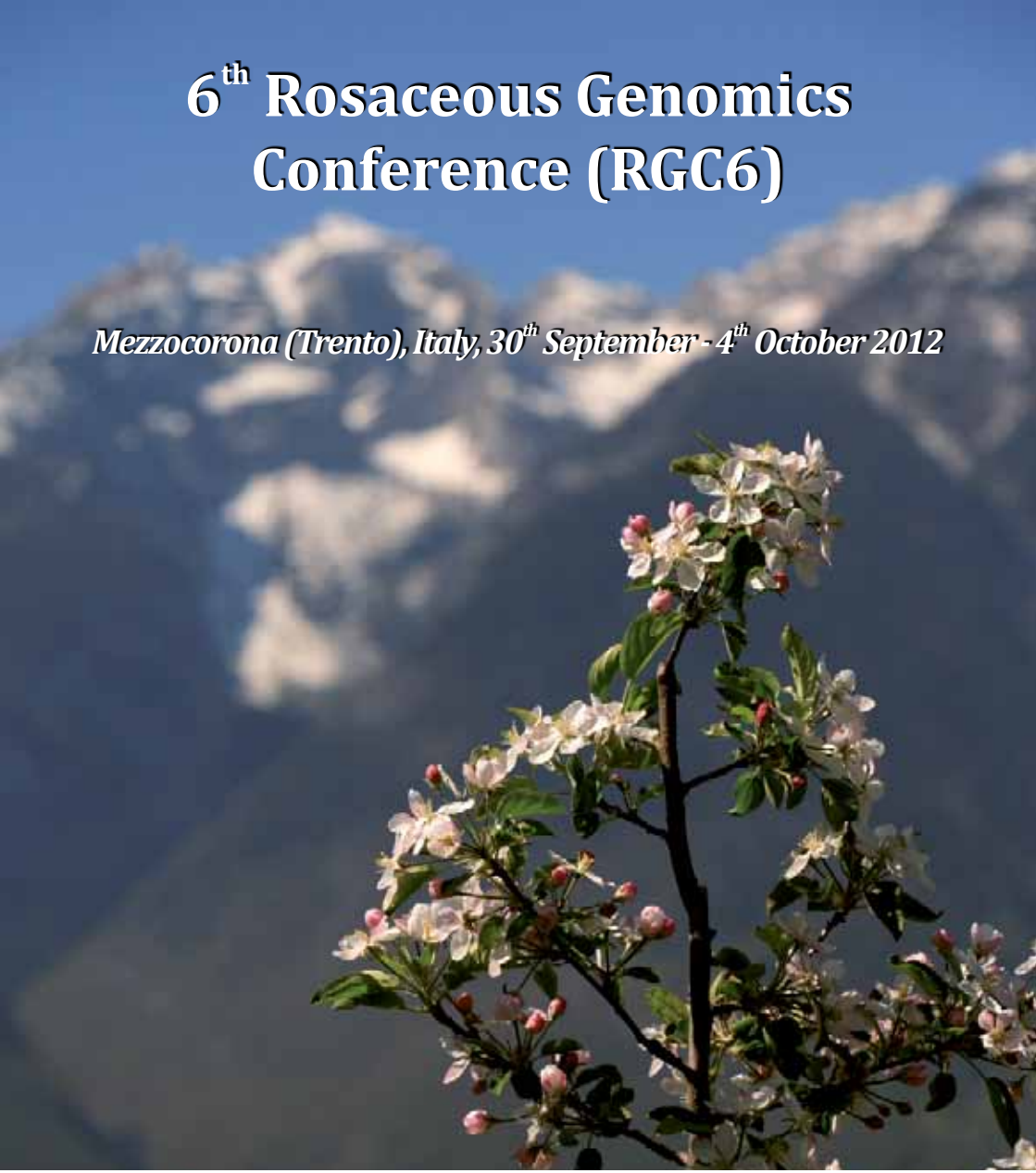
6th Rosaceous Genomics Conference (RGC6)

Mezzocorona (Trento), Italy, 30th September - 4th October 2012



FONDAZIONE EDMUND MACH

ISTITUTO AGRARIO
DI SAN MICHELE ALL'ADIGE

Sixth Rosaceous Genomics Conference
RGC 6

Under the Auspices of the
Autonomous Province of Trento

6th Rosaceous Genomics Conference (RGC6)

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PROGRAM AND BOOK OF ABSTRACTS

FONDAZIONE EDMUND MACH

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Under the Auspices of the Autonomous
Province of Trento

Event organized by

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Cover pictures: Flowering apples and Brenta Dolomites
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6th Rosaceous Genomics Conference (RGC6)

Program

Sunday, 30th September

Arrival and Accommodation

17:00 Registration at Palarotari, Mezzocorona

18:20 **Opening lecture:** *J. Salse* - Plant and animal genome structural, functional and evolutionary plasticity

19:00-21:30 Welcome dinner in the Historical Cellar

Monday, 1st October

8:00-09:00 Registration

8:30 *R. Viola* - Welcome address

9:00 **Plenary lecture:** *S. Myles* - Genotyping-by-sequencing in apple: Enormous promise and significant challenges

Session 1 – Structural Genomics

Chair: Sue Gardiner

9:30 *S. Gardiner* - A draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett')

10:10 *T.M. Davis* - Genome compositions in the octoploid and decaploid strawberries

10:25 *S. Montanari* - Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in European pear and inter-specific *Pyrus* hybrids

10:25 *J.M. Bradeen* - The RosaR80 system: a framework for cross-species comparative analyses of R-genes from Rosaceous species

10:55 Coffee break

11:15 **AFFYMETRIX SPECIAL EVENT.** *F. Brew* - How do you accelerate your genomics program from NGS to molecular breeding?

Session 2 - Functional Genomics

Chair: Herman Silva

11:45 *H. Silva* - A functional genomics approach to understand cracking susceptibility in sweet cherry (*Prunus avium*)

12:15 *G. Sanchez* - An integrative "omics" approach identifies new candidate genes to impact aroma volatiles in peach fruit

12:30 *T. Hytönen* - Interconnected molecular pathways control vegetative and floral development in *Fragaria vesca*

12:45 *R. Schaffer* - The complexity of apple fruit ripening

13:00 Lunch

Session 3 - Emerging Omics

Chair: Fulvio Mattivi

14:00 *R. Wehrens*- High-throughput metabolomics - challenges for bioinformatics

14:30 *V. Shulaev* - Harvesting the strawberry genome: from genes to metabolic networks

14:45 *A. Orellana* - Unravelling the proteome of the *Prunus persica* mesocarp during softening

15:00-17:00 First poster session (Even numbers)

17:00-19:00 Second poster session (Odd numbers)

15:00-17:00 SPECIAL EVENT. Illumina- Genome Studio workshop Session 1

17:00-19:00 SPECIAL EVENT. Illumina- Genome Studio workshop Session 2

15:00-19:00 SPECIAL EVENT. Global Crop Diversity Trust - Expert consultation workshop on the use of crop wild relatives for pre-breeding in apple

15:00-19:00 SPECIAL EVENT. Meeting of the COST Action on cherries

Tuesday, 2nd October

8:45 **Plenary lecture:** *J. Giovannoni* - Tomato as a model for analysis of molecular control of fruit ripening

Session 4 - Plant Genetics

Chair: Amy Iezzoni

9:25 *A. Iezzoni* - The genetic control of fruit size in cherry (*Prunus*): from phenotype to candidate genes

9:55 *S. Kumar* - Genome-wide association study of apple fruit traits in an experimental population

10:10 *A. Monfort* - A walk around the phenotype of diploid strawberry using a NILs collection

10:25 *C. Romero* - Self-compatibility associated with pollen modifier gene(s) in apricot

10:40 Coffee break

11:00-11:30 **ILLUMINA SPECIAL EVENT**. *R. Scavelli* - Genotyping by sequencing with Illumina technology

Session 5 – Fruit quality

Chair: Elizabeth Dirlewanger

11:30 *E. Dirlewanger* and J. *Quero-Garcia* - The genetic control of fruit quality traits in two prunus species: peach and cherry

12:00 *F. Costa* – Genomic approaches towards efficient assisted breeding for fruit quality in apple

12:15 *R. Pirona* - Genetic dissection of peach fruit quality traits

12:30 *E.J. Buck* - Genetic control of fruit sugar content across the Rosaceae

12:45 Lunch

Session 6 - Applied Molecular Breeding

Chair: Pere Arús

13:45 *P. Arús* - Towards the enrichment of the peach gene pool with alleles from wild species: the collection of peach-almond introgression lines

14:15 *S. Martens* - Genetic and molecular characterisation of apple-pear hybrids (*Malus domestica* × *Pyrus communis*)

14:30 *M. Davey* - Allelic-specific markers for fruit vitamin C breeding in apple

14:45 *K. Gasic* - Mapping QTLs Associated with Resistance to Bacterial Spot (*Xanthomonas arboricola pv. pruni*) in Peach

15:00 Coffee break

Session 7 - International 'Omics' Projects

Chair: Francois Laurens

15:20 *F. Laurens* - Progress in EU-FruitBreedomics

15:35 *M. Jänsch* - Precise mapping an identification of SNPs associated with the apple scab resistance genes *Rvi2*, *Rvi4* and *Rvi11*

15:50 *D. Micheletti* - Genetic variability description in a wide germplasm of domesticated peach through high throughput genotyping

16:05 *M. Bink* - Discovery & interpretation of multiple linked QTL

16:20 *E.van de Weg* - Towards a large sized Axiom SNP array for the allo-octoploid strawberry

16:35 *C. Peace* - Jewels in the apple genome: RosBREED's conversion of reported trait loci for fruit quality into DNA tests routinely used in breeding

19:30-23:30 Social Dinner at Castello di Toblino – Poster awards

Wednesday, 3rd October

8:30 **Plenary lecture:** *L. Han* - Strategy for identification of functionally important genes and marker-assisted plant breeding using NGS technology

Session 8 - Genetic Engineering

Chair: **Henk Schouten**

9:40 *H. Schouten* - New plant breeding techniques in the EU

10:00 *C. Gessler* - Can genetic engineering reduce pesticide use in apple orchards?

10:20 *H. Flachowsky* - Trans- and cis-genic approaches to improve apple breeding at JKI in Dresden-Pillnitz

10:35 *A. Chambers* - *Fragaria* Functional Genomics

10:50 Coffee break

Session 9 - Computational Biology and Bioinformatics

Chair: **Dorrie Main**

11:10 *S. Jung* - Ten years of GDR: Current resources and functionality GDR

11:40 *J. Ward* – Progress on the development of a genome sequence for red raspberry *Rubus idaeus*

11:55 *J. Jansen* - Statistical tools and software for the construction of integrated genetic linkage maps of outbreeding species using SNP markers with apple as a model

12:10 *J.M. Celton* - Re-annotation and transcript expression analysis of the apple genome

12:25 Lunch

Session 10 - Pests and Diseases

Chair: **Bert Abbott**

13:40 *B. Abbott* - Exploiting the peach genome sequence for identification of candidate genes for disease resistance in fruit and forest trees

13:55 *H. Duval* - Marker assisted breeding for peach seedling rootstocks resistant to root-knot-nematodes

14:10 *V. Decroocq* - Integrated approaches in *Prunus armeniaca* for resistance to sharka disease

14:25 *A. Peil* - Indications for a gene-for-gene relationship in the *Malus × robusta* – *Erwinia Amylovora* host pathogen system

14:40-15:40 Discussion

Conclusion and farewell

15:40-17:40 RosExec and RosIGI meetings

Thursday, 4th October

8:00-20:00 Dolomites bus trip. For those who want to experience one of the best mountain thrills of Europe, we have planned a trip on the Dolomites and surrounding areas. In case of bad weather we have an alternative programme that includes a visit of Bolzano historical centre and of the Ötzi-The Iceman Museum, with lunch in one of the renewed brewery of the town.

Abstracts

Keynote speakers

Tomato as a model for analysis of molecular control of fruit ripening

Jim Giovannoni[1]

[1]Boyce Thompson Institute for Plant Research and USDA-ARS, Tower Road, Cornell University campus, Ithaca, NY 14853 USA

Tomato is one of the most studied species for fleshy fruit development and climacteric ripening. Availability of a high quality tomato genome sequence has facilitated genetic inquiries via approaches that were not possible until now. Advances in understanding the genetic regulation of tomato fruit ripening to date have focused mostly on characterization of genes and gene expression patterns associated with ripening. Identification of specific genes underlying ethylene biosynthesis and downstream ethylene responses, cell wall metabolism and carotenoid accumulation have been more recently complemented with insights into transcriptional control. We have contributed with many others in the field to the identification and characterization of a number of tomato transcription factors necessary for ripening and associated ethylene biosynthesis and response. These include the RIN-MADS and NOR-NAC genes underlying the *rin* and *nor* mutations which completely block ripening. We have recently identified a number of additional transcription factors regulating fruit development and ripening and are building a network of regulatory control. Preliminary data on the role of the epigenome in tomato fruit development and ripening will also be presented. Specifically, we see changes in promoter methylation that parallel ripening phenomena and show that at least some of these changes can be associated with sites that bind important ripening regulators. In summary we provide evidence suggesting that both genetic and epigenetic factors regulate fruit development and ripening phenomena and that at least some of these factors operate in species beyond tomato suggesting possibilities for translational biology.

Strategy for identification of functional important genes and marker-assisted plant breeding using NGS technology

Lijuan Han[(1)]

[(1)]BGI-Shenzhen, Shenzhen, China

De novo genome sequencing has been widely used in generating reference genomes for plenty of economically and scientifically important plant species. With the assembly at hand, we can implement re-sequencing of a population of plant varieties, obtain the genetic variation information, further explore the population structure, domestication history, and detect regions or genes under artificial selection. For the past two years, we have published a series of investigations along with this strategy. We carried out genome-wide resequencing of 75 wild, landrace and improved maize lines, find evidence of recovery of diversity after domestication, and evidence for stronger selection during domestication than improvement. We re-sequenced the genomes of 40 cultivated accessions selected from the major groups of rice and 10 accessions of their wild progenitors, obtained 6.5 million high-quality single nucleotide polymorphisms (SNPs), and screened out a list of candidate regions selected during domestication which might be associated with important biological features. The re-sequenced of 17 wild and 14 cultivated soybean accessions revealed a high level of linkage disequilibrium in the soybean genome, identified a set of 205,614 tag SNPs that may be useful for QTL mapping and association studies. It is all part of an effort using a combination of sequencing and re-sequencing to identify those functional important genes controlling plant growth, development, productivity, reproduction, stress resistance, nutrition utilization and special metabolites formation, etc., thus potentially facilitate future marker-assisted plant breeding.

Genotyping-by-sequencing in apple: enormous promise and significant challenges

Sean Myles[1]

[1]Faculty of Agriculture, Dalhousie University, Nova Scotia, Canada

The majority of the international apple genomics community is currently using genotyping microarrays to collect genome-wide polymorphism data for the purposes of genetic mapping and genomic selection. Here I demonstrate that genotyping-by-sequencing (GBS) using next-generation DNA sequencing in apple presents an attractive alternative to genotyping microarrays. I show that a single lane of Illumina sequence data is sufficient to generate a saturated genetic map and enables the mapping of a mendelian trait in an F1 population of 95 apple trees. In addition, I present preliminary results from the analysis of tens of thousands of SNPs genotyped in ~2000 apple cultivars from the USDA apple germplasm collection. On a per genotype basis, GBS is clearly more cost effective than genotyping microarrays. However, significant statistical challenges remain in identifying genotyping errors and dealing with the large amounts of missing data produced.

Plant and animal genome structural, functional and evolutionary plasticity

Jerome Salse[(1)]

[(1)]EUMR INRA-UBP 1095 GDEC, Domaine de Crouelle - 234, Avenue du Brézet, 63100 Clermont-Ferrand. France

During the last decade, technological improvements led to the development of large sets of genomic resources permitting the emergence of high-resolution comparative genomic studies in both plant and animal lineages. In an attempt to unravel the structure and evolution of their ancestral founder genomes, we have re-assed the synteny and duplications of plant and animal genomes to identify and characterize paleo- and neo-duplications. We combined the data on the intra-genomic duplications with those on the colinear blocks that allowed us to propose a model in which the plant and animal genomes have evolved from a common ancestors with respectively a basic number of 5 to 13 chromosomes through whole genome duplications (i.e. paleopolyploidization) and translocations followed by lineage specific segmental duplications, chromosome fusions and translocations (Murat et al. 2012, Salse 2012). Based on the comparison of the plant and animal genome paleohistory, some major differences are unveiled in (i) evolutionary mechanisms (i.e. polyploidization processes), (ii) genome conservation (i.e. coding and non-coding sequence maintenance), and (iii) modern genome architecture (i.e. genome organization including repeats expansion and contraction phenomena). These data demonstrates how extant animal and plant genomes are the result of inherently different rates and modes of genome evolution resulting in relatively stable animal and much more dynamic and plastic plant genomes.
In order to investigate the plant genome plasticity deriving from surimposed rounds of polyploidies, we characterized Angiosperm ancestors comprising 5/7 ancestral chromosomes with 10000 protogenes providing a new reference for the plant chromosomes and new insights into their ancestral relationships that have led to arrange their chromosomes into concentric 'crop circles' of synteny blocks (Salse 2012). The established plant ancestor genome structure in term of chromosome structure and gene content offered the opportunity to study the impact of evolutionary shuffling events such as polyploidizations on (i) genome structure (i.e. mechanism driving the diploidization process), (ii) genome function (i.e. polyploidization resistant vs sensitive genes), (iii) genome expression (i.e. role of epigenetics on neo/sub-functionalization process), (iv) trait elaboration (i.e. conserved vs lineage specific traits), that will be discussed in details (Quraishi et al. 2011ab, Pont et al. 2011).

Abstracts

Oral presentations

A draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett')

Susan E. Gardiner[2], Ross N Crowhurst[1], Massimo Pindo[3], Amali Thrimawithana[1], Roy Storey[1], Cecilia Deng[1], Helge Dzierzon[2], Mark Fiers[4], Ashley Lu[4], Sara Montanari[3], Mareike Knaebel[2], Munazza Saeed[2], Yoon Kyeong Kim[5], Hilary Ireland[1], Andrew C Allan[1], Robert Schaffer[1], Alessandro Cestaro[3], Lester Brewer6, Riccardo Velasco[3], David Chagné[2]

(1) The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), Mount Albert Research Centre, Auckland, New Zealand
(2) Plant & Food Research, Palmerston North Research Centre, Palmerston North, New Zealand
(3) Istituto Agrarie San Michele all'Adige (IASMA) Research and Innovation Centre, Foundation Edmund Mach, San Michele all'Adige, Trento, Italy
(5) National Institute of Horticultural and Herbal Science (NIHHS), Suwon, Republic of Korea
(6) Plant & Food Research, Motueka Research Centre, Motueka, New Zealand -Eau, 15 rue Lainé-Laroche, 49000 Angers, France; abonyi@bieau.fr

We have sequenced the genome of European pear, *Pyrus communis* cultivar 'Bartlett'/'William Bon Chrétien' using second generation sequencing technology (Roche 454) A draft assembly was produced from single end reads, 2 kb, and 8 kb insert paired end reads using Newbler (version 2.7). The assembly contained 142,083 scaffolds greater than 499 bases (maximum scaffold length of 1.29Mb) covering a total of 577.3 Mb, representing 96.1% of the expected 600 Mb *Pyrus* genome. Preliminary analysis indicated that 549 SNP markers anchored 105 Mb (17.5%) of the assembled genome to a consensus genetic map of 'Old Home' x 'Louise de Bonne Jersey' and P019R054T042 x P037R048T081 segregating populations. Synteny with the apple genome primary assembly of 'Golden Delicious', and other rosaceous genomes is being used to extend putative anchoring. Gene prediction was performed and the use of the gene family of expansins will be reported as an example of assessment of the quality of the gene prediction. This 'Bartlett' genome sequence is a unique tool for identifying the genetic control of key horticultural traits and developing better pear cultivars, enabling wide application of marker-assisted (MAS) and genomic selection (GS).

Genome compositions in the octoploid and decaploid strawberries

T. Davis[1], Liu, B.[1], Zhang, Q.[1], Shields, M.[1], Mahoney, L.[1], Yang, Y.[1], Wood, D.[1], Zhang, H.[1], DiMeglio, L.[1], Lundberg, M.[2]

[1]University of New Hampshire, Durham, NH, USA
[2]Stockholm University, Stockholm, Sweden

The octoploid and decaploid Fragaria species are complex allopolyploids with as yet undefined subgenome compositions. Our aim is to elucidate their genome compositions and evolutionary histories. We are taking a multi-dimensional approach involving molecular cytogenetics, high throughput DNA sequence analysis, phylogeny reconstruction, genome size comparisons, interspecific hybridizations, comparative mapping, and others. The current state of knowledge about polyploid Fragaria genome compositions and diploid ancestries will be summarized, and new data will be presented indicative of subgenome-specific patterns of DNA sequence loss and the possible existence of as yet undiscovered diploid ancestors. The relevance of these findings to the Fragaria SNP array project (RosBREED) will be considered.

Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in european pear and inter-specific *Pyrus* hybrids

Sara Montanari[1], Saeed M.[2], Knaebel M.[2], Kim Y-K.[3], Troggio M.[1], Malnoy M.[1], Velasco R.[1], Fontana P.[1], Banchi E.[1], Won K-H., National Institute of Horticultural and Herbal Science (NIHHS), Suwon, Republic of Korea[3], Durel C-E.[4], Perchepied L.[4], Schaffer R.[5], Wiedow C.[2], Bus V.[6], Brewer L.[7], Gardiner S.E.[2], Crowhurst R.N.[8], Chagné D.[2]

[1]Research and Innovation Centre – Fondazione Edmund Mach, Via E. Mach, 1 – 38010, San Michele all'Adige, Italy
[2]The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), Palmerston North Research Centre, Palmerston North, New Zealand
[3]National Institute of Horticultural and Herbal Science (NIHHS), Suwon, Republic of Korea
[4]Institut National de la Recherche Agronomique (INRA), Angers, France
[5]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[6]Plant & Food Research, Hawke's Bay Research Centre, Havelock North, New Zealand
[7]Plant & Food Research, Motueka Research Centre, Havelock North, New Zealand
[8]Plant & Food Research, Mount Albert Research Centre, Auckland, New Zealand

Genomic resources are becoming increasingly available for Rosaceae crops, including pears. We have used next generation sequencing (NGS) technologies to identify single nucleotide polymorphism (SNP) markers from three European pear (Pyrus communis L.) cultivars to develop a set of high throughput markers useful for large-scale genotyping in the genus Pyrus. A set of 1096 pear SNPs was chosen and combined with 7867 apple SNPs in an Infinium II® array (Illumina). The 9K Infinium II array was evaluated using a segregating population of European pear parents ('Old Home' x 'Louise Bon Jersey') and three inter-specific families derived from Asian (P. pyrifolia and P. x bretschneiderii) and European pear. In total, 806 polymorphic pear markers were used for the construction of the first SNP-based genetic maps for pear. In addition, 785 SNP markers derived from apple (10% of the total apple SNPs included in the array) were polymorphic, including markers with null alleles. The study is first to assess SNP transferability across the genera Malus and Pyrus. The construction of high density SNP-based and gene-based genetic maps represents an important step in the process of identifying chromosomal regions associated with horticultural characters, such as pest and disease resistance, fruit yield and quality.

The ROSAR80 system: a framework for cross-species comparative analyses of R-genes from Rosaceous species

J.M. Bradeen[(1)], Luby, J.[(2)], Hokanson, S.C.[(2)], Zlesak, D.[(3)]

[(1)]University of Minnesota, Department of Plant Pathology, 1991 Upper Buford Circle / 495 Borlaug Hall, St. Paul, MN 55108 USA
[(2)]University of Minnesota, Department of Horticultural Science, 258 Alderman Hall / 1970 Folwell Avenue, St. Paul, MN 55108 USA
[(3)]University of Wisconsin—River Falls, Plant and Earth Science Department, 410 S. 3rd Street, River Falls, WI 54022 USA

Plants have evolved to recognize and respond to pathogens. Plant resistance (R) genes mostly encode nucleotide binding (NB) and leucine rich repeat (LRR) domains; typical plant genomes contain dozens to hundreds of NB-LRR genes. NB domains comprise conserved motifs and variable regions useful for studying R-gene relationships. We are conducting meta-analysis of R-genes from Rosaceous species using publicly available sequences. Adopting a strategy we optimized to study Solanaceous R-genes, here we outline plans for the RosaR80 (ROSAceae R-genes at 80% sequence identity) System for cross-species comparisons of Rosaceous R-genes. NB DNA sequences from Rosaceae species are assigned to homology groups using an 80% identity threshold. The resulting lineages are named RosaR80 groups and numbered sequentially (e.g., RosaR80.1, RosaR80.2, etc.). Next, phylogenetic analyses are conducted on translated protein sequences from each RosaR80 group. Finally, a table summarizing R-gene di stribution across Rosaceous species is constructed and ordered relative to the observed R-protein phylogeny. The resulting RosaR80 System enables visual discovery of patterns of R-gene distribution and allelic diversification across species and is useful for study of R-gene evolution, targeted R-gene mapping and cloning, and informed phenotypic survey of germplasm. The RosaR80 System is amendable and expandable and will be augmented as additional R-gene sequences become available. Community input is welcome.

A functional genomics approach to understand cracking susceptibility in sweet cherry (*Prunus avium*)

Herman Silva[(1)], Rios J.C.[(1)], Maldonado J.[(1)], Duchens H.[(1)], Lang E.[(2)], Carrasco B.[(3)]

[(1)]Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática, Universidad de Chile, Santiago, Chile.
[(2)]CEM, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[(3)]Facultad de Agronomía e Ingeniería Forestal, Departamento de Fruticultura y Enología, Pontificia Universidad Católica de Chile, Santiago, Chile.

The Chilean fruit industry has positioned itself as a key market for the country's development. To lead this market at the international level, it is necessary that Chile produce fruits and arrive to those markets with them in a very good condition/quality. Sweet cherries have become a fruit with a high value for the exporters and the growers. One of the problems associated with loss of production is fruit cracking of sweet cherries. Towards this end, it is essential to understand the molecular mechanisms that are involved in this disorder as well as identify genes and markers linked to this problem. In order to achieve this objective two approaches are under way. A biochemical approach was carried out to determine the role of alkenes in cracking using different varieties of sweet cherry (Lapins, Rainier and Bing). The cuticular wax composition was studied using nuclear magnetic resonance in one and two dimension. The analysis revealed that the saturated zone, range 1.18-1.38 ppm, correspond to alkanes with 28 to 30 carbons (C28 octacosano - C30 triacontane), which are present at different concentrations in each variety. The Lapins variety is less susceptible to cracking and contains a significantly lower concentration of this hydrocarbon compared to the other two varieties. On the other hand, RNA sequencing was performed for the three varieties as well as for one variety under water stress conditions. Several genes that might be involved in this disorder were identified. We are initiating a plum linkage map to validate and position the genes identified in sweet cherry.

This work is supported by CONICYT, FONDECYT/Regular Nº1120261 and Innova CORFO (07CN13 PBT-167); JCR is student of the Ph.D Biotechnology Program at Universidad Andres Bello.

An integrative "omics" approach identifies new candidate genes to impact aroma volatiles in peach fruit

Sánchez G.[(1)],. Besada C.[(3)], Monfort A.[(2)], Badenes M.[(3)], Granell A.[(2)]

[(1)]Instituto Nacional de Tecnología Agropecuaria (INTA). Ruta N°9 Km 170. 2930 San Pedro (Buenos Aires). Argentina
[(2)]Instituto de Biología Molecular y Celular de Plantas (IBMCP). Ingeniero fausto Elio, s/n. 46022 Valencia. España
[(3)]Instituto Valenciano de Investigaciones Agrarias. Carretera Moncada-Náquera Km 4,5. 46113 Náquera (Valencia). España

Aroma is one of the main traits affecting the quality of peach fruit. Since volatiles define the aroma, they have received much attention. Among over 100 volatile compounds described in peach, lactones have probably the largest effect in fruit aroma with esters, terpenoids, and aldehydes having minor but significant effects. The identification of key genes underlying the production of aroma compounds is of interest for any fruit quality improvement strategy.
Currently, a high quality genome sequence of peach is available at the Genome Database of Rosaceous (GDR). Nevertheless, experimental data that link genes to aroma is still missing. We have produced and integrated large sets of transcriptomic and metabolomics data aiming to identify genes associated to the main volatile compounds in peach fruit. The volatile accumulation (52 compounds) and genes expression (4348 genes) levels were obtained in maturity time-course series of two peach genotypes (melting and no-melting). By a combination of data analysis based on correlations (hierarchical cluster analysis, correlation network analysis, etc) we could identify a number of genes whose expression appear to be linked to lactones, esters, and phenolic volatiles contents, and therefore qualify as candidate genes for peach aroma. The gene expression profiles of these candidate genes were verified by qRT-PCR. The set of new candidate genes identified here could be of great interest for peach breeding or biotechnological purposes.

Interconnected molecular pathways control vegetative and floral development in *Fragaria vesca*

T. Hytönen[(1)], Mouhu K.[(1)], Koskela E.[(1)], Kurokura T.[(1)], Rantanen M.[(1)], Elomaa P.[(1)]

[(1)]University of Helsinki, Department of Agricultural Sciences, P.O.Box 27, FIN-00014 Helsinki, Finland

Short days and cool temperature in the autumn induce flowering in the Rosaceae model plant Fragaria vesca (L.) while vegetative reproduction through runners is enhanced by opposite environmental signals. We recently showed that FvTFL1 is the major floral repressor which controls photoperiodic flowering in F. vesca and demonstrated that mutation in this gene causes continuous flowering (Koskela et al. 2012. Plant Physiology 159: 1043-1054). However, neither over-expression nor RNAi silencing of FvTFL1 affected runner formation suggesting that other genes control this trait. Our studies in other floral regulators revealed that both FvFT1 and FvSOC1 are highly activated under long days compared to short days correlating positively with FvTFL1 mRNA levels in the shoot apex and negatively with flowering. Moreover, analysis of transgenic lines in both wild type and FvTFL1 mutant backgrounds suggested that FvFT1 activates FvSOC1 and FvSOC1 activates FvTFL1 in the shoot apex to r epress flowering. FvSOC1 miss-expression also strongly affected runner formation, probably through gibberellin pathway, suggesting that FvSOC1 is a branching point in the molecular pathways controlling the vegetative and generative developmental programs. We present a molecular pathway, which mediates both photoperiodic and temperature signals to control F. vesca development, and propose that this pathway may also control development in other rosaceous genera.

The complexity of apple fruit ripening

R. Schaffer[1], Ireland H.[1], Dayatalike D.[2], Diack R.[3], Gunaseelan K.[1], David K.[4], Chagné D.[5], Tustin S.[2], Johnston J.[1]

[1]Plant and Food Research, Auckland, New Zealand
[2]Plant and Food Research, Havelock North, New Zealand
[3]Plant and Food Research, Motueka, New Zealand
[4]University of Auckland, Auckland, New Zealand
[5]Plant and Food Research, Palmerston North, New Zealand

Apple ripening consists of a series of physiological changes including a change in skin colouration, a breakdown of stored starches to sugars, a softening of flesh and an increase in aroma compounds. Using transgenic apples that have the ethylene biosynthesis gene ACC OXIDASE 1, and MADS8 (a RIPENING INHIBITOR-LIKE gene) suppressed we have shown how each of these ripening characters are differentially controlled by developmental and hormonal signals. Using these unique lines, this research has also shown a role of cold temperatures in enhancing the rate of response to ethylene, and points to a requirement of a low auxin concentration for ripening to progress. At the molecular level, transcriptomics using mRNA-seq has given us insight into the way that apples respond to ethylene. Upon the detection of ethylene there are examples of very rapid expression changes in expression, while others such as cell wall genes have a much slower response. This complexity is further explo red in the variation of these ripening traits in 572 seedlings from a 'Royal Gala' x 'Braeburn' QTL mapping population grown at different locations. Combining these data shows a complexity in the regulation of the fruit ripening process.

High-throughput metabolomics - challenges for bioinformatics

R. Wehrens[(1)]

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, TN, Italy

For fruits and vegetables, the metabolome can be seen as the relevant phenotype, directly related to nutritional value and health effects. Worldwide, considerable efforts are being put in characterising the metabolomes of many different plant species. Even though the experimental work is labour-intensive and requires highly specialized people and material, the bottleneck very often lies in the elaboration of the data. The usual methods relying on hyphenated techniques lead to complex data, that even in small experiments need appreciable time for full analysis. Larger experiments, containing hundreds of samples, can no longer be analysed manually and automatic pipelines need to be developed. Several examples of such pipelines will be presented, focusing on LC-MS, GC-MS and HPLC-DAD data. These pipelines basically provide more abstract data summaries that are amenable for interpretation at a biological level. Fundamental differences with the classical manual analysis will be discussed. One should realize that the large amount of data obtained from typical untargeted experiments also has consequences for subsequent statistical analyses; also this aspect will be touched upon.

