SUNDAY 14 NOVEMBER 2010

16:00	**REGISTRATION @ WALLENBERG CENTRE, STIAS, STELLENBOSCH** Mounting of Posters Refer to poster numbers for numeric order
17:30 – 19:00	**WELCOME RECEPTION @ STIAS**

MONDAY 15 NOVEMBER

	FIRST AUTHOR LISTED, PRESENTING AUTHOR UNDERLINED	
08:00	**REGISTRATION & COFFEE**	
08:45	**OPENING :**	
08:55	**INDUSTRY PERSPECTIVE** **Chair:**	
08:55	Hugh Campbell, The South African deciduous fruit industry in perspective. Deciduous Fruit Producers Trust Research, SA	
09:20	Jim McFerson, WA Tree Fruit Research Commission, USA	
	SESSION 1: ORIGINS AND DIVERSITY **Chair:**	
09:45	Volk	O51. DIVERSITY AND DOMESTICATION OF APPLES
10:05	Micheletti/Troggio	O33. MOLECULAR GENETIC DIVERSITY AND ORIGIN OF THE CULTIVATED APPLE
10:25	Njuguna/Bassil	O36. PHYLOGENETIC ANALYSIS IN *FRAGARIA* USING COMPLETE CHLOROPLAST GENOME SEQUENCES
10:45	Volk	O52. PHYLOGENETIC RELATIONSHIPS AMONG MALOIDEAE SPECIES
11:05-11:45	**MORNING TEA & POSTERS**	
	SESSION 2: PHENOTYPING & QUANTITATIVE GENETICS **Chair:**	
11:45	Bink	O5. ON THE GENETIC DISSECTION OF QUANTITATIVE TRAITS USING DENSE MARKER DATA
12:05	Volk	O53. STANDARDIZED PHENOTYPING: A COORDINATED EFFORT FOR *MALUS, PYRUS, PRUNUS* AND *FRAGARIA*
12:25	Peart	O38. ILLUMINA TECHNOLOGIES; UNLOCKING AGRICULTURAL GENOMICS
12:45 - 14:00	**LUNCH + POSTERS**	
	SESSION 3: STRUCTURAL GENOMICS **Chair:**	
14:00	Sosinski	O46. A FIRST DRAFT OF THE PEACH GENOME SEQUENCE AND ITS USE FOR GENETIC DIVERSITY ANALYSIS IN PEACH
14:20	Verde	O49. WHOLE GENOME SNP IDENTIFICATION IN PEACH GERMPLASM AND *PRUNUS* GENUS USING NEXT GENERATION SEQUENCING PLATFORM
14:40	Jung	O28. COMPARATIVE ANALYSIS AMONG ROACEAE GENOMES AND BETWEEN ROSACEAE AND OTHER PLANT GENOMES
15:00	Dhingra/Oraguzie	O15. UNRAVELING ROSACEAE GENOMES
15:20	Illa/Sargent	O25. COMPARATIVE ANALYSIS OF ROSACEOUS GENOMES AND THE RECONSTRUCTION OF A PUTATIVE ANCESTRAL GENOME FOR THE FAMILY
15:40	**AFTERNOON TEA & POSTERS**	
	SESSION 4: GENOMIC TOOLS **Chair:**	
16:10	Edge-Garza	O19. ROUTINE MARKER-ASSISTED SEEDLING SELECTION IN WASHINGTON TREE-FRUIT BREEDING PROGRAMS
16:25	Bonet/Monfort	O6. THE NEAR ISOGENIC LINES COLLECTION IN *Fragaria vesca* VAR." REINE DES VALLÉES": AN IMPORTANT TOOL FOR THE STUDY OF AGRONOMIC INTERESTING TRAITS IN STRAWBERRY CROPS
16:45	Aldwinckle	O1. EFFICIENT TRANSFORMATION OF APPLE: A VITAL TOOL FOR FUNCTIONAL GENOMICS AND INTRAGENIC CULTIVARS
17:05		
17:20	Function	

TUESDAY 16 NOVEMBER

	SESSION 5 : FRUIT QUALITY **Chair:**	
08:30	Allan	O2. MYB TRANSCRIPTION FACTORS AS TOOLS TO GENERATE HIGH ANTHOCYANIN FRUIT
08:50	Denoyes-Rothan	O14. GENETIC CONTROL OF ORGANOLEPTIC QUALITY TRAIT IN THE COMPLEX ALLOPOLYPLOID STRAWBERRY
09:10	Longhi/Costa	O31. HIGH THROUGHPUT GENOTYPING AND HIGH RESOLUTION PHENOTYPING TOWARDS A COMPREHENSIVE QTL INVESTIGATION RELATED TO APPLE TEXTURE FRUIT QUALITY
09:30	Chagne	O10. AN ANCIENT DUPLICATION OF APPLE MYB TRANSCRIPTION FACTORS IS RESPONSIBLE FOR NOVEL RED FRUIT-FLESH PHENOTYPES
09:50	Zhu	O55. TRANSCRIPTOME PROFILING OF CULTIVAR-SPECIFIC APPLE FRUIT RIPENING AND TEXTURE ATTRIBUTES
10:10	Chagne/Davey	O11. GENETIC CONTROL OF ANTIOXIDANT CONTENT IN APPLE FRUIT
10:30-11:00	**MORNING TEA & POSTERS**	
	FRUIT QUALITY : CONTINUED **Chair:**	
11:00	Gonzalez Fernandez-Nino	O22. TEARING DOWN THE WALL – SHINING A LIGHT ON DIFFERENCES IN FRUIT TEXTURE BY STUDYING THE CELL WALL COMPOSITION OF FRAGARIA SPECIES
11:20	Khan	O29. GENETICAL METABOLOMICS STUDIES IN APPLE
11:40	Nilo	O35. TIME COURSE PROTEOMIC ASSESSMENT OF THE CHILLING-INJURY SYNDROME IN TWO SUSCEPTIBLE CONTRASTING *PRUNUS PERSICA* FRUIT VARIETIES
12:00	Tadiello/Trainotti	O48. HUNTING FOR GENETIC FACTORS INFLUENCING ETHYLENE BIOSYNTHESIS USING *STONY HARD* FRUIT
12:20	Quero-Garcia	O40. QTL DETECTION FOR FRUIT QUALITY TRAITS AND PHONOLOGICAL CHARACTERS IN SWEET CHERRY
12:40	Soeker	O45. GENETIC MAPPING OF FRUIT QUALITY TRAITS IN APPLE (*MALUS X DOMESTICA* BORKH.)
13:00–14:00	**LUNCH + POSTERS**	
	SESSION 6 : DORMANCY & ARCHITECTURE **Chair:**	
14:00	Guitton	O23. GENETIC DETERMINANTS OF FLOWERING REGULARITY IN APPLE TREES
14:20	Vizoso	O50. IDENTIFICATION OF PUTATIVE PEACH TRANSCRIPTIONAL REGULATORY NETWORKS ASSOCIATED WITH COLD AND/OR RIPENING RESPONSE GENES
14:40	Iwata/Foucher	O26. BLOOMING AND BLOOMING AGAIN: WHAT WE LEARN FROM ROSE AND STRAWBERRY
15:00	Celton	O8.QTL DETECTION FOR ARCHITECTURAL AND PHENOLOGICAL TRAITS USING TWO INDEPENDENT SEGREGATING F1 APPLE (MALUS X DOMESTICA BROKH) POPULATIONS
15:20	**AFTERNOON TEA + POSTERS**	
	SESSION 6 : DORMANCY & ARCHITECTURE Continued **Chair:**	
15:45	Dirlewanger	O16. QTLS DETECTION FOR PHENOLOGICAL TRAITS WITHIN THE ROSACEAE FAMILY
16:05	Zhebentyayeva	O54. FROM GENETICS TO EPIGENETICS IN CONTROL OF CHILLING REQUEREMENT AND FLOWERING TIME IN PEACH
	SESSION 7: HORTICULTURAL TRAITS **Chair:**	
16:25	Molina-Bravo/Sosinski	O34. CONSTRUCTION OF A GENETIC LINKAGE MAP OF RED RASPBERRY (*RUBUS IDAEUS* L.): QUANTITATIVE ANALYSIS OF HEAT TOLERANCE, PRICKLE DENSITY AND GROWTH HABIT
17:45	Celton/Costes	O9. QTL ANALYSIS OF FRUIT SELF THINNING CHARACTER IN AN APPLE PROGENY
17:05		
17:30	**ROS IGI**	
18:45	**ROS EXEC**	

WEDNESDAY 17 NOVEMBER

	SESSION 7 : HORTICULTURAL TRAITS Continued **Chair:**	
08:30	Pina	O39. STUDYING THE MOLECULAR MECHANISMS OF GRAFT INCOMPATIBILITY IN *PRUNUS* USING 454 SEQUENCING
08:50	Surbanovski	O47. *PIP* AQUAPORINS AND DROUGHT STRESS RESPONSE OF *F. VESCA*
09:10	Rubio-Cabetas	O42. A MICROARRAY ANALYSIS REVEALED ANP AND OXIDATIVE RESPONSE GENES UNDERLYING THE DIFFERENTIAL RESPONSE TO HYPOXIA OF TWO PRUNUS GENOTYPES
09:30	Razavi	O41. CLONING AND FUNCTIONAL ANALYSIS OF CANDIDATE GENES FOR RESPONSE TO WATER DEFICIT IN *FRAGARIA* DURING DROUGHT STRESS
09:50	Sanchez-Perez	O44. IDENTIFICATION OF TWO PRUNASIN HYDROLASES IN ALMONDS AND AND ITS RELATION WITH BITTERNESS
10:10	Cachi	O7. GENETIC MAPPING OF A NON-S-LOCUS ASSOCIATED WITH GAMETOPHYTIC SELF-COMPATIBILITY IN SWEET CHERRY
10:30-11:15	**MORNING TEA + POSTERS**	
	SESSION 8 : DISEASES **Chair**	
11:15	Joshi/Schouten	O27. FUNCTIONAL ANALYSIS OF THE APPLE GENES *HCRVF1* AND *HCRVF2* FOR DEVELOPMENT OF CISGENIC AND INTRAGENIC APPLE CULTIVARS WITH RESISTANCE TO SCAB
11:35	Debener	O12. POSITIONAL CLONING OF THE ROSE DISEASE RESISTANCE GENE RDR1
11:55	Decroocq	O13. KNOWLEDGE-BASED STRATEGIES FOR THE DIVERSIFICATION AND PYRAMIDING OF RESISTANCE MECHANISMS TO *PLUM POX VIRUS* IN STONE FRUIT TREES (*PRUNUS* SP.)
12:15	Durel	O17. IDENTIFICATION OF *PTO/PRF* RESISTANCE GENE ANALOGS IN THE FIRE BLIGHT RESISTANCE QTL OF THE ORNAMENTAL APPLE CULTIVAR 'EVERESTE'
12:35	Baldo/Malnoy	O3. GENOME-WIDE IDENTIFICATION OF NBS-ENCODING RESISTANCE GENES IN MALUS X DOMESTICA
12:55–13:45	**LUNCH + POSTERS**	
13:45	**SESSION 8 : DISEASES Continued** **Chair:**	
13:45	Durel	O18. GENETIC MAPPING AND CHARACTERIZATION OF DISEASE RESISTANCE FACTORS IN APPLE
14:05	Ruiz/Zhebentyayeva	O43. INITIAL PHYSICAL MAPPING OF THE APRICOT *PLUM POX VIRUS* RESISTANCE LOCUS AND BASIC COMPARATIVE ANALYSIS WITH THE PEACH GENOME SYNTENIC REGION
14:25	Gardiner	O21. DELINEATION OF THE APPLE SCAB RESISTANCE GENES *RVI6* AND *RVI18* USING SNP MARKERS BASED ON WHOLE GENOME SEQUENCE
	SESSION 9: COLLABORATIVE GENOMICS **Chair :**	
14:45	Main	O32. GDR: A COMMUNITY RESOURCE FOR ROSACEAE GENOMICS, GENETICS AND BREEDING RESEARCH
15:05	Bassil	O4. ROSBREED'S SNP DETECTION PIPELINE AND COMMUNITY-AVAILABLE GENOMIC RESOURCES
15:25	Evans	O20. DEVELOPING AN ONLINE TOOLBOX FOR TREE FRUIT BREEDING
15:45-16:05	**AFTERNOON TEA + POSTERS**	
16:05	Iezzoni	O24. ROSBREED'S APPROACH TO BRIDGING THE GAP BETWEEN GENOMICS KNOWLEDGE AND BREEDING APPLICATION
16:25	Laurens	O30. THE NEW EU PROJECT FRUITBREEDOMICS: AN INTEGRATED APPROACH FOR INCREASING BREEDING EFFICIENCY IN FRUIT TREE CROPS
16:45	Peace	O37. DNA-INFORMED BREEDING FOR HIGH-IMPACT FRUIT QUALITY AND PRODUCTIVITY TRAITS IN WASHINGTON, USA
17:05	Removal of posters	
19:15	**CLOSING DINNER**	

POSTER ABSTRACTS LISTED ALPHABETICALLY BY FIRST AUTHOR : PRESENTING AUTHOR UNDERLINED
Poster no's in sequence of order for mounting

NAME	**TITLE**
Akagi	P1. DEVELOPMENT OF A GENE EVALUATION SYSTEM WITH VIRUS-INDUCED GENE SILENCING IN *PRUNUS*
Amador/ Rubio-Cabetas	P2. PYRUVATE DECARBOXYLASE: KEY ENZYME IN THE WATERLOGGING TOLERANCE IN PRUNUS
Amador/ Rubio-Cabetas	P3. ALCOHOL DEHYDROGENASE: ESTRUCTURE AND FUNCTION IN THE WATERLOGGING TOLERANCE IN *PRUNUS*
Araya/Vizoso	P4. CONSERVED POLYMORPHISM BETWEEN *PRUNUS PERSICA* AND *PRUNUS AVIUM*

Ashkani	P5. STRUCTURAL EVOLUTION AND FUNCTIONAL DIVERGENCE OF *S*-RNASE IN *ROSACEAE*: A TUNNELING TO THE PAST
Cabrera/Iezzoni	P6. SNP DISCOVERY IN SWEET CHERRY FOR ROSACEAE COS FAMILY-WIDE MARKERS
Dare/Hellens	P7. PHENOTYPIC AND METABOLIC EFFECT OF REDUCED CHS ACTIVITY IN APPLE
De Beer	P8. ISOLATION AND CHARACTERISATION OF THE *SERK*/*RKS* GENE FAMILY IN STRAWBERRY (*FRAGARIA ANANASSA*)
Decroocq	P9. GENOMIC ORGANIZATION OF THE RESISTANCE TO *PLUM POX VIRUS* IN DISTINCT APRICOT PROGENIES THROUGH A QUANTITATIVE META-ANALYSIS
Decroocq	P10. TRANSPOSABLE ELEMENT ANNOTATION AND THE DEVELOPMENT OF INSERTION SITE-BASED POLYMORPHISM MARKERS IN *PRUNUS* SPECIES
Dreeson/Davey	P11. DEVELOPMENT, MAPPING AND VALIDATION OF MOLECULAR MARKERS FOR GENES REGULATING APPLE FRUIT TEXTURE
Du Preez	P12. INVESTIGATING ANTHOCYANIN PRODUCTION IN TWO PHENOTYPES OF 'BON ROUGE' *PYRUS COMMUNIS*, L. BY HIGH THROUGHPUT TRANSCRIPTOME SEQUENCING
Eduardo/Pirona	P13. PEACH VOLATILE COMPOUNDS QTL ANALYSIS AND CANDIDATE GENE COLOCATION
Esumi	P14. GENETIC RESOURCE OF JAPANESE FLOWERING CHERRY “SAKURA” IN SHIMANE UNIVERSITY, AN USEFUL COLLECTION FOR POST-GENOMIC RESEARCHES IN *PRUNUS* SPECIES
Fernández i Martí	P15. IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH SELF-COMPATIBILITY IN *PRUNUS*
Garkava-Gustavsson	P16. MOLECULAR TOOLS HELP TO IMPROVE THE STATUS OF FRUIT GERMPLASM COLLECTIONS IN SWEDEN
Giongo/Costa	P17. TEXTURE PROFILING FOR STRAWBERRY FRUIT DEVELOPMENT AND RIPENING
Gitonga	P18. QTL MAPPING OF MORPHOLOGICAL TRAITS IN TETRAPLOID ROSE
Greyvenstein	P19. PHENOTYPING HEAT TOLERANCE IN GARDEN SHRUB ROSES UNDER CENTRAL TEXAS CONDITIONS
Husselmann	P20. ANALYSIS OF THE PROTEOME RELATED TO APPLE (*MALUS* SPP) AND APPLE SCAB (*VENTURIA INAEQUALIS*) INTERACTION
Kawamura	P21. QUANTITATIVE TRAIT LOCI FOR FLOWERING TIME AND INFLORESCENCE ARCHITECTURE IN ROSE
Koning-Boucoiran	P22. GENERATION OF SNP MARKERS USING THE NEXT GENERATION SEQUENCING TECHNOLOGY TO SATURATE THE GENETIC LINKAGE MAPS OF TETRAPLOID CUT ROSES
Lasserre-Zuber/ Durel	P23. APPLE SCAB RESISTANCE GENE PYRAMIDING ASSESSED IN A FRENCH NETWORK OF EXPERIMENTAL ORCHARDS: COMBINATIONS OF MAJOR AND MINOR RESISTANCE FACTORS EXHIBIT DIFFERENTIAL EFFICIENCIES OVER TIME AND SPACE
Lasserre-Zuber/ Durel	P24. CHARACTERIZING THE GENETIC DIVERSITY WITHIN THE 1060 APPLE CULTIVARS OF THE INRA GERMPLASM COLLECTION INDICATES A WEAK STRUCTURATION ACCORDING TO THE AGE OR THE USAGE OF CULTIVARS
Lasserre-Zuber/ Durel	P25. GENETIC DETERMINISM OF SCAB RESISTANCE IN THE APPLE CULTIVAR ‘DISCOVERY’: COMPARISON OF QTL MAPPING RESULTS IN PEDIGREED POPULATIONS
Le Van/Durel	P26. ARE WILD HABITATS A THREAT FOR NEW SELECTED SCAB RESISTANT APPLE CULTIVARS?
Maliepaard/ Smulders	P27. MAPPING AND QTL ANALYSIS IN TETRAPLOID PLANT SPECIES: ROSE AS A MODEL SYSTEM
Mbandi	P28. A COMPUTATIONAL FRAMEWORK FOR *VENTURIA INAEQUALIS* GENOMICS
Micali	P29. A TRANSCRIPTOMIC PROFILING OF A BC_1 POPULATION FOR THE IDENTIFICATION OF GENES RELATED TO POWDERY MILDEW RESISTANCE IN PEACH
Molina-Bravo	P30. POLYPLOIDY, MUTATION RATES AND GENETIC RELATIONSHIPS OF IMPORTANT CENTRAL AMERICAN *RUBUS* GENOTYPES: BASELINE STUDIES FOR ESTABLISHING A BREEDING PROGRAM IN COSTA RICA
Moya/Carrasco	P31. MOLECULAR CHARACTERIZATION OF S-ALLELES ASSOCIATED WITH SELF-INCOMPATIBILITY IN JAPANESE PLUM (*PRUNUS SALICINA* LINDL.) BY PCR ANALYSIS
Nilo	P32. GEL BASED PROTEOMICS ANALYSIS OF THE *PRUNUS PERSICA* FRUIT SOFTENING OF FIVE COMMERCIAL PEACH AND NECTARINE VARIETIES
Persson Hovmalm	P33. SCREENING FOR LOW MAL D 1 CONTENT, HIGH CONTENT OF POLYPHENOLS AND RESISTANCE AGAINST APPLE SCAB
Porto/Revers	P34. DIFFERENTIAL GENE EXPRESSION OF TWO APPLE CULTIVARS WITH CONTRASTING CHILLING REQUIREMENT
Prat/Silva	P35. IDENTIFICATION OF THE METABOLIC PATHWAY FOR NONADIENOL, AN INTERESTING VOLATILE COMPOUND IN *FRAGARIA CHILOENSIS*
Rios/Silva	P36. CHEMICAL AND GENETIC ANALYSIS TO TRY TO UNDERSTAND CRACKING SUSCEPTIBILITY IN DIFFERENT VARIETIES OF CHERRY
Rowland	P37. ROUTINE MARKER-ASSISTED SEEDLING SELECTION IN THE WASHINGTON APPLE BREEDING PROGRAM PROVIDES RESOURCE SAVINGS

Rubio/ Martinez-Gomez	P38. GENE EXPRESSION ANALYSIS OF RESISTANCE TO *PLUM POX VIRUS*, "SHARKA", IN APRICOT BY TRANSCRIPTOME DEEP-SEQUENCING (RNA-SEQ)
Sanchez	P39. A NON-TARGETED APPROACH UNRAVELS A VOLATILE PRODUCTION NETWORK ON PEACH FRUIT
Sanchez-Perez	P40. INHERITANCE OF CHILLING AND HEAT REQUIREMENTS FOR FLOWERING IN ALMOND AND QTL ANALYSIS
Sargent	P41. IDENTIFICATION OF QTL FOR *VERTICILLIUM DAHLIAE* (VERTICILLIUM WILT) RESISTANCE IN THE CULTIVATED STRAWBERRY
Sehic	P42. WHAT CAN GENOMICS DO FOR APPLIED APPLE BREEDING?
Smulders	P43. HIGH PERFORMANCE ROSES: A NEW IMPULSE FOR GARDEN ROSES BY MOLECULAR MARKER TECHNOLOGY
Tao	P44. EST ANALYSIS OF DORMANT BUDS OF JAPANESE APRICOT (*PRUNUS MUME*) BY MASSIVELY PARALLEL PYROSEQUENCING
Tartarini	P45. RESEQUENCING OF A PEACH GENOTYPE AND SNP DEVELOPMENT FOR MAPPING AND GENETIC DIVERSITY ANALYSIS
Testone/Giannino	P46. *KNOTTED*-LIKE GENES OF PEACH: STRUCTURAL CHARACTERIZATION, MAPPING AND TRANSCRIPTIONAL PROFILING DURING DRUPE DEVELOPMENT
Vanblaere/Patocchi	P47. DEVELOPMENT AND CHARACTERIZATION OF CISGENIC GALA APPLES CARRYING THE APPLE SCAB RESISTANCE GENE *HCRVF2*
Van Dijk	P48. INCREASED EFFICIENCY IN GENOTYPING AND MAPPING IN OCTOPLOID STRAWBERRY THROUGH QUANTITATIVE INTERPRETATION OF SSR DATA
Vendramin	P49. SUPPRESSION SUBTRACTIVE HYBRIDISATION APPROACH TO IDENTIFY GENES INVOLVED IN THE STONY HARD TRAIT IN *P. PERSICA* (L.) BATSCH
Verde	P50. THE ITALIAN INITIATIVE FOR PEACH MIRNA ANALYSIS (MIRNITALY)
Verdu/Durel	P51. FINE MAPPING OF A LARGE-EFFECT QTL FOR APPLE SCAB RESISTANCE COLOCALISING WITH THE MAJOR RESISTANCE GENE RVI6(VF)
Villatoro/Monfort	P52. GENETIC ANALYSIS OF FRUIT NUTRITIONAL QUALITY CHARACTERS IN F2 POPULATION OF CULTIVATED STRAWBERRY
Volk	P53. DUPLICATION IN THE DOMESTICA APPLE COLLECTION WITHIN THE USDA-ARS NATIONAL PLANT GERMPLASM SYSTEM IN GENEVA, NEW YORK
Werlemark/Nybom	P54. THE DIFFICULTIES OF DOGROSES (*ROSA* SECT. *CANINAE* L.)
Werlemark, Gun	P55. STUDIES OF INTERSECTIONAL CROSSES BETWEEN PENTAPLOID DOGROSE SPECIES (*ROSA* SECT. *CANINAE* L.) AND TETRAPLOID GARDEN ROSES REVEALS NON-ADDITIVE INHERITANCE OF ITS GENE FAMILIES AND RDNA REARRANGEMENTS
Yang/Reighard	P56. DEVELOPMENT OF A GENETIC LINKAGE MAP FOR IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO BACTERIAL SPOT IN PEACH

ORAL ABSTRACTS:
ALL abstracts are listed alphabetically by first author; presenting author underlined

O1. EFFICIENT TRANSFORMATION OF APPLE: A VITAL TOOL FOR FUNCTIONAL GENOMICS AND INTRAGENIC CULTIVARS

Herb Aldwinckle and Ewa Borejsza-Wysocka
Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, New York 14456, USA, hsa1@cornell.edu

Confirming the putative function of genes requires demonstration of actual function by silencing or over-expression. Functional analysis of multiple genes depends on availability of low-cost, efficient and rapid transformation. Achievement of optimal transformation rate is obtained by rigorous attention to explant production and selection, media composition and processing, and regeneration protocols. Intragenics is defined as the use of genes from cross breeding plant species, driven by plant promoters, without use of marker genes. It is preferred for commercializing improved cultivars because of non-use of genes from other organisms, or of antibiotic resistance genes. This is expected to result in more ready acceptance of intragenic cultivars by consumers and growers, as well as a simplified approval process by regulatory agencies. It differs from cisgenics in greater flexibility in choice of promoter sequences. Use of markerless transformation technology (MTT) resulted in transformed plants with an increased level of resistance to the severe bacterial disease, fire blight. Because MTT can result in chimeric transformed plants, techniques to selected uniformly transformed, non-chimeric plants are being developed by use of the myb10 transcription factor, which acts as a visible, non-destructive reporter.

O2. MYB TRANSCRIPTION FACTORS AS TOOLS TO GENERATE HIGH ANTHOCYANIN FRUIT

Andrew C. Allan[1], Kui Lin-Wang[1], Richard V. Espley[1], David Chagné[2], Cyril Brendolise[1], Tony McGhie[2], Arnaud Bovy[3], Henk J. Schouten[3] and Roger P. Hellens[1]
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[3]Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Anthocyanin concentration is one major determinant of plant colour. Anthocyanin biosynthesis is controlled by a distinct clade of R2R3 MYB transcription factors. In apple, MYB genes have been isolated that activate fruit skin (MYB1/MYBA), fruit flesh and foliage anthocyanic colour (MYB10). We have isolated MYB transcription factors, with high sequence identity to these genes, from all the commercially important Rosaceous species (eg. apples, pears, plums, peaches, raspberries, rose, strawberry). We have shown that these transcription factors can induce the anthocyanin pathway. Efficient induction of anthocyanin biosynthesis by these MYBs is dependent on the co-expression of bHLH proteins. A strong correlation between expression of the MYB gene, and fruit and flower anthocyanin levels, suggests that these transcription factors are key components in the control of anthocyanin biosynthesis in these species. Furthermore, over-expression of these genes in the crop of origin elevates anthocyanins and other health-related compounds. We have studied the impacts of high levels of anthocyanins on plant performance and fruit quality. Characterisation of these genes within newly release genome sequences has implications for the development of new coloured fruit and flowers.

