ripening. Besides known changes in cell wall pectin composition during these events, the fate of hemicelluloses is yet to be studied as these polysaccharides contribute notably to the control of cell wall expansion. In a first study, the cell wall hemicellulose structural profile was assessed in developing and ripening Ariane and Rome Beauty apple by MALDI-TOF MS analysis of cell wall enzymatic digest. The results showed that the major xyloglucan (XgG), the minor galactoglucomannan (GgM) and the trace glucuronoarabinoxylan (GAX) hemicelluloses structures were significantly and differently affected during cell division and expansion phases. The two varieties significantly differed in their hemicellulose structure profile either during early development or during ripening. In a second study, cell wall hemicellulose structural profiling was coupled to gene expression in developing apple hybrids fruit. The results revealed that the shared early expressed genes between the hybrids mainly concern hemicellulose biosynthesis and modifications. In particular, the marked fine structural evolution of GgM was strongly correlated with mannan synthase, glucanase (GH9) and β-galactosidase gene expression.

The results question the function of the remarkable changes in cell wall hemicellulose composition and structure occurring at the cell division/elongation switch. Candidate genes are now available to further assess the role of these hemicelluloses structures on fruit growth and its impact on texture.

Genomic approach in identifying genes and gene networks that regulate strawberry fruit development

S4O2

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Fruits represent a key evolutionary innovation for seed disposal and are derived from successful development and fertilization of flowers. *Fragaria vesca*, the diploid strawberry, is emerging as a better model than the octoploid garden strawberry due to its diploidy and sequenced genome. We employed RNA-seq to investigate the molecular events during different developmental stages of flower and fruit development. The resulting transcriptome data on flower and fruit development (tissue and stage) can be accessed freely (http://bioinformatics.towson.edu/strawberry/). We then focused on comparing gene expression before and after fertilization to identify the earliest signals responsible for fruit initiation. Analysis of phytohormone biosynthesis and signaling genes confirm the critical roles of auxin and GA in fleshy fruit initiation. Further, the endosperm tissue was found to play a more prominent role than embryo in the biosynthesis of auxin and GA for fruit initiation. In addition, we examined genes specifically expressed in the receptacle, a unique fruit tissue of strawberry. Both receptacle-specific protein coding genes and miRNAs were identified and the functions of these genes are being determined. Our studies are beginning to reveal the molecular underpinnings of early stage fruit development. Insights into early stage fruit initiation have laid the foundation for investigations into mechanisms underlying morphological diversity in *Rosaceae* fruits. This new effort is recently funded by the US National Science Foundation and will contribute to the understanding of other *Rosaceae* fruits including apple, peach, and raspberry. Please refer to project website (http://www.clfs.umd.edu/CBMG/faculty/Liu/lab/Rosaceae/index.shtml) for further details.

From gene to phenotype: genetic control and modeling of sugar metabolism during peach fruit development

S4O3

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Fruit quality is a multi-criteria character with frequent antagonistic relationships. Among the three so-called major sugars in fruit, fructose is the sweetest and its concentration is the factor that most affects the fruit sweetness. The objective of the present study is to analyze the sugar metabolism in peach fruit from metabolic, enzymatic and genetic aspects and integrate into a mathematical model all information obtained. This work focuses on the identification of the effect of a low fructose concentration on the whole sugar metabolism and the understanding of the mechanisms responsible for this phenotype called 'low-fructose-to-glucose-ratio'. A nearly exhaustive biochemical characterization of sugar metabolism was conducted during peach fruit development. For this, 6 metabolites and 12 enzyme capacities were assayed in 106 genotypes of a population derived from an interspecific cross. This study revealed a high stability of the enzyme capacities despite large variations of metabolites. A QTL analysis performed on this dataset highlighted the instability of the effect of certain loci during fruit development. Co-locations of QTLs for metabolites and enzyme capacities and candidate genes were observed. In addition, the genomic region responsible for the 'low-fructose-to-glucose-ratio' phenotype was confirmed, and functional candidate genes were identified. An analysis of this gene has started to validate its function and its involvement in this particular phenotype. Based on data from 10 genotypes, a kinetic metabolic model that simulates the sugar accumulation in fruit was developed and validated. This model simulates contrasting phenotypes and helps in understanding the underlying mechanisms of the 'low-fructose-to-glucose-ratio' phenotype. In the future, the integration of the genetic control into the metabolic model will allow simulating virtual genotypes with different combinations of alleles and predict their sugar content.

Effects of exogenous application of GA4+7 and 1-Naphthaleneacetic acid on Sugar Accumulation and Related Gene Expression in Peach Fruits during ripening stages

S4O4

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In peach industry, one of the main goals is to improve the fruit quality which is determined by combinations of sugars, acids, volatile compounds. The mechanism of sugar biosynthesis has been paid an increasing attention by growers. Gibberellin and auxin have been reported to be efficiency to regulate fruit ripening, however, there is little information available on their role in the sucrose conversion. To better understand the molecular basis of GA and IAA interference with the sugar biosynthesis, two commercial cultivars with varying maturity time were selected. Three GA4+7 and four 1-naphthaleneacetic acid (NAA) concentration solutions were used as treatments to sprayed onto the fruit. Thirty fruits per treatment were collected for 10 times during fruit development stages. Sugar content was quantitated by HPLC. We performed a genome wide gene selection from three DGE profiling of previous research, finally thirteen differential expression genes in the sugars biosynthesis pathway were detected and testified by RT-PCR. The results showed that GA4+7 1.25 mM treatments significantly increased the fruit size and sucrose content comparing with the control fruits and the other treatments for both cultivars. While NAA 0.5 mM treatments significantly reduced the sucrose content. Ninety percent of the fruits deformed and fell off from the trees under the treatments of NAA 1 mM and 2 mM. The expression pattern of the same gene is observed to be different between these two cultivars, which might be related to the different growth curve. For sucrose synthesis, one important gene we found is sucrose-phosphate synthase gene (SPS2) which showed high express level and was significantly regulated by GA4+7 1.25 mM. We propose that GA4+7 1.25 treatment might be positive to increase the sucrose content. Endogenous hormone and related genes will be further investigated to get insight to the inherent association between phytohormone and sugar accumulation.

Understanding development, ripening and postharvest performance of peach fruit using a system biology approach

S4O5

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This study characterizes the impact of the environment and the post handling on peach fruit ripening and chill symptoms. 'June Gold' peaches grown at low (50 m) and high (550 m) altitude were sampled during different development stages (S2, green; S3, yellow-green, S4I, commercial harvest/before climacteric phase; S4II tree-ripe/climacteric phase) and then were ripened for up to 3 days (d) at 20 oC after harvest or following 20 and 40 d cold storage (0 oC). Fruit cultivated at high altitude exhibited superior quality traits at harvest (higher SSC:TA, higher skin red color coverage) and were characterized by higher ethylene production and increased softening rate, compared to those grown at low altitude. Fruit cultivated at low altitude were also more susceptible to the chilling injury syndrome, expressed as mealiness, lack of juiciness and internal bleeding. In a second experiment 'June Gold' peaches were harvested at the commercial harvest (CH) and at the on-tree ripe (ON-TR) stage and subsequently were cold stored (0 oC). A group of the CH fruits, immediately after harvest, were ripened off-tree (OFF-TR) at 20 oC for 3 days to the same level with ON-TR and then cold stored. After 20 and 40 days of cold storage, fruits were ripened at 20 oC for 3 days. It was observed that OFF-TR fruits were CI-free, CH fruit expressed moderate symptoms, while ON-TR fruit exhibited severe chilling symptoms. In these experiment we further used a systems biology approach by integrating transcriptomic (μPEACH 3.0 array platform and quantitative real-time RT-PCR), proteomic (2DE-PAGE coupled with nanoLC/MS/MS analysis) and metabolomic (GC-ToF-MS approach) data to understand regulatory networks underlying development, ripening as well as chilling injury symptoms in peach fruit. Overall, these combined physiological and systems biology analysis provides novel insights into peach fruit physiology.

Study of the regulatory mechanism controlling the climacteric ripening physiology in apple (*Malus x domestica* Borkh.) through an integrative approach combining transcriptomic assay with metabolite and physical analysis **S4P1**

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Ripening symbolizes the final stage of fruit development, during which the significant functional, biochemical and structural changes occurs. Apple (Malus x domestica Borkh.) is a model fruit species to investigate the metabolic modifications taking place at the onset of ripening and to elucidate the impact of ethylene in the physiological mechanisms. To dissect the climacteric interplay, a multidisciplinary approach was employed. A comprehensive analysis of gene expression together with the assessment of several physiological entities such as texture, volatilome and polyphenolic compounds was carried out over fruit development and ripening in apple fruit. Two genomic tools (microarray platforms) were employed in order to characterize the transcriptomic profiling. The first one is a custom array dedicated to fruit ripening pathways, while the second one is a whole genome array specifically enriched of ripening related genes for apple. We also studied the transcriptomic and phenotypic changes caused by the application of 1-methylcyclopropene (1-MCP), a molecule known to compete with ethylene at receptor level, interfering with the downstream signal cascade and largely used to extend the fruit shelf-life. The suppression of ethylene altered and delayed the receptor turnover, leading to important modifications in the overall fruit physiology. The integrative comparative network analysis showed both positive and negative correlations between ripening related transcripts and accumulation of specific metabolites or texture components. 1-MCP, beside to affect negatively the ethylene and texture control, stimulated the de-repression of transcription factors, photosynthethic and auxin related genes. These results showed the elucidation of the multi-layered control of ethylene, hypothesizing a possible hormonal cross-talk coupled with a transcriptional regulation.

Transcriptomic analysis and comparison of early and late harvest peaches and nectarines during ripening

S4P2

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The fruit ripening process in melting flesh type of Prunus persica implies several physiological, biochemical and morphological changes, being the most obvious modification the flesh softening. With the aim to understand the general and specific changes that participates in this process, the transcript expression profile in firm and soft fruit of early (Royal Glory and Magique) and late (Red Pearl and DU88) harvest peaches (Royal Glory and DU88) and nectarines (Magique and Red Pearl) melting flesh varieties were analyzed by RNA-Seq. A differential expression analysis determined that 394 sequences changed in all varieties during ripening, 607 transcripts changed only in peaches, 227 only in nectarines, 264 in late cultivars and 81 in early varieties. The principal component analysis (PCA) used 15,521 transcripts per library in order to supply a complete idea of the data profiles. The results exhibited a clear difference between genes in firm and soft conditions which expression depends on whether they are peaches or nectarines and also separate the data according harvest date. Both analyses showed that transcriptomic changes were greater in peaches compared to nectarines. On the other hand, when separately evaluated firm and soft conditions we found differences in 97 and 13 transcripts, respectively, showing that variances were greater at harvest moment than after ripening, independent of the harvest time or if they are peaches or nectarines. In spite of the differences in the transcript abundance, the transcriptome is conserved among varieties and during the transition from firm to soft flesh. These analyses agrees with the idea that commercial varieties in P. persica have a poor genetic diversity. This work was supported by Fondo de Areas Prioritarias Centro de Regulación del Genoma 15090007, PFB-16, FONDEF G13i1005. CONICYT D-21090737, Fondecyt 3140294, 3150538.

Stress responses in perennials; or how do trees "chill out"?

S5O1

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Due to the importance of phenological traits such as dormancy, chilling requirement (CR), bloom date (BD), response to abiotic and biotic stress, perennial plant breeders have traditionally focused on selections of breeding lines that exhibit a range of predictable variation for these important characters. In fruiting trees for example, having plant material that exhibits the range of genetic variation in traits such as CR, enables varieties to be optimized for fruit production in much of the temperate zone. These breeding materials also provide an excellent substrate to define and explore the links between phenological responses, many potentially driven by environmental stress, and control responses of the basic networks of gene activity (QTLs) underlying these perennial character states. However, to achieve this goal for phenological traits such CR, or biotic stress responses of perennials, we need to utilize simple perennial genome systems that have the genetic resources for the characters of interest and that facilitate the integration of the various levels of genetic control that modulate the important phenotypic states.

Others and we are utilizing the peach (*P. persica* (L.) Batsch) as a foundational genome species for comparatively exploring the genetic landscape associated with control of the perennial life cycle in trees and the gene networks associated with abiotic and biotic stress response. Currently, we are focused on understanding the gene network activities associated with winter dormancy (CR and BD) while exploring potential links with other stress responses that are known to impact these phenological characters (e.g. pathogen induced changes in floral timing). This introductory communication will present the conceptual framework that is emerging from comparative genomic studies of abiotic and biotic stress resistance in fruit and forest trees species highlighting significant advances in our understanding, as well as, significant questions that remain.

Effect of drought stress on gene expression, metabolites and phenotype in diploid and tetraploid apple (*Malus x domestica*)

S5O2

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Drought tolerance will become an important trait in fruit crops, mainly due to climate change. Hence to improve the knowledge on this topic we studied apple trees response to drought stress. The behavior of diploid and tetraploid one year old plants from 'Gala' and 'G40' cultivars under drought stress, was compared by measuring morphological traits (shoot growth, leaf number and area, number and size of stomata), physiological parameters (leaf water content, transpiration, stomata conductance, photosynthetic rate) and differential gene expression (RNA-seq). Tetraploid plants grew more slowly than diploids but under stress conditions all plants stopped growing after 10 days, when stress was still mild. Leaves of both ploidy levels had a similar size but tetraploid cells were larger. Tetraploids had larger stomata and stomatal aperture but lower stomatal density. Stomatal conductance was lower after drought stress in both genotypes. Tetraploids had lower stomatal conductance in control conditions but higher under stress conditions compared to diploids. This difference was also reflected in higher photosynthetic rate and leaf water content of tetraploid plants under drought stress compared to diploids. Differences were found in stress-related metabolites such as proline and different sugars but not for the antioxidants ascorbic acid and glutathione. Gene expression analyses were carried out on 'Gala' under mild stress. A relatively small number of genes was differentially expressed between diploid and tetraploid plants (<200; < 2%) both under control and stress conditions. Differences between control and stressed plants were much more pronounced: 2356 and 1437 genes in diploids and tetraploids, respectively. Differentially expressed genes were involved in metabolism (cell wall, sugar, nitrogen) and tetrapyrrole biosynthesis (chlorophyll). The differential gene expression was in line with the metabolic and physiological differences.

Transcriptional analyses of root responses under soil water stress of one-year old apple cultivars

S5O3

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Global climate change may lead to longer or more frequent episodes of extreme temperatures and drought, thus threatening plant crops. Thus, we focused on apple tree aiming to identify the molecular mechanisms that are triggered when trees face water soil deficit. We focused on differentially expressed transcripts (DET) in roots, between well-watered trees compared to trees grown under soil water deficit either moderate (MS) and severe (SS).

Two apple cultivars, 'Starkrimson' (ST) and 'Granny Smith' (GS), with contrasted behavior under water deficit, were grafted on M9 rootstock and grown in pots under controlled conditions. Roots were sampled when transpirable soil water (FTSW)=0.5 and FTSW=0.2 were reached, for MS and SS stress, respectively. RNA was extracted and analyzed using the AryANE micro-array apple chip to identify DET response to moderate and severe soil water deprivation. Among DET in roots, 209 up and 137 down-regulated genes were common to both cultivars under MS while 148 up and 542 down-regulated genes were common to both cultivars under SS. DET linked to the response to osmotic stress were observed at MS, especially signal sensors proteins kinases and phosphatases which were up-regulated. As expected, DET related to abscissic acid (ABA) were highly expressed under water deficit conditions, both ABA-dependent (ADP) and ABA-independent pathways (AIP) being stimulated. ABA biosynthesis was activated in both genotypes in MS, even though to a lesser extent in ST than in GS. However, these transcripts were no more differentially expressed in SS, suggesting that other mechanisms are involved under SS.

