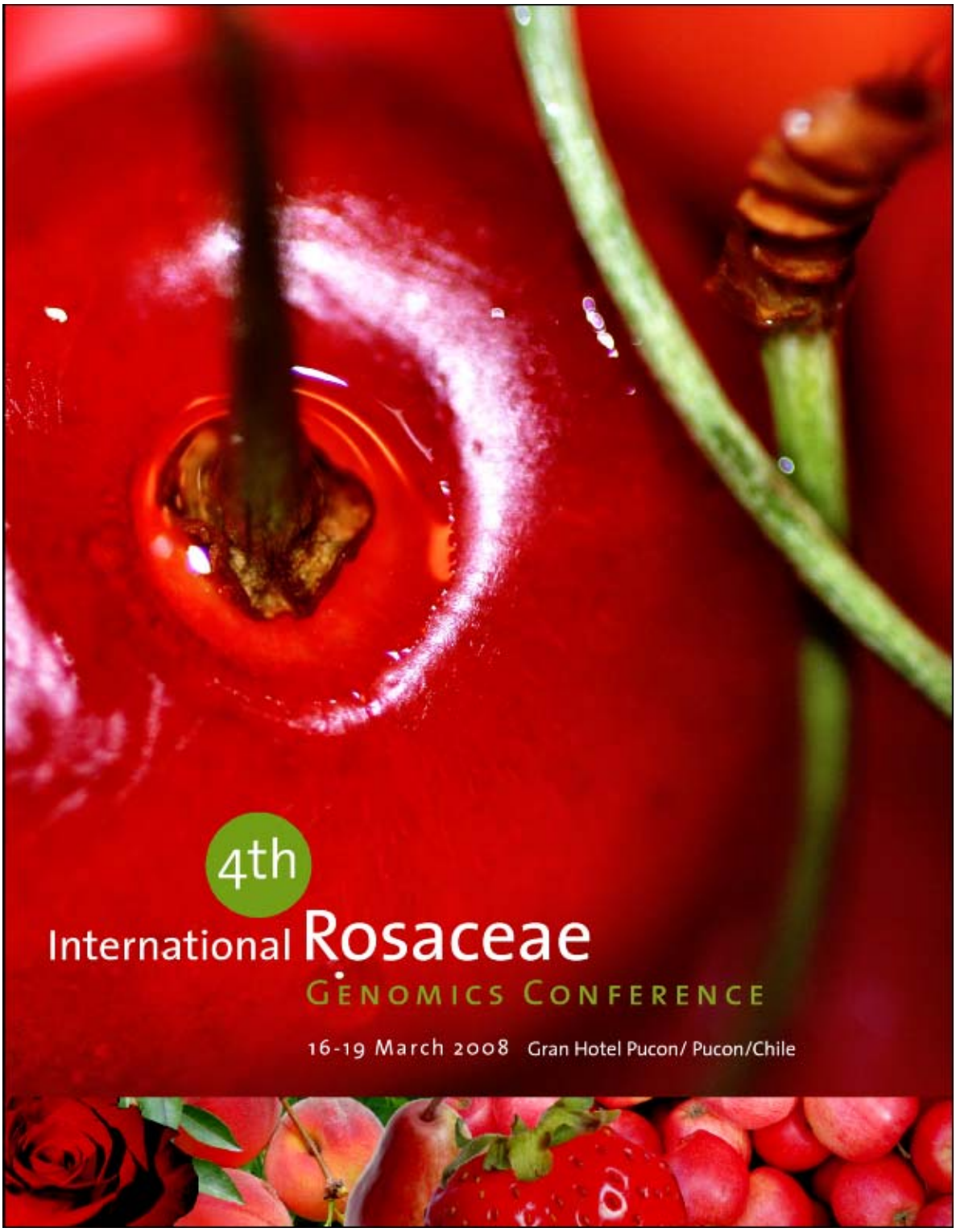
Pyramiding mono and polygenic resistances is one strategy to provide lasting control of the resistance to the green peach aphid and powdery mildew in peach

Patrick Lambert, Marie-Hélène Sauge, Jean-Luc Poessel, Thierry Pascal


4th
International Rosaceae
Genomics Conference
16-19 March 2008 Gran Hotel Pucon/ Pucon/Chile

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Session Chairpersons

Plenary Conference I
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Chair: **Ariel Orellana**

Session I – **Functional Genomics I**
Chair: **Sue Gardiner**

Session II – **Rosaceae Biology**
Chair: **Reinaldo Campos**

Session III – **Functional Genomics II**
Chair: **Vladimir Shulaev**

Session IV– **Molecular Markers and Quality Trait Mapping**
Chair: **Lee Meisel**

Session V – **Funtional Genomics III**
Chair: **Pere Arús**

Session VI– **The Future of Rosaceae Genomics**
Chair: **Kevin Folta**

Session VII– **Functional Genomics IV**
Chair: **Bryon Sosinski**

Session VIII & IX – **Genomics of Disease Resistance**
Chair: **Amy Iezzoni**

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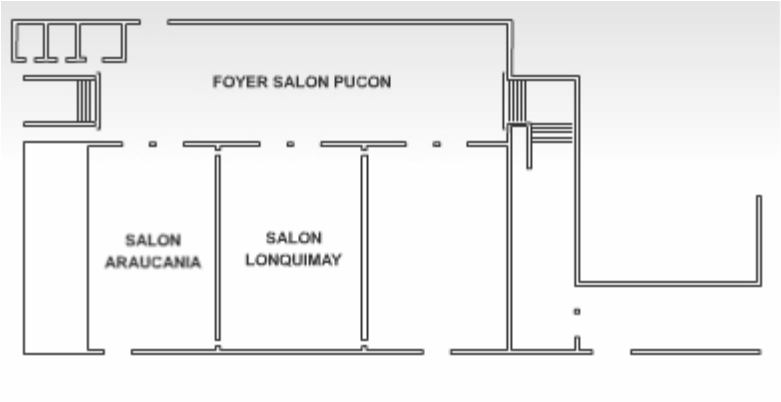

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Program

Sunday March16th

Registration Desk opens – Foyer Convention Center *11:00- 16:00*

Lunch- Caburga Restaurant *12:00- 15:00*

Session I – Araucania Salon *16:00- 17:40*
Functional Genomics I
Chair: **Sue Gardiner**

Kevin M. Folta (University of Florida, USA) 16:00- 16:20
Deep Sequencing the Fragaria Transcriptome

Arnaud Remay (INRA Cedex, France) 16:20- 16:40
Rose flowering: in the search for the recurrent blooming gene

John W. Forster (Victorian AgriBiosciences Centre, Australia) 16:40- 17:00
In vitro SNP discovery in the cultivated octoploid strawberry (*Fragaria x ananassa*)

Paula Pimentel (University of Talca, Chile) 17:00- 17:20
Searching for differentially expressed tags involved in softening of *Fragaria chiloensis* fruit

Vladimir Shulaev (Virginia Polytechnic Institute, USA) 17:20- 17:40
Mapping insertional mutants of the diploid strawberry, *Fragaria vesca*, through SNP discovery in flanking regions

Coffee break – Foyer *17:40- 18:10*

Session II – Araucania Salon *18:10- 19:10*
Rosaceae Biology
Chair: **Reinaldo Campos**

Pere Arús (IRTA, Spain) 18:10- 18:30
Variability and linkage disequilibrium in European and North-American peach cultivars

Amit Dhingra (Washington State University, USA) 18:30- 18:50
Cytoplasmic Fingerprinting of the U.S. Fragaria Core Collection

Donato Giannino 18:50- 19:10
(National Research Council of Italy (CNR), Italy)
Peach (*Prunus persica* [L.] Batsch) *KNOPE1*, a class 1 *KNOX* ortholog to Arabidopsis *BREVIPEDICELLUS/KNAT1*, is misexpressed during hyperplasia of leaf curl disease

Welcome – Araucania Salon *19:30*
Reception – Terrace
(Note: if it is raining, this will be held in the Calafquen Restaurant)

Monday March 17th

Breakfast – Caburga Restaurant from 7:30

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Chair: **Herman Silva**

Bryon Sosinski (NC State University, USA).
Sequencing of the Peach Genome

Session III – Araucania Salon *9:00- 10:20*
Functional Genomics II
Chair: **Vladimir Shulaev**

Abhaya Dandekar (University of California, Davis, USA) 9:00- 9:20
Functional Genomics of Apple Fruit Quality

Kevin M. Folta (University of Florida, USA) 9:20 - 9:40
Evaluating Novel Rosaceae Gene Function in Transgenic Strawberry and Arabidopsis

Ricardo Nilo (Andres Bello University, Chile) 9:40 - 10:00
Prunus persica fruit ripening assessment using a proteomics approach

Werner Howad (IRTA, Spain) 10:00- 10:20
Bin mapping candidate genes and SNP discovery in Prunus

Antonio Granell (IBMCP CSIC-U. Politécnica, Valencia, Spain) 10:20- 10:40
Genomics of the response of peach fruit to chilling temperaturas

Coffee break – Foyer *10:40- 11:10*

Session IV– Araucania Salon *11:10- 12:50*
Molecular Markers and quality trait mapping
Chair: **Lee Meisel**

Tetyana Zhebentyayeva (Clemson University, USA) 11:10- 11:30
QTL analysis of chilling requirement

Emily Buck (HortResearch, New Zealand) 11:30- 11:50
Transferability of Rosaceae Molecular Markers to Rubís

Ebenezer Ogundiwin (University of California, Davis, USA) 11:50- 12:10
Detailed QTL and candidate gene analysis of internal breakdown in peach fruit

Amy Iezzoni (Michigan State University, USA) 12:10- 12:30
Development of gene-based markers for linkage map construction and QTL analysis in sweet cherry

Andreas Peil (Julius Kühn Institute, Germany) 12:30- 12:50
High speed apple breeding – from vision to reality

Lunch – Caburga Restaurant *13:00- 15:00*

Poster Session I – Lonquimay Salon *15:00- 17:00*
Beer & Snack

rosIGI meeting – Araucania Salon *17:00- 18:00*

Dinner – Caburga Restaurant *19:00-22:00*

Tuesday March 18th

Breakfast – Caburga Restaurant from 7:30
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Chair: **Ariel Orellana**

Riccardo Velasco (IASM Research Center, Italy).
The Golden Delicious Apple Genome Sequencing Project: progress and perspectives

Session V – Araucania Salon 9:00- 10:20
Funcional Genomics III
Chair: **Pere Arús**

Amit Dhingra (Washington State University, USA) 9:00 - 9:20
International Consortium for Apple Genome Sequencing

Lee Meisel (Andres Bello University, Chile) 9:20 - 9:40
In fruta trans-activation of a cold-inducible peach promoter by a peach CBF functional ortholog - a platform for functional analyses of transcription factors in fruits

Daniel J. Sargent (East Malling Research, United Kingdom) 9:40- 10:00
Development of gene-specific markers from *Malus pumila* mRNA sequences and transferability and mapping in the other rosaceous genera

Thomas Davis (University of New Hampshire, USA) 10:00- 10:20
Subgenome Signatures: Identifying Diploid Contributions to the octoploid Strawberry (*Fragaria x ananassa*) Genome

Coffee break – Foyer 10:20- 10:50

Session VI– Araucania Salon 10:50- 12:00
The Future of Rosaceae Genomics
Chair: **Kevin Folta**

Amy Iezzoni (Michigan State University, USA) 10:50- 11:10
Pedigree Based Analysis: Our experiences using multiple pedigreed populations in sweet cherry

David Chagné (HortResearch, New Zealand) 11:10- 11:30
Single nucleotide polymorphism marker development in apple: Progress and applications

Henk Schouten (Wageningen University, The Netherlands) 11:30- 11:50
Cisgenesis and Intragenesis in Rosaceae Crops

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Functional Genomics IV

Chair: **Bryon Sosinski**

Elisabeth Dirlewanger (INRA, France) 12:00- 12:20
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Alberto Vecchietti (Parco Tecnologico Padano, Italy) 12:20- 12:40
Cloning and expression of peach aroma candidate genes and peach aroma microarray advancing

Akihiro Itai (Tottori University, Japan) 12:40- 13:00
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Poster Session II– Lonquimay Salon *15:00- 17:00*
Beer & Snack

Session VIII – Araucania Salon *17:00- 17:40*

Genomics of Disease Resistance

Chair: **Amy Iezzoni**

Véronique Decroocq (INRA, France) 17:00- 17:20
The SharCo consortium: Towards sharka containment

Angela M Baldo (USDA-ARS & Cornell University, USA) 17:20- 17:40
Resistance Gene Analogs in Rosaceae: Family-wide classification Including Raspberry, Cherry, and Wild Apples

Gala Dinner – Huife Hot Spring *18:00*

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Genomics of Disease Resistance
Chair: **Amy Iezzoni**

Derik Jiwan (Washington State University, USA) 8:30-8:50
Comparative and Functional genomics of *Powdery Mildew* resistance in Rosaceae

Patrich Lambert (INRA-Cedex, France) 8:50-9:10
Pyramiding mono and polygenic resistances is one strategy to provide lasting control of the resistance to the green peach aphid and *Powdery Mildew* in peach.

Veronique Decroocq (UMR INRA-Université Bordeaux) 9:20- 9:40
Allelic diversity of recessive resistance genes to sharka disease in Prunoideae

Final Discussion & Conclusions – Araucania Salon *9:40- 11:30*
Chair: **Members of the Scientific Committee**

Additional Activities:

ERGI (European Rosaceae Genomics Initiative) meeting
Contact: **Dr. Alberto Vecchietti**
Araucania Salon, Monday March 17th 21:00

Mini-workshop on Bin-set development (RosPOP activity)
Contact: **Dr. Cameron Peace, C.and/or Dr. Werner Howad**
Araucania Salon, Sunday March 16th 13:30-15:30

Plenary Abstracts

Sequencing of the peach genome

Sosinski, B.; Abbott, Albert; Main , Dorrie ; McCombie, Richard
NC State University
sosinski@ncsu.edu

In the dicots, the model *Arabidopsis* genome is clearly the research standard for identification and characterization of important plant genes, however, as a useful comparative genomics model for Rosaceous species, it has limitations. There are traits and genes, primarily those for fruit, growth habit, and life history traits (woody vs. herbaceous growth, annual vs. perennial) that may not have recognizable homologues in the *Arabidopsis* genome. Indeed initial EST efforts suggest that as many as 24% of the ESTs that we have characterized from peach fruit are orphans in the databases, showing no significant homology with genes from *Arabidopsis.* For this reason, it is now recognized that it is necessary to study and sequence model plants from other important families to develop a universally complete plant genome database. In this regard, there is little doubt that Rosaceae is an important family, and peach is ideally positioned as a model species.

Our approach for sequencing the peach genome will combine both a whole genome shotgun strategy with a minimal tile of the BACs comprising the peach physical map to provide a cost-effective, but effective means of obtaining this midsize genome. BACs from a tiling path will be pooled, and shotgun sub-libraries made for sequencing (Cai et al. 2001). The pooling approach will lower sheared library expenses by 10-fold over the course of the project, and the complexity of the genome strongly suggests that reconstruction will be achievable. Any pools of BACs that fail to assemble can be deconstructed for individual sequencing. While this approach will cost slightly more than a WGS approach, the data from other genome sequencing projects indicates that we will obtain a much more robust and accurately assembled genome. Moreover, this approach will let us selectively target certain areas of the genome for subsequent additional sequencing due to increased biological interest. It will likewise provide a more robust template for subsequent finishing if that becomes needed or feasible. Our goal is to obtain high-quality reference sequence for the coding regions of the peach genome. This resource will be of immediate utility to the Rosaceae community, as well as to those interested in comparative genomics and evolution. Based upon the current build of our physical map and the EST hybridization data, we estimate that we will have to generate pools for approximately 250 contigs, each with an average of 10 BACs in order to capture the majority of the gene space.

Keywords: Peach, genome sequencing

The Golden Delicious Apple Genome Sequencing Project: progress and perspectives

Velasco R R [1], Zharkikh A [2], Troggio M [1], Pruss D [2], Costa, F. [1], Bhatnagar S [2], Pindo, M. M [1], Lanchbury, J. [2], Micheletti, D. [1], Coppola, G. [1], Mraz, A. [5], Stormo, K. [5], Tao, Q. [5], Bogden, R. [5], Magnago P [1], Komjanc M [1], Harkins T [4], Malnoy M [1], Cestaro A [1], Baldi, P. [1], Salvi, S., S. [1], Fontana P [1], Gutin [2], Affourtit J [3], Salamini F [6], Egholm M [3], Skolnick M [2] and Viola, R [1]

[1]IASMA Research Center, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
[2]Myriad Genetics Inc, 320 Wakara Way, Salt Lake City, Utah 84108, USA
[3]454 Life Sciences Corp, 20 Commercial Street, Branford, Connecticut 06405, USA
[4]Roche Diagnostics Corp, Roche Applied Science, 9115 Hague Road, Indianapolis, Indiana 46250, USA
[5]Amplicon Express Inc., 2345 NE Hopkins Court, Pullman, Washington 99163, USA
[6]Technology Park Lodi, Località Cascina Codazza, Via Einstein, 26900 Lodi, Italy

Apple is one on the most diffuse fruit crop over the temperate climates, and one of the most important representatives of the Rosaceae large family. In our region of Italy, Trentino Alto-Adige, apple represents the most important agricultural resource. We have therefore concentrated our efforts on apple genome sequencing, selecting the elite cultivar Golden Delicious, grown worldwide and representing over 80% of apple in Trentino.
Our research has the multiple goals of genome assembly, gene identification and annotation, and identification of a maximum number of polymorphisms. Golden Delicious is highly polymorphic with two clearly distinguishable haplotypes, expecting to reveal several million SNPs and small indels. They represent a substantial resource for molecular breeding programs, as well as trait and QTL marker association. Based on our previous experience on the large heterozygous grapevine genome, we have used novel algorithms to address this challenge, which will be applied also to the apple genome. A total coverage of 4 genome equivalents of libraries of ascending size sequenced by the Sanger method, coupled with 10 genome equivalents of 454 Life ScienceTM sequences, will allow us to create an effective genome sequence. Assembly will be based on adding sequences of a BAC libraries and a fosmid library, end-sequenced to assemble large meta-contigs. Contigs will be oriented and ordered on appropriate chromosomes by high throughput marker development and genotyping in an F1 cross of Golden delicious x Scarlet.
Currently, over 3 billions of nucleotides and 1,2 billions of pyrosequencing, Sanger and 454 technique, have been produced. Further 6 billions of nucleotides will be developed to the final goal of 14 genome equivalents of apple (4x Sanger and 10x pyrosequencing, respectively). Following ESTs clustering in tentative consensus (TCs) and TC blast against the genome sequence, 2,000 SNPs of Golden Delicious are currently under development. Sequencing and mapping data will be public available at IASMA, NCBI and GDR databases.