Harvesting the strawberry genome: From genes to metabolic networks

V. Shulaev[1], *Holt, SH*[1], *Dan, Y*[2] [3], *Veilleux, RE*[2]

[1]Department of Biological Sciences, University of North Texas, Denton, TX
[2] Department of Horticulture, Virginia Polytechnic Institute & State University, Blacksburg, VA
[3]The Institute for Sustainable and Renewable Resources, The Institute for Advanced Learning & Research, Danville, VA

Sequencing efforts in different fruit species have led to the identification of thousands of new genes; the function of most remains however unknown. The development of tools for high-throughput gene discovery, validation, and translation into commercial crops is therefore critical for harvesting the fruits of these genome sequencing efforts. Strawberry, genus Fragaria, is an attractive model to study flavonoid biosynthesis due to localization of flavonoid pigments throughout the plant in different tissue and organs, i.e., fruits, achenes, petiole and stolon tissue, petals and leaf margins under different environmental conditions. The spatial expression is an indication that different regulatory gene combinations control the structural gene expression in the flavonoid biosynthesis pathway in different tissues. Our group is working on discovering genes involved in the biosynthesis and metabolism of flavonoids and anthocyanins in woodland strawberry, Fragaria vesca, and to validate their function. We use a combination of bioinformatics, metabolomics and functional genomics approaches to reconstruct flavonoid metabolic networks in strawberry using fully sequenced genome to identify a set of key genes coding for metabolic enzymes and transcription factors involved in these networks. Several examples of using this approach will be presented.

Unravelling the proteome of the *Prunus persica* mesocarp during softening

A. Orellana(1), Moraga, C.(1), Miyasaka, A.(1), Nilo, R.(1)

(1)Centro de Biotecnología Vegetal, U. Andres Bello and FONDAP Center for Genome Regulation, Santiago, Chile

Mesocarp softening in Prunus persica melting varieties involves a number of physiological changes. In order to expand our molecular understanding of this process, we looked at the quantitative and qualitative changes that occur in the pool of proteins during the transition from firm to soft fruit. To this end, we carried out a proteomic analyses based on 2D-gels. Thus, proteins from firm and soft fruits from 5 melting flesh varieties were analyzed. We consistently detected in all samples a total number of 621 protein spots. The transition from firm to soft fruit showed that depending on the variety, between 60 to 108 proteins changed in their content. In order to expand our identification of proteins involved in the transition from firm to soft a different approach was taken. Thus, protein samples from firm and soft mesocarp from the O´Henry variety were separated in 1D-gel (PAGE-SDS) and then subjected to trypsin digestion followed by LC/MS-MS analysis. Using the peach geno me information, data from the spectra could be matched to 2490 proteins. Interestingly, we found that around 2% of the proteins showed qualitative changes while 11% showed quantitative differences between firm and soft fruit. Our analyses could confirm that some proteins such as polygalacturonase, exhibit major changes in the transition from firm to soft fruit. The analysis of the 330 proteins showing different patterns of accumulation showed that different metabolic pathways are represented.
Funded by PB-16, FONDAP-CRG 15090007, ICM-P10-062-F

The genetic control of fruit size in cherry (*Prunus*): from phenotype to candidate genes

Iezzoni A.[1], Cabrera A.[2], De Franceschi P.[3], van der Knaap E.[2], Stegmeir, T.[1], Rosyara U.R.[1], Sebolt A.M.[1], Dondini, L.[3], Campoy, J.[4], Dirlewanger E.[4], Quero-Garcia J.[4]

[1]Michigan State University, East Lansing, Mich., USA
[2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, USA
[3]University of Bologna, Bologna, Italy
[4]INRA-UR419 Centre de Bordeaux, Villenave d'Ornon, France

Sweet cherry (*Prunus avium* L.) is a rosaceous fruit crop cultivated in temperate regions of the world for its highly valued fruits. In today's marketplace, large fruit is highly desirable and directly related to grower profitability; therefore, the development of new cherry varieties with large fruit is a major breeding goal. To elucidate the genetic control of fruit size in sweet cherry and thereby enable marker-assisted breeding, we undertook a fine mapping – candidate gene analysis of two previously identified fruit size QTLs located on linkage groups 2 and 6 (G2 and G6)(Zhang et al. 2010) in two sweet cherry populations. A total of 14 simple sequence repeats were developed from the peach genomic regions syntenic to the G2 and G6 fruit size QTLs and used to further narrow the sweet cherry QTL regions. Genome annotations of the peach sequence in these syntenic regions identified cell number regulator genes (CNR, Guo et al. 2010) as likely candidate genes for both the G2 and G6 fruit size QTLs. The CNR gene family, first described in maize based upon homology to the tomato fruit weight gene *fw*2.2, is hypothesized to contain genes involved in cell number regulation that ultimately affect plant growth and organ size. Sequencing of the CNR candidate genes from the parents of the mapping populations and a panel of diverse sweet cherry selections for G2 and G6 identified 3 and 2 allelic variants, respectively. These findings agree with the previously described functional haplotypes for these two fruit size QTLs. Comparisons of sweet cherry fruit weights based on their CNR genotypes further support our hypothesis that the phenotypic differences in fruit size associated with the G2 and G6 QTLs are caused by allelic variants of the CNR genes.

Genome-wide association study of apple fruit traits in an experimental population

K. Satish[1]

[1]Plant and Food Research, Havelock North, New Zealand

Understanding the genetic basis of various fruit quality traits and physiological disorders is important for the development of new apple cultivars. Availability of apple genome sequence and cost-effective high throughput genotyping platforms have helped develop genome-based crop improvement methods such as genome-wide association (GWA) mapping and genomic selection (GS). Preliminary studies on patterns of linkage disequilibrium decay in apple populations have been encouraging for implementing these genome-based methods. Both population-based and family-based designs have been used for GWA studies in different crops. Family-based designs have unique advantages over population-based designs, as they are robust against population stratification, and allow both linkage and association to be tested. Using a family-based population of about 1,200 seedlings that were genotyped using an 8K SNP chip, we are conducting GWA mapping of various fruit traits related to eating quality and physiological disorder susceptibility. We implemented single-SNP analysis models that accounted for population structure or not, and compared these with models fitting all markers simultaneously. Preliminary results will be presented that suggest major loci are segregating for many of the traits that have been studied to date.

A walk around the phenotype of diploid strawberry using a NIL collection

Urrutia-Rosauro M.(1), Bonet-Gigante J.(1), Follon I.(1), Arús P.(1), *A. Monfort*(1)

(1)IRTA, Centre for Research in Agricultural Genomics CSIC-IRTA-UAB-UB, Campus UAB, Edifici CRAG, 08193 Bellaterra (Barcelona), Spain

The wild strawberry (Fragaria vesca) has a small diploid genome and is a model for the Rosaceae and the genus Fragaria. Near-isogenic lines (NILs) are homozygous lines whose genome has a single introgression from a donor genotype, usually an exotic line or related species. NIL collections are a tool that allows a detailed genetic study of quantitative traits. The starting plant material for the strawberry NIL collection was PI 551824 (F. vesca cv. "Queen of the Valley"), chosen for its commercial quality as recurrent parent and the accession PI 657844 of F. bucharica ('FDP601') as donor or exotic parental. The NIL collection consists of 33 introgression lines that contain the complete genome of F. bucharica with overlapping homozygotic introgressions. The introgressions have an average size of 15.5 cM ranging from a minimum of 2 cM and a maximum of 55 cM. The number of NILs per linkage group (LGs) ranged from 9 in LG VI to 3 in LG I and IV. At least three plants of each individual NIL were phenotyped in a greenhouse at the Cabrils Center of IRTA for a set of agronomic characters. Reproducible QTLs for flowering time, presence of runners, time of fruit development, leaf and petal area, fruit diameter, ripening time and resistance to powdery mildew, were localized in the strawberry genome. The first strawberry NIL collection promises to be a useful tool for strawberry (diploid and octoploid) genetics and breeding.

Self-compatibility associated with pollen modifier gene(s) in apricot

Muñoz-Sanz, J.V.(1), Zuriaga, E.(1), Badenes, M.L.(1), *C. Romero*(1)

(1)Fruit Breeding Department, Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. Moncada-Náquera, Km 5,5. 46113, Moncada (Valencia), SPAIN

S-RNase and S-locus F-box proteins are essential for the gametophytic self-incompatibility (GSI) specific recognition in Prunus. However, accumulated genetic evidence suggests that other S-locus unlinked factors are also required for GSI. For instance, in the apricot (Prunus armeniaca L.) cvs. 'Canino' and 'Katy' GSI breakdown has been associated with pollen-part mutations (PPMs) unlinked to the S-locus. In 'Canino', this mutated modifier gene (M-locus) was fine-mapped, using a segregation distortion loci (SDL) based strategy, to the distal part of chr.3 flanked by two SSR markers within an interval of 1.8 cM corresponding to ~364 Kb in the peach (Prunus persica L. Batsch) genome. A bacterial artificial chromosome (BAC) contig was constructed for this region using overlapping oligonucleotides probes, and BAC-end sequences (BES) were blasted against Rosaceae genomes to perform micro-synteny analysis. In the integrated genetic-physical map of the region, BES wer e mapped against the peach scaffold_3 and BACs were anchored to the apricot map. Micro-syntenic blocks were detected in apple (Malus × domestica Borkh.) LG17/9 and strawberry (Fragaria vesca L.) FG6 chromosomes. Preliminary results indicate that the PPM causing self-compatibility (SC) in 'Katy' closely maps to that found in 'Canino'. Nevertheless, these two cultivars seem to be genetically unrelated suggesting that both PPMs (being or not the same) may have arisen independently. The M-locus fine-scale mapping provides a solid basis for SC marker-assisted selection and for positional cloning of the underlying gene, a necessary goal to elucidate the pollen rejection mechanism in Prunus. In a wider context, the syntenic regions identified in peach, apple and strawberry might be useful to interpret GSI evolution in Rosaceae.

The genetic control of fruit quality traits in two *Prunus* species: peach and cherry

E. Dirlewanger[(1)], Le Dantec, L.[(1)], Campoy, J.A.[(1)], Barreneche, T.[(1)], *Quero-Garcia, J.K.*[(1)]

[(1)]INRA-UR419, Centre de Bordeaux, Villenave d'Ornon, France

Peach (*Prunus persica* (L.) Batsch) and sweet cherry (*P. avium* L.) are two *Prunus* species cultivated in temperate regions. In peach, many fruit quality traits are controlled by a single or a small number of genes. On the opposite, in sweet cherry, most fruit quality traits are complex.
In peach, the D locus controls fruit acidity, low-acidity is determined by the dominant allele while the S locus (for saucer peach), controls the fruit shape, flat being dominant. In sweet cherry, one of the most important fruit quality traits for producers is the fruit cracking tolerance, especially in regions where rainy springs can destroy nearly all the production.
A positional cloning strategy using a large F2 population of 2161 individuals and differential expression analysis of the two parents of the F2 population (Ferjalou Jalousia and Fantasia) using RNA-seq, lead to the identification of candidate genes for the non acid fruit trait in peach. For the S locus, positional candidate genes were identified on the basis of their functional annotation. Using two sweet cherry F1 mapping progenies, Regina × Lapins and Regina × Garnet, many QTLs were detected for fruit cracking tolerance, each of them explaining a small percentage of the observed variation. Interestingly, one of them was detected in each year of evaluation. New deeper phenotypic studies will be developed to dissect this complex character.
We will compare the methodology used to study these simple and complex traits, and discuss their application in selection programs.

Genomic approaches towards an efficient assisted breeding for fruit quality in apple

S. Longhi[1], Hamblin M.T.[2], Trainotti L.[4], Cappellin L.[1], Biasioli F.[1], Gasperi F.[1], Guerra W.[3], Velasco R.[1], *Costa F.*[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (Trento), Italy
[2]Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853-2703, USA
[3]Laimburg Research Centre for Agriculture and Forestry, Vadena, (Bolzano), Italy
[4]Dipartimento di Biologia, Università di Padova, Viale G. Colombo 3, 35121 Padova, Italy

Fruit quality is characterized by a set of principal factors which are fundamental for the economic success of a cultivar. The quality components, in the last decades, have been considered by breeders as the major target in the constitution of novel apple accessions distinguished by superior quality features. This goal, however, is still normally achieved by traditional breeding strategies, mainly represented by rounds of cross and selection, making the finding of novel high quality varieties a laborious and expensive activity. The implementation of valuable molecular markers would certainly improve the efficiency of the seedling selection, making this process more precise and cost effective.
In this work, a comprehensive QTL mapping investigation for fruit quality is presented, focusing in particular on the apple fruit texture, to date considered a driver trait for fruit quality. Fruit texture was originally assessed by using a high resolution phenotyping equipment on t wo type of plant materials: two full-sib progenies and a cultivar collection. As genomic approaches, a QTL mapping investigation was initially chosen to genetically dissect the control of such complex trait, and a candidate gene based association mapping analysis was subsequently employed to fine map and develop the most valuable molecular markers suitable for this purpose. The potentiality of these markers, which were also tested in a breeding program simulation, are here presented and discussed.

Genetic dissection of peach fruit quality traits

R. Pirona[1],.Pacheco I.[2], Chietera G.[3], Eduardo I.[4], Da Silva C.[2], Troggio M.[5], Banchi E.[5], Dondini L.[6],Tartarini S.[6], Pozzi C.[5], Vecchietti A.[7], Rossini L.[1,2]

[1]Parco Tecnologico Padano, Lodi, Italy
[2]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Milan, Italy
[3]INRA, Versailles, France
[4]IRTA, Center for Research in Agricultural Genomics CSIC-IRTA-UAB-UB, Barcelona, Spain.
[5]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy.
[6]DCA, Department of Fruit Tree and Woody Plant Science, University of Bologna, Bologna, Italy
[7]Monsanto Vegetable Seeds Division, Bergschenhoek, The Netherlands

Genetics and genomics approaches are integrated to dissect key quality and agronomic traits in peach, such as fruit weight, composition of Volatile Organic Compounds (VOCs), resistance to pathogens and maturity date (MD). 213 peach accessions and five biparental populations were genotyped with advanced SNP platforms such as the 9K Illumina peach SNP array providing an ideal basis for GWAS and QTL analyses.
Here, we report results from QTL analyses of VOCs in an F1 cross between two peach cultivars differing in their aroma profiles, Bolero and OroA. Based on gas chromatography-mass spectrometry (GC-MS) analysis of fruit essential oil, 43 QTLs were uncovered for 23 different VOCs and candidate genes were identified for two major QTLs. In parallel, to gain insight into gene expression differences that might be associated with the aroma profiles of Bolero and OroA, a novel microarray (uPEACH2.0) was specifically designed: transcriptomic comparisons at three developmental sta ges allowed the identification of 12 genes putatively involved in VOC biosynthesis. Due to the strong impact of MD on a number of fruit quality traits, we also report progress on QTL analysis in three different populations segregating for MD: in addition to the Bolero x OroA cross, F2 populations derived from Contender x Ambra and NJ Weeping x Bounty were used to map QTLs providing a robust framework for genetic analysis of MD. Analysis of genetic variations in some CG will be discussed.

Genetic control of fruit sugar content across the Rosaceae

E.J. Buck[1], Chagné D.[1], Stephens M.J.[2], Bushakra J.M.[3], Boldingh H.[4], Sawyer G.[1], Dayatilake D.[5], Tustin S.[5], Diack R.[5], Oliver M.[5], Malone M.[5], Gardiner S.E.[1]

[1]The New Zealand Institute for Plant & Food Research Ltd (PFR), Palmerston North, New Zealand
[2]PFR, Motueka, New Zealand
[3]USDA-ARS, Corvallis, OR, USA
[4]PFR, Ruakura, New Zealand
[5]PFR, Havelock North, New Zealand

As the sugar content of fruit is a key component of flavour in commercial fruit species, increasing our knowledge of the genetics underlying fruit sugar content is a crucial step towards developing new cultivars with improved flavour. We studied the genetics of sugar content in raspberry and apple using QTL analysis and then compared the position of QTLs both between these species, as well as with two other Rosaceaous crops (strawberry and peach) for which syntenic relationships and QTLs for sugar content have been characterised. In raspberry, we used a genetic linkage map developed from a cross between black raspberry R. occidentalis 96395S1 and red raspberry R. idaeus 'Latham' comprising 155 F1 seedlings. In apple, we used a 'Royal Gala' x 'Braeburn' segregating population of 572 F1 seedlings. For peach, we used three populations segregating for the supersweet character, defined as having a Brix value above 20, consisting of 124 F1 seedlings from 'Candy Fl oss' crossed to three different breeding lines. We identified significant QTLs for glucose and fructose on raspberry linkage group 7. When the position of these QTLs and candidate genes involved in control of sugar levels was compared with QTLs for sugar content detected in octoploid strawberry, peach and the 'Royal Gala' x 'Braeburn' population, conserved genetic control was indicated among the species. This study illustrates the use of comparative genome mapping for advancing knowledge on an important commercial trait.

Towards the enrichment of the peach gene pool with alleles from wild species: the collection of peach-almond introgression lines

Donoso J.M.[(1)], Picaño l.R.[(1)], Eduardo I.[(1)], Howad W.[(1)], *Arús P.*[(1)]

[(1)]IRTA-Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB, Barcelona, Spain

The variability of the peach (*Prunus persica*) materials used for breeding in American and European programs is low, particularly when compared to other Prunus species. This is a serious limitation to peach breeding progress. Possible sources of variability are local European cultivars, Asian accessions, mainly Chinese, and wild relatives. While all these sources are useful, the largest variability reservoir is that of wild relatives. In this communication we will present our results on the process towards the construction of a near-isogenic line (NIL) collection of almond chromosome fragments coming from cv. 'Texas' in the background of peach 'Earlygold', based initially on a large backcross one population to the peach parent (T1E) from which several individuals with less than four introgressions have been selected with markers. The degree of success in extracting T1E lines with low numbers of introgressions, problems encountered with sterility barriers (male and female) and initial data on a few individuals of the BC2 progenies will be reported. A model for introgression of valuable characters from wild materials will be proposed.

Genetic and molecular characterisation of apple-pear hybrids (*Malus domestica* × *Pyrus communis*)

S. Martens[(1)], Ravi R.[(1)], Malnoy M.[(1)], Schimmelpfeng H.[(2)], Schwab W.[(3)], Fischer T.C.[(4)]

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach, 1 - 38010 San Michele all'Adige, Italy
[(2)]Hangenham, Germany
[(3)]Department of Biotechnology of Natural Products; Technische Universitaet Muenchen, Liesel-Beckmann-Str. 1, 85354 Freising , Germany
[(4)]Ludwig Maximilian Universitaet Muenchen, Fakultaet fuer Biologie, Lehrstuhl "Biochemie und Physiologie der Pflanzen", Biozentrum Großhaderner Str. 2 – 4, 82152 Martinsried, Germany

Apple (Malus x domestica) and pear (Pyrus communis) are two economically important fruit crops of the temperate zones that have been cultivated in Europe for more than 2.000 years. They belong to the Pyrinae, the former subfamily Maloideae of the Rosaceae. Both are well known for their unique texture and flavors but also by their nutritional qualities. The identical chromosome number (2n = 2x = 34) and similar genome size (apple 1,57 pg/2C; pear 1,11 pg/2C), as well as their supposed recent divergence date (33,9 to 55,8 million years ago) and DNA-marker analyses led to the assumption that their genomes might be highly co-linear. Recently, a putative hybrid between M. domestica and P. communis including five F1 seedlings became available giving a unique perspective not only for genomic, transcriptomic and metabolomic studies but also for advanced breeding strategies. Based on the recent (Malus) and ongoing (Pyrus) genome projects comparative genomic approaches were applied to identify the genetic differences of the putative hybrid and its five offsprings. Various SSR marker and characteristic ITS sequences gave clear evidence for a hybrid plant. The use of genomics and other -omics technologies (metabolomics, transcriptomics) will give further insight into the genetic reorganization of the hybrid but also enhance and accelerate the breeding process for the development of superior crops for producers and consumers by introducing the gene pool of Pyrus into Malus traits

Allele-specific markers for fruit vitamin C breeding in apple

Mellidou, I.[1], Chagné, D.[2], Laing, W.[3], Keulemans J.[4], M. Davey[4]

[1]Laboratory for Fruit Breeding and Biotechnology, Department Biosystems, Faculty of Bioscience Engineering, KU Leuven, B-3001, Heverlee, Belgium
[2]The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), Palmerston North Research Centre, Palmerston North 4442, New Zealand
[3]Plant & Food Research, Mount Albert Research Centre, Auckland 1142, New Zealand
[4]Laboratory for Fruit Breeding and Biotechnology, Department Biosystems, Faculty of Bioscience Engineering, KU Leuven, B-3001, Heverlee, Belgium

Vitamin C (L-ascorbic acid, AsA) performs multiple essential roles in both plant and human health, with roles in fruit post-harvest storage and disease resistance. To help understand the regulation and inheritance of fruit AsA concentrations, we identified major QTL for fruit AsA, and antioxidant concentrations in both flesh and skin tissues in a 'Telamon' x 'Braeburn' mapping population. Mapping of genes of AsA metabolism identified co-locations between orthologues of GDP-L-galactose phosphorylase (GGP, VTC2), dehydroascorbate reductase (DHAR) and nucleobase-ascorbate transporter (NAT) within these QTL clusters. Of particular interest are 3 paralogues of MdGGP, which all co-located within major AsA-QTL. Further, allelic variants of MdGGP1 and MdGGP3 derived from the 'Braeburn' parent were consistently associated with higher fruit total AsA concentrations both within the mapping population (up to 10-fold) and across a range of other apple germplasm (up to 6-fo ld). Differences in the expression of the 'Braeburn' MdGGP1 allele between fruit from high- and low-AsA genotypes, clearly indicate a key role for MdGGP1 in the regulation of fruit AsA concentrations. MdGGP allele-specific SNP markers are thus excellent candidates for directed breeding for enhanced fruit AsA concentrations. Co-locations between MdDHAR3 and a major QTL for browning in the 'Telamon' parent, highlights links between the redox status of the AsA pool and susceptibility to fruit flesh browning.

Mapping QTLs Associated with Resistance to Bacterial Spot (*Xanthomonas arboricola* pv. *pruni*) in Peach

K. Gasic[1], Yang N.[1], Reighard G.[1], Ritchie D.[2], Okie W.[3]

[1]SAFES, Clemson University, Clemson, SC 29634, USA
[2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695 USA
[3]ARS-USDA, S.E. Fruit and Tree Nut Research Lab, Byron, GA 31008 USA

Bacterial spot, caused by Xanthomonas arboricola pv. pruni (Xap), is a serious disease that can affect peach fruit quality and production worldwide. This disease causes severe defoliation and blemishing of fruit, particularly in areas with high rainfall, strong winds, high humidity and sandy soil. The molecular basis of its tolerance and susceptibility in peach is yet to be understood. To study the genetics of the peach response to Xap, an F2 segregating population between two peach cultivars, 'Clayton', a resistant phenotype, and 'O'Henry', which is very susceptible to Xap, was created. Phenotypic data for leaf and fruit response to Xap infection were collected over three years at two locations: the Sandhills Research Station, Jackson Springs, North Carolina (NC); the Sandhill Research and Education Center, Pontiac, South Carolina (SC). Sixty-three individuals exhibiting high tolerance/resistance to Xap were genotyped with an IPSC 9K peach SNP array v1 and 1,341 SNPs w ere used to construct a genetic linkage map. This map covers a genetic distance of 421.4 cM with an average spacing of 1.6 cM and is used for mapping QTLs responsible for Xap in peach. A QTL analysis revealed 14 QTLs involved in Xap resistance: 3 on linkage group (LG) 1; two each on LG2, 3, 4 and 8; and one each on LG5, 6, and 7. One major QTL, Xap.Pp.CO-4.1 on LG4 was associated with Xap resistance in leaf, and two major QTLs, Xap.Pp.CO-1.2 and Xap.Pp.CO-6.1 on LG1 and 6, respectively, were associated with Xap resistance in fruit. In addition, one major QTL, Xap.Pp.CO-5.1 on LG5, was associated with Xap resistance on both leaf and fruit. Haplotypes for the four major Xap QTL regions and breeding for bacterial spot resistance and marker assisted selection in peach will be discussed.

Precise mapping an identification of snps associated with the apple scab resistance genes *Rvi2, Rvi4* and *Rvi11*

M. Jänsch[(1)], Weger J.[(1)], Broggini G.[(2)], Bus V.[(3)], Gardiner S.[(4)], Bassett H.[(4)], Patocchi, A.[(5)]

[(1)]Agroscope Changins-Wädenswil Research Station, Phytopathology, Schloss 1, CH-8820 Wädenswil, Switzerland
[(2)]Swiss Federal Institute of Technology, Plant Pathology Group, Universitätstrasse 2, CH-8092 Zürich, Switzerland
[(3)]The New Zealand Institute for Plant & Food Research Limited, Private Bag 1401, Havelock North 4157, New Zealand
[(4)]The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North 4442, New Zealand
[(5)]Agroscope Changins-Wädenswil Research Station, Phytopathology, Schloss 1, CH-8820 Wädenswil, Switzerland

One of the objectives of the FruitBreedomics project is to identify SNP markers for the R-genes most used in apple breeding for application in MAB involving cheap SNP-based assays.
Here we present SNP markers for the apple scab resistance genes Rvi2 and Rvi4 derived from Russian apple R12740-7A, and Rvi11 from Malus baccata jackii. The populations and the genotyping data of the first mapping report for each of the genes were used for identification and mapping of the SNP associated to the genes. First, the genotyping data of each individual was critically checked. Missing data for SCAR and SSR were produced and data points for "double recombinants" were repeated. Markers causing too many tensions in the maps were removed. If the phenotype was the cause of the double recombinant, the plant, if still available was re-inoculated or was considered a "genotype-phenotype-incongruence" plant and not used for further mapping. To identify the region in which the SNPs had to be searched the sequences of the markers bracketing the R-gene were blasted against the 'Golden Delicious' genomic sequence. Pairs of primer were designed in the identified region and tested on the parents of the mapping populations. SNPs identified by sequencing the amplicons of the resistant parent were tested on a subset of resistant and susceptible progenies to verify the linkage to the R-gene and on the recombinants for precise mapping. The whole procedure and the obtained results will be presented.

Progress in EU-FruitBreedomics

F. Laurens [1]

[1] Institut de Recherche en Horticulture et Semences UMR1345 (INRA / Agrocampus-ouest / Université d'Angers), France

Genetic variability description in a wide germplasm of domesticated peach through high throughput genotyping

D. Micheletti[1], Aramini V.[2], Arús P.[3], Banchi E.[4], Barreneche T.[5], Bassi D.[6], Dirlewanger E.[5], Gao Z.[7], Gazza L.[2],Lambert P.[8], Laurens F.[9], Li X.[7], Pascal T.[8], Quilot-Turion B.[8], Rossini L.[6], Troggio M.[4], Verde I.[2], Aranzana M.J.[1]

[1]IRTA-Consortium CSIC-IRTA-UAB-UB. Centre of Research in Agricultural Genomics (CRAG), Barcelona, Spain
[2]CRA - Fruit Tree Research Center, Roma, Italy
[3]IRTA-Consortium CSIC-IRTA-UAB-UB. Centre of Research in Agricultural Genomics (CRAG), Barcelona, Spain
[4]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[5]INRA. UR419, Unité de Recherches sur les Espèces Fruitières, Villenave d'Ornon, France
[6]University of Milan, Milano, Italy
[7]Zhejiang University. Department of Horticulture, Hangzhou, China
[8]INRA. UR1052, Génétique et Amélioration des Fruits et Légumes, Montfavet, France
[9]INRA, Institut de Recherche en Horticulture et Semences, Beaucouze, France

Peach (Prunus persica (L.) Batsch) is one of the most economically important fruit crops in temperate areas. Classical fruit tree breeding is generally slow and inefficient. Molecular markers could improve its efficiency but, although nowadays many Mendelian traits are mapped in peach and SSR markers have been found to be linked to some of the key major genes, its use in breeding programs is still limited. Main reasons for that are insufficient linkage between the markers and the genes and the lack of markers suitable for medium-high degree of multiplexing. To address this limitation, about 1,300 peach cultivars were genotyped with the 9K peach SNP chip (Verde et al. 2012) in the frame of FruitBreedomics project. This germplasm was chosen to be representative of the genetic diversity present in five germplasm collection in Europe and in China. Out of the 8144SNPs present in the chip, about 4300 were positively genotyped and used for the further analysis. The average number of heterozygous loci in the genotyped accessions was 1186 (spanning from 13 to 2775). The preliminary results of the population structure reveal three main subpopulations and the presence of high number of admixed individuals. LD seems to decay at distance longer than ca. 1 Mb.
These results will be instrumental for implementing LD-based mapping of QTLs and genes in peach.

Discovery & interpretation of multiple linked QTL

M.C.A.M.Bink[1], Peace C.[2], Verma S.[2], Iezzoni A.[3], Rosyara U.R.[3], Wang D.[3], Jansen J.[4], Voorrips R.E.[5], Van de Weg W.E.[5]

[1]Biometris/Wageningen UR, Wageningen, the Netherlands
[2]Washington State University, Pullman WA, USA
[3]Michigan State University, East Lansing, MI, USA
[4] Biometris/Wageningen UR, Wageningen, the Netherlands
[5] Plant Breeding/Wageningen UR, Wageningen, the Netherlands

Consumers prefer excellent fruit quality and fruit quality traits are also high on the list of rosaceous breeders. Marker-assisted crop improvement of fruit quality traits like texture, acidity, sweetness and aroma is well feasible once the controlling loci have been identified and their magnitude validated. Two major international collaborative projects, i.e. USDA-SCRI RosBREED and EU- FruitBreedomics, pursue the promising pedigree-based QTL analysis to discover and characterize multiple QTL as to trace novel allelic variants from a wide range of ongoing breeding programs in several rosaceous crops.
In this presentation, we are revisiting the HiDRAS dataset to describe the pedigree-based analysis and QTL results for fruit firmness after two months of cold storage. We will focus on the interpretation of QTL results, especially when multiple peaks arise close together on the same linkage group (LG). In the latter case, there may be multiple linked QTL or multiple functio nal alleles for one QTL. In both cases, evidence for them being true rather than artifacts comes from their segregation in different parts of the germplasm which in turn results in different inferred QTL genotypes of mapping parents along the chromosome. A subsequent IBD analysis may reveal the founders that introduced the favorable QTL alleles into the breeding germplasm.

Towards a large sized Axiom SNP array for the allo-octoploid strawberry

Bassil N.[1], Amaya I.[2], Bellon F.[3], Mittmann M.[3], Pirani A.[3], Webster T.[3], Brew F.[4], Davis T.M.[5], Mahoney L.[5], Wood D.[5], Yang Y.[5], Zhang H.[5], Denoyes B.[6], van Dijk T.[7], Ficklin S.[8], Jung S.[8], Main D.[8], Peace C.[8], Iezzoni A.[9], Monfort A.[10], Sargent D.[11], W.E .van de Weg[12]

[1]USDA-ARS, NCGR, Corvallis, Oregon, USA
[2]IFAPA-Centro de Churriana, Málaga, Spain
[3]Affymetrix, Santa Clara California, USA
[4]Affymetrix UK Ltd, Wooburn Green, High Wycombe, UK
[5]University of New Hampshire, Durham, New Hampshire, USA
[6]INRA, Bordeaux, France
[7]Wageningen-UR Plant Breeding, Wageningen, The Netherlands
[8]Washington State University, Pullman, Washington, USA
[9]Michigan State University, East Lansing, Michigan, USA
[10]IRTA-CRAG, Barcelona, Spain
[11]Research and Innovation Centre – Fondazione Edmund Mach, S. Michele all'Adige, Trento, Italy
[12]Wageningen-UR Plant Breeding, Wageningen, The Netherlands

A central goal of the RosBREED consortium has been to establish SNP arrays for peach, cherry, apple, and strawberry, to facilitate QTL discovery and marker-assisted breeding. This goal has been advanced by the release of three Illumina® Infinium® arrays for apple, peach, and cherry (8K, 9K and 6K, respectively). Here, we report on the development of a 90K Strawberry Affymetrix Axiom® genotyping array.
The cultivated strawberry is an allo-octoploid. This level and type of ploidy creates challenges to overcome, which we address in several ways. First, the large size of the array permits success despite a lower conversion rate of candidate to functional SNPs than for diploid crops. Second, we exploit site-specific, biological reductions in ploidy resulting from subgenome-specific deletions. Third, we exploit designed reductions in ploidy by targeting probes to sites of subgenome-specific sequence motifs. Fourth, we include SNPs and/or probes specific to one sub-genome.
We are using a diverse germplasm discovery panel of 19 octoploids. The array will target several polymorphism types, including indels and di- and multi-allelic SNPs. Here we describe our approaches to reduce the effective ploidy level so as to choose subgenome-specific SNPs. We also report on a new bioinformatics pipeline, which includes local re-alignment around indels and polymorphism type-specific filt ering strategies. Production of the array starts in September 2012 and it will become commercially available.