Specific DET to each genotype were also observed. ST had a higher number of upregulated DET at MS and downregulated DET at SS than GS. Such results prove that even when grafted on the same rootstock, we notice a significant difference on the ST and GS roots system response to the soil water deficit evidencing that root systems (here M9) response to the soil water deficit depends on the grafted scion, probably because of different transpiration and regulation in response to soil water deprivation of the two scion genotypes. Further analyses are currently performed to decipher DET in aerial organs.

Keywords : differentially expressed transcript, gene expression, gene function, Malus x domestica, moderate and severe water deficit, hormonal signaling, osmotic regulation.

Flowering time response to temperature in sweet cherry across Europe: multi-cultivar and multi-environment analysis

S5O4

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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, and may lead to a wide range of problems, in relation to flower and fruit set, cross-pollination, formation of double fruits, sun-scald, or novel host-pest interactions.

In this context, one of the challenges will be to breed fruit trees adapted to future climatic conditions. Predictive models for flowering phenology based on genetic and phenology data will represent a valuable tool to assist in the process. In order to support the development of these models, we present an analysis of a wide dataset of flowering dates for sweet cherry cultivars, under different climatic conditions in Europe. These phenology data were provided through a French national network of experimentation (coordinated by CTIFL) and a European COST Action on cherries led by INRA-Bordeaux. Based on statistical analyses and hierarchical clustering, results revealed common and distinct patterns in the response to temperature for the different groups of cultivars. These information will be useful to better understand the main trends in flowering behaviour under different temperature conditions. Furthermore, such approaches will be applicable to many other important phenological traits, such as maturity or fruit set.

Identification of a new pear QTL associated with spring vegetative budbreak

S5O5

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European pear (Pyrus Communis) is among the three predominant fruit crops in temperate zones. Most of the high standard fruit quality cultivars require more than 800 chilling hours. Due to climatic changes over the recent years and the aspiration to grow quality cultivars in warmer areas, there is a growing demand for low chill cultivars. The aim of this study is to analyze the genetic factors that determine the chilling requirements in order to create efficient tools for breeders to select individuals with this trait at early stages. In this study we analyzed two F1 populations segregating in their chilling requirements. 180 progenies from a cross between 'Spadona' (low chill cultivar) and 'Harrow Sweet' (high chill cultivar) and 37 progenies from a cross between 'Spadona' and 'Burre Hardy' (high chill cultivar) were phenotyped for traits related to chilling requirements over two consecutive years (2014-2015). Two clones were planted in ARO, Beit Dagan (50m a.s.l), Israel and another two clones were planted in pots and transferred during each winter to Tzuba (720m a.s.l) from Beit Dagan and back, in order to examine budbreak time in the same conditions after being exposed to different amount of chilling hours. The two sites were significantly different in their chilling hours. Normalization was performed to evaluate the genotype effect and the interactions with the year and location (GXE interactions), due to the variance gaps between the two sites. The genotype effect together with the GXE interactions explains nearly 70% of the model variance. Based on the synteny between apple and pear, 14 microsatellites markers (SSR) in the location of the previously published QTL's in apple (LG8 and LG9) were selected to genotype the F1 population extremes ('tail analysis'). The SSR fragments detected and sized by the ABI PRISM™ 3730xl DNA Analyzer. Linkage between SSRs and QTL's associated to the time of vegetative budbreak was found (LOD score>3).

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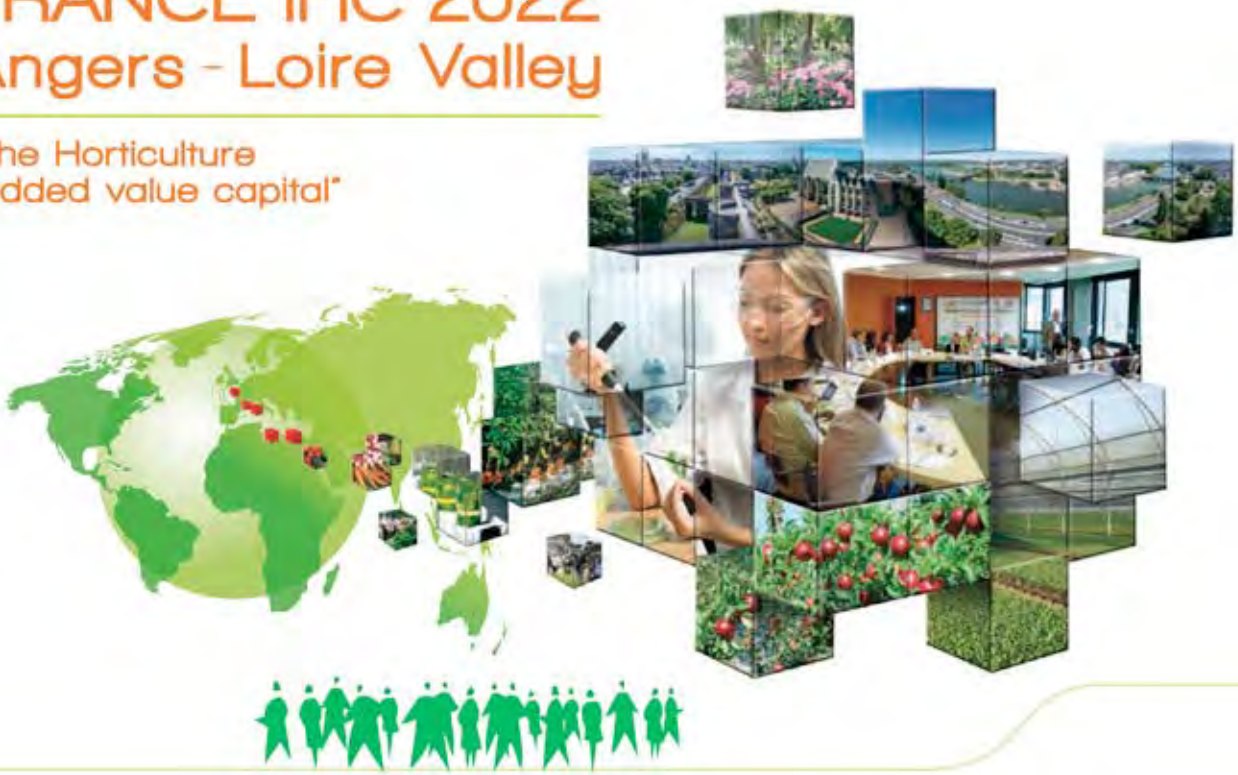

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Small RNA sequencing and DNA methylation analysis in floral bud reveal that RNA directed DNA Methylation (RdDM) participates during cold accumulation and dormancy release in sweet cherry (*Prunus avium* L.)

S5O6

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RNA directed DNA methylation (RdDM) is a plant epigenetic mechanism that involves several proteins and small/long non-coding RNAs. This process can generate *de novo* DNA methylation in every cytosine context (CG, CHG, CHH) and occurs mostly on transposons and repetitive elements. Usually, epigenetic modifications can be directly affected by environmental conditions like prolonged exposition to cold temperatures required for dormancy release and flowering in spring. It is known that in some members of the *Rosaceae* family, MADS-box genes involved in bud dormancy are negatively and epigenetically regulated by cold temperatures. In this work we analyzed RdDM participation during bud dormancy and cold accumulation through small RNA deep sequencing and bisulfite sequencing of two sweet cherry MADS-box genes (*PavMADS1* and *PavMADS2*). For small interference RNA analysis, adapters were removed from total reads and the range of size selected was between 18 and 24 nt long. Filtered reads were mapped with perfect match using Bowtie and *PavMADS1/2* sequences were used as reference. For *MADS1* first intron we observe siRNAs only before bud break, coincident with DNA methylation in this locus in all cytosine contexts. On its second large intron, the presence of complementary siRNA in every condition was also related with the maintenance of DNA methylation and the presence of a repetitive element. On the other hand, for *MADS2* no complementary siRNA were present and the methylation mechanism changes to CG methylation. Another RdDM component analyzed was the *DRM2* methyltransferases. Three putative *PavDRM2* were found in *Prunus avium* genome and their RNA level increased together with cold accumulation and bud break. Additionally, *MADS2* relative RNA level was higher than *MADS1* during dormancy, concomitant with the absence of siRNAs and methylations for the first gene. All together these results suggest that RdDM could be involved in dormancy regulation, producing a decrease in the RNA level of target genes.

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Inheritance of chilling and heat requirements for flowering in an inter-specific almond x peach progeny

S5P1

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Chilling (CR) and heat requirements (HR) and flowering time (FT) were studied in a in an inter-specific almond x peach F2 population (TxE) of 82 individuals obtained from the selfcrossing of 'MB 1.37' (an F1 hybrid from the cross by the almond 'Texas' and the peach 'Earlygold') at Gimenells (Lleida, Spain). Four different methods for estimating chilling requirements were evaluated and compared (below 7ºC, Utah, 0-7.2ºC and dynamic model), all of them showing a high significant correlation among them. The studied progeny showed a range of chilling requirements (chill units, CU) between 465 and 1294 CU, and a range of heat requirements (growing degree hours, GDH) between 372 and 5102 GDH, both between values observed for the parents and grandparents. However, flowering time showed a transgressive inheritance, observing some hybrids with later times than both founders. Correlations between chilling requirements, heat requirements and flowering date were also studied. Chilling requirements were negatively correlated to heat requirements, and positively correlated to flowering time. However, no significant correlation was found between heat requirements and flower time.

Preliminar QTL analysis identified a minor QTL for flowering time in LG1, two minor QTLs for chilling requirements in LG5 and LG6, respectively, and two minor QTLs for heat requirement in LG4 and LG5. QTLs for CR and HR on LG5 were closely located. Phenotyping will be carried out during next flowering season to confirm the results found.

Genotype x watering interaction of architectural and physiological characteristics in rose bush

S5P2

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Plant shape and, therefore, the architecture is a major component of the visual quality of ornamental plants in pot. Plant architecture is the result of growth and branching processes and depends on genetic and environment factors such as water supply, and their interaction. Genotypic responses of eight rose bush cultivars to alternation of water restriction (-20 kPa) and re-watering (-10 kPa) periods were analyzed at the architectural level through 3D digitalization using six architectural variables and at the physiological level by measuring stomatal conductance, water content, hormones, sugars and proline. Highly significant genotype and watering effects were revealed for all the architectural variables measured, as well as genotype x watering interaction, with two distinct genotypic architectural responses to water restriction - moderate and strong - represented by 'Baipome' and 'The Fairy', respectively. This difference in response could explain, for 'Baipome' compared to The 'Fairy', by: (i) the maintenance of the photosynthetic activity and budbreak during water restriction periods, probably due to a higher concentration in conjugated cytokinins (cCK) and to a lower concentration in salicylic acid (SA); (ii) a better resumption of budbreak during the re-watering periods, probably due to a lower concentration in abscisic acid (ABA). When associated with the six architectural descriptors, cCK, SA and ABA could be used as selection criteria for breeding programs aimed at improving plant shape and tolerance to water restriction.

Study on Abiotic Stress of *MdSBP20* Gene from *Malus*

S5P3

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To obtain the transcription factor related to stress conditions in apple rootstock, an apple *SBP* gene with the full-length cDNA was cloned in 'Qingzhen 1'(apomictic crabapple 'Pingyitiancha'(*Malus hupehensis* Rehder) ×columnar apple strain'CO' (*Malus*×*domestica* Borkh.) and 'M26' via homology-based cloning method. The gene cloned was named as *MdSBP20*. Bioinformatics analysis to the cDNA showed that the full-length cDNA was 1362bp, it's open reading frame possessed 1362bp, and encoded a polypeptide of 454 amino acids, which had obviously *SBP*-domain and two zinc finger structures (Zn-1, Zn-2) and the bothway nuclear location signal (NLS). Phylogenetic analysis suggested that *MdSBP20* gene had the closer relationship with *Pyrus bretschneideri* (XM_009339726.1) and *Malus domestica* (XM_008376435.1). Real-time PCR analysis showed that *MdSBP20* gene played a role in response to low temperature, drought, and salt tolerance. It was inferred that'Qingzhen 1' may has higher resistance than 'M26' no matter in stress of low temperature, drought, and salt. Based on the results in this experiment, *MdSBP20* gene had the significantly effects in apple rootstock response to abiotic stress.

Key words: Apple(*Malus*); Abiotic stress; *MdSBP20* gene; Gene clone; Expression analysis

A unique haplotype on chromosome 9 is associated with the ability to thrive under warm winter conditions, as revealed by characterization of old local apple accessions and their hybrids

S5P4

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Most commercial apple cultivars have moderate to high chilling requirements and therefore cannot produce under warm climate conditions. In addition, global climate changes raise concerns that some areas where commercial apples are currently grown might become unsuitable for those varieties. Apple production under warm climate conditions took place for many years in the Middle East, with local, well adapted varieties. These local apples, while having inferior fruit quality traits, served as a genetic source for the low chilling requirements trait in breeding programs in Israel in the middle of the previous century, and the 'Anna' cultivar, which spread into many warm areas around the globe, is their progeny. The apple germplasm collection at the Newe-Ya'ar Research Center contains about twenty accessions that have the ability to thrive under relatively warm climate conditions, most of them are not commercial varieties but old local accessions that were collected around Israel, cultivars that were bred in Israel, and one foreign cultivar. In addition, the germplasm collection includes approximately sixty accessions, local and foreign, with medium-to-high chilling requirements. The genetic basis of chilling requirements regulation in apples is not clear, however a major QTL for bud break time has previously been mapped to chromosome 9 by using a mapping population progeny of 'Anna'. Genetic characterization of the different accessions in our germplasm collection revealed that while the twenty warm climate accessions are quite diverse, they all share a unique, defined haplotype, inside the QTL region on chromosome 9. This haplotype does not appear in any of the medium-to-high chilling requirements accessions. Based on the polymorphism in this region we designed genetic markers that can be used in marker-assisted-selection in breeding programs aiming at developing apple varieties better adapted to warm climate conditions.

Transcriptome analysis of differentially expressed genes upon treatment with hydrogen cyanamide in peach

S5P5

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Peaches are a key alternative crop for growers affected by citrus greening in Florida, yet their temperate origins challenge optimal production in a subtropical climate. The majority of Florida's chill hours are accumulated in December and January, but in recent years accumulated chilling units continue to decline, posing a major challenge for growers. Hydrogen cyanamide (HC) is widely used where mild winters prevail with insufficient accumulated chill units. Timely application of HC can enhance uniform bloom and leaf emergence, increasing labor efficiencies; however, many growers have sustained significant crop injury because the application timing for different fruit crops is not well defined. In addition, the molecular mechanism of how HC works is still poorly understood. In order to find transcriptional pathways associated between dormancy release and HC application in peaches, we analyzed vegetative and reproductive buds in 'TropicBeauty' peach to identify gene expression triggered by HC application. 'TropicBeauty' was sprayed with 1% hydrogen cyanamide (BudPro, Green Trees & Plants II, LLC, Marietta, GA USA) when pollen grains were translucent (December, 2015). Single tree replicates (n=4) were sprayed with HC and compared with unsprayed trees. Vegetative and floral buds were collected from 4 branches per tree before application and 3, 5 and 7 days after application. RNA isolation was performed to carry out cDNA synthesis for real time PCR (RT-PCR). Ten genes involving dormancy release, cold regulation, and abscisic acid related genes were targeted for gene expression studies. The HC treatment resulted in uniform bud break and trees that bloomed 40 days earlier as compared to control. Control trees needed to accumulate the required chilling units and those with inadequate chilling displayed a prolonged bloom, uneven leaf emergence and fruit ripening. Gene expression analysis will be discussed, particularly in relation to dormancy-associated

Metabolites analysis of flower buds during dormancy in sweet cherry

S5P6

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Bud dormancy is the rest period that enables temperate fruit trees to protect sensitive tissue from unfavorable climatic conditions. This period is characterized by the accumulation of certain amount of cold temperatures - the chilling requirements - that are necessary for breaking dormancy and subsequent successful flowering.