Keywords: Apple, Sequencing genome

Oral Abstracts

Session I

Deep Sequencing the Fragaria Transcriptome

Clancy, M.; Kumar, D.; Chatterjee, M.; Wang, H.; Mishra, V.; Davis, T.Folta , K.

An extensive accounting of strawberry (Fragaria spp.) expressed sequences in strawberry as undertaken using 454 sequencing. A barcoding approach allowed deep sequencing of expressed sequences from various tissues. To increase coverage, tissues were pooled from separate plants exposed to a discrete enviornmental condition, such as darkness, heat, cold, far-red light, gibberellin treatment, spider mites, mechanical damage, in addition to 20 other treatments. Tissue was taken 1 and 24 h time points following treatment to identify primary and late response transcripts in these processes. Entire plants from *Fragaria vesca* and *Fragaria iinumae* (putative subgenome donors) were included to assess the allelic variation between these species and the octoploid. The results of this endeavor allow accounting of allele-specific expression in various plant tissues and developmental contexts, while greatly increasing the EST resources in the genus.

Keywords: Fragaria, 454 sequencing

Rose flowering: in the search for the recurrent blooming gene.

Remay, A.*, Lalanne, D., Thouroude, T., Hibrand-Saint Oyant, L. and Foucher, F.
UMR1259 Génétique et Horticulture (INRA, INH, UA) - BP60057 – 49071 Beaucouzé cedex – France
*Contact: arnaud.remay@angers.inra.fr

In perennial plants, genetic control of recurrent blooming remains unknown. Unravelling the molecular determinism of this character could allow its introduction in perennial plants of economic interest. Flowering control is well described in monocarpic model plants as Arabidopsis. Gibberellins (GAs) are key hormones in the control of flowering. In rose, GAs inhibit blooming only in non recurrent genotypes. Concerning the molecular control of recurrent blooming (RB), two hypotheses can be proposed: (i) the genes controlling this process are similar to those involved in floral initiation in annual plants (i.e. RB is a particular pathway of floral initiation); (ii) RB is a specific process to perennial plants and involves new unknown genes.
Our objective is to isolate and characterise the genes involved in the control of the recurrent blooming in rose.
Using a candidate gene approach, we have isolated in rose GA pathway genes or floral marker genes. The expression of the FT rose homologue is associated with the morphological changes observed during the floral initiation. The RoSPY and RoRGA, rose homologues of SPY and RGA, localise genetically with the RB locus; and three others genes of the GA pathway are differently regulated between recurrent roses and non recurrent roses. Moreover, different alleles of RoRGA and RoSPY exist within our genotypes. Functional studies are initiated in heterologous system for these candidates.
A second approach is developed using a 4.8kb Affymetrix® chip. New candidates for RB are looked for by differential display between floral and vegetative apices or between recurrent and non recurrent rose apices. Analyses of hybridization results are under progress.
Genes controlling flowering time in Arabidopsis are conserved in rose and the GA pathway play a key role in the control of recurrent blooming. Further analysis and functional validations should precise the role of those genes in recurrent blooming process and their common features with floral initiation of annual plants.

Keywords: Rose, Floral initiation, Recurrent blooming, Gibberellins, Transcriptomics

In vitro SNP discovery in the cultivated octoploid strawberry (*Fragaria x ananassa*)

Kaur, S.; Todorovic, M. ; Cogan, N.O.I ; Spangenberg, G.C. ; Forster, J.W.
[1]Biosciences Research Division, Department of Primary Industries, Victorian AgriBiosciences Centre, john.forster@dpi.vic.gov.au

The cultivated strawberry, *Fragaria x ananassa* (2n = 8x = 56), is an important fruit crop. Despite economic value and potential utility as an experimental system, little detailed information is available on genetic and genomic architecture. Different octaploid genomic composition models, such as AABBBBCC, AAA'A'BBBB and AAA'A'BBB'B, have been proposed, with a consensus toward the latter allopolyploid model based on origin from four diploid progenitors. The ancestors of the octoploid genome are poorly characterised, although the contemporary diploids *F. vesca* and *F. iinumae* have been implicated. Enhanced knowledge of genome structure will accelerate development of molecular markers and clarification of sub-genome identity. Nucleotide sequence variation between two cultivars of cultivated strawberry (Kalinda and Bunyarra) has been identified through amplicon resequencing, as a step towards the development of single nucleotide polymorphisms (SNPs) as highly informative functionally-associated genetic markers. A total of 86 potential candidate genes associated with day-neutrality and fruit quality were identified from public and proprietary DNA sequence databases. Five genes were selected for initial analysis: sorbitol transporter, aminotransferase, alcohol dehydrogenase, anthocyanidin synthase and a putative flowering time locus C orthologue. Primers were designed from the template sequences and fragments were amplified, cloned and sequenced. Sequence clustering analysis was performed to identify predicted paralogous sequence variants (PSVs), homoeologous sequence variants (HSVs) and allelic SNPs. Novel strategies for improved SNP discovery based on progenitor comparison and highly-parallel sequencing are being developed. Validated SNPs will provide valuable diagnostic markers for application in varietal development programs.

Keywords: strawberry, single nuceotide polymorphism, haplotype, candidate gene, homoeologous sequence variation.

Searching for differentially expressed Tags involved in softening of *Fragaria chiloensis* fruit

Pimentel, P., Figueroa, C., Salvatierra, A., Gaete, C., Herrera, R., Moya-León, M.A.
Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile.

The Chilean strawberry fruit (*Fragaria chiloensis* L. (Duch.)) is a promisory fruit product for Chile. Its postharvest quality attractiveness is associated to the higher sweetness and aroma properties than the commercial strawberry. Ripening of strawberry fruit is associated with changes in texture, leading to an extensive softening which limits its post harvest life. During softening a partial dissembling of the fruit cell wall takes place which is associated with the coordinated action of many cell wall degrading enzymes. So far, we have investigated the role of key enzymes during softening identifying potential candidate genes to assist strawberry breeding programmes. In order to broad our knowledge on fruit softening we have generated several suppression substractive hybridization (SSH) libraries. By contrasting different developmental and ripening fruit stages, including large green (firm fruit, stage 2), large white (stage 3) and ripe fruit (soft fruit, stage 4), six SSH libraries were built containing 1809 differentially expressed cDNAs (ESTs). BLAST analysis of the ESTs revealed that 90% showed similarity to known sequences within the plant kingdom. The global analysis of the cDNAs sequences revealed significant homology with genes involved in different plant processes, such as primary and secondary metabolism, development, signal transduction, cell wall loosening and genes induced in response to plant growth regulators or environmental stress. The expression analysis of interesting cDNAs related to fruit softening have revealed a correlation between the increase in expression of the gene and the loss of firmness observed in *Fragaria chiloensis* fruit.
Acknowledgements to Anillo ACT-41 project for financial support and Conicyt for a doctoral fellowship to P. Pimentel.

Keywords: Chilean strawberry, ESTs, supression substractive library, softening

Mapping insertional mutants of the diploid strawberry, *Fragaria vesca*, through SNP discovery in flanking regions

Ruiz-Rojas, J.J. ; Pattison, J. ; Sargent, D.; Oosumi, T.; Shulaev, V. ; and Veilleux, R.
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potato@vt.edu

Insertional mutant collections are essential tools in plant genomic research and functional genomics. Extensive collections in Arabidopsis and rice are used to gain intimate knowledge of gene function. These collections are less useful for understanding gene function and expression of native genes associated with the development of fleshy fruit that comprise the edible product of many horticultural crops. We have developed an insertional mutant collection of the diploid strawberry, *Fragaria vesca*, as a model crop for fleshy fruit. An accession of *F. vesca*, selected for photoperiod insensitivity, short life cycle and facility of Agrobacterium-mediated transformation, has been transformed with four different constructs resulting in several thousand independent knock-outs. Using TAIL PCR, we have generated sequences of the flanking regions adjacent to T-DNA insertions for 20 mutant lines. SNP discovery within these flanking regions between the parents of a strawberry mapping population and subsequent segregation analysis has allowed us to place 12 mutants on the genetic map yielding at least one insertional mutant for each of the seven linkage groups. Efforts are underway to map more of the mutants for broader chromosome coverage.

Keywords: Rosaceae, knock-out mutants, TAIL PCR, CAPS, fruit crops

Session II

Variability and linkage disequilibrium in European and North-American peach cultivars

Aranzana, M. J. ; Kadri, A. E.; Howad, W. and Arús, P.
IRTA. Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB
pere.arus@irta.es

Peach (*Prunus persica* (L.) Batsch) is one of the most economically important fruit crops that, due to its genetic and biological characteristics (small genome size, taxonomic proximity to other important species and short juvenility period), has become a model plant in genomic studies of fruit trees. Recent studies show a growing interest in using linkage disequilibrium (LD) as a tool for fine-scale mapping. The extent of LD around a gene has important implications in association mapping, since it determines the effectiveness of this approach. Thus, a good knowledge of LD extension in the working species is required for successfully attempting association mapping studies. Population Structure analysis based on data obtained with 46 SSRs covering the whole peach genome, show 3 main populations in our sample of 225 peach varieties, one mainly including peaches, one nectarines and one non-melting peaches and ancient cultivars. Indeed, these groups of varieties show different levels of variability, measured in terms of number of alleles (A) and observed heterozygosity (Ho), being the latter the group with highest A but lowest Ho. Further divisions of the varieties in groups attending to their fruit characteristics show, for example, that those with yellow flesh present lower A and higher Ho than the white flesh fruits studied here. According to this distribution of variability we have measured LD extension for the different groups of varieties and found it to be generally very high (up to 10-20cM). These data, combined with the phenotypic information available, will help us to select appropriate sets of genotypes for further association mapping studies.

Keywords: microsatellites, Prunus, association genetics, subpopulation structure.

Cytoplasmic Fingerprinting of the US Fragaria Core Collection

Dhingra, A.;Njuguna, W; Ramdial, J; Wildenstein, C; Bassil, N; Hummer, K
Washington State University and Oregon State University
adhingra@wsu.edu

The genetic material resides in three distinct compartments within a plant cell. The nuclear DNA undergoes recombination when transmitted to the progeny while mitochondrial and chloroplast DNA is inherited maternally in most cases. Thus cytoplasmic DNA information passes from generation to generation unaltered. Consequently this information can serve as tractable means to identify a species. We have had a long term interest in utilizing chloroplast genome sequence information for fingerprinting and resolving phylogenetic issues. The Fragaria core collection, which consists of accessions belonging to varied poloidy levels, has been analyzed for its cytoplasmic content using four distinct regions present in the inverted repeat of the chloroplast genome. Two of these regions are variable and the other two are stable present in the 16S operon. There are 46 Fragaria accessions and 2 closely related species namely Potentilla and Duschenea in the analysis dataset. The chloroplast regions were amplified and sequenced directly. Sequence editing was performed using Sequencher. We have identified unique cytoplasmic clades with in the collection that can serve as fingerprinting beacons along with other identification technologies.

Keywords: Fragaria, Chloroplast, Fingerprinting, Rosaceae, Genotyping.

Peach (*Prunus persica* [L.] Batsch) *KNOPE1*, a class 1 *KNOX* ortholog to Arabidopsis *BREVIPEDICELLUS/KNAT1*, is misexpressed during hyperplasia of leaf curl disease.

Testone, G. [1] ; Bruno, L. ; Condello, E.[1]; Chiappetta, A. ; Bruno, A. ; Mele, G.[1] ; Tartarini, A. ; Spanò, L. ; Innocenti, A. M. ; Mariotti, D.[1] ; Bitonti, M. B.; and Giannino , D. [1]

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Class 1 KNOTTED-like transcription factors (KNOX) control cell meristematic identity. We addressed whether they maintain this function in peach plants and might act in leaf curliness caused by the ascomycete Taphrina deformans. KNOPE1 function was assessed by overexpression in Arabidopsis and by yeast two hybrid assays with Arabidopsis BELL proteins. Subsequently, KNOPE1 mRNA and zeatin localisation were monitored in fully expanded leaves during leaf curl disease. KNOPE1 and Arabidopsis BREVIPEDICELLUS (BP) proteins fell into the same phyletic group and recognised the same BELL factors. 35S:KNOPE1 Arabidopsis lines exhibited altered traits resembling those of BP-overexpressing lines. In peach shoot apical meristem, KNOPE1 was expressed in the peripheral and central zones but not in leaf primordia, identically to BP expression pattern. These results strongly suggest that KNOPE1 must be down regulated for leaf initiation and that it can control cell meristem identity equally as well as all class 1 KNOX genes. Leaves attacked by T. deformans share histological alterations with class 1 KNOX-overexpressing leaves, including cell proliferation and loss of cell differentiation. Both KNOPE1 and a cytokinin synthesis ISOPENTENYLTRANSFERASE gene were found to be up-regulated in infected curled leaves. At early disease stages, KNOPE1 was uniquely triggered in the palisade cells interacting with sub-epidermal mycelium, while zeatin vascular localisation was unaltered compared with healthy leaves. Subsequently, when mycelium colonization and asci development occurred, both KNOPE1 and zeatin signals were scattered in sectors of cell disorders. These results suggest that KNOPE1 misexpression and de novo zeatin synthesis of host origin might participate in hyperplasia of leaf curl disease.

Keywords: Peach, KNOPE1, Arabidopsis

Session III

Functional Genomics of Apple Fruit Quality

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We investigated apple fruit quality by silencing key pathways like ethylene or sorbitol biosynthesis via gene silencing or chemical treatments such as 1-MCP. These alterations result in unique fruit quality phenotypes, most notably affecting sugar-acid accumulation in sorbitol-silenced fruit and changes in flavor metabolism in ethylene-silenced fruit. Phenotypically distinct tissues were obtained from fruit subjected to different ripening treatments that included wild type, transgenic, and chemically treated fruit (ethylene gas or 1-MCP), each displaying significant differences in phenotype and/or ethylene response. We compared a sorbitol-suppressed line with a control that translocated sorbitol normally to fruit. Differences in phenotypes and tissues were used to classify changes in gene expression patterns derived from microarray analysis of RNA isolated from these tissues and to identify ethylene- or sorbitol-regulated genes in apple fruit tissues. We designed a Combimatrix 12K custom microarray that contains 8,976 apple genes from the UniGene Build#14 which were derived from 34 fruit libraries, with an additional 163 genes of interest to us. A total of 12,395 probes were synthesized, of which 474 (4%) were control probes and 11,921 (96%) were apple probes. Oligo nucleotides were synthesized on the surface of the chip. Differential gene expression patterns identified 3204 genes significantly ($p < 0.05$) regulated by ethylene and 141 significantly ($p < 0.05$) regulated by sorbitol. Of these, 57 were regulated by both ethylene and sorbitol. A vast majority (67.5%) of the ethylene-regulated genes were expressed specifically in peel tissues: 20.5% were expressed in cortex and 12% were expressed in both tissues. To better define these genes, we categorized their expression patterns by treatment and functional categorization based upon MapMan, using Arabidopsis gene annotations to define the genes and pathways involved. We also performed a gene set enrichment analysis to identify functional categories that were significantly regulated by ethylene in peel and cortex.

Keywords: Apple, Fruit Quality, Gene Silencing , Microarray Analysis , Ethylene .

Evaluation of Novel Rosaceae Gene Function in Transgenic Strawberry and Arabidopsis

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The Rosaceae Family contains valuable fruit, nut and ornamental crops. Between 10-15% of the Rosaceae unigene is composed of transcripts that defy functional classification. It is possible that these encode the genetic elements that underlie the unique traits of the Rosaceae Family. Strawberry (Fragaria spp.) is a member of the Rosaceae with a rapid cycling and compact growth habit. It maintains a tremendously small haploid genome, it is routinely transformed and may be propagated by seeds or runners. For these reasons the strawberry is an outstanding system to study gene function, especially with regard to validation of rosaceous constructs. In this project 454 cDNA sequencing allowed deep description of the Fragaria transcriptome. Novel transcripts (lacking identifiable domains and no homology to transcripts outside of the family) were identified and shuttled into gateway-based vectors to overexpress the novel transcript in Arabidopsis and diploid strawberry (*Fragaria vesca*). The same constructs were suppressed in strawberry using RNAi. The resulting plants were examined in a series of revealing physiological tests. The results indicate that many of the previously unclassified transcripts have potent biological consequences upon overexpression- producing an array of phenotypes. Severe defects in leaf development, trichome emergence, rosette architecture, flower patterning and flowering time were observed among many other phenotypes. Here a genomics-level approach leverages the strengths of high-throughput sequencing, rapid cloning strategies, the most efficient transformation systems and careful physiological analysis to understand the roles for novel expressed genes in this valuable plant family.

Keywords: Arabidopsis, Rosaceae family, 454 sequencing

Prunus persica fruit ripening assessment using a proteomics approach.