Jewels in the apple genome: RosBREED's conversion of reported trait loci for fruit quality into DNA tests routinely used in breeding

C. Peace[1], Verma S.[1], Evans K.[1], Guan Y.[1], Luby J.[2], Clark M.[2], Schmitz C.[2], Brown S.[3], Orcheski B.[3], Bassil N.[4], Bink M.[5], van de Weg E.[6], Iezzoni A.[7]

[1]Washington State University, Pullman, WA, USA
[2]University of Minnesota, St Paul, MN, USA
[3]Cornell University, Geneva, NY, USA
[4]USDA-ARS, NCGR, Corvallis, Oregon, USA
[5]Wageningen-UR Biometris, Wageningen, The Netherlands
[6]Wageningen-UR Plant Breeding, Wageningen, The Netherlands
[7]Michigan State University, East Lansing, MI, USA

Delicious taste, excellent texture, and attractive appearance are attributes of apple fruit quality highly desired by consumers and targeted by U.S. breeders. RosBREED's powerful collaborative approach, with multi-institution standardized phenotyping, high-resolution genome-scanning with cutting-edge technologies, careful choice of breeding-representative germplasm, software capability to analyze QTLs across mixed pedigrees, and systematic conversion-to-application has not only detected but validated interesting QTLs with valuable alleles for apple fruit quality. If a trait is under genetic control and it has been phenotyped, chances are very good that we will find its controlling loci and translate them into the language of crop improvement. With our focus on determining functional alleles/haplotypes and their distribution in breeding germplasm, we are ensuring that, at long last, DNA information from QTL studies is applicable for breeding. In this presentation, functi onal alleles/haplotypes at trait loci such as Ma, Rf, Md-Exp7, Md-ACS1, and Md-ACO1 for flavor, texture, appearance, and other components of apple fruit quality will be described in a practical breeding context. Routine use of predictive DNA tests at the above-mentioned trait loci is already improving the efficiency of U.S. apple breeding programs

New plant breeding techniques in the EU

H.J. Schouten[(1)]

[(1)]Wageningen UR, Wageningen, The Netherland

17 years ago the first gm crops were commercially introduced in the US. In the following 17 years the area of gm crops expanded strongly, to more than 160 million hectares. This area is increasing steadily in 29 countries outside the EU. The number of farmers that grow GM crops is in the meantime about 17 million, which is comparable to the number of inhabitants in The Netherlands. This rise of cultivation of gm crops does not occur in the EU. In the EU, only one gm corn cultivar (MON810) is grown at a modest scale, mainly in Spain. The present procedures for approval of cultivation of gm crops in the EU are extremely slow and costly, preventing small and medium sized companies and universities from commercializing gm crops in the EU. Moreover, growing of gm crops can lead to co-existence problems when there is a gene flow to neighbouring fields via pollen or seed, or when admixture occurs in the chain. Moreover companies are afraid of actions by NGOs that may negatively impact the image of products from gm crops.
Simultaneously, the amount of knowledge of plant genes and DNA sequences of even whole plant genomes has increased exponentially. Breeders and researchers are searching for ways to use this knowledge in breeding processes. One way of applying this knowledge is marker assisted breeding, using DNA markers near or in genes that are responsible for phenotypic variation in offspring. Also new breeding techniques have been exploited, and open fascinating opportunities for breeding more efficiently and more precisely. Examples of new breeding techniques are reverse breeding, cisgenesis, fast breeding, and directed mutations using oligos, zinc fingers or TALENs. However, companies wonder whether commercial plants developed by means of these techniques have to be treated as GMOs. If plants would be covered by the GMO legislation in the EU, then these companies would stop their efforts in developing and applying these new techniques in the EU. Therefore it is critical to know whether new breeding techniques do lead to GMOs or not.
In 2006, we contributed to this discussion by means of a letter in Nature Biotechnology on regulation of cisgenic plants. In the same year, the Dutch Commission for Genetic Modification (COGEM) provided a report on environmental safety of cisgenic plants to the concerning Dutch minister. Soon after this report, the COGEM issued a report on other novel breeding techniques that use genetic modification during the process of developing or selecting plants. The Dutch Minister responded that the topic should be discussed at the European level, as the Dutch GMO regulation is based on European directives, such as Directive 2001/18/EC. In the mean time the Dutch Government discussed these techniques, especially cisgenesis, and adapted a series of motions, supporting excluding cisgenesis from the burden of the GMO regulation. The aim of the government has been to allow small and me dium sized companies developing disease resistant apples and potatoes for a strong reduction of pesticide input. The European Commission (EC) appeared to be willing to discuss the new breeding techniques at the European level, and initiated a working group that represented all European Member

States, to provide insight in the legal status of eight new breeding techniques. These techniques included zinc finger nuclease technology (ZFN-1, ZFN-2 and ZFN-3), oligonucleotide directed mutagenesis (ODM), cisgenesis (and intragenesis), RNA-dependent DNA methylation, grafting a non-GM scion on GM rootstock, reverse breeding, agro-infiltration, and synthetic biology. The working group finished it report in January 2012. This report describes the different techniques. Also it describes the working group's opinion whether new techniques lead to GMOs or not according to the definition of a GMO in the current European Directives.
Also the EC asked the European Food Safety Authori ty (EFSA) to evaluate the safety of cisgenic plants in comparison to transgenic or conventional bred plants. Moreover, the EC asked the Joined Research Centre to explore the potential economical impact of the novel breeding techniques. In view of the importance of the innovation from new breeding techniques, different companies and universities formed in 2011 and 2012 a platform, and provided in September 2012 to the EC their opinion on the legal status of the resulting plants, and whether these should be regarded and treated as GMOs according to interpretation of the GMO definition.
During the presentation, the results from these studies will be summarized.
The EC is now making up its mind, and will discuss this with the competent authorities of the member states. The EC hesitates to adapt the GMO Directive, but prefers to look only at the legal interpretation of the current GMO definitions in the European Directive 2001/18/EC. That should answer the question wh ether the resulting plants from new techniques give rise to GMOs or to non-GMOs.

Can genetic engineering reduce pesticide use in apple orchards?

C. Gessler(1), Broggini GAL.(1), Flachowsky H.(2), Hanke M.V.(2), Peil A.(2), Chevreau E.(3), Durel C.E.(3), Patocchi A.(4), Krens F.A.(5), Schouten H.J.(5)

(1)Plant Pathology, Institute of Integrative Biology (IBZ), ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland
(2)Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, Pillnitzer Platz 3a, 01326 Dresden, Germany
(3)UMR Institut de Recherche en Horticulture et Semences (IRHS) (INRA/Agrocampus-ouest/Université d'Angers) Centre Angers-Nantes, 42 rue Georges Morel – BP 60057, 49071 Beaucouzé cedex – France
(4)Agroscope Changins-Wädenswil (ACW) Research Station, Phytopathology, Schloss 1, 8820 Wädenswil, Switzerland
(5)Plant Research International, Wageningen University and Research Centre, PO Box 16, 6700 AA Wageningen, The Netherlands

Apple orchards in temperate climate with often wet springs need a substantial input of chemical crop protectants. These chemicals are mostly fungicides to control apple scab, caused by *Venturia inaequalis* and powdery mildew, caused by *Podosphaera leucotricha*. However, where the bacterium *Erwinia amylovora* is present and particular conditions during bloom are met, also antibiotics are used to avoid fire blight. Classical breeding has produced many scab resistant cultivars overwhelmingly based on the resistance from *Malus floribunda* 821 (*Vf*) and also some mildew resistant cultivars. In addition, efforts to breed fire blight resistant cultivars are currently undertaken. Such cultivars are used in organic production; however, they have little global impact. Marker assisted selection added a relevant quality by shortening breeding time and allowing pyramiding of resistance genes. If the development of markers for MAS was the primary goal of genetic analysis in the 90ies, identification and cloning of resistance genes is now the goal. The sole resistance gene which has been isolated and transferred into a susceptible apple cultivar and proven to induce apple scab resistance is the gene *HcrVf2*, responsible for the *Vf* scab resistance. Unfortunately, *Vf* resistance has been overcome by the pathotype vir-Vf of the pathogen in many production areas of North-Europe. Therefore, much effort is currently spent in the identification and positional cloning of other endogenous apple genes inducing resistance to apple scab and fire blight. We identified putative resistance genes of the *Rvil5* scab resistance (alias *Vr2*) and the genes for two fire blight resistances namely from cv. 'Evereste' and *Malus robusta* 5. The functionality of these candidate genes is currently under scrutiny by complementation experiments (*Vr2* at WUR, Evereste at ETH/Agroscope and Angers, M. robusta 5 at ETH/Agroscope and Dresden). However, the final goal is the creation of a product, e.g. an ameliorated apple cultivar by the addition of resistance to scab and fire blight, with advantages to the environment, producer and consumer, raising as little concern as possible. As the presence of any other foreign gene is highly questioned we opted for the cisgenic approach. We introduced by Agrobacterium transformation the scab resistance gene *HcrVf*2 with its own regulatory sequences into the highly susceptible apple cultivar Gala,

and eliminated post transformation all marker genes. As the used methodology allows the sequential addition of genes, e.g. through a second transformation, the putative Malus own fire blight resistance genes could be added to the primary cisgenic line. Several *HcrVf*2 lines were analyzed in detail, in trans and in cis for site of insertion, amount of DNA inserted, expression of the target gene and functionality. Each transformation event results in a very unique pattern of the analyzed factors. Three lines were selected for field trials. All three lines had an expression level of *HcrVf*2 clearly below the compared classical bred Vf cv. 'Florina' and showed chlorotic reaction with sparse sporulation of V. inaequalis (type 3a-3b) in glasshouse trials upon inoculation. Preliminary results from the field trials ranked the cisgenic lines between the mother cv. 'Gala' and the control Vf-containing cv. 'Santana' with a clear statistical difference to them. This collaborative work demonstrates that engineering a disease resistant cultivar using apple own genes and regulatory sequences is feasible. Once resistance genes are available it is relatively cheap and fast to transform a susceptible cultivar into a disease resistant one. Pyramiding resistance genes derived from wild *Malus* as strategy for durable resistance now appears as a practical solution and aid to classical breeding. If the public acceptance of such cisgenic apple cultivars can be obtained and the EU legal restrictions are lifted for such cultivars, then this approach may become one of the possible ways to drastically reduce chemical input and the uncertainty of damage by scab and fire blight.

Trans- and cis-genic approaches to improve apple breeding at JKI in Dresden-Pillnitz

H. Flachowsky[(1)], Wöhner, T.[(1)], Würdig, J.[(1)], Vogt, I.[(1)], Wenzel, S.[(1)], Peil, A.[(1)], Broggini, G.A.L.[(2)], Fahrentrapp, J.[(2)], Gessler, C.[(2)], Hanke, M.V.[(1)]

[(1)]Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, Dresden, Germany
[(2)]Plant Pathology, Institute of Integrative Biology (IBZ), ETH Zürich, Zürich, Switzerland

Trans- and cisgenic approaches represent promising tools to improve fruit tree breeding efficiently. Such technologies offer the opportunity to improve well established cultivars in individual traits in a manageable amount of time. Especially genes like the Rvi6 (HcrVf2) scab resistance gene, the MYB10 transcription factor as well as candidate genes for fire blight resistance are of particular importance. Using a vector system for producing marker-free genetically modified (gm) plants the Rvi6 gene from Malus floribunda 821 driven by its own regulatory elements was transferred into the commercially important cultivars 'Pinova', 'Mariri Red', 'Kanzi', 'Gala-Mitchgla', 'Novajo' and 'Gala-Brookfield'. Up to date transgenic lines are generated for four cultivars. Transgenic lines were investigated by PCR, RT-PCR as well as Southern Blot. Furthermore, they were evaluated in a greenhouse resistance test with scab races differing in their pathogenicity. First experiments to eliminat e the nptII marker gene from the genome of gm-apple plants are currently in progress. The results will be presented.
Another purpose is the transfer of the MdMYB10 gene from the red leave hybrid TNR 31-35 (Malus sieversii var. sieversii f. Niedzwetzkyana) into 'Pinova', which causes red flesh color. The MdMYB10 gene was isolated from TNR 31-35 and cloned into different vectors either driven by its own promoter or by the constitutive CaMV35S promoter. Transgenic apple clones were tested on integration and expression of the MdMYB10 gene. Most of the clones expressed a strong red coloration of all vegetative tissue as expected. First results of experiments aimed on the production of MdMYB10 cisgenic plants will be also presented.
Furthermore, we isolated the fire blight resistance QTL of Malus x robusta 5 using a map based cloning approach. The identified candidate gene was used for transformation of different apple cultivars.

Fragaria Functional Genomics

A. Chambers (1), Chatterjee M.(1), Brunings A.(1), Whitaker V.(2), Folta K.(1)

(1)University of Florida, Gainesville, FL, USA
(2)University of Florida, Wimauma, FL, USA

The grand challenge to plant genomics is to functionalize the last decade's sequence data. We now possess the strawberry (*Fragaria vesca* L) genome and expressed gene sequences, and must now return to fundamental analyses to determine how these genes contribute to plant biology. Our laboratory implements a high-throughput transgenic system to test gene contributions in planta. These trials allow us to discover the function of novel genes, test the roles of candidate genes and translate information from model systems to relevant crops of the Rosaceae family. Analysis of novel predicted sequences with RNAi revealed three genes that produce strong loss-of-function phenotypes that would have immense impacts to the strawberry industry, including larger fruits, more fruits, and open plant canopies. Comparison of fruits producing and not producing desirable flavor compounds has revealed a series of candidates that are now being tested in vivo. One candidate gene has a profound effect on flavor compounds upon over-expression. Tests of well described genes involved in leaf/plant architecture and photoperiodic flowering show that the rules described in the Arabidopsis model do not translate well to strawberry. The agile transformation system of strawberry, along with its compact size and rapid growth make it an ideal translational system for study of gene function in the Rosaceae, a valuable family of diverse crop plants.

Ten years of GDR: Current resources and functionality of the GDR

S. Jung[1], Lee T.[1], Ficklin S.[1], Cheng C.[1], Zheng P.[1], Blenda A.[2], Ru S.[1], Evans K.[1], Peace C.[1], Oraguzie N.[1], Abbott A.[2], Layne D.[2], Olmstead M.[3], Gmitter F.[3], Chen C.[4], Mueller L.[5], Main D.[1]

[1]Washington State University, Pullman, WA, USA
[2]Clemson University, Clemson, SC, USA
[3]University of Florida, Gainesville, FL, USA
[4]University of Florida - CREC, Lake Alfred, FL, USA
[5]Boyces Thompson Institute for Plant Research, Ithaca, NY, USA

Initiated in 2002, GDR, the Genome Database for Rosaceae (www.rosaceae.org) is now ten years old. In this update we report on new and future functionality of GDR. Rosaceae includes many economically important crops such as apple, peach, cherry, almond, strawberry, pear and rose. Whole genome sequences and annotation data for peach, apple, and strawberry can be accessed through GBrowse, a graphic genome browser. SNP markers included in the apple, peach and cherry illumina SNP arrays can also be searched, downloaded in excel and viewed in GBrowse. The genome data is integrated with other existing data so that users can go back and forth from the marker page, EST page, or CMap page to GBrowse. Recently, the predicted genes of the whole genome sequences have been annotated by homologous genes in other species, InterPro protein domains, GO terms and KEGG pathway terms, providing a glimpse of the pathways and traits in which they are involved. The annotated sequence data can be browsed through the species page or queried using various categories in the search sites. Synteny among the three sequenced genomes can be viewed through GBrowse_Syn. More functionality and data have also been added to the breeder's toolbox, providing breeders with tools to efficiently manage and query their data. The updated Breeders toolbox allows users to search for varieties using the molecular markers used for genotyping and molecular alleles as well as using variety names and trait thresholds.

Progress on the development of a genome sequence for red raspberry *Rubus idaeus*

J.A. Ward(1), Price J.C.(2), Clement M.(2), Schatz M.(3), Weber C.A.(4), Swanson J.D.(4), Bodily P.(2), Lewers K.S.(6), Fernandez F.(7), Burns P.D.(8), Velasco R.(9), Sargent D.(9), Udall J.(2)

(1)Cornell University, Geneva, NY
(2)Brigham Young University, Provo, UT
(3)Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
(4)Cornell University, Geneva, NY
(5)Salve Regina University, Newport, RI
(6)USDA-ARS , Beltsville, MD
(7)East Malling Research, East Malling, Kent, United Kingdom
(8)Georgia Tech, Atlanta, GA
(9)Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

A draft pseudo chromosome assembly of the highly heterozygous diploid red raspberry variety 'Heritage' (R. idaeus subsp. vulgatus Arrhen. x R. idaeus subsp. strigosus Michx) is presented. Approximately 90x coverage of the genome (~6x in pairs with insert greater than 2 kb) was used to assemble the sequence into 11,502 scaffolds. Detailed internal analysis of the assembly reveals two highly divergent haplotypes, consistent with previous linkage studies in raspberry, which likely contribute to the relatively high number of scaffolds. A genetic map was created using Genotyping by Sequencing and approximately 3400 markers from the map were used to assign scaffolds to linkage groups. Large-scale agreement between the scaffolds and the genetic map is apparent and only 0.2 percent of the scaffolds are mapped to by markers from more than one linkage group. Despite the high density of the genetic map it allowed assignment of only 60% of bases to pseudomolecules due to the relatively high number of scaffolds produced by the assembly. Efforts are now underway to improve the pseudomolecule assembly by incorporating synteny with the *Fragaria vesca* genome and additional paired data to assign more scaffolds to linkage groups. Gene finding with GeneMark ES+ estimates a gene content of 36009, which is approximately 1,200 more than F. vesca. Gene-family analysis and comparison to strawberry continues.

Statistical tools and software for the construction of integrated genetic linkage maps of outbreeding species using SNP markers with apple as a model

J. Jansen(1), Thissen J.(1), Troggio M.(2), Sargent D.(2), Bink M.(1), Peace C.(3), Schouten H.(4), Van de Weg E.(4)

(1)Biometris/Wageningen UR, Wageningen, the Netherlands
(2)Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
(3)Washington State University, Pullman, WA, USA
(4)Plant Breeding/Wageningen UR, Wageningen, the Netherlands

Integrated genetic linkage maps have become an indispensable tool in genetics and genomics. For outbreeding species with SNP markers, the construction of integrated genetic linkage maps is complicated by the fact that in different populations markers may either be segregating according to one of three types of segregation or be non-segregating. Further complications arise from the presence of unequal rates of recombination and from segregation distortion. In this presentation we will give a detailed description of the steps that have been made to construct an integrated genetic linkage map of apple using SNP data from three populations. We will also describe the tools that have been used in the process, which include tools for ordering markers, tools for selecting representative markers that can be used to construct framework maps, and tools for checking the quality of genetic linkage maps of individual populations as well as integrated genetic linkage maps. The integrate d linkage map of apple with a total length of 1278 cM contains 1199 SNP markers. We will present a comparison of the integrated map and the genetic linkage maps of the individual populations. The SNP positions on the integrated linkage map will also be compared with the corresponding physical positions of the Golden Delicious reference genome and will be used in following QTL discovery studies. This work has been performed in the framework of the projects EU-FruitBreedomics and USA-SCRI RosBREED.

Re-annotation and transcript expression analysis of the apple genome

J-M. Celton (1), Gaillard S.(1), Bruneau M.(1), Pelletier S.(1), Le Paslier M.C.(2), Rees D.J.G. .(3), Laurens F.(1), Renou J-P.(1)

(1)INRA, UMR1345 Institut de Recherche en Horticulture et Semences, SFR 4207 QUASAV, PRES L'UNAM, F-49045 Angers, France. AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, F-49045 Angers, France. Université d'Angers, UMR1345 Institut de Recherche en Horticulture and Semences, F-49071 Beaucouzé, France
(2)4INRA US1279, EPGV, 91057 Evry-Cedex, France
(3)Biotechnology Platform, Agricultural Research Council, Private Bag X05, Onderstepoort, 0110, South Africa

As encountered in many sequencing projects, genome annotation remains a complex endeavor despite advances in the quality of gene prediction software. In an effort to develop high-quality gene model for the apple genome (Malus x domestica), we aligned massively parallel cDNA sequencing reads of 19 apple tissues (representing over 700x) to the v.1 apple genome. Integration of RNA-Seq allowed us to identify hundreds of transcripts that had been missed by the automatic annotation procedure, to correct predicted gene structures, and to detect a multitude of alternative and trans-splicing events. Furthermore, data from the v.1 apple genome allowed us to develop a NimbleGen microarray chip with probes complementary to both sense and anti-sense transcripts. Natural anti-sense transcripts (NAT) have recently been shown to be a common regulatory mechanism for gene expression. Our expression analysis over eight apple organs show that NATs were identified in up to 20% of genes. Interesti ngly, the percentage of NATs identified varied greatly among gene and transcription factor families. Furthermore, results indicate that anti-sense strand transcription is likely specific to certain tissue and/or developmental stages. Analyses showed good correlation between normalized spot intensities, RPKM values (from mRNA-seq), and cycle threshold (Ct from RT-qPCR validations).
As shown by this study, sequencing projects require continual improvements and refinements. Our RNA-seq approach combined with large-scale transcript expression analysis by microarray provided a powerful method to rapidly build an improved version of the apple genome annotation.

Exploiting the peach genome sequence for identification of candidate genes for disease resistance in fruit and forest trees

A. G. Abbott[1], Zhebentyayeva T.[2], Decroocq V.[1], Badenes M.[3], Staton M.[2], Carlson J.[4], Nelson C.D.[5]

[1]INRA- Bordeaux, France
[2]Clemson University, Clemson, SC, USA
[3]IVIA-Valencia, Spain
[4]Penn State University, University Park, PA, USA
[5]USDA Forest Service, Southern Research Station, Saucier, MS, USA

Fruit and forest trees breeding programs continually face the challenge of improving varieties for important traits such as yield and quality while at the same time incorporating as much disease and pest resistance as is available in the native germplasm. Thus, many of the fruit and forest breeding programs focus on identification and marking of disease resistance genes as a primary goal. This has led to a significant number of molecular genetic maps developed in many different fruit and forest tree species focused on marking the genomic intervals that contain disease resistance genes. However, in tree genetics the difficulties associated with translating this genetic knowledge to candidate genes has significantly hampered progress in resolving these genomic "disease resistance" intervals into actual genes for study. With completion of the whole genome sequence assembly of the peach, we now have available all the requisite tools to identify and characterize candidate genes in genomic intervals that have previously been identified as contributing to disease resistance in trees. In this presentation, we will highlight two examples (Sharka disease in stone fruits, blight disease in nut trees) of how the peach genome is being leveraged to speed the candidate gene discovery in both fruit and forest trees. We will also highlight the importance and use of the peach genome as a comparative genomics tool both within and outside the Rosaceae.

Marker assisted breeding for peach seedling rootstocks resistant to root-knot-nematodes

H. Duval[(1)], Hoerter M.[(1)], Masse M.[(1)], Moretti A.[(1)], Esmenjaud D.[(2)]

[(1)]INRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes (GAFL) Domaine St Maurice, F-84143 Montfavet, France
[(2)]INRA PACA, UMR ISA, INRA1355-CNRS7254-Université de Nice, F-06903 Sophia Antipolis, France

Peach and apricot trees grown in France are mostly grafted with the peach seedling rootstock Montclar which is sensitive to damage by root-knot nematodes (RKN) Meloidogyne spp. Thus the INRA Prunus rootstock breeding program has two objectives: i) to introduce the genetic resistance to RKN conferred by the RMia gene from Nemared (homozygous resistant) in the Montclar background, and ii) to develop a marker-assisted breeding (MAB) method for RMia RKN resistance. The work was conducted using two segregating crosses created from 2008: the 4-way hybrid (Montclar x Nemared) x (Pamirskii x Rubira) and an F2 population from Montclar x Nemared. As RMia had been previously localized on the linkage group 2 (LG2) of the Prunus reference map, SSR markers of the gene region were selected from the peach genome 'release V1.0' in the GDR website. Two of the SSR markers, polymorphic between Nemared and the other peach accessions, were shown to flank the RMia gene in an interval of 29 8 kb in the peach reference genome. Out of the 949 total progenies used, four recombinant individuals are still available for a further reduction of this interval. For all of the 945 non-recombinant hybrids the predicted phenotype was confirmed on young seedlings by inoculation tests with the RKN M. incognita. As a result, both flanking SSR markers can already be used for MAB of the RMia gene. This strategy will be applied in order to obtain a seedling rootstock homozygous for RMia.

Integrated approaches in *Prunus armeniaca* for resistance to Sharka disease

V. Decroocq[(1)], Zhebentyayeva T.[(2)], Lambert P.[(3)], Geuna F.[(4)], Salava J.[(5)], Roch G.[(3)], Bassi D.[(4)], Audergon J-M.[(3)], Abbott A.G.[(1,2)]

[(1)]UMR BFP, INRA-Université de Bordeaux, 71 Avenue Edouard Bourlaux, 33140 Villenave d'Ornon (France)
[(2)]Department of Genetics and Biochemistry, 100 Jordan Hall, Clemson University, Clemson SC 29634 (USA)
[(3)]UR1052 GAFL, INRA, Domaine Saint Maurice, 84143 Montfavet (France)
[(4)]DISAA, University of Milano, Via Celoria 2, 20133 Milano (Italy)
[(5)]Division of Plant Medicine, Crop Research Institute, Drnovská 507, 161 06 Prague 6 (Czech republic)

Sharka disease significantly impacts the economics and productivity worldwide. Natural resistance sources have been identified in few Prunus species. Indeed, cultivars carrying resistance to Plum Pox Virus (PPV) were described in apricot (Prunus armeniaca), namely 'Goldrich', 'Harcot', 'SEO', 'Lito', 'Harlayne', 'Stella' ... In the frame of the FP7 SharCo project, these donors of resistance have been used by breeders to introduce PPV resistance into commercial cultivars. However, all these genitors have been shown to be genetically related (Zhebentyayeva et al., 2008); they likely share the same major QTL on the upper part of linkage group 1 (Marandel et al., 2009; Dondini et al., 2010), thus, underpinning future breeding germplasm on a single source of resistance. In the course of SharCo, fine-mapping of the resistance has been performed (Soriano et al., 2012); F1, F2 and BC1 populations originating from different donors have been tested in differ ent environments (Czech republic, Italy, France) in order to check for stability and durability of the resistance. This work has also allowed refining the genetic location of the host factor(s) underlying the resistance trait. These data are currently being incorporated into a whole genome association genetics study to explore the broad diverse apricot germplasm as a substrate for candidate gene(s) identification for Sharka resistance as well as for other important agronomical traits in Prunus (Project ANR ABRIWG 2012-2014).

Indications for a gene-for-gene relationship in the *Malus × robusta – Erwinia amylovora* host pathogen system

A. Peil[(1)], Vogt I.[(1)], Wöhner T.[(1)], Richter K.[(2)], Wensing A.[(3)], Geider K.[(3)], Hanke M-V.[(1)], Flachowsky H.[(1)]

[(1)]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural and Fruits Crops, Pillnitzer Platz 3a, 01326 Dresden, Germany
[(2)]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany
[(3)]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Schwabenheimer Straße101, 69221 Dossenheim, Germany

Whereas the interaction of a plant with a bacterium is well analyzed in the host pathogen system Arabidopsis thaliana - Pseudomonas syringae only little is known about the interaction of the bacterium Erwinia amylovora, causing the devastating disease fire blight on pome fruit, with Malus.
The wild species accession M. x robusta 5 (Mr5) shows high resistance to most E. amylovora strains. Nevertheless, the E. amylovora mutant ZYRKD3-1, lacking the effector avrRpt2EA, a homolog to avrRpt2 of Pseudomonas syringae, was able to overcome the resistance of Mr5. To investigate the role of avrRpt2EA in the host pathogen relationship Mr5 – E. amylovora the avrRpt2EA genes of 22 E. amylovora strains, some of which able to break down resistance of Mr5, were sequenced. Only one single nucleotide polymorphism (SNP) could be found resulting in an exchange of cysteine to serine in the deduced protein at amino-acid position 156. Inoculation experiments revealed that all strains able to overcome the resistance of Mr5 carry serine, all strains not able to infect Mr5 a cysteine at position 156. The two variants were determined as C- and S-allele. SNP-primers were developed to easily distinguish both alleles by PCR. Due to the fact that the mutant ZYRKD3-1 could break down resistance of Mr5 as well as E. amyolovora strains carrying the S-allele it is assumed that the recognition of the C-allele of the effector leads to resistance of Mr5. To confirm this theory ZYRKD3-1was complemented with either the C- or the S-allele. Inoculation of Mr5 with both strains showed that complementation of ZYRKD3-1 with the C-allele restored resistance of Mr5 whereas complementation of ZYRKD3-1 with the S-allele did not change virulence. Additionally RIN4 homologs were identified in Mr5 and sequenced. The importance of avrRpt2EA for resistance of Mr5 and the presence of RIN4 homologs are strong indications for a gene-for-gene relationship in Mr5 – E. amylovora similar to the s ystem Arabidopsis thaliana – P. syringae.

Breeding for sharka resistance: PaTl1, encoding a TRAF-like protein, as a candidate gene for PPV resistance in Apricot

Zuriaga E.(1), Soriano J.M.(1), Zhebentyayeva T.(2), Romero C.(1), Dardick C.(3), Cañizares J.(4), *M.L. Badenes*(1)

(1)Instituto Valenciano de Investigaciones Agrarias (IVIA), Apartado Oficial, 46113 Moncada, Valencia, Spain
(2)Department of Genetics and Biochemistry, Clemson University. 100 Jordan Hall, Clemson, SC 29634, USA
(3)USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430, USA
(4)Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

Sharka disease, caused by Plum pox virus (PPV), is the most important disease affecting Prunus species. A major PPV resistance locus (PPVres) was previously mapped to the upper part of apricot linkage group 1, within a 196 kb interval according to the peach genome syntenic region. In this study, a physical map of the PPVres locus in the PPV resistant cultivar 'Goldrich' was constructed. BACs belonging to the resistant haplotype contig were sequenced using 454 GS-FLX Titanium technology. Concurrently, whole genome sequences of 7 apricot varieties (3 PPV resistant and 4 PPV susceptible) and 2 related apricot species that are susceptible to PPV were obtained using the Illumina HiSeq2000 platform. Polymorphisms within the mapped interval were identified using reference assemblies against the recently sequenced peach genome. Searches for single nucleotide polymorphisms (SNPs) and deletion/insertion polymorphisms (DIPs) led to the identification of a TRAF-like gene, named PaTl1 (Prunus armeniaca TRAF-like gene 1).as a potential candidate for resistance to PPV in apricot. We hypothesize that this is a case of a natural 'dominant negative mutation' as heterozygosity is sufficient to confer PPV resistance in apricot.

Special events

1. Affymetrix Talk, 1st October, 11.15-11.45
2. Global Diversity Trust Workshop, 1st October, 15:00-19:00
3. Meeting Of The COST Action On Cherries, 1st October, 15:00-19:00
4. Illumina Genome Studio Workshop, 1st October, 15:00-17:00 Session 1
5. Illumina Genome Studio Workshop, 1st October, 17:00-19:00 Session 2
6. Illumina Genome Studio Talk, 2nd October, 11.00 – 11.30
7. FruitBreedomics, 2nd October, 15.20-15.50
8. RosExec Meeting, 3rd October, 15.30-16:30
9. RosIGI Meeting, 3rd October , 16:30-17:30

1. Affymetrix Talk, 1ST October, 11.15-11.45

Title: How Do You Accelerate Your Genomics Program From NGS To Molecular Breeding?

Presenter: *Fiona Brew*, Genotyping Specialist Senior Manager, Affymetrix Europe

Next Generation Sequencing (NGS) provides unprecedented opportunity to catalog Single Nucleotide Polymorphisms (SNPs) and other variants in diverse species. However, this is only the first step of an integrated workflow to develop raw SNPs into informative biomarkers for breeding. The issue of false positive SNPs in sequencing data must be resolved and downstream genotype-phenotype correlation studies require robust genotyping to reliably identify informative SNP biomarkers for breeding. Beyond this, there is the challenge of applying the biomarker panel in a scalable process and the risk of critical markers being lost if assay design or platform technology changes. For mid to high density biomarker panels, the prevailing strategy is to integrate NGS discovery into downstream microarray studies to take advantage of very high genotype accuracy, throughput, analytical efficiency and cost effectiveness offered by microarrays. We will present examples of best practice that is enabling investigators and breeders to advance from raw SNPs towards application of biomarkers in breeding. We will challenge the idea that arrays are inflexible by showing how our Axiom® Genotyping Solution enables a choice of strategies to eliminate sequencing errors and optimize array design. We will show how Axiom's flexibility enables arrays to be revised, affordably, as users advance through their workflow and how our technology avoids drop-out of critical biomarkers by ensuring that every requested SNP appears on every array, every time.