O3. GENOME-WIDE IDENTIFICATION OF NBS-ENCODING RESISTANCE GENES IN *MALUS* X *DOMESTICA*

Angela Baldo[2], Giulia Malacarne[1], Michele Perazzolli[1], Laura Righetti[1], A.G. Bailey[2], Alessandro Cestaro[1], Marco Moretto[1], Silvio Salvi[1], Robert Viola[1], Riccardo Velasco[1] and Mickael Malnoy[1]

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[2]USDA-ARS Plant Genetic Resources Unit, Geneva, NY

Plant R genes are known to confer resistance to a variety of pathogens in a gene-for-gene mode. A total of 868 NBS resistance Gene Analogues (RGAs) composed the apple genomes. Fifty eight percent of the RGAs are Non-TIR-type and thirty percent TIR-type RGAs. These RGAs are uneven distributed along the 17 chromosomes and 622 of these genes are clustering in 156 clusters. Approximately, eighty percent of these RGAs anchored in have been subjected to a recent duplication in the genome. This is due to a low cluster KS value which means that tandem gene duplication, transposition and genome duplication have shaped the evolution of the large NBS family in apple. The preliminary analysis phylogenic is comparing the RGAs of *Malus domestica* versus Wild apple (133 aa seq) or versus RGAs of the *Rosaceae* (18 RGAs of *Fragaria,* 236 RGAs from *Prunus,* 42 of *Pyrus, 75* RGAs of *Rubus,* 151 RGAs of *Rosa* species) shown some specific clade of resistance specifically present in the wild apple, or to some family of *Rosaceae.*

O4. ROSBREED'S SNP DETECTION PIPELINE AND COMMUNITY-AVAILABLE GENOMIC RESOURCES

Nahla Bassil[1], Barbara Gilmore[1], Dorrie Main[2], Cameron Peace[2], Todd Mockler[3], Larry Wilhelm[3], Eric van de Weg[4], Thomas Davis[5], David Chagne[6], Sue Gardiner[6], Ross Crowhurst[6], Ignazio Verde[7], Bryon Sosinski[8], Michele Morgante[9], Pere Arus[10], Riccardo Velasco[11], Michela Troggio[11], Alessandro Cestaro[11], Gennaro Fazio[12], Jay Norelli[13], Jasper Rees[14] and Amy Iezzoni[15]

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[8]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695, USA

[9]Istituto di Genomica Applicata, Parco Scientifico e Tecnologico, 33100 Udine, Italy

[10]Institut de Recerca i Tecnologia Agroalimentàries, 08348 Cabrils, Spain

[11]IASMA Research and Innovation Centre, San Michele a/Adige (TN) -38010, Italy

[12]USDA-ARS Cornell Univ., Geneva, NY 14456, USA

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One of the activities of the USDA-funded multi-institutional and trans-disciplinary project, "RosBREED", is to develop crop-specific SNP genome scan platforms for peach, apple, strawberry, and cherry at a resolution of at least one polymorphic SNP marker every 5 cM in any random cross. These genome scans will be used, via Pedigree-Based Analysis, to identify and

validate a multitude of marker-locus-trait associations for application in breeding. Accessions of apple, cherry, and peach chosen for re-sequencing with the Illumina Genome Analyzer IIx (GAIIx) using paired-end reads (2x80bp) were selected based on their use worldwide in breeding and for diversity of genetic background, geographical origin, and phenotype, with a focus on fruit quality. In apple, we have re-sequenced genomic DNA from 21 accessions coordinated between the U.S. and Plant and Food Research, New Zealand. Sequences from 11 apple accessions to also use for SNP detection are being generated. In peach, we have re-sequenced genomic DNA from fifty-one accessions coordinated between the U.S. and the International Peach Genome Initiative (IPGI). Sequences were also generated from 16 sweet cherry and eight tart cherry accessions. A SOAP/SOAPSNP-based pipeline was developed to identify SNPs after alignment to the 'Golden Delicious' genome sequence for apple and the double haploid 'Lovell' peach genome sequence for peach and cherry. The Golden Gate assay was used to validate subsets of these SNPs. In the octoploid strawberry, we have generated more than 20x sequence coverage of a highly heterozygous progeny of 'Holiday' x 'Korona', coordinated between the US and Plant Research International, The Netherlands. Sequences from two likely diploid progenitors, *Fragaria mandschurica* and *F. iinumae* were generated and are being used, in addition to available sequences from *F. vesca*, to identify genome-specific regions to target in SNP development. The genomic data and resulting peach, apple, cherry, and strawberry SNP platforms will be valuable resources made available to the worldwide Rosaceae research community.

O5. ON THE GENETIC DISSECTION OF QUANTITATIVE TRAITS USING DENSE MARKER DATA

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The release of the apple, peach and strawberry genome sequences will dramatically accelerate the availability of high-quality genetic markers across Rosaceae genomes. It will also reduce the cost of developing markers. Consequently, incomplete and or sparse genome coverage, hampering the genetic dissection of complex quantitative traits in the past may disappear soon. However, other factors will become limiting and will likely concern a) Reference populations; b) Phenotypes; c) Data analysis tools. First, the size and structure of the reference population dominates the power to identify true QTL and to reject false QTL. Secondly, phenotyping remains expensive and time consuming, especially for longitudinal traits. Thirdly, statistical analyses tools must be extended to allow vast numbers of markers, multiple correlated traits as well as complex QTL models. In this study, we will illustrate this shift in focus through a case study using a public available simulated dataset from the 13th QTLMAS workshop (www.qtlmas2009.wur.nl). The data comprised a population of multiple related full sib families in an outcrossing species, a longitudinal trait (measurements at multiple time points) and dense genome-wide marker data. The trait was statistically analyzed by first fitting individual curves and subsequently analyzing the estimated curve parameters in relation to underlying QTL. Such an approach can contribute to better biological interpretation of the genetic architecture of longitudinal traits.

O6. THE NEAR ISOGENIC LINES COLLECTION IN *FRAGARIA VESCA* VAR." REINE DES VALLÉES": AN IMPORTANT TOOL FOR THE STUDY OF AGRONOMIC INTERESTING TRAITS IN STRAWBERRY CROPS

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Fragaria vesca possesses several features that make it attractive as a model for the Rosaceae family, the diploid nature of its genome permits to circumvent the genetic complexity of the cultivated strawberry. Furthermore, wild strawberries are characterized by much higher concentrations of plant volatiles than the cultivated ones, being interesting donors for strawberry breeding programs. In order to obtain exotic introgressions in a *F. vesca* homogeneous background we are developed a Near Isogenic Line collection in diploid *Fragaria*, using an Asiatic variety, *F. bucharica*, as introgression donor (also used as a parent in the Fragaria reference map population) and *F. vesca* var. Reine des Vallées (PI551824), a French non-runnering variety commonly cultivated in Spain with industrial purposes, as recurrent parent. A Near Isogenic Line (NIL) is one identical to an original genotype, except for a single introgressed chromosome fragment from a donor line. A BC1 and BC2 population was performed and analyzed for the presence of *F. bucharica* alleles within the 7 linkage groups of the *Fragaria* reference map. 26 introgression lines have been selected for the NIL collection development. Up to now, phenotypical variation has been detected for agronomic interesting traits as: Flower development, Flowering time, Runner development, Leaf size, Plant size, Fruit size. The characterization of the selected recombinants will permit to locate and estimate the inheritance pattern of the genes involved on the described traits. A strawberry collection of NIL with introgressions covering the whole genome of the donor line is a potentially powerful tool for the study of Quantitative trait loci (QTL) involved in fruit quality and other important complex traits, besides the introduction of new genetic variability in modern cultivars from exotic sources.

O7. GENETIC MAPPING OF A NON-S-LOCUS ASSOCIATED WITH GAMETOPHYTIC SELF-COMPATIBILITY IN SWEET CHERRY

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Sweet cherry (*Prunus avium* L) is mostly self-incompatible. Like in other Rosaceae species a gametophytic self-incompatibility mechanism prevents self-fertilization. This system is genetically determined by a locus, *S*, with multiple alleles, where the specificity of the pollen and pistil interaction is determined by two linked genes, *S-RNase* and *SFB*, located in the *S* locus. Additional factors not linked to the *S* locus, are also needed for a full expression of the self-incompatibility response. `Cristobalina´ is a spontaneous self-compatible sweet cherry cultivar and previous studies revealed that self-compatibility in this cultivar is not linked to the *S* locus. A microsatellite marker linked to self-compatibility in 'Cristobalina' was recently identified. This marker had been previously mapped in Linkage Group 3 of sweet cherry genetic map and thus the objective of this work was to confirm the location of the self-compatibility locus in the same linkage group. With this aim, markers located in the surrounding area of the linked marker were analysed in `Cristobalina´ populations and genetic linkage was estimated to confirm its genetic location.

O8. QTL DETECTION FOR ARCHITECTURAL AND PHENOLOGICAL TRAITS USING TWO INDEPENDENT SEGREGATING F1 APPLE (*MALUS* X *DOMESTICA* BROKH) POPULATIONS

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In modern apple orchards, small trees obtained through a combination of natural tree structure and pruning, are desirable in order to optimize production efficiency. In addition to the need for adapted tree architecture, increasing awareness of the environmental impact of global warming, notably on dormancy release, has led to the demand for new apple cultivars better adapted to low chilling conditions. Here we describe the results of a quantitative trait loci (QTL) analysis performed to study the genetic control of growth traits and time of bud break on two segregating populations. The first F_1 progeny of 125 individuals derives from a cross between 'Starkrimson' and 'Granny Smith' (STKxGS), while the second is composed of 271 individuals and derives from a cross between 'X3263' and 'Belrene' (X3263xBel). Consensus genetic maps were constructed using 164 (STKxGS) and 175 (X3263xBel) markers, and covered 1037 and 1251,9 cM, respectively. Both populations were phenotyped during 3 to 6 consecutive years for architectural traits (internodes length, branch base diameter, branch angles) and phenological traits (time of vegerative and floral budbreak). Both stable and unstable QTLs were identified for a majority of traits, and common genomic regions were identified among traits and between the two populations. Results of this study will provide valuable clues about the biological processes that control tree architecture and phenology. Identification of major QTLs will allow us to develop robust markers for marker assisted selection, and enable the breeding of new cultivars better adapted to the changing environment. Sequence information from the apple genome will allow us to identify candidate genes controlling these traits, and enable us to study the expression of genes co-locating with QTLs.

O9. QTL ANALYSIS OF FRUIT SELF THINNING CHARACTER IN AN APPLE PROGENY

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In apple (*Malus* x *domestica* Borkh) fruit production, the main criteria for profitability are the total fruit load, the fruit caliber, and their gustative quality. In order to increase overall fruit quality, growers routinely make use of chemical thinning agents to reduce total fruit load. These agents present a serious threat for the environment, leading to the demand for new apple cultivars presenting self-thinning properties. The objective of this project is to study the genetic determinism of the self-thinning character using a F_1 progeny derived from the cross between the hybrid INRA X3263, assumed to possess the self-thinning character, and the variety 'Belrène'. Four main variables were considered on different shoot types and over three consecutive years: the total number of inflorescences per shoot type, the number of inflorescences with fruit set, the number of fruits per terminal inflorescence and return bloom rate per shoot type. Low to intermediate

heritability values were obtained for total number of inflorescences per shoot type and the number with fruit set (0,33 and 0,55 respectively), while high values were obtained for the number of fruit on the terminal inflorescence (h2=0.83) and the rate of return bloom (h2=0.73). Besides the significant genetic effects, variance analysis showed considerable effects for the observation year and the branch type factors, as well as an interaction effects between these two factors. Best Linear Unbiased Prediction (BLUP) was estimated from a linear mixed model with the genotype as random effect, and used for Quantitative trait locus (QTL) detection. This detection was performed on a saturated genetic map constructed using 175 molecular markers, and including SSR and SNPs. For most variables, QTLs were detected on several linkage groups (LG), either independent from the year of observation or specific to a particular year. Our results suggest a moderate genetic control of the character “one fruit per inflorescence”. Further analyses, possibly involving other progenies, will be necessary in order to develop robust molecular markers for this trait.

O.10 AN ANCIENT DUPLICATION OF APPLE MYB TRANSCRIPTION FACTORS IS RESPONSIBLE FOR NOVEL RED FRUIT-FLESH PHENOTYPES

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Anthocyanin accumulation is coordinated in plants by a number of conserved transcription factors. In apple (*Malus* x *domestica*, Borkh.), a R2R3 MYB transcription factor has been shown to control red fruit flesh and foliage anthocyanin pigmentation *(MYB10)*, and fruit skin colour (*MYB1)*. However, the pattern of expression and allelic variation at these loci does not explain all anthocyanin-related apple phenotypes. One such example is an open-pollinated seedling of ‘Sangrado’ that has green foliage and develops red flesh in the fruit cortex late in maturity. We use methods that combine plant breeding, molecular biology and genomics to identify a candidate MYB transcription factor for control of this phenotype. Furthermore, we demonstrate that the red-fleshed cortex phenotype is controlled by *MYB110a,* a paralogue of *MYB10*. The chromosomal location of *MYB110a* is consistent with a whole genome duplication event that occurred during evolution of apple within the Maloideae family. Both *MYB10* and *MYB110a* have conserved function in some cultivars; however, they differ in their expression pattern and response to maturity signals.

O11. GENETIC CONTROL OF ANTIOXIDANT CONTENT IN APPLE FRUIT

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Apple is popular with consumers for its healthy attributes including vitamin and antioxidant contents. Vitamin C is essential for a range of functions, including protection from oxidative stress and ensuring connective tissue health. It has popular recognition, and is rated highly for its desirability in fruit. Polyphenolic-related antioxidant properties are also well documented both scientifically and publicly. There is a wide variation in vitamin C and polyphenolic contents among apple varieties, indicating that these traits are under complex genetic control. We have analysed the vitamin C and polyphenolic contents of skin and flesh samples of fruit from 180 individuals of our 'Royal Gala' x 'Braeburn' QTL mapping population. Data from 2008 and 2010 showed considerable variation in vitamin C content, ranging from the 'low vitamin C' parent ('Royal Gala') to values almost twice as high as those of the nominated 'high vitamin C' parent ('Braeburn') - in total, ~7-fold variation. Fruit polyphenolics exhibited even greater variation. QTL analyses identified five genomic regions linked to vitamin C content. Alignment of these QTL regions with the whole genome sequence assembly of apple enabled the identification of putative candidate genes involved in the synthesis, transport and recycling of vitamin C within the QTL intervals. In addition, we identified 14 genomic regions associated with variation in polyphenolic compounds, including anthocyanins, catechins, epicatechins, phloridzin, p-coumaroyl quinic acid and procyanidins. A number of candidate genes related to polyphenolic biosynthetic pathways and gene regulation co-located with these QTLs. This information will allow us to target and focus future apple breeding programmes directed towards new varieties with higher vitamin C and polyphenolic contents.

O12. POSITIONAL CLONING OF THE ROSE DISEASE RESISTANCE GENE RDR1

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Black spot caused by the hemibiotrophic ascomycete Diplocarpon rosae is the most devastating disease of field grown roses. Therefore, research on the biology of rose black spot interaction received considerable attention over the last two decades. We were able to fine map a single dominant resistance gene to a telomeric position of the rose linkage group 1. With two BAC libraries that we constructed we were able to construct two contigs in two different rose species one of which spans the active resistance gene. For each contig we sequenced four BAC clones spanning the locus by 454 FLX and Sanger sequencing. After assembly of 260 kb at the Rdr1 locus we predicted 43 genes 9 of which belong to the TIR-NBS-LRR type of resistance genes which were the most likely candidates for Rdr1. All of the nine candidates carry intact motifs with known functions in active TIR-NBS-LRR genes as e.g. the GLPL, kinase 1 and kinase 2 motifs.

However, only five of the nine genes are expressed at low levels in leaves and petals, organs that display the Rdr1 resistance function. Therefore, we subcloned genomic DNA comprising the individual candidate genes into binary constructs and developed a transient complementation assay based on infiltration of transformation competent Agrobacteria harbouring individual genes. Although transformation efficiency in rose leaves was lower than in Nicotiana benthaminiana controls it was sufficient to clearly differentiate between the candidate genes and to identify RGA 8 as the functional Rdr1 resistance gene. Implications for future molecular breeding with the Rdr1 gene as well as the utilization of the infiltration assay to identify additional functional genes from other genetic backgrounds are discussed.

O13. KNOWLEDGE-BASED STRATEGIES FOR THE DIVERSIFICATION AND PYRAMIDING OF RESISTANCE MECHANISMS TO *PLUM POX VIRUS* IN STONE FRUIT TREES (*PRUNUS* SP.)

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A variety of genetical and biochemical approaches are currently available that allow us to gradually unravel the complex molecular interplay linking viruses and their host plants. This has lead to the identification of plant genes that either restrict or impair plant invasion by viruses (resistance determinants) or, on the contrary, contribute to the invasion process (susceptibility determinants). Remarkably, results of the past few years have demonstrated that in some cases allelic forms of susceptibility determinants can behave as resistance factors. This is illustrated in the case of *Plum pox virus* (PPV), the causal agent of the devastating Sharka disease of stone fruit trees, for which we have shown that functional eiF(iso)4E and eiF(iso)4G translation initiation factors are absolutely required for successful infection of the model plant *Arabidopsis thaliana*. Resistance mapping efforts in the natural *Prunus* hosts of PPV, peach and apricot, indicate that these or other isoforms may also contribute to natural polygenic resistance against this virus. It also provides us with copy-specific data of the peach orthologues and allows to set up different knowledge-based strategies to transform this key information into PPV-resistant *Prunus* crops.
Since most of the *Prunus* species are susceptible to PPV, we postulate that susceptibility alleles of those translation initiation factors are predominant and that natural variants for these genes are present naturally in *Prunus* germplasms. As a proof-of-concept, we surveyed 700 individuals from 20 Prunus species for *eiF4E* and *eIF(iso)4E* variants diverging from the PPV susceptible 'GF305' rootstock. Natural mutants were identified through resequencing of the candidate gene or/and HRM (High Resolution Melting) technology. Individuals presenting mutations in the translation initiation factors have been identified and are currently tested for resistance to PPV. A similar approach will be used to identify induced mutations among the 3,000 EMS mutagenised 'GF305' individuals under transfer in the field. Given the dominant nature of the susceptibility alleles of the translation initiation factors eIF4E and eIF4G, inactivation of the relevant endogenous copies is likely to interfere with the virus cycle and to result in resistance. To test this hypothesis, copy-specific RNAi-hairpin based constructs were obtained and transferred to peach and japanese plum by *Agrobacterium transformation*. The first transgenic lines are under test for resistance to PPV infection. We thank the CRG (INRA, France), MUAF (Lednice, Czech republic), ARS-USDA (Davis, USA), Inonu University (Turkey), AGRI (Bakou, Azerbaijan), JAAS (Nanjing, China) and Apricot repository in Xiongyue (China) for access to germplasm material.

O14. GENETIC CONTROL OF ORGANOLEPTIC QUALITY TRAIT IN THE COMPLEX ALLOPOLYPLOID STRAWBERRY

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Fruit quality traits are major breeding targets in the Rosaceae. Several of the major Rosaceae species are polyploids (e.g. the cultivated strawberry) or paleopolyploids (e.g. the apple). To dissect the inheritance of fruit quality traits in polyploid fleshy fruit species, we used a segregating population of cultivated strawberry constituted of a 213 full-sibling F1 progeny from a cross between the variety 'Capitola' and the genotype 'CF1116'. Towards this end, we further developed the strawberry linkage map already available which displays seven homoeology groups (HG). Each homoeology group, which is homologous to one chromosome in the diploid strawberry, comprises four linkage groups of at least two genetic origins (Rousseau-Gueutin et al. 2009). The map was used to identify quantitative trait loci (QTLs) for 19 fruit traits related to fruit development, texture, colour, anthocyanin, sugar and organic acid contents. Analyses were carried out over two or three years on field-grown plants. QTLs were detected for all traits analyzed. Because strawberry is a polyploid species ($2n = 2x = 56$), QTLs controlling a given trait and located at same positions on various linkage groups within one HG are considered as homoeo-QTLs. We found that, for various traits, about one third of the homoeo-QTLs were localized on 2 or more linkage groups. Several homoeo-QTLs controlling one trait and displaying same location within a HG could be detected the same year, indicating that several copies of the gene underlying the QTL are functional. Furthermore, the detection of some other homoeo-QTL was year-dependent. This result suggests that changes in allelic expression are taking place in response to modifications of environmental conditions. The mechanisms unraveled in the present study may play a crucial role in the variations of fruit quality in strawberry as well as in other polyploid fruit species.

O15. UNRAVELING ROSACEAE GENOMES

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The sweet cherry, double haploid pear and apple genome sequencing consortia

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Rosaceae is a unique plant family representing several important fruit and flower crops. In order to gain a holistic understanding of the structure and function of important genes in sweet cherry, pear and apple, we initiated sequencing the genomes of these plants. The genotypes used for genome sequencing include Stella, a founder genotype for most self-pollinated sweet cherry cultivars, genetically simpler double haploid (DH) Comice pear and golden delicious apple. We have obtained 4X genome coverage using next-generation sequencing approaches. Several assembly protocols have been implemented and the assembly has been validated by transcript mapping. Further, we have generated 8 X BAC libraries of DH Pear and Cherry and 6.4 X BAC library of the DH Apple. A fine genomic scaffold is being generated via "scaffold sequencing" – a novel approach that utilizes next generation platform sequencing of BAC superpools followed by computational de-convolution. The result is generation of a fine scaffold, a minimum BAC tiling path and genomic phase-specific assembly of BACs. A basic framework of this approach followed by results obtained from initial analyses will be presented along with progress on the status of genome sequences. Combined with the recently released apple (Velasco et al., 2010), peach and strawberry genome data, this information is expected to provide a framework for comprehensive comparative genomics studies.

O16. QTLS DETECTION FOR PHENOLOGICAL TRAITS WITHIN THE ROSACEAE FAMILY

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Plant ability to adapt to the global warming, either in natural or cultivated ecosystems, is becoming a crucial point for agriculture and environment. Within fruit species, an increasing evidence shows that advances in blooming dates, as well as phenological disorders, are linked to temperature increases during periods of dormancy (autumn, winter) and bud burst (spring). In order to anticipate the negative impacts and to safeguard fruit production in Europe, classical plant breeding programs must incorporate into their selection criteria traits related to adaptation to the climate change. Several phenological traits were evaluated on *Prunus* (peach, apricot and sweet cherry), *Fragaria* and *Malus* species during three to eight years according to the species. In peach (*P. persica* L. Batsch), two progenies were analyzed, one is an intraspecific F_2 issued from 'Ferjalou Jalousia®' × 'Fantasia' cross and the second is derived from an interspecific cross between the peach Summergrand and the clone P1908 of a wild species related to peach (*P. davidiana*). In apricot (*P. armeniaca* L.), two F_1 progenies were analyzed, one derived from the cross 'Lito' × 'BO81604311' was evaluated for maturity date, and one 'Goldrich' x 'Moniqui' was evaluated for blooming and maturity dates. In sweet cherry (*P. avium*), an F_1 progeny derived from the cross between varieties 'Regina' and 'Lapins' was evaluated for blooming and maturity dates. In *Fragaria,* blooming date was evaluated in the F1 population Capitola x CF1116. In apple, time of bud break was evaluated in two F1 progenies from the crosses 'Starkrimson' x 'Granny Smith' and ' X3263' x 'Belrene'. The stability of the detected QTLs across years will be examined. The QTLs detected in each species will be compared in order to evaluate the synteny level within the Rosaceae family for the QTL detected for phenological traits. Candidate genes and QTL colocalisation using the peach complete genome sequence will be examined. This will give preliminary arguments on the conservation on the putative processes involved in phenological traits within the Rosaceae.

O17. IDENTIFICATION OF *PTO/PRF* RESISTANCE GENE ANALOGS IN THE FIRE BLIGHT RESISTANCE QTL OF THE ORNAMENTAL APPLE CULTIVAR 'EVERESTE'

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Fire blight (*Erwinia amylovora*) is the most destructive bacterial disease in apple orchards worldwide. So far, no resistance gene against fire blight has been characterised in apple, despite several resistance regions have been identified. A major QTL for fire blight resistance has been previously identified on bottom of LG12 of the ornamental cultivar 'Evereste' (Durel et al., 2009). We initiated the cloning of this QTL both for using the resistance gene sequence in future putative cisgenesis/intragenesis approaches and for better understanding the resistance and defence mechanisms controlled by this gene by transgenic approaches. Using bulked segregant analysis (BSA) and amplified fragment length polymorphism (AFLP) techniques, the LG12 genomic region carrying the QTL was first accurately localised within 4 cM thanks to two flanking markers and one additional marker located very close to the QTL peak. The latter marker was used to screen a BAC library developed for Evereste. A BAC clone of 189 kb derived from the chromosome carrying the allele conferring fire blight resistance was isolated by chromosome landing and sequenced. New microsatellites markers were developed in this BAC clone and used for genotyping about 100 individuals exhibiting a crossing-over between the two flanking markers from a population of 2703 individuals. Thanks to accurate phenotyping of the recombinants individuals, the genomic region containing the resistance QTL could be limited to 78 kb carrying 23 genes predicted with Softberry software. A cluster of 8 genes with homologies to already known resistance genes against bacterial diseases was identified and their transcription verified. From this cluster, two genes were recognized *in silico* as the two most probable fire blight resistance genes showing homology with the *Pto/Prf* complex in tomato.