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The final step of fruit ripening in *Prunus persica* melting varieties is characterized by a number of physiological changes including softening, changes in sugar content, color, etc. All these processes involve many different metabolic pathways, however, we still know little about the different pathways involved in this process. In order to get a better understanding of these metabolic processes, a proteomic approach was used to examine the behavior of proteins that are expressed in mature and ripe fruits. Thus three peach and two nectarine melting type varieties were used to extract the proteins and then separated them in 2-DE. Deep purple staining allowed us to visualize an average of 700 spots on each variety. A two-way analysis of variance led us to find that nearly 18% of the 553 spots analyzed in the gels presented changes between these conditions for all the five varieties evaluated, including the already described endopolygalacturonase, pyruvate decarboxylase and the ACC oxidase 1, which are up-regulated, and the down-regulation of proteins such a Cu-Zn superoxide dismutase. Using multivariate analysis techniques, we were able to identify proteins that co-expressed closely with these proteins, which will help us to gain further insight in the metabolic pathways that are actively participating in the ripening process. On the other hand, we have determined the presence of a cluster of proteins that co-express in a variety specific fashion, which include proteins such as two small heat shock proteins, the eukaryotic translation initiation factor 5A and thioredoxin H. It is important to stress that the analysis of different varieties was crucial to discriminate changes that are related to the softening process or variety specific, which could not be done if only one variety was used in the assessment.
Supported by Millenium Nucleus in Plant Cell Biology ICM P 02-009-F, ICM P06- 065-F and UNAB DI 18-05/R

Keywords: Proteomics, *Prunus persica*, ripening

Bin mapping candidate genes and SNP discovery in Prunus

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The candidate gene (CG) approach is an efficient method to proof whether or not a certain gene sequence co-localizes with the position of mapped genes or QTLs for a trait of interest. In the European project ISAFRUIT more than 150 CGs, which are potentially involved in the expression of fruit quality traits, have been selected for mapping on the 'Texas' x 'Earlygold' (TxE) Prunus reference map. For that, we used the bin mapping strategy, where the genotypes of only eight plants (six selected individuals of the TxE F2 population plus two of its ancestors, the F1 hybrid plant and 'Earlygold') are sufficient to place any marker in a defined genome fragment (bin) of an average size of 7.8 cM. By using the bin mapping strategy, costs can be reduced to a minimum, which is of particular interest if a high number of CGs needs to be mapped. More than 100 CGs have already been placed on TxE bin map and an updated record of these results will be presented. Bin mapping has proven to be very efficient as about 90% of the analysed CGs could be mapped. At the same time the first data will be shown on the single nucleotide polymorphism (SNP) frequency in TxE, since most CGs were bin mapped by direct sequencing based on SNP variability. Another source for SNP discovery in Prunus derives from EST data that are available in the Genome Database for Rosaceae (GDR). By in silico sequence comparison of ESTs belonging to the same EST contig SNPs can be detected. Out of 716 SNP-containing EST contigs 19 were selected that encode for factors potentially involved in the expression of agronomic traits of interest. In order to evaluate the potential of these in silico SNPs, one or two per selected EST contig were chosen and the corresponding SNP genotypes were determined by pyro-sequencing in the TxE bin set as well as eight peach cultivars. Data on this in silico SNP analysis will be presented as well as final remarks on the outcome of different ways of SNP discovery in Prunus.

Keywords: Bin mapping, Candidate gene, SNP, Prunus, Reference map.

Genomics of the response of peach fruit to chilling temperatures

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We have developed a number of genomic tools that will contribute to the understanding of post-harvest quality-related processes in peach. The CHILLPEACH database includes a collection of 8,144 sequenced full-length enriched cDNAs from mesocarp of full-sib peach selections that are sensitive and tolerant to chilling injury, and corresponding bioinformatics information. Current stored data include: 1) EST processing and assembly results such as cDNA clone redundancy and unigene consensus sequences, 2) functional annotation such as BLAST results, GO terms, PFAM domains and EC numbers, etc., and 3) sequence features such as presence of SNPs or SSRs. This collection of cDNAs has the added value of being cloned in a GATEway vector that facilitates the rapid subcloning in a range of expression and gene silencing vectors to make assays of gene function much easier. Over 2,000 of these genes are new in Prunus and at least 379 have no Arabidopsis ortholog. Analysis of those sequences corresponding to an Arabidopsis ortholog indicated that more than 45% of them are likely to be full length. In addition, sequence information revealed the existence of 185 SSR, many of them new in Prunus. This information has been added to the currently available Peach EST sequences in order to design an oligo based array to be used by the Prunus community.

A CHILLPEACH™ microarray covering around 4,200 unigenes, developed in this project, has been printed. This microarray is currently used to identify changes at the transcript level which are associated with chilling injury during storage. Microarray hybridization using pool materials from normal fruit and fruit that were subjected to cold storage, already indicated the activation of a fruit specific program in response to cold that shares some components with cold aclimation in Arabidopsis but has also specific characteristics. Microarray analysis of the response of full-sib peaches to chilling temperatures will be reported and discussed.

Keywords: Peach, Microarray, chilling

Session IV

QTL analysis of chilling requirement

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The chilling requirement to release endodormancy in the vegetative and floral buds of temperate trees is an economically important trait that exhibits a continuous or quantitative variation. We are developing a genetic linkage map for chilling requirement in peach (*Prunus persica* L. [Batsch]). Two F2 populations from individually selfed F1 trees have been developed from two pairs of high and low chilling requirement parents. The cross currently used in mapping is Contender (high chill) × Fla92-2C (low chill) and consists of 378 F2 individuals. 83 SSR markers and 40 AFLP markers have already been used to construct a genetic linkage map which is largely in agreement with the general Prunus reference map. The qualitative trait showy/non-showy flower was mapped on linkage group 8 of this map. This is the first time that showy/non-showy flower has been genetically mapped. Blooming date of F2 individuals was scored in the spring of 2006 and 2007 and is segregating in a continuous fashion. Four significant QTLs for blooming date were detected: one in the middle of LG1 (LOD 15.22, 17.4% of phenotypic variance explained) and one at the end of LG1 (LOD 33.91, 36.3% of phenotypic variance explained); one in the beginning of LG4 (LOD 5.74, 7.1% of phenotypic variance explained); and one in the middle of LG7 (LOD 34.44, 36% phenotypic variance explained). Chilling requirement of F2 individuals was scored in year 2006/2007 (winter/spring) and its scoring in year 2007/2008 is ongoing.

Keywords: QTL analysis, genetic map, chilling requirement, peach.

Transferability of Rosaceae Molecular Markers to Rubus.

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Within the Rosaceae there is a wealth of genetic research available particularly for crops such as apple (*Malus X domestica*) and peach (*Prunus persica*). Through the use of comparative mapping, this information could be transferred to other species within the family, such as Rubus, where the research, in comparison, is less extensive. We present here the transferability of markers from several key Rosaceae species to Rubus, with future comparative mapping in mind. Over 150 EST-SSRs and genomic SSRs from peach, apple, pear and strawberry were screened over a subset of seedlings from two Rubus mapping populations; R. idaeus cv 'Latham' crossed with R. occidentalis derivative population (HortResearch, New Zealand) and a R. idaeus x parvifolius hybrid cross with R. idaeus cv 'Qualicum' population (North Carolina State University, US). Orthologous markers were identified from each major taxonomic group and their potential as framework markers for genetic mapping and trait dissection in Rubus will be discussed.

Keywords: Rubus, Raspberry, transferability, microsatellites, comparative mapping

Detailed QTL and candidate gene analysis of internal breakdown in peach fruit

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A major challenge to peach and nectarine industry is extending fruit shelf life while maintaining quality. Internal breakdown (IB) or chilling injury is the term used to describe a collection of physiological disorders occurring in peach fruit stored at cold temperatures. These disorders include mealiness, flesh browning, internal bleeding, loss of flavor, and development of off flavors. A dedicated genomics toolkit has been developed from two full-sib peach genotypes contrasting for IB for functional analysis of IB and for identification of IB-controlling genes. This toolkit, consisting of a ChillPeachTM EST database and cDNA microarray, is being used in combination with the candidate gene approach to identify IB-controlling genes via a fully characterized peach progeny population (Pop-DG) segregating for IB resistance. Previously, some QTLs were identified for IB using phenotypic data collected over three years on 51 progeny of Pop-DG. An additional year molecular marker and IB phenotypic data collection was made in the summer of 2007 on a larger population size of Pop-DG (150 progeny) to increase the statistical power of QTL detection and to identify new QTLs. Because mealiness, a key IB symptom, occurs only in freestone melting flesh (FMF) genotypes of Pop-DG and not in clingstone non-melting flesh genotypes (CNMF), 100 of the 150 progeny were FMF genotypes. These resulted in an expanded Pop-DG linkage map covering ~95% of the peach genome, validation of previously localized QTLs, and localization of additional QTLs controlling IB. Pop-DG map is collinear with TxE Prunus reference map. Seventy of the 77 ChillPeach SSRs that were novel to Prunus were mapped directly to the expanded Pop-DG map or bin-mapped to the TxE reference map. Over 80 additional ChillPeach unigenes differentially regulated in normal peach mesocarp tissue versus cold-treated tissue were similarly mapped to Prunus genome. Several ChillPeach unigenes were mapped to the vicinity of IB QTLs in addition to previously mapped candidate genes. Detailed analysis of a gene encoding leucoanthocyanidin dioxygenase enzyme (PpLDOX) that mapped to a major QTL for browning (qP-Brn5.1m), revealed strong evidence for its control of this IB symptom. Ongoing efforts on further mapping and functional characterization of IB-controlling genes through extensive IB microarray analyses, and the validation of IB QTLs obtained from Pop-DG in other peach progeny populations with different genetic backgrounds will be presented.

Keywords: ChillPeach, Internal Breakdown, chilling injury, microarray, candidate gene.

Development of gene-based markers for linkage map construction and QTL analysis in sweet cherry (*Prunus avium* L.)

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Linkage maps of the sweet cherry cultivar 'Emperor Francis' (EF) and the wild forest cherry 'New York 54' (NY) were constructed using primarily simple sequence repeat (SSR) markers from other Prunus linkage maps. However, as only 26% of the SSR markers could be placed on the EF or NY maps, we initiated an effort to develop gene-based markers for cherry using 170 previously mapped Prunus ESTs. From these 173 ESTs and 2 sequences of poplar homologous to tomato FW2.2 gene, we developed 27 CAP, dCAP and In/Del markers of which 25 were placed on the EF and NY linkage maps. Fruit size was evaluated from the EF × NY progeny in 2006 and 2007 and QTLs were identified on linkage groups two and six. The significant regions for both QTLs contained at least one of the newly derived EST-based markers. Because of our continuing need for more markers not only in cherry, but for comparative mapping within the Rosaceae, we initiated the development of Conserved Ortholgous Set (COS) markers. From a total of 412,827 Rosaceae ESTs, 30,801 putative COS were pre-selected after BLAST search against Arabidopsis single copy genes. The assembly of the Rosaceae COS candidates resulted in 7,573 Rosaceae COS of which 2,386 corresponded to Arabidopsis COS. These 2,386 Rosaceae-Arabidopsis COS will form the basis of our future gene-based marker development for comparative mapping within the Rosaceae.

Keywords: Cherry, linkage map, Conserved Ortholgous Set

High speed apple breeding – from vision to reality

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The most time-consuming factor in the apple breeding process is the long juvenile phase of seedlings which can take six to ten years and some times even more. During the juvenile phase apple seedlings are not competent to develop flowers and fruit. This is harmful for breeders and causes high expenses because the examination of fruit quality is the most important selection step. Furthermore, the seedling material needs to be maintained for years in the orchard occupying a large area. Shortened juvenility and precocious flowering are, therefore, important breeding goals. Beside floral-inducing techniques like retarding growth of the seedlings or the application of plant growth regulators, transgenic early flowering plants could help to overcome this problem. Recently it was shown that the BpMADS4 gene of silver birch induces early flowering in apple. Based on this fact we started an innovative breeding approach combining the advantages of transgenic early flowering trees and molecular markers as an efficient tool to speed up the selection process. Plants of BpMADS4 transgenic 'Pinova' apple lines were pollinated with pollen of the fire blight resistant wild species Malus fusca under greenhouse conditions in winter 2005/2006. Fruits were harvested in fall 2006 and seeds were sown in spring 2007. All seedlings were screened by PCR for the presence of the BpMADS4 gene and the non-transgenic seedlings were discarded. Transgenic seedlings were further cultivated and the first flowers were obtained within a few weeks after seeding. These seedlings were pollinated again with pollen of the Vf resistant cultivar 'Topaz'. The fruits of these crosses were harvested in fall 2007. These seeds will be sown in spring 2008 and the seedlings will be selected for the presence of BpMADS4 and of Vf by PCR. The present study demonstrates the proof-of-concept showing that one crossbred-generation per year is feasible in apple.

Keywords: juvenility, early flowering trees, BpMADS4.

Session V

International Consortium for Apple Genome Sequencing

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A public initiative to sequence the doubled haploid apple has been established at Washington State University. Sequence generation from the doubled haploid material will enable rapid genome assembly and identification of alleles when compared with heterozygous genome sequence. The genome sequence information will also facilitate research into the phenomenon of haploidization. It will enable us to ask questions regarding this intriguing biological process that was not possible earlier. Importantly, it will enable genome-wide functional genomics research. An update on the sequencing progress of the unique apple material will be provided.

Keywords: Malus, Genome Sequencing, Double Haploid, Pyrosequencing

In fruta trans-activation of a cold-inducible peach promoter by a peach CBF functional ortholog - a platform for functional analyses of transcription factors in fruits

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Transcription factors play a key role in altering gene expression in response to changes in environmental conditions. Therefore, transcription factors and their target genes are promising candidate genes for crop improvement. However, rapid and cost effective systems are necessary to characterize these transcription factors and their target promoters in fruits. In order to establish a homologous system in which the trans-activation of candidate promoters and candidate transcription factors may be analyzed in fruit tissue, we have isolated and characterized a peach functional ortholog of the Arabidopsis CBF transcription factor as well as a putative downstream target promoter, PpDX2, a putative ortholog of AtXero2. Since Xero2 is downstream of CBF in Arabidopsis and since the promoter of PpDX2 contains three DRE/CRT domains, PpXero2 is a likely candidate gene downstream of PpCBF. Agro-injection of peach fruits with a PpCBF over expression construct with a PpDX2 promoter/reporter construct demonstrates that PpCBF is capable of trans-activating this promoter in fruits. Therefore, our results demonstrated that PpCBF is a functional ortholog of Arabidopsis CBF and that PpCBF is able to activate this cold-inducible promoter in fruta. Therefore, we have established a transient homologous system in which the functionality of transcription factors and target promoters may be analyzed rapidly and cost-effectively.

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Keywords: Peach, CBF,Transcription factors, cold

Development of gene-specific markers from *Malus pumila* mRNA sequences, mapping in Malus and transferability and mapping in other rosaceous genera.

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A set of Malus mRNA sequences that were putatively single-copy in the apple genome were selected from the EMBL database. The sequences were blasted against the Arabidopsis TAIR website and *A. thaliana* genes with the close homology to the Malus sequences were identified. Where there was strong identity between a Malus and an Arabidopsis gene homologue, full-length genomic DNA and cDNA Arabidopsis sequences were downloaded. The Malus and Arabidopsis sequences were aligned using the MegAlign software package and putative intron sites were identified in the Malus mRNA sequences. Primer pairs were designed to flank these intron sites and used to amplify PCR products from genomic DNA from the parents of the East Malling Malus mapping population 'Fiesta' × 'Totem' (F×T). All primer pairs amplified a product larger than the size expected from mRNA sequence alone, indicating introns were indeed amplified from the Malus genomic DNA. Eleven loci, representing ten genes (39%) were polymorphic in the 'Fiesta' × 'Totem' population and mapped to seven Malus linkage groups. Transferability to other rosaceous genera was high, with primer pairs representing 85% of genes, amplifying products from both Fragaria and from Prunus genomic DNA. These primers were screened in the Fragaria and Prunus mapping bin sets and 38% of the genes were successfully located on both maps. Analysis of the markers mapped in more than one rosaceous genus revealed patterns of synteny between genera, whilst a comparison with the physical positions of homologous genes on the Arabidopsis genome revealed high sequence conservation but only fragmentary patterns of macrosynteny.

Keywords: Malus pumila, mapping, macrosynteny

SUBGENOME SIGNATURES: IDENTIFYING DIPLOID CONTRIBUTIONS TO THE OCTOPLOID STRAWBERRY (*FRAGARIA ×ANANASSA*) GENOME

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The cultivated strawberry (*Fragaria ×ananassa*) maintains an octoploid genome composed of as many as four independently segregating subgenomes. Classical studies of meiotic configurations have suggested that at least one of the subgenomes descended from *Fragaria vesca*, a wild diploid strawberry species. The identities of the other subgenome donors have not been elucidated. Here, Gene Pair Haplotypes (GPHs) were examined to inform description of strawberry's reticulate ancestry. GPHs are genomic signatures defined by a series of intergenic characters, useful as molecular markers in polyploid organisms. In this study the order and nature of single nucleotide polymorphisms (SNPs) and insertion-deletion events (indels) was assessed across the hypervariable regions of several strawberry intergenic sequences in a set of cultivars. Two trends are evident. The first indicates that at least one of the subgenomes most resembles the diploid *Fragaria iinumae*, suggesting that this Asian diploid is an ancient contributor to the polyploid. Most surprisingly, while haplotype matches between *F. vesca* and octoploid *F. virginiana* were observed, they were lacking in preliminary assessment of several *Fragaria ×ananassa* cultivars. Thus, molecular evidence indicates that *F. iinumae* can be confidently ascribed as a subgenome donor, yet demands careful reassessment of the extent to which *F. vesca* alleles are represented as subgenomic signatures in *F. ×ananassa*. The classical studies and some other molecular studies of the octoploid clearly indicate an *F. vesca* genomic component, so a possible interpretation of our results is that subgenome composition of cultivated strawberry may not be uniform between cultivars. The results emphasize the utility of the GPH marker, not only as a tool for molecular mapping, but also for a means to identify complex variable regions for comparative genomics studies across ploidy levels in *Fragaria*.