The mechanisms involved in dormancy induction, maintenance and release are still poorly understood. In order to identify key pathways controlling the dormancy process in sweet cherry flower buds, we investigated several key metabolites, including sugars and amino acids, during the dormancy period in a low chill cultivar ('Cristobalina') and a high chill cultivar ('Regina').

Upon dormancy release, a complex metabolic shift takes place, and the time course of several metabolites enables describing different metabolic phases of dormancy. In particular, the time lag between the decrease /increase of some metabolites in the two cultivars suggests that they are good markers for dormancy release.

Molecular and epigenetic mechanisms during dormancy in sweet cherry flower buds

S5P7

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In perennial species, dormancy is a period of repressed growth that acts to protect sensitive tissues during unfavorable climatic conditions. Dormancy and bud burst are strongly affected by temperature. The mean surface temperature of the earth is increasing and this climatic change may have serious negative consequences on the dormancy release, potentially resulting in lower cherry production. Despite this strong effect of temperature on dormancy, the molecular events regulating dormancy and the effect of temperature on dormancy are still not very well understood. It has been shown that DORMANCY-ASSOCIATED MADS-box (DAM)-related genes are up-regulated in dormant buds in a certain number of plants (peach, raspberry, leafy spurge, poplar). Recent studies also highlighted the presence of epigenetic mechanisms in the regulation of bud dormancy. To better understand the mechanisms underlying the effect of temperature on dormancy we are assessing genome-wide dynamics of several chromatin marks and expression during dormancy in floral buds of sweet cherry (Prunus avium L.) varieties with contrasted bud burst dates. To validate the role of epigenetic mechanisms in dormancy and the dormancy release in cherry floral buds, we are also studying the effect of epigenetic drugs on the dormancy cycle of floral buds. We observed a distinct change in histone methylation in floral buds during the dormancy cycle. We could also detect transcriptional dynamics of dormancy-related genes and we are currently analyzing their interplay. The results of floral bud treatment with epigenetic drugs also confirmed the crucial role of chromatin dynamics in bud burst. Ongoing and future studies will help to identify mechanisms and gene markers involved in dormancy in sweet cherry in order to create new varieties of Prunus species that are adapted to future climatic conditions.

Synergy and success: Industry stakeholders and the Rosaceae Genomics Conference S6O1

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Since the first Rosaceae Genomics Conference in Toreilles, France in 2000, the international research community has clearly achieved its original intent: "to promote scientific interaction among the major research groups working on genetic and physical mapping research of important fruit tree crops in Rosaceae." In fact, RGC8 manifests a breathtaking expansion in technical scope, range of rosaceous crops, diversity of participants, and impact of rosaceous research activities worldwide. To scientists, the promise of genomics has been fulfilled almost beyond comprehension. What seemed hopelessly aspirational in 2000 is now routine. Widely available DNA sequencing technologies, analytical tools, database resources, and well-trained scientists make earlier genetic and physical mapping work seem quaint and the notion of a model species nearly frivolous. At the same time, to many industry stakeholders and breeders, genomics applications in rosaceous crops have appeared only somewhat successful, exorbitantly expensive, and routinely oversold. Fortunately, many other industry stakeholders and breeders worldwide have become vigorous advocates for investment in genomics research. Thus, community-based projects like FruitBreedomics and RosBREED, as well as numerous smaller-scale regional and national efforts, received critical support and funding opportunities. Outcomes of these projects are beginning to contribute directly to produce the most tangible of outcomes at the industry stakeholder level: superior new cultivars. The first generation of students trained in breeding yet fresh with substantial competencies in applied genomics – another tangible outcome – are now entering into private and public sector breeding programs worldwide. The RGC has become an increasingly popular and meaningful event – one more tangible outcome. RGC1 was based on the somewhat modest aim of promoting scientific interaction among major programs. It is now apparent that initial catalytic event, complemented by the community's commitment to deliver impact to plant breeders and crop producers, began a process that energized the global rosaceous research community, attracted industry support, and still guides us on our current pathway.

Towards gene pyramiding for apple scab resistance following the working results at VNIISPK

S6O2

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Involving of marker assisted breeding (MAB) methods in breeding for apple scab resistance has been recently started at the All-Russian Research Institute for Fruit Crop Breeding (VNIISPK).

At the first stage of work, evaluation of important germplasm from the VNIISPK collection on the presence of scab resistance genes by means of DNA-markers has been done. Scab resistance genes Vf, Vm, Va1, Vh2 have been identified at VNIISPK germplazm collection. In the most cases previously known the presence of Vf and Vm genes was supported by DNA-markers data. Exception has been found for two scab resistant columnar cultivars 'Poezia' and 'Priokskoye' obtained by open pollination of maternal parents carried no Vf and thus 'Poezia' and 'Priokskoye' had not been expected having this gene. They have shown the presence of Vf according to the related DNA-markers and most likely have inherited it with pollen. Gene Va1 has been identified in old and new Russian cultivars. Cultivars and selection having markers of several genes have been found (Vf and Vm – 'Poezia'; Va1 and Vf – 'Svezhest'; Vf and Va1 – 'Zarianka', 'Patriot' and 'Sokovinka'; Vf and Vh2 – 27-1-222). These genotypes may be used in further breeding for combining genes of apple resistance to scab, and they are perspective for cultivating from the point of view of long-term resistance.

The MAB for scab resistance based on Vf and Vm genes has been carried out in the seedling nursery since 2012. Hybrids combining Vf and Vm associated markers have been found. Fructiferous hybrids homozygous for marker allele in coupling with the Vf have been revealed. The best of them will be used for further breeding.

MAB for scab immunity complementing other breeding methods would contribute in development of new adaptive and environmentally-friendly apple cultivars.

Validation of genetic markers for fruit quality and disease resistance in apple breeding germplasm using the openArray® technique

S6O3

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Genome mapping and analyses have aided fruit tree breeders during the last 10 years, yet one of the greatest challenges remaining to fruit tree geneticists is the translation of QTLs and whole genome sequences into diagnostic assays that are both efficient and cost-effective for use by breeders in making decisions on parental choice and seedling selection. We chose a set of 128 apple SNPs linked to fruit quality and pest and disease resistance trait loci for design of the apple International RosBREED SNP Consortium openArray v1.0 assay (IRSC oA v1.0). The Thermo Fisher Scientific / Life Technologies openArray® technology enables multiplexed screening of genetic markers using the fluorescent probe-based Taqman® assays and a real-time PCR instrument. The apple IRSC oA v1.0 was used to screen 240 individuals from the Plant & Food Research apple breeding programme. These individuals included cultivars, elite selections, and families segregating for the numerous breeding-relevant trait loci. In total, 78 SNP markers (60%) in the IRSC oA v1.0 assay were validated for use in marker-assisted selection. A subset of these markers were subsequently transformed into single tube Taqman® assays for use in apple breeding internationally. This work was enabled by the European Commission's 7th Framework Programme, Fruit-Breedomics, USDA's National Institute of Food and Agriculture Specialty Crop Research Initiative, RosBREED, and New Zealand's Ministry of Business, Innovation and Employment and PREVAR Ltd Pipfruit Research Consortium 2 projects.

From QTLs to routine DNA-informed breeding: prospects, advances, and needs

S6O4

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Once, marker-assisted breeding in Rosaceae meant genetic mapping and QTL discovery, at least to lab-based scientists. Now, researchers and funders of this model horticultural crop family have embraced the understanding that breeding impact from genomics requires focused attention on translating discoveries into breeder-friendly tools and information. Translational steps to achieve routine DNA-informed breeding include breeder confidence in the benefits of DNA information, systematic validation of QTLs in germplasm representative of specific breeding programs, development of robust and versatile DNA tests that differentiate high-value contrasts in genetic potential, identification of cost-efficient DNA testing schemes that also consider genetic gain, availability of cost-effective and timely DNA testing services and streamlined tissue-sampling operations, and obtaining experience in routine practice to identify shortfalls and opportunities. Translation to practice in the apple and cherry breeding programs of Washington State University and other demonstration breeding programs of the RosBREED project have demonstrated what works and what doesn't. Compelling needs are improved accuracy of trait performance predictions from DNA information, evaluation of multi-trait genetic potential that is locus-specific and genome-wide, breeding information management tools, and training of the next generation of professionals in horticultural breeding and genetics.

Using SNP arrays to leverage historic data sets for improved prediction accuracy and estimation of GxE of fruit maturity in sweet cherry

S6O5

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Improved prediction accuracy of the genetic potential of advanced selections and cultivars for commercially relevant traits in commercially relevant conditions will lead to increased response to breeding. While a large number of loci might influence quantitative traits, QTLs with major trait effect have been identified, and can be used for selection if the effect of alleles on commercial performance can be accurately predicted. However, selection response will be compromised if the effect of major trait loci are confounded with polygenetic effects from the background genome - if not accounted for, and external factors such as management practices or environment conditions also influence the trait. Here, we propose a novel approach to improve prediction accuracy of genetic potential under commercial conditions by using SNP arrays to characterise variation at major effect QTLs and estimate realised additive and dominance relationships among individuals that have also been evaluated for traits of interest. This approach enables data from otherwise disconnected historical data sets to be combined into a single analysis, thereby increasing prediction accuracy, without the need for replication of individuals. In addition, the different locations from which the performance data have been collected represent samples of the variability of external factors. As such, this analysis can be also used to investigate the influence of these factors on the stability of both major-effect QTLs and polygenetic effects. As part of the RosBREED project, we demonstrate this approach and results using 800 sweet cherry individuals that have were genotyped using the 6K SNP sweet cherry array and evaluated in the USA (Prosser, Washington),Canada (Summerland British Columbia), France (Boudreaux), and Italy (Forli).

Character integration, breeding goals compatibility and selection indexes using genome wide breeding values: a study case

S6O6

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Breeding in apple is characterized by a high number of characters concerned, related to several biological processes. The main characters are fruit quality, disease resistance and yield. In addition, the will to reduce the use of chemical products in agriculture and the changing climatic context causes the need to breed for new complex characters such as yield regularity and adaptation to temperature increase. This work is a study case of trait integration for fruit quality, yield level and regularity, and bud phenology related traits. Compatibility of breeding objectives, according to one fixed ideotype, were analysed and a strategy for the introduction of new characters in breeding schemes using molecular markers, breeding values estimation, and selection indexes was proposed. Two unrelated families were phenotyped, QTL analyses were run with the Bayesian software FlexQTL™ and the individual genetic breeding values were computed trait by trait by adding the genetic effect of loci estimated during the QTL mapping process. An ideotype integrating all traits was fixed and principal component analyses were realized in order to study the breeding goals compatibility. Traits were integrated in two selection indexes computed with breeding values. Synergistic correlations between fruit size and yield regularity was identified and antagonistic correlation was observed between fruit size and yield level, and yield regularity and level and partially explained by colocalization of QTLs with high opposite effects according to the ideotype. In case of antagonistic correlation between important traits, a genetic ideotype could be a tool to monitor precisely the choice of genitors and identification of progenies compatible with the ideotype. Principal component analyses and selection indexes values revealed a differential genetic potential of the two families depending on traits and their correlations, and interesting individuals could be identified in both families even if selection indexes values were in favor of one of the families. In conclusion, the use of molecular markers, genetic effects estimation in a genomic selection approach, combined with a selection index strategy would be an efficient way to introduce simultaneously new complex characters in breeding schemes.

Integration of Genomic Selection into the University of Florida strawberry breeding program

S6O7

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Genomic Selection (GS) aims to increase genetic gains for complex, polygenic traits by predicting performance of individuals when phenotypic data for those individuals has not been obtained. The objective of this study was to evaluate the potential of GS in the UF strawberry breeding program and to define a breeding strategy for its operational implementation. Four clonally replicated field trials, two in each of two years comprised of a total of 1628 genotypes, were established in 2013-14 and 2014-15. Four traits, average fruit weight (AWT), total marketable yield (TMY), proportion of cull fruit (TC) and soluble solids content (SSC) were assessed weekly in each season. Individuals were genotyped using the IStraw90 Axiom® SNP array, from which 17,479 markers were chosen for analysis after marker quality control. Several GS methods including Genomic BLUP (GBLUP), Bayes B, Bayes C and Reproducing kernel Hilbert Space (RKHS) were explored and compared based on their predictive ability (PA). True validations of GS were performed using the four trials as training and validation populations. The efficiency of GS was assessed as the ratio of genetic gain when selecting the top 5% and 10% of individuals obtained by using a GS model trained in one test/year (T1) to predict breeding values in another test/year (T2) compared to the gains obtained from a model using T2 marker and phenotypic data to estimate breeding values. The different GS methods showed similar PA for a given trait, but PA varied among traits, from 0.306 for TMY to 0.515 for AWT. Accuracy of prediction ranged from 0.363 for TC to 0.766 for SSC. The efficiency of GS ranged from 28% to 74% for the four traits, indicating that at as much as three quarters of the gains obtained using phenotypic information could be obtained by selection using markers only. The UF breeding program aims to implement GS during the 2016-17 breeding cycle by selecting a proportion of parents for crossing before they have been clonally tested, thus reducing the average length of the breeding cycle. If whole-genome genotyping costs can be sufficiently reduced, seedling selection would also become a viable GS approach in strawberry.

Prediction of apple fruit phenotypes using genome-wide markers: progress and challenges

S6O8

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Genomic selection (GS) is successfully being applied in breeding programmes of commercial plant and animal species. Non-additive genetic effects may have an important contribution to total genetic variation of complex traits, so genomic prediction of both the additive and non-additive effects may be desirable if both breeding value for parent selection and genotypic value for commercial cultivar selection are to be obtained. The influence of non-additive genetics and genotype-by-environment interaction on GS accuracy are not well understood for apple fruit quality traits. Understanding the persistence of GS accuracy over generations is also essential for recalibration of GS models. Plant & Food Research (PFR) have phenotyped and genotyped a series of experiments to investigate these aspects of GS technology. Averaged over several traits, the estimated non-additive genetic variance was about 20% of the total phenotypic variance. However, the accuracy of GS were almost identical for models with or without including non-additive effects. Genetic relationship between the training and the validation population, along with trait heritability, were among the key factors influencing GS accuracy. Accuracy of predicting performance at untested sites was largely influenced by trait heritability. Results on the prediction accuracy of GS models across breeding cycles, along with their implications on the accelerated breeding of novel cultivars, will also be presented.

Main achievements of COST Action FA1104 'Sustainable production of high-quality cherries for the European market'

S6P1

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The aim of COST Action FA1104 'Sustainable production of high-quality cherries for the European market' was to create a stable network of scientists and other professionals interested in sweet or sour cherry production, both from academic institutions and the private sector. This network has addressed, in a highly multi-disciplinary way, all aspects related to cherry production, commercialisation, and consumption. A special emphasis was placed on key EU priorities such as the promotion of sustainable agriculture, adaptation to climate change, and the development of high-quality fruits from a nutraceutical point of view. The Action covered the period April 2012- April 2016 and was organised in four working groups: 'Genetic resources and breeding, Genetics and Genomics', 'Crop Production', 'Crop Protection' and 'Socio-economics'. The cherry COST was particularly active, involving over 300 members from 44 countries in Europe and abroad. Of these, over 200 participated to 25 meetings, five training schools and were involved in 30 short-term scientific missions. Some of the main deliverables of the project were the exchange of data between research teams, the adoption of common experimental protocols, the implementation of predictive models in the fields of epidemiology and tree phenology, and the establishment of coordinated European marker-assisted selection strategies, including multi-location field trials. Here we synthesize the main scientific achievements of this Action, with a particular focus on those having direct implications on sweet or sour cherry breeding programs.