O18. GENETIC MAPPING AND CHARACTERIZATION OF DISEASE RESISTANCE FACTORS IN APPLE

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Scab (*Venturia inaequalis*) and fire blight (*Erwinia amylovora*) are two major diseases in apple orchards. INRA-Angers research group is conducting research to identify and characterize resistance factors against these two diseases with special concern for resistance durability. For scab, the position of the major resistance QTL (QRL) deriving from the cultivar 'Antonovka 172632' into the hybrid TN10-8 was shown to precisely colocalise with the major resistance gene *Vf* on LG1 (poster 1). This QRL was efficient against most but not all *V. inaequalis* strains. By crossing 'Worcester Pearmain' and 'Beauty of Bath' cultivars, we reproduced a 'Discovery' sisters-

brothers progeny (D-sibs). Genetic mapping of D-sibs combined with pathological tests using a mixture of *V. inaequalis* strains confirmed the broad-spectrum of QRLs located on LG11 and LG17, previously identified in cvr 'Fiesta' (poster 2). The combination of these two QRLs from 'Fiesta' with *Vf* and *Vg* R-genes was shown to significantly improve resistance efficiency in three unsprayed orchards (North, West, and South-West of France) especially when Vf-virulent strains were present (poster 3). Finally, challenging various combinations of the previous resistance factors with *V. inaequalis* strains from wild *Malus* species (*M. sylvestris* and *M. sieversii*) in controlled conditions indicated a maintained efficiency of the 3 QRLs over all the tested strains (poster 4). Combining scab major genes with broad spectrum QRLs is seen as a valuable strategy for breeding new more-durably-resistant cultivars. For fire blight, the position of the QRL detected on the LG12 of the ornamental cvr 'Evereste' was accurately defined within 3 cM in collaboration with ETH-Zürich (CH). Resistance candidate genes found during the QTL cloning process will be soon tested thanks to genetic transformation (oral communication, INRA-ETHZ). The QRL FBF7 detected on LG7 of 'Fiesta' was challenged with three different strains of *E. amylovora* and proved to be efficient whatever the strains. To broaden the germplasm representation in resistance genetic studies, French genetic resource of dessert and cider apple cultivars (~1000 cvrs) was analyzed with 12 SSR and exhibited a low level of structuration (poster 5). A core collection will be soon built up and will allow association genetic studies for disease resistance and other agronomical traits in the frame of the FruitBreedomics European project (oral communication, F. Laurens).
Financial supports: ENDURE EU network, PomFruitHealth EU COST action, CTPS national project, FRB national project, INRA multi-division project

O19. ROUTINE MARKER-ASSISTED SEEDLING SELECTION IN WASHINGTON TREE-FRUIT BREEDING PROGRAMS

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Breeding new cultivars of tree fruit is slow and incurs huge operational costs for growing and maintaining inferior seedlings in the field. New genetic screening tools exist that might minimize these costs, although these tools are currently used rarely in tree-fruit breeding. We have explored feasibilities and developed protocols for marker-assisted seedling selection (MASS) to assist the Washington State University's apple and sweet cherry breeding programs in identifying cultivars with superior eating qualities, large fruit size, and self-compatibility. These protocols are designed to save the programs unnecessary operational costs of maintaining and evaluating inferior seedlings. A spreadsheet-based decision support tool is now used to predict cost efficiency of routine MASS schemes. Sensitivity analyses consistently show that for the apple breeding program, MASS is best implemented before nursery bud-grafting, but after culling for low vigor and disease susceptibility. For sweet cherry, the best stage is prior to field planting and also after cheap visual culling for low vigor and disease susceptibility. MASS is now routinely used in both programs. In 2010, three genetic tests enabled selection for texture and flavor in apple seedlings, and four genetic tests were used in sweet cherry for selecting for large fruit and self-compatibility. Further benefits of the genetic tests include establishing genetic identity, verifying parentage and detecting mislabeled plants. Incorporating DNA information into first test phase selection decisions currently saves each breeding program a third or more of operational costs.

O20. DEVELOPING AN ONLINE TOOLBOX FOR TREE FRUIT BREEDING

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There is currently no publicly available online database of any real practical use for tree fruit breeding. The team at Washington State University is collectively addressing this problem and adding the appropriate data and tools to secure site in GDR using their apple and cherry breeding programs as the models. The provision of breeder focused datasets and analysis tools integrated within GDR will significantly aid decision making within the breeding programs. This database will also allow the collection, storage and analysis of appropriate DNA, RNA, phenotype and germplasm datasets which can then be linked to traits that are of interest to breeders and industry stakeholders. In this presentation we will outline the progress made to date for the Apple breeding program using open source software.

O21. DELINEATION OF THE APPLE SCAB RESISTANCE GENES *Rvi6* AND *Rvi18* USING SNP MARKERS BASED ON WHOLE GENOME SEQUENCE

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Single Nucleotide Polymorphism (SNP) markers are an excellent choice of genetic markers for use by molecular geneticists working with apple breeders in the 'post-genomic' age. Occurring at a frequency of 4.4/Kb in the 'Golden Delicious' whole genome sequence, not only are SNPs plentiful, but they are easily identified and screened at medium throughput using High Resolution Melting (HRM) analysis. We demonstrate the use of such markers for locating a long-known, but unmapped resistance to a cluster on apple Linkage Group 1 (LG1). The highly scab-resistant accession Russian apple R12740-7A has been used as a progenitor in many apple resistance breeding programmes internationally. Although it was argued that it carries three major gene resistances, *Rvi2* (*Vh2*), *Rvi4* (*Vh4*) and *Vr*, the last-named gene has remained elusive for mapping because its identity was not clear, with the name *Vr* often having been associated with what is now *Rvi2*. While *Rvi2* and *Rvi4* both have been located to opposite ends of apple LG2 by mapping in differential hosts derived from Russian apple, the location of *Vr* has not been previously demonstrated, since it was not assigned to a specific phenotype when the name was coined in 1968. We report on the use of SNP markers developed from whole genome sequence of 'Golden Delicious' to map *Vr* to a location in very close proximity to a new marker developed originally for high-throughput mapping of *Rvi6* (*Vf*) in the Plant and Food Research cultivar

breeding programme, using the population 'Royal Gala' x A248R012T025. Re-mapping in a second, validation population 'Royal Gala' x G17 has enabled us to delineate more closely the relationship of *Vr* to *Rvi6* and further research is being carried out to determine the genetic relationship between them.

O22. TEARING DOWN THE WALL – SHINING A LIGHT ON DIFFERENCES IN FRUIT TEXTURE BY STUDYING THE CELL WALL COMPOSITION OF *FRAGARIA* SPECIES

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The fruit softening during ripening occurs primarily due to the loss in the cell wall (CW) structure. The major changes in texture of the fruit flesh have been attributed to the partial or complete solubilization of polysaccharides that constitute it. Numerous studies have characterized the changes in polymers related to the CW in the maturation. Demonstrating increased activities of hydrolases CW polymers during ripening. From the commercial point of view, this becomes more important for those fruits that are known for having a short postharvest life, such as strawberries. Two species of strawberry, *Fragaria chiloensis* (*Fc*) (local accession) and *Fragaria* x *ananassa* cv. Camarosa (*Fa*), show differences in the firmness of their receptacles, in similar periods of development. In the degradation of the cell wall of *Fc* and *Fa*, like in other fruits such as tomato, melon, apples, peach and grapes, the pectins were shown as one of the major polysaccharides involved in the change in texture during fruit ripening. Where the solubilization of pectins would be the process that contributes the most to softening of the pulp. Our results suggest that rhamnogalacturonan I is the polysaccharide that changes the most during development and maturation of *Fc* and *Fa*. Additionally, we observed significant differences in the amount of the wall monosaccharide galactose between both investigated species of strawberry, which may indicate differences in pectic polymers such as galactans, results that coincide with the Beta-galactosidase activity. Also, we observed the distribution of antibody LM6, LM5 and CCRC-M1 in the receptacle tissue of strawberries. The immnunofluorescence results showed different tissue distribution, similar to what happens in tomato mesocarp, where galactose is decreased near the epidermal layer, also were differences in the signal of these antibodies in accordance with the measures of CW sugar residues analyzed.
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O23. GENETIC DETERMINANTS OF FLOWERING REGULARITY IN APPLE TREES

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Perennial plants have a potentially long juvenile phase. When mature, flowering is recurrent but can be strongly inhibited by concurrent fruiting, leading to alternate bearing. In fruit trees and particularly in apple, this generates major agronomic issues by decreasing yield during "off" years and fruit quality during "on" years. Alternate bearing can be reduced by pruning and chemical fruit thinning, although these increase orchard management costs and environmental impact. Therefore, the development of novel cultivars with intrinsic regular bearing is highly desirable.

Our aim is to identify the genomic determinants of alternate bearing in apple trees. Our strategy combines quantitative genetics, Quantitative Trait Locus (QTL) mapping, candidate gene mapping and transcript expression. The experimental population, a F_1 progeny of 125 individuals, exhibits segregation of bearing behavior. The number of inflorescences, the number and the mass of harvested fruit were observed over six years, and summarized using indices such as biennial bearing index (BBI), precocity index (PI) and cumulative yield (CY). We then used these indices to estimate genotypic effects and to detect QTL. Floral genes and hormone-related genes were identified in the apple whole genome sequence assembly and markers were designed within candidate genes to compare their positions with QTLs for alternate bearing. The expression of the candidate genes co-locating with QTLs was investigated during floral induction and differentiation in a range of cultivars known as regular or alternate bearing. Transcript levels of trees carrying heavy crop, "on" trees, and trees carrying light crop, "off" trees, were compared using a cultivar exhibiting the alternate bearing trait. Candidate gene mapping indicated that known flowering genes may not be responsible for alternate bearing, as they do not co-locate with QTLs, whereas several hormone-related genes are located within the QTL intervals. RNA profiling between "on" and "off" trees exhibited contrasting expression patterns, confirming the possible regulation of flowering by hormones such as gibberellins and auxins. Based on our results, we propose a regulatory network for floral induction and alternate bearing in apple that highlights the involvement of phytohormones. These results raise questions concerning flowering control in perennials, which will be investigated by further experiments. Allelic variation of the candidate genes co-locating with QTLs will be confirmed in different genetic backgrounds to validate their involvement in alternate bearing in apple. Finally, these candidate genes will be used to develop markers for marker-assisted selection.

O24. ROSBREED'S APPROACH TO BRIDGING THE GAP BETWEEN GENOMICS KNOWLEDGE AND BREEDING APPLICATION

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"RosBREED" is a large-scale initiative of the U.S. Rosaceae genomics, genetics, and breeding community with strong international involvement, dedicated to genetic improvement of rosaceous crops by targeted application of genomics and socio-economics knowledge and tools to increase the efficiency of breeding programs, engage stakeholders, and train the next generation of plant breeders. Rosaceae genomics resources are developing rapidly but have not been translated to routine practical application. Our goal is to sustainably integrate modern genomics tools with traditional breeding approaches initially focusing on three fruit-bearing genera of Rosaceae: *Malus* (apple), *Prunus* (peach and cherry), and *Fragaria* (strawberry). Specific objectives are to: (1) enhance the likelihood of new cultivar adoption, enlarge market potential, and increase consumption of rosaceous fruits by using socio-economic knowledge of stakeholder values and consumer preferences to inform breeding; (2) establish sustainable technical infrastructure for an efficient Marker-Assisted Breeding (MAB) Pipeline in Rosaceae, including crop-specific single nucleotide polymorphism genome scan platforms for breeding-relevant germplasm exploiting the shared ancestry of Rosaceae crops; (3) integrate breeding and genomics resources by establishing a user-friendly U.S.-wide standardized statistical framework and breeding information management system; (4) implement MAB in RosBREED demonstration breeding programs with a common focus on fruit quality traits; and (5) enhance sustainability of cultivar development by transferring MAB technologies to the public and private community of U.S. Rosaceae breeders through training current and future breeders as well as engaging the production, processing, and marketing sectors, allied scientists, and consumers. RosBREED funding is provided from the USDA-SCRI, award number 2009-51181-05808.

O25. COMPARATIVE ANALYSIS OF ROSACEOUS GENOMES AND THE RECONSTRUCTION OF A PUTATIVE ANCESTRAL GENOME FOR THE FAMILY

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Comparative genome mapping studies in Rosaceae have been conducted until now by aligning genetic maps within the same genus, or closely related genera and using a limited number of common markers. The growing body of genomics resources and sequence data for both Prunus and Fragaria permits detailed comparisons between these genera and the recently released Malus × domestica genome sequence. We have generated a comparative analysis using 129 molecular markers that are anchored genetically to the Prunus and Fragaria reference maps, and physically to the Malus genome sequence. The correspondence between marker positions was high and conserved syntenic blocks were identified among the three genera in the Rosaceae. The findings have allowed us to develop an hypothesis for genome evolution within Rosaceae, and to reconstruct the ancestral genome of the family.

O26. BLOOMING AND BLOOMING AGAIN: WHAT WE LEARN FROM ROSE AND STRAWBERRY

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Understanding flowering process in horticulture is an important issue in term of fruit and flower production. In Rosaceae, wood strawberry (*Fragaria vesca*) and rose (*Rosa* genus) are two closely related species with genotypes having the ability to flower continuously. In both species, this trait is controlling by a recessive gene. Our objective was to identify the molecular basis of continuous flowering (CF). Here we show that the continuous flowering genes is encoded by a floral repressor of the *TERMINAL FLOWER 1* (*TFL1*) family. In rose, the CF phenotype is due to

the insertion of a *copia*-like retrotransposon in the second intron of a *TFL1* homologue, *RoKSN*. The insertion of the retrotransposon blocks the maturation of the mRNA and no *RoKSN* mRNA is accumulated. Frequently in rose, CF rose gave once-flowering climbing vegetative mutant. We characterized six independent vegetative climbing mutant pair. In these mutants, the retrotransposon recombines, and only the LTR (Long Terminal Repeat) element is remaining, restoring a function *RoKSN* allele. Furthermore, using large F1 progeny, no recombinant was found on 670 individuals between the CF locus and *RoKSN* gene. In once-flowering rose, *RoKSN* is weakly expressed in spring in vegetative shoots that will become floral. Later in the season, the new arising shoots present a high *RoKSN* accumulation and remain vegetative. In opposite, in CF genotypes, *RoKSN* is not accumulated and all new shoots are becoming floral, whatever the season. We also investigate the function of *RoKSN* in the wood strawberry. The *RoKSN* homologue, *FvKSN*, present a 2bp deletion in the coding region leading to the translation of a non functional protein. This deletion is associated with the CF phenotype in a collection of wood strawberry from Alpine origin. Our results demonstrate a new role of *TFL1* in perennial plants in maintaining vegetative growth and modifying flowering seasonality. It offers new perspectives to control flower and fruit production in perennial plants.

O27. FUNCTIONAL ANALYSIS OF THE APPLE GENES *HCRVF1* AND *HCRVF2* FOR DEVELOPMENT OF CISGENIC AND INTRAGENIC APPLE CULTIVARS WITH RESISTANCE TO SCAB

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The majority of the apple cultivars are susceptible to apple scab caused by the fungus *Venturia inaequalis.* In view of development of cisgenic and intragenic cultivars with resistance to apple scab, we functionally analyzed the apple genes *HcrVf1* and *HcrVf2.* These genes were isolated, including their native promoters, coding and terminator sequences. Two fragment lengths of the native gene promoters of both *HcrVf* genes were tested for expression and their effect on resistance. The highly active apple rubisco promoter was also used to express both *HcrVf* genes. The scab susceptible cultivar 'Gala' was used for plant transformations. After selection of transformants, the *in vitro* propagated shoots were micrografted onto apple seedling rootstocks in the greenhouse. The expression of the genes was measured by quantitative RT-PCR (qRT-PCR). The apple rubisco promoter proved to give the highest expression of both the genes. The resistance spectra of the two genes were evaluated by testing resistance to six *V. inaequalis* isolates. *HcrVf1* did not give any resistance to any of the isolates tested. *HcrVf2* provided resistance at comparable levels and according the same spectrum as Santana, which has *Vf* resistance through conventional breeding. *HcrVf2* did not provide any resistance to a *Vf* virulent strain, even not in case of over expression by the apple rubisco promoter.

O28. COMPARATIVE ANALYSIS AMONG ROACEAE GENOMES AND BETWEEN ROSACEAE AND OTHER PLANT GENOMES

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We present genome duplication and conserved synteny analysis among the three sequenced Rosaceae genomes: peach, strawberry and apple. Our study detected some triplicated regions in these genomes in addition to some duplicated regions indicative of the ancestral paleo-hexaploidization. The comparison with grape and poplar showed that the hexaploidization predates the separation between the Rosaceae species and grape and poplar, and also the whole genome duplication in the poplar lineage. The conserved syntenic regions among the Rosaceae genomes, as well as between Rosaceae and other plant genomes, will be presented. The visualization of the syntenic regions in GBrowse_syn will also be presented.

O29. GENETICAL METABOLOMICS STUDIES IN APPLE

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Apples are among the main contributors of flavonoids in the human diet. The current research is focused on finding the genetic base of quantitative and qualitative variations of potentially health beneficial polyphenolic compounds in apple. The segregating population 1988-001 derived from the cross 'Prima' X 'Fiesta' was used for mapping metabolite QTLs (mQTLs). The integrated genetic linkage maps contained 801 markers (mainly DArT, AFLP, and SSR), spanning 1348 cM. Metabolites were detected by means of Liquid Chromatography-Mass Spectrometry (LC-MS). Processing of raw LC-MS data led to the detection of 418 metabolites in peel and 254 metabolites in flesh, which were used in mQTL mapping. In the untargeted mQTL mapping using MetaNetwork, 669 mQTLs were detected, 488 in peel and 181 in flesh. Four linkage groups (LGs) i.e. LG1, LG8, LG13 and LG16, were found to contain mQTL hotspots. For peel, 68 metabolites and for flesh 29 metabolites were annotated. 80% of the annotated metabolites belong to the

phenylpropanoid pathway. The genetics of the annotated metabolites was studied in detail using a targeted mQTL approach using MapQTL6.0™. The different quercetin glycosides have mQTLs on LG1 except for quercetin rhamnoside which has an mQTL on LG8 while methoxy-quercetins have mQTLs on LG13. The octane di-ols have mQTLs on LG8. The most significant mQTL hotspot was detected on LG 16 containing mQTLs for 32 metabolites in peel, including procyanidins, flavan-3-ols, phenolic esters, phenolic glycosides and kaempferol glycosides. In flesh, mQTLs were detected in the same hotspot on LG16 for 18 metabolites including procyanidins, phenolic esters, flavan-3-ols and glucuronic acid. All these metabolites except glucuronic acid belong to the phenylpropanoid pathway. We located the structural genes of this pathway on the chromosomes of apple, using the known sequences of these genes in *Arabidopsis* and the whole genome sequence of the apple cultivar 'Golden Delicious'. The structural gene LAR *(leucuanthocyanidin reductase)* was in the genetic window of the mQTL hotspot on LG16. Also some transcription factors were found here. We study whether LAR or any of the detected transcription factors is responsible for the mQTL hotspot on LG16.

O30. THE NEW EU PROJECT FRUITBREEDOMICS: AN INTEGRATED APPROACH FOR INCREASING BREEDING EFFICIENCY IN FRUIT TREE CROPS

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An international consortium gathering European and non European teams has designed FruitBreedomics, an ambitious research project, to improve the efficiency of fruit breeding by bridging the gap between scientific genetics research and application in breeding. The project will focus on apple and peach, two major fruits in Europe, but the tools and the knowledge gained will also benefit other species of the *Rosaceae* family *via* the strong ancestral relatedness among these species. The aim of FruitBreedomics is to provide the European fruit tree sector with cutting-edge breeding tools for the efficient and accelerated creation of new apple and peach varieties with excellent fruit quality characteristics, improved resistances to diseases and pests, and that can be grown in sustainable agriculture systems in the context of climate change. A major breeding tool to be developed is a validated pipeline for Marker Assisted Breeding and its implementation in ongoing commercial breeding programs. Towards this, the efforts will be directed to improve our understanding of the genetics of some major horticultural traits, develop innovative research tools to accelerate the breeding cycle, and efficiently find marker trait associations in breeding and GeneBank germplasm. Additionally, the project aims at increasing the accessibility of breeders to the genetic diversity present in GeneBank germplasm collections, thus contributing to widening the genetic basis of cultivated fruit trees. The collected data will provide precious genetic information on the pool of genitors and founders to be used in future breeding programmes. FruitBreedomics will use a multidisciplinary approach that includes genetics, genomics, ecophysiology and bioinformatics, and will liaison international partners with complementary expertises. From its start, the consortium aims at setting up a collaborative

European network of breeders, GeneBank curators and industry representatives with the aim of rapidly and widely disseminating and implementing the obtained results among all interest European stakeholders.

O31. HIGH THROUGHPUT GENOTYPING AND HIGH RESOLUTION PHENOTYPING TOWARDS A COMPREHENSIVE QTL INVESTIGATION RELATED TO APPLE TEXTURE FRUIT QUALITY

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Texture is one of the main principal factors defining fruit quality, and plays fundamental roles in consumer's judgment related to fruit enjoyment. Texture is constituted by a series of sub-traits rather than a single feature, and a possible reason of this behavior could be explained by the coordinated genetic process leading the changes occurring during the ongoing of ripening, as well as by the cortex architectural constitution. Among all the textural traits crispness is the most important, accounting for 90% of the general fruit appreciation. For a better investigation of fruit texture, with a particular emphasis to crispness, we employed a high resolution phenotyping strategy, combining simultaneously a mechanical and a acoustic profiling in order to dissect the textural complexity in apple. A statistical multivariate analysis (such as PCA), computed on the texture variability assessed on a collection of 86 cultivars (assembled as proof of concept), distinguished the parameters here defined in three main categories, and distributed the cultivars in a 2-D plot based on their textural performance. This novel combined strategy was further exploited to phenotype in a high resolution fashion two mapping populations high-throughput genotyped with two type of molecular markers. The first type is represented by SSRs assembled in new triplex set, as well as based on candidate genes derived from a translational genomic program specific for apple. The second group of marker is based on SNPs discovered during the sequencing project of the heterozygous genome of Golden Delicious, and genotyped through two technologies: SNPlex (Applied Biosystem) and GoldenGate assay (Illumina). The analysis performed on the two mapping populations allowed a broad QTL mapping investigation towards the identification of the main genomic regions involved in the control of these textural sub-traits. The genomic intervals related to the QTLs targeted on both maps were then anchored on the Golden Delicious assembled genome, and the identified predicted genes underlying the QTL-LOD profiles were manually annotated. These genetic elements might represent (previous validation) an important candidate gene set to be used in future programs of marker assisted breeding, for the selection of novel cultivars distinguished by a superior fruit texture performance.

O32. GDR: A COMMUNITY RESOURCE FOR ROSACEAE GENOMICS, GENETICS AND BREEDING RESEARCH

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The Genome Database for Rosaceae is an established community resource housing the integrated genomics, genetics and breeding data for Rosaceae. Accessible through easy-to-use and intuitive web interfaces developed in Drupal, GDR provides a wealth of comparative genomics tools including CMap, GBrowse, GBrowse-Syn and the custom designed genome annotation and curation tool GenSAS. In this presentation we provide the community with an update of the current GDR trait, diversity, transcript, pathway, and genome sequence data available for several

Rosaceae species, together with a description of browser functionality and analysis tools, and describe future developments of this federally funded community resource.

O33. MOLECULAR GENETIC DIVERSITY AND ORIGIN OF THE CULTIVATED APPLE

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We assembled a collection of about 200 apple accessions, including more than 100 cultivars and representing almost all the wild apple species. The collection was genotyped using several hundreds SNPs originally discovered within the FEM-IASMA Golden Delicious genome sequencing project and 35 publicly available SSRs. For portions of the collection, re-sequencing of 23 genic regions were also obtained. The transferability of *M.* x *domestica* SNPs within the cultivated germplasm ranged between 12% and 41%, for unselected and selected SNPs for higher MAF, respectively. A much lower rate was observed for most of the *Malus* wild species. Molecular phylogeny of the genus *Malus* based on our molecular data broadly agreed with the standard taxonomy of the genus and confirmed the tight relationships of *M.* x *domestica* with accessions of the wild apple species *M. sieversii*.

O34. CONSTRUCTION OF A GENETIC LINKAGE MAP OF RED RASPBERRY (*RUBUS IDAEUS* L.): QUANTITATIVE ANALYSIS OF HEAT TOLERANCE, PRICKLE DENSITY AND GROWTH HABIT

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Despite the high level of interest for growing raspberries (*Rubus idaeus*) in the southeastern US, production is limited by the lack of adapted, high quality cultivars. Breeding efforts are underway for increasing cultivar availability, however breeding improvement in *Rubus* is a slow and time-consuming process. In order to expedite this process, molecular breeding tools are being developed. Cultivars adapted to the southeastern US must tolerate warm, humid summers, so to address this issue, a genetic mapping population has been developed from a cross between (*R. parvifolius* × 'Tulameen') × 'Qualicum'. We constructed a genetic linkage map and conducted quantitative trait loci (QTL) analysis for a number of traits segregating in this population. Seven linkage groups were identified and were anchored to the already existing raspberry map (Graham et al., 2004). The majority of the linkage groups identified were of similar length, and anchor markers were located at similar genetic distances relative to other markers for all linkage groups when compared to the existing map. Chlorophyll fluorescence was used to assess heat tolerance in the mapping population. Three QTL were found and each explained 15.9, 10.4 and 8.8% of the variation. Other variably important horticultural traits segregated in the (*R. parvifolius* × 'Tulameen') × 'Qualicum' cross, and were evaluated for QTL analysis. These traits were growth habit, and prickle density. Two field evaluations were performed for growth habit and several regions were identified as significant. One QTL co-localized to the same region of the linkage map for growth habit in both growing seasons. Important QTL have been mapped for heat tolerance and should be considered for further molecular studies. This research has drawn a baseline foundation for the development of molecular technologies in improving heat tolerance in *Rubus*. Future research will focus on these regions to develop closely linked molecular markers for marker assisted breeding.