Keywords: strawberry, genome, haplotype

Session VI

Pedigree Based Analysis: Our experiences using multiple pedigreed populations in sweet cherry

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Sweet cherry is an out-crossing species with a long generation time. Therefore, QTL analysis using recombinant inbred lines is not a possibility. Instead, we are using a new QTL statistical approach called pedigree based analysis (PBA) (syn. pedigree genotyping) that utilizes existing pedigreed populations and ties together the many segregating crosses through their common ancestors in the pedigree. This approach adopts the Identity by Descent concept to express the identity of an allele of a modern selection in terms of alleles of founder cultivars. In our project, the plant material consisted of our sweet cherry NY × EF mapping population, a "pedigree set" of 44 sweet cherry varieties, and parental selections. These individuals were phenotyped for fruit size and cell number and genotyped for 67 SSRs and gene-based markers with known positions on our sweet cherry linkage maps. In addition, 40 progeny individuals from three segregating populations that are genetically related to the individuals in the pedigree set were also phenotyped for fruit size in 2007 and genotyped for 28 mapped markers known to segregate in these populations. We utilized PBA to: (1) validate the fruit size QTLs on LG2 and LG6 that were found in our mapping population, (2) identify two additional fruit size QTLs on LG 1, (3) estimate the effects of these alleles, and (4) determine that the QTL alleles contributing to increases in fruit size are not "fixed" in large fruited cultivars.

Keywords: sweet cherry, SSRs, pedigreed

Single nucleotide polymorphism marker development in apple: progress and applications

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Single Nucleotide Polymorphisms (SNPs) are excellent candidates for marker development as they are the most common DNA sequence variations found in genomes of most organisms, including apple. SNPs are found in most genomic regions, including coding regions, making them attractive markers for mapping candidate genes. We developed a set of 93 “reference” SNP markers located within expressed genes identified in public apple EST collections, and these were located on a ‘Royal Gala’ genetic map using selective (or bin) mapping. This resource will be invaluable for applications in reverse genetics and in apple breeding, and for understanding the evolution of the apple genome within the family Rosaceae. We will present the new markers and discuss the application of SNP based markers for candidate gene mapping in relation to apple fruit quality traits.

Keywords: SNPs, Malus, candidate genes.

Cisgenesis and Intragenesis in Rosaceae Crops

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Introgression of traits from wild germplasm into pip fruit cultivars by means of classical breeding is painstakingly slow. Introgression of, for example the apple scab resistance gene Vf from Malus floribunda 821 into marketable top quality apple cultivars took more than 50 years. In the mean time Vf resistance has been compromised by new virulent races of Venturia inaequalis in northern Europe. For durable resistance more than one resistance gene should be combined. However, this may take many years. This slow tempo is caused mainly by the long juvenile period and by linkage drag of hundreds of undesired alleles. The process would be much faster if only the allele of interest were inserted, without the other alleles from the wild germplasm. This process is named "cisgenesis". Cisgenesis would allow rapid accumulation of resistance genes or other desired alleles from wild sources.

We have defined cisgenesis as genetic modification of plants, inserting genes of the plant species itself or from crossable relatives. The gene should contain its native introns and be flanked by its native promoter and terminator in sense orientation. A cisgenic plant does not contain genes from outside the gene pool of the conventional breeder. If the plant does contain foreign genes, the plant is named transgenic. Scientific inquiries indicate that acceptance by consumers is better for cisgenic plants than for transgenic plants.

As the phenotypic traits from cisgenesis can in principle also be obtained by means of conventional breeding, induced translocation breeding or mutation breeding, cisgenic plants are at least as safe as conventionally bred plants, or plants from induced translocation breeding or mutation breeding. Therefore we propose to add cisgenesis of plants to the list of GM technologies that are exempted from the GMO regulation in the European Union (Annex 1B of Directive 2001/18/EC).

"Intragenic plants", like cisgenic plants, contain no foreign genes. However, intragenic plants may contain novel combinations of native promoters and native coding sequences. This provides more possibilities to the molecular breeder compared with the very strict approach of cisgenesis. As intragenic plants do not harbour foreign genes or promoters, we propose that in the EU these plants be regulated less stringently than transgenic plants, which do contain foreign genes.

The number of functionally analysed genes in the Rosaceae family is increasing, and will be boosted further by combining whole genome sequences of apple and peach with known genetic loci for interesting traits, gene expression data, and ESTs. Also technologies are available for either introduction of alleles without use of marker genes, or for later excision of marker genes, such as kanamycin resistance gene, the so called "marker-free" technologies. Cisgenesis and intragenesis combine the knowledge of gene sequences and their functions with marker-free technologies.

Cisgenesis and intragenesis are approaches for utilizing the growing wealth of knowledge of plant genes to the benefit of the society in a fast, safe and acceptable way.

Keywords: Rosaceae crop, cisgenesis

Session VII

Fine mapping of the D gene controlling fruit acidity in peach and screening of a new BAC library

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Peach (*Prunus persica* (L.) Batsch) is the second most important fruit tree crop in Europe after apple. It is a diploid species (2n=16) with a short juvenile period (2-3 years) and a small genome (262 Mb) about twice the size of that of *Arabidopsis thaliana*. Therefore, peach is considered as a model for Rosaceae. A peach F2 progeny (JxF), obtained from a cross between Ferjalou Jalousia® and Fantasia, segregating for several mendelian traits, was analyzed for fruit quality traits and used for the construction of a genetic linkage map. The D gene controlling the "non-acid" character of peach fruit was mapped on linkage group 5 and co-localized with QTLs with major effects involved in the control of pH, titratable acidity, organic acid contents and with QTLs with low effect for sugar contents. To understand the molecular and physiological bases of the D gene, a positional cloning strategy is in progress.

Thirty tree new markers were added on linkage group 5 using a BSA-AFLP method. Among them, 11 AFLP markers were located within 10 cM containing the D gene. Two SSR markers and four AFLP markers transformed into SCARs were used to identify recombinants among 1510 F2 additional individuals obtained for the fine mapping of the D gene and planted in 2005 and 2006. A new BAC library was realized for the isolation of the D gene using F1 hybrid DNA (obtained from the JxF cross) which is heterozygous for this character. This new BAC library was realized at URGV and contains 52 000 clones with a mean insert size of approximately 90 kb corresponding to 15-16x coverage of the peach haploid genome. The BAC library was screened with nearest codominant markers in order to identify clones containing the D and d alleles. Positive clones were already identified for each allele with the D-Scar0 marker cosegregating with the phenotype. A physical map will be constructed for each allele and the two sequences will be compared in order to identify the D gene. According to the high level of synteny reported within the Rosaceae, the results should be transferred to the other species of this family.

Keywords: BSA-AFLP, fine mapping, BAC library, fruit acidity, peach.

Cloning and expression of peach aroma candidate genes and peach aroma microarray advancing

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Peach flavour consists of a huge variety of volatile compounds. Esters, alcohols, aldehydes, terpens, lactones, C6 compounds are the main components of the peach aroma and their relative abundance is a fingerprint of a particular variety. Volatiles compounds encompassed shikimic acid derivates (eugenol, isoeugenol, chavicol, methyl benzoate) are present in a high concentration. An enzyme involved in phloroglucinol biosynthesis belongs to the family of O-methyl transferase. O-methyl transferase (OMT) enzymes catalyse the transfer of a methyl group to an hydroxyl group of an acceptor molecule with the formation of its methyl ether derivates. Among other volatiles compounds produced during ripening, esters and lactones are a quantitatively important part of the peach aroma. A wide range of esters are produced from alcohols and acyl-CoAs. The last step in the ester production is catalysed by alcohol acyltransferase (AAT) enzymes. Lactones are supposed to derive from fatty acid degradation and an epoxide hydrolase (EH) is suggested to be involved in their production. Here we report the cloning and expression of genes coding for OMTs, ATTs and EHs and the phylogenetic analysis of their families derived from ESTree DB and biochemical assay were conducted on recombinant proteins. On the other hand a different approach using microarray has been conducted on two different peach varieties (Bolero, melting variety and OroA non-melting variety). The expression profiles from four different ripening stages were compared within each variety and between the two varieties. Advancing on the gene clusters analysis is reported.

Keywords: peach, aroma, candidate genes, expression, microarray.

Characterization of expression and cloning of ripening-related MADS box genes in pear fruit

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MADS box genes have been known to be involved in the formation of floral organs in plants. Recently, it was reported that the MADS-box gene (LeMADS-RIN) was necessary for fruit ripening in tomato. They also suggested that a common regulatory network by MADS box genes exists in climacteric and non-climacteric fruit. However, there is no report on MADS box genes in pear. The characterization and functional analysis of MADS box genes present in ripening fruit has been conducted to clarify the ripening mechanism of pear. Three cDNAs (PpMADS1, 3, 5) encoding MADS box have been isolated by screening of cDNA library from 'Nijisseiki' ripe fruit. The deduced amino acid sequence of PpMADS1, 3, 5 has about 50% identity with LeMADS-RIN. PpMADS1and 3 belong to class A gene and PpMADS5 belongs to class E gene. Accumulation of mRNAs for three MADS box genes was examined during fruit development and ripening. The expression of PpMADS1 and PpMADS5 mRNA was high during fruit development and decreased during fruit ripening. By contrast, PpMADS3 gene was expressed low during development, but was expressed highly particularly during fruit ripening. The accumulation of PpMADS3 transcript was inhibited by 1-MCP, an inhibitor of ethylene action, suggesting that PpMADS3 is subject to positive regulation by ethylene. The PpMADS3 gene was introduced into the ripening-impaired mutant, rin of cv. 'Ailsa Craig' tomato, to determine whether PpMADS3 had the same function of LeMADS-RIN in pear fruit. Transgenic rin plants showed abnormal fruit shape, but normal ripening processes were not restored by the introduction of PpMADS3. This indicates that PpMADS3 plays an important role in fruit development more than fruit ripening in pear.

Keywords: Pear, MADS box Genes, development

Session VIII

The SharCo Consortium : Towards sharka containment

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Within the 7th European Framework, the concept of the European small collaborative "SharCo" project is to combine prophylactic and genetic solutions to prevent or limit the spread of the sharka disease caused by a virus, the *Plum pox virus* (PPV). The project scope covers the entire chain from seedling production, grafted material production, to orchard management. It is aimed at providing tools such as marker-assisted selection, PPV resistant plant materials, guidelines, warning systems, decision-support system. On that purpose, the project will, in the field of epidemiology, identify driving factors of PPV spread and diversification and develop novel and highthrough-put detection systems warning sharka outbreaks. In the field of genetics, it will provide molecular markers for the implementation of marker assisted selection of PPV resistant fruit varieties. In the field of biology, we will assess innovative biotechnological approaches to broaden resistance to PPV in different fruit tree species. All knowledge and tools developed by the project will be widely disseminated all over Europe with special attention made to PPV endemic countries.

Keywords: *Plum pox virus*, MAS (Marker Assisted Selection), Prunus

Resistance Gene Analogs in Rosaceae: Family-wide Classification Including Raspberry, Cherry, and Wild Apples

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Genetic studies have shown that NBS-LRR Resistance Gene Analogs (RGAs)tend to occur in clusters and often map to major resistance genes or QTLs. The identification and use of specific RGAs as molecular markers among plant material displaying different resistance phenotypes has the potential to directly identify the genes/genomic regions responsible for disease resistance. We undertook a comprehensive analysis of the RGAs found in Rosaceae to revise our earlier classification and identify classes associated with disease resistance. 500 Rosaceae RGA amino acid sequences were downloaded from NCBI. Additional conceptually translated sequences from our studies include 75 RGAs from raspberry germplasm, 90 from cherry germplasm, and almost 300 from various wild apple species. These RGA's represent both major classes (TIR and non-TIR subfamilies) and some have striking sequence similarity to genes previously associated with pest resistance in other rosaceous species. For example, two TIR RGA's from one of the cherry leaf spot resistant parents match an apple sequence associated with apple scab resistance. Another cherry NTIR RGA sequence matches a peach sequence that maps near a nematode resistance gene. Finally, another RGA sequence from the cherry *Powdery Mildew* resistant parent is nearly identical to a peach sequence that co-locates on the peach map with *Powdery Mildew* and sharka resistance QTLs. These results suggest that using an RGA approach in fruit crops has the potential to not only "tag" resistance gene regions but also identify potential parents among germplasm collections and provide insight into the evolutionary conservation of RGA's and their location within the Rosaceae.

Keywords: NBS-LRR, RGA, HMM, Disease, Resistance.

Session IX

Comparative and Functional genomics of *Powdery Mildew* Resistance in Rosaceae

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The Rosaceae family contains economically important fruit species of great nutritional value. Pathogen attack poses a significant risk of pre- and post-harvest losses for these crops. *Powdery Mildew* is a devastating disease which can cause widespread losses if not managed with repeated fungicide applications in a timely manner. The goal of our research is to identify the gene(s) responsible for *Powdery Mildew* resistance in Rosaceae and develop functional markers for a marker assisted breeding program. We have identified, isolated and cloned a putative *Powdery Mildew* resistance gene, mlo from peach. A similar gene has been amplified in plum, apricot, strawberry (diploid and octaploid varieties) and cherry (sweet and tart varieties). Comparative genomic tools have revealed that the currently sequenced mlo genes from Rosaceae segregate into a unique clade separate from well studied mlo genes in Arabidopsis, rice, maize and barley. In peach, strawberry and cherry the gene is present as a single copy and plum and apricot have two copies of mlo. Two splice variants of this gene were identified in peach. They differ in their exon number and relative expression in leaves, stem and fruit. Several cherry cultivars with differing levels of resistance to *Powdery Mildew* were evaluated for genotypic differences of this gene. A cherry transformation system is being developed for functional validation of genes involved in the *Powdery Mildew* resistance pathway.

Keywords: *Powdery Mildew*, Resisance, Rosaceae, Functional Marker.

Pyramiding mono and polygenic resistances is one strategy to provide lasting control of the resistance to the green peach aphid and *Powdery Mildew* in peach.

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Peach (*Prunus persica* L. Batsch) is the stone fruit crop for which breeding efforts are the most important worldwide. However, works for the improvement of resistance to pests and diseases appear limited compared to those dealing with fruit quality while the current trends are to reduce the chemical inputs and limit pesticides residues on fruits.
At the INRA-Avignon, we have developed for several years important breeding programs aimed at selecting new peach cultivars introgressed with genetic resistance factors against several pests and diseases important in peach orchards, particularly the green peach aphid (*Myzus persicae*), *Powdery Mildew* (*Sphaerotheca pannosa var. persicae*), peach leaf curl (*Taphrina deformans*) and sharka disease (*Plum pox virus*). Major monogenic resistance sources for the green peach aphid and *Powdery Mildew* have been identified in the peach rootstocks varieties 'Rubira' and 'Pamirskij' respectively. P. davidiana (clone 1908), a wild species closely related to peach, has proven to bear factors involved in polygenic resistance to all above-mentioned enemies. Several F1 and F2 progenies have been obtained from crosses involving these genitors. Genetic maps have been constructed based on transferable molecular markers anchored to the 'Texas' x 'Earlygold' general map for Prunus. Studies involving biochemical tools and plant phenotyping including observation of the aphid behaviour in controlled conditions have been conducted. Such studies would allow finding the different genome regions involved in these resistances and thus to develop molecular markers linked to both types of resistance in order to use it for Marker Assisted Breeding. Building lasting resistance is an important challenge and the release of cultivars with good agronomical features concurrently bearing mono and polygenic resistance factors against these enemies appears a valuable strategy.

Keywords: Peach, resistance, *Powdery Mildew*

Allelic diversity of recessive resistance genes to sharka disease in Prunoideae

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Viruses are obligatory parasites with a very small genome and which rely on the host cellular machinery to complete the different steps of their cycle. Recent results suggest that recessive resistances against viruses are more likely to correspond to a passive mechanism due to the absence or to the inapropriate nature of a host factor specifically required by the virus to complete its cycle. The dominant allele can then conceptually be envisionned as encoding a susceptibility factor needed by the virus to be able to infect the host plant. Indeed, one of the major findings in the last few years was the identification of natural recessive resistance genes against several important potyviruses in vegetable crops, all of them encoding the translation initiation factor eIF4E or one of its isoforms. Interestingly, we also showed that a knock-out (KO) mutant for eIF(iso)4E or for eIF(iso)4G in *Arabidopsis thaliana* is resistant to *Plum pox virus* (Decroocq et al., 2006; Nicaise et al., 2007). These results suggest that many if not all Potyviruses probably use identical or closely related host factors in widely different plants to complete their life cycle.

As a consequence, the identification of a plant susceptibility factor in any given potyvirus-plant pathosystem is very likely to be transferable to a number of other pathosystems and, in particular, to stone-fruit trees since *Plum pox virus*, the causal agent of sharka disease, belongs to the potyviruses. We postulate that most of the Prunus species bear a susceptibility allele of eIF4E and eIF4G and that some rare variants might occur in the natural Prunus population and germplasm collection. The purpose of this project is therefore the detection of polymorphism in the Prunus eIF4E and eIF4G homologues and isoforms and to correlate natural polymorphisms with a PPV resistance phenotypic trait.

Acknowledgements: We thank collegues for giving access to the live reference Prunus collections of ARS-USDA (Davis, USA), Mendel University for Agriculture and Forestry (Lednice, Czech rep.), IVIA (Valence, Spain), Mustafa Kemal University (Antakya, Turkey) and INRA (Bordeaux and Avignon, France).