Improving the stability of high-quality traits of berry in different environments and cultivation systems for the benefit of European farmers and consumers

S6P2

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The main objective of GoodBerry is to provide the necessary knowledge and procedures to facilitate the development of highly productive and top quality berry fruits, even under multiple suboptimal growth conditions, at competitive costs. The project is based on an integrative multi-actor approach, from cultivation techniques to molecular studies, aiming at the development and validation of a range of tools to improve competitiveness of the European berry production, and eventually the attraction and confidence of consumers. The selection of the model species can be considered as strategic since strawberry is the most important berry crop in Europe and the production of raspberry and blackcurrant are increasing strongly in recent years.

The project will apply the most recent technical advances in:

- The identification of berry germplasm exhibiting advantageous balance of production vs nutritional quality throughout the EU,
- The search of innovative production systems to maintain high yield in a range of European-wide environments,
- The development of standardized and reliable analytical tools to evaluate berry production and fruit quality.

As result, it is expected:

- The implementation of modern breeding strategies to accelerate the release of new berry cultivars;
- The adoption by EU-growers of high quality production systems to improve fruit quality.

The project establishes as obligatory to disseminate and communicate the results to the scientific community, industry, the broad public and interested stakeholders' user. The final impact will be to consolidate the emerging needs of high-quality berries, and to boost consumer and market confidence supported by an improved competitiveness of producers. It is a multidisciplinary, collaborative project based on complementary expertise and skills of internationally recognized berry research institutions, and highly involved key berry SMEs that will combine their effort to secure the robustness of the results.

RosBREED: Combining disease resistance and horticultural quality in new rosaceous cultivars

S6P3

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Rosaceous crop breeders have identified resistance to important diseases from wild and/or unadapted germplasm, but resistant cultivars have only rarely achieved commercial success due to shortcomings in horticultural quality or other critical attributes. RosBREED 2 is addressing this need through a nationally-coordinated multidisciplinary effort, empowering breeding programs of rosaceous crops to routinely apply modern genomics and genetics tools and to more efficiently and effectively deliver cultivars with producer-required disease resistances, productivity, and market-demanded horticultural quality. The project builds on the recently concluded USDA NIFA SCRI project "RosBREED: Enabling marker-assisted breeding in Rosaceae", which enabled the first routine application of DNA-based information for horticultural quality improvement in U.S. apple, peach, cherry, and strawberry breeding programs. Four crops are newly included in RosBREED 2 (blackberry, pear, rose, and Prunus rootstocks) and 16 disease threats are targeted. The use of DNA information is being expanded from individual QTL/major trait loci to develop a genome-wide understanding of the effects of minor and major loci and to enrich breeding families. Additionally, genomic and statistical tools are being applied to account for non-genetic effects on trait variation. This project, like the prior RosBREED project, is committed to strong collaborations with the international Rosaceae community. Current progress and plans will be highlighted to continue fostering international partnerships. RosBREED 2 is funded by the USDA NIFA SCRI Award number 2014-51181-22378.

Cornell Apple Breeding and Genetic Diversity Studies

S6P4

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Cornell's program has been studying wide hybridizations, characterizing germplasm in the USDA collection for phenolic compounds, and conducting studies on genes of interest. Learning objectives are to see germplasm characterization within a breeding program and why it is of interest and how results may be leveraged. Recent studies have included the elucidation of the pale green lethal from a genetic, genomic and physiological perspective. Breeding ornamental crabapples has lead to interesting discoveries about the genetics of leaf size, leaf-lobing and coloration. These studies were part of our summer scholars research projects, emphasizing the use of progenies for short-term studies. Methods to enhance the development of double flowered offspring will be discussed. Interspecific hybridizations have been made to study epigenetics, using the columnar gene as one indicator. Breeding for varieties suited to fresh use and as a base for alcoholic ciders is on going as is the characterization of apple germplasm for phenolic compounds. The commercialization and marketing of the two most recent apple varieties from the Cornell program also will be discussed. New challenges to breeding will be detailed, such as new invasive pests and diseases usually problematic in more southern regions. While this study prevents diverse topics, their interactions and applications to apple breeding will be stressed.

The development of scab immune (vf), triploid (3x) and columnar (co) apple cultivars

S6P5

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In 1976, a large-scale breeding program for developing scab immune (Vf) apple cultivars in Russia was started at the All Russian Research Institute for Fruit Crop Breeding (VNIISPK). By 2015, 23 apple cultivars were included in the State Register of Breeding Achievements Admitted for Use. The most well-known cultivars are 'Bolotovskoye', 'Veniaminovskoye', Imrus', 'Candil Orlovsky', 'Orlovskoye Polesie', 'Rozhdestvenskoye', 'Svezhest', 'Solnyshko', 'Stroevskoye' and 'Yubilar'. At the first stage of work, Scab immune hybrid seedlings 814, 1924, etc. were used as donors of immunity (V_f). Now, marker-assisted selection is used for breeding scab immune cultivars.

Apple breeding with using polyploidy was started at VNIISPK in 1970. The most promising method for developing hybrid pools of triploid seedlings was from crossings of orthoploidy type: diploid x tetraploid and tetraploid x diploid.

By now, for the first time in Russia, 10 triploid apple cultivars were obtained from these crosses and regionalized: 'Avgusta', 'Aleksandr Boiko', 'Bezhi Lug', 'Vavilovskoye', 'Dariona', 'Maslovskoye', 'Orlovskiy Partizan', 'Osipovskoye', 'Patriot' and 'Yablochny Spas', with four having scab immunity ('Aleksandr Boiko', 'Vavilovskoye', 'Maslovskoye', 'Yablochny Spas'.

Columnar apple breeding started in 1984. Six columnar (Co) and scab immune (Vf) apple cultivars were developed, 'Priokskoye', 'Poezia' and 'Vostorg' were regionalized and 'Orlovskaya Yesenia', 'Girlianda' and 'Sozvezdie' are passing State Trials.

Developing triploid columnar and scab immune apple cultivars is a focus. The selection 30-47-88 genotype (V_f + 4x) is both a donor of scab immunity and diploid gametes. Twelve columnar scab immune triploid seedlings were developed using selection 30-47-88.

Development and evaluation of a temporary immersion system for mass propagation of sweet cherry cultivars and cherry rootstocks

S6P6

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Sweet cherry varieties and cherry rootstocks form part of an important fruit industry worldwide and clonal mass propagation of relevant genotypes are an opportunity for the use of temporary immersion systems (TIS). We describe the establishment of tissue culture procedures for TIS in four trendy genotypes: rootstocks Maxma-14 and Colt, and varieties Van and Rainier. Liquid immersion assays were started using internodal segments from 30 d sub-cultured seedlings grown in semi-solid DKW-modified propagation medium. Preliminary assays were set using five different modifications to PM and the best two combinations selected (medium A and medium B). A TIS protocol was set for cultures of 14 d in length, and comparisons between TIS and semi-solid were carried out evaluating number of plants (Px), biomass (Qx) and sucrose consumption. Immersion time (T; for T=1 and 3 min), immersion number (N; for N=2-4) were evaluated in addition for TIS. Results showed that Maxma 14, Colt, and 'Van' TIS cultures improved performances compared to semi-solid; 'Rainier' did not showed differences. The best Qx and Px were obtained by rootstocks Maxma and Colt at N = 4 and medium B. In Maxma, Px increased 120% over semi-solid culture. 'Van' explants were also responsive to these TIS conditions, although some hyperhydricity in bigger leaves was observed. Low performance in 'Rainier' explants was accompanied of important extents of vitrification. Stomatal opening microscopy showed that 14-d TIS-produced plantlets had an intermediate stage between semi-solid (14-d) and greenhouse (adult) plants. Photosynthetic efficiencies of these TIS-produced materials depicted a lack of autotrophic capability at this rate. The successful genotypes were conducted to whole plants in a 65-70 d pipeline, including 14-d in TIS, 30-d for rooting in rooting semi-solid media, and 15 d for acclimatization in greenhouse. Funding: FONDEFG09I1008.

Conversion of DNA tests to high-throughput technologies supporting apple breeding decisions

S6P7

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DNA-informed breeding is conducted routinely in U.S. Rosaceae breeding. However, to remain a feasible and efficient strategy for multiple programs that vary in access to DNA-based diagnostic services, trait-predictive DNA tests need to be adapted to a range of markers and platforms. Although simple PCR-based markers such as SSRs and SCARs have been the markers of choice in the last five years, they can sometimes provide ambiguous results requiring subjective scoring and leading to inconsistent interpretations between laboratories and even among personnel within a laboratory. Even with adequate quality control in technical operations, genotype calling is often time-intensive. New genotyping platforms, combined with existing decision-support tools have reduced the ambiguity of genotype calls and streamlined the process of scoring, organizing, and delivering results to breeders for timely crossing and selection decisions. Within the context of the USDA-NIFA project RosBREED, five DNA tests routinely used in several U.S. apple breeding programs (SSR or SCAR markers) were converted to SNP-based assays for the High Resolution Melting and Taqman platforms. The University of Minnesota's breeding program used DNA test information in the spring of 2016, saving the program more than 50% of projected operational costs for the first round of seedling selection in the orchard. To enable use of these updated DNA tests to more apple breeding programs, descriptions of their essential features were posted online at www.rosbreed.org/breeding.

Marker Assisted Selection (MAS) in apple: case studies for red skin coloration and Rvi12 (Vb) scab resistance

S6P8

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Marker Assisted Selection (MAS) has become an essential tool for plant breeders. MAS uses molecular markers to assemble desirable allelic combinations in new varieties. In Rosaceous fruit trees, MAS can be applied at the young seedling stage, reducing costs and time. A crucial step in MAS is the choice of cost-effective DNA markers associated with the traits of interest, such as disease resistance and fruit quality.

MAS was applied in the apple breeding program at the Foundation Edmund Mach (FEM) over the past years. Originally microstaellites were chosen for MAS, based on published loci. However new markers, such as SNP markers using real-time PCR assay, were developed and evaluated. A functional genetic marker was developed in collaboration with Plant & Food Research (New Zealand) for red skin colouration. This marker predicts apple colour in a wide range of germplasm, including breeding populations growing in warm summer environment. Another example of SNP-based marker developed for MAS is for the apple scab resistance gene Rvi12 (Vb) derived from the 'Hansen's baccata #2'.

Integration of a molecular marker for the highlighter (red skin color suppression) trait in a peach breeding program

S6P9

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IRTA-ASF-FruitFutur peach breeding program started the integration of molecular markers in 2010. Since then marker-assisted selection has been applied to several monogenic traits including fruit shape (flat/round), subacid (acid/subacid), slow ripening (slow ripening/normal ripening) and male sterility (fertile/sterile). Here we present the development of a new marker for the highligther trait, characterized by the suppression of the red skin color, which is controlled by a single recessive gene (H/h). For the development of a molecular marker, an F2 progeny of 276 individuals segregating for this character was created and phenotyped. Two-hundred and five individuals presented red color covering fully or partially its surface, corresponding to HH and Hh genotypes, and 71 presented absence of red color (hh). The highlighter trait mapped to linkage group 3, co-locating with a MYB10 transcription factor. A codominant marker based on the sequence of this gene was used to genotype the F2 progeny and cosegregated with the trait. To further validate the marker it was tested in a collection of 87 peach cultivars. The nine cultivars with no red skin color were homozygous for the marker allele associated with the highlighter trait, confirming the association marker-character, and allowing its use as a diagnostic marker for parent and progeny selection.

Identification of almond genomic regions in four 3-way interspecific hybrid progenies **S6P10**

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The challenge in rootstock breeding programs is the combination of abiotic stress tolerances in new interspecific hybrids including crosses combining almond, peach and plum genotypes in order to obtain rootstocks adapted to a wide range of soil conditions. Marker Assisted Introgression (MAI) can efficiently be used to identify almond regions in several progenies. We analyzed four 3-way interspecific hybrid progenies and their parental genotypes (two myrobalan plums 'P.2175' and 'P.2980', the almond-peach hybrids 'Garnem' and 'Felinem', 'Garfi' almond and 'Nemared' peach). Forty-eight polymorphic SSRs in the parental genotypes were screened along the eight linkage groups obtained from several Prunus reference maps. The UPGMA dendrogram generated using the genetic variability observed, classified the genotypes in five different clusters allowing us to differentiate the almond genomic regions from the peach and plum background in our progenies. The study of some candidates genes involved in drought stress tolerance in those regions will be accomplished as well as comparative genomic analysis once the almond genome will be available.

An apple amiRNA efficiently silences the phytoene desaturase gene in apple

S6P11

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Artificial miRNA (amiRNA) is a powerful technology to silence genes of interest with a high specificity, either to validate their function or to create new traits. To set up such a gene regulation tool for apple, we designed two amiRNA constructs based on an apple endogenous miRNA backbone that was previously characterized (Md-miRNA156h), and we checked their efficiency on an easily scorable marker gene: the phytoene desaturase gene (PDS). Two pairs of miRNA:miRNA* regions were designed according to the recommendations published by Whartman et al. (2008). The monocistronic Md-miRNA156h with these PDS targets was placed under the CaMV 35S promoter and cloned using the Gateway recombination method in the destination plasmid pK7WG2D, generating the two plasmids pAmiPDS-h and pAmiPDS-w. Two *Agrobacterium*-mediated transformation experiments were performed on the cultivar 'Gala', with a rate of transformation of 2 % for pAmiPDS-w and 3.4 % for pAmiPDS-h. In total, 5 and 10 independent transgenic clones were recovered, respectively. Most transgenic lines had a typical albino and dwarf phenotype. However, three clones had a wild type green phenotype. Molecular analyses are underway to correlate the phenotype with the degree of expression of the amiRNA gene and of the PDS gene. This study is the first demonstration in apple of the functionality of an amiRNA based on an endogenous miRNA backbone. It provides important opportunities for apple genetic functional studies as well as apple genetic improvement projects.

An unexpected candidate gene may be involved in the control of fruit acidity in peach

S7O1

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Acidity is one of the major components of fleshy fruit taste and was attributed to the D locus in peach. The D locus was mapped to the top of linkage group (LG) 5 by using a progeny derived from the cross between the sub acid cultivar 'Ferjalou Jalousia®' (D/D) and the acid cultivar 'Fantasia' (d/d). The D locus was delimited to a genetic interval of 0.4 cM corresponding to a DNA fragment of 100 kb. Two BAC clones, each corresponding to an allele were sequenced. Using the peach annotated genome sequence as a reference, 9 potential genes were located in this region. To our knowledge, none of them are known to be involved in the control of fruit acidity. Differential expression analysis of RNA-Seq data generated from fruits sampled at different stages of development from 'Ferjalou Jalousia®' and 'Fantasia' revealed 17 genes with significant differential expression on LG5, but only one of them located in the D locus. The expression of this gene was significantly higher in the 'Ferjalou Jalousia®' than in the 'Fantasia' fruits. This gene potentially encodes a protein with high similarity to Glucan endo-1,3-beta-glucosidase (GebG). Comparative analysis of the two allele sequences of the GebG gene and its surrounding intergenic sequences revealed an insertion/deletion in a tandem repeat sequence localized upstream of the transcription initiation start site. Analysis of this region in a panel of peach trees producing acid and sub-acid fruits showed a highly significant association between the insertion and the low acidity of the fruit. This tandem repeat sequence could be -involved in the transcriptional regulation of GebG. qRT-PCR analysis of GebG expression along the development of peach fruits confirmed the results of RNA-Seq analysis and revealed spatial and temporal control of GebG expression. All these results strongly suggest that GebG may play a major role in the control of fruit acidity in peach.