2. Global Diversity Trust Workshop, 1ST October, 15:00-19:00

Expert Consultation Workshop On The Use Of Crop Wild Relatives For Pre-Breeding In Apple

Workshop Expected Outcomes:

- Synthesis of past experiences and challenges of using CWRs in apple pre-breeding.
- Synthesis of expert opinions on the best way forward with regard to the use of CWRs in apple pre-breeding.
  1) Key traits
  2) Taxa of key interest
  3) Access to desirable taxa
  4) Overcoming biological obstacles and challenges
  5) Logistical efforts (who, where, what)
  6) Needs for developing countries

15:00 Introduction to the Workshop
Presenter: *Hannes Dempewolf*

15:15 Diversity, taxonomy, and origins of wild apple species
Presenter: *Gayle Volk*

15:45 Discussion topics

1) Current use of CWR in breeding programs (discussion among participants)
2) Challenges of using CWR in breeding programs
3) Strategies to meet the challenges of using CWR in apple breeding programs
4) Genomic/biotech opportunities
5) Current needs (gaps) in breeding programs that could be addressed by CWRs
6) Taxa known to possess traits of interest
7) Availability/access to key taxa
8) Survey of existing materials vs Need for additional collections
9) Screening new materials for desirable traits/alleles
10) Access of material to breeding programs and genebanks (long term preservation)
11) Implementation of apple pre-breeding programs

3. **Meeting Of The COST Action On Cherries, 1st October, 15:00-19:00**

4. **Illumina Genome Studio Workshop, 1ST October, 15:00-17:00 and 17:00-19:00**

An Introduction To SNP Data Analysis Generated With The Illumina Infinium Genotyping Array Using GenomeStudio

A hands-on session to learn the basics of Infinium data analysis using genome studio

- Essential participation for all those needing to analyze SNP data generated using the Illumina Infinium genotyping platform
- Bring your laptop

5. **Illumina Genome Studio Talk, 2nd October, 11.00 – 11.30**

Title: Genotyping By Sequencing With Illumina Technology

Presenter: *Rossana Scavelli*

6. **FruitBreedomics, 2nd October, 15.20-15.50**

7. **RosExec Meeting, 3rd October, 15.30-16:30**

The mission of the US Rosaceae Genomics, Genetics and Breeding Executive Committee (RosExec) is to serve as a communication and coordination focal point for the US Rosaceae genomics, genetics and breeding community; to define research priorities based on input from the industry and research community; to facilitate scientific interaction and foster dynamic research teams; to promote research priorities; and to coordinate educational efforts from the research community to the industry and the public.

8. **RosIGI Meeting, 3rd October , 16:30-17:30**

RosIGI is an organization that serves to ensure communication among Rosaceae researchers and organize the biennial Rosaceae Genomics Conference; coordinate international efforts on several key initiatives within the areas of Comparative Genomics, Structural Genomics, and Functional Genomics; and facilitate scientific interaction among members of the research community.

Abstracts

Poster presentations

Phenotypes of AGAMOUS2-RNAi galaxy apple flowers

H. Aldwinckle[1], Borejsza-Wysocka E.[1], Adhikary D.[1]

[1]Department of Plant Pathology and Plant Microbe-Biology
Cornell University, Geneva, New York 14456 USA

With the goal of developing an ornamental apple with polypetalous flowers, AGAMOUS2 (AG2) (MdMADS15) was silenced by RNAi in several lines of Malus pumila Miller cv. Galaxy. Grafted AG2-RNAi Galaxy plants were grown in a greenhouse and began flowering five years after transformation, with seven transformed lines flowering in the 6th year. The C- Class gene function was altered, with phenotype varying among the transformed lines. Four lines had a strong phenotype with no stamens and 22-24 petals; the shape of locules was irregular, with an increased number of ovules per carpel. The number of petals in the AG2-RNAi Galaxy flowers was 19-25 petals vs. 5 in the non-transformed Galaxy flower. The number of ovules increased from 10 in the non-transformed Galaxy flower to as many as 16 in the AG2-RNAi Galaxy flowers . The phenotype was not altered in two AG2-RNAi Galaxy lines. These results add to our understanding of regulation of floral development in apple, and may lead to de velopment of polypetalous ornamental apple cultivars.

Odour profiling of apple cultivars and correlation with volatile compounds

E. Aprea[1], Corollaro M.L.[1], Betta E.[1], Endrizzi I.[1], Demattè M.L.[1], Biasioli F.[1], Gasperi F.[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

We are using a trained sensory panel to define the sensory attributes profile of a wide number of apple commercial cultivars and new selections under evaluation at FEM (Fondazione Edmund Mach). The same fruit are evaluated through instrumental determinations as well. Here we present the correlations found between perceived odours (by trained panel) and volatile compounds (by SPME-GC-MS) in 18 apple cultivars.
It is known that perceived odours are mainly the result of mixture of odorants (more than 300 compounds that can contribute to apple odour and flavour have been identified) and the single components of a mixture may lose their individual identity and a new mixture with a specific odour quality could emerge.
Thus the correlations between odours and volatile compounds in apples were investigated by a multivariate approach. Regression models allowed the identification of compounds highly contributing to the odours arising from the complex mixture of volatile compounds released by apples. For example acetate esters strongly contribute to different fruity attributes and the results suggest that perceived odours are due to the relative proportions among esters rather than their presence/absence.

In conclusion, sensory and instrumental profiling in combination with appropriate chemometric analyses can help to elucidate the relationships between the perceived odours in real food and the complex mixture of released volatile compounds.

Domestication bottleneck as a signature of history diffusion of apricot species

Hedia, Bourguiba[(1)], Lamia, Krichen[(1)], Neila, Trifi-Farah[(1)], Bouchaib, Khadari [(2)], Ali, Mamouni[(3)], Samia, Trabelsi[(4)], Claudio, D'Onofrio[(5)], José, Egea-Caballero[(6)], Bayram-Murat, Asma[(7)], and *Jean-Marc. Audergon*[(8)]

[(1)] Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Campus universitaire, 2092 El Manar, Tunisie
[(2)] INRA, UMR 1098 Développement et Amélioration des Plantes, Campus CIRAD, TA A96/03 Avenue Agropolis, F-34398, Montpellier cedex 5, France
[(3)] INRA, Unité d'Arboriculture Fruitière, Centre Régional de Meknès, Maroc
[(4)]Université Saad-Dahlab, Blida, Algérie
[(5)] Dipartimento di Coltivazione e Difesa delle Specie Legnose, Università di Pisa, Via del Borghetto, 80, I-56124 Pisa, Italy
[(6)] CEBAS-CSIC, Departamento de Mejora Vegetal, 30100 Campus Universitario de Espinardo, Murcia, Spain
[(7)] Department of Biology, Inonu University, Malatya 44280, Turkey
[(8)] INRA, UR 1052 Génétique et Amélioration des Fruits et Légumes, Domaine Saint-Maurice, BP 94, F-84143 Montfavet, France

Domestication generally implies a loss of diversity in crop species relative to their wild ancestors because of genetic drift through bottleneck effects. The comparison of genetic diversity in Mediterranean areas was assessed. 207 native apricot accessions representatives of the local variability from different Mediterranean countries: Algeria, France, Italy, Morocco, Spain, Tunisia and Turkey in each country were selected excluding those issued from breeding programs. This material was analysed for genetic diversity and structure using a common set of 25 monolocus microsatellites distributed throughout the Prunus genome. According to the geographic origin of the material and using a model-based clustering method, four main gene pools were revealed, namely 'Irano-Caucasian', 'North Mediterranean Basin', 'South Mediterranean Basin', and a more transversal one named 'Adapted Diversity'. A significant gradient of decreasing diversity and allelic richness from the east to the south-west of the Mediterranean Basin was highlighted. Overall, results suggested that from the Irano-Caucasian area, apricot was introduced into the Mediterranean Basin through at least two different routes: the first one through the South of Europe and the second one through the North Africa. On the present bases relevant elements have been obtained useful for future management of apricot genetic resources as well as for genetic selection programs related to adaptive traits.

Developing genomic resources in black raspberry

N. Bassil[1], Gilmore B.[1], Dossett M.[2], Mockler T.[3], Filichkin S.[4], Lewers K.[5], Peterson M.[6], Finn C.[5]

[1]USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333, USA
[2]Agriculture and Agri-Food Canada - Pacific Agri-Food Research Centre, 6947 Hwy #7, P.O. Box 1000, Agassiz, B.C. V0M 1A0, Canada
[3]The Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132, USA
[4]Center for Genome Research and Biocomputing, Oregon State University, 3054 Cordley Hall Corvallis, OR 97331, USA
[5]USDA-ARS Horticultural Crops Research Unit, 3420 NW Orchard Ave., Corvallis, OR 97330, USA
[6]USDA-ARS BARC, Genetic Improvement of Fruits and Vegetables Lab, Rm. 210, Bldg. 010A, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

Since the early 1900s, the black raspberry industry in the US has steadily declined due in large part to a lack of adapted and disease resistant cultivars. News regarding potential health benefits of black raspberries has revived interest in production and breeding new cultivars. We are developing the genomic infrastructure for black raspberry. Two mapping populations, ORUS 4305 and ORUS 4304, were propagated and planted in grower's and research fields across five US production areas. Each segregates for a new source of resistance to the large raspberry aphid, an important vector to the black raspberry necrosis virus. This virus is a leading cause for the short life of plantings. Polymorphism screening of over 200 Rubus microsatellite markers in parents of ORUS 4305 identified 42 polymorphic microsatellite that are being used to construct a linkage map. Six cDNA libraries generated from five tissue types of 'Jewel' produced 704 Gbp of sequences. Initial assembly of the genome from a highly homozygous accession yielded an assembly of 268 Mbp. The low percent of SNP bases (0.06%) confirmed low heterozygosity. The transcript sequences will be assembled next and used to develop gene models for genome assembly. These developing genomic resources will be instrumental in building the infrastructure needed for identification of candidate genes or markers responsible for many traits of interest for development of improved black raspberry cultivars.

Marker validation for *Rpf*1 red stele resistance in strawberry

M.M. Mathey[(1)], Jamieson A.R.[(2)], van de Weg E.W.[(3)], N.V. Bassil[(4)], Hancock J.F.[(5)], Finn C.E.[(4)]

[(1)]Oregon State University, Corvallis, OR, USA
[(2)]Agric. & Agri-Food Canada, Kentville, NS, Canada
[(3)]Wageningen-UR Plant Breeding, The Netherlands
[(4)]USDA-ARS, NCGR, Corvallis, OR, USA
[(5)]Michigan State University, East Lansing, MI, USA

Red stele is a devastating root rot disease in strawberries. Several sources for genetic resistance are exploited in breeding, and several race-specific R-genes were distinguished. Recently, a tightly linked SSR marker was found for the Rpf1 gene at Wageningen-UR, The Netherlands. One hundred and forty nine individuals with known and unknown response to this pathogen, Phytophthora fragariae, were tested in bench tests for response to two races of this disease: Canadian race 4 (A-3) isolate ONT-3, and Cdn-5 (A-5) isolates BC-23 and NOV-77 where Rpf1 confers resistance to race 4 and is ineffective against race Cdn-5. Twenty-nine individuals consisting mostly of wild accessions or recent derivatives of crosses with wild relatives were identified as having other, potentially new factors of resistance by exhibiting resistance to race A-5 and may be valuable for widening the genetic base of resistance in commercial cultivars. To avoid epistatic effects, these individuals were e xcluded from validation of the Rpf1 marker. For the120 individuals that showed high disease scores for Cnd-5, 41 showed and 79 lacked the marker allele. Preliminary analysis for correlations between marker and disease scores revealed 30 deviations, 17 individuals being susceptible despite having the marker and 13 individuals having low disease scores despite lacking the marker. Causes for the lower (75%) than previously observed (99%) marker to disease correlations are being investigated.

Towards high throughput phenotyping of the multisensory space of apple quality

Biasioli F.[(1)], Aprea E.[(1)], Betta E.[(1)], Cappellin L.[(1)], Corollaro M.L.[(1)], Costa F.[(1)], Dematte L.[(1)], Endrizzi I.[(1)], Romano A.[(1)], Ting V.[(1)], F. Gasperi[(1)]

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige (TN), Italy

The perceivable quality of apple is one of the most important aspects to be considered in breeding, for the selection as well as the commercialization of new accessions. Sensory science allows the definition and control of the multidimensional space behind the perceived quality of apple, but it is usually expensive and time consuming. For this reason the implementation of sensory attributes as phenotypic descriptors for genomic investigation is, in general, difficult.
In 2010 we started a large sensory/instrumental phenotyping activity on several apple based on innovative technology and multidisciplinary know how to define models that,

on the basis of rapid instrumental characterization, can provide reliable quantification of key sensory traits on large data sets to be used in genome wide association analysis, particularly elucidating the genetic basis of fruit texture and flavor related traits. Here we describe the general frame of our project and the results obtained as follows: i) the set up of a comprehensive sensory methodology to provide reliable, unified and competitive sensory characterization, ii) the setting and validation of models to estimate sensory attributes based on instrumental data, iii) the study of the complexity of the interaction between product and consumer during apple fruits consumption by nose space analysis and psycho-physical experiments and iv) the investigation of the link with genomic information.

Use of genotyping-by-sequencing for quantitative trait loci mapping of chilling requirement and bloom date in peach

Rauh B.(1), Fan S.(2), Abbott A.G.(3), Gasic K.(1), Okie W.R.(4), D.G. Bielenberg(1)

(1)School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, South Carolina 29634 USA
(2)JC Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, South Carolina 29646 USA
(3)INRA-Bordeaux, France
(4)USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, Georgia 31008 USA

Bloom date (BD) and chilling requirement (CR) are economically important traits for breeding fruit and nut tree species adapted to local climate conditions. We have developed a peach F2 mapping population with 57 genotypes from a selfed F1 progeny of a cross between 'Hakuho' (high CR) and 'UFGold' (low CR). We scored BD in seven years; CR was evaluated over two winter seasons by scoring bud break of forced cuttings. Initial marker screening identified 37 polymorphic simple sequence repeat (SSR) markers distributed across all linkage groups (G). In order to saturate our genetic map with markers, we tested a genotyping-by-sequencing (GBS) approach to discover single nucleotide polymorphisms (SNP) present in the population. Genomic DNA was ApeKI restricted, ligated to barcoded adaptors, and pooled and amplified for multiplex sequencing on the Illumina HiSeq 2000 platform. Approximately 160 million sequence reads were processed using TASSEL 3.0. SNPs were filtered to retain only loci homozygous within each parent, but polymorphic between parents. This conservative genetic linkage map consisting of 37 SSRs and >380 SNPs in eight linkage groups was constructed using JoinMap v4.1. Several quantitative trait loci (QTL) were detected for BD and CR on G4 and G7. GBS provided a rapid and relatively low cost method to produce a SNP-based map in a novel progeny.

Proteomic of resistance of B-9 apple genotype to *Erwinia amylovora* (fire blight)

E. Borejsza-Wysocka[(1)], Aldwinckle,Herb[(1)]

[(1)]Cornell University,Geneva,NY,USA

Fire blight (Erwinia amylovora) infection of the rootstock of apple trees is an increasing threat to productivity and longevity in high-density orchards of susceptible high-quality cultivars. Resistant rootstocks are the only reliable option to prevent development of rootstock blight. The Budagovsky 9 (B9) dwarfing rootstock displays a substantial increase in resistance to E. amylovora) as it ages – young shoot growth is susceptible, 2-yr-old shoots are resistant. A proteomic approach was used to study this unusual resistance phenotype. Using the iTRAQ technology protein profiles of young and older B.9 tissue were identified. By blasting an apple database 2674 proteins showing differential expression were identified. Proteins were characterized by up-regulation or down-regulation in samples of first-year and second-year tissue at intervals after E. amylovora inoculation: 0h, 96 proteins; 12h, 94 proteins; 24h, 81 proteins.

Genetic determinism and candidate genes for chilling requirement and flowering date in sweet cherry (*Prunus avium*)

Castède, S. [(1)], Campoy, J.A[(1)], Lafargue, M. [(1)], Quero-Garcia, J. [(1)], Le Dantec, L. [(1)], Wenden B. [(1)], Dirlewanger, E. [(1)]

[(1)] INRA-UR419, Centre de Bordeaux, Villenave d'Ornon, France

Phenology and especially reproductive timing is crucial for fruit tree species as yield and fruit quality are directly linked to an adequate flower development. In sweet cherry, flowering process is induced by specific chill and heat requirements. As temperatures become milder, this process is disrupted.
Two intraspecific F1 mapping progenies, Regina × Lapins (R×L) and Regina × Garnet (R×G), were genotyped using the 6K cherry chip SNP developed in the RosBREED project. For the detection of quantitative trait locus (QTL) controlling chilling requirements, the R×G progeny was evaluated during 3 years. For QTL controlling flowering date the two progenies were evaluated during 5 years. QTL analyses were performed separately for each year and combined for all years together.
For chilling requirements, QTLs were detected on linkage groups (LG) 1, 2, 4, 5, 6 and 7. Whereas for flowering date, QTLs were detected on all LGs. For both traits, a major QTL was detected on LG4 for each year of evaluation. Candidate genes were identified by combining functional annotation of the peach genome and QTLs' localisation for both traits.
Functional studies using RNAseq and qRT-PCR are in progress to select the most promising candidate genes. Our results provide a basis for the identification of genes involved in chilling requirements and flowering date that could be used to develop cultivar ideotypes adapted to future climatic conditions.

Fire blight-induced phytoalexin biosynthesis in apple

Chizzali C. [1], Liu B. [1], Richter K. [2], Flachowsky H. [2], Peil A. [2], Hanke M.V. [2], Beerhues L. [1]

[1] Technische Universität Braunschweig, Institute of Pharmaceutical Biology, Braunschweig, Germany.
[2] Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany.

Fire blight, caused by Erwinia amylovora, is the most threatening bacterial disease of pome fruit trees worldwide. Although apple and pear (Pyrinae) have enormous economic importance, their disease resistance mechanisms are poorly understood. In response to pathogen attack, Pyrinae species form biphenyls and structurally related dibenzofurans as phytoalexins. Four biphenyls and two dibenzofurans were exclusively found in the transition zone between necrotic and healthy segments of stems of 'Holsteiner Cox'. The key enzyme of phytoalexin formation is biphenyl synthase (BIS), a type III polyketide synthase. BIS of apple is encoded by a gene family consisting of four subfamilies. In response to inoculation with E. amylovora, BIS3 was expressed in stems of 'Holsteiner Cox', with highest transcript levels in the transition zone (4000-fold increase vs. control). The BIS3 protein was immunohistochemically detected in the transition zone at the junctions between neighboring corti cal parenchyma cells. BIS2 was transcribed in leaves which, however, failed to form immunodetectable amounts of BIS protein and phytoalexin compounds. Expression of BIS1 to BIS3 was observed in E. amylovora-treated apple cell cultures. Thus, members of the BIS gene family are differentially expressed in response to fire blight infection, which is regulated at the transcriptional and translational levels. The product of BIS, 3,5-dihydroxybiphenyl, which serves as the biosynthetic precursor of the variety of biphenyls and dibenzofurans, exhibited in vitro highest antibacterial activity against the fire blight pathogen. Thus, engineering phytoalexin metabolism may provide a new tool for fire blight control in fruit trees.

Towards unravelling the genetics of resistance to european canker in apple. Current stage: Phenotyping

Garkava-Gustavsson L. [1], Zborowska A. [1], Ghasemkhani M. [2], Sehic J. [2], Nybom H. [2], Lateur M. [3],van de Weg E. [4]

[1]Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology, Box 101, 230 53 Alnarp, Sweden
[2]Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden
[3 Walloon Agricultural Research Centre, Breeding & Biodiversity Unit, Rue de Liroux, 4 – 5030 Gembloux, Belgium

(4)Plant Breeding, Wageningen University & Research Centre, Box 16, 6700AA, Wageningen, Netherlands

European canker, caused by the fungus Neonectria ditissima (Nectria galligena), is a large problem in the apple production in Sweden and many other countries. Major canker damage occurs almost yearly in nurseries and orchards, and destroys large amounts of trees. No protective measures are available that can prevent occurrence of epidemics. To date, complete resistance to N. ditissima is not known in apple, and therefore attention is directed to cultivar differences in partial resistance.
In order to set up a standardized phenotyping procedure, 16 apple cultivars were screened for resistance to N. ditissima. One-year-old trees were wound-inoculated in the greenhouse with conidia suspension. Colonization rate was assessed by measuring the length of the occurring cankers at regular time intervals throughout a period of four months. In addition, 'natural' infection at high infection pressure in the field was assessed. Cankered wood was put on top of the trees in the fall in 2011, and high moisture was maintained during 5 days by daily showering of the trees. Infection percentage was assessed in June 2012 and calculated as a proportion of infected leaf scars.
Cultivars showed differences in colonization rate and infection percentage that correlated well with experiences of growers as well as published data. For instance, in cultivars known to have a high degree of resistance, i.e. Golden Delicious, Liberty and Santana, lesions progressed much slower compa red with notoriously susceptible cultivars like Cox's Orange Pippin and James Grieve. These results validate the reliability of our phenotyping procedures. These procedures will now be applied on already available and to be generated mapping populations. The aim is to dissect the genetics of resistance of some cultivars that show high levels of partial resistance like Jonathan, Santana and Aroma. This research will be performed within the framework of national and international (FruitBreedomics, NordApp) projects.

Using genetic markers of the self-incompatibility fertilization system in Rosaceae for increasing fruit quality and yield

Goldway M, Stern R, Annat Zisovich A, Raz A, Schnieder D, Niska R, Gerty K, Yaron A, Zlil Baras Z.

The Molecular Genetic lab. in Agriculture, MIGAL-Galilee Technological Center, Kiryat Shmona, Israel.
Dep. of Biotechnology, Tel-Hai College, Upper Galilee, Israel

The Rosaceae present the S-RNase-mediated gametophytic self-incompatibility (GSI) fertilization system which is controlled by a single multi-allelic locus (S-locus). The S-locus contains a haplotype-specific S-RNase gene, which is expressed in the pistil and a few F-Box gene named SFB or SLF (S-haplotype-specific F-Box, S-Locus F-Box), which are expressed in the pollen tube. Thus, fruit trees of the Rosaceae depend on cross-pollination and therefore commercial orchards contain at least two cultivars that flower synchronically. Semi-compatibility between apple pear and plum cultivars in semi-

optimal conditions for pollination and fertilization (such as parts of the Mediterranean basin) leads to a reduction in fruitset and fruit size. Thus, full compatibility is beneficial for ensuring satisfactory yields with big size fruit. Following this notion, in Israel all cultivars are S-genotyped and orchards are planned taking into consideration full compatibility between adjacent ly planted varieties. This approach is applied ubiquitously also in other countries. Nonetheless, despite the significant amount of varieties, still there are occasions in which a suitable fully compatible pollinator is lacking. This is the case with 'Spadona', the main European pear in Israel. Therefore, seedlings of the wild Syrian pear (Pyrus syriaca) and Japanese pear cultivars were S-genotyped and examined for their fertilization abilities for 'Spadona' and some of them turned out to be very efficient.

Gene expansions and conserved clades in R2R3 – MYB subfamily across Rosaceae and *Arabidopsis* genomes: an *in silico* approach to regulation and synthesis of flavonoids compound

M. González[(1)], Salazar E.[(2)],Moya M.[(1)],Gebauer M.[(3)], Carrasco B.[(1)]

[(1)]Facultad de Agronomía e Ingeniería Forestal. Departamento de Fruticultura y Enología; Pontificia Universidad Católica de Chile, Santiago, Chile
[(2)]Unidad de Recursos Genéticos, Instituto de Investigaciones Agropecuarias (INIA), Santiago, Chile
[(3)]Facultad de Agronomía e Ingeniería Forestal. Departamento de Ciencias Vegetales, Pontificia Universidad Católica de Chile, Santiago, Chile

Flavonoids synthesis and anthocyanin accumulation depends on the control exerted by transcription factors of the MYB R2R3 subfamily. In Rosaceae studies have focused primarily on the identification and characterization of MYB 10 and MYB 1 factors, both involved in regulating the synthesis of flavonoids, however studies of the other factors involved are scarce. Using BLAT and BLAST tools in strawberry-genome and phytozome databases we find putative R2R3 MYB subfamily genes for each Rosaceae species with sequenced genome. For apple, peach and strawberry 111, 115 and 116 gene models have been found, respectively. These gene models were analyzed together with 126 R2R3 MYB genes described for Arabidopsis thaliana. Clades associated with the regulation of flavonoid synthesis were identified. To clarify the role of gene models found, the construction of a new phylogenetic tree was performed. This tree incorporated the R2R3 MYB transcription factors previously described for Rosaceae, Arabidopsis and grapevine allowing the association of potential orthologous genes to each gene model. Some of the identified gene models were tested in Japanese plum (Prunus salicina). Some of the candidate genes were isolated and their expression patterns in skin and pulp, at different stages of fruit development, were determined. Events of expansion, loss, or retention of genes in Rosaceae are discussed.
This work was supported by fellowship Conicyt 21090118, Tesis de Postgrado en la project Fondecyt 24110179 and Consorcio Tecnológico de la Fruta SA.

Identification of genomic regions and candidate genes involved in fruit ripening in two apricot (*Prunus armeniaca*) cultivars

Lambert P. [(1)], Ruiz D.[(2)], Gouble B. [(3)], Bureau S. [(3)], Buendia L. [(1)], Reich M. [(3)], M.G.C. Renard C. [(3)], Audergon J.M.A. [(1)]

[(1)] INRA, Unité de Génétique et d'Amélioration des Fruits et Légumes, UR1052, Domaine St Maurice, CS60094, F84143 Montfavet, France
[(2)] Department of Plant Breeding, CEBAS-CSIC, PO. Box 164, Murcia, Spain
[(3)] INRA, Université d'Avignon et des Pays du Vaucluse, UMR408, Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon

In apricot, there are major difficulties in keeping the ripening under control and predominantly at the post-harvest stage. Consequently, the knowledge of mechanisms involved in fruit ripening is particularly important. It is the reason why the identification of genomic regions involved in apricot ripening has been targeted.
An apricot F1 population of 183 off springs has been constituted from a cross between two parents contrasting for their ripening features: 'Goldrich' (large, firm, orange fruit with a slow evolution during ripening, before and after picking), and 'Moniquí' (mean, soft, white fruit with a very rapid evolution and high ethylene production, particularly at the post-harvest stage). The parents and the off springs were characterized for fruit maturity date and ethylene production during two consecutive years. One SSR-based genetic linkage map anchored to the general map for Prunus was established for each of the parents and QTL analyses were performed for these traits. QTL stability was stated between years. A very large variability was observed among the off-springs and QTLs were detected in several linkage groups. One common region for ethylene production and maturity date was detected in both maps. Candidate genes were identified in most of the QTL regions when c ompared to the annotated peach genome sequence. The sequences of the most likely genes were compared for polymorphism between parents as well as their expression and informative SNPs were identified.

Isolation of genes differentially expressed during refrigerated storage involved in internal breakdown of japanese plum (*Prunus salicina* lindl.) Fruits

M. Moya [(1)], González-Arroyo M.[(1)], Defilippi B. [(2)], Salazar E. [(3)], Gebauer M. [(4)], Silva H. [(5)], Carrasco B.[(1)]

[(1)]Facultad de Agronomía e Ingeniería Forestal, Departamento de Fruticultura y Enología, Pontificia Universidad Católica de Chile, Santiago, Chile
[(2)]Laboratorio de Postcosecha, Instituto de Investigaciones Agropecuarias (INIA), Santiago, Chile
[(3)]Unidad de Recursos Genéticos, Instituto de Investigaciones Agropecuarias (INIA), Santiago, Chile
[(4)]Facultad de Agronomía e Ingeniería Forestal. Departamento de Ciencias Vegetales; Pontificia Universidad Católica de Chile, Santiago, Chile

(5)Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática, Universidad de Chile, Santiago, Chile

Chile is the main exporter of Japanese plum fresh fruit in the world. One of the key issues for the industry is the post harvest conditions of the fruits that allow reaching the market with a good quality product. The fruits are highly perishable and suffer physiological disorders associated to the long time storage under low temperature. One of these disorders is the Internal Breakdown (ID). Toward the identification of genes differentially expressed related to ID in plum fruit, a subtractive suppressive hybridization (SSH) methodology was employed. Two libraries were generated from transcripts obtained from different storage treatments. A set of 387 genes were isolated, sequenced and characterized. BLASTN algorithm and public databases (NCBI and TIGR) were used to determine similarity scores between the cDNA clones and known sequences. Functional categorization was manually determined using the FunCat catalogue from the Munich Information Center for Protein Sequences (MIPS) . Approximately 90% of the partial cDNAs showed significant similarity to proteins registered in databases and 10% presented problems with the sequence therefore were not considered for the analysis. Proteins related to protein fate (folding, modification, destination) were identified when a treatment of two days at 20 ºC and storage at 0 ºC for 42 days (fruit library T2) was used. Moreover, genes that codify by proteins related to cellular transport, cell-wall-related proteins and transcription factors were identified after a treatment of two days at 10 ºC and storage at 0 ºC for 42 days (fruit library T4). Four genes were analyzed by qRT-PCR.
This work was supported by fellowship Conicyt 2109018, Tesis de Postgrado en la Industria 78111112, project Fondecyt 11080192 and Consocio Tecnológico de la Fruta SA.

Bioinformatic identification of a putative microrna-transcription factor network motif in the regulation of laccase genes in peach (*Prunus persica*)

A.M. Cattaneo(1), Grzeskowiak L.S.(1), Colaiacovo M.(2), Faccioli P.(2)

(1)Research and Innovation Centre - Fondazione Edmund Mach, San Michele all'Adige (TN), Italy
(2)CRA - Genomics Research Centre, Fiorenzuola d'Arda (PC), Italy

Laccase proteins are multicopper glycoprotein oxidases expressed in plant tissues under biotic and abiotic stress conditions. They are able to catalyze oxidation of a broad range of substrates including phenols and amines. The regulation of expression of such genes is crucial for proper reaction to stress. At the DNA level, this modulation is mediated by the recruitment of specific transcription factors (TF) to suitable transcription factor binding sites (TFBS), usually located upstream of a gene. At the RNA level, the short microRNAs molecules (miRs) interfere with the translation of target proteins through base-pairing with messenger RNAs. Complex regulatory circuits combining those interactions fine-tune protein expression and enhance plant responses to environmental change.

In this case study we performed a phylogenetic analysis of peach laccases and characterized specific peach miRs (miR397a and miR408), reported previously as post-transcriptional regulatory elemen ts of laccase genes. Using a bioinformatic approach we identified unique TFBS for abscisic acid (ABA) response elements in promoter regions of both miR and laccase genes. The signaling molecule ABA plays a major role in plant responses to stress. We propose a feed-forward loop motif in the stress response network involving ABA action in peach by integrating the TF-mediated regulation of miR and laccase genes at the transcriptional level with the miR regulation of laccase target genes at the post-transcriptional level.

Genetic dissection of fruit aroma in apple

F. Costa[1], Dunemann F.[2], Cappellin L.[1], Longhi S.[1], Ulrich D. [2], Soukoulis C.[1], Velasco R.[1], Gasperi F.[1], Biasioli F.[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (Trento), Italy
[2]Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Quedlinburg, Germany

The quality of a fruit is represented by a specific set of primary and secondary metabolites which make the fruit edible and desirable. The aromatic "bouqet", in particular, contributes to the final appreciation by consumers, being the last factor non-destructively perceived after appearance. The aroma in apple is controlled by a series of important physiological pathways leading to the formation of key compounds, such as terpenes, alcohols, aldehydes and esters, the latter one the most abundant in the apple aroma.
In order to genetically dissect such complex control, two full sib progenies and two analytical technologies have been employed in this investigation. As phenomics technology for aroma profiling a novel PTR-ToF-MS was employed, in order to fingerprint the VOCs production of the Fuji x Delearly progeny after a postharvest storage. The QTL profile was further compared with the one already available for the C3 population (Discovery x Prima), for which a dataset of volatiles assessed by HS-SPME-GC equipment was already available. The alignment of the two maps allowed the detection of a common set of QTLs, highlighting those positioned on LG2 and co-located with an AAT gene cluster, known to be involved in acetate ester production in apple. Association mapping based on the AAT candidate genes allowed the characterization of a set of markers specifically associated with the ester accumulation in ripe fruit. The results presented here discuss about the utility of these new markers as a valid tool for a molecular breeding towards the creation of new high-quality aromatic apple varieties.