Keywords: Sharka, Prunus, resistanse

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Poster abstract –session 1

1

Improving Tissue Culture, Micropropagation and Biotechnological Applications in Rosaceaous Crops

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Present rosaceaous tissue culture and micropropagation practices often rely on traditional and often non-optimized media compositions and procedures. In order to address such issues, an effort must be made to develop proficient universal tissue culture and micropropagation methodologies within the Rosaceae family. Diverse aspects of our efforts to improve plant biotechnology in this field are further discussed. One of these approaches focuses on the integration of a temporary immersion system for the ideal growth of rosaceous crops. Additionally, due to the importance of light quality and composition on plant growth, we are studying the effect of assorted light treatments upon rosaceous plant growth and development. Various treatments of hormones and physiologically active small molecules are being studied to optimize the production of directed organogenic differentiation from cultured explants. With further focus directed to the development of robust tissue culture and micropropagation methods, there promises to be an increase in the quality and speed of in vitro generated horticultural crops for scientific and commercial purposes.

Keywords: Micropropagation, Rosaceae, Phytohormones, Photobiology

3

Bitterness in almonds (*Prunus dulcis* (Miller) D. A. Webb)

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Bitterness in almond (*Prunus dulcis* (Miller) D. A. Webb) is determined by the content of the cyanogenic diglucoside amygdalin. The ability to synthesize and degrade prunasin and amygdalin in the almond kernel was studied throughout the growth season using four different genotypes for bitterness. LC-MS analyses showed a specific developmentally dependent accumulation of prunasin in the tegument of the bitter genotype. The prunasin level decreased concomitant with the initiation of amygdalin accumulation in the cotyledons of the bitter genotype. By administration of radiolabelled phenylalanine, the tegument was identified as a specific site of synthesis of prunasin in all four genotypes. A major difference between sweet and bitter genotypes was observed upon staining of thin sections of teguments and cotyledons for β-glucosidase activity using Fast Blue BB salt. In the sweet genotype, the inner epidermis in the tegument facing the nucellus was rich in cytoplasmic and vacuolar localized β-glucosidase activity, whereas in the bitter cultivar, the β-glucosidase activity in this cell layer was low. These combined data show that in the bitter genotype, prunasin synthesized in the tegument is transported into the cotyledon via the transfer cells and converted into amygdalin in the developing almond seed, whereas in the sweet genotype, amygdalin formation is prevented because the prunasin is degraded upon passage of the β-glucosidase rich cell layer in the inner epidermis of the tegument. The prunasin turn-over may offer a buffer supply of ammonia, aspartic acid, and asparagine enabling the plants to balance the supply of nitrogen to the developing cotyledons.

Keywords: almonds, LC-MS, prunasyn

5

Dissecting apple tree architecture into genetic, ontogenetic and environmental effects

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Tree architecture varies with climatic conditions and tree ontogeny. The present study aimed to dissect this plasticity into genetic, ontogenetic, and environmental effects over the 4 first years of growth of an apple F1 progeny. Traits related to growth and branching processes were annually assessed on different axes of the trees implanted in a staggered-start design. This allowed us to distinguish consecutive years of growth from climatic years and to build mixed linear models that included genotype (G), tree age (A), and climatic year (Y) effects, and the interactions between genotype and age (GxA) and genotype and year (GxY). In this model, data consisted in sequences of repeated data. Both spatial repetitions, i.e. different axis types, and temporal repetitions, i.e. successive ages of trees, were accounted by modelling the residuals with variance-covariance structures. For each studied trait, predicted values (BLUPs) of the G, GxA and GxY effects were extracted from the models, and used for QTL mapping. A significant G effect was found for most studied traits and numerous QTL were detected. Some particularly robusts co-localized in common genomic regions, for internode lengthening, top diameter, and number and percentage of axillary shoots. GxA and GxY effects were also detected which allowed us to distinguish among the traits that were under genetic control, those for which this control is exerted differentially throughout tree life or depending on climatic conditions. QTL detected for BLUPs of GxY effects were thus interpreted as resulting from the interaction between genetic maximal potential of growth and climatic factors, while those for GxA effects were interpreted in relation to tree ontogeny. Most of them were found to be concomitant with key development stages during which the traits started to decrease, but with different intensities depending on genotype. The 2-step approach proposed in the present study also improved QTL detection power and appears generic enough to apply to other traits measured as repeated data which are common in perennials.

Keywords: phenotypic plasticity, growth and branching, repeated data, mixed linear model, QTL analysis.

7

Identification of new resistance gene analogs in 33 wild Malus species.

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Plant R genes are known to confer resistance to a variety of pathogens in a gene-for-gene mode. 800 unique apple genome samples were amplified and sequenced from a variety of wild material and rootstocks using degenerate primers for NBS-LRR Resistance Gene Analogs (RGAs). Forty five differents Malus accessions from the USDA apple gerplasm collection at Geneva, representing 33 Malus species (*MalusXdomestica*, asiatica, baccata, coronaria, florentina, floribunda, fusca, halliana, honanensis, hupehensis, ioensis, kansuensis, micromalus, ombrophila, orientalis, prattii, prunifolia, pumila, robusta, sargentii, sieboldii, sieversii, sikkimensis, sublobata, sylvestris, transitoria, toringoides ,zumi, yunnanensis, zhaojiaoensis), hybrid white angel, and the rootstock Geneva41 were used in this study. Sequences were screened and cleaned of vector sequences and compared with resistance genes previously identified among the Rosaceae and other green plants. Approximately 90 of these matched RGAs previously identified among Malus while an additional 200 are similar to RGAs found in other Rosaceae genera and other plant families. Roughly 30% of the RGA's identified in this study are TIR-type RGAs, while the remaining 70% are Non-TIR-type. These genes will be used to identify novel sources of disease resistance among the wild apples in the USDA apple collection.

Keywords: Apple, gene resistance

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Linkage disequilibrium analysis to enable more efficient gene and QTL mapping in apple

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The estimation of marker-trait association based on linkage disequilibrium (LD) analysis across germplasm collections can provide an efficient alternative for gene/QTL mapping and cloning. This is particularly true for species with a long generation time such as apple (*Malus X domestica*) where mapping based on experimental crosses is very time-consuming. In order to better implement this mapping strategy, the average nucleotide diversity, the LD level across the genome and the existence of hidden population subdivisions should be investigated. For these aims, we assembled a collection of 185 apple accessions, including a comprehensive set of wild apple species as well as cultivars representative of the elite apple germplasm. The collection was genotyped using publicly available SSRs and several hundreds SNPs originally discovered within the IASMA Golden Delicious genome sequencing project. Markers were selected for being distributed evenly across the genome. A preliminary survey of our results will be presented. Faster discovery of useful alleles using LD-based approaches should lead to a more efficient marker-assisted breeding.

Keywords: apple, QTL, Linkage disequilibrium

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A genomics approach to fruit softening in apples

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Fruit softening is a key factor in fruit quality which directly affects commercial returns to growers. Softening occurs in mature apple fruit after the detection of the ripening hormone ethylene. We have used a 16,000 feature microarray hybridised with labelled RNA from a number of ripening treatments1,2, to identify changes in gene expression that occur with apple fruit softening. Genes that showed a large increase in expression with ethylene included a number of cell wall-related enzymes such as polygalacturonase (PG), xyloglucan endotransglucosylase/hydrolase and B-galactosidase. As the level of PG expression seemed to correlate with softening, the transcription factors that activate this gene were further investigated. Members of the AP2 transcription factor family of genes were initially targeted as they have been implicated in the first steps of ethylene signal transduction as well as the transcription factors that were up regulated during ripening. Using high throughput qPCR we identified 18 ethylene-induced transcription factors 4 of which were AP2 like genes. To establish which transcription factors directly activated the PG gene, a previously published 2.8 kb PG promoter sequence was cloned3 and screened against the HortResearch transcription factor library using a transient assay system4. Combining the results from these two screens we have identified candidate transcription factors that may be involved in the regulation of fruit softening.

1. Schaffer et al. (2007) Plant Phys. 144:1899-912
2. Janssen et al. (submitted)
3. Atkinson et al. (1998) Plant Mol. Biol. 38:499-60
4. Hellens et al. (2005) Plant Methods 18:1-13

Keywords 1: *Malus domestica*, Fruit softening, Genomics, Transcription factor, Polygalacturonase

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Dwarfing apple rootstocks: a single genetic source?

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Apple rootstocks (*Malus domestica* Borkh and Malus spp.) have been selected for centuries for their ability to control tree size as well as other traits such as disease, pest and cold resistance. At the beginning of the 20th century, all the apple rootstocks grown in Western Europe were grouped and classified 'M.1' to 'M.25' at East Malling (UK) according to their dwarfing capacity (Webster and Wertheim, 2003). Since then, most of the apple rootstock varieties bred throughout the world have used parents from this series, particularly the dwarfing 'M.9' selection. SSR markers linked to a major QTL influencing dwarfing (DW1) were identified and mapped in a population from the cross 'M.9' (dwarfing) × 'Robusta 5' (vigorous). Two of these SSR markers mapping about 1.5 cM away from the dwarfing QTL DW1 were screened over 58 rootstock accessions that confer a range of effects on scion growth.
The majority of the dwarf and semi-dwarf rootstock accessions screened carried the locus DW1. The tight linkage of both SSRs to the DW1 locus indicates that these markers should be highly effective in identifying dwarfing rootstocks. The results of this analysis indicate that there may be only one genetic source of dwarfing in apple rootstocks.

Keywords: Apple rootstock, dwarfing, QTL

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Identification of QTLs for Bud Break Traits in Low Chilling Apple Families (*Malus X domestica* Borkh.)

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The use of dormancy breaking chemicals form part of standard orchard management in the Western Cape region of South Africa where mild winter temperatures result in the inefficient release from dormancy in apple trees during spring. Environmental concerns, as well as Global warming, directed local breeding programs towards focusing on apple cultivars with a lower chilling requirement that are better adapted to local climatic conditions.
Previous work has shown an important heritable component in the control of bud break traits in apples. In this work we have used 4 mapping populations, 'Golden Delicious' x 'Anna', 'Golden Delicious' x 'Prima', 'Sharpe's Early' x 'Anna' and 'Austin' x 'Anna'. Phenotypic trait assessment, involving time of initial vegetative and reproductive budbreak as well as number of buds, were done for 3 to 4 years on juvenile trees from all 4 populations and on adult trees from the latter 3 populations. Bud break QTLs, showing consistency over several years and in different genetic backgrounds, have been identified.
This work will facilitate the application of marker-assisted-selection (MAS) in the breeding of apple cultivars with a lower chilling requirement that will be beneficial for local production, ensuring that South Africa remains competitive in the global apple export market.

Keywords: Apple, QTL, marker-assisted-selection

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Making RNAi mutants of apple with high-efficiency by use of multi-vector transformation.

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To facilitate high-efficiency generation of RNAi-mutants for determination of function of candidate genes in resistance of apple to Erwinia amylovora (fire blight), the M.26 apple genotype was transformed with a mixture of five RNAi EST-silencing vectors in each transformation experiment to allow selection of up to five types of mutants from a single experiment. ESTs associated with response to E. amylovora, identified by bioinformatics analysis, were used to create RNAi-silencing constructs. These constructs were transferred to Agrobacterium tumefaciens strain EHA105pCH32. The five transformed A. tumefaciens strains were mixed, and the mixture used to transform leaf-slice explants. Regenerants were selected on M.26 regeneration medium with 100 mg/L kanamycin, and screened by PCR using universal primers to determine integration of a silencing construct. In most lines PCR showed only single genes had been inserted. Because amplicons from some transgenics co migrated, to better determine the identity of the ESTs contained in the silencing-insertion, the PCR fragments were cut with 4-cutter restriction enzymes. Thus far ESTs from genes in six functional categories, general metabolism (1), photosynthesis (2), nucleic acid metabolism (1), protein metabolism (3), signaling (4), and defense/stress (4), have been subjected to this protocol. Young plantlets will be inoculated with E. amylovora to assayed phenotype reaction.
This project is supported by a National Research Initiative Competitive Grant 2005-35300-15462 from the USDA Cooperative State Research, Education, and Extension Service.

Keywords: RNAi, apple, *Erwinia amylovora*

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A distinct recognition mechanism in the gametophytic self-incompatibility system in Prunus

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S-RNase and F-box genes are commonly found as the pistil and the pollen determinants, respectively of the S locus of three plant families, Rosaceae, Solanaceae, and Plantaginaceae. Therefore, a common working hypothesis for the self and non-self recognition in the Rosaceae, Solanaceae, and Plantaginaceae was proposed involving the ubiquitination and subsequent degradation of non-self S-RNases. In this model, SFB/SLF functions as a component of SCFSFB/SLF, which ubiquitinates all non-self S-RNases to make them for degradation (general interaction) but interacts specifically (S haplotype-specific interaction) with the self S-RNase to leave it active, leading to the arrest of self pollen tube growth. However, recent molecular studies on self-compatible (SC) pollen-part mutant (PPM) S haplotypes in Prunus (Rosaceae) suggested that the recognition mechanism of the S-RNase-based GSI in Prunus differs from the mechanism(s) in the Solanaceae and Plantaginaceae. In this report, we summarize the differences in the recognition system of self-incompatibility in Prunus and others.

Keywords: Prunus, self-incompatibility

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Sweet cherry breeding in Summerland: history and current initiatives

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The sweet cherry breeding program in Summerland has been an unbroken effort since the 1930s. This unique continuity is arguably one of the main factors in its success. The program has typically produced a thousand new seedlings per year from 15-20 parents (with ~25% seed set and 50% germination). The breeding goals are self-fertility, resistance to splitting, improved fruit size, growth habit, time of maturity and improved fruit quality (including taste, firmness, stem quality and postharvest characteristics). Seedlings of inferior quality have usually not been kept. Efforts are currently underway to evaluate segregating populations for fruit size, time of maturity and *Powdery Mildew* resistance utilizing published resources of Prunus SSR markers. All new seedlings are also being screened for the S4' allele for self-fertility. Many aspects of fruit development and how traits are expressed remain only roughly understood. We are using gene expression patterns in ripening cherry fruit to study the triggers of the rapid expansion phase of growth. GA / ABA growth hormone balance appears to control anthocyanin pathway gene expression early in this growth phase. This suggests that embryo / fruit hormone levels might influence time of maturity in cherry.

Keywords: Self-fertility, Time of maturity, GA/ABA, Anthocyanins, Gene expression

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Flowering, production and fruit quality of eleven sweet cherry cultivars in an experimental orchard at central Chile.

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This publication reports four years the results of dates, crop load, productive efficiency and fruit quality of cultivars ´Brooks', 'Lapins', 'Bing', 'Sweetheart', 'Stella', 'Cristalina', 'Santina', 'Garnet', 'Newstar', 'Summit' and 'Sylvia' on 'Pontaleb' (*Prunus mahaleb*) rootstock taken in 4th to 7th leaf at a grower's experimental orchard, located at Central Chile (33º52' S and 70º41 W at 700 m altitude) and planted at 1481 trees/ha. Flowering periods, floral density and intensity has been determined for these cultivars. Accumulated production in 2003 to 2006 has been between 10.5 T/ha for Summit and 91.4 T/ha for 'Lapins', Production efficiency between 3.5 and 200 g/cm2 TCA, with higher levels in 'Sweetheart', 'Lapins', 'Santina' and 'Stella' (self fertil cultivars). During the second season, this index ranged between 8.2 g/cm2 in 'Summit' and 243 g/cm2 in 'Lapins'. Fruit quality has been measured by fruit weight, firmness, soluble solids and acidity, and local adaptability through cracking incidence and bird damage. This is part of a project that studies sweet cherry productivity and quality potential with modern techniques in different winter chilling locations in Chile.

Keywords: Cultivars, Productive efficiency, Flowering, Sweet cherry, Fruit quality

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Construction of the first loquat (*Eriobotrya japonica* (Thunb.) Lindl.) linkage map

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Loquat (*Eriobotrya japonica* (Thumb.) Lindl.) was introduced in Europe in the 18th century, but it only started to be grown in regular orchards since the beginning of the 20th century. It adapted well to the Mediterranean climate and, currently, more than 50% of total European crop production is located in South East of Spain. Moreover, a growing interest in this species, as an alternative to the main fruit crops, is rising in recent times. In this context, the 'Instituto Valenciano de Investigaciones Agrarias' (IVIA), in collaboration with the grower association from 'Callosa d'En Sarrià', began in 2002 a breeding program aimed at extending the crop season of the main cultivar 'Algerie' by means of new loquat varieties with higher quality and productivity. In the framework of this breeding program, a loquat genetic linkage map has been constructed as a tool that may facilitate MAS in the future. The mapping population used was an F1 comprising 80 individuals derived from the cross between 'Algerie' and 'Zaozhong-6', a Chinese cultivar. A total of 455 microsatellites derived from Eriobotrya, Malus, Pyrus and Prunus genera have been tested for polymorphism, 300 gave amplification and more than 100 were mapped. Additionally, 170 AFLP markers have been analyzed. The seventeen linkage groups corresponding to the basic chromosome number of the species have been defined on the basis of SSRs held in common with Malus and Pyrus maps. Self-incompatibility trait has been mapped in the 17 linkage group, the same group were had been previously located in Malus, reinforcing the high degree of synteny observed with shared SSRs. This is the first loquat linkage map available and represents a starting point to improve germplasm management and a useful tool for future assistance on loquat breeding.

Keywords: *Eriobotrya japonica*, linkage map, microsatellite, self-incompatibility, synteny.

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Identification of a minimal SSR marker panel useful for fingerprinting and paternity tests in the *Prunus persica* (peaches and nectarines) Chilean germplasm.