Transcriptional, genetic and chemical approaches to understand tolerance to cracking in sweet cherry fruits

S7O2

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The Chilean fruit industry has positioned itself as a key market for the country's development. To lead this market at the international level fruits must arrive in a very good condition/quality. Sweet cherries have become a fruit with a high value for the exporters and the growers. One of the problems associated with loss of production is cracking of sweet cherry fruit. Towards this end, it is essential to understand the molecular mechanisms that are involved in this disorder as well as identify genes and markers linked to this problem. In order to achieve this goal two approaches are under way. A chemical approach was carried out to determine the role of alkenes in cracking using five different varieties of sweet cherry. After removal of cuticular wax, fruit cracking was significantly increased. Nuclear magnetic resonance analysis (1H- and 13C-NMR), revealed that fruits of different sweet cherry varieties contain primarily n-alkane with 29 carbons and no iso-alkane. Gas chromatography–mass spectrometry (GC-MS) enabled identification and quantification of n-alkanes. Varieties with significantly higher concentrations of nonacosane (Kordia, Regina and Lapins) were more tolerant to cracking compared to varieties with lower amounts (Bing and Rainier).

On the other hand, we used 454 Roche technology (454 GS-FL and 454 GS-FLX) to sequence the transcriptome of mature fruit from three sweet cherry varieties (Bing, Lapin and Rainier). Then, using CLC Genomics Workbench de novo assembly, we obtained a reference transcriptome of 20,349 contigs over 200 bp with a mean length of 919 bp. Illumina sequencing was performed for Bing variety under cracking water stress conditions. Several genes that might be involved in this disorder were identified. We have constructed a plum linkage map to validate and position the genes identified in sweet cherry.

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Analysis of genetic control of fruit size in apple using both multiple, pedigree-related and single full-sib families

S7O3

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Fruit-size is an important component of both appearance quality and productivity in apple. Identifying QTL controlling this trait will provide tools for marker-assisted selection which should enhance breeding efficiency. We present results on genetic control of fruit size in a large and diverse population composed of 24 pedigree-related full-sib families and in two single full-sib families, D1980-15-25 x D1973-01-41 (DLO-12) and 'Telamon' x 'Braeburn' (TeBr), all genotyped with the apple 20K array in the frame of the EU FP7 FruitBreedomics project (www.fruitbreedomics.com). Phenotypes for fruit size were obtained on a 1-5 scale between 2003 and 2005 for the pedigree-related families whereas fruit weight was recorded in the two single families in two or three growing seasons. Three QTL for fruit size were detected on LG1, LG11 and LG12 in the pedigree-related families when the three years were analyzed together. The first two regions were described for the first time in this study while the last one could confirm a previously reported QTL for fruit weight. Additional QTL regions on LG16 and LG17 were expressed only in one year. In the DLO-12 family, we detected three QTL regions stable over two years, on LG9, LG 12 and LG14, and three QTL regions expressed only in one year, on LG15, LG16 and LG17. In the TeBr family, one QTL on LG10 was stable over the three years and two additional QTL on LG2 and LG15 were detected only in one year (2006). The QTL identified on LG12, for fruit size in the pedigree-related families and for fruit weight in the DLO-12 family, were located in similar regions and the unfavorable allele originated from 'Golden Delicious'. On LG1, the unfavorable allele for fruit size was associated with the haplotype carrying the resistance allele at the Rvi6 locus. Recombinants between the QTL for fruit size and Rvi6 would be an interesting material for breeders who still wish to use the resistance allele.

Unraveling the dynamics of QTLs associated to fruit firmness in apple over postharvest storage using a multi-family Pedigree Based Analysis (PBA) approach

S7O4

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In apple, firmness plays a critical role in the definition of fruit quality. Although a loss of firmness is necessary to enable the fruit to become more palatable and attractive, an excessive softening leads to severe fruit decay (especially during postharvest storage). This phenomenon negatively affects the consumer appreciation, since soft apples are generally associated to an overall lower fruit quality. To dissect the genetic control of fruit firmness in apple during postharvest storage a QTL discovery based on a multi-family Pedigree Based Analysis (PBA) approach was employed, consisting of 26 bi-parental families (1109 individuals), connected by a common pedigree structure, and 102 progenitors. The direct parents of the FS families together with their offspring were phenotyped with a penetrometer.

The association between fruit firmness assessed at four time points (harvest, two and four months of postharvest storage and two weeks of shelf-life after two months of storage) and genotypic data (7109 SNPs assembled into 1113 haploblocks), resulted in a total of 34 QTLs mapped in 17 genomic regions spanning over 12 chromosomes. Three genetic intervals, located on linkage groups 10, 14 and 15, showed a QTL for all four traits, identified with strong evidence based on Bayes Factor values. The comparison of the QTL patterns unraveled a QTL dynamics over storage, shedding light on the specific genetic control ongoing over postharvest storage and shelf-life. Besides the presence of three common QTLs, other QTL with strong evidence were specifically associated to one or a few particular postharvest ripening stages. Moreover, the Bayesian statistic (MCMC simulation) and IBD (Identity by Descent) analysis allowed to trace the QTL-allelic flow over pedigrees, thereby defining QTL genotypes for a series of progenitors, founders and closely related cultivarsproviding a comprehensive overview of the genetic control of fruit firmness in apple at different

Deciphering the genetic determinism of flowering/harvest period and several fruit sensory quality traits in apple by a Genome-Wide Association approach

S7O5

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Elucidating the genetic control of important agronomical traits in apple is essential for breeding cultivars adapted to their growing environments and thus potentially producing high quality fruits. In the frame of the EU-FP7 Fruit-Breedomics project, we performed a high-density Genome Wide Association (GWA) study to investigate the genetic determinism of important agronomical traits in apple, by exploiting the Axiom®Apple480K array in a population of 1,168 cultivars. GWA analyses were conducted using a multi-locus mixed model (MLMM), which handles the confounding effect of background loci that may be present throughout the genome at the GWA scan step. We identified two significant associations with flowering period, both on chromosome 9, and six associations with harvest period, four on chromosome 3, one on chromosome 10, and one on chromosome 16. The two SNPs significantly associated with flowering explained 8.9% of the phenotypic variation and the six SNPs associated with harvest period 17.2%. Concerning fruit sensory traits, variable numbers of association were identified: i) two for juiciness on chromosomes 1 and 16 explaining 10.2% of the phenotypic variation, ii) two for firmness on chromosomes 3 and 10 (17.6% of the phenotypic variation) and iii) one for crunchiness and one for meltiness, each on chromosome 3 (8.0% and 16.7% of the phenotypic variation, respectively). The significant SNPs for firmness and meltiness located on chromosome 3 were coincident with the significant SNPs identified for harvest period, suggesting the presence of at least one causal gene that may control both harvest period and sensory traits. Cultivars carrying alleles associated to early harvest period also showed softer and meltier texture. A GWA for textural traits with a modified model controlling for the effect of harvest period resulted in the retention of significant SNPs for firmness on chromosomes 3 and 10, but showed a lower effect, jointly explaining only 6.4% of the phenotypic variation. Associations for crunchiness and meltiness were not significant any more.

Mealiness in apple is associated with changes in specific ACC synthases genes

S7O6

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Mealiness in apple develops during storage and reduces the apples quality, and ethylene has been shown to be associated with this disorder. The main enzymes involved in ethylene production are ACC synthase (ACS) and ACC oxidase (ACO) and they are encoded by gene families which were not accurately referenced in genes bank(s). We organized the available data on ACS and ACO genes and determined their chromosome location, showing some redundancy in identification. In addition, we analyzed the expression of ACS and ACO genes during fruit development, and storage in three cultivars: Golden Delicious, Galaxy and Ana with different storage capacity. Only in Ana cultivar, with the lowest storage capacity, ACS3a expression increased prior to ethylene production peak, however, the expression of the genes ACS5b, ACS6, ACS8 and ACS9 remain constant during development and storage. Interestingly, there was no change in expression of two MADS-box genes MADS4 or MADS9, but MADS8, is temporarily increased prior to ACS3a only in Ana, in contrast to Galaxy and Golden Delicious. Our data suggest that ACS3a has an important contribution to ethylene auto catalytic production peak which is the highest in Ana fruit, making this gene as a good candidate for poor storage capacity marker.

Identification of a candidate gene for fruit flat shape in peach

S7O7

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Peach is one of the fruit species economically more important in temperate regions. Most of commercialized varieties are round to oval shaped, however commercial interest in flat shape fruits is increasing. Many genetic studies have aimed to unravel the genetic bases of fruit pattering, especially in the model species Arabidopsis and in tomato. In peach, no candidate genes for fruit shape have been identified so far. The flat shape of the peach fruit is determined by a single dominant gene S mapped to the distal part of chromosome 6. The allele conferring the flat appearance is partly dominant: s/s fruits are round and S/s flat, while S/S fruits abort few weeks after anthesis. Up to now several markers have been identified around the S locus by the analysis of mapping populations and germplasm, and one of them works efficiently in MAS. By association analysis of the candidate region for flat shape we have identified a putative causal gene for this trait. The allele associated with the flat shape contains a 10Kb deletion affecting the 5'UTR, the first exon and the intron of the gene. This deletion, as well as two small indels in the second exon of the gene have been evaluated in a panel of more than 200 peaches (including flat and round fruits) indicating a unique origin of this allele. Expression analysis of this gene in flower buds and ovary RNA reveals no transcription of S. Peach is recalcitrant for genetic transformation, thereafter functional validation is complicated by using this mechanism. Thereafter, we have used a sport mutant of a flat fruit variety that reverted to round fruit to validate the function of this gene. In this sport mutant we detect a modification affecting S, while s remains intact, which confirms a putative role of this candidate gene in peach fruit shape.

Genetic and biochemical characterization of fruit from different apricot accessions highlights apricot as a rich source of phytoene and phytoflouene, and indicates Carotenoid cleavage deoxygenase4 (Ccd4) gene as a potential regulator of fruit color

S7O8

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Carotenoids are tetraterpene molecules produced by all photosynthetic organisms as well as some bacteria and fungi. They are red, orange and yellow pigments, providing fruit tissues with bright colors. Carotenoids also serve as precursors to important volatiles, contributing to the distinctive aroma of many fruits. Moreover, carotenoids elevate the nutritional value of our food as they are essential in our diets, and are known to protect against various chronic disorders. Carotenoids and their metabolism have been studied extensively in model plants. However, very little is known about the carotenoids in apricot (Prunus armeniaca), their profile and its variation, and the regulation of their accumulation in the fruit tissue. In this study we analyzed the carotenoid content and composition in fruit from more than one hundred different apricot accessions, originating from all over the world and grown in the Newe Ya'ar germplasm collection. Our results show that apricot fruit contains a unique profile of carotenoids consisting of large amounts of beta-carotene, phytoene and phytofluene, and small amounts of other intermediates of the biosynthesis pathway, including cis-lycopene. These findings point out apricot as one of the richest natural sources of the colorless carotenoids phytoene and phytofluene, whose health benefits were recently highlighted. To understand how carotenoids are synthesized and accumulate in apricot we characterized the fruit transcriptome during ripening, in three different apricot accessions exhibiting contrasting carotenoids profiles. In addition, the high diversity in fruit carotenoid composition and content, among apricot accessions, served us in genetic association studies. The results highlight the complex nature of the regulation of carotenoid accumulation in apricot fruit, and suggest a role for the gene Ccd4, encoding a carotenoid degrading enzyme, as a potential regulator, hence influencing apricot fruit color.

Mapping the distinctive aroma of «wild strawberry» using a NIL collection

S7O9

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Fragaria vesca is a simplified model for the study of the strawberry genetics. The availability of a quality reference genome and the recently developed near isogenic line (NIL) mapping collection are powerful tools for the study of any genetic trait. Recent transcriptomic studies have also contributed to the re-annotation of the species. In this study we pursue the mapping of the wild strawberry-like aroma performing detailed profile and QTL map of the volatile composition of F. vesca NIL collection.

We studied the volatilome of diploid strawberry ripe fruits using 42 lines from NIL collection. Fully ripe berries from all NILs were collected and analyzed by gas chromatography-mass spectrometry (GC-MS). In order to assess which regions of the genome could be responsible for the variations in volatile ratios, a QTL analysis was performed with all samples collected each year independently.

We were able to map a total of 126 QTL from 81 different compounds including esters, aldehydes, alcohols, ketones, terpenoids and furans. The QTL mapping revealed 50 major stable QTL that accounted for a high proportion of the variability of 47 compounds, including 14 major QTL for 13 'key compounds' responsible of strawberry aroma. Many QTL co-locate in few genetic regions according to clusters suggesting a control by a reduced number of loci. The transcriptome of fully ripe fruits from selected NILs and from the recurrent parental of the NIL collection, F. vesca var. 'Reine des Vallées' (RV), were characterized by RNASeq in an Illumina HiSeq2000 platform. Two genetic regions that harbor interesting QTL involved with strawberry aroma perception has been selected to perform a whole transcriptomic study. The differential expression analysis of the red-ripe fruits allowed to highlight differentially expressed genes between the NILs and the recurrent parental RV that might be contributing to the observed QTL.

Apple breeding for the improvement of biochemical composition of fruit. the inheritance of sugar, ascorbic acid and phenolic compound contents

S7P1

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Apple breeding for the improvement of biochemical composition of fruit has been carried out at the VNIISPK since 1970. The hybridization volume made up 454 100 flowers, 98 500 seedlings were grown.

Long-term genetic studies showed that sugar and AC content, as well as phenolic compounds content in fruit are polygenically inherited. Both positive and negative transgressions have been observed. For instance, the new cultivar 'Dariona' contains more sugar (10.7%) than its two parents: 'Melba' and 'Papirovka' (9,9 and 9,1%, respectively). Average AC content in the Renet Chernenko' x 4-14-78 ('Severny Sinap x 'Pomon-kitaika') family is 25.1mg/100g and 66 seedlings have AC content in range from 21 mg/100g to 46 mg/100g whereas the AC content of the two parents is not higher than 20,1 mg/100 g.

Many crosses have been performed between the hybrids with the highest level of AC or between this hybrids and high vitamin cultivars.

Weak correlations have been observed between ascorbic acid (AC) in fruits and scab susceptibility on leaves. This allows to breed cultivars combining high AC contents in fruit and scab resistance.

While the average AC content of the cultivars recommended for cultivation in the Central and Central Volga regions of Russia is 12,6 mg/100 g, some new cultivars issued from our breeding program contain 15-20 mg/100 g ('Pepin Orlovsky', 'Maslovskoye', 'Veteran', 'Ivanovskoye', etc.).

The development of apple cultivars with higher contents of sugars, ascorbic acid and phenolic compounds is very desired, since their introduction into industry allows better nutritional and therapeutic-prophylactic values.

Primary metabolite fruit profile is altered in response to source-sink imbalance and can be used as early quality predictors innectarine

S7P2

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Peaches and nectarines are among the most exported fresh fruits from Chile. Fruit acceptance by final consumers is defined by quality parameters as size, taste, color and juiciness. In these fruits the balance between soluble sugars present in the mesocarp and organic acids determines the taste. Biomass production and metabolites accumulation by fruits occur during different developmental stages and depend on photosynthesis and carbon exportation by source leaves. Carbon supply to fruits can be potentiated through field practices of thinning (removal of flowers/fruits) that lead to change in source-sink balance. It is well known that thinning leads to fruits with increased size, but it is not known how this practice could influence fruit quality in terms of metabolite composition. In this work, we analyzed primary metabolite of nectarine cv'Magique' at different developmental stages and from trees submitted to thinning. Fruit mesocarp primary metabolites were analyzed across the whole development until harvest and subsequently postharvest ripening. Sugars, amino acids and organic acids were measured by 1H-NMR,HPAEC-PAD and HPLC-DAD. In addition, harvest and ripening quality parameters as size, juiciness and brix was performed. We observed that fruits from thinned trees had better quality parameters than unthinned trees. Our results also indicated that thinning affects metabolic composition from early through late developmental stages. Principal component analysis of phenotypic and metabolic data at different stages of development and thinning revealed that glucose, fructose, sucrose, inositol, galactose and succinate concentrations at early stages of development (S1 and S2 stages) can be used to segregate fruits with higher quality. In conclusion, we suggest that profile of these metabolites in early stages of development could be a metabolic predictor of final fruit quality in nectarines. Funding: FONDECYT1130197, MetaboHUB-ANR-11-INBS-0010.