High or low fructose? Consequences for sugar metabolism in peach fruit

Desnoues, E. [1], Gibon, Y. [2], Baldazzi, V. [3], Signoret, V. [1], Génard, M. [3], Quilot-Turion, B.[1]

[1] INRA, UR1052 Génétique et Amélioration des Fruits et Légumes F-84143 Montfavet, France
[2] INRA, UMR1332 Fruit Biology and Pathology F–33883 Villenave d'Ornon, France
[3] INRA, UR1115 Plantes et Systèmes de culture Horticoles F-84914 Avignon, France

Fruit taste is largely affected by the content of sugars and acids. Fructose is the sweetest tasting sugar. In commercial peach fruit, sucrose is the main sugar, followed by fructose and glucose which have similar levels. Interestingly low fructose accessions have been described in wild peaches. Two hypotheses can explain this fructose deficiency: reduced synthesis or increased degradation. Through an extensive profiling of metabolites and enzymatic activities, this study aims at i) describing sugar metabolism in peach fruit at different developmental stages and ii) comparing two genotypes with contrasted fructose/glucose ratios. We have measured 12 metabolites and 12 enzyme activities during fruit growth, for two genotypes over two years. Genotypic and year effects were observed for some metabolites, whereas the enzyme activities were stable between genotypes and years. More specifically, we did not detect any difference in the activities of the enzymes responsible for s ynthesis or degradation of fructose. Finally, our results show a highly regulated system in which a major perturbation in a central compound has only slight repercussions on sugar metabolism. Further explanations for the low fructose phenotype are discussed, such as different substrate affinities between iso-enzymes, limited fructose storage resulting in higher degradation or a differential consumption of the two hexoses for respiration, cell wall or synthesis of other carbon compounds.

Candidate gene functional profiling during fruit development and ripening in apple

S. Longhi[1], Tadiello A.[1], Scholtz M.[1], Wehrens R.[1], Franceschi P.[1], Biasioli F.[1], Velasco R.[1], Tononi P.[2], Ferrarini A.[2], Delledonne M.[2], Costa F.[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (Trento), Italy
[2]Department of Biotechnology, University of Verona, Italy

The physiological process of fruit development and ripening is the result of a functional gene interplay governing the formation of the features characterizing the fruit quality properties. Among the several physiological processes, the modifications occurring in the cell wall are the most relevant and important, for their impact in the maintenance of the general fruit quality as well as the management of the postharvest storage. Beside

the specific gene activation over the fruit cycle, also the particular genetic constitution can play important controlling roles.
To investigate the functional machinery involved in the control of fruit texture and ethylene production during fruit ripening, two different cultivars, such as Golden Delicious and Granny Smith, were selected and a specific set of samples were collected, processed and hybridized over an ad hoc designed custom microarray platform. The specific regulation of approximately 3800 genes involved in fruit ripening and regulatory process, together with their relative anchoring on a set of QTL intervals, will allow the simultaneous analysis of the genes differentially regulated among the different samples and their putative genetic control on these fruit quality traits. This study can thus represent a step forwards into the comprehension of the several mechanisms underlying the apple fruit quality, offering new opportunity for supporting the constitution of the most favourable apple ideotypes.

Identification of a cell number regulator (CNR) gene family in *Prunus*

P. De Franceschi [1], Cabrera A.[2], van der Knaap E.[2], Iezzoni A.[3]

[1]University of Bologna, Bologna, Italy
[2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, USA
[3]Michigan State University, East Lansing, Michigan, USA

In natural and breeding cross populations fruit size varies continuously, behaving as a typical quantitative trait with multiple loci incrementally contributing to and largely influenced by environmental conditions. However, in some cases, a high proportion of the phenotypic variation can be explained by a few quantitative trait loci (QTL), corresponding to genomic regions that likely contain one or more major genes with a significant effect on fruit size. A gene known to regulate fruit size, tomato fw2.2, and two of its maize homologs, named CNR (Cell Number Regulators), have been shown to exert their effect on organs size by modulating cell number. In the present study, the CNR gene family in the recently released peach (Prunus persica) genome was characterized. A total of 23 CNR gene sequences, spanning the eight Prunus chromosomes were identified and their sequences were compared to the 13 CNR gene family members identified in maize. Moreover, two of the Prunus CNRs l ocate in close proximity of two known cherry (P. avium) fruit size QTLs, one of which has been shown to be associated with differences in mesocarp cell number. Even though the functional characterization of CNR genes in Prunus is still at the beginning, they provide a set of candidate genes of great interest for understanding the genetic bases underlying important traits related to plant and organ (e.g. fruit) size.

QTLs for brown spot resistance in European pear

P. De Franceschi[1], Cappai F.[1], Ciriani A.[2]., Collina M.[2]., Brunelli A.[2]., Dondini L.[1]

[1]Dept of Fruit Tree and Woody Plant Sciences, University of Bologna Bologna, Italy

(2)Dept of Agri-food Protection and Improvement, University of Bologna

Brown spot is the most serious fungal disease of pear in the Po valley and many of the most grown pear varieties in Emilia-Romagna (i.e Abbé Fétel) are susceptible. It is caused by Stemphylium vesicarium and it requires numerous fungicide applications throughout the period between flowering and harvesting times. A sustainable alternative to this traditional approach may be offered by the molecular breeding once the pathogen resistance genes already present in the pear germplasm will be identified.
Young leaves and fruits from seedlings derived from the cross Abbé Fétel x Max Red Bartlett (susceptible and resistant to the pathogen respectively) have been evaluated for resistance to S. vesicarium for two consecutive years; the results have been analysed for QTL identification in an upgraded version of the linkage maps available on this progeny. Analyses evidenced the presence of a significant QTL on linkage group 2 of the resistant parent Max Red Bartlett and another Q TL for susceptibility in linkage group 15 of Abbé Fétel. Markers located in the QTL regions represent a first step to set up a MAS approach aimed to select new genotypes resistant to brown spot.

Quality control improves resource savings in routine marker-assisted seedling selection for the Washington apple breeding program

D. Edge-Garza(1), Rowland T.(1), Konishi B.(2), Brutcher L.(2), Evans K.(2), Peace C.(1)

(1)Washington State University, Pullman, WA, USA
(2)Washington State University, Wenatchee, WA USA

Marker-assisted seedling selection (MASS) is in routine use for apple breeding at Washington State University for the last four years. Our experiences have revealed challenges and prompted solutions to prevent critical failures during the high-throughput DNA testing phase of MASS. Unsuccessful DNA extraction or genotyping of whole batches of samples are counter-productive to the resources savings that MASS otherwise provides a breeding program. Dedicated attention to quality control is essential for our breeders to use the resulting data to make confident breeding decisions. We try to mitigate those limitations outside our control. Here we describe some challenges experienced at different steps in routine MASS application, how they influence breeding resource savings, and the control measures we take to ensure quality results.

Integration of molecular markers into a peach breeding program: examples of marker-assisted selection for fruit shape and fruit acidity

I. Eduardo(1), Batlle I.(2), Arús P.(1)

(1)IRTA. Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB. Campus Universitat Autònoma de Barcelona. 08193 Bellaterra, Spain
(1)IRTA Mas de Bover. Ctra. Reus-El Morell km 3,8. 43120 Constantí, Spain

Marker-assisted selection (MAS) is one of the main applications of genomic research to breeding. We describe the initial steps taken by the IRTA-ASF peach breeding program towards its routine selection for fruit shape (round, flat and aborting) and fruit acidity (acid vs. subacid).
Integration of MAS into a breeding program requires: a) the identification of alleles tightly linked to target traits, b) the integration of the genotyping step to the work pipeline of the breeding program and c) the adaptation of high-throughput protocols for DNA extraction and genotyping.
We decided to start MAS with fruit shape and fruit acidity, two simply inherited traits already mapped in the Prunus reference map and that are key breeding targets. Linked alleles to these genes were found based on single-family analysis and later validated using wide germplasm collections. In very few cultivars the identified molecular markers were not useful. Once the appropriated alleles were identified, molecular markers were genotyped during two years in different progenies of the breeding program. We started with plants that were already in the field producing fruits to estimate selection efficiency. After genotyping and phenotyping more than 600 individuals for each trait, the prediction was more than 95% accurate. The second year MAS was applied at the seedling stage with 192 individuals from different crosses. All plants were transferred to the field ordered by phenotype predictions. Also in this plant case prediction was correct more than 95% of the times. We are currently starting to use MAS for discarding unwanted genotypes without any further testing.

Functional characterization of fire blight resistance in malus fusca - fine mapping, cloning and characterization of Fire Blight resistance genes from *Malus fusca*

O. Emeriewen[1,2,3], Zini E.[1], Kilian A.[4], Malnoy M.[1], Peil A.[2]

[1]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach, 1 – 38010 San Michele all'Adige (Trento), Italy
[2]Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural Fruit Crops, Pillnitzer, Platz 3a, 01326 Pillnitz, Dresden, Germany
[3]Food and Agricultural Science, Technology and Biotechnology School, University of Modena and Reggio Emilia, Via Universita, 4 41121, Modena, Italy
[4]Triticarte P/L, PO Box 7141, Yarralumla, Canberra, ACT 2600, Australia

Fire blight (FB), caused by the gram-negative bacterium, Erwinia amylovora (Ea), is the most important bacterial disease on pome fruit worldwide including apple. Although the use of antibiotics as a control measure has been successful to some degree, their application is unwanted, forbidden or strictly regulated in many countries due to the threats they pose to the environment. Hence, there is the need for an alternative means of control. Recently, research has shown that genetic resistance can play a vital role in the management of fire blight disease. In different wild species of Malus, several potential sources to FB resistance have been identified and as a result, some wild apple accessions have been utilised as sources of resistance. Consequently, different quantitative trait loci (QTLs) for FB resistance have been identified by Peil et al. 2007;

Durel et al. 2009, and Parravicini et al. 2011 and also by Calenge et al. 2005; Khan et al. 2006; in wild apple accessions and cultivated apple (Malus x domestica) respectively. These QTLs exhibit different levels of resistance to the disease. With the aim of identifying, isolating and characterizing resistance to fire blight from the wild species M. fusca, we report here the first results of QTL analysis and marker development for fine mapping. F1 progenies of Malus fusca x Idared were grafted and inoculated in the greenhouse with E. amylovora strain Ea222 in two consecutive years. DArT, SSR and SNP markers were developed and used to establish a genetic map and to perform QTL analysis. A major QTL could be localised on LG10. Two DArT markers were closely linked to the QTL. Four SSR markers linked to resistance were developed using the reference sequence of Golden Delicious for fine mapping the QTL. This research is in progress and additionally a bacterial artificial chromosome (BAC) library of M. fusca will be screened with SSRs flanking the QTL.

Analysis of flavonoid regulation in Rosaceae

R.V. Espley[(1)], Ravaglia, D.[(2)], Henry-Kirk, R.[(1)], Lin-Wang, K.[(1)], Palmer, J.[(1)], Costa, G.[(2)], Allan, A.C.[(1)]

[(1)]Plant and Food Research, Auckland, New Zealand
[(2)]Department of Fruit Tree and Woody Plant Sciences, University of Bologna, Bologna, Italy

The flavonoid pathway provides phenolic compounds that play major roles in Rosaceous fruit colour. These compounds also determine many of the dietary health-related properties in both the peel and cortex. Regulation is primarily conducted by a complex of transcription factors (TFs), central to which are the MYB TFs. Using examples from peach and apple, we provide functional analysis for the control of flavonols, proanthocyanidins (PAs) and anthocyanins by the MYB family. In peach, the pattern of UFGT gene expression correlates with TFs which up-regulate anthocyanin biosynthesis (MYB10 and bHLH3), or repress (MYB111 and MYB16) transcription of biosynthetic genes. The expression of a potential PA-regulating transcription factor, MYBPA1, corresponds with PA levels. Functional assays show that MYB10 positively regulates the promoters of UFGT and DFR but not LAR. In contrast, MYBPA1 trans-activates the promoters of DFR and LAR, but not UFGT, suggesting exclusive roles of antho cyanin and PA regulation. In apple the flavonoid biosynthetic pathway is most active in the skin, with the flavan-3-ols, catechin and epicatechin, acting as the initiating units for the synthesis of PA polymers. We have examined the genes involved in PA production in different apple cultivars and show that flavan-3-ol biosynthesis is under the control of biosynthetic enzymes ANR and LAR1. These steps are under the control of developmental and environmental stimuli, such as temperature and light. Heating fruit rapidly reduces expression of the transcriptional activation complex responsible for red skin colour, while a single night of low temperatures is sufficient to elicit a large increase in transcription of MYB10 and consequently the biosynthetic pathway.

Analysis of a TFL1 homologous gene and its relationship to the inflorescence variation in Japanese flowering cherries

T. Esumi[(1)], Ohtani, H.[(1)], Yoshibayashi, R.[(1)], Kobayashi, N.[(1)], Asao, T.[(1)], Itamura, H.[(1)]

[(1)]Department of Agriculture, Faculty of Life and Environmental Science, Shimane

University. 1060 Nishi-kawatsu, Matsue, 690-8504, Japan

Japanese flowering cherries called "Sakura" is a symbolic flower in Japan. Sakura consists of several different species, such as Prunus 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., in a genus. A number of their hybrids and mutants have so far been developed for ornamental use in Japan. In Prunus genus, the structures of inflorescence are varied widely. The inflorescences of Sakura species show morphologically gradual variation from corymb to umbelliform. TFL1 (TERMINAL FLOWER 1) gene, a flowering related gene originally found from inflorescence structural mutants in Arabidopsis and Antirrhinum, is an interesting target gene for the inflorescence development. TFL1 homologous gene has been characterized in peach (Prunus persica L.) and apricot (Prunus mume Sieb. et Zucc.). The TFL1 homologous gene was suggested to be involved in the development of juvenile and young vegetative organs in apricot. However, its involvement in the inflorescence development is hard to study in peach or apricot because they produce pure flower buds with single flower. In contrast, Sakura species produce flower buds consisting of 2-5 flowers with an inflorescence and are suitable candidates to elucidate the relationship between TFL1 homologous gene and the morphological development of inflorescence. In this study, first, we measured the size and shape of each inflorescence of 132 Sakura cultivars and determined the morphological variation, and secondly, we analyzed the promoter region of a TFL1 homologous gene in Sakura species. We discussed the correlations among several parameters acquired from the measurement and the promoter analysis. The next step of this study will need to use genome-wide comparison techniques to develop various genetic markers for the breeding. We pro mote genome-based approach for the study of Sakura genetic resource in Japan.

The logistical challenges of marker-assisted seedling selection in an apple breeding program

K. Evans[(1)], Konishi B.[(1)], Brutcher L.[(1)], Edge-Garza D.[(2)], Rowland T.[(2)], Peace, C. [(2)]

[(1)]Washington State University, Wenatchee, WA, USA
[(2)]Washington State University, Pullman, WA, USA

Considerable advances have been made in developing predictive DNA tests for use in apple breeding programs over recent years. Now that we are applying these tools routinely in seedling selection, we have identified the need for improved logistics of interfacing with various on-the-ground breeding program operations in order to improve efficiency and accuracy. Outcrosses and seedling mix-ups are generally a minor problem for breeders who are relying strictly on phenotypic selection, some of these even yielding possible new releases. 'Stray' seedlings need to be handled differently when breeders apply DNA-based screening; DNA tests may be uninformative for these individuals and the breeder needs to decide whether or not to cull. The breeder also needs to be fully confident that data can be connected to seedlings with no room for

error; once a seedling is identified for culling, the result is terminal and any error in matching data points to seedlings cannot be corrected. The team at Washington State University has identified several possible sources of error in the application of DNA-based tools to our apple breeding program; here we present some of the logistical solutions developed to overcome these issues.

Abscisic acid plays a role in the regulation of sugar transport and accumulation in apple fruit

R. Falchi[(1)], Zanon L.[(1)], Noferini M.[(2)], Costa G.[(2)], Vizzotto G.[(1)]

[(1)]University of Udine, Dipartimento di Scienze Agrarie e Ambientali, Udine, Italy
[(2)]University of Bologna, Dipartimento di Colture Arboree, Bologna, Italy

Apple, similarly to other Rosaceae tree fruits, synthesizes sucrose and sorbitol in source leaves, which are translocated and utilized in fruit. Metabolites transport represents a critical step in partitioning of photosynthetic products in sink organs and is a major determinant of yield and quality. Previous studies provided clear evidence for an apoplasmic phloem unloading pathway in apple fruit, involving sugar carriers.
Basing on the observation that abscisic acid (ABA) is a potential regulator of fruit development and that during ripening apple fruit produce high levels of ABA, we performed several trials in order to study its effect on sugar transport and accumulation in fruit. To this purpose, trees of cv. Gala (Brookfield) were treated with ABA at different fruit ripening stages (expressed as IAD stated by DA-Meter device), and molecular analysis on the expression of genes encoding sorbitol and sucrose transporters (SOT and SUT) were carried out on fruits. Results pointed out that the time of application plays a crucial role and, in detail, the early application of ABA produced the major effect at molecular level. Moreover, treatments differently affected the expression of considered genes, showing up-regulation, both in the short- and in the long-term. In addition, when compared to unsprayed fruits (control), treated samples reached higher size, lower flesh firmness and a more advanced ripening stage and these effects were concentration related.
The ABA-associated regulatory processes in carbohydrate metabolism are discussed, but it is reasonable to suppose that the hormone could promote sugar accumulation in apple fruit also through the transcriptional regulation of carriers involved in this event.

Three sucrose transporters are differentially expressed in mesocarp of developing peach fruit

Zanon L.[(1)], *R. Falchi*[(1)], Santi S.[(1)], Vizzotto G.[(1)]

[(1)]University of Udine, Dipartimento di Scienze Agrarie e Ambientali, Udine, Italy

The partitioning of sugars in sink organs such as fruits is regulated by several complex physiological processes, including phloem unloading and post-phloem transport and

metabolism of imported sugars in sink cells. Phloem unloading has been studied in various fleshy fruits, but the relationships between growth and sugar metabolism have attracted several studies both in edible and in non-edible organs because, besides their relevance in plant growth and fruit quality, sugars act as essential signaling molecules.
Aim of this study is to elucidate the roles that the three sucrose transporters (named PpSUT1, PpSUT2, PpSUT4 in accordance with Arabidopsis classification), identified in peach genome, play in sucrose partitioning in fruits. Experiments carried out by a phloem mobile symplastic tracer (CFDA) showed the absence of connection between vascular bundles and parenchyma cells in peach mesocarp, in the early and middle phases of fruit development, highlighting the requirement of an active transport driven by specific carriers present in the plasma membrane of phloematic system and parenchyma cells. Consistently with these results, data obtained by real-time PCR allowed to outline the expression pattern of different transporter isoforms in flesh tissues. In detail, transcripts related to PpSUT4 gene exhibited the highest levels in all mesocarp samples collected throughout development (from 57 to 110 days after full bloom, DAFB). In contrast, PpSUT2 and PpSUT1 expression appeared bare ly detected and almost absent, respectively, in samples assayed.
Afterwards, more exhaustive information was obtained taking advantage of Laser Capture Microdissection (LCM) technique, allowing single cell types isolation and then gene expression analysis in selected tissues. This effective approach led to discover that both in the early (57 DAFB) and in the middle-late (92 DAFB) phases of fruit development PpSUT4 was expressed mainly in parenchyma cells, whereas PpSUT2, characterized by low transcript levels when analysis were carried out on the whole fruit, showed high expression levels, limited to vascular bundles. Taken together data provide evidence for a role of PpSUT2 in active phloem unloading and sucrose accumulation in peach fruit.

Identification and characterization of early pathway genes of ellagitannin biosynthesis in strawberry (*Fragaria vesca*)

A. Feller[(1)], Martens S.[(1)]

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

Ellagitannins are polyphenolic antioxidants found in certain fruits, trees, tea and medicinal plants. In many fruits, such as strawberries, raspberries, blackberries or pomegranate, ellagitannins, besides anthocyanins, are the most abundant antioxidants. The high amount of antioxidants present in these fruits have been associated with a reduced risk of cardiovascular disease, Diabetes mellitus (type 2) or cancer and these properties, together with the pleasant taste, have made berries one of the favorite fruits on the fresh food market but also as source for nutraceuticals or functional foods.
Due to the previously described bioactivities associated with ellagitannins, studies at the molecular level and identification of genes involved in this biosynthetic pathway are mandatory for further engineering strategies in planta to modulate the amount of metabolites (transgenic strawberry; breeding programmes). We show here the

identification and characterization of four putati ve shikimate dehydrogenase (SDH) encoding genes from strawberry. SDH has recently been shown to convert 3-dehydroshikimate (3-DHS) to gallic acid (GA), the first intermediate in the ellagitannin biosynthetic pathway. Until this finding, SDH was mainly known to catalyze the reversible reduction of 3-DHS to shikimic acid (SA), an essential intermediate for the production of aromatic amino acids. In higher plants the shikimate pathway is present in plastids but has been proposed to exist as a second pathway in the cytoplasm. Both tobacco (N. tabacum) and tomato (L. esculentum) have two SDH encoding genes, one localizes to the chloroplast, where it participates in the production of aromatic amino acid for protein synthesis, and one is localized in the cytoplasm, possibly involved in synthesis of natural products. However, the function of this cytoplasmic SDH is still not completely clear. We propose here that the cytoplasmic SDH is involved in GA formation in strawberry (F. vesc a) and that it catalyzes the first step in the ellagitannin biosynthetic pathway.

Mapping quantitative trait loci for nut quality in almond

A. Fernández i Martí[(1)], Font i Forcada C.[(2)], Socias i Company R.[(3)]

[(1)]Plant breeding & molecular biology Laboratory. Parque Cientifico Aula dei (PCTAD) Zaragoza Spain
[(2)]Unidad de Pomología, EEAD-CSIC, Zaragoza, Spain
[(3)]Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain

Almond breeding is increasingly taking into account kernel quality as a breeding objective. Information on the parameters to be considered in evaluating almond quality, such as protein and oil content, as well as oleic acid and tocopherol concentration, has been recently compiled. The genetic control of these traits has not yet been studied in almond, although this information would improve the efficiency of the almond breeding programs. A map with 56 simple sequence repeat or microsatellite (SSR) markers was constructed for an almond population showing a wide range of variability for the chemical components of the almond kernel. A total of 12 putative quantitative trait loci (QTL) controlling these chemical traits have been detected in this analysis, corresponding to seven genomic regions of the eight almond linkage groups (LG). Some QTLs were clustered in the same region or shared the same molecular markers, according to the correlations already found between the chemic al traits. The logarithm of the odds (LOD) values for any given trait ranged from 2.12 to 4.87, explaining from 11.0 to 33.1% of the phenotypic variance of the trait. The results produced in the study offer the opportunity to include the new genetic information in the almond breeding programs. Increases in the positive traits of kernel quality may be looked for simultaneously whenever they are genetically independent, even if they are negatively correlated. We have provided the first genetic framework for the chemical components of the almond kernel, with twelve QTLs in agreement with the large number of genes controlling their metabolism.

Sensory profiling of apple cultivars

Corollaro M.L.(1), Aprea E.(1), Endrizzi I.(1), Demattè M.L.(1), Costa F.(1), Biasioli F.(1), F. Gasperi(1)

(1)Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, (TN), Italy

Sensory quality of apples, recognised as a key factor driving consumer choice, is frequently indirectly measured using basic instrumental or pomological descriptors. Several studies so far investigated the correlations between instrumental measurements and sensory properties, these latter not always analysed by trained panel according to proper sensory science principles.
Here, we present the setting up and the application of a trained panel sensory evaluation tool for the characterization of apple. Fruit physical and chemical parameters related to sensory descriptors of flavour and texture (basic composition, volatile metabolite profiling and texture profiling) were instrumentally measured as well.
The proposed methodology, validated on a wide selection of apple cultivars (more than 20 commercial varieties and 11 new FEM accessions) over 2 years of production, allows to discriminate among different cultivars and highlights the perceivable changes developed during postharvest. Multivariate regression models show that it is possible to predict by instrumental measurements most of the textural sensory properties together with some flavour attributes.
The opportunity to monitor several important sensory attributes makes the proposed sensory/instrumental approach a valuable tool for cultivar evaluation in breeding programs to assist the genetic improvement of new apple accession characterised by a better fruit quality, oriented towards the consumer preferences.

Agrimoniin the most important ellagitannin in human diet: elucidation of its identity in strawberry fruits and the influence of fruit ripening on its concentration

M. Gasperotti(1), Guella G.(2), Palmieri L.(1), Martinatti P.(1), Pojer E.(1,3), Mattivi F.(1), Vrhovsek U.(1)

(1)Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, (TN), Italy
(2)University of Trento, Department of Physics, via Sommarive 14, 38100 Trento, Italy
(3)University of Padova, Department of Pharmaceutical Sciences, Via Francesco Marzolo 5, Padua, Italy

Of the most commonly consumed berries, strawberries (Fragaria ananassa Duch.) are the most popular choice with consumers, being eaten both fresh and frozen, as well as in different processed products. Although the composition of strawberry fruit has been extensively studied, especially for the most abundant phenolic compounds, agrimoniin

has been only recently univocally identified as one of the most abundant phenolic compounds in the fruit (Vrhovsek et al. 2012). In this study agrimoniin was isolated in the fruit of Fragaria vesca and its structure fully characterized, reporting for the first time the full NMR assignments for this dimeric ellagitannin. Agrimoniin is a known bioactive compound, which has been used for treatment of diarrhea and haemorrhaging and reported to have antitumor properties. Its presence as the main ellagitannin in both F. vesca and Fragaria ananassa D. fruit is therefore noticeable.
The establishment of a new HPLC protocol for the separation of the strawberry ellagitannins, and the isolation and characterisation of other ellagic acid derivatives, allowed us to produce an accurate quantification of the main ellagitannins and ellagic acid conjugates in 6 different varieties of strawberry and in 2 woodland strawberry at four different ripening stages from the green stage up to overripe fruit.
Of fruit containing ellagitannins, strawberries are the most widely consumed, and agrimoniin is suggested to be one of the most widely present ellagitannins in the human diet. Agrimoniin, together with the other strawberry ellagitannins and ellagic acid derivatives characterised in this study, deserve further attention since they are expected to play an important, yet still largely unexplored, role in the beneficial health effects associated with the consumption of strawberries by humans.
Literature:
Vrhovsek u., Guella G., Gasperotti M., Pojer E., Zancato M., Mattivi F. Clarifying the identity of the main ellagitannin in the fruit of the strawberry, Fragaria vesca and Fragaria ananassa Duch. Journal of agricultural and food chemistry volume, 60, 10, 2507-2516.

The 'Knotted'-like '*Knope1*' gene regulates stem elongation and lignification during primary growth of peach stem

Testone G. (1), Nicolodi C. (1), Mele G. (1), Giannino D. (1), Condello E. (2), Verde I. (2), Caboni E. (2), Dettori M.T. (2), Vendramin E. (2), Bruno L. (3), Bitonti M.B. (3)

(1) Institute of Agricultural Biology and Biotechnology, National Research Council of Italy (CNR), Rome, Italy.
(2) Fruit Tree Research Centre, Agriculture Research Council (CRA), Rome, Italy.
(3)Department of Ecology, University of Calabria, Cosenza, Italy

The 'KNOTTED'-like genes ('KNOX') encode homeodomain transcription factors and regulate several processes of plant organ development. The peach ('Prunus persica' L. Batsch) 'KNOPE1' was assessed to link to a QTL for the internode length in the Peach x Ferganensis population. The 'KNOPE1' expression decreased progressively from stem primary (elongation) to secondary growth (radial expansion) of adult shoots. During primary growth, the 'KNOPE1' mRNA was localised in the cortex and in the procambium/metaphloem zones, whereas it was undetected in incipient phloem and xylem fibres. 'KNOPE1' over-expression in the arabidopsis 'bp4' loss-of-function background ('35S:KNOPE1/bp' genotype) restored the rachis length, suggesting, together with the QTL association, a role for 'KNOPE1' in peach shoot elongation. Several lignin biosynthesis genes were up-regulated in the 'bp4' internodes but

repressed in the '35S:KNOPE1/bp' lines similarly to the wild type. Moreover, the lignin deposit ion pattern of '35S:KNOPE1/bp' and the wild type internodes were the same. The KNOPE1 protein was found to recognize in vitro one of the typical KNOX-DNA binding sites that recurred in peach and arabidopsis lignin genes. 'KNOPE1' expression was inversely correlated with that of lignin genes and lignin deposition along the peach shoot stems and was down-regulated in lignifying vascular tissues. These data strongly support that 'KNOPE1' prevents cell lignification by repressing lignin genes during peach stem primary growth.

Development of a premium variety of apple seedlings with greater sweetness

M. Handford[(1)], Díaz, F.[(1)], Aguayo, F.[(1)], Mandujano, P.[(1)], Zamudio, S.[(1)], Araya, J.[(1)], Arcos, Y.P. [(2)], Arce, P.P.[(2)], Norambuena, L[(1)], Stange, C[(1)]

[(1)]Universidad de Chile, Santiago, Chile
[(2)]Universidad Católica, Santiago, Chile

Sorbitol is the major phloem-translocated carbohydrate in Rosaceae species. In fruits, sorbitol dehydrogenase (SDH) catalyses the reversible oxidation of sorbitol, which has low sweetening power, to fructose, a sugar with greater sweetness. This makes SDH an excellent candidate for modulating the sugar composition of fruit, if specifically over-expressed in this organ. With the aim of generating a new apple variety with sweeter fruits, different binary vectors were generated in which SDH was cloned under the control of two different promoters, one constitutive and the other fruit-specific. To achieve our aim, we have used tomato plants as a model system to test the functionality of our vectors. Firstly, using biochemical analyses, we show that there is greater SDH activity in extracts of fruits which have been transiently transformed with our vectors compared to control-transformed fruits. Secondly, tomato plants were stably transformed with Agrobacterium tumefaciens carr ying the vectors, and molecular analyses such as PCR and RT-PCR, have been performed to determine the expression of the transgenes. Finally, we are successfully implementing a system for the stable transformation of apples, and have transformed explants with A. tumefaciens harbouring a control vector, pBI121 carrying the uidA reporter gene. We are also using the same transformation platform to increase the vitamin A and antioxidant properties of fruits. Funding: Innova-Corfo 07CN13PBD-19, Fondef D10I1022.

RosBREED #2: extending marker-assisted breeding capability to a wider range of rosaceous crops, traits, and germplasm

Iezzoni A. (1), Peace, C. (2), Bassil, N. (3), Coe, M. (4), Fazio, G. (5) , Gallardo, K. (2), Gasic, K. (6), Luby, J. (7), Main, D. (2), McFerson, J. (8), van de Weg, E. (9), Weebadde, C. (1), Yue, C. (7)

(1) Michigan State University, East Lansing, MI, USA
(2) Washington State University, Pullman, WA, USA
(3) USDA-ARS National Clonal Repository, Corvallis, OR, USA
(4) Cedar Lake Research Group, Portland, OR, USA
(5) USDA-ARS Plant Genetics Resources Unit, Geneva, NY, USA
(6) Clemson University, Clemson, SC, USA
(7) University of Minnesota, St. Paul, MN, USA
(8) Washington Tree Fruit Research Commission, Wenatchee, WA, USA
(9) Plant Research International, Wageningen, Netherlands

RosBREED is a multi-state, multi-institutional, multi-national project dedicated to genetic improvement of rosaceous crops by targeted applications of genomics knowledge and tools to accelerate and increase the efficiency of breeding programs. The current RosBREED project focuses on fruit quality traits for five rosaceous crops: peach, apple, strawberry, sweet cherry, and tart cherry. Socio-economic surveys of breeders, growers, and marketing intermediaries have uncovered knowledge on trait values to help direct marker-assisted breeding (MAB) toward highest priority traits. Genome-scanning SNP arrays were developed and are being used to characterize functional genetic diversity in genomes of U.S. breeding germplasm. The same germplasm is undergoing three years of comprehensive, standardized fruit quality evaluation across 14 breeding programs. Previously reported and newly discovered marker-locus-trait associations are progressing through our systematic MAB Pipeline for t ranslation into ready-to-use information for immediate breeding decision support. The overwhelming amount of data generated is being assembled in an online Breeding Information Management System with interpretive software-based tools. Discussions are underway to extend these resources and increase their impact by developing a second RosBREED project that will extend the array of crops, traits, and germplasm.

A gene expression analysis of *Fragaria vesca* 'alta' in relation to low temperature

Kurokura T. (1), Koskela E. (1), Hytönen T. (1)

(1) Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

Fragaria vesca L. is a useful diploid model to analyse genetic mechanisms of physiological responses of octoploid garden strawberry (F. x ananassa Duch.). Low temperature is known to promote flower induction in seasonal short-day flowering Fragaria and thus a better understanding of molecular mechanisms of temperature regulation of flowering is important to control flowering time. To clarify how low temperature controls flowering we studied flowering gene expression levels in a normal

short-day accession and in a subarctic accession 'Alta' which has deep chilling requirement for flowering. In addition, we analysed the role of the major flowering suppressor FvTFL1 by RNA interference in 'Alta'.
In the short-day accession, the combination of low temperature (11°C) and long day (18h light/6h dark) down regulated the FvTFL1 and up regulated downstream genes for floral morphogenesis. On the other hand, the same treatment was not effective to down regulate FvTFL1 in Alta', so that the downstream genes were not expressed. The gene knock-down of FvTFL1 in 'Alta' resulted in continuous long-day flowering phenotype similar to everbearing F. vesca accessions which has non-functional FvTFL1. These results indicate that low temperature alone is sufficient to regulate flowering genes, 'Alta' has defects in the down regulation of FvTFL1 in response to normal low temperature, and that 'Alta' can be used as a system to study the regulation mechanisms of FvTFL1 by environmental signals.