O35. TIME COURSE PROTEOMIC ASSESSMENT OF THE CHILLING-INJURY SYNDROME IN TWO SUSCEPTIBLE CONTRASTING *PRUNUS PERSICA* FRUIT VARIETIES

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Peach and nectarine that belongs to the melting varieties are characterized by displaying a short postharvest shelf life due to the rapid loss of flesh firmness at the end of the softening process. Hence, several strategies have been adopted to enhance fruit storage, being the use of low temperature one of the most frequently employed. However, this process induces several symptoms that affect the fruit quality and, consequently, the consumer acceptance. On the other hand, the time frame and temperature range that triggers the irreversible fruit damage depends on the genetical background of each variety. In order to gain a global perspective of the chilling-injury syndrome, a time course proteomic based analysis was set. Fruits of two *Prunus persica* varieties that showed a contrasting susceptibility to this phenomenon were stored for different lengths at 5°C, followed by shelf life at 20°C to induce its normal fruit softening or the development of cold injury related symptoms, such as fruit mealiness. Proteins were extracted from these samples and analyzed by means of fluorescence two-dimensional difference gel electrophoresis followed by Principal Component Analysis and Bayesian Estimation of Temporal Regulation evaluation. Through the use of this methodology, it could be established that the cell wall and carbohydrate metabolism, stress and abiotic stimulus response as well as ethylene biosynthesis were modified during the fruit softening under normal conditions, and considerably altered by the low temperature storage. Particularly, the accumulation pattern of proteins such as endopolygalacturonases, alpha-1,4-glucan-protein synthases, isocitrate dehydrogenases, monodehydroascorbate reductases, hydroperoxide lyases, dehydrins and thaumatin-like proteins were crucial to discriminate normal from chill injured fruit. This information opens the opportunity to breed new peach and nectarine varieties that displays the desired organoleptical properties and lower susceptibility to the chilling-injury.
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O36. PHYLOGENETIC ANALYSIS IN *FRAGARIA* USING COMPLETE CHLOROPLAST GENOME SEQUENCES

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Species evolution in *Fragaria* L. is characterized by interspecific hybridizations and auto- and allo-polyploidizations. Previous use of a small number of chloroplast regions for phylogenetic resolution included intergenic spacers (*trn*L-*trn*F and *psb*A-*trn*H) as well as the *trn*L intron and uncovered limited variation. The objective of this study was to obtain complete chloroplast genome sequences using the Illumina Genome Analyzer and use them to evaluate phylogenetic relationships in *Fragaria*. Chloroplast genomes from 22 *Fragaria* species and one *Potentilla* were sequenced in multiplex using modified Illumina adapters containing 3 bp barcodes. Sequencing chloroplast PCR fragments and genomic DNA preparations resulted in genome coverage of 78-99% (mean=82%) of 21 *Fragaria* chloroplast genomes. Phylogenetic analysis confirmed previously identified relationships of clades A, B and C, maternal inheritance in *Fragaria* and polyphyly of *F. vesca*. Calculations of divergence time from Bayesian analysis resulted in

discovery of the young age of the genus, 2.7 million years, a contrast from previous hypothesis of the existence of the genus long before the Pleistocene era. Species in unresolved clade C consisted of diploid and tetraploid Himalayan strawberries and evolved only 1.3 mya. The octoploids and decaploid *F. iturupensis* are monophyletic. A close phylogenetic relationship between *F. vesca* ssp. *bracteata* with octoploid and decaploid species was observed supporting a possible North American origin of the octoploids. The octoploid clade is only 450 thousand years old explaining low differentiation of the American subspecies. Validation of these relationships is under way by Sanger sequencing in three parsimony informative sites and taxon sampling in these three regions.

O37. DNA-INFORMED BREEDING FOR HIGH-IMPACT FRUIT QUALITY AND PRODUCTIVITY TRAITS IN WASHINGTON, USA

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Routine marker-assisted breeding (MAB) in tree fruit is rare. For the apple and sweet cherry breeding programs of Washington State University, two of the largest programs in the world for their crops, MAB is a reality. DNA information supports breeding decisions and genetic screening is integrated into ongoing breeding operations. Applications are diverse, including marker-assisted parent selection, parentage verification, assessment of crossing success, marker-assisted seedling selection, marker-assisted advanced selection description, genetic identity confirmation during repropagation, and fingerprinting of new cultivar releases. Targeted loci include both Mendelian trait loci and QTLs and involve known genes to flanking markers to single linked markers. SSRs and SCARs have proven the most versatile marker types for predicting breeding value of parents, high-throughput seedling screening, and describing genetic potential of advanced selections. We use the “MAB Pipeline” and Pedigree-Based Analysis to prioritize, adapt, validate, assess utility, optimize, trial, and apply reported marker-locus-trait associations to address breeding needs. This approach, at the core of the RosBREED project (www.rosbreed.org) that involves ten further U.S. Rosaceae demonstration breeding programs, provides a systematic procedure for channeling genomics research into breeding applications. Local industry funding is a vital ingredient, signifying stakeholder engagement and leveraging national and international resources. These efforts are aiding development and deployment of self-fertile sweet cherry cultivars for reliable cropping and efficient orchard design, and, following the motto of “put the consumer first”, advancing genetic improvement in fruit texture and flavor of apple and fruit size, firmness, and flavor of sweet cherry.

O38. ILLUMINA TECHNOLOGIES; UNLOCKING AGRICULTURAL GENOMICS

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Illumina's goal is to apply innovative technologies to the analysis of genetic variation and function. We have developed a comprehensive line of products and services addressing the scale of experimentation required to enable the use of genomics in agricultural research and production. These products include leading-edge platforms for genome-scale sequencing, gene expression profiling and array-based SNP genotyping. This talk will provide an introduction to the Illumina product portfolio and describe how it can be used to enhance the quality and quantity of agricultural production.

O39. STUDYING THE MOLECULAR MECHANISMS OF GRAFT INCOMPATIBILITY IN *PRUNUS* USING 454 SEQUENCING

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Plant grafting is a widely used means of plant propagation and growth control that is of considerable importance in the adaptation of interesting cultivars in appropriated areas. However, when divergent genotypes are grafted, they do not always constitute a successful graft and show their disagreement in the form of incompatibility. While several studies have described the morphological and physiological changes associated to compatible and incompatible unions in herbaceous and woody plants, there is limited information on the molecular basis for the incompatibility reaction. For a deeper knowledge of this topic, the main goal of this work was to compare the transcriptome of apricot/plum graft interfaces with different degree of compatibility at early stages of graft union development. The molecular approach used was 454 sequencing technology, this technique allows the transcriptome survey of organisms with unknown genomes. Two normalized cDNA libraries were constructed, one from the compatible graft interface Paviot grafted on Marianna 2624, and other from the incompatible graft interface Moniqui grafted on Marianna 2624 at 10 days after grafting. 454 derived sequences from both samples (compatible and incompatible) resulted in a total of 642,923 and 668,538 high quality reads respectively, being the total reads 1,311,461 with an average length 385,9 nucleotides. Assembly was performed and incorporated sequence reads into 19,927 contigs with an average length 908,3 nucleotides. 92.7 % of original reads went into contigs and 7.3 % became singlets. In order to assign putative functional roles to the contig sequences, these were blasted against different databases finding high percentage of contigs with a match. 18,989 contigs were assigned a total of 76,577 gen ontology (GO) terms covering a broad range of GO categories. The library-specific contigs were filtered from the overall result set for convenience. While the number of contigs with only reads from the compatible library was 207, the number of contigs with only reads from the incompatible library was 2949. Incompatible-only contigs had a very high proportion of GO terms not found in compatible-only contigs. These results will contribute to a molecular-level understanding of the complex process of graft incompatibility between graft partners that potentially affect the fate of the graft.

O40. QTL DETECTION FOR FRUIT QUALITY TRAITS AND PHENOLOGICAL CHARACTERS IN SWEET CHERRY

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Three-year (2006, 2008 and 2009) QTL detection studies were conducted at INRA-Bordeaux (France) on a sweet cherry F_1 progeny derived from the cross between varieties 'Regina' and 'Lapins'. 'Regina' is a very late blooming variety, with a late ripening date and a low fruit cracking susceptibility. 'Lapins', one of the first widely commercialised self-fertile varieties, blooms relatively early, has an intermediate ripening date, and is relatively susceptible to fruit cracking. The progeny is composed of 124 hybrids planted in their own roots in orchard and grafted on Tabel® Edabriz and planted in pots. A map was constructed for both parents, 'Regina' and 'Lapins', with respectively 89 and 72 SSR markers. Traits were measured during 3 years: bud break date, blooming and, ripening dates, cracking susceptibility, fruit weight, and fruit firmness. Fruit cracking was evaluated on individuals planted in orchard and on individuals planted in pots, according to different tests, based on the observation of 100 randomly selected fruits (% of cracked fruits). The strongest and most stable QTLs were found for the following traits: blooming date (LG4), mean fruit weight (LG2 and LG3), and fruit firmness (LG2). QTLs for cracking susceptibility were relatively weak and variable across years. The construction of a sweet cherry unigene in order to search candidate genes is already initiated and preliminary results will be presented. Implications for future sweet cherry programs and the use of marker-assisted selection will be discussed.

O41. CLONING AND FUNCTIONAL ANALYSIS OF CANDIDATE GENES FOR RESPONSE TO WATER DEFICIT IN *FRAGARIA* DURING DROUGHT STRESS

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With increasing evidence of the effect of climate change on water resources, drought stress and its effects on plant productivity is receiving more and more interest. Strawberry (*Fragaria* x *ananassa* Duch.) with a shallow root system and a large leaf area is very susceptible even to short periods of water deficit. In this study the quantitative expression pattern of some responsible genes in plant reaction to water deficit is being investigated in *Fragaria*. Twenty-three *Fragaria* genotypes were already characterized for their physiological response under drought conditions by measuring two eco-physiological characters: leaf relative water content (RWC) and water losing rate (WLR). Accordingly, two *F*. species together with one tolerant and one sensitive cultivar were selected and exposed to drought stress for gene expression analysis. The selection of candidate genes was based on previous studies on plant biochemical reactions under drought. These genes are commonly regulated by water deficit and encode the key enzymes of different pathways like osmotic adjustment by sugar and proline metabolism, ascorbic acid metabolism along with the genes encoding for some anti-oxidant enzymes. Genomic/cDNA sequences of *Fragaria* corresponding to these putative genes were cloned and sequenced. The cloned sequences shared a high homology with the functional genes as proved by blast comparison with public nucleotide databases. The most critical step for the whole quantification process was the selection of the suitable housekeeping genes. In previous analyses of gene expression in *Fragaria* with different purposes, different housekeeping genes were applied, but at least two or three

housekeeping genes are recommended. Reliable housekeeping genes were identified by investigation of variability in transcript accumulation of 10 cloned genomic/cDNA sequences corresponding to frequently used housekeeping genes. For this, proper primers for real-time PCR were developed on these 10 *Fragaria* sequences and all genes were tested in real-time PCR. The geNorm software was applied to determine the most stables from the set of tested genes and could state how many housekeeping genes will be sufficient for RT qCR normalization in *Fragaria* during drought stress. Finally the expression pattern of all genes of interest will be analyzed using RT qPCR in different *Fragaria* genotypes treated with drought stress. Based on the results obtained in this study we will be able to conclude which plant pathways are inducible in response to drought stress in *Fragaria.* More functional analysis of up/down regulated genes during drought stress in this study will be performed by RNAi.

O42. A MICROARRAY ANALYSIS REVEALED ANP AND OXIDATIVE RESPONSE GENES UNDERLYING THE DIFFERENTIAL RESPONSE TO HYPOXIA OF TWO *PRUNUS* GENOTYPES

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Plants have developed metabolic, structural and molecular mechanisms that allow them to survive during prolonged hypoxic stress. The molecular response program to hypoxia involves many genes, and at least 20 anaerobic polypeptides (ANPs) have been described in different sugar metabolism pathways. Transcriptional regulation in response to hypoxia treatment of two Prunus rootstocks was investigated. A microarray representing 4261 ChillPeach unigenes was hybridized with cDNA of root tissues of Myrobalan 'P.2175' *(P.cerasifera)* and 'Felinem' hybrid *(P.amygdalus x P.persica)*, which correspond to tolerant and sensitive plants respectively to waterlogging. "In vitro" rooted plants were submitted to hipoxia and normoxia conditions. The hypoxic treatment was carried out in airtight chambers with 3% O2, 0.03% CO_2 and 97% N_2 gas composition for 2 h and 24 h. The normoxia group was treated similarly, except that they were kept aerobic. For each sampling time (0, 2h and 24 h) three set of roots were taken for each genotype. To analyze globally the effect of hypoxia over treated roots compared to control roots at different times a SAM multiclass analysis was used. A total of 2442 genes are found differential when all samples are considered using a cut-off od FDR< 5% and q-value < 0.05. Out of them 916 genes are different between genotypes at time 0 and 1549 genes are not different at time 0. From the 916 genes, 482 are more highly expressed in the sensitive genotype and 434 in the tolerant genotype. Clustering and principal component analyses confirmed that both treatment and genotype contribute equally to the sample variance. To identify the genes most responsible for separating samples by treatment (PC1) and genotype (PC2) we select the genes with highest loading values. From the PC1 a total of 41 genes were selected as the genes that most contribute to separate samples according to the treatment: 33 were repressed and 8 were induced under the hypoxia treatment. And only 38 of the total were differential between genotypes (contributing to the PC2;) 19 were repressed and 19 were induced according the genotype. In relation to time evolution (PC3) we could observe that, while normoxia does not produce large changes from not treated roots with time, exposure to hypoxia resulted in dramatic changes in the transcriptome, especially at 24h. Clustering analysis revealed a number of genes encoding ANPs, as well as genes previously reported as induced by H2O2, indicating a possible crosstalk between the signaling pathways for hypoxia and oxidative stress.

O43. INITIAL PHYSICAL MAPPING OF THE APRICOT *PLUM POX VIRUS* RESISTANCE LOCUS AND BASIC COMPARATIVE ANALYSIS WITH THE PEACH GENOME SYNTENIC REGION

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Sharka disease, caused by the plum pox virus (PPV), is one of the major limiting factors for stone fruit crops in Europe and America. Unfortunately, attempts to block disease spread through eradication of the infected trees have been unsuccessful to date. In consequence, PPV resistance has become the most important trait for the apricot breeding programs currently in progress. Previous studies have reported the presence of a major PPV resistance locus (*PPVres*) on the apricot (*Prunus armeniaca* L.) linkage group 1 (LG1). However, mapping accuracy was not reliable enough and *PPVres* position was predicted within a low confidence interval. In this study, a total of 50 and 36 SSRs were incorporated into the LG1 maps corresponding to 'Lito' and 'Goldrich' PPV resistant cultivars, producing high density maps with 0.70 and 0.68 markers/cM, respectively. Part of the integrated markers belong to a set of 102 new SSRs developed in this study. Using these maps, and excluding those hybrids showing phenotype-genotype incongruence (GPI), a new binary trait locus (BTL) analysis for PPV resistance was performed and support intervals for *PPVres* narrowed down to 7.3 and 5.9 cM in 'Lito' and 'Goldrich', respectively . To develop a draft physical map for *PPVres* locus and the surrounding area, 71 overgo probes were hybridized against an apricot BAC library identifying 914 single BACs from which 340 were finally anchored onto a map region covering ~30-40 cM. Contigs corresponding to the two allelic haplotypes (resistant/susceptible) were built by high-information content fingerprinting (HICF) using BACs anchored onto the *PPVres* locus region. A total of 300 BAC-end and internal sequences were obtained and 257 out of them (assembled into 35 sequence contigs and 177 singletons). As a result we have developed high-density SSR maps for the apricot LG1 upper part.. Furthermore, these maps have been used to provide anchoring points for ordering a draft physical map. Anchored BAC clones will facilitate the isolation by positional cloning of the PPV resistance underlying gene(s). Preliminary results on synteny-based comparative mapping with the recently released peach genome sequence, suggest that this tool will be extremely useful for that purpose.

O44. IDENTIFICATION OF TWO PRUNASIN HYDROLASES IN ALMONDS AND AND ITS RELATION WITH BITTERNESS

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Prunasin is a monocyanogenic glucoside present in different genus such as *Prunus*, *Davallia* or *Eucalyptus* within the *Rosaceae*, *Davalliaceae* or *Myrtaceae* families respectively. Cyanogenic glucosides are toxic compounds found in more than 2500 plant species. More specifically, prunasin has been detected in all parts of an almond tree (*Prunus dulcis* (Miller) syn. *Prunus amygdalus* (D. A. Webb Batsch)) as roots, shoots and young fruits. Prunasin, the precursor of amygdalin that is accumulated in bitter almonds, can be degraded to HCN, glucose and benzaldehyde by prunasin hydrolase (PH) and mandelonitirile lyase. In this study, we have searched in the genome database of *Rosaceae* for ESTs with high similarity to PHs, obtaining four accessions, which maximum identity with the five PHs previously characterized in *P. serotina* was more than 80%. Fruit of RACE experiments, two different 5' UTR regions were obtained, meaning that there were, at least, two PHs, called *Ph691* and *Ph692* in the two almond phenotypes assayed. Both cDNAs had 86% of identities and 78% of identities at a protein level. Moreover, *Ph691* and *Ph692* were showing a 92% and 86% of identity to *Ph1* from *P. serotina*. However, at a protein level, PH691 and PH692 were more similar to PH5 from *P. serotina* with a 93% and 78% of homology respectively. The genomic DNA of the two *Phs* showed that these genes contained twelve introns, in the same splicing site as the ones present in the *Phs* from *P. serotina*. Both proteins contained, with a high score, a signal peptide between residues 39-40 for PH691 and between residues 31-32 for PH692. To check the tissue and cellular localization of these two proteins, we collected samples during fruit ripening and performed immunolocalization studies in two sweet and two bitter almonds with an antibody recognizing specifically PHs. Confocal studies have shown that these proteins can be localized in the apoplast, cell wall, inside or outside the vacuole or, surrounding the nucleus. Moreover, *in situ* studies with specific primers for *Ph691* and *Ph692* have revealed that not all the cells expressed these genes, what agrees with the immulocalization studies. Finally, these results point out that the expression patterns and the localization of these genes are specific for each variety, what implies that there will be different levels of prunasin available to be transformed to amygdalin, the bitter compound accumulated in heterogeneous amounts in almond varieties.

O45. GENETIC MAPPING OF FRUIT QUALITY TRAITS IN APPLE (*MALUS X DOMESTICA* BORKH.)

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Apple fruit quality is of utmost importance to apple farmers and breeders in the selection and commercialization of new cultivars. Fruit size, colour, texture, firmness and taste are all traits that affect the quality of fruit. In this study the genetic contribution of these traits is being evaluated in order to generate the genetic markers required for the application of marker assisted selection in fruit quality breeding. Three mapping populations, 'Prima' x 'Anna', 'Golden Delicious' x 'Priscilla' and 'Golden Delicious' x 'Anna', consisting of 87, 87 and 141 respectively, were used in the study. Fruit samples were analysed, using a range of visual, physical and sensory measurements, over a period of three years, and the data was then correlated using statistical analysis. The genetic maps for these populations were generated using both published microsatellites and new EST-SSR and DART markers, using JoinMap 4.0™. The location of quantitative trait loci (QTL) was detected using MapQTL 5.0™. Comparative genome analysis and the role of various genes on the outcome of fruit quality can then be investigated, using the integrated genetic map, and the QTLs identified can be used for marker assisted selection, to increase the speed and efficiency of the apple breeding program.

O46. A FIRST DRAFT OF THE PEACH GENOME SEQUENCE AND ITS USE FOR GENETIC DIVERSITY ANALYSIS IN PEACH

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Peach (*Prunus persica*) is one of the most important fruit crops in the world and ranks fourth in fruit production in temperate regions after apple, orange and pear with a world production of about 18 million of tonnes per year. China leads the world production with 8 million tonnes followed by Italy with 1.6 millions of tonnes, USA 1.3 million tonnes, and Spain with 1.15 million tonnes. Peach is considered one of the best genetically characterized species in the Rosaceae, and it has distinct advantages that make it suitable as a model genome species for *Prunus* as well as for other species in the Rosaceae. An international consortium of scientists, The International Peach Genome Initiative (IPGI) has recently completed a first high quality draft sequence of the peach genome. The initial assembly consists of approximately an 8 fold coverage of whole genome shotgun Sanger sequence derived from the doubled haploid 'Lovell'. The assembly is arranged in 202 scaffolds larger than 1Kb, equalling approximately 227 Mbp rather than the 280-300 Mbp that was previously predicted. The first 8 scaffolds (218 Mbp) represent chromosome scale molecules (or pseudochromosomes) that have been aligned to the *Prunus* reference map (T x E). The pseudochromosome molecules are named according to their corresponding linkage groups. To date, 99.2% of the sequence has been anchored to the reference map while 95.6% of them have been also orientated. Automated gene prediction and annotation produced 27,851 genes, 838 of which show alternative splicing giving a total of 28,689 predicted protein-coding genes. This first

draft assembly (Prunus Persica V1.0) is available at: www.peachgenome.org; www.phytozome.net/peach; http://services.appliedgenomics.org/gbrowse/prunus_public/ and was publicly released on April 1, 2010. IPGI is currently employing the peach genome for comparative analysis with the other available genome sequences. The genetic diversity within the species is also being investigated through the resequencing of different *Prunus* accessions.

O47. PIP AQUAPORINS AND DROUGHT STRESS RESPONSE OF *F. VESCA*

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Aquaporins are transmembrane proteins which facilitate the movement of water down an existing water potential gradient. The aquaporin family is particularly diverse and abundant in plants comprising of ~35 genes clustered in four subfamilies identified by phylogenetic analyses. The *plasma membrane intrinsic protein (PIP)* is the largest subfamily of aquaporins and is considered to be involved in water transport through tissues and plant water-balance in general. Here we report ten isoforms of *PIP* aquaporins identified in *F. vesca*, three belonging to PIP1 and seven to PIP2 group. Physiological responses of *F. vesca* was monitored and expression patterns of four *PIP* isoforms were analysed in leaf and root tissue during severe drought stress and re-watering and also in leaves during diurnal changes following moderate drought stress. During drought stress *F. vesca* showed dehydration postponement mechanisms involving leaf area reduction as well as reducing transpiration per leaf area but no significant response was observed regarding root mass. Three of the investigated genes (*FvPIP1;1*, *FvPIP2;1* and *FvPIP2;2*) showed a distinct diurnal pattern of expression and responded to water stress in one or both tissues whilst one (*FvPIP1;2*) was constitutively expressed. Expression in the root system of one of the genes (*FvPIP2;1*) showed a strong correlation with changes in soil moisture.

O48. HUNTING FOR GENETIC FACTORS INFLUENCING ETHYLENE BIOSYNTHESIS USING *STONY HARD* FRUIT

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Ethylene is the key hormone in controlling fruit ripening and, to better understand the regulation of its synthesis and its specific action during the syndrome, extensive gene expression profiling has been carried out on fruit carefully selected at different stages from preclimacteric to full climacteric. The ethylene pathway has been altered by either the application of its inhibitor 1-methylcyclopropene (1-MCP) or genetically, by using fruit carrying the *STONY HARD* (*SH*) locus. *SH* peach varieties lack the ability to synthesize 1-aminocyclopropane-1-carboxylic acid (ACC) because the expression of ACC synthase 1 (ACS1) is blocked while that of ACC oxidase 1 (ACO1) is not. ACS1 is strongly induced by auxin but also by 1-MCP. 1-MCP blocks ethylene perception, thus ethylene responsive genes, such as the melting polygalacturonase (*PG*) and *ACO1*, are repressed in 1-MCP-treated fruit. *PG* is repressed also in the *SH* genotype, as it is *ACS1* while *ACO1* is transcribed as in climacteric varieties. 1-MCP, besides inducing *ACS1*, induces also auxin-responsive genes, such as *GH3*. The auxin-responsiveness of ripening-induced genes has been tested on auxin-treated *SH* fruit. Many genes of the auxin domain are repressed in the *SH* fruit but their expression, as that of *ACS1*, could be rescued by an auxin treatment. The auxin treatment of *SH* fruit led to the production of ethylene which, consequently,

induced ethylene-regulated genes. Surprisingly, many genes belonging to the ethylene domain, as *ACO1*, *ACO2*, *ETR1* and *ETR2* are transcribed in *SH* as in normal fruit. Transcription factors (TFs) are keystones in regulating plant developmental processes as fruit ripening. The expression of peach TFs orthologs to *NOR* (*non ripening*) and *RIN* (*ripening inhibited*), know to be responsible for ripening regulation in tomato and other systems, increases with ripening in normal and *SH* genotypes. On the contrary, the expression of other TFs mediating hormone responsiveness, as an Aux/IAA, an auxin response factor (ARF) and an ethylene response factor (ERF), is reduced in the *SH* genotype. Assuming that these TFs are not altered in primary sequence, it is possible to speculate that the alteration causing the *SH* phenotype resides downstream *RIN* and *NOR* and before *ACS1* and possibly involves genes whose products are responsible for the increase in the free auxin necessary to start *ACS1* transcription. The peach genome sequence of the Yumyeong cultivar will help to verify if among the candidate genes presented there are alleles that might be the genetic basis of the *SH* phenotype.

O49. WHOLE GENOME SNP IDENTIFICATION IN PEACH GERMPLASM AND *PRUNUS* GENUS USING NEXT GENERATION SEQUENCING PLATFORM

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The next-generation sequencing technologies coupled with the growing number of genome sequences provide new opportunities to redesign strategies for more effective genome analyses. Massive parallel sequencing may be applied in whole genome resequencing of unrelated accessions for variant discovery (as SNPs, small in/dels and structural variants), aligning the reads to a reference genome. In the frame of the peach genome sequencing initiative, a panel of *Prunus* accessions was resequenced with the Illumina Genome Analyzer IIx to investigate nucleotide variation in the peach germplasm and detect useful SNPs. Peach accessions have been selected for their broad variability according to previous molecular studies. The chosen accessions also display a broad range of geographical origins and phenotypical variations. In total 28 peach accessions were resequenced with GAIIx at 4-5X coverage using paired-end reads (2x100bp) and indexing methodology. Seven *Prunus* genotypes [*P. persica* L. cv. Earligold, *P. persica* L. cv. Yumyeong, *P. persica* L IF7310828, *P. persica* L F_1(C x A), *P. persica GF305, P. ferganensis* and *P. davidiana*] were resequenced with a 20-30X coverage at 2x75bp or 2x100bp to deeply investigate single individual variation and fine scale linkage disequilibrium. *P. dulcis* (cv. Texas) was resequenced with a 60X coverage with the aim of building a *de novo* assembly. A total of more than 1 billion of high quality pair-end reads (about 100 Gbp) were obtained. All the reads were aligned against the peach whole genome sequence (Peach V1.0) recently released by IPGI (The International Peach Genome Initiative). Preliminary analysis within the peach accessions resequenced at the highest coverage showed a low level of variation (SNPs and structural variants) within the species. The peach genotype with highest level of variation compared to Lovell was the Korean cv. 'Yumyeong'. *P. ferganensis* resulted quite similar to *P. persica* suggesting a revision in its taxonomy, likely being a sub-species of *P. persica*. *P. davidiana,* conversely, showed the highest level of diversity in comparison to the peach sequence. Heterozygosity was in general

quite high within the peach accessions with the exception of *P. ferganensis* and GF305. This is in agreement with the pedigree of the resequenced accessions and with the flower biology of the analyzed species.