Impact of defoliation treatments on apple fruit quality before and after cold storage

S7P3

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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 and the size of bourse leaves during the growing season. Several studies suggest a possible direct relationship between dry mater accumulation and high quality texture. To study the impact of trophic competitions on fruit quality, the sink-source equilibrium was modulated at the level of the fruit bearing branch and inflorescence by different defoliation treatments.

The experiments were carried out on different apple varieties with contrasting sensory phenotypes. Apple quality was investigated using morphometric, mechanical, biochemical and transcriptomic analyses.

The impact on fruit development and quality was dependent on the timing of the defoliation treatment and on the variety. Data suggested an important role of bourse and bourse shoot leaves in early stages of fruit development. Transcriptomic analyses revealed a differential stress response to defoliation treatment.

Transcriptional regulation of carotenoid and vitamin C contents in apple fruits during postharvest and shelf-life storage

S7P4

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Consumers have a growing interest in foods with an enhanced composition in nutrients and bioactive compounds. Vitamin C and carotenoids (provitamin A) are essential micronutrients and also play an important role as antioxidants in humans, therefore both types of metabolites possess properties that are desirable in the composition of food. Fruits and vegetables are good sources of vitamins and in fact their concentrations have been already described in a number of plant species. However, the effects of storage conditions on the concentration of vitamin C and carotenoids and the transcriptional regulation of their metabolic pathways are still rather unknown, especially in apple fruits. The effects of storage and shelf-life in altering fruit composition should not be disregarded in apple fruits since they have good storability in terms of organoleptic properties and hence can be stored for several months. Thus, in the present work we have evaluated the evolution of vitamin C and total carotenoid contents in apple fruits of selected commercial apple varieties during storage at 1°C and also after shelf-life. The varieties selected displayed different amounts of vitamins at harvest time and distinct accumulation patterns while they were stored. The transcriptional regulation of key genes of vitamin C and carotenoid pathways have been evaluated. The results highlight the relevance of the regulation of these metabolic pathways at the transcriptional level and also the different behaviors of commercial varieties. The potential bottlenecks and key steps in the regulation of vitamin C and carotenoids metabolism during apple fruit storage will be discussed.

Genetic structure based on EST-SSR: A promising tool for fruit color selection in Japanese plum (*Prunus salicina* L.) breeding programs

S7P5

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Prunus salicina is one of the most economically important stone fruit species. However, there is scarce genetic information available, which makes it difficult to implement Marker-Assisted Selection (MAS). Recently, next-generation sequencing (NGS) has greatly improved breeding program strategies, generating information associated with the identification of simple sequence repeats (EST-SSRs) and single nucleotide polymorphisms (SNPs). Few studies have focused on developing EST-SSR markers considering both gene expression levels of contrasting phenotypes and specific transcription factors of metabolic pathways. This study investigated the transcriptome profile of P. salicina in fruits with contrasting skin colors, obtaining 54,224 unique contigs. From this data set 44 EST-SSR have been generated, considering gene expression levels of contrasting phenotypes and specific transcription factor from three metabolic pathways: citric acid, carbohydrate metabolism and flavonoid pathways.

In this work, we report the first set of EST-SSR markers for P. salicina developed from specific genes associated to fruit color skin by anthocyanin accumulation. Three EST-SSR markers developed from the putative flavonoid pathway transcription factors PsMYB10, PsMYB1 and PsbHLH35 were selected to determine a pattern of grouping based on the phenotype of the 29 cultivars. This structure was contrasted with the genetic structure generated using genomic SNPs obtained by genotyping-by-sequencing (GBS) in the same cultivars. The analysis using SNPs identified two groups, while the use of selected EST-SSR identified three. In contrast to the structure given by the SNPs, the EST-SSR grouped all the yellow varieties in one cluster, which was composed mainly of varieties of this color. The EST-SSR developed in this study may be considered as candidate markers to be evaluated in MAS strategies in genetic breeding programs.

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Identification of QTLs for aesthetic properties in apple using image analysis

S7P6

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The aesthetic properties (AP) of apple fruits are important features because they affect consumer's choice and therefore they are considered as desirable traits in most breeding programs. The development of DNA-based markers for AP is considered an efficient tool to improve the efficiency of breeding programs. Currently, most of the DNA-based markers for AP are based on visual assessments, which could be biased, considering the subjectivity of human perception. The variation of fruit appearance found in a 'Telamon' x 'Braeburn' population was used to carry out a QTL analysis using visual parameters assessed through image analysis. Fruits were harvested at commercial maturity and then images were taken around the equator of the fruit at four different angles. Subsequently the images were analyzed with MATLAB®. QTLs associated with percent skin surface covered by red coloration were detected on LG9 and LG15 explaining 5.2 and 68.5% of the variation, respectively. Moreover QTLs for the ground color of the peel were identified on LG2, LG9, LG13 and LG16, which explained 9.4, 19.7, 8.9 and 15.9% of the total variation respectively. Finally there were also QTLs detected for the intensity of the red skin color on LG6, LG9 and LG15 explaining respectively 9.9, 69.3 and 8.5% of the variation. In these experiments we confirmed previously reported QTLs on LG9 near the location of MYB1, master regulator of anthocyanin accumulation, and we were able to detect novel QTLs for AP.

QTL mapping for phytochemical compounds in peach [*Prunus persica* (L.) Batsch] S7P7

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Genetic control and location of QTLs associated with phytochemical compounds in peach were evaluated using bi-parental mapping and genome wide association study (GWAS). The bi-parental mapping was conducted in an F2 population (ZC2) derived from cross between 'Zin Dai' x 'Crimson Lady'. GWAS was performed on an association panel representing modern peach cultivars available and/or produced for the U.S. market. Antioxidant capacity and phenolic compound accumulation (total phenolics, flavonoids and anthocyanin) were evaluated for two years on all material. The ZC2 progeny were genotyped using IPSC 9K peach SNP array v1., and the association panel was genotyped using genotyping-by-sequencing. The genetic linkage map, constructed with 908 SNP markers distributed among eight linkage groups, covers a genetic distance of ~ 336 cM, with an average marker density of 1.07 cM/marker. Total of 10 QTLs associated with phytochemical traits were identified on 5 linkage groups (LGs). Two major QTLs were observed on LG 6 and 8. qPC.ZC-6.1 was associated with all phytochemical compounds, while qPC.ZC-8.1 exhibited association only with total phenolics and anthocyanin content. GWAS, performed on a dataset of 35,198 SNPs and all phytochemical compounds, revealed a significant association ($P < 0.05$) for 94 SNPs covering the entire genome. Majority of SNPs (90) were associated with anthocyanin accumulation and spread across all LGs, while 4 SNPs on LG7 were associated with both antioxidant capacity and flavonoid content. Although none of the SNPs associated with phytochemical content detected via GWAS overlapped with the single QTL identified using the bi-parental population, many were found in flanking regions. Furthermore, a single SNP associated with anthocyanin on LG3 flanked the major blush QTL PprMYB10 detected previously in the same progeny. Feasibility of enabling marker-assisted breeding for phytochemical composition will be discussed

Mapping the human taste experience on the apple genome

S7P8

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Apples are a diverse and complex crop that can require more than twenty years to breed a successful cultivar. Paramount to the success of an apple cultivar is exceptional fruit quality which meets consumer demand for sweet, juicy and crisp apples. To increase breeding efficiency toward this goal, recent advances in apple genomics have led to the development of DNA markers that enable early selection for favourable fruit quality traits. These DNA markers have been based on instrumental assessments of fruit quality traits such as titratable acidity, volatile composition and flesh firmness. However, such instrumental methods provide only an approximation for the human experience of apple taste, flavour and texture. In this study, we present a first attempt to directly map the human sensory perception and taste experience of apple fruit onto the apple genome, in order to develop practical DNA markers for apple breeding. Using a diverse collection of 85 heritage and modern apple cultivars at the Vineland Research and Innovation Centre in Vineland, Ontario, Canada, we found that a trained human sensory panel could quantify 17 taste, flavour and texture attributes, and that many of these attributes could be mapped onto the apple genome. We employed 52,000 SNP markers developed through genotyping-by-sequencing to conduct a genome-wide association study for each trait, and identified previously unreported loci controlling sensory fruit quality in apple. We discovered a new major locus for juiciness and crispness on chromosome 13 which shows strong promise for application in DNA marker-assisted apple breeding. To our knowledge, this is the first study that combines a genome-wide association analysis with the human perception of a plant product in order to breed better fruit. Beyond apples, this approach has broad application in horticulture plant breeding, and we are currently pursuing similar sensory-genomic mapping studies in other Rosaceae species.

Identification of candidate genes associated with fruit softening rate in nectarine (*Prunus persica*) using QTLs and expression QTL **S7P9**

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Melting flesh (MF) and non-melting flesh (NMF) are the two main phenotypic flesh classes in peach and nectarine. MF varieties show a higher softening rate in postharvest than NMF. However, there are significant differences in the MF class on softening speed. In some populations derived from crosses between MF varieties, it is possible to observe a normal distribution for this trait indicating a multiple-gene control. The aim of this work was to identify candidate genes involved in flesh softening through QTL and expression QTLs (eQTL) analysis. An F2 population (n= 152) derived by selfing 'Venus' nectarine was phenotyped at harvest + 3 days at room temperature during three consecutive seasons. Eight individuals showing contrasting softening rate were submitted to total RNA isolation, sequenced using RNA-seq. Based on a previous genetic map a conventional QTL analysis was performed. From the RNA-Seq analysis we found 2,822 differentially expressed genes between high and low softening rate groups. Three co-localized QTLs were detected on linkage group 4 (mean LOD score equal to 9.7 and 58% of variation explained) using phenotypic data from three season. Eight eQTLs were detected co-localizing on LG4 (7 trans-eQTL and cis-eQTL) with a LOD score between 3.5 and 12.0. These genes (eQTL) are related to remodeling cell wall, sensing and ethylene biosynthesis and auxin synthesis. This work helps to unravel the molecular mechanism responsible for softening rate in nectarine. This work was supported by CORFO Consorcio Biofrutales 13 CTI-21520-SP03 & 13 CTI-21520-SP04, Fondo de Areas Prioritarias Centro de Regulación del Genoma 15090007, FONDECYT 1160584, FONDEF Genoma G13i10005 and CORFO-Innova 09PMG7240.

What do evolutionary histories of pathogens teach us about their various capacities to overcome plant resistances?

S8O1

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Understanding how populations of pathogens overcome plant resistance factors is essential to optimize plant breeding and subsequent deployment of plant resistance genes. In phytopathology, studies carried out on adaptive processes involved in resistance breakdowns considered only populations already infecting crops. Indeed, the rapid emergence of new virulent isolates is hypothesized to arise from de *novo* adaptive mutations within avirulent populations already present in the agrosystem, as a response to the introduction of new resistant hosts. Standing genetic variation occurring in non-agricultural reservoirs is neglected and is then rarely considered as a source of virulence and consequently a threat for crops. Working on *Venturia inaequalis*, the ascomycete responsible for apple scab, we showed that populations responsible for *Rvi6* resistance breakdown in orchards originated from non-agricultural reservoirs. We described a situation -that we call The Trojan Horse emergence- in which the introgression of *Rvi6* from the wild apple tree *Malus floribunda* into *Malus x domestica* cultivars allowed the invasion of a virulent population from non-agricultural habitats to orchards bringing together environmental and agricultural pathogen lineages that diverged in allopatry several thousand years ago. More importantly, we showed that hybrids in orchards may even exhibit higher aggressiveness on susceptible apple cultivars than parental lineages highlighting the risks of evolutionary and epidemiologic changes in pathogens. We showed that wild plants do not consist in "passive" reservoirs of pathogens but can also be potent drivers of their genetic diversity, with dramatic consequences for the emergence of new pathogen variants. We will discuss on the crucial need for plant breeders to have the most exhaustive view as possible on diversity and evolutionary history of pathogens, on existence of disease reservoirs in the wild and we finally propose some practical measures to avoid rapid virulence spreading in agrosystems.

Using 'omics to understand the genetic mechanisms of the *R-Avr* model between *Maleae* hosts and *Venturia* species

S8O2

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Scab disease caused by *Venturia* species is a worldwide problem in pipfruit orchards. The fungus exhibits host specificity, with *V. inaequalis (Vi)* and *V. pirina (Vp)* infecting apple and European pear, respectively. Within each host-pathogen system, the interactions follow the gene-for-gene model, with 17 pairs of avirulence *(Avr)* and resistance (R) genes identified in *Vi* and its host apple. To investigate the genetic interaction between *Malus* and *Venturia*, we carried out gene differential expression experiments on two *Malus* accessions, the susceptible 'Royal Gala' (RG) and the resistant A248R04T010, a progeny of a host (5) carrying the *Rvi5* resistance gene, accession 9-AR2T196, at multiple time points. At one day post-inoculation (dpi) of *Vi* isolate MNH120, 2995 genes were differentially expressed (up- or down-regulated) in A248R04T010, but only 31 in RG. At 2 dpi, the number of differentially expressed genes increased to 5427 and 419 in A248R04T010 and RG, respectively. Our results revealed that the action point of transcription factor responses to *Vi* varies, with the response of MYBs in A248R04T010 commencing as early as 1 dpi, whilst WRKY genes were expressed mainly at 2 dpi. In addition to investigating the host response, we also sequenced four *Vi* isolates, three specific for *Malus* but differing in race profile, one a pathogen of loquat, and one isolate of *Vp*. Through transcriptome profiling and comparative genomics, we identified *Venturia* genes that are species- and race-specific amongst ~600 small secreted candidate effectors. Our analysis also shows that the candidate effectors are frequently found in low gene-density regions and adjacent to repeats. Among them, candidates were identified for *AvrRvi1, AvrRvi2, AvrRvi8* and *AvrRvi9*. Our studies provide valuable resources for research into understanding the genetic mechanisms of the *R-Avr* models between *Maleae* hosts and *Venturia* species.

Intergeneric transfer and functionality of a major apple scab resistance gene

S8O3

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Scab is the most important fungal disease of apple and pear. Apple (Malus x domestica) and European pear (Pyrus communis) are genetically related (strong co-linearity between the two genomes), but they are hosts of two different fungal species: Venturia inaequalis for apple and V. pirina for European pear. The apple/V. inaequalis pathosystem is quite well known, whereas knowledge about the pear/V. pirina pathosystem is still limited. The aim of our study is to analyze the mode of action of a major resistance gene of apple (Rvi6) in transgenic apple and pear plants interacting with the two scab species (V. inaequalis and V. pirina), in order to determine the degree of functional transferability between the two genera. Transgenic apple clones carrying the Rvi6 gene under the control of the CaMV35S promoter have been produced by the PRI team in Wageningen. We have produced about 50 transgenic pear clones of the variety Conference carrying the same construct. After inoculation in greenhouse with V. pirina, strong defense reactions and very limited sporulation were observed on all transgenic pear clones tested. However, this resistance proved variable according to the strain of V. pirina, and one of the strains tested (VP 98) overcome the resistance of most of the transgenic pear clones. Microscopic observations after solophenyl flavine staining revealed frequent aborted conidiophores in the Rvi6 transgenic pear/V. pirina interaction. Transcriptomic analyses of all the susceptible, resistant and non-host interactions are underway to identity the underlying molecular mechanisms. This study is the first example of a successful intergeneric transfer of R gene among Rosaceae, with a resistance gene functioning towards another species of pathogen.