Identification of the biosynthetic keystep leading to the biosynthesis of dihydrochalcones in apple (*Malus × domestica* borkh.)

S. Martens[(1)], Herrera A.L.[(1)] , Ibdah M.[(2)], Lewinsohn E.[(2)], Gang D.R.[(3)]

[(1)] Fondazione Edmund Mach, Centro Ricerca e Innovazione, Department of Food Quality and Nutrition, Via E. Mach, 1 - 38010 San Michele all'Adige (TN), Italy.
[(2)] NeweYaar Research Center, Agriculture Research Organization, P.O.Box 1021, Ramat Yishay, 30095, Israel.
[(3)] Institute of Biological Chemistry, Washington State University, PO Box 646340, Pullman, WA 99164-6340, USA.

The apple tree is an agriculturally and economically important tree commonly used in food and beverages. Apple has also drawn attention in recent years due to its potential pharmaceutical and nutraceutical applications which are correlated with secondary metabolites. The major phenolic compounds found in apple belong to the class of dihydrochalcones, represented by various phloretin derivatives (e.g. phloridzin, sieboldin, trilobatin). Beside their contribution to the bitter taste of cider and the colour of apple juices due to oxidation products and they were also associated with health effects of apple fruits, and their processed products. The specific reaction that leads to the synthesis of dihydrocoumaroyl-CoA, the direct precourser of dihydrochalcones has not yet been determined. The availability of apple genomic and transcriptomic resources make apple an ideal plant to elucidate this key reductase activity that leads to the production of many valuable dihydrochalcone s in apple but also in other plants. To identify genes involved in the synthesis of dihydrophenolic compounds the existing genome database of the Rosaceae was screened for apple genes with significant sequence similarity to Arabidopsis alkenal double-bond reductase. The functionally expressed apple double bond reductase exhibits p-coumaroyl-CoA reductase activity generating dihydrocoumaroyl-CoA. This finding contributes significantly to our understanding of dihydrophenol formation in plants.

Identification of a *Skp1*-like protein interacting with SFB, the pollen *S* determinant of GSI in *Prunus*

D. Matsumoto[(1)], Yamane, H.[(2)], Abe, K.[(3)], Tao, R.[(2)]

[(1)]Laboratory of Plant Producton Control, Graduate School of Agriculture, Kyoto University, Takatsuki, Osaka, Japan
[(2)] Laboratory of Pomology, Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto, Japan
[(3)] Apple Breeding and Physiology Research Team, National Institute of Fruit Tree Science, Morioka, Iwate, Japan

Many species in Rosaceae, Solanaceae and Plantaginaceae exhibit S-RNase-based self-incompatibility (SI). In this system, the pistil and pollen specificities are determined by S-ribonuclease (S-RNase) and the S locus F-box protein, respectively. The pollen S determinant F-box protein is the S haplotype-specific F-box (SFB) protein in Prunus (Rosaceae) while it is called the S locus F-box (SLF) in Solanaceae and Plantaginaceae. Prunus SFB is thought to be a molecule indispensable for its cognate S-RNase to exert cytotoxicity and to arrest pollen tube growth in incompatible reactions. However, how SFB participates in the Prunus SI remains to be elucidated. Here we report the identification of Prunus avium SFB-interacting Skp1-like protein 1 (PavSSK1) using a yeast two-hybrid screening against the pollen cDNA library. Phylogenetic analysis showed that PavSSK1 belongs to the same clade as solanaceous and plantaginaceous SLF-interacting Skp1-like. In yeast, PavSSK1 interacted n ot only with PavSFBs from different S haplotypes and Cullin 1-likes (PavCul1s), but also with S-locus F-box likes (PavSLFLs). A pull-down assay confirmed the interactions between PavSSK1 and PavSFB and between PavSSK1 and PavCul1s. These results collectively indicate that PavSSK1 could be a functional component of the SCF complex and that PavSFB may function as a component of the SCF complex in self/nonself-recognition in the GSI of Prunus.

Apple quality: relationships among fruit maturation, fruit firmness at harvest, post-harvest fruit softening and susceptibility to storage diseases

H. Nybom[(1)], Sehic J.[(1)], Tahir I.[(2)], Hertog M.[(3)], Ahmadi-Afzadi M.[(1)]

[(1)]Balsgård, Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Fjälkestadsvägen 459, SE-29194 Kristianstad, Sweden
[(2)]Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Box 101, SE-23053 Alnarp, Sweden
[(3)]BIOSYST-MeBioS, Katholieke Universiteit Leuven, Willem de Croylaan 42, bus 2428, BE-3001 Heverlee, Belgium

Various factors involved in fruit maturation are important for initial firmness and for amount of softening during cold storage and when kept at ambient temperature. Some of these factors may also affect the susceptibility to fungal storage diseases. When

analysed in 127 apple cultivars, maturation time was positively correlated with firmness at harvest and negatively correlated with fruit softening rate (difference between firmness at harvest and after storage, divided by number of weeks in storage). Alleles previously described as responsible for good texture, were associated with significantly lower softening for Md-ACS1 and Md-PG1, but the opposite was noted for Md-EXP7. Results were non-significant for Md-ACO1. The predictive power of these four genes was also calculated with a partial least squares discriminant analysis (PLS-DA); genotypes accounted for only 15% of the observed variation in initial firmness and for 18% of the variation in softening rate. Inclusion of maturation time, initial firmness and storage time (6 or 12 weeks) into the model increased the predictability of softening rate to 38%. Freshly harvested fruit from 92 cultivars were inoculated with spore suspensions of blue mould, Penicillium expansum. Regression analyses showed that fungal lesion diameter evaluated after cold storage, was positively affected by fruit softening and negatively affected by maturation time and by firmness at harvest. A PLS-DA showed that 43% of the variation in lesion diameter could be explained by these variables.

Effect of cool storage duration on ripening initiation of Angelys® pear fruit

A. Jajo[(1)], Rahim Md. A.[(2)], Serra S.[(3)], Musacchi S.[(3)], Trainotti L.[(2)], Bonghi C.[(1)]

[(1)]Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35120 Legnaro (PD), Italy
[(2)]Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy
[(3)]Department of Fruit Tree and Woody Plant Science, University of Bologna, Viale Fanin 46, 40127 Bologna

Certain pear cvs (winter pears) require a chilling period before ripening can initiate at room termperature (RT). Cool storage requirement ranges from one up to four months, however extended cool storage does not guarantee ripening onset. In this study fruit at the same maturity stage (measured by IAD, an index based on chlorophyll content) were separated into three groups: harvest point (without chilling), fruit after one and three months of chilling. Structural and biochemical measurements, performed after one week of RT holding for each of the three groups, showed that ripening is halted in the non chilled pears and delayed following the three months chilling, while ripening is initiated after one month chilling. This behaviour is paralleled by the expression profile of genes known as ripening-induced. In which their transcripts were strongly accumulated in the one month group, and almost undetectable in the non chilled fruit. However, the transcript accumulation in the th ree months group is partially initiated when compared to the one month group. These differences can only be attributed to the time the fruit has spent in the cool storage, because the fruit genome, maturity stage and storage conditions are all the same. To understand these differences and identify the gene network involved in the initiation of pear ripening we have applyied an RNAseq approach.
This study is supported by the Progetto AGER INNOVAPERO, grant n° 2010-2107.

Impact of tree training system, branch type and position in the canopy on the ripening homogeneity of abate pear fruit

Rahim Md.A.(1), A. Jajo(2), Serra S.(3), Musacchi S.(3), Bonghi C.(2), Trainotti L.(1)

(1)Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy
(2)Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35120 Legnaro (PD), Italy
(3)Department of Fruit Tree and Woody Plant Science, University of Bologna, Viale Fanin 46, 40127 Bologna

In pear, fruit ripening is not homogeneous, which leads to problems in harvest and storage management. To identify factors affecting the ripening homogeneity, structural, biochemical and molecular parameters were investigated. Fruit were sampled from trees trained with three different systems; spindle, V shaped and Bibaum®, which were further grouped on the basis of canopy position (top or bottom) and branch type (spurs, 2 and 3 year branches and twigs). Ripening homogeneity was assayed by using a spectrophotometric index (IAD) based on chlorophyll content. Homogeneity was higher in spindle in which no significant differences among branch types was observed, while in V shaped and Bibaum® IAD values were more dispersed. Focusing only on the main IAD class at harvest (1.8 to 2.0 IAD); structural and biochemical parameters (weight, flesh firmness, titratable acidity and sugar content) were similar independently by training systems and branch types, suggesting that IAD can be u sed to harvest homogenous fruit. However, the trend of homogeneity degree of the 2 and 3 year branch fruits was higher than those harvested from spurs and twigs. This is further supported by analyzing the expression of genes involved in ethylene biosynthesis and perception, and in cell wall metabolism. Future research will address the expression threshold of marker genes that better correlate with the ripening process after harvest.
This study is supported by the Progetto AGER INNOVAPERO, grant n° 2010-2107.

Biennial bearing in apple – expression patterns of several floral genes revealed by in-situ hybridization

El-Kholti N.(1ac), Wünsche J.N.(2), J.J. Kelner (1b), Verdeil J.L. (1c), Costes E. (1a)

(1a)INRA, UMR AGAP, AFEF team (Architecture and Functions of Fruit Trees), F-34060 Montpellier, France
(1b)Montpellier SupAgro, UMR AGAP, AFEF (Architecture and Functions of Fruit Trees) team, F-34060 Montpellier, France
(1c)CIRAD, UMR AGAP, MRI, PHIV (Plate-forme d'Histo-cytologie et Imagerie cellulaire Végétale), F-34060 Montpellier, France
(2)Universität Hohenheim, Institute of Crop Science, Section Crop Physiology of Specialty Crops, 70599 Stuttgart, Germany

Biennial bearing habits are annual cyclical changes in cropping characterized by "on" and "off" years with "heavy" and "light" fruit loads, respectively. Within the domesticated apple, there is a large variation among cultivars in the amplitude of cycling and tendency to fruitfulness and regular cropping behavior, suggesting genetic control of this phenomenon. Based on previous investigations (Guitton et al., 2011), we focused on 3 floral genes, GA 20-oxidase (MdGA20ox1a), TERMINAL FLOWER1 (MdTFL1) and SOC1 (MdSOC1), assumed to have a major role in the control of flower induction (FI) of apical shoot meristems in apple. GA20ox1 is involved in the later steps of the gibberellin biosynthetic pathway. MdTFL1 is assumed to repress flowering, thus maintaining the apical meristem vegetative. SOC1 integrates multiple flowering signals derived from photoperiod, temperature, hormone, and age-related signals. The aim of this study was to reveal the temporal and spatial expression patterns of these 3 genes during floral bud induction and initiation by in-situ hybridization.
Apical meristems from 2-year-old spurs of Royal Gala/ M.9 "on" and "off" trees were collected between 19 April to 14 June 2012, at weekly intervals, as well as on 28 June and on 30 July. Single-stranded antisense and sense RNA probes of the genes were transcribed with T7 polymerase and labelled with digoxiginin (DIG). At sampling, meristems were immediately fixed. Prior to in-situ hybridization, samples were washed, dehydrated, embedded in paraffin, and cut in 8 µm sections. In-situ hybridization was performed at 55°C (for genes GA20OX1a and SOC1) and at 57.6°C for TFL1a overnight with 0.2 µg ml-1 of the digoxigenin-labelled RNA probe. Several post-hybridisation washes were then carried out before signal detection using an antiDIG antibody coupled with a phosphatise alkaline and VectorBlue as its substrate.
Distinct temporal and spatial changes were revealed in the expression patterns of the 3 studied floral genes. All genes showed initially no or little expr ession in meristems. Thereafter, their expression level drastically increased over time, and each gene exhibited a characteristic spatial distribution, irrespective of subsequent induction or repression of flowering. Even though differences between on and off trees could be visually distinguished in mid and late June samples, these results must be further confirmed by quantitative analyses, such as qRT-PCR.

Construction and comparative analyses of highly dense linkage maps of two sweet cherry intra-specific progenies of commercial cultivars

C. Klagges[(1)], Campoy J.A.[(2)], Quero-García J.[(2)], Guzmán A.[(3)], Mansur L.[(3)], Gratacós E.[(3)], Silva H.[(4)], Rosyara U.R.[(5)], Iezzoni A.[(5)], Meisel L.A.[(1,6)], Dirlewanger E.[(2)]

[(1)]Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, República 217, 837-0146 Santiago, Chile
[(2)]INRA, UR419, Unité de Recherches sur les Espèces Fruitières (UREF), Centre de Bordeaux, F-33140 Villenave d'Ornon, France
[(3)]Estación Experimental La Palma, Facultad de Agronomía, Pontificia Universidad Católica de Valparaíso, Quillota, Chile
[(4)]Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile

(5)Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA, 6INTA-Universidad de Chile, Santiago, Chile.

Sweet cherry (Prunus avium) is an important fruit tree crop. The construction of a sweet cherry genetic linkage map is important to facilitate quantitative trait locus analyses and gene tagging for marker assisted breeding programs. In this work, high-density genetic maps for four parents and two F1 populations obtained from two intra-specific crosses between 'Black Tartarian' × 'Kordia' (BT×K) and 'Regina' × 'Lapins'(R×L) were constructed using the recently designed RosBREED 6K SNP chip. A total of 5,696 SNP markers were tested in each progeny, mapping 723 SNPs and 701 SNPs in the BT×K and R×L linkage maps, respectively. The resulting maps spanned 752.9 and 651.6 cM with an average distance of 1.1 and 0.9 cM between adjacent markers. The maps displayed high synteny and co-linearity between each other and with the peach genome v1.0 for all eight linkage groups (LG1-LG8). However, we identified inversions in sweet cherry genome when compared to the peach genome at LG1, LG5 and LG7. The highly dense linkage maps developed from these two segregating populations of contrasting cherry cultivars will be useful for further research in development of markers for economically important traits, based upon the haplotype segregation in the progeny.
Funded by 07CN13PBT-167 INNOVA Chile CORFO project, INRA's Department of 'Genetic and Plant Breeding' and the Conseil Régional d'Aquitaine 2011 project 'convention 2011-12 01 00122000568', UNAB DI-78-12/I project, the USDA's National Institute of Food and Agriculture – Specialty Crop Research Initiative project "RosBREED: Enabling marker-assisted breeding in Rosaceae" (2009-51181-05808), Fondecyt Nº1120261 and N°1121021. CONICYT doctoral fellowship (N°21120115 and N°24121484). Postdoctoral fellowship from the "Programa de Formación Postdoctoral de Personal Investigador" of the "Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia", Spain.

To flower or not - from photoperiodic signals to flowering responses in the woodland strawberry (*Fragaria vesca* l.)

E. Koskela[(1)], Kurokura T.[(1)], Hytönen T. [(1)]

[(1)] Department of Agricultural Sciences, P.O.Box 27, FIN-00014 University of Helsinki, Finland

Sensing photoperiodic cues and modifying developmental processes accordingly is a prerequisite for successful plant reproduction. In the Rosaceous model species woodland strawberry, day-length affects flowering by regulating the expression of a major floral repressor, FvTFL1. Our recent study showed that flowering in seasonal flowering F. vesca accessions is repressed by the activity of FvTFL1 in long days, allowing the plants to flower only under short days when FvTFL1 is strongly down regulated. A frameshift mutation in the gene eliminates the requirement for short days and leads to a perpetual flowering phenotype (Koskela et al. 2012). As photoperiodic control of TFL1 homologs has not been shown in other species, it would be of great interest to elucidate the genetic factors upstream of FvTFL1. The genomic region around

FvTFL1 contains several CArG box-like sequences both 5' and 3' of the coding region. CArG boxes are typically recognized by MADS box transcripti on factors, and have been shown to be crucial for proper expression of many genes involved in e.g. flower development. Moreover, our experiments with FvSOC1 transgenic plants have suggested that FvTFL1 is activated by FvSOC1 under long days. As SOC1 homologs are MADS box transcription factors, it is conceivable that FvTFL1 could be regulated directly by FvSOC1. Clarifying the regulatory network around FvTFL1 could further our understanding on fine-tuning of floral commitment in response to changing photoperiod.

De novo assembly of sweet cherry (_Prunus avium_) transcriptome from 454 sequencing data

L. Le Dantec(1), Quero-Garcia, J.(1), Dirlewanger, E.(1)

(1)INRA UR 419, Centre de Bordeaux, Villenave d'Ornon, France

Very few genomic resources are actually available for sweet cherry. Emergence and generalization of next-generation DNA sequencing technologies that reduce cost, labor, and time, provide the opportunity to conduct large-scale genomic projects at lower cost even for non-model organisms like prunus avium. In order to obtain a panel of prunus avium expressed genes for functional studies and candidate gene strategy, a normalized complementary DNA library has been sequenced using high throughput 454 technology (GS FLX Titanium) yielding 1175627 reads after cleaning. Clustering and assembly generated a total of 19574 contigs and 149688 singletons. After functionnal annotation of clusters and singletons, a web portal has been created to query the prunus avium transcriptome database. This annotated transcriptome constitute a versatile resource for candidate gene selection based on functional annotation and will be used soon as a reference for short read mapping in differential ex pression studies by RNA-seq.

Molecular events in DAM6 chromatin during dormancy release of peach (_Prunus persica_ [L.] Batsch.)

C. Leida(1), Badenes M.L.(1), Rìos G. (1)

(1)(IVIA) Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia (Spain)

Dormancy is one of the most important mechanism developed by perennial plants in order to adapt to the environment. From this mechanism it depends the survival of the whole plant under unfavourable conditions, and consequently the stability of the production for the next years.
MADS box transcription factors encoded by DORMANCY ASSOCIATED MADS-box (DAM) genes in peach are the main genes involved in this pathway and the regulation of DAM gene expression is not well known at the molecular level.

In order to unravel the molecular mechanisms involved in bud dormancy process we studied the histone modifications and expression of DAM6 gene in peach during dormancy release in two different varieties, "Big Top" and "Red Candem".
We focused on the molecular mechanisms of DAM6 down-regulation which is concomitant with dormancy release in flower buds. A ChIP analysis of DAM6 promoter and structural gene revealed chromatin modification events similar to those observed in vernalization of Arabidopsis and cereals. We showed that DAM6 is transcriptionally active in dormant buds collected in October, when a short chromatin region around its ATG was trimethylated in histone H3 at K4 (H3K4) and acetylated at the N-terminal tail of H3. Concomitantly with DAM6 repression, H3K4 became demethylated and H3 deacetylated. Later, H3K27 was found trimethylated along a genomic region larger than 4kb, including promoter, coding sequence and intron.
The analisis of chromatin modifications reinforced the role of epigenetic mechanism in DAM6 regulation and bud dormancy release and highlighted common features with the vernalization process in Arabidopsis thaliana and cereals.

Transposon based activation tagging in diploid strawberry

N. Lu[1], Upham K.[1], Pereira A.[2], Davis C.[1], Holt S.[3], Shulaev V.[3]

[1]Virginia Tech, Department of horticulture, Blacksburg, VA, USA
[2]University of Arkansas, Department of crop, soil, and environmental sciences, Fayetteville, AR, USA
[3]University of North Texas, Department of biological sciences, Denton, TX, USA

The diploid strawberry, Fragaria vesca, is a potential model plant in Rosaeceae for studying fruit crops. Its short life cycle, prolific seed production, and easy transformation protocol make it a good candidate for studying functional genomics. Transposon tagging is an efficient method of gene discovery by using the transposase gene from maize. Here we developed a population of 66 T0 lines by transforming F. vesca PI 551572 with a two-component Ac-Ds activation-tag (ATag) construct Ac-Ds:ATag-BAR_gosGFP using Agrobacterium-mediated transformation. The Ac element carries hygromycin resistance and GFP as selectable markers as well as the tranposase gene whereas the Ds element has both a 4x 35S enhancer element and basta herbicide resistance. T0 plants were screened based on GFP expression in the roots. For the T1 progeny, seedlings were screened by first selecting those without GFP expression, then by Basta painting on GFP negative plants; subsequent multiplex PCR confirme d the Basta positive/hyg negative plants, indicative of transposed Ds-ATag elements. In the 52 lines that we have screened, a total of 78 candidate T1 transposants was found from 14 lines that show transposition. Flanking sequences of insertion sites from both T0 launch pad and T1 transposants were amplified by TAIL PCR and localized in the strawberry genome by BLAST search. The genome browser identified genes near the insertion sites. All of the mutants will be incorporated as part of an on-line database.

Development of genomic resources for *Fragaria iinumae* – a second diploid ancestor to the octoploid cultivated strawberry

L. Mahoney[1], Sargent D.[2], Ficklin S.[3], Zhang, H.[1], Davis, TM[1]

[1]University of New Hampshire, Durham, NH, USA
[2]Research and Innovation Centre, Fondazione Edmund Mach, S. Michele all'Adige, Trento, Italy
[3]Washington State University, Pullman, WA, USA

The cultivated strawberry, Fragaria x ananassa, and its immediate ancestors F. chiloensis and F. virginiana, are octoploids (2n=8x=56). The subgenome compositions of these octoploid species have yet to be fully determined. An abundance of evidence indicates that at least one of the octoploids' subgenomes is derived from a diploid ancestor that resembles contemporary diploid Fragaria vesca. On this basis, the strawberry genomics community has adopted F. vesca as a model species. Transformation systems and linkage maps have been developed for it, and the genomic sequence of F. vesca subsp. vesca "Hawaii 4" was published in early 2011. In recent years, phylogenetic and other evidence has accumulated implicating another diploid species, F. iinumae, as a second subgenome contributor to the octoploid strawberries. Intriguingly, a mitochondrial marker is shared uniquely between F. iinumae and all octoploids examined, suggesting that F. iinumae may be the source of the octo ploids' mitochondrial genome. We are developing germplasm and genomic resources for F. iinumae, including high throughput genomic sequence data, molecular markers, and the first F. iinumae linkage mapping population. This map is based on segregation data from an F2 population of 150 seedlings derived from a cross two F. iinumae accessions collected in Hokkaido, Japan by Tom Davis and Kim Hummer in 2004. The status of knowledge and resource development in F. iinumae will be described.

Identification of self(in)-compatibility genotypes and microsatellite marker based fingerprinting of traditional italian sweet cherry accessions

A. Marchese[1], Marra F.P.[1], Giovannini D.[2], Leone A.[2], Mafrica R.[3], Pangallo S.[3], Caruso T.[1]

[1]Dipartimento DEMETRA, Università degli Studi di Palermo, Italy
[2]Unità di Ricerca per la frutticoltura di Forlì (CRA-FRF), Italy
[3]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università degli Studi Mediterranea di Reggio Calabria, Italy

Italy is one of the main European sweet cherry producers and it has a rich germplasm, including many minor local varieties that have not been well studied or used in breeding programmes. Sweet cherry is self-incompatible, with few exceptions; its incompatibility is controlled by a multi-allelic S locus, which is gametophytically expressed. The knowledge of S-alleles and cross-incompatibility groups of sweet cherry genotypes and

cultivars is important for growers and breeders. In this work we analysed 48 traditional sweet cherry varieties and cultivars, mainly from Calabria and Emilia Romagna regions, together with eight Sicilian cultivars and the standard set of reference genotypes proposed by the European Collaborative Programme for Genetic Resources (ECPGR) Prunus group (Clarke and Tobutt, 2009), using molecular markers. Two consensus primer pairs for the incompatibility (S) locus, and eight microsatellite (SSR) primers, recommended by the ECPGR, were used in multiplexe d reactions to characterise the accessions. Twelve different S-alleles were detected. S3, S6 and S13 alleles were the most common. Accessions were assigned to incompatibility groups. The chosen SSRs were able to discriminate most of the accessions and to discover duplicates and synonyms. Our results are useful for efficiently design orchards and for planning crosses for future breeding programmes. The usefulness of some accessions for breeding is discussed.

Metabolite profiling of dihydrochalcone compounds in apple germplasm collection

S. Martens(1), Herrera A.L.(1), Malnoy M.(1)

(1)Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach, 1 - 38010 San Michele all'Adige, Italy

The major phenolic compounds found in apple (Malus x domestica) belong to the class of dihydrochalcones, represented by various phloretin derivatives (e.g. phloridzin, sieboldin, trilobatin). Phloridzin (phloretin 2'-O-glucoside) and the other derivatives are found not only in the apple fruits (peel and seeds) but also in flowers, leaves, roots and bark of the entire tree. Furthermore, significant variations between genotypes and between orchards have been described. Due to the supposed health benefits of these metabolites, knowledge on biochemical and molecular level of the involved proteins/genes is of major importance to support ongoing molecular breeding programmes. The Malus germplasm collection at FEM-IASMA includes around 300 accessions which will be screened for variation in dihydrochalcone profile and content in leaves. From a preliminary screen of the metabolic profile of around 70 accessions it became apparent that three classes can be distinguished: 1. Phlor idzin group, 2. Trilobatin, and 3. Sieboldin group. Beside some intermediates all accessions of one group are clearly characterised by the major compound belonging to one or the other group. This knowledge will not only the basis for more detailed characterisation of the biosynthetic pathway but also for isolation of different dihydrochalcones and to prepare specific extracts rich in various combinations of dihydrochalcones for in vitro studies of their function and their bioavailability.

Saturation of the 'Texas' x 'Earlygold' prunus reference map using genotyping by sequencing

C. Meneses[1,2], Vera, G.[1], Tonda, R.[1], Arús, P.[1] and Howad, W.[1]

[1]IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra (Cerdanyola del Vallès), 08193, Barcelona, Spain
[2]Universidad Andrés Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal, República 217, Santiago, Chile.

Peach (Prunus persica (L.) Batsch) is after apple the second temperate fruit tree species of economic importance and has been used as a model species for genetic and genomic studies within the rosaceous family. Furthermore, Next Generation Sequencing (NGS) technologies have evolved rapidly in the last few years and made available innovative strategies for molecular marker identification and genetic linkage map construction. The aim of this study is to take advantage of this NGS technology for SNP discovery and genetic linkage map saturation using the 'Texas' x 'Earlygold' (TxE) Prunus reference population.
For this purpose, Randomly Amplified DNA Fingerprinting (RAF) fragment libraries were sequenced by the 454 technology (Roche) and a software was developed (Bin Mapping Program) in order to identify SNPs and indels, select the most informative ones and genotype and bin map the segregating RAF fragments. The Bin Mapping Program allows the configuration of s everal parameters to define a genotype (homozygous or heterozygous) according to the read redundancy, and mapping of SNPs and indels with incomplete or missing data for one or more individuals of the TxE bin set (6 individuals of the TxE F2 population plus 2 parents, Earlygold and the F1 Hybrid). To saturate the TxE linkage map, 3,340 contigs were obtained from the assembly of reads from the TxE bin set RAF libraries sequenced on the 454 GS-FLX sequencer. In total, 5,712 indels and 6,678 SNPs were detected in 2,155 contigs, revealing a frequency of 1 SNP/170 bp. Using the Bin Mapping Program of SNPs and indels, 984 of the polymorphic contigs were bin mapped; additionally, dominant markers identified as an assembly sub-product (presence or absence of reads) were used to map 143 additional contigs. Thus, a total of 1,127 RAF contigs were bin mapped on the Prunus reference map.
The combination of NGS, bioinformatics and bin mapping has proven to be an efficient strategy for SNP and indel discovery and linkage map saturation in peach in order to develop tools for marker assisted selection.

Genetic relationships of Central American *Rubus* genotypes by SSR and RAPD markers

R. Molina-Bravo[1,2], Hilje-Rodríguez I.[1], García-Pinares A.[1], Orozco-Rodríguez R.[1], Argüello-Delgado F.[1]

[1]Universidad Nacional de Costa Rica, Escuela de Ciencias Agrarias, Apartado Postal 86-3000, Heredia, Costa Rica
[2]Universidad de Costa Rica, Centro de Investigación en Biología Celular y Molecular, San José Costa Rica

Costa Rica possesses abundant diversity in Rubus with desirable economic traits, but little is known about the genetics and phylogenetics of this material. We are currently researching this material with the ambition to start a breeding program and provide farmers with clean, high quality cultivars. As an initial draft of the genetic relationships among these genotypes, we used 10 RAPD primers previously tested on Rubus as well as SSR and SSR-EST markers developed in Rubus and other rosaceous crops. Nei's genetic distances were calculated for both marker types. Results concur with morphological data in all cases except for R. urticifolius, when genotypes were analyzed using RAPD. When genotypes were analyzed with SSR and SSR-EST markers, a similar relationship was found. The genotype Castilla has been suggested to be the result of a natural raspberry-blackberry cross in South America. This genotypes is most closely related to raspberry genotypes in the analyses, thus fu rther supporting this hypothesis. 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.

Towards a first draft of the octoploid strawberry genome sequence

Tonda R.[1], Alioto T.[2], Arús P.[1], *Monfort A.*[1]

[1]IRTA, Centre for Research in Agricultural Genomics CSIC-IRTA-UAB-UB, Campus UAB, Edifici CRAG, 08193 Bellaterra (Barcelona), Spain.
[2]Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain

Assembly of sequences from genomes of polyploid species is challenging due to the abundant presence of highly homologous regions. Strawberry (Fragaria x ananassa) is an octoploid fruit of economical importance. It has been estimated to have a genome size of approximately 600 Mb, though its sequence remains to be determined yet. Generation of a consensus sequence of the genome is capital for the development of new markers and their association to relevant QTL. The goal was to generate a consensus sequence of the octoploid strawberry genome. A highly homozygous individual from an F2 population was selected and two pair-end libraries with insert sizes of 490 and 520 nucleotides and two mate-pair libraries (2.5 kb and 4 kb) were constructed and

sequenced in an Illumina GenomeAnalyzer II. Several trials were conducted to determine the best combination of assembly software and kmer size and several assemblers were tested. Additional scaffolding and gap closing steps allowed us to reduce by 60% the total number of contigs/scaffolds, increase the N50 value from 2,071 bp to 5,589 bp and reach a max scaffold size of 183.6 kb. To test the goodness of the assemblies, 49,132 ESTs of all the Fragaria genus and 13,289 ESTs specific for F. x ananassa were blasted against each assembly, obtaining a high rate (>95%) of positive hits. Homology studies with F. vesca, one of the diploid ancestors, are being conducted to facilitate a first draft of F. x ananassa genome sequence.

Deciphering apple fruit mealiness, a transcriptomal approach

Mikol S., Bruneau M., Gourdon C., Celton J-M., *M. Orsel*, Laurens F., Renou J-P.

INRA, UMR1345 Institut de Recherche en Horticulture et Semences, SFR 4207 QUASAV, PRES L'UNAM, F-49045 Angers, France.
AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, F-49045 Angers, France.
Université d'Angers, UMR1345 Institut de Recherche en Horticulture and Semences, F-49071 Beaucouzé, France.

Apples appetence and conservation are depending on fruit quality. It is therefore a main issue for breeding. Fruit quality is built on complex traits such as taste, texture, colours and aromas, developed during fruit maturation and conservation. The genetic and molecular bases of these traits are still mainly unknown. The recent release and annotation of the apple genome (Malus x domestica) allow the development of genome wide strategies and the identification of genes and regulations network involved in fruit quality.
Taking advantage of the v.1 annotation, AryANE-v1, a whole genome transcripts microarray chip was constructed with the NimbleGen technology. Specific probes were designed and synthesised for the 60 000 identified coding sequences and some miRNA precursors. On the bases of sensorial analysis for apple mealiness, several hybrids with contrasted phenotypes were identified within a segregating population. A transcriptional analysis was then performed with AryANE-v1 microarray in order to compare the different genotypes along the fruit maturation and conservation process. Global analysis of the results highlights genes belonging to 4 different biological processes (cell wall synthesis, carbohydrate metabolism, defences genes and hormonal pathways). Most interesting expression profiles were confirmed using RTqPCR. Candidate genes are selected in order to develop a functional approach and investigating their roles in establishment of mealiness.

Gene families: Clarifying the role of individual members through real time qPCR expression studies, the case of *Mal d 1/ pr-10* genes in *Malus domestica*

Pagliarani G. [1], Paris R. [2], Tartarini S. [2], van de Weg E. [1]

[1] Plant Breeding, Plant Research International, Wageningen, The Netherlands
[2] Department of Fruit Tree and Woody Plant Sciences, University of Bologna, Bologna, Italy

In plant genomes many genes exist in multiple copies as members of gene families. The high number of similar genes and their co-localization on the same genomic region hamper the elucidation of the genetic base of related traits. We demonstrate how PCR based expression studies may be of help to clarify the role of the individual genes. This qPCR approach is robust, cheap and easy to apply. As case we use the Mal d 1/PR10 family of apple, composed by 31 genes. This family is involved in food allergy (referred to as Mal d 1) as well as in the defence system of the plant (referred to as PR-10) whereby the biological function is still unclear. We developed gene specific primer pairs for all the 31 genes. Specificity was validated both in silico and in vivo. Expression was studied firstly, on young apple leaves from susceptible and resistant genotypes after challenging with the fungus Venturia inaequalis. The observed differences in expression profiles (intensity & time-li nes) indicate differentiation in biological functions. Secondly, expression was studied on peel and flesh of fruits from cultivars that differ in allergenicity. Only part of the family was expressed in fruit and a clear differential expression was found between tissues as between cultivars. This allowed to trim down the number of genes that can be involved in apple allergy. We hereby demonstrate that qPCR based expression studies are useful to further clarify the role of individual members of large gene families.