O50. IDENTIFICATION OF PUTATIVE PEACH TRANSCRIPTIONAL REGULATORY NETWORKS ASSOCIATED WITH COLD AND/OR RIPENING RESPONSE GENES

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Previously, we have reported the identification of clusters of genes that are co-expressed under different postharvest conditions using digital expression analyses (Vizoso et al., 2009). *In silico* analyses of the upstream regulatory regions of these co-expressed genes in the recently completed peach genome has enabled us to identify conserved cis-regulatory elements in these co-expressed genes. Candidate transcription factors responsible for this co-regulation are being identified using comparative analyses with Arabidopsis. Additionally, we have begun functionally characterizing these candidate transcription factors using transient over-expression analyses in peach fruits. We have also analyzed the expression of several transcription factors in cold treatment time course experiments in peaches.
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O51. DIVERSITY AND DOMESTICATION OF APPLES

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Species within *Malus* are genetically diverse. Individuals within the USDA-ARS National Plant Germplasm System have been identified with ploidies ranging from diploid to hexaploid. Chloroplast sequence data from seven regions have revealed genetic relationships among apple species and has aided in the assignment of individuals to specific species. We have also used both chloroplast sequence data and nuclear microsatellite data to determine genetic relationships among European cider apples and wild apple species including *M. sieversii*, *M. orientalis*, and *M. sylvestris* as well as species of Chinese origin. The integration of genetic data types over large populations of individuals will serve to understand the complex and reticulate history of apple domestication.

O52. PHYLOGENETIC RELATIONSHIPS AMONG MALOIDEAE SPECIES

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The Maloideae is a highly diverse sub-family of the Rosaceae containing several agronomically important species (*Malus* sp. and *Pyrus* sp.) and their wild relatives. Previous phylogenetic work within the group has revealed extensive intergeneric hybridization and polyploidization. In order to develop a framework for estimating diversity above the species level within the accession holding within USDA-ARS National Plant Germplasm system, we sampled 150 species representing 16 genera within the Maloideae from the Plant Genetic Resources Unit Geneva, NY and National Clonal Repository in Corvallis, OR. Sequence data taken from 7 chloroplast regions representing ~2.5 kb were collected from up to 10 individual genotypes for each of the nearly 800 accessions. Results indicate high levels of diversity of haplotypes with over 200 SNPs identified. These data can be used not only to organize and validate this important collection but can serve as the basis for further comparative studies aimed at integrating nuclear, plastid and quantitative data.

O53. STANDARDIZED PHENOTYPING: A COORDINATED EFFORT FOR *MALUS, PYRUS, PRUNUS* AND *FRAGARIA*

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The availability of genomic sequence and maps for multiple genera in the Rosaceae family has led to a need for widely transferable phenotypic data. The Genome Database for Rosaceae (GDR) has initiated efforts to include phenotypic data within its data tables. As part of this project, we are assembling and classifying key trait performance and morphological descriptors for diverse Rosaceae crops. In addition to using phenotypic information available for multi-insitutional projects such as RosBREED, we have included information from additional U.S. and international breeding and genebank programs with the goal of designing data tables that are as useful as possible. Frameworks will be developed such that data can be compared across crops, to facilitate the development and application of marker-trait associations within, and perhaps between, genera of the Rosaceae family.

O54. FROM GENETICS TO EPIGENETICS IN CONTROL OF CHILLING REQUIREMENT AND FLOWERING TIME IN PEACH

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Failure in accumulation of sufficient chilling by dormant buds has severe impact on fruit crops productivity and limits their geographic distribution. In spite of considerable progress in characterizing genetic pathways and epigenetic mechanisms involved in vernalization response in model plants, inherent mechanism of response to cold treatment in fruit trees is poorly understood. Based on our recent QTL mapping data in two Prunus species (peach and apricot) and the peach genome assembly, we developed a comprehensive program for identifying genetic pathways and potential epigenetic mechanisms involved in control of chilling requirement and flowering time in deciduous fruit trees. Three complementary experimental systems have been used to address these complex phenomena: 1) diversified peach and apricot germplasm for a “within-QTL interval” association analysis; 2) high- and low chill individuals from a peach F_2 mapping population with fixed parental haplotypes for functional analysis; 3) sequenced di-haploid genotype Lovell for seasonal monitoring of a global methylation status and miRNA profiling using high-throughput platforms. As a starting point, peach genomic regions harboring temperature-dependent QTLs were inspected for presence of genes controlling vernalization response and flowering in other plants such as Arabidopsis, rice and wheat. This analysis pointed out the Polycomb group (PcG) genes involved in epigenetic regulation of flowering in Arabidopsis as potential candidates for control of a temperature-dependent network in peach. We extracted all PcG-like genes from the peach genome assembly v1.0 (http://www.phytozome.org/peach) and identified peach PcG orthologs via phylogenetic interference with precomputed sets of plant gene families from PLAZA (http://bioinformatics.psb.ugent.be/plaza). Twenty PcG-targeted SSR markers were generated for narrowing QTL intervals on the peach CxFla map. New markers were tested and used as co-factors for QTL detection if they localized within the 95% confidence interval for at least a single chilling requirement, heat requirement or bloom date QTL. In addition, a set of gene-specific primers was used for analyzing the expression of candidate genes during the chilling fulfilling time course in vegetative and floral buds in two CxFla individuals (high- vs low chill extremes). In parallel, global DNA methylation status was analyzed in vegetative buds using methylation-sensitive AFLP analysis. Results from all these analyses and the utility of the Arabidopsis model for deciphering cold acclimation regulatory networks in peach and other Prunus species will be discussed.

O55. TRANSCRIPTOME PROFILING OF CULTIVAR-SPECIFIC APPLE FRUIT RIPENING AND TEXTURE ATTRIBUTES

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Transcriptome analysis, scanning electron microscopic (SEM) examination and systematic physiological characterization were performed on two apple cultivars, 'Honeycrisp' (HC) and 'Cripps Pink' (CP), which have distinct ripening features and texture attributes. Based on weekly maturity data at comparable ripening stages, substantial differences of fruit firmness and crispness were observed between these two cultivars. SEM images of fruit cortex tissues showed distinguishable cell wall thickness, which may contribute to the phenotypic variations of cultivar-specific fruit firmness and crispness. A high-density long-oligo apple microarray consisting of duplex 190,135 cross-hybridization-free 50-70-mer isothermal probes, and representing 23,997 unigenes, was designed for and manufactured on a Nimblegen array platform. Cortex tissues from both HC and CP at three maturation stages, 4, 2 and 0 week(s) before harvest, were utilized for transcriptome profiling and each sample is represented by 4 biological repeats. A total of 1793 and 1209 differentially expressed unigenes were identified from HC and CP, respectively, based on ANOVA analyses with a cutoff value of 2-fold change of normalized signal intensity and a non-adaptive false discovery rate (FDR) of 0.01. Unigenes implicated in hormone metabolism and response, cell wall biosynthesis and modification and those encoding transcription factors were among the major transcriptomic changes during ripening. Between two cultivars, most of the identified unigenes were similarly regulated during fruit ripening. A short list of gene families or specific family members exhibited distinct expression patterns, which may represent the candidates controlling cultivar-specific fruit ripening patterns and quality attributes. Our data suggested that a deficiency of auxin transport and homeostasis occurred in late-ripening CP with extreme firm fruit; while the elevated expression patterns for a number of unigenes related to auxin and JA functions as well as for hemicelluloses-degrading XTHs were observed in early-ripening HC with outstanding crispness. For 12 randomly selected unigenes, more than 85% of values showed consistent expression patterns between microarray data and RT-qPCR results. Selected candidate genes are being further investigated in wider selections of apple germplasm, including a cross population of 150 siblings between HC and CP, to validate their specific associations with apple fruit ripening and texture attributes.

POSTER ABSTRACTS:

ALL abstracts are listed alphabetically by first author; presenting author underlined

P1. DEVELOPMENT OF A GENE EVALUATION SYSTEM WITH VIRUS-INDUCED GENE SILENCING IN *PRUNUS*

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Apple latent spherical virus (ALSV) vectors have been shown to effectively induce stable virus-induced gene silencing (VIGS) in a wide range of plant species including roseaceous fruit tree species, such as apple and pear. In this study, we attempted to develop a VIGS-based gene evaluation system for *Prunus* using the ALSV viral vector system. A partial sequence of *phytoene desaturase* (*PDS*) of Japanese apricot (*Prunus mume*) was cloned and ligated into the T-DNA region of a binary vector pBICAL2. The T-DNA region of pBICAL2, designed based on the RNA2 of ALSV, contains a single ORF for the ALSV polyprotein under the control of double CaMV35S promoter sequences. The partial *PmPDS* sequence was ligated in frame with the coding sequences for the movement protein and the capsid protein Vp25 flanking the cloning site. The resultant pBICAL2-PmPDS was introduced into a disarmed *Agrobacterium* strain EHA105. The pBICAL1 constructed based on the RNA1 of ALSV was also introduced into EHA105. To amplify and produce recombinant ALSV particles, leaves of *Nicotiana benthamiana* were infected with pBICAL1/EHA105 and pBIDAL2-PmPDS/EHA105 at the same time. The amplified ALSV in *N. benthamiana* was then isolated and used to infect peach (*P. persica*) and Japanese apricot (*Prunus mume*) seedlings. We also constructed ALSV vectors for the VIGS of *PmDAM*s (*Prunus mume Dormancy Associated MADS-box* genes) to investigate the possible involvement of *PmDAM*s in lateral bud endodormancy of Japanese apricot. We will discuss the possible use of VIGS-based gene evaluation system for fruit tree species.

P2. ALCOHOL DEHYDROGENASE: ESTRUCTURE AND FUNCTION IN THE WATERLOGGING TOLERANCE IN *PRUNUS*

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The oxidative phosphorylation is a metabolic pathway located in mitochondrial membrane, whose function is to obtain energy and to reduce power. This pathway is locked as a consequence of low or null availability O_2 as a terminal electron acceptor when plants are exposed to low or free oxygen atmosphere. Under this condition plants trigger the fermentative pathways to get ATP. Ethanol fermentation is considered the most efficient pathway to obtain ATP and NAD^+ regeneration in oxygen deficit conditions. This fermentation is a relatively simple enzymatic process involving two enzymes: Pyruvate decarboxylase (PDC) and Alcohol dehydrogenase (ADH). Plant Alcohol dehydrogenase (ADH, E.C 1.1.1.1) belongs to a small multigene family and catalyzes the conversion of acetaldehyde to ethanol as a final step in the ethanol fermentation pathway. In the present study we have found the 3' and 5' ends of Myrobalan 'P. 2175' and 'Felinem', tolerant and sensitive to waterlogging respectively from very conservative ADH sequence. Gene expression by qRT-PCR and enzymatic activity were also measured in order to establish the differences at molecular and biochemical levels between the two genotypes subject to hypoxia and anoxia conditions In 'silico' analysis were carried out showing no differences between deduced ADH aminoacids sequence compared with others ADH from diverse species

and related with anaerobiosis. The maximum homology was found with ADH2 from *Vitis vinifera* in both genotypes. Gene expression studies revealed the accumulation of transcripts along the entire treatment with higher activity in the sensitive genotype. While the enzymatic activity revealed basal activity at 0 hour in both genotypes, after 24 h decreased in the sensitive genotype. The difference in enzymatic activity profile between Myrobalan 'P.2175' and 'Felinem' could be an indicator of the lower sensitivity of 'Felinem' to low oxygen concentration.

P3. PYRUVATE DECARBOXYLASE: KEY ENZYME IN THE WATERLOGGING TOLERANCE IN *PRUNUS*

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Waterlogging is an abiotic stress occurring in poorly drained soils after flooding either for short or long-term. As consequence, oxygen levels fall in soils producing an anaerobic environment around roots that creates hypoxia and subsequently anoxia conditions in soils. Under this situation, the oxidative phosphorylation is blocked, triggering other alternatives pathways to obtain energy, like the fermentative pathway. In plants there are three fermentation pathways: Lactic fermentation, ethanolic fermentation and alanine fermentation. Ethanolic fermentation is the most important pathway which is a simple pathway involving only two enzymes, namely Pyruvate decarboxylase (PDC) and Alcohol dehydrogenase (ADH). Pyruvate decarboxylase 2-oxo-acid carboxylase, (PDC) (EC 4.1.1.1) catalyzes the conversion of pyruvate to acetaldehyde and it is considered to be a key enzyme in switching to fermentative metabolism, since pyruvate is located at the branching point between anaerobic an aerobic metabolism. In plants PDC is part of a gene family being mainly the PDC1 gene that is activated under anaerobic conditions. In this work we present the 5' and 3' ends in Myrobalan 'P. 2175' and 'Felinem', tolerant and sensitive to waterlogging respectively from very conservative PDC sequence. We carry out studies to determinate the deduced aminoacid sequence and gene expression by qRT-PCR and enzyme measure in two genotypes under hypoxia and anoxia conditions. The comparison of the PDC deduced sequence from Myrobalan 'P. 2175' and 'Felinem' with other PDC related with anaerobiosis showed no differences among aminoacids. The maximum homology was found with PDC and PDC1 of *Prunus Armeniaca* and *Fragaria ananassa*. Gene expression studies revealed the accumulation of transcripts along the entire treatment but it was higher in the tolerant than in the sensitive genotype. While the enzymatic activity showed possible presence of regulatory posttranscriptional mechanisms and an acclimation of plants to more stringent conditions of oxygen deficit.

P4. CONSERVED POLYMORPHISM BETWEEN *PRUNUS PERSICA* AND *PRUNUS AVIUM*

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Because of the economic interest of exporting improved quality peach and cherry fruits, early identification of varieties with phenotypic features beneficial to the Chilean fruit economy are needed. Currently, Chile is implementing Marker Assisted breeding programs in several fruit species of high commercial interest. Due to the high degree of synteny found in member of the Prunus genus, our laboratory has been working towards identifying conserved SSR and SNPs polymorphisms between *Prunus avium* and *Prunus persica*, to be used in these breeding

programs. In this context, *in silico* analyses of DNA sequences of several peach and cherry varieties have enabled us to identify putative SNPs and SSR. The putative SNPs are being validated with HRM (High Resolution Melting) analyses; and the putative SSRs with GeneScan technology. The association between these polymorphism and genes involved in fruit quality will be analyzed so they can be used in Marker Assisted breeding programs for early identification of improved quality fruit varieties.
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P5. STRUCTURAL EVOLUTION AND FUNCTIONAL DIVERGENCE OF *S*-RNASE IN *ROSACEAE*: A TUNNELING TO THE PAST

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A major goal for the biochemists and evolutionary biologists is to understand the mechanisms and dynamics by which changes in gene sequence generate shifts in function and therefore phenotype. A complete understanding of this process requires the analysis of how changes in protein structure mediate the effects of mutations on function. The Rosaceae contains three subfamilies, one in Rosoideae, and two in Spiraeoideae (Prunoideae and Maloideae). The ribonuclease activity of S-RNases is essential for pollen rejection in the Rosaceae. Fossil records have shown representation of Prunoideae in the Eocene, 44.3 million year ago (MYA). These evidences along with the comparative analysis of S-RNase extant proteins would provide indirect insights into the diversification of S-RNase 3D-structure. Our findings from this study will contribute to further protein-engineering studies with the aim of clarifying the structure-function relations that shape the evolutionary process of S-RNase.

P6. SNP DISCOVERY IN SWEET CHERRY FOR ROSACEAE COS FAMILY-WIDE MARKERS

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A Rosaceae Conserved Orthologous Set (RosCOS) of genes was recently developed and 613 of these genes were bin-mapped in the *Prunus* reference genetic map of T×E (almond × peach). As the RosCOS provide a set of gene-based, orthologous, genome-wide, anchor markers for the Rosaceae, identifying SNPs for these genes in cherry breeding germplasm would provide useful markers for comparative genomics in Rosaceae and for high-throughput genome scanning of cherry. As it is expected that many RosCOS primer pairs, designed primarily from peach DNA sequences, would produce amplification products in the closely related cherry, SNP discovery in cherry was accomplished by the amplification, sequencing, and nucleotide alignment of the resulting PCR products from a set of sweet cherry selections predicted to represent the range of genetic diversity in breeding material. Using 627 previously designed primer pairs, 95% (596) of the RosCOS amplified a product in sweet cherry. Of these, 284 were monomorphic and not included in subsequent analyses. A total of 273 (44%) of the RosCOS were polymorphic among

the six cultivars, including 268 that had known *Prunus* T×E bin map locations. When the RosCOS haplotypes were evaluated for each of the eight *Prunus* linkage groups, the mean cM distances between markers ranged from 1.2 for Group 1 to 2.9 for Group 2 and Group 7. A set of 77 of the RosCOS markers are currently being added to existing sweet cherry segregating populations to facilitate QTL discovery.

P7. PHENOTYPIC AND METABOLIC EFFECT OF REDUCED CHS ACTIVITY IN APPLE

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In apple (*Malus x domestica*) there appear to be three distinct gene sequence with sequence similarity to functionally characterised chalcone synthase (CHS) genes. A construct was designed to generate dsDNA to a region of sequence common to all three apple CHS genes, and transformed into the cultivar 'Royal Gala'. Regenerated plants that were transformed with the dsRNA construct showed sever developmental abnormalities; leaves were small, internodes were shortened and axilary buds were less dormant in transgenic plants where the levels of all three CHS transcript were very low. Plants with reduced levels of transcript were developmentally normal although anthocyanin accumulation in the stem, but not the axillay buds, was affected. Microscopic analysis of the leaves revealed gross changes in the leaf ultrastructure with fewer palisade cells and quite different patterns when hybridised with the JIM7 antibody. Metabolic analysis confirmed a reduction in known CHS-derived metabolites such as flavonols and anthocyanins. Flouritin anad florizidin were also reduce to less than 1% of the levels in control plants, indicating the synthesis of these apple-specific compounds may required CHS activity. Taken together, these data implicates a phenoyl propoanoid derivation may have a key role in apple plants development.

P8. ISOLATION AND CHARACTERISATION OF THE *SERK*/*RKS* GENE FAMILY IN STRAWBERRY (*FRAGARIA ANANASSA*)

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Somatic embryogenesis receptor kinases (SERKs) are not only involved in plant development but also play a role in plant defence responses. These receptors form part of the LRRII subfamily within the leucine rich repeat receptor-like kinase (LRR-RLK) super family, as described for *Arabidopsis thaliana*. Here we report the isolation and characterisation of seven members of this subfamily within *Fragaria ananassa*. Deduced protein sequence analysis of the strawberry receptor kinase like SERK (FaRKS) family reveals high sequence similarity to other RLKs including SERK(s) from *Arabidopsis thaliana*, *Daucus carota* and *Vitis vinifera*, as well as nuclear interacting kinases (NIKs) from *Lycopersicon esculentum* and *Glycine max*. Similar to these RLKs, the strawberry RKS proteins shared common structural motifs, which include a signal peptide, followed by a leucine zipper sequence, five leucine rich repeats, a transmembrane region and a serine/threonine kinase domain. Expression analyses showed that the *FaRKS* family in strawberry are differentially expressed within the different plant tissues and respond differentially to treatment with various biotic and abiotic stimuli. The *FaRKS* family members showing highest homology to *SERK1* (*FaRKS0*, *FaRKS10* and *FaRKS2*) showed similar expression patterns in most tissues, with the highest expression levels observed in leaves. In addition, *FaRKS0* showed high

abundance of transcripts in the crown of strawberries. The other *RKS* genes (*FaRKS1*, *FaRKS5*, *FaRKS4* and *FaRRKS7*) were most abundant in the leaf stems, roots and flowers. *FaRKS0* and *FaRKS1* genes are significantly upregulated in response to mechanical wounding, jasmonic acid application and *Botrytis cinerea* infection, while being down regulated in response to salicylic acid, brassinosteroids and cold treatment. *FaRKS2*, *FaRKS5*, *FaRKS7* and *FaRKS10* on the other hand are upregulated with *B. cinerea* infection and the application of jasmonic acid only, with minimal to no response upon mechanical wounding. Furthermore, they do not respond to the application of brassinosteroids and only *FaRKS2* is significantly down regulated upon cold treatment. Lastly, *FaRKS4* expression remained relatively constant throughout *B. cinerea* infection, wounding and even after the application of salicylic acid, jasmonic acid and brassinosteroids. These results support the idea that the SERKs are not only involved in developmental processes, but they and possibly the extended LRRII family may also play a role in defence responses of plants.

P9. GENOMIC ORGANIZATION OF THE RESISTANCE TO *PLUM POX VIRUS* IN DISTINCT APRICOT PROGENIES THROUGH A QUANTITATIVE META-ANALYSIS

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The *Plum pox virus* (PPV), the causal agent of the sharka disease, is the most detrimental virus on stone-fruit trees, worldwide. To date, few sources of resistance have been identified and mapped or are being mapped in several apricot progenies issued from the genitors 'Stark Early Orange' (SEO), 'Goldrich', 'Harcot' 'Harlayne' and in *Prunus davidiana* clone P1908. However, whether those sources of resistance are distinct or not is still questioned. To answer this question, a meta-analysis of the apricot and *P. davidiana* resistances to PPV was performed, using both the Biomercator and MetaQTL softwares. The purpose of this study was i) to integrate all PPV resistance QTL information available in the literature in a QTL meta-analysis in order to detect consensual, most probable genomic regions linked to resistance to sharka disease, ii) to compare the genomic organisation of those resistance loci in different genitors, sometimes not genetically related. The first step consisted in merging the genotypic and phenotypic data in an extensive QTL meta-analysis. Starting from a consensus genetic linkage map, a statistical algorithm based analysis enabled to pinpoint the best model fitting the observed QTL. Data were projected on the *Prunus* TxE reference map as well as on the physical map. The QTL meta-analysis provided evidence on the occurrence of three Meta-QTL in the upper part of LG1 in Apricot and one on linkage group 3 (Marandel et al., 2009). While the Apricot cultivar 'Goldrich' bears one single QTL, 'Harlayne' and 'SEO' share two QTLs, explaining the higher resistance level of those two cultivars. Indeed, this analysis enabled to refine the boundaries of the genomic region controlling PPV resistance in the current genitors of resistance to PPV. The next step will be, in a near future, to integrate the QTL and meta-QTL maps with the peach full genome sequence using the BioMercator V3 software (ANR project 08GENO126). BioMercator V3 offers to perform within a user-friendly graphical interface, genetic map compilation, map comparison and MetaQTL-analysis using various state-of-the-art methods from previous versions of BioMercator (Arcade, et al., 2004) and MetaQTL (Veyrieras, et al., 2007) . BioMercator V3 will be available freely by early 2011 (http://moulon.inra.fr/index.php/fr/equipestransversales/atelier-de-bioinformatique/projects/74).

P10. TRANSPOSABLE ELEMENT ANNOTATION AND THE DEVELOPMENT OF INSERTION SITE-BASED POLYMORPHISM MARKERS IN *PRUNUS* SPECIES

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Complete genome sequencing is leading to important advances in genome annotation including the determination of the whole genome repeat sequence content of which transposable elements (TEs) are a key feature. Repeats in genomes are classified on the basis of their unique sequence signatures and the mechanism by which they are formed. For example, one category of repeat consists of tandemly duplicated sequences including any sequence found in consecutive copies along a DNA strand. In contrast, TE's, on which we focused our study, are repeat elements that are found dispersed across the whole genome. TEs can be classified according to the mechanism and intermediate they use to move (e.g. RNA (ClassI) or DNA (ClassII). Within each of these classes, TEs are further subdivided on the basis of the structural features of their sequences. Interestingly, in addition to their relative copy number importance in genomes, TEs are now known to play major roles in genome evolution, and gene regulation, thus linking global genome function to whole genome structure. Furthermore, TEs are ubiquitous in nature, often found in high-copy number, distributed throughout the genome in both hetero- and euchromatin, and show insertional polymorphism both among and within species. It has also been proposed that TE-induced genomic rearrangements and mutations tend to promote local genetic diploidization in polyploid genomes. Because of these characteristics, TE-based molecular markers have great utility for: establishing phylogenies; studying biodiversity; generating dense linkage maps; and they are ideal tools for studying the structure and evolution of the diploid and polyploid *Prunus* genomes. In order to develop insertion-site based polymorphism (ISBP) markers in *Prunus*, we first screened the peach genome sequence (http://www.rosaceae.org/peach/genome) for transposable elements, identifying and annotating them with the analysis program REPET TEdenovo and TEannot pipelines (http://urgi.versailles.inra.fr/development/repet/index.php). TEdenovo results were compared with the outputs of several other different programs such as LTRFinder, MUST-MITE and others . Data were verified manually to generate a full-length TE peach sequence database (named PTED for 'peach TE database') that was, in the last step, cross matched with the whole genome sequence of peach through the ISBP Finder software (Paux et al., Plant Biotechnology Journal, 2010). The design of TE-based markers is currently under validation using a subset of these ISBP markers in order to confirm that predicted ISBP markers correspond to single genomic loci in a collection of *Prunoideae* species and segregating *Prunus* populations.

P11. DEVELOPMENT, MAPPING AND VALIDATION OF MOLECULAR MARKERS FOR GENES REGULATING APPLE FRUIT TEXTURE

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The maintenance of firmness and texture during transport and post-harvest storage is considered to be one of the most important fruit quality characteristics in apple. Texture is recognized to be an important determinant governing consumer choice, and as such it is a major target of modern fruit breeding programs. However the complex physiology and polygenic nature of this trait inheritance, combined with the long juvenile period of apple means that this is a problem that is ideally suited to the application of molecular marker technology. Changes in fruit texture are intimately linked to fruit metabolism during ripening and development These ultimately lead to fruit softening and the loss of crispness, juiciness and the occurrence of mealiness. In the present study, candidate genes (CGs) potentially involved in regulating fruit firmness were selected from both the literature as well as from the results of in-house research programs which identified genes that are differentially expressed during fruit ripening. These CGs belong to functional classes involved in cell wall degradation, ethylene biosynthesis, signal transduction and perception as well as regulatory genes that are up-regulated around the climacterium. We have developed genetic markers for these CGs and have screened available germplasm for allelic diversity. Where possible these CGs were also mapped onto genetic linkage maps of the cultivars Telamon and Braeburn to examine their association with QTLs for fruit firmness. In parallel, a Bayesian belief network was inferred from the full 257 progeny of the Telamon x Braeburn mapping population. The inferred Baysian network visualizes the probabilistic relationships between the different allelotypes and the observed fruit softening. As such, the network can be interpreted in terms of the underlying gene regulatory network and it can be used to show the genetic potential for fruit softening of any particular genotype.