Targeted Mutagenesis of MLO-Homologous Genes in the Rose Genome

S8O4

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Powdery mildew caused by *Podosphaera pannosa* is one of the most severe diseases of rose. Especially in greenhouse production it causes great economical losses. The diurnal production of wind-borne conidia as well as a high genetic diversity within the population of this race-forming pathogen limit the effectiveness of race-specific, monogenic resistances. An alternative to overcome this problem can be the mildew resistance locus o (*mlo*)-mediated resistance. Until now the *mlo*-based resistance is characterized in barley, A. *thaliana*, tomato and pea where the loss-of-function of specific members of the *MLO* gene-family leads to a recessive broad-spectrum resistance. This resistance displays a high durability since it has been used in barley for over thirty years without being broken. The MLO proteins are heptahelical transmembrane proteins which probably manipulate or suppress the SNARE protein-dependent and vesicle-associated defense mechanisms of the cell and by that confer susceptibility towards the pathogen. In rose four *MLO* homologs (*RhMLO1, RhMLO2, RhMLO3* and *RhMLO4*) closely related to the functional ones in *A. thaliana*, pea and tomato have been sequenced and mapped. The functionality of these four rose *MLO*s regarding the mediation of susceptibility towards *P. pannosa* is investigated using different approaches. Stable transgenic plants of the tetraploid *Rosa hybrida* cultivar 'Pariser Charme' expressing RNAi constructs to silence the four *MLO* genes have been obtained. These plants were analyzed for a down regulation of the genes as well as for a change in susceptibility. TALEN and CRISPR/Cas constructs were used to create stable transgenic 'Pariser Charme' plants with a knock-out of each of the four *MLO* genes. Here the effectiveness of the different constructs is additionally analyzed in a transient assay using petals. Moreover the transient over-expression of the four RhMLO genes will be analyzed in a detached rose leave assay as well as in a heterologous expression system.

Mapping black spot resistance in autotetraploid rose using genotyping-by-sequencing

S8O5

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Landscape roses, Rosa hybrida, are a globally important ornamental plant and are a preferred choice by both professional and hobbyist gardeners. A severe challenge for rose growers across the world is the black spot pathogen, Diplocapon rosae. When the fungus infects a rose plant it results in unsightly black marks on leaves which is often followed by defoliation. Over time black spot weakens and can eventually kill a rose bush. Genetic resistance to races of black spot exist but due to the complex autotetraploid nature of landscape roses (2n=4x=28) developing easy to use genetic markers linked to black spot resistance has been slow going.

Here we present our work to map resistance to two races of black spot in autotetraploid rose. The mapping population of 333 F1 hybrids was SNP genotyped using genotyping-by-sequencing (GBS). A detached leaf assay was used to phenotype members of the population for resistance to both races of black spot, each of which mapped as a single dominant gene. A GBS-derived SNP linked to black spot resistance was converted to a high resolution DNA melting assays and has been deployed to Vineland's cold hardy landscape rose breeding program. This project will serve as a framework for future development of markers across a number of traits important for developing superior landscape roses.

Exploring horticulturally important traits in an apple population using genome-wide association studies

S8O6

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Apple (Malus x domestica Borkh.) is one of Canada's most important horticulture crops, and is grown across the country from coast to coast. With all crops, there is a need to develop new cultivars to meet the consumer and production demands of the future. Current methods of apple cultivar development demand much time and resources, but genomics-assisted breeding could help alleviate some of these demands via marker-assisted selection (MAS). Genome-wide association studies (GWAS) are emerging as a method to pinpoint genetic regions associated with horticulturally important traits. In this study, GWAS were conducted on a population of over 170 different apple cultivars grown in Kentville, Nova Scotia, Canada to explore traits ranging from storage disorders to scab resistance. Phenotype data were collected over two years and included measurements taken at harvest, as well as after three months of storage in refrigerated air. Through the use of the next-generation sequencing (NGS) genotyping-by-sequencing (GBS) approach, over 50,000 single nucleotide polymorphism (SNP) markers were discovered for these analyses. This work helps shed light on the genetic basis of traits important to the apple industry, and we discuss the potential utility of this type of study for a MAS apple breeding program.

Comparison of the transcriptomes of a partially resistant and highly susceptible apple cultivars in response to *Neonectria ditissima* infection

S8O7

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European canker, caused by the fungus *Neonectria ditissima*, is a serious economic problem for apple production in countries with a cool and wet climate. Information on the genetic control of resistance towards this pathogen would greatly enhance the prospects for breeding resistant cultivars.

The aim of this work was to clarify the mechanisms involved in resistance responses. Hereto an RNAseq analysis was applied to identify differentially expressed genes (DEGs) in response to *N. ditissima* between the partially resistant cultivar 'Jonathan' and the highly susceptible cultivar 'Prima'. Samples of *N. ditissima*-inoculated and water-inoculated (control) wood were taken from three biological replicates at three different time points, i.e., 5, 15, and 30 days after inoculation (dai) when measurable lesions have not developed yet. At later stages statistically significant differences in canker size were obtained in favor of 'Jonathan'.

The number of DEGs increased in time from 4 (3 from 'Prima', one from 'Jonathan') to 7,251 to 14,020. Also, the contribution of the susceptible cultivar increased in time, from 46% to 64% for the 15dai and 30dai treatments/samples respectively, which may indicate that a more susceptible response leads to an earlier, wider range of secondary processes, which may include cell death. For the latter two treatments, the number of genes that showed opposing expression patterns decreased from 11 to eight.

The upregulated genes belonged to a surprisingly similar set of gene ontology classes ("molecular functions", "cellular components" and "biological processes", etc) in the two cultivars. This result indicates that there is no major difference between the two cultivars in their overall susceptibility response.

In contrast, the down-regulated genes did show a differentiation between the two cultivars. For 'Prima', these belonged mostly to the "membrane" and "membrane parts" classes, and for 'Jonathan' to the "cell" and "cell parts" classes. From this it can be hypothesized that a more efficient defensive strategy is associated with a local shutting down of molecular processes associated with for instance cellular component organization and/or biogenesis of specific compounds. Verification of current DEGs using RT PCR and examination of additional cultivars is currently in progress.

NBS-LRR resistance genes polymorphism in genus *Malus* revealed by NBS profiling **S8P1**

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NBS profiling is a PCR-based technique that efficiently targets disease resistance genes (R-genes) and their analogs and allows to develop molecular markers. NBS profiling was used to study NBS-LRR resistance genes polymorphism and phylogeny in 89 Malus accessions (including 48 species and interspecific hybrids, 32 M. domestica varieties, and 9 Antonovka landraces) from the collection of the Vavilov Research Institute of Plant Industry. Standard NBS2 and NBS5 primers (Van der Linden et al., 2004) were used for the analysis and enabled the identification of 165 polymorphic (79%) NBS fragments. PCO-analysis of the NBS profiling data revealed four groups formed by Malus species, with hybrid species occupying an intermediate position. First group (A) comprises species of the Section Malus and includes two subgroups: A1 formed by 31 M. domestica varieties, A2 includes 16 other studied accessions of the Section Malus (M. sylvestris, M. sieversii, M. turkmenorum, M. orientalis, M. asiatica) with 7 Antonovka landraces, in intermediate position. These results confirm that Antonovka landraces have specific NBS patterns and might be a useful source of the new R-genes for apple breeding. Group B includes mainly species of the Section Gymnomeles (M. baccata, M. sachalinensis, M. hupehensis, M. mandshurica), as well as M. sieboldii and M. sargentii previously referred to as the Section Sorbomalus and the apple landrace Yakutskaya that may be the result of the domestication of one of the Gymnomeles species. Group C includes species from the Section Sorbomalus (M. kansuensis, M. toringoides, M. transitoria, M. florentina). American species M. coronaria and M. ioensis (Section Chloromeles) form group D. The results suggest that NBS profiling is effective not only for evaluation of resistance genes variability and detection of new R-gene donors for apple breeding, but also for phylogeny reconstruction in Malus.

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Effector-Mining in the genome of Diplocapon rosae

S8P2

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The hemibiotrophic ascomycet Diplocarpon rosae is the causing agent of the black spot disease, one of the most common and damaging fungal diseases on roses. Understanding the molecular mechanisms controlling the infection is one way to find new targets for resistance breeding.

Effector proteins are the key factors in the interaction of a pathogen with its host. These proteins are secreted into the host to suppress the plant immune response or to acquire nutrients. Besides these functions as virulence (vir) factors they can also be recognized by the plant immune system and become avirulence (avr) factors. This process is called effector-triggered immunity (ETI), where R-proteins of the plant recognize the fungal effector proteins or their effect and activate the plant immune response.

To identify effector candidates in the genome of D. rosae we sequenced the isolate DortE4 with a combination of Illumina- and 454-sequencing and added data of a long Mate Pair library. This resulted in 7820 scaffolds with a N50 of 243kb. We used the BUSCO pipeline to be sure that the genome contains the complete gene space. With the MAKER pipeline we predicted and annotated 14.007 gene models.

By combining different programs we identified 110 gene models which possess typical characteristics of effector protein. With generated transcriptome data it is possible to analyze the expression of these candidates during the infection, which allows the identification of the most promising ones for further analysis.

Exploring the genetic basis of host specificity in *Pseudomonas syringae* of *Prunus*

S8P3

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The globally important phytopathogen, *Pseudomonas syringae*, includes pathovars that infect over 180 plant species. Individual pathovars only infect one or a few hosts. However, despite this specialisation, host jumps have occurred frequently within *P. syringae*. It is believed that effector repertoires are linked to host range and therefore genetic alteration of these repertoires may enable host range expansion or host jumping events. This topic was explored using three divergent clades that have convergently evolved to cause bacterial canker on *Prunus species: P. syringae* pv. *morsprunorum (Psm)* (which is differentiated into two races, based upon host response) and *P. syringae* pv. *syringae (Pss)*. Three reference isolates of Psm R1, R2 and Pss were sequenced with PacBio sequencing and the genomes of a further fifteen genomes were sequenced using 2x250bp Illumina reads. Comparative genomics of the *Prunus* strains has revealed highly divergent effector repertoires within and between the different clades infecting *Prunus* species, with a small number of conserved effectors, some of which may play a key role in specialisation to *Prunus*. Clades also differed in the presence of phytotoxin genes and those associated with survival in woody plant tissue. This analysis indicates that they may utilise different virulence mechanisms to cause similar disease outcomes. Transfer of effectors from strains from plum that were weakly pathogenic on cherry revealed the action of possible avirulence genes in cherry.

Positive selection acting on cherry (*Prunus avium* L.) resistance gene analogs (RGAs) S8P4

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Sweet and wild cherry (Prunus avium L.) is an important tree species which is however intensively plagued by a plethora of phytopathogenic fungal species. Fungi recognition is the first crucial step of defense reactions in plants which is often mediated by a plethora of rapid-evolving receptors, many of which containing ligand-binding and signal transduction domains, such as Leucine-Rich Repeats (LRRs) and NB-ARC domains, respectively. Furthermore, resistance gene analogs (RGAs) are the largest class of potential resistance (R) genes depicting highly conserved domains and structures. Therefore, RGAs are crucial components of breeding projects concerning improved disease resistance, serving as useful functional markers linked directly to R genes. In order to assess the evolutionary pressures acting upon P. avium RGAs candidates, we mined their 173 homologues being previously deposited in Genbank. Their proteins were clustered according to their blast(p) similarities in 12 MCL (Markov Cluster Algorithm) tribes resulting in unique and well supported paralogous gene groups (PGGs). The extent to which these RGAs genes exhibit signs of diversifying selection were determined using a series of maximum likelihood analyses. The results obtained showed intensive evidence of positive selection, acting in almost all of the clustered PGGs across their phylogenies. Furthermore, analyses revealed that the majority of positively selected residues sites are localized widely across the RGAs sequences. We speculate that the clustered distribution of these genes might also be pronounced of high birth and death rates with diversifying episodes acting on their NB-ARC domains, putatively affecting the ligand-binding specificities. These results would provide a critical foundation for the ongoing P. avium disease resistance breeding efforts in the future.

BAC library screening for the identification of Dp-fl resistance gene to *Dysaphis plantaginea* in the apple cultivar Florina

S8P5

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Rosy apple aphid (*Dysaphis plantaginea*, Passerini) is one of the most damaging insect affecting cultivated apple (*Malus* x *domestica*). The aphid causes severe damages on shoots, leaves and fruits, the latter remaining smaller and deformed thus losing their economic value. By now, no resistance gene against the rosy apple aphid has been characterized in apple. Recently, thanks to phenotypic characterization of several segregating F1 progenies of the resistant cultivar 'Florina', the resistance locus (denoted *Dp-fl*) has been mapped. The *Dp-fl* region is about 300 kb flanked by two single nucleotide polymorphism markers (SNPs). The aim of the work was to complete the chromosome walking in the *Dp-fl* gene region with a minimum tiling path of BAC clones. The two SNP markers combined with new markers developed within the *Dp-fl* region have been used for screening a 'Florina' BAC library. So far, five overlapping BAC clones that fully cover the resistance locus have been identified. This region is under sequencing to identify possible candidate resistance genes. The identification of candidate genes will open the way for a functional characterization of the resistance mechanism. By now, the markers developed during the chromosome walking are promptly available for an efficient marker-assisted breeding selection.

Key words: Apple, aphid resistance, candidate genes, chromosome walking

Screening candidate genes for resistance to Sharka disease in *Prunus* species

S8P6

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Sharka disease caused by the Plum Pox Virus (PPV) is one of the most devastating diseases in Prunus species resulting in heavy losses of fruit production. As an obligatory parasite to complete its infectious cycle, potyviruses such as PPV require interactions between its viral proteins and host factors.

Very few resistances against PPV have been reported so far. However, in the past few years, natural recessive resistances were identified against several important potyviruses in vegetable crops. Most of them encode one of the translation initiation factors of the eIF4F complex, i.e. eiF4E or its isoform, eiF(iso)4E. Experimentally, a knock-out mutant for eiF(iso)4E in Arabidopsis thaliana is resistant to PPV infection. More recently, the chloroplast phosphoglycerate kinase (cPGK2) was identified as a candidate to reduce drastically PPV accumulation in Arabidopsis and variants of protein disulfide isomerase (PDI) can induce natural resistance to plant viruses. This suggests that PPV is using one of those susceptibility factors to complete its life cycle in stone fruit trees as it does in Arabidopsis. This prompted us to search for allelic variation among those candidate genes in Prunus species and link it to resistance or susceptibility to PPV. Due to their large susceptibility to PPV, we postulate that most of the Prunus cultivated species bear susceptibility alleles and that natural variants for these genes could naturally be present in germplasm collections and/or wild Prunus populations.

Through the Plant-KBBE European project COBRA, we screened 1,300 individuals from peach, apricot & almond trees. Plant material originates both from cultivated and wild compartments (Caucasus, Central Asia, Asia). Individuals presenting mutation(s) in the amino acid sequence(s) are currently tested for PPV resistance.