New SNP markers for raspberry germplasm genotyping

L. Palmieri[1], Giongo L [1]

[1]Research and Innovation Centre. Fondazione Edmund Mach, Via E. Mach, 1 - 38010 San Michele all'Adige, Italy

Since 2003 more then 250 raspberry (Rubus idaeus) accessions were collected at FEM in order to generate a germplsam collection, which needed to be genotyped. To do so, ten new SNP markers were developed on 10 raspberry varieties, starting from SCAR markers transferred from diploid strawberries and apple (Sargent et al.,2007; Sargent et al., 2009). As results of sequencing analysis, new raspberry sequences of anthocyanidin synthase (ANS), lipoxygenase (LOX), ent-kaurene oxydase (EKO), cynnamyl alcohol deydrogenase (CAD), exspansin (EXP), dihydroflavonol 4-reductase (DFR), cytosolic ascorbate peroxidase (APX), spermine synthase (ACL5), alpha amylase (AMY), maltose transporter (MEX), soluble inorganic pyrophosphatase (SIP), polyamine oxidase (PAO), pectate lyase (PL) enzyme regions were obtained and the polymorphisms presence was

assessed. Finally, the germplasm genotyping was done on about half of the accessions number using the new markers. The data obtained were analysed with PowerMarker v.3.25, NTSY and STRUCTURE softwares to determine the genetic diversity of the whole collection and to obtain a preliminary cluster analysis. These molecular markers might be helpful not only for germplasm characterization but also, such as candidate genes, for mapping, synteny studies within the Rosaceae family and as a starting point functional to marker-assisted selection for raspberry breeding programs.

Silencing Mlo-like susceptibility genes to achieve broad-spectrum resistance to powdery mildew in apple

Pessina S. [1,2], Bai Y. [1], Malnoy M. [2], Schouten H. [1]

[1] Wageningen UR Plant Breeding P.O. Box 16, 6700 AA, Wageningen, The Netherlands,
[2] Fondazione Edmund Mach, Centro Ricerca e Innovazione, Department of Genomics and Biology of Fruit Crop, Istituto Agrario San Michele all'Adige–IASMA, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

Powdery mildew is a disease caused by about 650 obligate biotrophic fungi species, capable of colonizing around 10,000 plant species, including many crops. A particular kind of resistance to this pathogen, characterized by durability, broad-spectrum effectiveness and recessive inheritance, was obtained for the first time in a barley mlo mutant. Mlo genes encode for susceptibility factors and are therefore called susceptibility genes (S-genes). Loss-of-function mutations in these genes lead to lack of susceptibility, which means resistance. Mlo genes have been found and studied in many plant species, including several crops, while there are no studies on fruit trees yet. Our work aims to study Mlo genes in apple, in order to achieve broad spectrum resistance. Previous works on different species (tomato, arabidopsis, grape) show how the expression levels of some of the Mlo genes increased in response to the inoculation with the pathogen. By means of a bioinformatic approach, we looked for mlo-like genes in apple genome and we tested their expression after inoculation with Podosphaera leucotricha. Three apple cultivars have been tested: Golden Delicious, Gala and Braeburn. Preliminary analysis conducted on Golden Delicious show that 4 Mlo genes are up-regulated because of the interaction with the pathogen; one of these genes co-localize with a QTL. Further we are silencing mlo-like genes using a RNA interference approach, in order to deprive the pathogen of its target and, therefore, to obtain resistance.

Complementary strategies for the breeding of cultivated strawberry for high level of antioxidants

A. Petit[1], Tallès K.[1], Jousseaume G.[1], Chartier P.[1], Denoyes B.[2]

[1]Ciref, Douville, France
[2]INRA, UMR 1332, Université Bordeaux, Villenave d'Ornon, France

Strawberry (*Fragaria x ananassa*) is a good source of antioxidants (AO) due to its high levels of anthocyanins (ACY), flavonoids (FL) and phenolic acids (PH). These compounds are essential for fruit nutritional and organoleptic qualities. Two complementary strategies for breeding varieties rich in AO are set up: (i) optimizing the choice of parents in breeding programs by screening genetic resources (GR) showing high level of AO, and (ii) development of a Marker-Assisted Selection (MAS) using markers identified in Quantitative Trait Loci (QTL) approach.
Material represented by 28 genotypes for GR and one segregating population, were cultivated in a soilless system and assessed during one and three consecutive years, respectively. AO content was evaluated by measuring by colorimetry the total PH, total FL and ACY contents and the total antioxidant capacity with the Trolox equivalent antioxidant capacity (TEAC) assay.
Statistical analyses showed a strong correlation between TEAC, PH and FL contents. Screening of GR by using Principal Component Analysis showed that the cultivar Ciflorette is very rich in AO and could be chosen in breeding programs. A large number of minor QTL was identified. Among them, a cluster of QTL and some putative homoeo-QTL were observed in the same homoeology group, which could be further studied for the development of MAS. These two complementary strategies seem to be the best way to create strawberry variety rich in AO.

MAB enabling DNA markers for red stele root rot resistance in strawberry

A. Pikunova[1,3], Pagliarani G.[1], van Thijs D.[1], Meulenbroel B.[2], Koehorst-Van Putten H.[1], Noordijk Y.[1], van de Weg E.[1]

[1]Plant Breeding, Wageningen University and Research Centre, Wageningen, The Netherlands
[2]Fresh Forward Breeding B.V., Eck en Wiel, The Netherlands
[3] All-Russian Research Institute of Horticultural Breeding (VNIISPK), p/o Zhilina, Orel region, Russia

Red stele root rot is a fungal, soil borne disease caused by Phytophthora fragariae var. fragariae. In Europe and USA it is a quarantine disease that may cause serious losses to nurseries and growers. Here we report on the successful fine mapping of the major, dominant, race-specific resistance gene Rpf1 and the development of linked molecular markers that can be used in Marker Assisted Breeding.
Our first linked SSR marker was discovered through a Pedigree Based Analyses (PBA) approach on a series of pedigreed cultivars and breeding selections. Knowing Rpf1's position on the genetic map of octoploid strawberry, other published markers for this region have been used to define a chromosome segment within which this gene should be located. Next, Rpf1 was fine-mapped by making use of the reference genome sequence for the diploid F.vesca Hawaii-4, that gave us the opportunity to develop, and map new polymorphic markers. As a result, the genomic region for Rpf1 was narrowed down to 1.3Mbp of the physical map. DNA-markers were identified that are easy to score and that flank Rpf1 at opposing sites, of which the ones nearest to Rpf1 are at just 1cM

from each other. Also, we noticed several inconsistencies between our genetic maps and the physical map with regard to position and orientation of contigs.
Currently the best SSR markers are being used in a commercial Dutc h breeding program, and one of these is under test in the USA within the framework of the USDA-SCRI RosBREED project.

Genetic, genomic and breeding research on Rosaceae at Vniispk, Russia

Sedov E.(1), Knuasev S.(1), Dzhigadlo E.(1), A. Pikunova(1)

(1)All-Russian Research Institute of Horticultural Breeding (VNIISPK), 302530,p/o Zhilina, Orel region, Russia

The All-Russian Research Institute of Horticultural Breeding (VNIISPK) at Orel is one of the oldest pomological centers of Russia. Here, research is performed on a number of crops from Rosaceae family including apple, pear, raspberry, plum, apricot, sweet and tart cherry, strawberry. More than 130 new varieties have been bred here, such as apple cultivars combining resistance to scab with high fruit quality meeting consumer preferences, and cherry cultivars resistant to leaf spot.
VNIISPK has one of the biggest horticultural germplasm collections in the country. It counts more than 85 species, 6500 varieties and around 80 000 hybrid seedlings.
VNIISPK is the first Russian institute where scab resistant cultivar were bred. Today over 19 scab resistant varieties have been released, and also several varieties with high content of ascorbic acid and P-active substances in fruit as well as 15 new triploid varieties. Triploid varieties are remarkable thanks to large fruit size, good commercial quality and regular fruiting.
There are cytoembryological, micropropagation and PCR laboratories at the VNIISPK. Selection under artificial infection has been practiced. Lately molecular genetic studies started at VNIISPK. Genetic polymorphism of native cherry cultivars have been estimated by means of DNA-markers in collaboration with Mexican scientists. Native apple cultivars have been tested on presence of some published DNA-markers associated with scab resistant genes like Vf, Vm.

Evaluation of oxidative stress in homo- and- heterografts from pear and quince

Irisarri P.(1), Martens H.J.(2), Errea P.(1), A. Pina(1)

(1)Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av Montañana 930, 50059, Zaragoza, Spain
(2)Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Reactive oxygen species (ROS) are generated when the plants are exposed to various biotic and abiotic stress factors such as temperature extremes, drought, mineral deficiency, chemical imbalances, wounding etc. The oxidative stress response is triggered by an imbalance in the production and metabolism of ROS and the harmful

effects of ROS on cellular components are well documented. Plants have developed antioxidant mechanisms to protect themselves against oxidative damage by scavenging of ROS. These mechanisms employ antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). These antioxidant enzymes inactivate the cytotoxic compounds and minimize their ability to diffuse into the intracellular space. Recently, it has been hypothesized that oxidative stress could trigger cell and tissue degradation processes in incompatible grafts. The aim of this study was to determine the activity of antioxidant enzymes (SOD, APX and CAT) in callus unions with different degree of compatibility throughout two weeks after grafting as well as in vivo quantification of ROS over time using the ROS-indicator dye CM-H2DCFDA and confocal laser scanning microscopy. The preliminary analysis showed that antioxidant enzymes activities were lower at the rootstock/scion interface in incompatible combinations at early stages of development. The results will be discussed in terms of the possible involvement of ROS levels in rootstock-scion interactions and graft incompatibility in fruit trees.

Evolution of diversity in *Prunus avium*: Assembly of its genome using next generation sequencing

Pinosio S.[(1)], Cattonaro F.[(2)], Felice N.[(3)], Vendramin G.G.[(1)], Morgante M.[(3)]

[(1)] CNR, Istituto di GeneticaVegetale, Sezione di Firenze, Sesto Fiorentino, Italy
[(2)] IGA Technology Services, Udine, Italy,
[(3)] Dipartimento di Scienze Agrarie e Ambientali, Università di Udine, Udine, Italy,

Sweet cherry (Prunus avium) is an economically important crop having a diploid genome (2n=16) with an estimated size of 338 Mb. In the framework of a national project aimed at the study of the effects of domestication on genetic variability in cherry, we set out to build a first draft genome sequence of P. avium by means of next-generation sequencing. We sequenced the sweet cherry variety Big Star at a coverage of approximately 100X using the Illumina HiSeq 2000 platform. Short reads were de novo assembled into 43,011 contigs covering 204 Mb and having an average size of 4.7 Kb and an N50 size of 14 Kb. We used mate-paired libraries of 2.5 Kb to perform the scaffolding and we obtained a preliminary assembly composed by 30,204 scaffolds with an average size of 6.8 Kb and an N50 size of 29 Kb. We compared the P. avium assembly with the Prunus persica reference genome: overall, 105 Mb of P. avium were aligned to the P. persica genome suggesting a high level of conservation b etween the two species. Conserved sequences showed in general a high gene content and a low rate of repetitiveness. The P. avium genome assembly will be further refined and improved and will provide a new resource for biological research and breeding of this species. The future aim of the project will be the resequencing of several local landraces, commercial varieties and wild P. avium genotypes to explore the genetic mechanisms influencing domestication in this species at a genome-wide level.

Generation of a bacterial artificial chromosome-base physical map of the red raspberry genome

I. Punsod[(1)], Paterson A.[(1)], Ellis E.M.[(1)], Lutz E.M.[(1)], Graham J.[(2)], Kassim A.[(3)]

[(1)]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK
[(2)]James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
[(3)]Faculty of Civil & Environmental Engineering, Universiti Tun Hussein Onn, Johor 86400, Malaysia

The red raspberry (Rubus idaeus) has morphologic diversity and a small genome size (275 Mb), and is a good model for applications in molecular breeding programmes. Significantly, raspberry serves as a valuable source of anti-oxidants, cyaniding and pelargonidin anthocyanins that confer nutritional health benefits as well as colour to fruits. The biosynthetic pathway producing anthocyanins involves type III polyketide synthase (PKSs), and has been studied in two varieties of red raspberries. PKS1 has been mapped to chromosome 7 (LG7). This study aimed to produce a physical map of part of chromosome 7 of the red raspberry (Rubus idaeus cv. Glen Moy). Two bacterial artificial chromosome (BAC) clones originating from chromosome 7 were digested by restriction endonucleases and subcloned into fosmids to assist 454 genome sequence assembly. This BAC-based physical map consists of 10 contigs with a total physical length covering at 290 kb. Several open reading frames (ORFs) have been identified in the BACs. Two ORFs encode polyketide synthase and chalcone synthase (PKS1 and PKS5). Three other ORFs encode ATP-binding transporter (ABC), Cycloartenol synthase (CAS) and Cytochrome P450. All of theses sequences were compared with red raspberry Rubus strigosus cv. Lathaim. The map produced represents an important genomic resource for the completion of the red raspberry genome. Furthermore, this physical map will be a useful tool for the comparative analysis of soft fruit genomes and applications in molecular breeding strategies.

Fosmid library construction of aromatic polyketide synthase in red raspberry *Rubus idaeus*

I. Punsod[(1)], Paterson A.[(1)], Ellis, E.M.[(1)], Lutz E.M.[(1)], Kassim A.[(3)], Graham J.[(2)]

[(1)]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK
[(2)]James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
[(3)]Faculty of Civil & Environmental Engineering, Universiti Tun Hussein Onn, Johor 86400, Malaysia

A large family of polyketide synthases (PKSs) catalyzes a diverse polyketide biosynthetic pathway producing significant compounds acting a broad range of biological activities including pharmacological properties, in particular type III polyketide synthase

involving in biosynthesis of higher plant anthocyanins serving as significant antioxidants for human healthy. To understand the organization and evolution of Aromatic Polyketide synthase (PKSs) in red raspberry, in this research, a random sheared fosmid library of red raspberry Rubus idaeus cv. Glen Moy was constructed from the positive Bacterial Artificial Chromosomes (BACs) screened with chalcone synthase probe (CHS 11). The fosmid library consisting of 2,311 clones with average inserted size of approximately 35 kb was screened by PKS primers and probes resulting in 52 positive clones. Fosmid stability assays showed that red raspberry DNA was stable during continuous propagation. To provide a preliminary assessment of the genome, a minimum of 84 randomly selected clones were generated and end-sequenced to check the overlap between the fosmid clones. These sequences presented a total length of 134.4 kb covering 0.084 % of red raspberry genome (275 Mb). The fosmid end sequence (FESs) in conjunction with 454 BAC sequence data has been used for assembly of BAC clones to facilitate the sequence completion. Further, the red raspberry fosmid library could be a beneficial resource for construction of a physical mapping and position cloning including provide a further understanding of red raspberry genome.

Correlation between cracking, CER6 gene expression and alkane concentration in sweet cherry fruits

J.C. Rios[(1)], Lang E.[(2)], Silva H.[(1)]

[(1)]Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática, Universidad de Chile, Santiago, Chile
[(2)]CEM, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Cracking of the fruits is the major cause of losses in the cherry's industry worldwide. This phenomenon is mainly caused by rain fall during the harvest period and is related to osmotic factors and fruit water permeability. Studies in cherry tomato showed that the silencing of the gene which encodes for β-Ketoacil-Coenzyme A Synthase Elongase (lecer6) enhances dehydration 3 to 8 fold on those fruits. The quantification of the wax components in these tomatoes, showed a considerable decrease in the concentration of n-alkanes with 28-30 carbon length. On the other hand, qualitative and quantitative analysis of waxes from sweet cherry fruits showed that their components are mainly triterpenes (76%), alkanes (19%) and alcohol (1%). This could suggest that the different expression levels of the othologous lecer6 gene in sweet cherry fruit might play a key role in the development of cracking between varieties with different susceptibility. Epicuticular waxes NMR analysis for alkanes from Lapins, Bing and Rainier cherry fruit varieties showed significant differences within n-alkanes concentrations. Lapins showed the lowest concentration compared to Bing and Rainier. The expression analysis of the cherry lecer6 ortholog gene showed a higher expression in Rainier than Lapins. These results are correlated with the fact that Lapins, in our study, is the less susceptible variety to cracking.
This work is supported by CONICYT, FONDECYT/Regular Nº1120261 and Innova CORFO (07CN13 PBT-167); JCR is student of the Ph.D. Biotechnology Program at Universidad Andres Bello.

Routine marker-assisted seedling selection in the Pacific Northwest sweet cherry breeding program provides resource savings

T. Rowland[1], Edge-Garza D.[1], Athanson B.[2], Oraguzie N.[2], Peace C.[1]

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

WSU's sweet cherry breeding program is routinely conducting marker-assisted seedling selection (MASS). The Pacific Northwest Sweet Cherry Breeding Program focuses on "consumer" traits of fruit quality while seeking improvements in some key production traits. Since 2010, 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. The Silica Bead Method for DNA extraction, with its simple tissue sampling procedure integrating easily into breeding operations and enabling >3000 samples/week by one technician, was tweaked for cherry. Genotyping is conducted for fruit size and self-fertility DNA tests. Field planting in fall 2010 was eased after 850 seedlings were tested and 500 culled that were predicted to have small fruit or be self-incompatible. In spring 2011, another 1000 seedlings were culled from 1900 tested, elimin ating subsequent resource expenditures on inferior trees for planting, tree maintenance, and fruit assessment. Quality control was emphasized from 2011 to increase reliability and help streamline the DNA testing process, from tissue sampling to DNA extraction and genotyping to timely provision of results for enacting culling. To date, MASS has provided an estimated net savings of $80,000 in present and future costs for the Pacific Northwest Sweet Cherry Breeding Program.

Expression of genes related to the response of water stress in stone fruits (*Prunus spp.*)

Bielsa B.[1], Leida C.[2], *M.J. Rubio-Cabetas*[1]

[1]Unidad de Fruticultura. CITA - Gobierno de Aragón, Avda Montañana, 930, 50059, Zaragoza, Spain
[2]Departamento de Fruticultura. Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada a Náquera Km. 4,5, 46113 Moncada, Valencia, Spain

Stone fruits have a different behaviour depending on environmental stresses they are subjected. In order to understand the response of drought, physiological and molecular parameters of three Prunus hybrid rootstocks, the almond x peach hybrid (P. amygdalus x P. persica) 'Garnem' and their descendents 'P.2175' x 'Garnem'-3 trihybrid and 'P.2175' x 'Garnem'-9 trihybrid (P. cerasifera x [P. amygdalus x P. persica]) were investigated. Plants in pots were subjected to water stress conditions during one month. Subsequently, plants were submitted to re-watering period. For each sample time (0, 10, 15 days of treatment and 15 and 30 days of re-watering) two set of roots and floem were

taken for each genotype. Physiological responses were monitored and relative expression patterns of two genes coding for proteins related to ABA pathway and abiotic stress, a dehydrin (ppa005514m) and A20/AN21 zinc finger (ppa012373m), was analyzed by qPCR. During water stress, all genotypes showed a decrease in leaf area as well as transpiration and leaf water potential, existing significant differences along the experiment and among the genotypes. The expression in root and floem systems of dehydrin and A20/AN21 zinc finger genes showed a correlation with physiological parameters of drought response. The expression of both genes was higher in roots than floem at 15 days of drought stress. Thus the transcript level of dehydrin gene as the A20/AN21 zinc finger gene were higher in the two trihybrid genotypes than in the parent 'Garnem', thereby trihybrid genotypes could be more tolerant to drought stress than 'Garnem' genotype.

Differences on mealiness incidence in early and late season varieties of *Prunus persica* and cell wall modifications by postharvest treatments

D. Sanhueza[(1)], Covarrubias M.P.[(1)], Meneses C.[(1,2)], Paredes R.[(3)], Defilippi B.G.[(4)], Orellana A.[(1,2,a)], Campos-Vargas R.[(1,a)]

[(1)]Universidad Andres Bello, Fac. Ciencias Biológicas, Centro de Biotecnología Vegetal, Santiago, Chile
[(2)]FONDAP-Center for Genome Regulation, Santiago, Chile
[(3)]Universidad Andres Bello, Fac. de Ecología y Recursos Naturales, Escuela de Medicina Veterinaria, Santiago, Chile
[(4)]Instituto de Investigaciones Agropecuarias, INIA La Platina, Santiago, Chile
[(a)]Basal Project PFB-16

Mealiness is an important problem for the peach industry. It is well known that early and late varieties behave differently when they are exposed to cold storage, aiming to extend their postharvest life. In general, early cultivars do not have many problems, but late varieties are more prone to a damage known as "Chilling injury" (CI) whose main consequence is a mealy flesh. The basis of CI are still unknown, but different evidences suggests that the cell wall metabolism is altered, in particular the homogalacturonan chains, that are not disassambled normally, due to changes in the levels of degrading enzyme activities. Seeking for solutions to this problem, technologies such as "Controlled atmosphere (CA)" and "Conditioning" have been developed. However, these postharvest treatments do not work for all varieties, which rather have a differential behavior. Based on this information we evaluated the fruit quality after cold storage and postharvest treatments of four peach varieties, two early (December) and two late varieties (January). The results indicated that early varieties were always juicy. In contrast, late varieties were mealy after cold storage for 21 days at 4°C; however, the use of CA or conditioning reduced the mealy phenotype. In order to visualize changes in the cell wall we used periodic acid schiff (PAS) staining. The results showed that cells from mealy fruit mesocarp became more rounded and the expansion of intercellular spaces caused separation of adjacent cell walls in comparison to cells from juicy flesh. In order to look for differences in the methylation pattern of the homogalacturonan chains,

inmunolabeling analysis were performed using antibodies that are able to discriminate between different methylation patterns. Regarding the uronic acid content, no differences were observed among early peach varieties exposed to different treatments; however in the late cultivars it was possible to observe difference s in the content of total uronic acid, and in one of the cultivars the highest uronic content correlated with the lowest juice percentage. PACE analysis showed that early cultivars do not have differences in their methylation pattern, however, changes were observed in late cultivars. These results confirm the hypothesis that mealiness is associated to changes in the cell wall.(CONICYT-Doctoral Fellowship D-21090737). Acknowledgements: Funded by UNAB DI-64-12/1; Doctoral thesis project 24121174 from CONICYT, PFB-16, FONDAP-CRG 15090007.

Survey and conservation of old regional cherry cultivars in Burgenland, Austria

E. Schüller(1), Pilz V.(1), Spörr T.(1), Spornberger A.(1), Keppel H.(1)

(1)University of Natural Resources and Life Sciences, Vienna - Department of Crop Sciences, Division of Viticulture and Pomology. Vienna, Austria

While cherry cultivation has a long tradition in Burgenland, Austria's easternmost state, today its economic importance is continuously declining. The cultural landscape with old cherry trees in vineyards and old cherry cultivars are therefore in danger to disappear. The conservation of fruit- and biodiversity is of great importance, especially regarding future challenges in fruit production because of climate change.
In two projects 2011 and 2012/13 old cherry trees in two regions of Burgenland, Noplerberg-Biri (meadow orchards) and Leithaberg, are investigated.
The projects include surveying and mapping of cherry trees, phenotypical characterization and, if possible, also identification of the cultivars as well as the propagation of the most promising cultivars through grafting. Parameters of blossom, tree and fruit are evaluated. Future plans include genetic characterization via microsatellite markers (SSRs) as well as S-Allele analysis.
30 different cherry cultivars were found in the region of Noplerberg-Biri. 11 of those could be identified; 19 cultivars are not yet identified local cultivars. 20 cultivars were chosen to be propagated and conserved.
In the region Leithaberg the identification of cultivars is additionally needed for a certificate of origin and quality. The value chain of organic cherry production is already well developed in this region and this certificate shall help to fully utilize the existing capacity.

Application of microsatellite markers for determination of trueness-to-type of selected apple varieties

A. Storti[1], Dalla Via J.[2], Baric S.[1]

[1]Research Centre for Agriculture and Forestry Laimburg, 39040 Vadena (Bolzano – Italy)
[2]Autonomous Province of Bozen/Bolzano, Andreas Hofer Strasse 18, 39100 (Bolzano – Italy)

Apple (*Malus × domestica*) is globally one of the most important fruit crops, which displays a large varietal diversity. Many regional and national programmes for the collection and maintenance of the remaining genetic resources of cultivated apple are underway, and the description and characterisation of these resources rely on both, the application of pomological descriptors as well as on molecular markers. In the present study, accessions of three cultivars from different origins ('Antonovka', 'Laxton's Superb' and 'Worcester Pearmain') were analysed at 14 variable microsatellite loci and subjected to a comparative analysis involving also a database with molecular genetic profiles of reference cultivars. The advantages of the comparative molecular database approach for the reliable determination of trueness-to-type of apple cultivars maintained in germplasm collections are exemplified.

Genetic resources of Rosaceae fruit crops in Southern Russia and their use in the breeding

Suprun I. [1]; Eremin G. [2]; Ulyanovskaya E. [1]; Lugovskoy A. [1]; Kochetkov V. [3]

[1] State scientific organization North Caucasian Regional Research Institute of Horticulture and Viticulture, Krasnodar, Russia
[2] Krymsk Experimental Breeding Station, Krymsk, Russia
[3] Maikop Experimental Station of VIR, Maikop, Russia

South of Russia is the main fruit producing region in Russian Federation and the greatest germplasm collection of Rosaceae is concentrated there. North Caucasian Regional Research Institute of Horticulture and Viticulture coordinates the management of these genetic resources as well as fruit crop breeding. Collections of genetic resources include about 12 000 genotypes. It consists of species and varieties belonging to three genera: Prunus (ca. 7000 accessions) with majority of P. domestica, P. persica, P. cerasifera, P. armeniaca, P. avium, P. cerasus; Malus (ca. 3000 accessions) – mainly M. domestica; and Pyrus (ca. 1200 accessions) - majority of P. communis. Diversity of species is also presented in the large scale. There are classes of donors for different traits and trait complexes among represented genotypes.
Pre-breeding material and breeding selections (~17000 genotypes) have been developed from these genetic resources. They derived from different genetic stock material and serve as donors for complex traits. Breeding collections includes

intervarietal, interspecific hybrids, mutants, and polyploids. Genetic stock material is thus used for the development of well adapted and high quality varieties. Several rootstock sets were breed in the institute for different fruit crops. The common breeding directions are high temperature and drought tolerance in summer period, low temperature tolerance in winter and spring time. As for biotic stress resistance main directions are following: for apple – resistance to Venturia inaequalis, Podosphaera leucotricha; for pear - resistance to Venturia pirina and Erwinia amylovora; for cherry – resistance to Monilia cinerea and Coccomyces hicmalis; for plum – resistance to Clasterosporium carpophilum. Large scale utilization of wide gene tic resources of fruit crops in the breeding gave opportunities for breeding varieties having the above mentioned desired traits.

Use of DNA-markers on Russian genetic resources collections and breeding of rosaceous fruit crops

I. Suprun[(1)], Tokmakov S.[(1)], Ilnitskaya E.[(1)], Stepanov I.[(1)]

[(1)]State scientific organization North Caucasian Regional Research Institute of Horticulture and Viticulture, Krasnodar, Russia

There are several research lines at our institution make use of molecular markers. The three main are:
Evaluation of genetic relationships among genetic resources using SSR markers. Hereto, highly polymorphic SSR-markers are searched for. The most informative ones are further used for genotyping of Russian varieties. As a start, 18 SSR markers were selected from the HIDRAS database for Malus and seven for Prunus from published works. The SSR genotyping of the varieties confirmed their genealogy. For large scale investigation of genetic diversity amount of SSRs will be increased.
Estimation of germplasm for allelic composition of the self incompatibility locus. At the present moment 84 accessions of apple have been analyzed for the alleles S1, S2, S3, S5, S7, S9, S10, S19. Groups of compatible and incompatible genotypes were identified. Alleles S2, S3, S7, and S10 are the most distributed among modern Russian varieties and advanced breeding selections. Investigation of old and autochthonous varieties is on its way. Among already genotyped 14 varieties of sweet cherry bred in Russia, allele S3 is the most common.
Marker-assisted breeding is carried out for apple scab resistance genes Vf and Vm, as for genes involved in fruit firmness (Md-ACS1 and Md-ACO1) by testing cultivars and breeding selections, several of which have both Vf + Vm. The common allelic combination for Md-ACO1 gene is 1/2 in all members of a set of 46 Russian varieties, while for Md-ACS1 all allelic combinations occur (1/1, 1/2, 2/2) with the majority of 1/2. The results of marke r-assisted identification of target genes are useful for development of scab resistant varieties with better firmness, better storability and long shelf life of the apple fruit. Large scale DNA-marker based assessment of Russian genetic resource would clarify genomic polymorphism of Rosaceae by the comparing with germplasm from other regions.

In-vitro and in-vivo flavour relase from six intact and fresh-cut apple cultivars in relation to their textural and physico-chemical parameters

V.J.L. Ting[1,2], Soukoulis C.[1], Aprea E.[1], Romano A.[1], Silcock P.[2], Bremer P.[2], Cappellin L.[1,3], Gasperi F.[1] and Biasioli F.[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, (TN), Italy
[2]Department of Food Science, University of Otago, Dunedin, New Zealand
[3]Institut für Ionenphysik und Angewandte Physik, Leopold-Franzens Universität Innsbruck, A-6020, Innsbruck, Austria

To gain a better understanding on the genetic basis of apple quality, the volatile organic compound (VOC) profile of six commercial apple cultivars (Fuji, Golden Delicious, Granny Smith, Jonagold, Morgen Dallago and Red Delicious) was determined using Proton Transfer Reaction Quadrupole Mass Spectrometry (PTR-MS). The textural and physicochemical (pH, acidity and water content) properties of the six cultivars were also measured.
Cultivar type strongly influenced volatile release: Fuji and Granny Smith apples had the lowest total concentration of VOC (esters, aldehydes, alcohols and terpenes) whereas Red Delicious had the highest. Differences in VOC release enabled cultivars to be grouped based on their genetic/inherited traits such as Jonagold and Golden Delicious. Dynamic in-vivo nose space analysis allowed cultivars to be characterized on mastication time and in-nose concentration based on four VOC measured (esters m/z 43, 61; acetaldehyde m/z 45; ethanol m/z 47) . Firm cultivars (Fuji, Granny Smith) had a longer consumption time and a lower VOC concentration. Softer cultivars (Golden Delicious, Morgen Dallago) were consumed faster, released more VOCs and reached a maximum VOC intensity faster.
Nosespace VOC data on the cultivars, collected with a novel PTR-Time of Flight Mass Spectrometer, PTR-TOF-MS enabled isobaric compounds to be differentiated and provided a more accurate profile solely based on cultivar variation highlighting its potential to rapidly screen samples.

Towards a large axiom SNP array for tetraploid rose

Smulders M.J.M.[1], Koning-Boucoiran C.F.S.[1], Esselink D.G.[1], van 't Westende W.P.C.[1], Voorrips R.E.[1], *W.E. van de Weg*[1], Bellon F.[2], Mittmann M.[2], Pirani A.[2], Webster T.[2] Brew F.[3], Maliepaard C.[1]

[1]Wageningen-UR Plant Breeding, Wageningen, The Netherlands
[2]Affymetrix, Santa Clara California, USA
[3]Affymetrix UK Ltd, Wooburn Green, High Wycombe, UK

Rose, as many other important ornamental, vegetable and field crops, is polyploid. This poses constraints in genetic analyses, due to, amongst others, the occurrence of multiple alleles at marker and trait loci, and the existence of multiple allele dosages. As a

consequence, development of genetic and molecular tools for breeding is slow. Most frequently marker development and the construction of a linkage maps are carried out on the diploid level whereby the translating of this knowledge back to the tetraploid level is done only to a limited extent.
The availability of a large-sized SNP array would allow extensive genetic studies on tetraploid roses, especially as we have recently developed software for automated genotype calling of bi-allelic SNPs in tetraploid species, enabling scoring of allele dosage and thereby of the five alternative genotypes (aaaa, baaa, bbaa, bbba and bbbb; nulliplex to quadruplex) (fitTetra; Voorrips et al. BMC Bioinformatics 2011, 12:172).
Here, we report on the development of a 60K Rose Affymetrix Axiom® genotyping array. We have used a diverse germplasm SNP discovery panel of cut rose and garden rose cultivars. To obtain SNPs in coding sequences we harvested mRNA from leaves and flowers in various developmental stages. The SNPs were identified in Illumina Hiseq reads using QualitySNP. Production will start before the end of 2012.

Genomic rearrangements, duplications and signatures of breeding in the allo-octoploid strawberry as revealed through an allele dose based SSR linkage map.