P12. INVESTIGATING ANTHOCYANIN PRODUCTION IN TWO PHENOTYPES OF 'BON ROUGE' *PYRUS COMMUNIS*, L. BY HIGH THROUGHPUT TRANSCRIPTOME SEQUENCING

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The production and stability of fruit skin colour in red and blush pears are desirable traits in many export cultivars under commercial production in the Western Cape region of South Africa. To investigate the underlying molecular mechanism controlling the production, stability and loss of red pigment, anthocyanin, which is produced in response to a variety of stress conditions including pathogen and UV light stress, we aim to characterise the extreme differences found between red leaved 'Bon Rouge' *Pyrus communis*, L. pear trees and their green leaved sports, using high throughput Solexa/Illumina sequencing. The cultivar 'Bon Rouge' was derived from a rare, spontaneous bud mutation of the green pear cultivar William's 'Bon Chretien' ('Bartlett') and is characterised by red skinned fruit resulting from anthocyanin production. 'Bon Rouge' was observed to revert at a high frequency, producing green tissues in clonal stripes on stems and fruit. The production of both phenotypes on the same tree presents a system in which to study the control of colour development under the same set of environmental variables in an identical genetic background. Gene expression differences were measured by high throughput next

generation sequencing using the mRNAseq module on Illumina/Solexa's GAII sequencing platform. Data analysis was performed with a number of different Bioinformatics tools. Sequences were assembled with Velvet (Zerbino et al., 2008) and further investigated using various propriety and publicly available software packages for genomic analysis. We report the most significant differences in gene expression levels between red and green phenotypes of 'Bon Rouge'.

P13. PEACH VOLATILE COMPOUNDS QTL ANALYSIS AND CANDIDATE GENE CO-LOCATION

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Volatile organic compounds (VOCs) are involved in aroma and pest resistance. Aroma is an essential factor influencing peach fruit quality. The study and understanding of aroma is hampered by its complexity and because the knowledge on the molecular, genetic and physiological mechanisms underlying its formation is limited. The aims of this study were to identify QTLs for VOCs in peach, to further understand the genetic basis of these components and to pave the way towards breeding-assisted selection programs by identifying molecular markers linked to some of these QTLs. QTLs were identified for 19 out of 23 VOCs analysed, including three major QTLs for nonanal, linalool and for p-menth-1-en-9-al. Based on the comparison with the Prunus reference map, hosting the map position of a high number of fruit quality candidate genes, two putative candidate genes were identified. A sequence similar to a lipoxygenase (LOX) that could be involved in nonanal biosynthesis and one similar to a geranylgeranyl diphosphate synthase (GGPPS) that could be involved in the biosynthesis of linalool and p-menth-1-en-9-al. Experimental evidence for the co-localization between these sequences and major QTLs for nonanal, linalool and p-menth-1-en-9-al is provided. Here we report for the first time QTLs involved in VOCs content in peach. Co-localization with putative candidate genes was found for three major QTLs.

P14. GENETIC RESOURCE OF JAPANESE FLOWERING CHERRY "SAKURA" IN SHIMANE UNIVERSITY, AN USEFUL COLLECTION FOR POST-GENOMIC RESEARCHES IN *PRUNUS* SPECIES

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Japanese flowering cherries called "Sakura" is a symbolic flower in Japan and deeply related to the tradition and culture. Sakura trees consist of several different species in *Prunus* genus, such

as *P. jamasakura* Sieb., *P. lannesiana* Wils., *P. sargentii* Rehd., *P. verecunda* Koehne, *P. pendula* Maxim., *P.* x *yedoensis* Matsum., *P. maximowiczii* Rupr., *P. nipponica* Matsum., *P. incisa* Thunb., *P. apetala* Fr. et Sav., and *P. campanulata* Maxim.. A number of their hybrids and mutants have been developed and found for ornamental use. Shimane University has collected the Sakura genetic resource since 1963. Now, the collection becomes one of the big genetic resource centers in the world as well as in Japan. The trees were propagated by grafting and maintained in the experimental station of university with regular care every year. The collection consists of more than 200 individual trees of 150 different varieties (cultivars). Several of them are our originally created cultivars named 'Kezou-no-Taki', 'Fumai', 'Homatsuri', 'Honjo-Akebono', 'Beni-Izumo', 'Yakumo-Shidare', and 'Inata-Hime'. Here, we can propose the usage of this genetic resource for post-genomic researches in *Prunus* species. The selective and cross breeding of Sakura with long history in Japan contributed their extremely differentiated phenotypes in bud-break time (including less dormant type), petal colors (white, pink, red, purple, green-yellow), number of petals (single, double, multiple whorls for petals), flower size, inflorescence type (umbel, raceme, corymb), and aromatic compounds (coumarin). Currently, our interests are in molecular genetics for the flowering, flower and inflorescence morphogenesis, and bud dormancy. Moreover, the self-incompatibility, dwarf/vigorous growth habit, weeping branch habit, rooting capability, and ploidy variation are interesting characteristics for research. We introduce our collection here and believe a huge variety of genetic resources will provide research platforms for the 'Structural', 'Functional', and 'Comparative' genomics.

P15. IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH SELF-COMPATIBILITY IN *PRUNUS*

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Self-compatibility in Rosaceous fruit species is based on a single-locus qualitative trait. However, the evidence observed in different species has indicated the presence of modifier genes outside the *S* locus affecting the expression of self-compatibility/self-incompatibility. The study of a progeny obtained from the cross of the almond genotypes 'Vivot' × 'Blanquerna' has allowed the construction of a genetic map based on microsatellite markers and the identification for the first time in the Rosaceae family of two additional loci located outside the *S*-locus and affecting the expression of self-compatibility/self-incompatibility. A quantitative trait locus (QTL) was located relatively close to the *S* locus, on linkage group 6 (G6), whereas the second one was located on G8. These QTLs appear to be involved in conferring self-compatibility to genotypes not possessing the S_f allele. These results are consistent with almond being a self-incompatible species with a genetic background of pseudo-self-compatibility controlled by modifier genes. The effect of the S_f allele and the two QTLs may contribute to explain the wide range of fruit sets observed when self-pollinating different almond genotypes.

P16. MOLECULAR TOOLS HELP TO IMPROVE THE STATUS OF FRUIT GERMPLASM COLLECTIONS IN SWEDEN

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In Sweden as well as in many other European countries, preservation of genetic resources in fruits crops in directed mainly to old and presumably indigenous cultivars. The 'National Program for Diversity of Cultivated Plants' has defined a set of mandate cultivars aimed for publically funded conservation. These mandate cvs are maintained in numerous clone archives with the aim to preserve fruit trees as part of the Swedish cultural history. In addition, many of these cultivars are also included in a more research- and breeding-oriented germplasm collection at Balsgård, Swedish University of Agricultural Sciences. However, origin of the plant material in these collections is often uncertain and thus potential problems with cultivar identification do exist. Molecular markers, SSR (Simple Sequence Repeats) were used to provide mandate cultivars with 'molecular profiles' in order to detect duplicates and mis-labellings in germplasm collections (Balsgård and clone archives around Sweden), to verify pedigrees of some cultivars and to evaluate the extent of genetic diversity in apple, pear and cherry collections. The results indicate that trees of many of the Swedish mandate cultivars are correctly identified in clone archives and at Balsgård. Still, approximately 30% of the fruit trees in these collections appear to have labels with either the wrong name or with a name that is synonymous with another name. This is especially problematic when these trees are used for expensive and time-consuming research projects or when there is a high risk of providing mis-labelled material to growers and the general public. Superfluous duplicates and mis-labelled trees should be removed from the gene banks and thus money can be saved. Obviously, molecular markers is an important and useful tool in verification of the status of germplasm collections. The marker data must, however be complemented with careful phenotypic re-examination of all problematic cases, possibly also including those cvs where only one sample was available. Genetic diversity in the analysed plant material of all three crops was high and in agreement with previously reported results. Analysis of relatedness among apple cvs indicate that Swedish mandate cvs overlap considerably with cvs from other countries, and do not represent unique germplasm. In addition, more recently released apple cvs seem to represent the same level of variability as found among older cvs.

P17. TEXTURE PROFILING FOR STRAWBERRY FRUIT DEVELOPMENT AND RIPENING

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Strawberry is a non-climacteric fruit, characterized by a continuous decreasing in respiration rate during ripening and by the absence of the ethylene burst. During the ongoing of ripening one of the most relevant changes is the loss of firmness. In strawberry the fruit softening phenomena occurs very rapidly, causing the major loss during the short-term post-harvest storage, thus strongly affecting the commercial chain and the fruit economic value. Textural attributes vary significantly within strawberry cultivars and these are largely affected by the genetic constitution as well as environmental and cultural factors. To support future breeding programs aimed to improve strawberry fruit texture we have applied a texture analyzer TA.XT plus in order to improve the phenotyping resolution, profiling the mechanical response of the samples during the compression phase. An efficient and more precise phenotyping strategy is a fundamental step for a more reliable and focused future QTL mapping towards the identification of possible genomic regions

involved in the texture multi-traits control in strawberry. This advanced phenotyping technology was employed in this context in order to profile the texture evolution during strawberry fruit development, ripening and post-harvest in three short day responsive cultivars: Elsanta, Darselect and Candonga. In addition, this equipment was also exploit to assess the fruit texture behaviour carried out over 28 varieties, both junebearing and everbearing. For each stage per variety 10 fruits were processed, thought as replications, and the analyses were carried out profiling the mechanical response detected by the instrument. Analyses were replicated three times during 2010 in the same location with the same cultural practices (soilless conditions). Fruit quality attributes were monitored over the experimental time course. For the fruit developmental texture profiling were considered ten stages on the three main cultivars, while only full ripe and shelf life stage were considered for the cultivar comparison. Furthermore, physiological measurements were weekly examined during the autumn growth season in order to evaluate variety response. From each mechanical profile a series of parameters have been defined in order to characterize the texture in strawberry. Multivariate statistical approach, like Principal Component Analysis, was employed to analyze the data set related to the textural parameters and the 2D plot showed the distribution of the variability existing among the cultivars, allowing the clustering of the different strawberry varieties. In addition, critical variation time points in profiling were defined during fruit development and correlations to organoleptic traits were analysed.

P18. QTL MAPPING OF MORPHOLOGICAL TRAITS IN TETRAPLOID ROSE

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Selective breeding in cut roses requires a better understanding of biological mechanisms and knowledge of the inheritance of the major target traits which can lead to new or improved screening methods. A segregating progeny set of 184 F_1 hybrids ($2n=4x=28$) from a cross between tetraploid parents P867 and P540, was used to construct a genetic linkage map of the rose genome by Koning-Boucoiran et al., (2009). A total of 428 markers (107 NBS, 175 AFLP and 146 SSR) were used. Eleven horticulturally interesting quantitative traits, plant height (H), plant vigor (V), bending time (B), stem length (SL), stem width (SW), prickles on the stem (PS), prickles on the petioles (PP), number of petals (NP), chlorophyll content (Chl), side shoots (SS), and relative water conductance (RWC) were analyzed in the progeny in order to map quantitative trait loci (QTLs) controlling these traits. Phenotypic data indicated that most of the traits exhibited transgressive segregation suggesting the action of multiple QTLs. A total of 11 QTLs have been identified although the number of separate loci may be less. A QTL for prickles on petioles was identified on linkage group B2-3 which explained 27.8% of the phenotypic variance. The QTLs for vigor, height and number of petals are found on the linkage group B1-3. The QTLs for stem length, prickles on stem and number of petals were found on group 30 consisting of 2 markers, and can not yet be included in the linkage maps. The total variation explained by the combined markers multiple linear regression analyses per trait ranged from 7.5 – 44.1%. All the traits scored had moderate to high heritabilities: 53 -97%. The observed correlation between height and vigor may be partially explained by the presence of markers on linkage group B1-3 that are linked to both traits. The other correlated traits, prickles on stem and stem length, share linked markers.

P19. PHENOTYPING HEAT TOLERANCE IN GARDEN SHRUB ROSES UNDER CENTRAL TEXAS CONDITIONS

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Heat stress is a major limiting factor in the growing of ornamental garden roses in sub-tropical climates like central Texas and the southeastern USA. Heat stress is often associated with a decrease in flower number and quality. Within cultivated and wild *Rosa* there exists a vast amount of genetic variability for adaptation to different abiotic growing conditions. Utilisation of the available variation in breeding programs depends on the development of accurate and repeatable phenotyping techniques. Three rose cultivars ('Belinda's Dream', 'RADrazz', and 'Sea Foam') were pruned to two-shoot plants, and were then subjected to two week periods of heat stress (36ºC/28ºC, day/night) during an eight week cycle to determine the stage of shoot development that flower development is most sensitive to heat stress. Measurements on flower and shoot dry weight, number of nodes, and days to flower were recorded for the first flowering shoot on each plant on the day the first flower opened. Flower dry weight, shoot dry weight, and days to flower but not the number of nodes to the terminal flower were significantly affected by the timing of the heat stress period during flower and shoot development. Flower buds visible to the naked eye are the most sensitive to the heat stress treatments, resulting in flowers aborting and a continuation of vegetative growth. 'Belinda's Dream' had the highest occurrence of flower abortion and 'RADrazz' had the least flower abortion. All cultivars had a significant decrease in flower dry weight between plants grown in continuous heat and continuous optimal conditions (24ºC/17ºC). Leaf abaxial stomatal density and transpiration rate will be examined and related to the heat stress tolerance of the three cultivars used. The techniques described in this presentation are a starting point in the quantification of heat tolerance in garden roses.

P20. ANALYSIS OF THE PROTEOME RELATED TO APPLE (*MALUS* SPP) AND APPLE SCAB (*VENTURIA INAEQUALIS*) INTERACTION

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Apple of the genus *Malus* is the largest deciduous fruit crop in the Western Cape province of South Africa. It represents the fourth most important crop in the world with approximately ± 750 000 tonnes produced in South Africa. Apple scab [*Venturia inaequalis* (Cke.) Wint] is one of the most important apple pathogen and causes millions of dollars worth of damage to orchards. The disease weakens trees and damages fruits and could ultimately wipe out an entire orchard if left untreated. It requires chemical control, thus increasing production costs and environmental pollution. *Venturia inaequalis*, a hemibiotrophic ascomycete, infects its host 10-15 days after inoculation. A number of major resistance genes have been mapped in apple, of which the *RVi6* (*Vf)* gene is the first to be introgressed into commercial cultivars. However, virulent apple scab races have already overcome some apple scab resistant cultivars as well as the commonly used fungicides, leaving the apple industry at risk of a major apple scab outbreak. To address this issue

we have embarked on a genomic-proteomic study to unravel the host-pathogen interaction. Young (one week post germination) leaves of fully susceptible 'Golden Delicious' open pollinated seedlings were infected with apple scab (strain WS1). These were then collected at different intervals postinoculation, up to 14 days, for proteomic analysis. Proteome maps have been developed for uninfected apple and *Venturia inaequalis* grown in culture, as well as for infected apple leaves. Two-dimensional electrophoresis analysis, of leaf protein extracts, was done using Melanie 7.0 software package to investigate differentially regulated proteome expression profiles. Matrix-assisted laser desorption/ionization was used for the identification of protein spots of interest. A comparative spot analysis was done on 4-days post treatment sample collections, this considered being an ideal time-point for optimal early molecular effects. Out of 150 protein spots visualised on Coomassie stained gels, 50 were reproducibly responsive to the pathogen treatment. Some of the proteins responsive to the interaction between host and pathogen were identified. The combined genomics-proteomics study will give a better understanding of the proteins expressed/suppressed following scab infection on the host. Ultimately proteomics data will be used to gain a better understanding of the molecular mechanism for resistance.

P21. QUANTITATIVE TRAIT LOCI FOR FLOWERING TIME AND INFLORESCENCE ARCHITECTURE IN ROSE

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Inflorescence development pattern is an important ornamental character. The genetic of which is little known. The objective is to understand the genetic basis of inflorescence. In a diploid garden rose population, we investigate the genetic architectures of 10 traits associated with inflorescence developmental timing, architecture, and flower production. There is substantial genetic variation for inflorescence development traits, with broad sense heritabilities ranging from 0.82 to 0.93. Genotypic correlations are significant for most (78/90) pairs of traits, suggesting either pleiotropy or tight linkage among loci. Non-significant correlations reveal two independent developmental pathways controlling inflorescence architecture: (1) inflorescence node production increases branch number and flower production: (2) internode elongation is associated with intensive branching and flower production. QTL mapping indicates 6 common QTL regions (cQTL) for inflorescence developmental traits. Several QTL associated with different developmental traits map to the same cQTL, in agreement with the strong genetic correlations observed. Several candidate genes that are known to control inflorescence developmental traits and gibberelling signaling in Arabidospsis were mapped in rose. Rose orthologs of *FLOWERING LOCUS T* (*RoFT*), *TERMINAL FLOWER 1* (*RoKSN*), *SPINDLY* (*RoSPINDLY*), *DELLA* (*RoDELLA*), and *SLEEPY* (*RoSLEEPY*) colocalized with cQTL for relevant traits. This is the first report on the genetic basis of inflorescence developmental traits in rose.

P22. GENERATION OF SNP MARKERS USING THE NEXT GENERATION SEQUENCING TECHNOLOGY TO SATURATE THE GENETIC LINKAGE MAPS OF TETRAPLOID CUT ROSES

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Tetraploid Hybrid Tea roses, displaying large flowers and long stems represent most of the commercial cultivars for cut-roses. They were obtained after many years of interspecific hybridizations between about 10 different diploid and tetraploid rose species. Therefore, inheritance patterns still remain difficult to predict, hampering the use of molecular markers in breeding programmes in these complex tetraploid species. Transmission of the genes can follow disomic inheritance keeping both diploid genomes separate but it can also follow tetrasomic inheritance allowing the four homologous chromosomes to pair during meiosis, resulting in multiple recombinations, and possibly leading to double reduction. Therefore, our goal was to use molecular tools to study the inheritance mode, and construct genetic linkage maps of a tetraploid population of cut-roses. The K5 population was investigated, consisting of a cross between two tetraploid genotypes P540 and P867 from a cut-rose Hybrid Tea breeding programme. Its segregating progeny comprises 184 genotypes. Nucleotide-binding site (NBS) profiling was performed as well as a screening of the K5 population with microsatellites (SSR) from Rosaceae to generate molecular markers. In total, 428 AFLP, NBS profiling, and SSR markers were used to construct two parental genetic linkage maps using Joinmap. Only uni- and bi-parental simplex markers were included because JoinMap is designed to analyze populations with a disomic inheritance and not for populations with a tetrasomic inheritance. For this reason, both parental maps were constructed separately and homologous groups were manually identified through shared multi-allelic SSRs. This resulted in two linkage maps: one for P540 comprising 166 loci distributed over 28 linkage groups covering 1004 cM, and one for P867 comprising 199 loci distributed over 32 linkage groups covering 1170 cM. However, the map density is still rather low (1 marker every 6 cM), therefore, Next Generation Sequencing technology such as the Illumina platform was used to identify a large number of single nucleotide polymorphisms (SNPs). Consequently, RNA was isolated from flowers at three developmental stages for each parent. The synthesized cDNA was then sequenced, and SNPs between both parents were identified. These SNPs are currently further investigated to determine their suitability as molecular markers in order to screen the offspring of the K5 population using a SNP array. We expect that these newly generated markers will allow saturation of both parental genetic linkage maps, identification of haplotypes in tetraploids, and alignment between the already existing diploid rose linkage maps.

P23. APPLE SCAB RESISTANCE GENE PYRAMIDING ASSESSED IN A FRENCH NETWORK OF EXPERIMENTAL ORCHARDS: COMBINATIONS OF MAJOR AND MINOR RESISTANCE FACTORS EXHIBIT DIFFERENTIAL EFFICIENCIES OVER TIME AND SPACE

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Genetic resistance to diseases is an alternative to chemical control which use leads to ecological and human health risks. But breeders are facing the frequent occurrence of quick breakdown of monogenic resistance due to the emergence of virulent races in the pathogen population. Combining major resistance genes with quantitative resistance factors is nowadays considered as

a way to extend resistance durability of the former genes. In the present study we tested different genetic combinations of apple scab resistance factors over three different environmental conditions in France to evaluate their differential efficiencies in scab disease reduction over time and space. Apple hybrids cumulating zero to four resistance factors (two major genes *Vf* and *Vg* from cv. 'Prima' and two broad-spectrum QRL F11 and F17 from cv. 'Fiesta') were used to evaluate the efficiency of gene pyramiding as regard resistance to apple scab in space and over time. Apple hybrids chosen within the 'Prima x Fiesta' mapping population (kindly made available by PRI-Wageningen) and commercial cultivars were planted in experimental orchards in three contrasted regions of France. Trees were scored in springtime over a period of four years for disease severity with an ordinal scale evaluating the percentage of infected leaves. Inoculum pressure and makeup evolved over the four year trials leading to the breakdown of resistance factors, especially monogenic factors. Genetic constructions combining the two quantitative resistance factors and the two major genes conferred a significantly higher resistance than other constructions over the years. Favourable synergic (epistatic) effects between major genes and QRLs or between both QRLs could be observed according to the site. When coupled with other disease-control strategies like prophylaxy, pyramiding both quantitative and qualitative resistance genes is seen as a promising strategy for future breeding programs to get higher level of resistance and improve resistance durability.

P24. CHARACTERIZING THE GENETIC DIVERSITY WITHIN THE 1060 APPLE CULTIVARS OF THE INRA GERMPLASM COLLECTION INDICATES A WEAK STRUCTURATION ACCORDING TO THE AGE OR THE USAGE OF CULTIVARS

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Apple is the fourth most important fruit crop worldwide after citrus, grapes, and banana and it is the most ubiquitous and well-adapted of the temperate fruit crop species. Despite a large interest in saving old varieties and the large number of germplasm conservatories in France, French apple genetic resources remain poorly studied and exploited. To perform breeding for durable disease resistance, it is interesting to have deeper knowledge about the available genetic diversity. Here we investigated the genetic diversity of approximately 1060 apple varieties of which 680 were old French dessert cultivars, 230 were old French cider cultivars and 150 were recent French or foreign dessert cultivars. In addition, eight genetically well known genotypes were used as positive controls: 'Golden Delicious', 'Fiesta', *Malus floribunda* 821, 'Michelin', 'M9', 'Prima', 'Robusta 5', and 'Worcester Pearmain'. The entire collection was genotyped using 12 unlinked simple sequence repeat (SSR) markers, belonging to the ECPGR SSR set (Evans et al, 2007) and considered to be genetically neutral. The characterization of the collection through descriptive statistics based on molecular marker data revealed a large allelic diversity with an average of 19 alleles per marker, and high level of heterozygosity (~80%). The genetic structure of the collection was then studied based on several complementary methods. The results showed a structuration of the collection into three subgroups according to the age or the usage of cultivars. However the observed structure appeared to be quite weak and further investigations will be conducted with a better coverage of the genome, using 22 additional SSR.

P25. GENETIC DETERMINISM OF SCAB RESISTANCE IN THE APPLE CULTIVAR 'DISCOVERY': COMPARISON OF QTL MAPPING RESULTS IN PEDIGREED POPULATIONS

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Apple scab, caused by the fungus *Venturia inaequalis*, is one of the most widespread diseases of *Malus* x *domestica*. Durable scab resistance is a major goal for apple breeding programs. Increased knowledge on the genetic architecture of scab resistance in the most durably-resistant apple genotypes is a good way to identify efficient gene combinations. In previous studies, the genetic architecture of the highly resistant apple cultivar 'Discovery' was explored from F1 progenies (Liebhard *et al* 2003, Calenge *et al* 2004). In such experiments, no contrast is observed among offsprings in case of a homozygous parental genotype for a possible quantitative trait loci (QTL). Such a situation is suspected about 'Discovery' since the QTL detected in the F1 progenies may not fully explain its strong resistance. Therefore the genetic architecture of this cultivar was explored by reproducing the cross between its parents ('Worcester Pearmain' x 'Beauty of Bath'). The obtained progeny (260 individuals) has been inoculated in a greenhouse with a mixture of five isolates of *V. inaequalis*. Two main significant QTLs were detected on linkage groups (LG) 11 and 17, explaining 26.6 % and 5 % of the phenotypic variation respectively. Two weaker QTLs were also detected on LG 9 and LG14. These two genomic regions have already been identified for partial resistance to apple scab in other studies. In a 'Prima' x 'Fiesta' progeny, a QTL on LG11 was detected in both heterozygous parents and was supposed to follow a recessive pattern (Durel *et al* 2003). The same genomic region was shown to carry a QTL in a 'Fiesta' x 'Discovery' progeny for 'Discovery' (Liebhard *et al* 2003) but not in a 'Discovery' x 'TN10-8' progeny (Calenge *et al* 2004). To go further in the understanding of the genetic pattern of these QTLs, epistatic background effects were investigated through a two- way ANOVA model with an interaction component between all pairs of markers. An important epistatic effect was detected between genomic regions bearing the QTLs on LG11 and LG17, but also between six other genomic regions. These QTLs could be also studied concerning interactions with environment.

P26. ARE WILD HABITATS A THREAT FOR NEW SELECTED SCAB RESISTANT APPLE CULTIVARS?