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The possibility to establish the unequivocal genetic identity of a plant variety is an issue to protect breeding rights and to trace the right genotypes during plant prepagation. However, in the case of peaches and nectarines (*Prunus persica*), their very narrow genetic background can severely hamper the results. So, a simple and efficient fingerprinting scheme for this self-pollinating diploid (2n=16) species remains as a challenge. This genetic bottleneck comes from the beginning of this species breeding, based on a small number of genotypes. An additional problem is the very large number of cultivars available from a number of breeding programs from around the world.
Aranzana et al. (2003) differentiated the 200 cultivars most commonly planted in Spain using 16 SSRs. However, when comparing those genotypes with the 90 cultivars registered in Chile, only eight were shared. By this reason, it was needed to evaluate the usefulness of this SSR set on the cultivars grown in Chile, also trying to reduce the number of markers in order to improve the economical aspects of the analyses. The plant material used in this work where obtained from the Chilean Phytosanitary Agency, RVP-SAG, plus some materials of the public domain. Until now, the results are confirming the low genetic diversity described for the species. For example, the most informative marker exhibits just seven alleles along the entire collection of cultivars, being 3-4 alleles per marker the most common result. Moreover, some markers were not polymorphic at all. Also, some synonim cultivars have been identified, not differentiable by any of the tested markers. The genetic relationships among the hole collection will be presented to illustrate this result. By other side, 30 SSRs were used to run paternity tests from a local breeding initiative. In this case, the parents for 13 selected lines were confirmed, discarding other putative progenitors managed in the same crossing block, which had less than 50% of shared alleles with the selected lines.

Financed by FONDEF-Chile, Grant # D04I-1060.

Keywords: SSR Marker, Peach

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Mapping of peach KNOTTED-like transcription factor genes and unravelling the KNOPE1 member's role in stem development and lignin deposition.

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Plant KNOTTED-like homeodomain containing transcription factors (KNOX) play important roles in development and fall into two classes, distinguished by: the identity grade of the residues within the homeodomain, the intron position, and the expression patterns. Class 1 genes are required in different aspects of organ development, particularly in shoot apical meristem (SAM) formation and maintenance, whilst class 2 gene functions are still undefined. We cloned five class 1 members (KNOPE1, KNOPE2, STM-like1, STM-like2, and KNOPE6) and two class 2 members (KNOPE4 and KNOPE5) in the genome of *Prunus persica* (cultivar 'Chiripa'). Both class 1 and 2 genes tightly conserved intron positions as compared to the Arabidopsis orthologues. Class 1 KNOPE1 and STM-like1 mapped on linkage group (LG) 1 and 3 of the Prunus ('Texas' x 'Earlygold') reference map, respectively; class 2 KNOPE3 and KNOPE4 lay on LG 1 and 7, respectively. The KNOPE1 member fell in the region where a QTL affecting internode length was placed in the *P. persica* x P. ferganensis linkage map. We previously evidenced that KNOPE1 shared functional equivalence with the Arabidopsis BREVIPEDICELLUS (BP/KNAT1), the latter also regulates stem length and lignin deposition. Consequently, we addressed whether KNOPE1 might maintain a role in these functions. In 5 month old shoots, the KNOPE1 transcript abundance decreased from top to basal stem portions, concomitantly with the up-regulation of lignin biosynthesis genes, suggesting that KNOPE1 activity and lignin deposition were inversely correlated. The KNOPE1 transcript localisation varied along the shoot axis: it featured in the cortex and encircled the petiole bundles in stem portions at 0.2 mm below the shoot apical meristem, while it just marked the phloem and intra fascicular cambium in internodes at 4 mm. In addition, Arabidopsis overexpressing KNOPE1 exhibited altered pattern of lignin deposition similarly to those overexpressing BP. Moreover, the KNOPE1 protein recognised the KNOX typical DNA-binding motif, which was harboured in the promoter regions of Arabidopsis lignin biosynthesis genes, such as COMT1 and CCoAOMT encoding lignin methyl-transferases. Taken together these results suggest that KNOPE1 may have role in regulating peach stem development and maturation.

Keywords: KNOPE1, Peach, transcription factors

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Genetic analysis of host resistance to postharvest brown rot and sour rot in *Prunus persica*

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Postharvest brown rot (BR) caused by *Monilinia fructicola* and sour rot (SR) caused by *Geotrichum candidum* are serious diseases of peach and nectarine in California. Current disease management is primarily by pre- and postharvest application of fungicides. Resistance to both fungi was surveyed among 80 (for BR) and 34 (for SR) commercial peach and nectarine cultivars as well as a few old cultivars, landraces, and closely related accessions. Of these, 22 cultivars were tested with both fungi. A total of 204 individuals of two peach segregating populations ('Loadel' × 'UCD96,4-55' and 'Dr. Davis' × 'F8,1-42') were evaluated for genetic analysis of resistance to BR. Two methods – wounded vs. nonwounded fruit - were used for inoculations with *M. fructicola*. All SR inoculations involved wounded fruit. BR lesion size varied among cultivars and among the progeny of both populations. SR lesion size also varied among cultivars tested. Yellow fleshed cultivars were significantly less susceptible than white cultivars to BR nonwounded inoculation ($P < 0.05$) but no significant difference was observed between both colors for wound inoculation. Nectarines were significantly less susceptible to BR wound inoculation than peaches ($P < 0.01$) but no significant difference was observed between the two fruit types for nonwounded inoculation. Lesion size was determined to be under genetic control from analysis of cultivar differences. A weak but significant linear relationship was observed between wounded and nonwounded BR inoculation methods (R2 = 6-27%; $P < 0.01$). However, several cultivars and progeny that displayed resistance to nonwounded inoculation were susceptible to wound inoculation. This indicated that similar as well as different resistance mechanisms may be present for wounded vs. nonwounded fruit. Host resistance also varied between SR and BR as t-test analysis showed significant differences between the reactions of the cultivars to both fungi. A number of wild peach accessions and old cultivars showed a high level of resistance to BR suggesting that these may be untapped sources of resistance to the fungus. Scaffold linkage maps are being constructed for both populations and QTL analysis will follow. A set of Prunus candidate genes in the cutin, lignin, chlorogenate, and caffeic acid biosynthesis pathways as well as resistance gene analogs has been assembled and will be mapped to both linkage maps. Results of molecular genetic analysis will be presented.

Keywords: Brown rot, Sour rot, *Monilinia fructicola*, inoculation, genetic analysis.

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Mapping the nuclear genomic region associated with the Peach Tree Short Life Syndrome using microsatellite/SSR markers

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Peach Tree Short Life (PTSL) is a complicated disease syndrome, which occurs commonly in the southeastern U.S. It causes peach tree death at an early stage (e.g., the third or fourth year) and thus causes large economic losses for growers. However, recently the rootstock Guardian® 'BY 520-9' was selected for its tolerance to PTSL, and was released commercially in the 1990's. Guardian® selection 3-17-7 and a PTSL susceptible rootstock cultivar, Nemaguard, were crossed (15 F1s). Each F1 was self-crossed to create segregating F2 populations. For example, F1-11 has 100 F2 trees, each replicated at least three times. The trees have been rated on their susceptibility and response to PTSL every spring since 2004. Genomic DNA was isolated and purified from 1 gram of fresh leaf tissue of population of F1-11 using an SDS-based mini-prep protocol. One hundred and seventy microsatellite (Simple Sequence Repeats, SSRs) markers, originally developed from different Prunus sp. uniquely mapped to chromosomal locations on the Prunus reference genome, were used to screen the two parents and F1-11. Forty SSR markers showed polymorphism among parents, and F1-11 was heterozygous at each SSR locus. These 40 polymorphic SSRs were screened on the segregating F2 population of F1-11. Furthermore, three other F1s (F1-2, F1-6 and F1-7), have been screened by 90 of the 170 SSR markers in order to identify potential polymorphic SSRs for use in the corresponding F2 populations. Segregation data for PTSL-response and SSR markers will be complied and subjected to Analysis of Variance (using SAS) to determine differences in genotype means. Our results will define the genetic component controlling response to PTSL syndrome. Furthermore, it is anticipated that we will identify one or more regions of the peach genome where genes controlling PTSL susceptibility and tolerance reside.

Keywords: Peach, SSR markers, PTSL susceptibility

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Chilling injury development, enzyme activities, and proteomic approach evaluation in delayed cooling 'Roysun' plums.

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To extend their postharvest life, stone fruits are commonly cold stored at low temperature. Nevertheless, after long storage periods, chilling injuries symptoms like woolliness, browning, flesh translucency are developed in these fruits. To study these phenomena, the effects of delayed cooling (DC) at 20 ºC before cold storage for 42 days at 0ºC were studied in 'Roysun' plums. Mature-green and ripe fruits were compared to delayed cooled fruits. Quality parameters, chilling injuries development, PG and PME enzymes activity were evaluated. A proteomic approach was also used in order to evaluate the behavior of proteins that are expressed in ripe plums at harvest and after 21 and 42 days postharvest times at 0ºC plus 3 more days at 20ºC for a simulated commercialization process. Deep Purple stained 2-DE gels with an average of 700 spots were generated for each evaluation and some candidates were selected for further characterization due to their differential accumulation. Extractable juice content was higher in all treatments up to 55% in all evaluations. Firmness decreased constantly in the 3 treatments during the 42 days of storage. Chilling injuries like browning and flesh translucency developed at ripening at 20ºC after 28, 35 and 42 days at 0ºC. A subtle change in total soluble solids was observed in the plums with a variation between 13 to 14,5 ºBrix. In the near future we hope to be able to establish correlations between the quality parameters, chilling injuries development, the enzymatic activities and the corresponding protein accumulation profiles. Supported by: Millenium Nucleus in Plant Cell Biology ICM P 06-065-F and UNAB DI 18-05/R CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministério da Ciência e Tecnologia, Brasil.

Keywords: *Prunus salicina* Lindl, Roysun, postharvest, PG, PME, proteomics.

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Dormancy-associated MADS genes in the EVG locus: phylogeny and expression analysis

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The evg mutant of peach is a naturally occurring dormancy-incapable genotype. We have mapped cloned and sequenced the genomic locus of EVG in wild-type trees. The locus contains a duplicated array of six MIKC-type MADS box transcription factors which we have designated DAM1 through 6 (dormancy-associated MADS). Expression of all six DAM genes is lost in the evg genotype. The six DAM genes are members of the SVP/StMADS11 clade of MADS box genes and make up a monophyletic group when aligned with Arabidopsis and Populus sequences. The tandem array of genes appears to have resulted from successive duplications of individual genes from a single common ancestor. We have quantitatively determined the expression of the six DAM genes in wild-type germplasm throughout an annual cycle and in controlled photoperiod conditions. Five of the six genes show distinct seasonal expression patterns that can be correlated to photoperiodic responses in controlled conditions. Differential expression patterns suggest the six tandemly duplicated genes are not redundant in function.

Keywords: MADS, Prunus, photoperiod, phylogeny, duplication, dormancy

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Linking phenotype to genotype in red raspberry for major quality traits; a genetic, sensory and compositional approach.

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The UK raspberry industry was traditionally a processed fruit industry which has been in decline for the past two decades, in order to prevent a further decline in this profitable soft fruit crop it is evident that alternative strategies must be employed A mapping population constructed from two phenotypically different cultivars, the European c.v Glen Moy and a North American c.v Latham, were analysed across three different environmental locations (one open field, and two protected sites) to link those deemed the sweetest, brightest most enjoyable, to the actual underlying genotype. Phenotypic data regarding size, colour, sweetness and sourness has been collected and analysed, over two separate seasons, which has indicated towards genetic control rather than environmental influence as the main factor involved in quality trait development. Initial comparative analysis from other Rosaceae species has identified several candidate genes involved in the biological pathways relating to quality traits. As a result several individual single nucleotide polymorphisms (SNP's) within various genes have been identified and sequenced in the red raspberry Rubus ideaus. Construction of a cDNA library for both parents is underway and this will be utilised to identify and probe for further candidate genes relating to quality traits which are expressed at various stages in developing fruit. By selectively screening for the presence of specific genes within potential breeding cultivars, the possibility of linking genotype to phenotype for selected traits becomes more of a reality.

Keywords: Red Raspberry, Quality traits, Genetic analysis, molecular assisted breeding.

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Differential softening rates between *F. chiloensis* and *F. × ananassa* fruits revealed a different pectin degradation pattern

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The Chilean strawberry (Fragaria chiloensis) is one of the wild parents of the commercial strawberry (Fragaria × ananassa), and it has the potential to become a new exotic fruit species that could add to the diversity of berries that Chile can export. Fruit softening is a factor that can affect the shelf-life and reduce the keeping quality of strawberries. In strawberry fruit, softening is characterized by the increase in solubilization and the reduction in molecular mass of cell wall components, specially pectin. In other fruits, these modifications have largely been attributed to the activity of polygalacturonase (PG). However, studies have highlighted the role of pectate lyases (PL) as the main pectinase in strawberry. The objective of this work was to analyse and compare the major changes in cell wall metabolism and gene expression between both strawberry species during fruit development. Strawberry fruit from both species were harvested and classified into 4 stages according to weight and color: stages 1 and 2 correspond to small, unripe and hard fruit; while stages 3 and 4 correspond to color-breaker and ripening fruit. Changes in firmness, pectin solubilization and depolymerization, and the expression of PG and PL genes were analyzed. F. chiloensis fruit presented a higher level of softening than F. × ananassa between stages 2 and 3. This phenomenon is associated in F. chiloensis with a great solubilization of covalently bound pectin, although no reduction in pectin size was observed. In the case of F. × ananassa a high degree of pectin depolymerization was observed during softening. In F. chiloensis higher levels of PG transcripts accumulation than in F. × ananassa were observed between stages 2 and 3, while a higher increment in PL transcript accumulation between the same stages was observed in F. × ananassa. The faster softening rate observed in F. chiloensis correlates well with the increase in solubilization of covalently bound pectin and with the higher and earlier PG gene expression.

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Keywords: softening, cell wall metabolism, Chilean strawberry, polygalacturonase, pectate lyase.

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The Fragaria Genome: Structure, Organization, and Transmission

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Phylogenetic and other analyses support emphasis on *F. vesca* as a diploid (2n = 2x = 14) model for *Fragaria*, and point to *F. iinumae* as a second genome donor to the octoploid (2n = 8x = 56) strawberries. Sequencing of clones from an *F. vesca* fosmid library generated a total of ~1.75 Mb of sequence from the 200 Mb *F. vesca* genome. Thirty clones were chosen at random, while twenty were selected using probes for four anthocyanin pathway genes, four other metabolic genes, seven flowering-related genes, and five resistance-like genes. Analysis of the data set has provided insight into the organization and content of strawberry gene space. Protein-encoding genes occurred at an average density of one per 6 kb. Several tandem gene duplications were found, including five of the twenty genes targeted by probes: *Adh*, *Chs*, terpene synthase, pistillata, gRGA1. SSRs also occurred at a frequency of one per 6 kb. Most fosmids displayed regions of microcolinearity, encompassing two to five genes, in comparisons with *Arabidopsis*, *Populus*, *Vitis*, and other sequenced genomes. Both Copia and Gypsy retrotransposon-related sequences were found. The expanding *Fragaria* EST database provides an invaluable resource, complemented by the overall Rosaceae EST database, in relation to ongoing genomic annotation efforts. Intergenic distances were often quite low (500 bp to 5 kb) in strawberry, providing a basis for the PCR amplification of products spanning highly polymorphic intergenic regions while achieving robust priming in conserved coding regions of adjacent genes. Resequencing at these “gene pair loci” is being used to define gene pair haplotypes at sites on each *Fragaria* chromosome, enabling the potential differentiation of up to eight haplotypes in a single 8x individual, and providing insight into the octoploids’ subgenome composition and diploid ancestry. To simplify the analysis of gene pair and other marker transmission patterns in the progeny of an octoploid, a novel, pentaploid mapping population was derived from a diploid x octoploid (*F. vesca* x *F. virginiana*) cross.

Keywords: Fragaria, strawberry , genomics , gene pair, pentaploid.

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Anthracnose Resistance Marker Evaluation and Development in Florida Strawberry Cultivars

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Diseases caused by Colletotrichum species lead to large-scale crop loss and substantial decreases in strawberry production worldwide. In many regions, Colletotrichum acutatum is the causal agent of anthracnose fruit rot. Symptom development is favored by wet conditions and manifested as brown sunken lesions on fruit and as black lesions on runners and petioles. Many cultivars possess inherent resistance to C. acutatum and a single dominant SCAR marker segregated with anthracnose resistance in a substantial cross section of strawberry genotypes from different geographical regions (Lerceteau-Kohler et al, 2005). Since anthracnose fruit rot is a particularly deleterious problem in Florida, USA, the presence of the RCA2 markers (RCA240 and RCA417) was assessed in regional cultivars. The markers were also applied to the wild octoploid core collection. Unlike the previous study, we found no correlation between the presence of the SCAR marker and anthracnose resistance in Florida cultivars and the core collection. In fact, the most susceptible cultivars maintained the dominant marker that segregates with anthracnose resistance in other populations. These findings indicate that the marker is not useful for prediction of anthracnose susceptibility in Florida cultivars. These results may be due to other genetic sources of resistance and susceptibility in this germplasm or to local variants of C. acutatum that remain attack strawberry cultivars in the presence of the marker. A separate approach utilized a modified cDNA-AFLP method to identify differentially expressed transcripts in bulked pools of susceptible or resistant tissue from a ‘Sweet Charlie’ (resistant) x ‘Treasure’ (susceptible) cross. These sequences are being evaluated as markers for resistance as well as for direct participation in modulating disease expression.

Keywords: strawberry, Gene resistance, markers

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Translation of Photoperiodic Flowering Models to Cultivated Strawberry

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The transition from vegetative to floral growth is a critical reproductive process in most plant species and a vital process to both natural evolution and crop production. Regulation of this developmental progression has been elegantly dissected in model systems using physiological, molecular and biochemical techniques. The mechanisms underlying photoperiodic flowering control are of intense interest to strawberry breeders, as flowering habits dictate the timing of fruit set. A wide variation in the timing of the floral transition has been documented, with short day, day neutral, long day and other flowering habits observed within the cultivated octoploid species, Fragaria ×ananassa. In this study we translate the findings from model systems to the diploid and culativated strawberry. This study characterizes components of the photoperiodic flowering pathway in strawberry, including CONSTANS (CO) and the CRYPTOCHROME blue light sensor. The CO gene family consists of at least four distinct members with high similarity those in other plant species. Fragaria CO is a single copy gene per haploid genome with multiple, differentially-expressed alleles, and has been assigned to Group VI of the Fragaria linkage map. Overexpression of the Fragaria CO restores normal flowering habits to the Arabidopsis co mutant, and contributes to an uncharacteristic long hypocotyl phenotype in developing seedlings. Expression analyses indicate that unlike other plants, Fragaria CO transcripts peak only at dawn on short days. Expression patterns are similar in octoploid and diploid accessions, in both short-day and day-neutral cultivars. Limited accumulation is observed under long day conditions in all genotypes tested. Both FaCO and FaCRY have been introduced into plants in overexpression and RNAi configurations. This report provides an initial characterization of central components of the photoperiodic pathway and its role in regulated flowering in short day and day neutral strawberry cultivars.