Van Dijk, T. [(1)], Pagliarani, G. [(1)], Pikunova, A. [(1)], Yilmaz, H. [(1)], Dubos, T. [(1)], Noordijk, Y. [(1)], Van de Weg, E.[(1)]

[(1)]Wageningen UR Plant Breeding, Wageningen, Gelderland, Netherlands

In this study, we developed an integrated genetic map of two octoploid strawberry cultivars (Holiday and Korona) that, for the first time for a polyploid plant species, included comprehensive haplotype information, even for the homozygous regions. It was possible to do this because we used the MADCE method for determining the allelic configuration of our parental genotypes and offspring. This approach allowed us to give accurate estimations on the level and distribution of homozygosity and haplotype sharing which could be indications of a breeding signature. In addition it allowed us to reveal differences between the homoeologues more accurately than previously published maps. The completed map revealed several interesting features, including a possible rearrangement on one of the homoeologous genomes, and strong signs of breeding signatures in the parental cultivars.

Quality of 'Santana', 'Golden delicious', 'Liberty' and 'Topaz' apple cultivars grown under organic and integrated production

Stopar M.[(1)], Vrhovsek U.[(2)], *A. Vanzo*[(1)]

[(1)]Department of Fruit and Vine Growing and Central Laboratory, Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana, Slovenia
[(2)]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach, 1, 38010 San Michele all'Adige, Italy

Apple fruit quality was investigated on scab-resistant apple cultivars 'Santana', 'Liberty' and Topaz' and on scab-nonresistant cultivar 'Golden Delicious' when they were grown according to either organic (ORG) or integrated production (IP). Trees subjected to

similar crop load were used to asses physicochemical and sensorial parameters of fruits on both production systems. ORG produced 'Santana', 'Liberty' and 'Golden Delicious' had on the average smaller fruits and more soluble solids (with exception of 'Topaz') and higher flesh firmness (equal in case of 'Golden Delicious'). Higher total phenol content in ORG fruits was found on the average in all investigated cultivars, however only in case of 'Golden Delicious' the difference was statistically significant. The change of phenol content in 'Golden Delicious' might be related to either its higher synthesis or to changes in fruit size. In order to elucidate which classes of phenols were affected by the production management, both ORG and IP cultivars were further analyzed by targeted metabolomics method for multiple classes of phenolics. In conclusion, the sensorial evaluation indicated significant better overall flavor and more acceptable overall appearance of IP produced apples.

AGER Project on Apple Advanced Research. Apple fruit quality in the post-genomic era, from breeding new genotypes to post-harvest: nutrition and health

R. Velasco[(1)], Cipriani G.[(2)], Costa G.[(3)], Gianfranceschi L.[(4)], Pellegrino S.[(5)], Ramina A.[(6)] *representing the AGER TEAM of the six Institutions*

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach, 1, 38010 San Michele all'Adige, Italy
[(2)]University of Udine, Department of Agricultural and Environmental Science
[(3)]University of Bologna, Department of Fruitculture
[(4)]University of Milano, Department of Biotechnology and Biomolecular Science
[(5)]Consortium for Research, Experimentation and Divulgation for Piedmontese horticultural and fruit crops
[(6)]University of Padova, Department of Environmental Agronomy and Crop Production

International competition and the pressing request of the consumers oblige italian fruticulture to be at the forefront of innovation. Thanks to the most recent advances on apple "omics" approaches, a wealth of in-field experience, interaction with nutritionists and experts of human health, modern tools for apple orchard management and fruit conservation, six Italian institutions joined in 2010 to implement Italian apple cultivation: A) By developing fundamental technological genetic/genomics tools for apple breeding and technologies and tools for non-invasive fruit quality assessment; B) by elucidating the main structure of the apple fruit metabolome and its relationship with standard fruit quality parameters to improve the knowledge about the molecular bases of fruit quality with emphasis on nutritional and health-related properties. C) By applying the above powerful molecular genetics tools and knowledge to molecular breeding and to the production chain in order to 1) improve apple resistance to pathogens, 2) enhance fruit quality (mainly in terms of nutritional aspects), 3) reduce environmental impacts and increase sustainability, and 4) optimize the production chain.
This proposal has therefore set the following specific objectives:

1. Production of genomic, transcriptomic and metabolomic tools and knowledge to lay the foundation of efficient marker-assisted breeding for varietal innovation and better comprehension, in molecular terms, of the key quality traits of apple fruit.
2. Creation of genetic lines and selections aimed to improve fruit quality and disease resistance.
3. Improvement of the key steps of the pre-harvest production phase, achieved by means of the following actions:
   -Development of new thinning strategies based on environment-friendly chemicals and mechanical methods.
   -Development of new self-thinning cultivars.
   -Water management improvement to control fruit quality.
   -Real time management of crop load.
   -Innovative measuring tools for monitoring the onset and evolution of fruit ripening.
4. Optimization of the production chain, its traceability, and fruit quality assessment, pursued by:
   -New methods to enhance fruit homogeneity.
   -Advanced traceability system.
   -Comparison between instrumental and sensorial fruit quality assessment.
5. Characterization of the key nutraceutical profile and allergenic properties of apple fruit by: A) Identification of bioactive molecules and analysis of their roles as modulators of risk factors for dismetabolic diseases and their anti-neoplastic activity; B) Identification of the master determinants of apple allergenicity and search for hypoallergenic apple cultivars.

The genome sequence of peach, a key diploid tree species, reveals unique patterns of genetic diversity, domestication and genome evolution.

I. Verde[1], and The International Peach Genome Initiative

[1]CRA Centro di Ricerca per la Frutticoltura, Rome, Italy

The plant family Rosaceae is the most important fruit producing clade and its key commercially relevant genera (Fragaria, Rosa, Rubus, Prunus) display a broad diversity in growth habit and fruit type as well as compact diploid genomes. Peach [Prunus persica (L.) Batsch], a key diploid species, is one of the best genetically characterized deciduous trees. We report the high quality genome sequence of peach obtained from a completely homozygous genotype. A complete chromosome-scale assembly was obtained by Sanger whole genome shotgun methods. We predicted 27,852 protein-coding genes as well as non-coding RNAs. Analyses about the expansion in the peach lineage of gene families related to sorbitol metabolism and to phenylpropanoid network individuated cornerstone features in the evolution of the Spiraeoideae subfamily and a Prunus-specific mode of genome evolution likely associated with unique production of the lignified stone in these species. We investigated the path of pea ch domestication through whole genome resequencing of 14 Prunus accessions. The analyses suggest major genetic bottlenecks that have significantly shaped the peach genome diversity.

Furthermore, comparative analyses show that peach has not undergone recent whole genome duplication (WGD) and even though the ancestral triplicated blocks in peach are fragmentary compared to those in grape, all seven paleosets of paralogues from the putative paleoancestor are detectable.

A second generation peach linkage map using the IPSC 9K SNP chip for advanced QTL identification

I. Verde(1), Gazza L.(1), Aramini V.(1), Taller D.L.(1), Dettori M.T.(1)

(1)CRA - Centro di Ricerca per la Frutticoltura - Roma, Italy

Peach [*Prunus persica* (L.) Batsch] is one of the most important fruit crops in temperate area. Peach breeding is costly and time consuming due to the size of the plants and the relatively long (3-4 years) intergeneration period. Marker assisted breeding can help in reducing the cost and time in obtaining improved cultivars. The availability of the complete peach genome sequence (International Peach Genome Initiative) allowed the development of a powerful genetic tool, a 9K SNP chip delivered by the International Peach SNP Consortium. The chip is being employed within the EU funded project FruitBreedomics to dissect QTL and identify SNPs linked to important agricultural traits. Here we report a peach linkage map obtained with 1279 SNPs of the IPSC 9K chip using 232 individuals of a BC1 progeny. The map is distributed in 8 linkage groups and covers 610.8 cM with an average distance between adjacent markers of 0.5 cM (1.2cM excluding co-mapping markers). The map aligns compl etely with the eight peach V1.0 pseudomolecules, a few incongurences highlight putative misassembly occurred in Peach v1.0 release. A QTL analysis was performed for the flowering time recorded in spring 2012 (2 years old plants) using MapQTL6. A single major QTL was identified in Group 4 (SNP_IGA_420819 at 13939118 bp in Peach v1.0). The variance explained was 39.7% with an additive effect of -4.035 days.

QTL mapping for content of phenolic compounds extracted from fruit and juice in a cider apple progeny

C.F. Verdu(1,2,3,4), Guyot, S.(5), Childebrand, N.(1,2,3), Lasserre-Zuber, P.(1,2,3), Boucourt, M.(1,2,3), Le Pautremat, E.(1,2,3), Celton, J.-M.(1,2,3), Gaillard, S.(1,2,3), Gatto, J.(4), Troggio, M.(6), Dupuis, F.(1,2,3), Guilet, D.(4) and Laurens, F.(1,) , (2), (3)

(1) INRA, UMR1345 Institut de Recherche en Horticulture et Semences, SFR 4207 QUASAV, PRES L'UNAM, F-49045 Angers, France,
(2) AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, F-49045 Angers, France,
(3) Université d'Angers, UMR1345 Institut de Recherche en Horticulture and Semences, F-49071 Beaucouzé, France,
(4) Université d'Angers, EA 921 laboratoire Substance d'Origine Naturelle et Analogues Structuraux, SFR 4207 QUASAV, 49045 Angers, France,

(5) INRA, UR117 Recherches Cidricoles et Biotransformation des Fruits et Légumes, 35653 Le Rheu, France,
(6) Research and Innovation Centre,Fondazione Edmund Mach, S. Michele all'Adige, TN, Italy

Polyphenols have favorable antioxidant potential on human health suggesting that their high content in apple is responsible for the beneficial effects of apple consumption. They are also related to the quality of ciders as they predominantly account for astringency, bitterness, color and aroma. Five groups of phenolic compounds are described in the apple fruit: flavanols, hydroxycinnamic acids, dihydrochalcones, flavonols and anthocyanins. So far, only two studies have been published on the genetic basis of the phenolic content of dessert apples. As cider apples are commonly described to be much more concentrated in phenolic compounds than dessert varieties, the present study focuses on a cider apple progeny. 32 compounds belonging to the five groups were identified and quantified by HPLC-UV and UHPLC-UV-MS/MS in fruit extracts and juices. 53 QTL controlling phenolic compounds concentration were detected on nine linkage groups (LG) on the integrated linkage map, for all p henolic groups except anthocyanins. QTL clusters located on LG1, 12, 14, 15 and 17 were stable across the year or the studied material. QTL detected on LG1, 14 and 17 for quercitrin, p-coumaroylquinic acid, rutin and chlorogenic acid confirmed results of previous studies. However, no significant QTL was obtained on the LG16 where a major locus for flavanols was previously located. With the two previous studies, this study shows the diversity of genomic regions controlling traits of interest in apple.

Genetic diversity of wild European and Mediterranean pear species

G.M. Volk(1), Richards C.(1), Henk A.(1), Bassil N.(2), Postman J.(2)

(1)USDA-ARS-National Center for Genetic Resources Preservation - 1111 South Mason St, Fort Collins, Colorado 80521, USA
(2)USDA-ARS National Clonal Germplasm Repository - 33447 Peoria Road, Corvallis, Oregon 97333, USA

Many pear species are native to Europe, the Middle East, and Northern Africa. These seemingly distinct species readily hybridize resulting in nomenclatures that do not reflect their phylogenetic history. We have used microsatellite and chloroplast sequence markers as well as phenotypic traits to differentiate between European and Mediterranean wild pear species in the world pear collection at the USDA-ARS National Clonal Germplasm Repository in Corvallis, Oregon. Species include *Pyrus communis*, *P. eleagrifolia*, *P. gharbiana*, *P. mamorensis*, *P. regelii*, *P. sachokiana*, *P. salicifolia*, *P. spinosa*, and *P. syriaca*. We took a population genetic approach when evaluating the diversity within and among these described species by using a model based clustering method to distinguish genetic lineages. These estimated lineages often contained individual genotypes that were from different species. These groups were then assessed geographically and genetically to better understand specie s distribution and differentiation. Our data revealed hybridization among species as well as diagnostic

traits that could be used for species identification. By integrating population genetic and phylogenetic analyses we are able to more completely describe the historical relationships and diversity within these important crop wild relatives.

The USDA-ARS national plant germplasm system malus collection: diversity of cultivars and wild species

Volk G. [1], Richards C. [1], Gross B. [2], Forsline P. [3] (retired), Fazio G. [3], Chao C.T. [3]

[1] USDA-ARS National Center for Genetic Resources Preservation, 1111 South Mason St., Fort Collins, Colorado 80521
[2] University of Minnesota Duluth, 207 Swenson Science Building, 1035 Kirby Drive, Duluth, Minnesota 55812
[3] USDA-ARS Plant Genetic Resources Unit, Geneva, New York 14456-0462

The USDA-ARS National Plant Germplasm System (NPGS) Plant Genetic Resources Unit (PGRU) apple collection in Geneva, NY conserves over 2500 trees as grafted clones. We have compared the genotypes of 1131 diploid Malus × domestica Borkh. cultivars to a total of 1910 wild and domesticated samples representing 41 taxonomic designations in the NPGS collection to identify those that are genetically identical based on nine simple sequence repeat (SSR) loci. A total of 238 M. × domestica and 10 samples of other taxonomic groups shared a genotype with at least one other M. × domestica individual. We identified examples of genotypes for cultivars that matched genotypes of known rootstocks, and indicated that these accessions may not accurately represent the indicated named clones. Twenty three sport families, comprised of 104 individuals, were identified that could not be differentiated using the nine SSR loci. SSR markers as well as phenotypic traits were used to compare the di versity of the currently designated core collection to that of the entire diploid grafted collection. We have identified a set of individuals that augment the diversity of the existing core collection, thus capturing more than 95% of the allelic and phenotypic diversity. We have also identified sets of 100 individuals that also capture the desired diversity within the collection. Five of the selected markers (CH01h01, CH02d08, CH01f02, G12, GD147) overlap with sets of markers that have been used to fingerprint European apple collections, thus making it possible to compare and coordinate collection inventories on a world-wide scale.

A targeted metabolomics method for the rapid quantification of multiple classes of phenolics in the fruits of Rosaceae

U. Vrhovsek[1], Masuero D.[1], Gasperotti M.[1], Franceschi P.[1], Caputi L.[1], Viola R.[1], Mattivi F.[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

In recent years, the interest in phenolic compounds has been increasing due to compelling evidences of their beneficial health properties and to their impact on food

quality. The complexity and remarkable diversity of phenolics has challenged the analytical performances of separation and detection methods in terms of resolving power, selectivity and sensitivity for the identification and quantification of these compounds in different matrices. Targeted metabolomics is a strategy based on the use of predefined metabolite-specific signals, such as MRM transitions, that can be used to accurately determine the concentrations of a wide range of known metabolites.
We developed a rapid and versatile UPLC-MS/MS based method for the quantification of >150 phenolics, such as benzoates, phenylpropanoids, coumarins, stilbenes, dihydrochalcones and flavonoids in fruits. Compounds commonly occurring in plants were included in the method together with metabolites specific of a single species or family. Reverse-phase chromatography was optimised to achieve separation of the compounds over 15 min, reducing possible ion suppression effects and resolving many isomeric compounds. The optimal fragmentation conditions for each analyte were studied and MRM transitions were selected for accurate quantification. The effectiveness of the method was validated by studying the limits of detection and quantification, the linearity ranges of the instrumental response and the repeatability of the analysis.
The method was successfully applied and validated for the analysis of apples, cherries, raspberries, strawberries, as well as grape, wine and green tea, and was shown to represent a valuable tool for the quantitative evaluation of the chemical phenotype, measuring the presence, amount and natural variance in phenolics composition of these fruits.

Overexpression of a peach *Cbf*-transcription factor gene in apple regulates both dormancy and freezing tolerance in apple: lab and field studies

Wisniewski, M. [(1)], Artlip, T. [(1)], John Norelli[(1)]

[(1)]USDA-ARS, Kearneysville, WV 25430 USA

Economic production of fruit trees in a temperate climate is dependent upon seasonal changes in cold acclimation and dormancy. Evidence indicates that these processes will be greatly affected by climate change (higher atmospheric carbon dioxide and temperatures). This problem may also be exacerbated by erratic weather patterns. Weather events in the USA in the spring of 2012, resulting in devastating losses to fruit crops, are an example of the potential danger. Wisniewski, et al. (2010. Planta 233: 971-983) previously demonstrated that a transgenic 'M.26' apple line (T166) overexpressing a peach CBF gene increased the freezing tolerance and induced earlier dormancy (Wisniewski, et al. 2010. Planta 233: 971-983). Since that study, the field performance of T166, has been monitored in comparison to wt ('M.26'), and apple lines in which expression of a native CBF has been suppressed (CBF-Si). Self-rooted trees were planted on October 7, 2010 and several phenotypic c haracteristics monitored. The T166 line exhibited an immediate response to cool temperatures and short photoperiod. Trees exhibited a large increase in anthocyanins in their leaves followed by rapid senescence. By November 4, 2010, T166 trees had lost all their leaves while wt, and CBF-Si trees still had green leaves. In spring of 2011, the CBF-Si line was the first to break bud, prior to

the wt trees. T166 trees leafed out last. Mean date of leaf emergence varied by about 2 weeks between the three lines. Growth (shoot growth and main stem diameter) was lowest in the T166 line. In 2011, the fall pattern of leaf senescence and drop was similar to 2010. Thus it appears that overexpression of a peach CBF gene in apple has significant, long-term effects on several phenological events in apple. Observations for 2012 support these conclusions.

One practice to use the available resistant genetic markers for marker-assisted selection in apple breeding

H. Xuan[1], Rueß F.[2], Peil A.[1], Flachowsky H.[3], Bantleon G.[1], Radek C.[1], Mayr U.[1]

[1]Kompetenzzentrum Obstbau-Bodensee (KOB), Schuhmacherhof 6, D-88213 Ravensburg, Germany
[2]LVWO-Weinsberg, Traubenplatz 5, D-74189 Weinsberg, Germany
[3]Julius Kühn-Institut (JKI), Pillnitzer Platz 3a, D-01326 Dresden, Germany

Apple (Malus x domestica) is the most important fruit species in the temperate zone, ranks second to banana in terms of total global production as a major fruit source for the world's population. Apple breeding is carried out on a large scale in several scientific institutes throughout the world. Breeders worldwide intensively develop new selections annually, though only a few dozen types are widely produced in commerce today. Breeding of apple varieties is time consuming because of protracted generation cycles, requires substantial space for the planting of seedling populations in the field, and is labour and cost consuming.
Apple is host to a wide range of pests and diseases, a number of which need to be controlled for profitable commercial production. Scab disease, caused by the ascomycete fungus Venturia inaequalis; fire blight, caused by bacterium Erwinia amylovora; powdery mildew, caused by the obligate biotrophic ascomycete fungus Podosphaera leucotricha, are the three most damaging diseases in economic terms. Therefore, the work of resistant apple breeding is still very important in practice. The approaches to shorten breeding period by use of marker-assisted selection and to improve apple resistant breeding have changed markedly in the past two decades. Different markers for scab, fire blight and powdery mildew resistant breeding are available in the world gen bank.
In this study, two markers for powdery mildew; two markers for fire blight; eleven markers for scab, were chosen to characterize 35 cultivars. The resistant gens which every cultivar could carry will be evaluated, and the comparisons with phenotypes will be discussed.

Deep resequencing the QTL intervals for chilling requerement and bloom date in peach, a model genome system for woody tree species genetics

Zhebentyayeva T.[(1)], Fan S.[(1)], Olukolu B.[(1)], Hughes-Murphree S.[(1)], Chandra A.[(1)], Barakat A.[(1)], Bielenberg D.[(1)], Reighard G.[(1)], Okie W.[(2)], Abbott A.[(1)(3)],

[(1)] Clemson University, Clemson, South Carolina, USA
[(2)] ARS-USDA, Southeastern Fruit and Tree Nut Research Laboratory, Georgia, USA
[(3)] INRA-Universite Bordeaux II, Villenave d'Ornon, France

Peach represents advanced genetic system for studying a chilling requirement (CR) for bud dormancy release in woody species. Capitalizing on a high quality genome assembly (www.rosaceae.org) and robust QTL mapping results (Fan et al. 2010), we exploit a combined genetic and genomic approach to elucidate molecular mechanism controlling bud dormancy release and flowering time in peach. We resequenced 2 high- vs. 2 low-chill individuals from phenotypic extremes in the QTL mapped cross using the Illumina 100bp paired-end reads platform. Reads were imported into the CLC Genomics Workbench (http://www.clcbio.com) and aligned against the reference genome represented by a high-chill doubled haploid Lovell genotype. A haplotype configuration of individuals allowed reconstruction of homozygous high- and low-chill alleles at the three strongest QTLs on G1, G4 and G7 that collectively explain up to 70% of phenotypic variation for CR and bloom date in the C x Fla background. Using the CLC tools we detected SNP, DIPs and structural variants within QTL intervals in high- vs. low-chill haplotypes, eliminated non-informative variation (common in both alignments against the reference) and created a prioritized list of low-chill specific polymorphisms at the three most significant QTL loci. The SNP verification via Sanger resequencing was done in a set of 24 peach cultivars with CR variation from 150 to 1250 hours. Also, we used electrophoresis on acrylamide and agarose gels to confirm presence of DIPs and large insertions respectively. We will report these results and other data that are in agreement with our working hypothesis on the potential network of candidate genes for control of CR and bloom date in peach.

Characterization of the apple (*Malus domestica*) ROP GTPase machinery: gene expression in response to postharvest storage and ethylene inhibition

E. Zonin[(1)], Zermiani M.[(1)], Vezzaro A.[(1)], Begheldo M.[(1)], Ramina A.[(1)], Nonis A.[(1)], Ruperti B.[(1)]

(1) Università di Padova - Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente – DAFNAE, Viale dell'Università 16, Legnaro (Padova), Italy

Rho-related GTPases of Plants (ROPs) are molecular switches regulating responses of plants to environmental changes through modulation of reactive oxygen species (ROS) homeostasis. The interplay between ROPs, ROS homeostasis and the action of the plant hormone ethylene is poorly characterised. We have investigated the expression patterns of apple (Malus domestica) genes encoding ROPs and their ancillary proteins ROP-GEFs,

ROP-GDIs, ROP-GAPs during postharvest storage of fruits in controlled atmosphere (CA - 1% O2,and 1°C), and in response to the inhibitor of ethylene perception 1-MCP (1-methylcyclopropene). BLAST sequence similarity searches run on the apple genome database (www.rosaceae.org) using the A. Thaliana proteins as queries allowed the identification of 11 ROP, 14 ROP-GEF, 10 ROP-GAP, and 10 ROP-GDI encoding genes. The expression of these genes, studied by Real-time PCR on peels of apples (cv Granny smith) after 0, 1, 3 and 6 months of storage in CA with or wit hout treatment with 1-MCP, evidenced different transcript accumulation patterns. Remarkably, some ROP and ROP-GEF genes showed an increasing trend in 1-MCP-treated samples, suggesting that the inhibition of ethylene perception may result in the specific co-regulated derepression of genes encoding some ROPs and ROP regulatory proteins. The data suggest a complex interplay between ethylene perception and the transcriptional regulation of the molecular machinery of ROP GTPases.

NADPH oxidase (NOX) activity and gene expression in apple (*Malus domestica*) fruits subjected to hypoxia

Zermiani M.(1), Begheldo M.(1), *E. Zonin*(1), Nonis A.(1), Mercadini M.(2), Tonutti P.(3), Ruperti B.(1)

(1)Università di Padova - Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente – DAFNAE, Viale dell'Università 16, Legnaro (Padova), Italy
(2)MARVIL engineering S.r.l. - Via Nazionale, 46/48, Salorno (Bolzano), Italy
(3)Scuola Superiore Studi Universitari Sant'Anna, Piazza Martiri della Libertà, 33, Pisa, Italy

NADPH oxidases (NOXes) are ROS producing enzymes involved in response to pathogens and to abiotic stresses in eukaryotic organisms. We have identified, by BLAST searches on the apple genome, 16 genomic sequences encoding proteins displaying similarity to Arabidopsis NADPH oxidases (RBOH). Ten genes resulted to encode bona fide NOXes displaying the conserved domains typical of NADPH oxidases. Gene expression profiles on different tissues (whole flowers, anthers, petals, developing fruits, seeds, and young, fully expanded and senescing leaves) evidenced the preferential expression of apple NADPH oxidases in distinct tissues, with one gene being almost exclusively expressed only in anthers and two genes mainly expressed in developing seeds.
To evaluate NADPH oxidase enzymatic activity in apple fruits, a protocol has been optimized for the isolation of microsomal fractions from apple cortex and epidermal tissues. DPI-sensitive activity was analyzed on microsomal fractions ob tained from fruits (Granny smith) subjected to three different levels of oxygen and two degrees of hypoxic stress (normoxic: 20% O2; hypoxic: 1% O2; ultra low oxygen – ULO – 0.4% O2) in a time course experiment at 1, 2, 3, 4 and 5 weeks of storage at 1°C. NOX activity in the apple fruit cortex appeared to be highly regulated in response to oxygen availability only when O2 concentration reached levels below 1%. The expression of apple NOX encoding genes in response to oxygen deprivation is in progress.

Preliminary characterization of the molecular factors associated with bitter pit development of apple (*Malus domestica*) fruits

Nonis A.(1), E. Zonin(1), Bassi G.(2), Ramina A.(1), Bonghi C.(1), Ruperti B.(1)

(1)Università di Padova - Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente – DAFNAE, Viale dell'Università 16, Legnaro (Padova), Italy
(2)Servizio sperimentazione frutticola e vitivinicola della Provincia di Verona, Via della Pieve, 64, San Floriano VR, Italy

Bitter pit is a physiological disorder of apple fruits, which is manifested with necrosis on hypodermal tissues, evidenced by depressed areas on the fruit's surface, possibly due to perturbations of calcium homeostasis, believed to be necessary for membrane stability. The molecular aspects responsible for bitter pit induction and development are largely uncharacterized. To gain a first insight into molecular factors associated with bitter pit development we have undertaken a deep sequencing approach on the transcriptomes of epidermal and outer cortical layers of fruits with manifested symptoms of bitter pit versus healthy fruits. From preliminary analyses (30*106 of 50 bp single reads) 290 genes resulted to be upregulated and 3 downregulated, adopting a threshold of 20 fold induction/repression. Among these, Mapman pathways were obtained indicating that concurrently with bitter pit development a significant upregulation takes place for genes belonging to “biotic stress” a nd “development” (cellular response) and to the phenylpropanoid and flavonoid pathways (metabolism). As far as regulatory genes are concerned, a number of transcription factors, receptor-like kinases and calcium response proteins appeared to be induced in fruits with bitter pit, confirming the role of calcium in bitter pit development.
"Supported by Progetto AGER, grant n° 2010-2119"

Gene expression profiles of auxin metabolism in maturing apple fruit

Shin, S.(1), Mattheis, J.(1), Evans, K.(2), Zhu, Y.(1)

(1)USDA ARS Wenatchee, WA, USA
(2)Washington State University, Wenatchee, WA, USA

Variation exists among apple genotypes in fruit maturation and ripening patterns that influences at-harvest fruit firmness and postharvest storability. Based on the results from our previous large-scale transcriptome profiling on apple fruit maturation and well-documented auxin-ethylene crosstalk, the current experiment was designed to understand the molecular events related to auxin metabolism during fruit development and their interactions with ethylene pathways. Maturity-defined weekly apple fruit samples of four cultivars with distinct ripening phenotypes were aligned according to their physiological maturity. The expression of eight candidate genes with annotated functions of auxin transport, GH3 and auxin response were profiled by qPCR for at least 10 consecutive weeks; and the weekly samples with similar Ct values selected as calibrator of relative expression level for better across-genotype comparability. Most of

these genes showed dynamic regulation during fruit development and differential expression among genotypes. The peak expression of auxin transporter MdPIN1-1 correlated with the detection of the transcripts of a pre-climacteric ethylene biosynthesis gene MdACS3. Both transcript profiles during maturation and tissue-specific expression features for these genes suggested that auxin transport and homeostasis are important in regulating the timing of ethylene pathway activation and therefore may contribute to distinct ripening processes among apple genotypes.

Phenolic profile of different red and yellow raspberry varieties during ripening

Elisabete Carvalho[(1)], Pietro Franceschi, Stefan Martens

[(1)]Research and Innovation Centre, Fondazione Edmund Mach, S. Michele all'Adige, Italy

Rubus species, including raspberries, have been used since ancient times for the treatment of wounds, diarrhea, colic pain, diabetes, etc.[1]. Being among the fruits with higher antioxidant contents, raspberries are receiving increasing attention as a source of potentially healthy compounds that can help prevent cardiovascular disease and diabetes mellitus [2]. A large part of the health effects attributed to berries is supposed to be due to polyphenolic compounds. The majority of raspberries polyphenols are ellagitannins, but they also contain large amounts of anthocyanins (in red raspberries) and smaller amounts of hydroxycinnamic acids, flavonols, flavan-3-ols and proanthocyanidins [3]. Yellow raspberries, which lack anthocyanins at all, seem to be as effective or even more effective than their red counterparts at inhibiting enzymes with potential impact on chronic diabetes or hypertension [4, 5]. From the biosynthetic point of view there is as yet no information on where the block of the anthocyanin pathway in yellow raspberries could be. In this study a targeted UPLC-MS/MS method recently developed by our lab was used to screen 140 phenolic compounds including benzoates, phenylpropanoids, coumarins, stilbenes, dihydrochalcones and flavonoids, using MRM transitions for accurate quantification. The fruits of different plants of 4 red and 6 yellow raspberry varieties, at different ripening stages (green, turning and ripe) where analyzed. Around 34 phenolic compounds where detected above the quantification limit at the different ripening stages.
This allowed to obtain not only a profile of the phenolic composition of the different raspberry varieties, at different stages, but also to highlight the different tendencies in the variation of concentration of each of the different compounds during ripening. Furthermore, a general biosynthetic scheme and a prediction of the mechanism underlaying the loss of anthocyanisn in yellow raspberries can be predicted

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The Role Of MYB Factors In Fruit Color

Fouche M. (1), *Denoyes B. (1)*, Rothan C.(1), Vignes M. (2), Mangin B. (2), Petit A. (3), Chartier P. (3), Schwab W. (4), Monfort A. (5), Villatoro C. (5)

(1)INRA, UMR 1332, Université de Bordeaux, Villenave d'Ornon, France
(2) INRA,BIA, Toulouse, France
(3) Ciref, Douville, France
(4) TUM, Munich, Germany
(5)IRTA, Barcelone, Spain

Fruit quality traits included color are major breeding targets in the Rosaceae. In order to have better knowledge on genetic control of fruit color, we analysed a segregating population and genetic resources in the cultivated octoploid strawberry for skin fruit color. In addition, color of the juice was measure with a spectrophotometer. Results highlighted one region, in which in several QTL linked to fruit color co-localized. Based on the genome *Fragaria* sequence, the QTL region included two MYBs that were not yet studied. Expression patterns and transitory expression of these MYB were performed. Results suggested the role of one these MYB in the control of fruit color. Considering the level of ploidy of strawberry, only one allele out of the eight present in the parent may control the QTL linked to color. In addition, using a marker linked to the region that included the MYB, we showed an association between this marker and fruit color assessed visually in genetic resources. We believe that the mechanisms unraveled in the present study may play a crucial role in the variations of fruit color in other Rosaceous species.

AGER Project: Development and use of molecular markers and selection of new varieties with multiple resistance traits

G. Cipriani[1], Tartarini S.[2], Gianfranceschi L.[3], Pellegrino S.[4], Berra L.[4], Falginella L.[1], Gregori R.[2], Troggio M.[5], Velasco R.[5]

[1] Dipartimento di Scienze Agrarie e Ambientali, University of Udine, via delle scienze 208, Udine, Italy
[2]Dipartimento Colture Arboree, University of Bologna, viale Fanin 46, Bologna, Italy
[3]Dipartimento di Bioscienze, University of Milano, via Celoria 26, Milano, Italy
[4]Creso – Centro ricerche per la frutticoltura piemontese, Cuneo, Italy
[5]Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy

One of the main objectives of apple breeding programs worldwide is the introgression of disease and pest resistances into cultivated apples in order to reduce the use of chemicals thus reducing human health and environment concerns. Apple scab has always been the most important disease but nowadays other diseases deserve our attention: powdery mildew, fire blight and, more recently, Apple Proliferation (AP). In the frame of the Italian AGER project funded by a group of Italian Bank Foundations, some research topics have been addressed with the following aims: (a) to identify molecular markers bracketing disease resistance genes to improve breeding efficiency; (b) to create breeding selections combining different resistances; (c) to produce parental genotypes which combine resistance to the common apple diseases with elevated fruit quality standards, comparable, if not superior, to those of the current commercial cultivars. All the research activities will take advantages from the recent availability of the apple whole genome sequence. Resistance genes from new sources of resistance are going to be characterized and mapped. Marker-assisted selection is going to be applied to allow selecting the most promising genotypes in segregating progenies specifically prepared to "pyramid" resistance genes from different sources as well as to combine resistances to different pests, in the same genotype.

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The event is supported by

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