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Evaluation of plant resistance efficiency to pathogens is rarely done with isolates from wild habitats although the heterogeneity of such habitats may generate pathogen diversity that may be a source of virulences for the cultivated habitat. The aim of this study was to evaluate if resistance factors to apple scab identified and characterized with isolates of *Venturia inaequalis* from a cultivated habitat were still efficient against isolates from wild habitats. Three *V. inaequalis* core collections originating from *M.* x *domestica* (cultivated apple), *M. sieversii* and *M. sylvestris* (wild apples) were built to maximise pathogen diversity. For each core collection, ten isolates were inoculated in mixture on 51 genotypes from an apple progeny segregating for two major resistance genes (*Rvi6* - *Vf* - and *Rvi1* - *Vg* -) and six Quantitative Resistance Loci (located on linkage groups 1, 2, 5, 11 and 17). The results showed that isolates from the wild habitats were able to develop on the susceptible apple genotypes, but these isolates were never more aggressive than isolates from the cultivated habitat on the resistance factors tested in the present study. As a

consequence, these resistance factors identified with isolates of *V. inaequalis* from apple orchards were shown to be also efficient against isolates from *M. sylvestris* and *M. sieversii*.

P27. MAPPING AND QTL ANALYSIS IN TETRAPLOID PLANT SPECIES: ROSE AS A MODEL SYSTEM

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Many important ornamental, vegetable and field crops are polyploids, *e.g.* cut rose and garden rose, potato, alfalfa, chrysanthemum etc. Mapping and QTL analysis in these crops is far from straightforward due to several constraints, for example:

- Multiple alleles at marker loci *and* trait loci
- Allele dosages can vary and can be hard to distinguish
- More unobserved marker information for dominant markers than in a diploid
- Possibly bivalent and multivalent pairing during meiosis
- Possibly preferential pairing of different homologs
- Possibly different recombination frequencies of different homolog pairs
- More possibilities for the linkage phase between markers
- Genetic phenomena such as double reduction
- Many polyploids are also cross pollinators and do not allow repeated selfing: homozygous parents unavailable
- Sequence information often not available

Due to these constraints, development and application of markers, linkage maps and QTL analysis tools for aiding the breeding of these crops, is slow. However, next-generation sequencing techniques in combination with new statistical tools can offer a possibility to overcome some of these constraints. Much larger numbers of markers could be generated. We will use next-generation sequencing technologies to detect and score a large number of SNP markers in mapping populations of tetraploid cut rose and garden rose and will use these to generate tetraploid linkage maps that can be used for QTL analyses. Four scenarios will be considered:

1. only simplex segregating SNP markers are used to develop dense genetic maps for each of the homologous chromosomes separately
2. simplex segregating SNP markers are integrated with multi-allelic microsatellite markers to integrate the homologous chromosomes
3. Multi-SNP haplotypes are constructed from SNPs that reside in the same sequence
4. Multi-SNP haplotypes are constructed from linkage analysis of individual linked SNPs on the basis of recombination estimates in the segregating population

Where possible, we will use mixture models according to Voorrips et al. (2010, in preparation) to derive SNP allele copy number from the combined information of segregation and signal of the individual SNPs. We have experience with this methodology in a tetraploid potato population.

P28. A COMPUTATIONAL FRAMEWORK FOR *VENTURIA INAEQUALIS* GENOMICS

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Venturia Inaequalis is an Ascomycete fungus and the causative agent of the economically important apple scab disease on apples ('Golden delicious'). Scab is a serious problem in apple producing nations, requiring approximately 20 fungicide sprays per growing season and thus increases agricultural inputs and substantially diminishes apple production worldwide. Therefore understanding V. inaequailis pathogenesis could serve as a plant pathogen model organism for the biological research community. The approximately 40 Mb genome of V. inaequalis has been sequenced on the Illumina GAII and assembled into 3088 contigs using velvet. We have also sequenced the fungal transcriptome, and exploiting this wealth of genetic data requires an integrated system that allows us to visualize and interrogate our assembly data in the context of an underlying annotated genome and interspecies genomic data. We describe an open source data management and visualisation system that integrates both genome and transcriptome data for V. inaequalis. Extension to this genomic resource includes annotations and functional characterization of pathogenecity with V. Inaequalis. This resource will provide a computational framework for biologist to engage in functional and comparative genomics, promoting hypothesis-driven research towards an understanding of the molecular interaction in this pathosystem; furnishing clues to efficiently control apple scab.

P29. A TRANSCRIPTOMIC PROFILING OF A BC_1 POPULATION FOR THE IDENTIFICATION OF GENES RELATED TO POWDERY MILDEW RESISTANCE IN PEACH

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Powdery mildew caused by *Sphaerotheca pannosa* (Wallr.) var '*persicae'*.is one of the most important fungal disease in peach. Being the pathogen an obligate parasite that cannot be maintained *in vitro*, the strategy for evaluating resistant genotypes is to test them in the field under the pressure of natural contamination by a highly susceptible peach cultivar and in multiple environmental conditions. One of the breeding goals is to obtain resistant peach cultivars in order to reduce chemical inputs necessary to keep the fungus under control thus contributing to the safeguard of both the environment and human health. With the aim of identifying genes involved in powdery mildew resistance, a whole genome approach was undertaken by means of cDNA-AFLP technique. An interspecific BC_1 population (PxF) segregating for the resistance was used. RNA was extracted from the leaves of plants grown in open field after the spontaneous onset of the infection, when the symptoms of the disease were clearly scorable. Expression patterns were obtained from the parents of the population (the susceptible peach selection IF7310828, the resistant *P. ferganensis* accession and the F_1 hybrid) and from two bulks, each made up of 7 individuals that over a period of 10 years had been always scored as resistant (Bulk R) and highly susceptible (Bulk S), respectively. A total of 16 primer pairs from the AseI / TaqI enzymatic combination were used with an extension of 2 selective nucleotides. Twelve combinations

produced an average of 404 transcript derived fragments (TDF) for each genotype, while 4 gave very poor transcript profiling and were therefore discarded from the analysis. In the comparison between the peach selection and the *P. ferganensis* expression patterns a total of 49 differential fragments were revealed, which are not all necessarily related to the resistance to powdery mildew. In order to focus on the genes more likely to be involved in the resistance, only the fragments whose expression pattern was confirmed by the respective bulk of the progeny were taken into account. By following this criterion 10 differentially expressed fragments were isolated that are putatively related to the resistance to powdery mildew. Six of them were re-amplified and the sequences obtained were aligned with those present in public databases. Similarity analysis has revealed for four of them a role in the mechanisms involved in the resistance to biotic stresses.

P30. POLYPLOIDY, MUTATION RATES AND GENETIC RELATIONSHIPS OF IMPORTANT CENTRAL AMERICAN *RUBUS* GENOTYPES: BASELINE STUDIES FOR ESTABLISHING A BREEDING PROGRAM IN COSTA RICA

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In the past year, the production of Central and South American blackberries (*Rubus* spp) has increased in Costa Rica (Pima-Cenada 2010, Aprocam, 2010). Costa Rica possesses abundant diversity in *Rubus* with desirable economic traits, but little is known about the genetics and phylogenetics of this material (Flores-Mora and Argüello-Delgado 2005; Jennings 1988). We are currently researching this material with the ambition to start a breeding program and provide farmers with clean, high quality cultivars. The genetic material grown in Costa Rica consists of different *Rubus* genotypes (principally, types of the *R. adenotrichus*) and can be found in southern Mexico, Central and South America (Flores-Mora and Argüello-Delgado 2005). We are conducting several experiments to establish baseline information about the diversity and genetic complexity of this material. As an initial draft of the genetic relationships among these genotypes, we used 10 RAPD primers previously tested on *Rubus* (Graham et al. 1995). Nei's genetic distances were determined, and results concur with morphological data in all cases except for *R. urticifolius.* To ensure propagule quality for production, and preserve the genetic diversity of Costa Rican material, we examined different *Rubus* genotypes for somaclonal variation during five micropropagation stages using the same 10 RAPD primers. This molecular screen will allow us to make decisions on the stringency of micropropagation steps in *Rubus* spp as well as view potential differences in mutation rates of *Rubus* in Costa Rica. The findings of this research can be further expanded to other important *Rubi* currently grown in other countries, such as *Rubus laciniatus*, as there has not been a study that has examined the in vitro mutation rate in *Rubus.* Lastly, polyploidy is prevalent in *Rubus*, thus we plan to examine ploidy levels in these genotypes using flow cytometry. This information is crucial to breeders for determining crossing compatibly among the genotypes, and initializing a competent breeding program.

P31. MOLECULAR CHARACTERIZATION OF S-ALLELES ASSOCIATED WITH SELF-INCOMPATIBILITY IN JAPANESE PLUM (*PRUNUS SALICINA* LINDL.) BY PCR ANALYSIS

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Diploid Japanese plum (*Prunus salicina* Lindl.) cultivars are generally self-incompatible. Self-incompatibility (SI) is genetic systems that hermaphroditic plants have adopted as a reproductive strategy, by which inbreeding is prevented and outcrossing is promoted. Self-incompatibility allows the pistil to distinguish between genetically related and unrelated pollen. This intraspecific breeding barrier is controlled by the interaction of several alleles of a single genetic locus termed S. The molecular allelic variation was characterized for gametophytic self-incompatibility in 29 Japanese plum cultivars. Primers were designed with reference to the conserved amino acid sequence of SI according to the sequence published to the GeneBank database of NCBI. Additionally, degenerate primers were used according to sequences published to genus *Prunus.* The size of bands ranged between 500- 2000 pb which were cloned and sequenced; the identification of nucleotide sequences from clones was established using the NCBI blast program. According on S-allele patterns, all of the studied genotypes were identified as self-incompatible.

P32. GEL BASED PROTEOMICS ANALYSIS OF THE *PRUNUS PERSICA* FRUIT SOFTENING OF FIVE COMMERCIAL PEACH AND NECTARINE VARIETIES

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The fruit integrity of *Prunus persica* melting varieties is constrained by its fast mesocarp softening, restraining the time frame for fruit consumption. Therefore, a broader knowledge of this phenomenon is mandatory to improve the shelf life of peaches and nectarines. A quantitative protein profiling using two-dimensional gel electrophoresis, followed by a multivariate statistical analysis, was chosen as strategy to obtain a global assessment of the fruit softening process from five commercial yellow flesh melting peach and nectarine varieties. Fourteen proteins related to the cell wall metabolism, ethylene synthesis, oxidative phosphate pentose pathway, fruit senescence and organic acid and nitrogen metabolism displayed the same pattern in all varieties evaluated, whereas proteins associated with aspects such as enzymatic ROS scavenging and metabolite transport, exhibited an intraspecific variation pattern. Particularly, two endopolygalacturonase isoforms were detected, with one of them showing a direct relationship with the freestone and clingstone trait. Additionally, a NADH dehydrogenase subunit F increment during the fruit softening could lead to the induction of the *P. persica* carotenogenesis, as recently seem in tomato.
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P33. SCREENING FOR LOW MAL D 1 CONTENT, HIGH CONTENT OF POLYPHENOLS AND RESISTANCE AGAINST APPLE SCAB

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In Sweden, apple breeding is carried out at SLU-Balsgård. One of the goals is to develop new apple cultivars that combine important traits like low content of the allergenic protein Mal d 1, high levels of polyphenols with antioxidant capacity, and durable resistance to apple scab. Such cultivars would be truly healthy for growers, consumers and environment. A substantial part of the population in the northern and central European countries is allergic to birch pollen. Many birch pollen-allergic patients become sensitised also to fresh apples, resulting in IgE-mediated symptoms like itching and swelling of lips, tongue and throat after ingestion, i.e. oral allergy syndrome (OAS). Several allergens have been identified in apple fruits, with the protein Mal d 1 being most well-known. There are differences in the allergenic potency of different apple cultivars. In addition, environmental factors, like different types of cultivation and storage, may affect Mal d 1 content. Polyphenols are secondary metabolites, which are widely found in apples. They have numerous important roles in the human diet and their beneficial effects have been attributed partly to their significant antioxidant capacity. The phenolic composition varies greatly between different cultivars, and between peel and pulp of the same cultivar. Apple scab, caused by the fungus *Venturia inaequalis*, is the most detrimental disease in commercial apple orchards. Several applications with fungicides per year are usually required in orchards with scab susceptible cultivars. Both dominantly inherited resistance and polygenically controlled so-called field resistance is available in the *Malus* germplasm. In this project, we screened cultivars, selections and progeny groups for content of Mal d 1 and polyphenols, using ELISA and HPLC-MS, respectively. Some of the individuals were also screened for resistance against apple scab using molecular markers. We found large differences in total content of Mal d 1 and polyphenols within as well as between progeny groups. In general, the levels of both Mal d 1 and polyphenols were lower in the pulp than in the peel. We also found quite pronounced variation between years. Some selections showed low Mal d 1 content and high polyphenolic content and may be marketed as new healthy cultivars. Some seedlings combined all three characters, since they had a low level of Mal d 1 and a relatively high level of polyphenols, and showed the marker for *Vf*-resistance.

P34. DIFFERENTIAL GENE EXPRESSION OF TWO APPLE CULTIVARS WITH CONTRASTING CHILLING REQUIREMENT

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Temperate fruit crops are of great economic importance worldwide, and its production depends on developmental processes, mainly the shift from juvenile to reproductive phase, dormancy transitions and flowering. Apple tree development is subjected to regulation by environmental inputs, specially chilling temperatures, which are needed to dormancy establishment and release. In this work, we aimed to investigate the differential gene expression between Gala and its derived bud sport Castel Gala, which are apple cultivars displaying medium and low chilling requirement, respectively. Bud samples were collected in 2007 at the beginning (May) and end (August) of the dormancy period. Total mRNA was isolated by LiCl precipitation, and suppressive subtractive

hybridization assays were performed using the PCR-select kit (Clontech Laboratories, Inc.) according to manufacturer instructions. Differentially expressed cDNA tags were sequenced by the Sanger method (ABI3100 Genetic Analyzer, Applied Biosystems). Sequences were manually processed and assembled with CodonCode software. BLAST searches and GO classification assignments and statistics were performed using the Blast2GO suite. 'Gala' buds showed increased number of transcripts related to stress and cold response (metallothioneins, dehydrins, etc.) at both dates. August 'Gala' samples contained several transcription factors associated to dormancy in the literature, like Kelch repeat-proteins, GRAS family and dormancy-associated MADS box genes. 'Castel Gala' samples were generally enriched in sequences related to cytoskeleton and photosynthesis. Our findings will be useful information to help unveil the molecular mechanisms of bud dormancy establishment and release in apple.

P35. IDENTIFICATION OF THE METABOLIC PATHWAY FOR NONADIENOL, AN INTERESTING VOLATILE COMPOUND IN *FRAGARIA CHILOENSIS*

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Several volatiles compounds were identified in *Fragaria chiloensis* including a series of C9-aldehydes and alcohols. The green and fresh flavour of white strawberries detected by GC-MS and GC-O was attributed to E-2-Z-6-nonadien-1-ol. The identification of these compound, combined with enzymatic evidence, suggest that nonadienol are biosynthesized via unsaturated aldehydes from linolenic acid. A search for genes involved in the biosynthesis of nonadienol was carried out in a population of ESTs from *F. chiloensis* (MIFAB) and other Rosaceae databases. We were able to identified putative sequences of LOX and HPL, key enzymes in this pathway. In addition LOX and HPL activities were followed along strawberry development and ripening, from green fruits to white ripe fruits. Expression of these genes was studied by qPCR at different tissues and development stages of *F. chiloensis* fruits. The expression profiles along fruit receptacle development and ripening showed that *FcLOX2* and *FcHPL* reaches their maximal level of expression in the stage S3 and decrease in the full ripening stage (S4).
This research was supported by PBCT R-11, ICM P06-065-F and UNAB DI-51-06/R.

P36. CHEMICAL AND GENETIC ANALYSIS TO TRY TO UNDERSTAND CRACKING SUSCEPTIBILITY IN DIFFERENT VARIETIES OF CHERRY

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Chile is the main exporter of sweet cherries (*Prunus avium*) from the south hemisphere; unfortunately these fruits suffer a series of superficial problems such as the cracking. This problem is one of major reason of losses in the worldwide production. The damage is produced when the cherry-tree became in contact with water rain for long periods of time. Analyses of the cuticular wax of cherries and tomatoes showed that their components and ratios are very similar and that

the n-alkanes could play an important role in the impermeability of the cuticle. This information allows us to hypothesize that the susceptibility to cracking in sweet cherry fruits could be related to structural components of the exocarp and diferencial expression of genes associated with this physiopathology. Three varieties of cherry were analyzed (Bing, Lapins and Rainier) using cracking assays *in vitro* and these assays indicate that Bing and Rainier are more susceptible to the cracking, than Lapins. Analysis of ^{1}H in nuclear magnetic resonance (NMR) spectrum for alkanes extracted from fruit epicuticular waxes shows the existence of an alkane in high concentration. The 2D NMR alkane's analysis showed significant differences between the concentrations of this hydrocarbon. Bioinformatics studies of differential gene expression between the varieties studied, revealed interesting candidate genes that together with the alkanes might explain the resistance/susceptibility to cracking in these varieties.

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P37. ROUTINE MARKER-ASSISTED SEEDLING SELECTION IN THE WASHINGTON APPLE BREEDING PROGRAM PROVIDES RESOURCE SAVINGS

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WSU's apple breeding program is routinely conducting marker-assisted seedling selection (MASS). Several Rosaceae crop breeding programs claim to be using marker-assisted breeding strategies, but regular practice in breeding operations is rare except for assisting decisions in parent selection. WSU's apple breeding program focuses on better eating quality, where fruit flavor and texture are the primary targets for improvement. In recent years, the program has saved money, labor, and time by not wasting resources on inferior seedlings through relatively small investments in developing and implementing DNA-based genetic tests for high impact traits. MASS involves deciding on genetic tests to include, genetic screening at optimal stages within traditional breeding operations, and timely culling of inferior seedlings. Genetic tests to include are chosen by economically weighted prioritization of reported associations, increasingly determined by objective means. Genetic screening involves tissue sampling, DNA extraction, genotyping, and provision of data in the form of "keep or cull" for each seedling. We use the Silica Bead Method for simple tissue sampling that integrates easily into breeding operations and enables high-throughput DNA extraction of >3000 samples a week by one technician. Genotyping is currently conducted on a family-specific basis for two functional genes predicting genetic potential for fruit storability via ethylene production (*Md-ACS1* and *Md-ACO1*). Field planting in spring 2010 was eased after screening 2600 seedlings for *Md-ACS1* and *Md-ACO1*, which resulted in the culling of 1690 predicted inferior trees and avoids their future resource-consuming tree maintenance and fruit assessment. Integrating into an earlier stage of the breeding scheme, expensive nursery propagation and subsequent maintenance and assessment was avoided by culling 2900 seedlings, of 5300 screened in summer 2010, that carried inferior alleles for *Md-ACS1* and/or *Md-ACO1*. Quality control was emphasized in 2010 to increase reliability and help streamline the genetic screening process. This year, by spending $10,000 on genetic screening, MASS provided an estimated net savings of $62,000 in present and future costs for the WSU apple breeding program.

P38. GENE EXPRESSION ANALYSIS OF RESISTANCE TO *PLUM POX VIRUS*, "SHARKA", IN APRICOT BY TRANSCRIPTOME DEEP-SEQUENCING (RNA-SEQ)

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"Sharka", caused by *Plum pox virus* (PPV), is currently the main disease menacing apricot (*Prunus armeniaca* L.) culture in affected areas. For this reason, the development of new resistant varieties with adequate agronomical characteristics is the main objective of breeding programs. However, despite the fact that resistance to PPV has been studied for many years in apricot, the genetic control and molecular basis are still unknown. Transcriptome analysis could be a good option for new studies on PPV resistance in apricot. First works on this topic have been reduced to the study of several candidate genes, including resistance gene analogs (RGA); nucleotide binding site-leucine-rich repeat (NBS-LRR); eukaryotic translation initiation factor (eIF4E); RNA helicase SDE3 (Cd93); and Argonaut AGO1 protein, although no conclusive results have been found. For this reason, transcriptome studies using new strategies of massive sequencing analysis (high-throughput), which can produce larger amounts of data in terms of expression of a large number of genes in a single experiment, could be a better alternative for new transcriptomic studies. One of these systems is massive transcriptome analysis using cDNA biochips (microarrays) to analyze thousands of genes by hybridization of mRNA labeled with fluorescence. However, the recent emergence of a massive sequencing methodology ("deep-sequencing") of the transcriptome (RNA-Seq), based on lowering the costs of DNA sequencing, could be more suitable than the application of microarrays. A recent project is being developed by our group in collaboration with other groups from France, Italy and USA to study the gene expression analysis of resistance to PPV in two apricot genotypes with different behavior against PPV. We are using an approach based on RNA-Seq technology to analyze two apricot genotypes from the same cross ['Orange Red' (resistant to PPV) x 'Currot' (susceptible)] with similar characteristics, although one is resistant ('Rojo Pasión') and the other susceptible ('Z506-7'). In this presentation we show the results of the complete sequencing of the first two transcriptomes. Differential analysis of these RNA-seq data has great potential for application in the identification of ESTs (Expressed Sequence Tags) and SNPs (Single Nucleotide Polymorphisms) that may be correlated with the differential response of apricot against PPV and in the identification of differentially expressed genes.

P39. A NON-TARGETED APPROACH UNRAVELS A VOLATILE PRODUCTION NETWORK ON PEACH FRUIT

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Volatile compounds represent an important part of the plant metabolome and are of particular agronomic and biological interest since they contribute to the aroma and flavor of the fruit and therefore affect fruit quality. Although, more than 100 volatiles fruit have been described in peach, this is far from the complete picture. In addition, very little is known about the metabolic network underlying the production of aroma volatiles. In this work, we have applied a non-targeted data

analysis approach to describe the volatile compounds complement of peach fruit. We have also analyzed metabolite-metabolite correlations to get an insight into the peach biochemical pathways directly affecting fruit quality. To increase the robustness of the metabolite network, metabolite-metabolite correlations were obtained from a diverse (heterogeneous) set of samples including 4 different genotypes (2 melting and 2 non-melting peaches), 4 maturity stages and one postharvest treatment thus covering a wide range of physiological responses. Volatile compounds were analysed by mean of HS-SPME-GC-MS (Head Space-Solid Phase MicroExtraction-Gas Chromatography-Mass Spectrometry). We followed a non-targeted data analysis approach based on Multivariate Mass Spectra Reconstruction (MMSR). This method basically consists of 1) a GC-MS chromatograms alignment by MetAlign software and 2) a Hierarchical cluster analysis of the aligned fragment for metabolite recognition. Thus, a total of 93 volatile metabolites were putative identify and relatively quantified over the sample set. The reliability of our identification procedure was confirmed for 100% of a set of 39 volatiles with commercially available standards. A hierarchical cluster analysis of the entire dataset (85 samples x 93 metabolites) classified the compounds according to their chemical structure (lactones, no-cyclic esters, carboxylic acid and long chain aldehydes) and by the metabolic pathway they belong to (i.e. lipid derived metabolites and terpenoids biosynthesis). Moreover, a correlation network analysis of the complete data set revealed unexpected interactions between different groups of volatiles. In addition, several volatile compounds that have not been described in peach fruit so far could be readily assigned to co-regulated groups and/or putative metabolic pathways. These results contribute to define the peach volatile map including the regulatory and interaction patterns which we believe can be useful for breeding purposes.

P40. INHERITANCE OF CHILLING AND HEAT REQUIREMENTS FOR FLOWERING IN ALMOND AND QTL ANALYSIS

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Chilling and heat requirements for breaking dormancy and flowering were studied in an almond progeny from the cross between the late flowering French selection ‘R1000’ and the early flowering Spanish cultivar ‘Desmayo Largueta’ for two years. The seedlings evaluated showed a wide range of chilling requirements, between both progenitors with a narrower range of heat requirements. Flowering time, chilling and heat requirements showed a quantitative inheritance although in the case of chilling requirements and flowering time a major gene could be involved being modified quantitatively by other minor genes. In addition, the results indicated that flowering time in almond is mainly a consequence of the chilling requirements, having heat requirements a smaller effect. QTL analysis for flowering time allowed the identification of one significant QTL on the linkage group 4 (G4) that explained most of the phenotypic variation together with other QTLs located in G1, G6 and G7. In addition, a significant QTL for chilling requirements was found in G4 together with other QTLs located in G1 and G3. In the case of heat requirements only a QTL located in G2 was identified.

P41. IDENTIFICATION OF QTL FOR *VERTICILLIUM DAHLIAE* (VERTICILLIUM WILT) RESISTANCE IN THE CULTIVATED STRAWBERRY

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Wilt, caused by the soil borne fungus *Verticillium dahliae* is one of the most economically important pathogens affecting modern strawberry production worldwide, and with the removal of methyl bromide as a fumigant, it is forecast to become even more costly to the strawberry industry over the coming years. Existing cultivated strawberry germplasm shows a range of responses to pathogen infection, from almost complete resistance, to severe susceptibility. Breeding and growing of resistant cultivars is one of the ways of combating widespread *Verticillium* infestation and ensuring the future sustainability of strawberry production. To this end, we have identified quantitative trait loci (QTL) for resistance to *Verticillium* wilt from a mapping progeny of 188 plants developed from a cross between the resistant cultivar 'Redgauntlet' and the susceptible cultivar 'Hapil'. A linkage map was constructed using transferrable microsatellite markers that spans the cultivated strawberry genome. Phenotypic data was collected from a replicated trial of the mapping progeny planted on soil heavily infested with *Verticillium* wilt. Symptom expression was scored on a 1 - 9 scale and the data obtained were used to identify resistance QTL.

P42. WHAT CAN GENOMICS DO FOR APPLIED APPLE BREEDING?