De novo transcriptome sequence assembly in apricot using PPV infected and healthy plants

S8P7

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Sharka disease, caused by *Plum pox* virus (PPV), is the most important viral disease affecting *Prunus* species. The best long-term solution is to breed new PPV-resistant varieties from some few resistance sources already reported in apricot (*Prunus armeniaca* L.). Although peach (*Prunus persica* L.) is considered as a reference for *Prunus* species, increasing available apricot genomic and transcriptomic resources is still desirable in order to optimize research effort in this species. In this study, de novo transcriptome from leaves samples of 3 apricot varieties ('Goldrich', 'Stella', and 'Canino') has been analyzed by next-generation sequencing. Samples were obtained from healthy and PPV-inoculated plants. HiSeq200 Illumina sequencing using pair-ends resulted in more than 490M of 101 bp raw sequences reads, with a mean of 30,63M per sample. The assembly was made using the Trinity (v.20140413p1) software after the normalization using 30X k-mer (k=25) coverage, and later was refined using Cap3 software. In order to check the quality of the transcriptome assembly, the number of assembled transcripts that seem to have a complete length was estimated by comparison with the peach transcriptome (v.1.0). Structural and functional annotation was performed by sequence comparison with public databases: Swiss-Prot, TAIR10 and UniRef90. Also a functional classification of the unigenes following the Gene Ontology (GO) scheme was made. A bidirectional blast search comparison was performed in order to obtain a set of putative orthologs between apricot and peach using the peptides contained in the peach database (GDR), and also between apricot and *Arabidopsis thaliana* using the TAIR10 database. For molecular markers identification, SSRs were annotated using the ngs_backbone software. Sequences containing di-, tri- or tetra-nucleotide repeats were selected. In conclusion, this transcriptomic dataset increases the genomic resources of this species for future molecular genetic studies in apricot and also related species.

Apple scab and Powdery Mildew: from Applied Genomics and NBTs the Ultimate Solution?

S8P8

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Apple scab (Venturia inequalis) and powdery mildew (Podosphaera leucotricha) are, together with fire blight (Erwinia amylovora), the most devastating diseases in apple orchards. They lead to significant economic losses in apple production and their control requires significant plant protection input. Breeding programs are attempting to introgress scab and powdery mildew resistance genes originating from wild apples into commercial cultivars for the control of the disease. 18 scab and 5 mildew resistance genes (R) have been identified and mapped, recently 6 of them have been fine-mapped and candidate resistance genes have been identified. Furthermore, susceptibility genes (S) that encode for negative regulators of the plant immunity system have been characterized as suppressors of plant defense and their impairment led to resistance. Having the apple genome available, and thanks to joint efforts of several institutions, many of these genes become available to implement different approaches than classical breeding. Cis-genic approaches and, even more, genome editing has been successfully used to obtain genotypes which may not fall under the umbrella of GMO legislation, opening new horizon for "non-GMOs" varieties.

The holy grail for plant geneticists: good phenotyping data!

S8P9

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Molecular markers for monogenic disease resistances are routinely used in the breeding of many different crops although eventual breakdown of resistance is common. For a woody crop like apple, durability of resistance is especially important since the trees are expected to remain in the same orchard for many years. Durable resistance or tolerance to many devastating apple diseases is, however, usually under polygenic controlled. For these diseases, markers have not yet been developed or are associated with QTL that explain only a minor part of the variation.

A Public Private Partnership for Prebreeding was initiated by the Nordic Council of Ministers in 2012. One of the three funded projects is a collaboration between partners in Finland, Norway and Sweden, and is focused on the analysis of resistance to some of the economically most important apple diseases in the Nordic countries; fruit tree canker caused by *Neonectria ditissima*, and storage rots caused by, e.g., *Neofabraea* spp. and *Penicillium expansum.*

Within this project, a genetically diverse set of old and/or local cultivars are screened together with some modern cultivars and breeding selections with valuable traits for yield and fruit quality. Degree of resistance to the diseases is evaluated after inoculation with fungal spores or hyphae. The goal is twofold, (1) informed selection of optimal parents for the applied apple breeding carried out by the three partners, and (2) application of GWAS to identify candidate genes and develop markers for future breeding.

Acquiring high-quality phenotyping data has unfortunately proven very difficult due to, e.g., contamination with other fungi, and high sensitivity to environmental conditions affecting both the plant material itself and the experimental procedures. Efforts are continuously being made to improve the experimental procedures and to refine evaluation of data, in the hope to achieve the Grail, eventually…

Identification of genomic regions for virulence in the fruit canker fungus *Neonectria ditissima*

S8P10

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The apple industry is the most important fruit sector in Sweden. One of the most serious diseases in Swedish apple production is Fruit Tree Canker (FTC) caused by *Neonectria ditissima*, which is favoured by our relatively cool and rainy climate. FTC is also a large and urgent problem in many other European countries. The damage by FTC can be extensive and very costly, since diseased wood must be removed. *N. ditissima* is able to infect throughout the year through wounds and can spread aerially and by rain splashing within the same tree and to nearby trees. Branches and even the trunk can be girdled, leading to death of all proximal parts. *N. ditissima*, is also known to attack several forest hardwood tree species (e.g., *Acer, Betula, Fagus, Fraxinus, Populus, Quercus* and *Salix*).

Haploid single-spore *Neonectria* isolates (39) collected from susceptible and partially resistant apple cultivars from Sweden and other parts of Europe has been cultured and DNA has been extracted and sent for sequencing with Illumina chemistry. Thus far, the sequenced β-tubulin gene and ITS1 show a very highly conserved pattern for both genomic regions between all isolates.

Currently, the virulence (i.e. the degree of pathogenicity) of the isolates is being tested by using a detached twig assay (Garkava-Gustavsson et al., 2013) on a susceptible apple cultivar 'Elise', and a more resistant cultivar 'Aroma' to associate the phenotype of the pathogen (lesion length) with the genotype by a genome wide association mapping. We expect the pathogenic fungus to harbour broad-spectrum virulence factors in the genome. A fungal transformation system to generate knockout mutants in order to test some of these virulence factors is also being established.

Phenotyping pathogen resistance in cultivated strawberry roots using hyperspectral imaging

S8P11

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Verticillium dahliae is a major soil-borne pathogen that causes vascular wilt disease in cultivated strawberry (*Fragaria x ananassa*) leading to substantial crop loses. The root lesion nematode *Pratylenchus penetrans* has been reported to form a disease complex with *V. dahliae* on the host plant strawberry. Specifically, the presence of *P. penetrans*, activates the disease potential of *V. dahliae* at levels below the threshold that would normally cause disease. Thus far, eleven *V. dahliae* resistance loci have been identified through aboveground phenotyping. The development of a belowground phenotyping system using hyperspectral imaging will allow a faster and more comprehensive assessment of disease symptoms. Experiments have determined a root reflectance ratio that can quantify the infection level of *V. dahliae* inside in vitro strawberry roots. Screening of a mapping population segregating for resistance to *V. dahliae* and *P. penetrans* will assist with the confirmation of known quantitative trait loci (QTL) and also the identification of novel QTL controlling resistance to both pathogens. Ultimately, the natural resistance alleles present in cultivated strawberry and their wild relatives may be pyramided into high quality fruit lines in order to produce commercial cultivars with robust resistance to *V. dahliae* and *P. penetrans*.

Improving disease resistance in strawberry

S8P12

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The global strawberry industry faces major challenges, with growers encountering increasingly unpredictable and variable weather conditions, as well as the withdrawal of many fungicides and soil fumigants. These challenges are resulting in increased strawberry crop losses due to soil-borne diseases such as strawberry crown rot and strawberry red core, caused by the oomycete pathogens *Phytophthora cactorum and Phytophthora fragariae*, respectively. This project aims to identify and map resistance quantitative trait loci (QTL) in strawberry (*Fragaria* spp.) and examine the mechanisms of resistance.

There has been extensive research investigating qualitative (major gene) resistance to *Phytophthora* species, however, much less is known about quantitative resistance (multiple genes, each of partial effect). We present our progress to date in identification of the genetic basis of quantitative resistance to *P. cactorum* in the octoploid strawberry (*Fragaria* x *ananassa*). A mapping population (n= 181) of 'Emily' x 'Fenella' was assessed for resistance/susceptibility to *P. cactorum*, by scoring the disease severity in the crowns and multiple QTLs for resistance were identified. The QTL were almost exclusively additive in nature with no significant epistasis detected between loci. ANOVA analysis revealed a strong correlation between predicated and observed phenotypic differences. Testing a subset of the mapping population progeny (n= 15) against a range of *P. cactorum* isolates revealed no major differences in host response, however, some lines showed higher susceptibility than predicted, indicating that additional undetected factors may affect the expression of some quantitative resistance loci. Pedigree analysis revealed the presence of the identified resistance loci across a range of cultivated strawberry germplasm, indicating the utility of these markers in crop improvement.

Identification of one major QTL associated with gummosis disease in peach (*Prunus persica*) using the 9K SNP array

S8P13

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Peach gummosis, caused by Botryosphaeria spp. infection, is one of the most destructive diseases during planting process in the South part of China. It causes significant growth stunting, yield loss, branch trunk and tree death, and has been becoming a restrictive factor for healthy and sustainable development of peach production. The knowledge of the genetic mechanism of gummosis disease (GD) is necessary to develop new varieties with durable disease resistance in the peach breeding programs. In the present study, a segregate F1 population which comprised of 94 seedlings from a cross between a yellow peach cv. 'Jin Xiu Huang Tao' (female parent, 'JX', resistant to gummosis disease) and a white flat peach cv. 'Yu Lu Pan Tao' (male parent, 'YL', sensitive to gummosis disease). GD for each seedling was scored in the field on a 0-4 scale (0 = no visible symptoms to 4 = very severe infection on branches and the main trunk) in 2015 and 2016. The 9K SNP chips (Illumina Infinium) which include 8144 SNP markers were used to genotype all samples. Three Genetic maps ('JX'-map, 'YL'-map, 'JX' ×'YL'-map) were constructed with JoinMap 4.0: one for each parent and one for the linkage groups that could be integrated. QTL analysis was carried out by WinQTLcartographer 2.5 software under the LOD value >3. After the SNP quality filtration, the map-'JX'was composed of 426 SNPs distributed in eight linkage groups, and the map-'YL'was composed of 298 SNPs distributed in seven linkage groups. The map-'JX' ×'YL' was composed of 538 SNPs distributed in eight linkage groups, spanning a total genetic distance of 528 cM. One putative QTL was detected to be located on the bottom of LG6 in the integrated map-'JX' ×'YL', which will be useful in MAB programs for the genetic improvement of peach cultivars carrying the resistance locus.

Keywords: Peach, gummosis disease, linkage map, SNP array

Polygenic inheritance of resistance to *Cacopsylla pyri* in a *Pyrus communis* x P. ussuriensis population explained by four QTLs and an epistatic interaction

S8P14

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Pear psylla (Cacopsylla pyri) causes severe damage on European pear cultivars, resulting in high yield losses. Its control has become difficult since it has developed resistance to a wide range of pesticides and the number of molecules authorised has been decreased. Identifying pear psylla resistance factors should help breeding new resistant pear cultivars. We here analyzed the quantitative resistance to psylla inherited from the resistant genotype 'NY 10355' deriving from Pyrus ussuriensis. Quantitative trait locus (QTL) analysis was carried out by numbering the nymphs and estimating the nymphal development using a free-choice test performed on a large mapping population. We identified and mapped three new resistance loci against pear psylla on the linkage groups (LG) LG01, LG04 and LG12 of 'NY 10355' and confirmed the QTL previously detected on LG17. A strong epistatic interaction between the two QTLs detected on LG01 and LG17 appeared to be the major factor controlling the psylla infestation in the genotype 'NY 10355'.

Two large effect QTL identified and characterized for soft scald incidence in apple

S8P15

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Soft scald is a postharvest storage disorder of apple (*Malus* x *domestica*) characterized by distinct, usually slightly sunken, dark brown lesions often extending in a ribbon pattern across the apple skin surface. Susceptibility to the development of this disorder varies among varieties, locations, and storage conditions. The cultivar 'Honeycrisp', which is widely planted in North America, has proven especially prone to developing this disorder. The identification of loci controlling soft scald development would be useful in marker assisted breeding to select against high soft scald incidence in future apple cultivars. The purpose of this study was to identify and characterize quantitative trait loci (QTL) for soft scald incidence to facilitate marker-assisted parent and seedling selection in apple breeding programs. Towards this goal, four populations with 'Honeycrisp' as a common parent and 11 smaller pedigree connected populations from the University of Minnesota portion of the RosBREED germplasm set were phenotyped for soft scald incidence using 5 to 20 fruits stored at 1°C for 20 weeks in 2014 and 2015. All populations and the individuals available in their pedigrees were genotyped using the International RosBREED SNP Consortium apple 8K SNP array. QTL analyses were performed using the Bayesian statistics and pedigree-based QTL detection software FlexQTL™ and a highly curated genetic map. QTL were identified at the beginning of linkage group (LG) 2 and the middle of LG16 in both years with consistent effects within families. The QTL on LG2 is of larger effect with a consistently deleterious and identical-by-state haplotype present in the heterozygous state in many parents and is homozygous in 'Honeycrisp'. The QTL on LG16 has higher haplotype diversity and explains a large proportion of the variation in soft scald incidence within some families. This study suggests that two stable QTL are responsible for a majority of soft scald incidence.

Identification of new genomic regions for rose resistance to black spot

S8P16

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Black spot disease (caused by the fungal pathogen Diplocarpon rosae) is the most severe disease of roses in the outdoor landscape. New rules on pesticide use (Ecophyto 2018, Ecophyto II, J. Labbé and energy transition French laws) encourage breeders to develop roses with a high resistance level to black spot. Most current cultivars are susceptible to this disease because of a narrow genetic diversity in the cultivated germplasm. This project aimed at detecting resistance genes of rose germplasm to D. rosae that was slightly or not yet studied, and at evaluating the stability of these genes in different genetic backgrounds. Two progenies connected by the male parent, Rosa wichurana, known to be black spot resistant under different environmental conditions, were studied: Rosa hybrida 'H190' x Rosa wichurana (referred to as HW; 209 hybrids) and Rosa chinensis 'Old Blush' x Rosa wichurana (referred to as OW; 151 hybrids). These progenies were scored for black spot resistance after natural infections in field over three years (2012/2013/2014) in three French locations (Angers/Bellegarde/Diémoz) for HW and over two years (2014/2015) in only one location (Angers) for OW. Genetic maps based on microsatellite and SNP markers were developed for HW and OW progenies, respectively. One quantitative trait locus (QTL) was localized on linkage group 3 (LG3) of the male maps; it was revealed in the two progenies for several years and in different locations. Another QTL was identified but only in the HW progeny in 2013 in Angers and Bellegarde; it was mapped on LG5. The effectiveness of these QTL should be confirmed against a widest range of pathogen isolates. According to the precision and effect of detected QTL, searching for candidate genes underlying QTL would be planned thanks to rose genome sequence.

Update on molecular characterization of aphid resistance in black raspberry germplasm

S8P17

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USDA-ARS National Clonal Germplasm Repository

Black raspberry is a minor but lucrative crop with most of the acreage in the U.S. grown in Oregon. One of the challenges for black raspberry growers is the rapid decline of plantings resulting from aphid-borne virus infection. The North American large raspberry aphid is a vector of Black raspberry necrosis virus (BRNV) and other viruses in the Raspberry mosaic virus complex, to which all available cultivars are susceptible. BRNV spreads rapidly in the field resulting in plantings that decline in as few as two or three growing seasons. Aphid resistance was discovered in each of three separate wild black raspberry populations collected from Simcoe, Ontario, Canada (ON), Gardiner, Maine, USA (ME), and Bath, Michigan, USA (MI). Three full-sib black raspberry populations, designated ORUS 4305 (ON), ORUS 4304 (ME), and ORUS 4812 (MI), were used to study the inheritance of the aphid resistance from the three sources. We have successfully mapped the locus for the ON source of resistance, designated Ag4, on Rubus Linkage Group 6. Association analysis suggests that sequences from black raspberry genome Scaffolds 99, 525 and 684 are important for determining aphid resistance in ORUS 4305. We have identified simple sequence repeat (SSR) loci throughout these three scaffolds and have screened the loci in a subset of each population. To date, ten SSR loci on Scaffold 99 (S99) that segregate with aphid resistance have been identified, while screening of the loci for Scaffolds 525 and 684 is in progress. Preliminary results suggest that all three resistance loci segregating in each of the three populations are linked and strongly associated with S99 but each represents a unique locus. All markers will be validated in populations with mixed sources of resistance.

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