Keywords: strawberry, photoperiodic flowering, expression analyses

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Analysis of cell wall components in Fragaria cultivars with different firmness.

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An important aspect in the study of the development and ripening of the fruit is related to the change in the texture, which lead to have soft fruits. Form the commercial point of view this becomes more important for those fruits that are known for having a short life of postharvest or "soft fruits", such as strawberries. While some articles indicate that the hydrostatic pressure (turgor) and the dehydration have an influence, another important aspect that needs to be considered is the degradation or changes in architecture of the cell wall, which has an important influence in the fruit firmness. Two species of strawberry, *Fragaria chiloensis* (Fc) and *Fragaria x ananassa* (Fa), shows differences in the firmness of their receptacles, in similar periods of development. Studies in *Fragaria x ananassa*, have shown that cell wall component like pectin and hemicellulose, change as the fruit softens, obtaining an increase in the soluble fraction of their sugars. Microarray experiments from other laboratories, using mature strawberry fruits, have shown an increase in the expression of some genes coding for proteins and enzymes involved in the modification of the cell wall (alpha- xylosidase, expansin, pectate lyase and polygalacturonase). In our laboratory, we have analyzed the presence of different pectic polysaccharides using immunofluorescense in both species of strawberry and in different developmental stages (E2 and E3). Our results show higher levels of low esterified homogalacturonan in E3 compared to E2 for both species of strawberry, using the antibody JIM5. Additionally, polymer structures such as (1-5)-alpha-L-arabinan are accumulated in epidermal and parenchymal cells, whereas other structures such as (1-4)-beta-D-galactan, xylogalacturonan, highly esterified homogalacturonan and partially de-esterified homogalacturonan, recognized by the monoclonal antibodies LM5, LM8, JIM7 and LM7, respectively, are preferentially accumulated in cells from the epidermis of the receptacle, observing very little or no fluorescence in parenchymal cells. On the other hand, 2-DE analysis of total proteins, showed differences in protein accumulation between the two states of maturation as well as for both species of strawberry studied. The results support the idea that, these two species exhibit a different behavior during the development due to their differences in the cell wall degradation.
Supported by Millenium Nucleus in Plant Cell Biotechnology ICM P 06-065-F and grant UNAB DI-01-06/I

Keywords: Fragaria, cell wall, proteomics

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Study of the aroma metabolic pathway in *Fragaria chiloensis*

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Volatiles compounds from the native Chilean strawberry (*Fragaria chiloensis*) and cultivated strawberry (*Fragaria x ananassa*) were analyzed through gas chromatography-mass spectrometry and gas chromatography-olfatometry. A high concentration of 3-oxo-α-ionol, a terpenoid that belongs to a C13-nonisoprenoid family was isolated only in fruits of the Chilean native strawberry. This compound contributes to floral and citric attributes of the native fruits and possesses interesting flavor aroma properties together with low aroma thresholds. The 3-oxo-α-ionol is a product of oxidative carotenoids cleavage. A potential Carotenoid Cleavage Dioxygenase (FcCCD1) gene was identified in a 9,000 *Fragaria chiloensis* ESTs collection (MIFAB, Millennium Institute of Fundamental and Applied Biology). The open reading frame of the FcCCD1 consists of 1,565 bp encoding a 521 amino acid protein. The FcCCD1 is 78 % identical to LeCCD1A, an enzyme responsible for formation of ß-ionone in tomato (Lycopersicon esculentum), that has been shown to be the second most important volatile contributing to tomato fruit flavor. Expression of the gene was studied by RT-PCR at different developmental stages of fruits from Chilean strawberry ecotypes. An induction of the gene expression approaching to the last stage of ripening was observed. This research was supported by ICM P06-065-F; Proyecto regular UNAB DI-51-06/R

Keywords: Strawberry (Fragaria chiloensis), Aroma biosynthesis, Bioinformatics, Flavour, Functional Genomics.

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Exploration of the Malus NBS-R Gene family by saturation sequencing and genetic Mapping

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To date five classes of R proteins have been identified as e-LRR, LRR-TM, toxin reductase, protein kinases and NBS-LRR (major class). The NBS-LRR class is made up of a centrally located nucleotide binding site (NBS) separated by a hydrophobic GLPL motif from the Cterminal leucine rich repeat (LRR) domain. The LRR domain has been implicated in pathogen recognition and the NBS domain hydrolyses ATP molecules and supplies energy to trigger a cascade of signal transduction pathways that lead to a hypersensitivity response. We have sequenced 661 NBS domain fragments of candidate NBS-LRR genes (RGAs) using Sanger sequencing and an additional 5260 using the 454 technology. The Sanger dataset contained sequences with an average length of 520 bases and was used for phylogenetic analyses. These genes were broadly divided into TIR and non-TIR subfamilies in which a total of 14 clusters were obtained. RGA sequence data showed a large number of pseudogenes containing nonsense or frame-shift mutations. Analysis of coding to silent mutation rates showed clusters undergoing diversifying, neutral and purifying selection pressures. Sequence assemblies containing both Sanger and 454 data generated a total of 278 contigs with 54 containing at least one sequence from Sanger and 224 from 454. A total of 841 singletons were obtained and thus complicating estimation of the RGA copy number in apples. Analysis of such estimates and verification of genuine to sequence artefacts in the singleton dataset remain questions that require completion of the apple genome sequencing to evaluate. Targeted sequencing of RGA transcripts from apple seedlings before and after inoculation with scab and *Powdery Mildew* was used as a technique to analyse the NBS-LRR transcriptome. This technique allowed for the identification of genes whose transcription is either induced or suppressed by pathogen infection, with the later also including pseudogenes. This technique also enabled the identification of genes that appear to be
constitutively transcribed and possibly playing a role in the plant basal defence systems.
Candidate SNPs were identified in re-sequenced RGAs and these were subsequently genotyped in six seedlings of the Anna x Golden Delicious bin mapping population. SNaPshot™ assays were used to verify segregation of these SNPs in the mapping population. Using this approach one of the twelve RGA clusters identified through phylogenetic analyses was subsequently mapped onto the Anna x Golden Delicious genetic linkage map.

Keywords: apple, 454 sequencing*, Powdery Mildew*

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Functional Genomics Resources for Malus ESTs

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Publicly available databases contain in excess of 250,000 Malus sequences, which when clustered using a 95% threshold yields 17,460 tentative consensus sequences and 25,478 singletons. HortResearch has contributed over 150,000 EST sequences, from some 43 cDNA libraries, to this dataset. The cDNA libraries were derived from a variety of tissues, developmental stages, tissues and cell lines subjected to biotic and abiotic stresses and a variety of apple cultivars and rootstocks. A custom-built, Bioview interface allows researchers to select ESTs, derived from the HortResearch sequencing effort, and request complete sequence determination and subsequent channelling into a functional genomics pipeline. The pipeline offers a number of options for the generation of transformation constructs and transformation of plants for determining gene function, including: • Conventional and Gateway-adapted plant transformation vectors for gene expression. • Gateway-adapted plant transformation vectors for gene silencing. • Transient expression in Nicotiana benthamiana. • Stable transformation of Arabidopsis. • Stable transformation of Malus x domestica cultivars ‘Royal Gala’ and ‘Bolero’. • Maintenance of the plants under containment glasshouse conditions to promote flowering and fruiting. In addition, researchers have the ability to request transcript profiling experiments using our 15,723 Malus EST data-based oligonucleotide microarrays and facilities.

Keywords : Malus, EST, Functional Genomics, vectors, transgenic plants.

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INVESTIGATION OF CYANIDIN AND PELARGONIDIN CONTENTS IN THE GENUS *FRAGARIA* L.

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Cultivated strawberry (*Fragaria* × *ananassa*) fruit has been reported to contain primarily pelargonidin and little cyanidin. The antioxidant potential of cyanidin is reportedly twice that of pelargonidin. Using a modified HPLC method, fruit cyanidin (Cyn) and pelargonidin (Plr) contents were assayed in 73 *Fragaria* accessions, and % Cyn (Cyn as % of Cyn-plus-Plr) values were calculated. In a time course study, Plr content increased during fruit ripening, while Cyn content held steady. Consistent with previous studies, % Cyn was comparatively low (5-20%) among twelve *F.* × *ananassa* cultivars, but was > 40% in diploid *F. vesca*. Importantly, three *F. chiloensis* accessions had greater than 50% Cyn, and subsp. *lucida* CFRA1691 had the overall highest Cyn content: 707 µg/g FDW. Of the *Fragaria virginiana* subspecies, subsp. *virginiana* from Ontario had the highest Cyn contents of 293 µg/g FDW. The data suggest that the relatively low % Cyn in *F.* × *ananassa* may be the consequence of breeding, as the accessions from the reputed provenances of *F. chiloensis* from Chile and *F. virginiana* from the south in the United States all contained unremarkable fruit Cyn content. An octoploid F_1 population derived from hybridizations of 'Bountiful' and 'Pink Panda' segregated for both Cyn and Plr contents. Our investigation identifies useful germplasm, offering promise for increasing Cyn content and antioxidant potential through introgressive breeding and provides a foundation for future efforts to identify the gene(s) responsible for high cyanidin content in strawberry.

Keywords: strawberry, anthocyanidins, anthocyanins

Poster abstract –session 2

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Genes of the climacteric fruit softening pathway in Rosaceae tree fruit – a review

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Ethylene plays numerous roles in plant development. The climacteric phase of ripening that occurs for many fruit crops is associated with a rapid production of ethylene, which triggers a cascade of many ripening processes associated with fruit quality. This ethylene burst switches on many cell wall modifying enzymes that are part of the process of fruit softening. Genes encoding ethylene biosynthesis (e.g. ACS and ACO) and signal transduction (e.g. ERS) and certain cell wall modifying enzymes (e.g. endoPG and Exp) are therefore key genetic control points for fruit softening. Considerable evidence has accumulated for Rosaceae tree fruit – stone fruit and pome fruit – that genetic variation within these genes contributes to important horticultural differences in fruit texture. Due to their hierarchical arrangement, the role of any one of these genes is better understood in the context of the larger biochemical pathway. These genes are excellent primary targets for characterizing the genetic predisposition of current cultivars and advanced selections, marker-assisted breeding using natural variation, and genetic manipulation using created alleles. Genetic improvement of tree fruit texture via an understanding of the climacteric fruit softening pathway in this model family offers a stable means of increasing fruit consumption for improved public health. We present a review of advances made so far and prospects and challenges for future exploration of this important biochemical pathway for the benefit of stone and pome fruit industries.

Keywords: fruit quality, ethylene, endoPG, apple, peach.

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Almond genetic linkage map and quantitative trait loci for flowering time in a "Nonpareil x Lauranne" population

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Almond (*Prunus dulcis*) is the only nut crop of the Rosaceae and until recently has received marginal attention in relation to its cultivation and breeding. As one of the earliest species to bloom in spring, almond trees can experience severe crop loss due to late frosts and unfavourable climatic conditions, every other year on average. The Australian almond breeding program is one of the world's major almond breeding programs and has developed a genetic linkage map. One of the objectives of the project is to investigate the inheritance of flowering time. The flowering time of a "Nonpareil x Lauranne" population
was evaluated for two years. Seven stages were defined to evaluate flowering time and observations for each stage were recorded following the same procedures for each year.
The data collected were analysed, followed by Principal Component Analysis (PCA) to assess the categories that contain most information on tree differences. This reduced the number of categories by unifying those most similar into one category. Using a form of Generalised Linear Model (GLM), called the proportional odds logistic model, the data were analysed considering the development of buds and flowers as latent variables. The analysis was able to distinguish between early and late flowering trees by assigning a point on a scale ranging from minus to positive values. These values were used in QTL analysis of flowering time. It is hoped that our work will lead to the identification of genetic markers useful in breeding for altered flowering time

Keywords: flowering time, QTL, almond

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Mapping of apple genes found to be expressed during the *Erwinia amylovora:Malus* interaction

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The necrogenic enterobacterium, Erwinia amylovora (Ea) is the causal agent of the fire blight (FB) disease of many Rosaceae species, including apple and pear. During the infection process, the bacteria induce an oxidative stress response with kinetics similar to those induced in an incompatible bacteria-plant interaction. In order to understand the mechanisms that distinguish resistant and susceptible responses in FB resistant and susceptible apple genotypes after inoculation with Ea, differentially expressed genes were identified by cDNA-AFLP analysis. cDNA was isolated from M.26 (susceptible) and G.41 (resistant) apple tissues collected 2 and 48h after challenge with a virulent Ea strain or mock (buffer) inoculated. To identify differentially expressed transcripts, electrophoretic banding patterns obtained from cDNAs treated with the Licor cDNA-AFLP kit. In the AFLP experiments, using both kits, M.26 and G.41 showed different patterns of expression, including genes specifically induced, not induced, or repressed by Ea. In total, 190 EST's differentially expressed between M.26 and G.41 were identified using 42 pairs of AFLP primers. cDNA-AFLP analysis of global EST expression in a resistant and in a susceptible apple genotype identified three major classes of genes. Sequencing data for the EST's showed that genes linked to resistance, encoding proteins involved in perception, signaling, defense and apoptosis, were modulated by Ea in its host plant. The time course of expression of some of these EST's selected via a bioinformatic analysis has been determined. Some of these EST's have been also mapped

Keywords: apple, Erwinia *amylovora*, AFLP

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Self incompatibility in Malus: modelling and structure studies of S-RNase and SLF proteins

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Self incompatibility (SI) is a widespread mechanism in many flowering plants, which was identified in Rosaceae, Solanaceae and Scrophulariace. In these families, SI is gametophytically controlled and retains inter-specific genetic variation by out-crossing promotion. In Malus SI prevents self-fertilization through growth suppression of pollen tubes in the pistil with the same S alleles. Self/non-self discrimination in SI is expected to occur as a result of the interaction between the S-determinants of pollen (SLF/SFBB) and pistil (S-RNase). However, previous studies showed that the interaction between hypervariable (HV) region/s in SLF/SFBB and S-RNase is important to recognize self and non-self pollen, but how SLF/SFBB and S-RNase interact to trigger SI reaction is still largely unclear. These regions are usually exposed on the molecule's surface, and are positively charged. Knowledge of the three-dimensional structure may aid our understanding of Rosaceae S-RNase molecular recognition between S-alleles and, recognition between S-RNase(s) and the pollen S-gene product(s). Unfortunately the 3D-structures of any S-RNases and SLF/SFBBs in Malus has not solved, so we used the homology modelling to predict of them, whoever, it was difficult to model the full length SLF/SFBB containing about 400 amino acid as a close template could not be found. We also used docking method to find the residues, which involved in interaction between these proteins. This study aims to get a clearer understanding of the ligand-receptor binding mechanism of SI using molecular evolutionary, homology modelling and protein-protein docking methods focussing on the hypervariable (HV) regions of S-RNase and SLF/SFBB although other regions of the molecule may also be involved.

Keywords: Self incompatibility, Malus, Homology modelling, Protein protein docking.

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Allergens Expression in Apple Fruits

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Roseceae fruits are regularly consumed for their positive influence on human health. However, they are also responsible for allergic reactions in susceptible individuals due to the presence of specific proteins called allergens. Apple allergy is due to four major allergens families: Mal d 1, Mal d 2, Mal d 3 and Mal d 4. For each family, many iso-allergens have already been mapped and characterized. In the framework of the Isafruit UE-Project some researches has started to identify factors such as genotype, tissue or stage of fruit development that influence allergens expression and hypothetically apple allergenicity. As genotypes, two cultivars were chosen: Gala and Florina. Gala apples are known as highly allergenic while the fruits of Florina are not tested yet. Some genomic sequences within the four allergens were cloned from both cultivars and specific primers were designed for each gene, in order to study the allergen expression in apple peel and flesh, at different stages during fruit development. The expression studies were performed at the mRNA level in two different ways: semi-quantitative and quantitative PCR. The results showed high variability in expression level between the four allergens and different trends of expression during the fruit development. In general, the higher expression levels were detected in fruits at harvest. Mal d 1 was the most expressed allergen, followed by Mal d 3, Mal d 2 and finally, Mal d 4 that was expressed at very low levels in each sample. Because Mal d 1 seems the main allergen it will be important to study the expression of each Mal d 1 iso-allergen. Moreover, allergen expression appeared to be tissue-specific: Mal d 1 and 3 transcripts were more abundant in fruit peel than flesh. The opposite trend was observed for Mal d 2. The allergen expression in Florina was higher than Gala therefore Florina fruits could be presumed more allergenic than Gala. However, to obtain more comprehensive and accurate information on the role of different allergens in the allergenicity further in vivo and in vitro analysis are needed.

Keywords: Mal d 1, Mal d 2, Mal d 3, Mal d 4, apple allergen

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Comparing high-throughput DNA extraction methods for marker-assisted selection in Malus

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A fundamental requirement of practical marker-assisted selection is high-throughput DNA extraction. To date, few such extraction methods are established for tree fruit. We explored three methods: one with magnetic manipulation of metal beads for mixing, another using silica beads that serve to desiccate and grind the samples, and one employing a robot that completely automates the process. Equipment, reagent, and labor costs were compared, as were throughput levels and quantity of PCR-quality DNA produced from Malus leaf tissue. Two of the extraction techniques are suitable for tens of thousands of seedlings per season, although the silica bead method is more streamlined for sample collection. The robot method is most efficient for extracting DNA from hundreds of thousands of seedlings. Efficient high-throughput DNA extraction is now being integrated into the Washington apple breeding program. These efforts can be expanded to other tree fruit breeding programs that seek to routinely apply genetic markers to assist with new cultivar production.