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Worldwide, apples constitute one of the economically and culturally most important crops among fruit and berries, and may - due to their high accessability and comparatively low price - have a major, positive impact on human health. Even in Sweden, where imported fruit covers about 80% of the fresh apple consumtion, we do love our home-grown apples! A small apple breeding program conducted at Balsgård since the late 1940s has resulted in, e.g., the well-known ’Aroma’ and ’Katja’ (’Katy’) and the new and promising ’Fredrik’ and ’Frida’. Potential parents for our crosses as well as advanced selections are evaluated for economically important traits like yield, disease resistance, chemical contents (antioxidants and allergenic proteins) and fruit quality. As an example, we have recently started to screen levels of tolerance for fungal storage diseases through inoculation with *Penicillium expansum* and *Colletotrichum gloeosporioides*, and for fruit tree canker through inoculation with *Nectria galligena*. Together with colleagues in other European countries, we are also involved in inoculation-based testings of tolerance for the bacterial disease fire blight (*Erwinia amylovora).* The above-mentioned characterizations are to an increasing extent supplemented with DNA-based screenings. Previously published single-gene markers and QTL markers have been used to determine type and level of resistance towards apple scab in approx. 200 apple cultivars. QTL markers that have shown an association with fire blight tolerance, especially in cultivars descending from ‘Cox’s Orange Pippin’, have also been screened. Once we have determined levels of tolerance for the major storage diseases and for fruit tree canker, we hope to develop QTL markers for these diseases. Another important area is fruit texture since this has emerged as one of the major determinants within the concept of fruit quality in apple. We have therefore screened about 200 apple cultivars for their allelic composition in two loci that appear to be involved in fruit ripening and/or fruit texture, namely *Md-ACS1* and *Md-Exp7*. Self-sterility allele constitution has also been determined, and this data is now used both as a tool in designing crosses and to provide growers with information on cross-pollination relationships. However, utilization of presently available DNA markers is time-consuming, costly and sometimes of uncertain value since the prediction ability, especially of QTL markers, may be limited to certain

genetic backgrounds. So what can the new genomic reseach do for a low-budget apple breeding program in a marginal apple production area? More genes responsible for economically important traits must be identified, and interactions within and between gene complexes must be elucidated. DNA-based analyses should be more robust within and between laboratories, and valid in all genetic backgrounds. At the same time, economical and technical demands should not be totally prohibitive. Maybe we hope for miracles…

P43. HIGH PERFORMANCE ROSES: A NEW IMPULSE FOR GARDEN ROSES BY MOLECULAR MARKER TECHNOLOGY

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In recent years the market for garden roses has seen a strong growth in Central and Eastern Europe. Unfortunately, the continental climate in these regions limits rose cultivation as the plant may freeze during the cold winters or stop with recurrent flowering during the hot summers. We aim to enable marker-assisted breeding (MAB) in this tetraploid crop, starting with two essential traits for these new markets: winterhardiness and recurrent flowering. *Winterhardiness* is the result of a combination of physiological processes, including early closure of growth and buds, frost tolerance, and a delay in bud break in spring (so that damage due to late spells of frost can be avoided). Canadian cultivars have been bred for winterhardiness, so that they can withstand up to -45 C. Depending on the parental hardiness level very hardy offspring can be obtained in one to three generations of breeding, which suggests that winterhardiness in roses is controlled by a very few major genes or closely linked genetic factors. Unfortunately, the Canadian roses otherwise have limited value, as many are climbers and the diversity in flower color is very limited. *Recurrent flowering* is one of the key characters crucial for the success of roses as ornamental crops as it leads to superior genotypes flowering throughout the whole growing season. This trait was introgressed from *R. chinensis* and *R. odorata* in the early 19th century. It affects the vernalization requirement of rose shoots. Recurrent flowering is based on a single recessive gene. However, the phenotype depends also on other physiological processes, as recurrent flowering stops at high temperatures, as is also the case in cultivated strawberry, where additional QTLs have been identified that are necessary to protect from heat stress in order to maintain the expression of the recurrent flowering gene. The methods by which we will achieve marker-assisted breeding include: quantitative assessment of allele dosages in tetraploids; Pedigree Based Analysis (PBA) for QTL identification and allele mining based on multiple populations, cultivars and breeding lines; and use of statistical approaches and software dedicated to trace and quantify putative inter-locus interactions in the QTL mapping procedure.

P44. EST ANALYSIS OF DORMANT BUDS OF JAPANESE APRICOT (*PRUNUS MUME*) BY MASSIVELY PARALLEL PYROSEQUENCING

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Bud dormancy in temperate woody perennials is a complex process necessary for plant survival under unfavorable conditions. Bud dormancy consists of three different states, paradormancy (summer), endodormancy (autumn), and ecodormancy (winter). Endodormant buds are different from the other dormant buds in that they are incapable of resuming growth under favorable conditions, which suggests the possible existence of internal factors inhibiting bud burst and

outgrowth in endodormant buds. Because endodormant buds require a certain amount of chilling accumulation for the transition to the ecodormant state, the genes exhibiting chilling-mediated differential expression patterns would be the candidates for the internal factors controlling endodormancy. To search for the internal factor candidates, we monitored gene expression patterns of several different stages of dormant buds of Japanese apricot by massively parallel pyrosequencing technology. Although data analysis is still ongoing, to date, a total of 14027 contigs and 19928 singlets have been found from more than 200,000 reads. When we searched for the genes showing seasonal differential expression, we found that 677 genes were up-regulated in endodormant buds as compared with paradormant or ecodormant buds. However, only 49 of them showed chilling-mediated suppression in ecodormant buds. Thus, these 49 genes are the candidates for the inhibitory factors controlling endodormancy. Among 49 genes, two *SVP*-like MADS-box genes, *PmDAM5* and *PmDAM6*, were included. These results support our idea that DAM genes are possibly involved in endodormancy maintenance by inhibiting bud burst and outgrowth.

P45.RESEQUENCING OF A PEACH GENOTYPE AND SNP DEVELOPMENT FOR MAPPING AND GENETIC DIVERSITY ANALYSIS

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The advent of the sequencing era has opened new frontiers in plant genomics. In this context the International initiative lead by US Institutions, together with the Italian project “DRUPOMICS”, recently delivered the peach whole genome sequence (available at the web site http://www.rosaceae.org/peach/genome). This deeper knowledge of the peach genome could facilitate the development of polymorphic markers in intra-specific crosses. In this sense, since now, the low level of heterozygosity of this species was a limiting factor. In fact, thanks to this very powerful tool, putative SNPs could be identified by re-sequencing the parents of some mapping progenies. To this extent, a peach accession derived from the cross ‘Contender’ x ‘Ambra’, named F1CxA, was re-sequenced by Illumina technology with about a 20X coverage. By aligning the derived sequences with the peach reference sequence it was possible to identify several thousand SNPs. Among these, about one hundred of genome-wide distributed SNPs have been used to genotype a peach progeny of 317 seedlings derived from the self-pollination of the F1CxA as well as about 50 peach accessions by a Sequenom platform. A large proportion of the developed SNPs were confirmed to be polymorphic in the CxA progeny as expected from the re-sequencing data. The marker segregation results allow to construct a molecular map spanning the peach genome perfectly anchored to the whole sequence. Linkage group denominations were confirmed by SSR mapping. Markers order substantially confirms their expected positions on the first draft of peach sequence and just a few of them mapped in a different region. Moreover it was possible to anchor and, in some case, orientate some small super-contigs. The same SNP markers were used to preliminarily characterise the genetic diversity among peach varieties giving a first indication about their transferability within the peach germplasm. For its relative low cost, re-sequencing demonstrates to be a very solid tool for the development of molecular markers in specific genomic regions with the perspective to speed up the marker assisted breeding for several agronomic traits in a next future.

P46. *KNOTTED*-LIKE GENES OF PEACH: STRUCTURAL CHARACTERIZATION, MAPPING AND TRANSCRIPTIONAL PROFILING DURING DRUPE DEVELOPMENT

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Plant KNOTTED-like homeodomain containing transcription factors (KNOX) take part in the correct development of both vegetative and reproductive organs. The peach genome contains ten *KNOX* genes (*KNOPEs*), six of class1 (*KNOPE1*, *KNOPE2*, *KNOPE2.1*, *KNOPE6*, *STMlike1* and *STMlike2*), three of class 2 (*KNOPE3*, *KNOPE4* and *KNOPE7*) and one of class M (*KNOPEM*), which were located on diverse linkage groups of the *Prunus* reference map. To inquire about the *KNOPE* roles in drupe development, the expression was monitored in the cultivar 'Chiripa' mesocarp during the S1-S4 stages. Both class1 *KNOPE1* and *KNOPE6* transcript levels were high at S1 and dropped subsequently, whilst those of the other class1 and class M members were undetected. The expression of both class 2 *KNOPE3* and *4* was unvaried in S1-S2 and declined subsequently, whereas that of *KNOPE7* diminished progressively along the growth. The aforesaid patterns were maintained in the 'Fantasia' and 'Bolero' cultivars. The monitor of class 1 *KNOPE1* transcription was focused on 'Chiripa' S1, during which the drupe size increased from ca. 6 to 35mm average diameter and the cell division occurred as marked by the *HISTONE3.2* gene expression. The *KNOPE1* mRNA abundance raised gradually, peaked in 30mm drupes and decreased later on. In ca. 5mm drupes, the *KNOPE1* mRNA localized in pericarp sectors such as sub-epidermal cells, mesocarp unexpanded cell clusters, vascular bundles and endocarp cells bordering the ovary cavity. Histological staining showed that *KNOPE1* transcript localisation overlapped with districts of dividing, un-vacuolated and cytoplasm-rich cells, likely to have meristematic nature. We suggest that *KNOPE1* may play a precocious role in drupe development, by maintaining the meristem (or quasi-meristem) identity of cell niches within the diverse tissue layers or by preventing cell expansion. Both functions are consistent with those assessed for the Arabidopsis *BREVIPEDICELLUS*, which was previously shown to share functional equivalence with *KNOPE1*. Finally, based on synteny/co-linearity among *Prunus spp.* genetic maps, the *KNOPE1* genetic position was compatible with QTLs of drupe size, further supporting a putative involvement in the trait control.

P47. DEVELOPMENT AND CHARACTERIZATION OF CISGENIC GALA APPLES CARRYING THE APPLE SCAB RESISTANCE GENE *HcrVf2*

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Apple scab, caused by the fungal pathogen *Venturia inaequalis,* is a disease of great economical importance in most temperate climates. To control the disease a high number of fungicide

applications per growing season are necessary. However, the use of fungicides is a controversial issue due to their potential environmental impacts. Genetic transformation offers the possibility to introduce new traits into a cultivar. However, most of the genetically modified apples produced so far contain genes foreign to the species as well as selectable markers such as antibiotic or herbicide resistance genes. In the attempt to increase the acceptance of genetically modified plants, Schouten et al. (2006) developed the concept of "cisgenesis". A cisgenic plant is defined as a genetic modified plant containing only genes, including introns and flanking regions such as promoter and terminator in a sense orientation, derived from a crossable donor plant. Applying the system described by Schaart et al. (2001), which combines an inducible site-specific recombinase, for excision of the T-DNA sequence coding for *cod*A-*npt*II hybrid gene for positive and negative selection of recombinant cells, ETH-Zürich managed to introduce the scab resistance gene *HcrVf2*, controlled by its own promoter (242bp in length) and its terminator (220bp in length) into the scab susceptible apple cultivar Gala. Absence of selectable markers in the plants regenerated after *Agrobacterium* mediated transformation and activation of the recombinase protein was proved by PCR. Transcription of *HcrVf2* in cisgenic lines has been demonstrated by RT-PCR. Currently three lines are available as grafted plants in greenhouse (lines CG7.1.49, CG11.1.53 and CG12.1.49) and are under evaluation for scab resistance and possible unwanted side effects at ACW-Wädenswil. To do so, the *HcrVf2* cisgenic 'Gala' lines are compared with the untransformed Gala. Following parameters are under study: 1) Phenotypic growth characteristics (*e.g.* shoot length, average leaf number and leaf size); 2) Possible aberration in photosynthetic activity (assessed using an infrared gas analyzer); 3) Protein profiling (2D-PAGE); 4) Qualitative and quantitative changes of the Mal d isoforms (at DCA-Bologna); and 5) Effects on non-target disease: Fire blight.

P48. INCREASED EFFICIENCY IN GENOTYPING AND MAPPING IN OCTOPLOID STRAWBERRY THROUGH QUANTITATIVE INTERPRETATION OF SSR DATA

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Genetic mapping in cultivated strawberry (*Fragaria x ananassa*) is complicated because of it's allo-octoploid (amphi-diploid) genome composition. Recent mapping studies relied on using only the information of markers that could be mapped through dominant (presence/absence) scoring. Because many SSR markers share alleles between homoeologous genomes, a lot of information is lost using this methodology. We have developed a new approach that allows for estimating the dosage of alleles in parents and individuals. This information is then used to identify co-segregating alleles and to reconstruct the haplotype configuration of each homoeologous chromosome pair for each parent. The approach is based on quantitative interpretation of SSR data, making use of the area under allele peaks (allele intensities) in ABI electropherograms. As result, more loci can be mapped per primer pair, a larger number of loci can be integrated between parents, and accurate information on homozygous regions is obtained. This approach has been applied on ±200 SSR markers that were tested on the Holiday x Korona mapping population. This resulted in the mapping of ±800 loci on 28 integrated linkage groups, which number is equal to the haploid chromosome number of the *F.ananassa* genome. This approach also allows tracing of marker alleles over pedigrees, even through homozygous regions. The development of this procedure and reference linkage map is a first step towards the identification of QTL for horticultural traits through the use of multiple breeding populations, cultivars and breeding lines following a Pedigree Based Analysis (PBA) approach. Proof of concept of the PBA approach and the methodology of the developed software has been delivered in apple through the European project HiDRAS, and is now a key element in new consortia projects like the USDA/SCRI project RosBREED (http://www.rosbreed.org/), in which strawberry is one of the core crops. In this

presentation, the procedure is presented and results on map construction and pedigree analysis are presented.

P49. SUPPRESSION SUBTRACTIVE HYBRIDISATION APPROACH TO IDENTIFY GENES INVOLVED IN THE STONY HARD TRAIT IN *P. PERSICA* (L.) BATSCH

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In climacteric fruits, the ripening process, such as flesh softening and epicarp coloring appears to be due to the transition from basal ethylene production (System 1) to the autocatalysis of the hormone (System 2). Two key enzymes, ACC synhtase (ACS) and ACC oxidase (ACO) are involved in the ethylene biosynthetic pathway. The stony hard (Hd) peaches are unable to make the transition from System 1 to System 2. The transcripts encoding the two key enzymes (ACS, ACO) are unexpressed in the mesocarp of the stony hard peaches. Moreover the stony hard fruits soften in the presence of ethylene but they are not able to synthesize the hormone unless they are treated with its immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). The present work focused on the genes involved in the conversion of ACC into ethylene. A subtracted library was constructed using stony hard peach flesh (cv. Yumyeong, stage S4, Y) properly treated.
Two different treatments were applied to Hd fruits: 1.000 ppm of ethylene for 48 hours and 1.000 ppm of ACC for 72 hours. The decreasing of flesh firmness in both treatments confirmed that the ripening process had occurred. Total RNA was extracted from ACC treated flesh (Y+ACC) and from ethylene treated flesh (Y+Eth). The library was constructed with the BD PCR-Select cDNA Subtraction Kit (Clontech) using Y+ACC RNA as driver and Y+Eth RNA as tester. Thirty putative differentially expressed clones were isolated and sequenced. The strings were trimmed and assembled to obtain 8 contigs and 9 singlets with a redundancy reduction of 40 %. The seventeen sequences were compared with different databases searching for high similarity to attribute putative functions, all show a correspondence with others belonging to Rosaceae family and *A. thaliana*. The transcripts were putatively assigned to functions such as elongation factors, allergens and proteases. To validate the putative differentially expressed transcripts a quantitative Real Time-PCR (qRT-PCR) was performed on the cDNA derived from the ACC treated flesh and the ethylene treated flesh using one specific primer pair for each transcript and 25S-5S interspaced sequence as housekeeping. Twelve transcripts out of the seventeen isolated show the looked-for expression patterns, indeed they are over expressed from 1 to 9 fold (average 4,4) in the ACC treated flesh in comparison with the ethylene treated mesocarp. Through the first peach genome assembly (Peach V 1.0) recently released by IPGI (www.peachgenome.org), the genes differentially expressed have been successfully localized in the 8 chromosome of the species.

P50. THE ITALIAN INITIATIVE FOR PEACH MIRNA ANALYSIS (MIRNITALY)

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The peach genome sequence, recently released by the International Peach Genome Initiative (IPGI), has become a valuable resource that started to foster functional genomic studies in peach and related species. One of the most interesting area is the study of the role of miRNA in the regulation of several important physiological process such as organ development, stress response and bodyguard functions . An Italian network for studying miRNA in peach(miRNitAly) was recently established among several Institutions. The goal of the network is to investigate the role of miRNA in the regulation of critical processes having important impact on the peach industry. The network will cover several crucial aspects in which miRNA regulation might be involved:

1) Developmental aspects such as juvenility, fruit ripening, quality and abscission,
2) Stress related responses (drought, nutrient deficiencies, salt)
3) Disease resistance in particular to viruses and viroids such as PPV and PLMVd

A combination of bioinformatics ,Next Generation Sequencing and microarray approaches is being developed for extensive investigation of miRNA populations and their putative targets in peach.

P51. FINE MAPPING OF A LARGE-EFFECT QTL FOR APPLE SCAB RESISTANCE COLOCALISING WITH THE MAJOR RESISTANCE GENE *RVI6*(*VF*)

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Apple scab, caused bythe fungus*Venturiainaequalis*, is one of the major diseases in apple orchards. The major resistance gene*Rvi6*(*Vf*), widely used in apple breeding worldwide, was overcome by new races of the fungus about 20 years ago. It is therefore necessary to find new genetic combinations which are more durably resistant. A large-effect scab resistance QTL (called T1) was identified at INRA on the linkage group 1 of the apple genome in the vicinity of the *Rvi6* locus (Calenge et al., 2004, Phytopathology). It was found in the hybrid 'TN10-8' and was shown to be inherited from the cultivar 'Schmidt's Antonovka P.I. 172632'. For an efficient use in marker-assisted selection, it was necessary to reduce the confidence interval of QTL T1 on the linkage group 1. Individuals recombining in the vicinity of the *Rvi6* locus were selected within three families deriving from 'TN10-8' thanks to *Rvi6*-flanking markers and phenotyped for their scab resistance level. Additional markers (mainly SSR) were genotyped to get a denser genetic map in the region of the *Rvi6* locus. Fine mapping and meta-analysis approaches developed in our study helped to significantly reduce the confidence interval of QTL T1, which localized very close to the *Rvi6* locus.

P52. GENETIC ANALYSIS OF FRUIT NUTRITIONAL QUALITY CHARACTERS IN F2 POPULATION OF CULTIVATED STRAWBERRY

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The cultivated strawberry *Fragaria* ×*ananassa* (2*n*=8*x*=56) belongs to the Rosaceae family and is one of the most consumed berries. Strawberry fruit is an important source of health-related compounds; in particular they are a rich source of phenolic compounds such as flavonoids and phenolic acids thus having high antioxidant capacities. To acquire genetic knowledge in *Fragaria* and to apply it for fruit quality improvement we are developing a new octoploid map in a F2 population generated with two genetic distant lines selected for having contrasted features of fruit nutritional quality and plant health quality parameters. The number of markers analysed are a minimal set of 60 common SSRs selecting to cover the entire strawberry octoploid map from the IRTA collection and public SSRs mapped in the diploid strawberry genetic map. The nutritional quality parameters studied in the progeny are the quantity of the main sugars in ripe strawberry fruit (sucrose, glucose and fructose), the total polyphenol content and total antioxidant capacity. A 92 progeny of F2 population segregate for content in nutritional parameters studied compared to the parental lines. The genetic map is being used to analyze Quantitative Trait Locis (QTLs) related with the production of nutritional quality parameters. The QTLs obtained will be converted to genetic markers to be used in breeding programs for improved fruit nutritional quality.

P53. DUPLICATION IN THE DOMESTICA APPLE COLLECTION WITHIN THE USDA-ARS NATIONAL PLANT GERMPLASM SYSTEM IN GENEVA, NEW YORK

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The USDA-ARS National Plant Germplasm System maintains more than 1300 named accessions of *Malus x domestica* in a field collection in Geneva, NY. Seven microsatellite markers (GD12, GD15, GD96, GD103, GD142, GD147, GD162) were used to identify duplicates within a set of 1240 domestica accessions within the USDA collection. Each of the SSR loci contributed enough allelic variation that a multilocus random match probability among duplicates could be calculated with high confidence. One hundred twenty-seven genotypes represented 348 of the accessions within the collection. Among the matches, we identified cultivars that matched to rootstocks, sets of sports, accessions with the same name in different languages, and accessions with similar names from different countries. Visual observations and phenotypic data will confirm the similarity between accessions that was identified using the genotypic markers.

P54. THE DIFFICULTIES OF DOGROSES (*ROSA* SECT. *CANINAE* L.)

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Species of dogroses (*Rosa* section *Caninae*) are mainly pentaploid, although tetraploid and hexaploid species also occur. All species within this section are characterized by their odd meiosis; only two of the genomes pair as bivalents while the rest of the chromosomes remain unpaired as univalents and are transmitted only through the female side. This means that the majority of the genetic material originates from the seed parent, and the offspring has a strong morphological resemblance to this parent. Depending upon what taxonomist is consulted, 30–50 (or more…) dogrose species are recognized and the section is often referred to as taxonomically difficult. Dogroses have been studied genetically and morphologically at Balsgård for two decades, and here we present some highlights. Athough these species are very similar to each other, some inter-specific differentiation can be ascertained by morphological and molecular markers. Our studies have indicated three rather distinct groups; the *canina* group (mostly glabrous leaves), the *villosa* group (mostly tomentose leaves) and the *rubiginosa* group (apple-smelling leaves). Within groups, species however overlap considerably, especially with the DNA markers! The *canina* meiosis appears to be highly regulated. Each SSR locus in a pentaploid species generally contains a maximum of four different alleles, never five. With MAC-PR (Microsatellite DNA Allele Counting – Peak Ratios, comparison of ratios for allele peak areas in each locus) we showed that bivalents are formed by two highly similar genomes, whereas univalent chromosomes differ in many alleles and are also more species-specific. Just as other *Rosa* species, the dogroses contain one rDNA site in each genome; one very large, one very small and three intermediate. Two of these last three are involved in bivalent formation. One of the rDNA families, defined by sequencing of the ITS region, is known as ‘beta’, is specific for sect. *Canina*e and appears to be located mainly in bivalent-forming genomes. The other rDNA families are more common on univalent-forming chromosomes and occur also in other sections of the genus *Rosa*. Some thousands of years ago, a ‘beta’-containing proto-*canina* species probably hybridized with several other *Rosa* species, thus giving rise to the allopolyploid hybrid complex presently known as section *Caninae*. The integrity of the ‘beta’-containing genome was preserved by evolvement of the *canina* meiosis. Molecular studies of interspecific crosses among dogrose species show that the offspring plants inherit all – or nearly all – of the seed parent’s markers, whereas only half of the pollen-specific markers are transferred to the offspring. However, in wide intersectional crosses, i.e. when the dogroses are pollinated with diploid pollen from tetraploid garden roses, the new alleles in the F1 offspring are so different that there is a change in the morphology, in that the offspring plants assume more ornamental characters, with e.g. more petals and more waxy leaves. Seedlings originating from open pollination of these ornamental hybrids, often loose some of the seed parent’s alleles (both bivalent and/or univalent alleles as ascertained with MAC-PR). Probably the hybrid environment disturbs the otherwise highly regulated *canina* meiosis.

P55. STUDIES OF INTERSECTIONAL CROSSES BETWEEN PENTAPLOID DOGROSE SPECIES (*ROSA* SECT. *CANINAE* L.) AND TETRAPLOID GARDEN ROSES REVEALS NON-ADDITIVE INHERITANCE OF ITS GENE FAMILIES AND rDNA REARRANGEMENTS

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The well-circumscribed but taxonomically difficult section *Caninae* in the genus *Rosa* has received much attention lately as a potential donor for disease resistance as well as human health-promoting fruit contents. An informal research group network now seeks to elucidate the origination of this section and its relationships with other sections. Intersectional crosses were performed between the pentaploid dogrose species *Rosa rubiginosa* (sect. *Caninae*) as seed parent (producing 4x egg cells due to the unique *canina* meiosis) and the tetraploid garden rose *R. hybrida* 'André Brichet' as pollen parent (producing 2x pollen cells after normal meisois). From 838 crossings and with approximately 26% germination, 128 seedlings were obtained, F1. These F1s were open-pollinated and the resulting seeds produced the F2 generation. To examine inheritance of rRNA (18S-5.8S-26S) gene families in hybrids, we sequenced the ITS1 (18S-5.8S intergenic spacer) sub-region and determined the chromosomal position of rDNA loci in two F2 hexaploid individuals and their grandparents. Cloning and sequencing revealed the presence of different ITS1 (18S-5.8S intergenic spacer) classes in the parents: *R. rubiginosa* carried *gamma* (major), *beta* (major) and *epsilon* (minor) families while the *R. hybrida* ITS1 pool was dominated by two major families, *eta* and *theta*. Thus, there was a clear separation of ITS1 types indicating a relatively large evolutionary distance between the hybridizing species. In the two F2 hybrids, we observed a reduction of gene families from both genome donors and some families (*theta*) were not transmitted at all. By contrast, the *beta* family had the highest penetrance and appears to have been enriched in both hybrid individuals. Fluorescence in situ hybridization (FISH) showed a reduction of rDNA loci (4 and 5 instead of 6) while maintaining the hexaploid karytoype (2n=42). Together, these results indicate that the intersectional hybridization was associated with a substantial reduction of ITS1 types probably due to array and locus elimination. Furthermore the high penetrance of the section *Caninae*-specific *beta* family indicates its likely occurrence on bivalent-forming chromosomes of *R. rubiginosa*. We hypothesize that the bivalent chromosomes of this species may eventually pair with homeologous chromosomes and could be successfully transmitted throughout the hybridogenic F1 meiosis. By contrast, the univalent-forming chromosomes of *R. rubiginosa* tend to be lost when exposed to a hybrid environment.

P56. DEVELOPMENT OF A GENETIC LINKAGE MAP FOR IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO BACTERIAL SPOT IN PEACH

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Bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni*, is a serious disease that can affect peach fruit quality and production worldwide. The molecular basis of its tolerance and susceptibility is yet to be understood. To study the genetics of the peach in response to bacterial spot, a segregating population between two peach cultivars, Clayton, a resistant phenotype, and O'Henry, which is very susceptible to bacterial spot, was created. Four hundred and thirty-two SSR markers already mapped in *Prunus* were tested for their polymorphism. Only 25% (108) were informative and were used in development of a genetic linkage map. The F_2 population was planted at three locations: the Sandhills Research Station, Jackson Springs, NC; the Sandhill Research and Education Center, Pontiac, SC; and the ARS-USDA Southeastern Fruit and Tree Nut Laboratory at Byron, GA. Field data of leaf response to bacterial spot infection were collected three times a year from two locations, NC and SC. Preliminary data indicate involvement of one or two major genes in peach having resistance to bacterial spot leaf infection. The genetic map in combination with field data will be used to locate the region(s) in the genome associated with bacterial spot resistance. Marker-assisted selection for bacterial spot resistance will be discussed.