Keywords**:** efficiency, automation, cost, PCR, breeding.

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Assessment of the genetic diversity and disease resistance of wild Malus orientalis seedlings from Turkey and Souther

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Genetic diversity and disease resistance are described for 496 seedlings from wild populations of Malus orientalis collected in southern Russia and Turkey in 1998 and 1999. Eighty five half-sib families were genotyped using seven microsatellite markers and disease resistance was determined for apple scab (Venturia inaequalis Cooke), cedar apple rust (Gymnosporangium juniperi-virginianae Schwein) and fire blight (Erwinia amylovora Burrill). Of the M. orientalis seedlings assayed for disease resistance, 76 were resistant to scab and fire blight, 16 were resistant to scab and cedar apple rust, 11 were resistant to fire blight and cedar apple rust, and 20 were resistant to all three diseases. Seedlings genotyped from the four Turkish collection locations were more diverse than those genotyped from the two Russian Caucasus locations. Bayesian analyses of the population structure revealed six distinct clusters. Most of the individuals segregated into two clusters, one containing individuals primarily from southern Russia and the other containing individuals from both Russia and northern Turkey. Individuals in the four small clusters were specific to Turkish collection locations. These data suggest wild populations of M. orientalis from regions around the Black Sea are genetically distinguishable and show high levels of diversity.

Keywords: Malus orientalis, Genetic diversity, Disease Resistance.

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Cytoplasmic Characterization of the US Malus Core Collection

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In a plant cell, genetic material resides in three distinct compartments. The nuclear DNA undergoes recombination when transmitted to the progeny while mitochondrial and chloroplast DNA is inherited maternally in most cases. We have had a long term interest in rapidly accessing chloroplast genome sequence information and utilizing it for resolving phylogenetic issues. In our recent studies we had identified a unique hypervariable region that may be informative about evolutionary time scales and inter-relationships between genotypes in a taxonomic group. The US Malus core collection was screened for 45 unique genotypes to survey the chloroplast genome content in these species. The 1.5 kb chloroplast hypervariable region was amplified and sequenced directly. Sequence editing was performed using Sequencher. We have arrived at a maternal phylogenetic lineage for the US Malus core collection utilizing the sequence information.

Keywords: Chloroplast, maternal lineage, Malus, Rosaceae, genotyping

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EST-SSR Mapping Focussed on the Analysis of Synteny within the Rosaceae.

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The availability of new technologies for fast and effective generation of sequence data enabled the rapid expansion of the genomic resources available for molecular breeding techniques. Currently available EST databases for Prunus and Malus provide the resources needed for comparative genomic analysis between these two closely related Rosaceae. As part of a bigger project involving the development of EST-SSR markers for Malus, the development and implementation of EST-SSRs showing homology to EST sequences from Prunus are prioritized. Being able to align the Malus genetic linkage map to the already available high density consensus genetic linkage map for Prunus, onto which many candidate genes and QTLs have been mapped, will facilitate the localization of candidate genes and putative QTLs for a variety of economically important traits on the Malus genetic linkage map. Comparative mapping among these two species will also facilitate a better understanding regarding evolutionary processes of chromosomal rearrangement.

Keywords: Rosaceae, Synteny, EST-SSRs

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MOLECULAR AND PHYSIOLOGICAL BASES OF AROMA BIOSYNTHESIS IN APRICOT FRUIT (Prunus armeniaca L.)

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A salient genetic attribute of tree fruits is the unique blend of sugar, acid, phenolic and volatile components that determine their flavor. This complex genetic trait is manifested in ripe fruit through a complex interaction of metabolic pathways and regulatory circuits that results in the unique fruit flavor composition, a key to fruit consumption. Loss of flavor, particularly the aroma attribute, is a limiting factor in apricot quality. In spite of its significance, very little is known at the molecular, genetic and biochemical level of the genes and pathways that are responsible for the synthesis, accumulation and regulation of volatile compounds. In order to understand the biological basis of aroma biosynthesis we characterized and differentiated four stages in terms of maturity parameters, aroma-related volatile compounds, and gene expression levels. We cloned and quantified by qPCR the gene(s) encoding: alcohol acyl transferase (AAT), alcohol dehydrogenase (ADH), lipoxygenase (LOX) and pyruvate decarboxylase (PDC), key enzymes involved in alcohol and aldehyde synthesis. As fruit ripening progressed, we observed an increase in adh and aat transcript levels simultaneously with a decrease in aldehydes (i.e. hexanal and (E)-2-hexenal) and alcohols (i.e. 1-hexanol). We think that further studies to be performed in terms of identifying and characterizing these genes in P. armeniaca will contribute to understand overall aroma development during fruit ripening (Funded by FONDECYT 1060179).

Keywords: Prunus armeniaca, Ripening, Aroma, Volatile compounds, Gene expression.

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Isolation and characterization of *PmFT*, an *FT* homolog from *Prunus mume*

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PmFT, a homolog of FT in *Arabidopsis thaliana* (L.) Heynh., was isolated from a genomic library constructed from Japanese apricot (Prunus mume Sieb. et Zucc. cv. Nanko). Genomic DNA blot analysis showed that PmFT is a single copy gene encoding a total of 174 amino acid residues, 2 residues longer than FT. PmFT is comprised of four exons and three introns, as is *Arabidopsis thaliana* FT. Expression analysis by RT-PCR showed that PmFT is expressed in all tissues tested including floral bud, leaf bud, sepal, petal, stamen, pistil, leaf, and fruit with the highest expression level being observed in floral bud. Overexpression of PmFT results in precocious flowering in both the wild type and the ft-1 strains of *A. thaliana*, indicating that PmFT promotes flowering, as does FT

Keywords: *Prunus mume* , expression analysis

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SYNERGY BETWEEN AGRONOMY AND MOLECULAR GENETICS TO ESTABLISH A CHERRY BREEDING PROGRAM IN CHILE

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Sweet cherry is one of the most profitable fruit exports with a great potential. Chile is the largest cherry fruit exporter of the Southern hemisphere having only 1.6% of the world's production. Therefore, a large demand is unsatisfied given that most of the consumption in the Chilean summer is concentrated in the Northern hemisphere. One major concern is the future access to new varieties which is becoming more restrictive. Not having its own varieties threatens the Chilean competitiveness and viability of the sector. The high profitability of the crop can sustain a program of genetic improvement for the cost of the program would be a small part of the amount that is exported annually. We have set out to establish a platform in which agronomic science and molecular genetics converge under the following objectives: Creation of sources of genetic variation establishing a collection of germplasm of foreign varieties and Chileans eco-types. Creation of at least 20 segregating populations where selections can be made for adaptation to diverse agro-ecological zones of Chile established previously. Construction of 2 genetic linkage maps with SSR and SNP molecular markers to position QTLs and to develop strategies of marker assisted selection for important agronomics characters (fruit colour, yield and adaptation to agro-climatic zones, quality of post harvests, among others). To develop a genomic platform of functional genetics, sequencing 30,000 ESTs and analyzing the differential expression of genes between varieties with contrasting characteristics, with the purpose of identifying candidate genes related to important characteristics for the success of the cultivar. Accomplishing these objectives will set the foundation of a genetic improvement program using modern molecular tools. If successful the program will allow Chile to have varieties own having good quality and yield and adapted to different zones which can travel long distances. This in turn will enhance the profitability of the crop for farmers and make Chile a better competitive in the world's fruit market.

Keywords: Sweet cherry, Breeding program, Molecular markers, Germplasm, Segregating populations.

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Identification of Genomic Factors Regulating Flower Density in Sweet Cherry

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Floral bud initiation and development in Rosaceae controls fruit yield and quality. In sweet cherry, Prunus avium, the rootstock directs scion floral bud density. Determining the genetic controls and mechanisms for the interaction of the rootstock on fruit production can be extremely important for fruit production. Utilizing information gained from this project will aid in improving fruit quality while reducing thinning requirements and maintaining high production. A systems biology approach combining transcriptomics, proteomics and translational genomics is being undertaken to dissect this developmental program. Floral buds collected from different rootstock/scion combinations are being probed to identify the repertoire of genes that may be involved in determining the number of floral bud primordia. In the future, protein-protein interactions of the gene products will be examined to ascertain the major players in floral bud density determination. Functional validation of the identified genes will be performed via either strawberry or sweet cherry transformation systems.

Keywords: Rosaceae, Prunus avium, Systems biology , Flowering, Transcriptomics.

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Changes in the expression of polygalacturonase, pectate lyase and acc-oxidase genes in mealy peaches during postharvest.

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Peaches [(*Prunus persica* L. (Basch.)] destined to far markets should be maintained in cold storage in order to reduce their metabolism and prolong their commercial value. During this process they are exposed to suffer physiological disorders that affect the final quality. Among these physiological alterations, the chilling injury that is evident by the development of mealy flesh is one of the main problems. This phenomenon is caused by the uneven proportion of enzymes that affect the mesocarp's cell wall, however the molecular base involved in this process is not fully understood. The analysis of gene expression changes during postharvest handling offers an alternative strategy to understand the molecular bases of mealiness in stone fruit, In this work, we evaluated the changes in the relative abundance of polygalacturonase (endoPG), pectate lyase(PL) and acc-oxidase (ACO) transcripts in juicy and mealy fruit for two melting fleshed cultivars ('Elegant Lady' and 'O'Henry') and one non melting fleshed cultivar ('Ross'). The results showed that the expression levels of the genes encoding these three enzymes diminished in mealy fruits and further support the previously reported ethylene regulation of endoPG. Moreover, our data is consistent with a role of PL in the degradation of high molecular weight during fruit ripening.

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Keywords: Peach, expression analysis, polygalacturonase

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QTL analysis of metabolites involved in fruit quality in Prunus species and co-location with candidate genes in the European Project ISAFRUIT – Preliminary results obtained on *P. persica* related species

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ISAFRUIT is an integrated European project aimed at increasing fruit consumption with the goal to improve health of the consumers. One of the objectives of the work package GENFRUIT is to set the genetic basis of fruit quality by mapping the major genes and QTLs involved (Work package 6.1). This is performed on two peach (*Prunus persica* L. Batsch) and two apricot (Prunus armeniaca L.) progenies. Only results obtained on peach will be presented. One is an intraspecific F2 progenies issued from Ferjalou Jalousia® x Fantasia cross (JxF) and the second derived from an interspecific cross between the peach Summergrand and the clone P1908 of a wild species related to peach, Prunus davidiana (BC2). The composition of the fruit flesh was evaluated on 130 individuals in JxF and 120 in BC2. The major metabolites were evaluated using 1H-NMR metabolic profiling at INRA Bordeaux and the phenolic compounds were determined by HPLC-DAD at INRA Avignon. The genetic maps derived from the progenies have been constructed or saturated using anchor markers mapped to the reference Texas' x 'Earlygold' (TxE) map. Preliminary results on QTL detection concerning major metabolites and phenolic compounds will be presented. The positions of these QTLs were compared with those of more than 100 candidate genes (CGs) implicated in physiological pathways of sugar/acidity metabolism or flavonoid biosynthesis, previously bin mapped in TxE regions at IRTA (Cabrils). First colocalisations of QTLs with candidate genes were identified and the latter are currently being mapped in both progenies. The colinearity of the CGs and QTLs detected on the two populations will be examined.

Keywords: fruit quality, Prunus, metabolic profiling, QTL, synteny

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Identification of clusters of co-regulated *Prunus persica* genes under different post-harvest conditions.

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The distance that fruits must be shipped during export and delays in reaching the market, due to sanitary regulations or transport from distribution warehouses to the supermarket and then to the final consumer, have increased the need to improve the shelf-life of fresh fruits. In order to identify candidate genes that may be incorporated into a peach marker assisted breeding program for improved fruit post-harvest quality, we have performed comparative analyses of the abundance of expressed sequence tags from peaches under four different post-harvest conditions: 1) non-ripe fruits, 2) ripened fruits, 3) non-ripe cold-treated fruits, 4) ripened cold-treated fruits. These analyses have revealed 13 clusters of co-expressed or co-regulated genes under different post-harvest conditions. These clusters of co-regulated genes include clusters of genes that show induction/repression in ripe fruits, induction/repression during cold-storage, or induction/repression in woolly fruits. Further characterization of these co-regulated genes should provide insight into the transcriptional regulatory networks that lead to the characteristics present in fruits under different post-harvest conditions.

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Keywords: Peach, expressionanalysis, co-expressed genes

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An integrated physical genetic map of peach genome and its application for Prunus genetics and breeding

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Using high-information content fingerprinting (HICF) we developed a first generation physical map for peach, a model rosaceous species. The map is composed of 2,138 contigs containing 15,655 BAC clones. These BACs represent 4.3x peach genome equivalent. In total, 2.633 markers, i.e. peach unigene EST set (PP_Le), cDNAs, RFLPs and overgo probes, are integrated into physical framework. Altogether, 60% BACs in FPC database have hybridization hits. Therefore, the peach physical map is biased to the expressed portion of the peach genome. According to conservative estimate, the physical length of the HICF-based physical map is 303 Mb that corresponds to an estimated size of the peach genome of 290-300 Mb (Baird et al., 1998). The physical framework is anchored to the Prunus genetic map using 152 core genetic probes derived mainly from the reference T x E (Texas x Early Gold) cross. The anchored contigs span over 44.5 Mb (or 15%) of the peach genome. To provide additional anchor points for the map, we exploit currently a set of ~400 of bin-mapped peach unigene EST-SSRs using overgo hybridizations. This will allow us to assign floating contigs to the Prunus genetic map and collapse physical framework into mega-contigs.

The integrated physical/genetic map serves as a tool for microsyntheny analysis and map-based cloning of the regions of interest across the Rosaceae family. Our current efforts are aimed at the genomic regions conferring resistance to plum pox virus in Prunus species, peach fruit and almond seed quality characters and metabolic pathways leading to accumulation of phenylpropanoid compounds of a consumption value. Using combined genetic/ genomic approach, we are also targeting genomic regions involved in blooming time and showy flower (SH) phenotype in Prunus.

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Genetic relationships of *Pyrus* species based on analysis of a ripening specific O-methyltransferase gene (*PpOMT1*)

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PpOMT1 was isolated as a ripening specific gene by cDNA differential display. Database searches revealed that PpOMT1 belongs to the O-methyltransferase family, not be involved in DNA, RNA and protein methylation and lignin biosynthesis. PpOMT1 hybridized to an RNA in ripe and overripe fruit, but not in other tissues. Transcripts accumulated to much higher levels in overripe fruit, expression being fruit specific. Interestingly, while high expression of PpOMT1 was detected in all Japanese and European pear cultivars, no expression was observed in any of the Chinese pear cultivars. Moreover, this gene is expressed during fruit ripening in apple, quince and Chinese quince. The absence of expression in Chinese pear cultivars, suggests that PpOMT1 is a specie specific gene, revealing a difference in transcriptional activation mechanisms between Chinese pear and other Pyrus species. Southern analysis with PpOMT1 as a probe against several restriction enzyme digests detected a high degree of RFLPs among Pyrus species. Primers derived from sequences of PpOMT1 cDNA produced no fragment, 2.1kb and 1.7kb for Chinese pears, Japanese pears and European pears, respectively. These markers have been successfully used for genetic relationships of wild species and cultivars in Pyrus. PCR amplification using the same primers produced fragments of 2.5kb, 2.2 kb and 3.4kb for apple, quince and Chinese quince, respectively. The differences in fragment size are mostly due to length differences in second intron. The genomic sequence revealed that more than 7kb sequences are deleted in 5' regions of PpOMT1 gene in Chinese pears and some wild species. It is possible that the PpOMT1 gene is an effective molecular marker distinguishing not only Pyrus species, but also other subfamily Maloidaeae.

Keywords: Pirus, O-methyltransferase , lignin biosynthesis

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Genetic Engineering of Drought and Salt Tolerance in Peach Tree

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Genetic improvement of woody fruit plants using conventional breeding requires tremendous efforts because of the long generation time of the plants. The obstacles of high heterozygosity, long juvenile periods and auto-incompatibility impose more limitations on the use of traditional breeding methods for genetic improvement of the plants. Thus, application of biotechnology heavily relying on transgenic techniques in peach tree genetic improvement would certainly accelerate the breeding process, leading to new cultivars with greatly enhanced performance in agricultural production. Drought and salt tolerance in plant is important in agriculture facing the big challenge of diminishing water resources globally. We conducted research to genetic engineer peach plant with enhanced drought and salt tolerance by overexpression of AVP1 gene from Arabidopsis. The AVP1 encodes the vacuolar H+-pyrophosphatase (H+-PPase) and its overexpression in transgenic Arabidopsis, tomato and cotton has been demonstrated to result in (i) greater pyrophosphate-driven cation transport into root vacuolar fractions, (ii) increased root biomass, and (iii) enhanced recovery of plants from an episode of soil water deficit stress. Using the transformation protocol established in our lab, we have produced putative transgenic peach plants harboring AVP1 gene. Molecular analyses are being conducted to evaluate transgene integration into the host genome and transgene expression in transgenic plants. Experiments will also be conducted to test whether the expression of AVP1 gene in transgenic plants will enhance plant tolerance to drought and salt conditions. Genetic engineering of enhanced drought and salt tolerance in peach will lead to new cultivars that can be used directly in production. It can also provide new genetic resource for enhancing peach conventional breeding efforts.

Keywords: Drought tolerance, Genetic Transformation, Salt tolerance, Transgenics, Vacuolar proton pyrophosphatase

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Genes controlling the waterlogging response in Prunus Rootstocks

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Prunus rootstocks confer an ensemble of phenotypic traits upon the scion/rootstock complex. Some are rootstock-specific, such as waterlogging tolerance. The breeding strategy for adaptative traits is based upon the understanding of the physiological and molecular aspects involved in such adaptative response. In an attempt to identify genes which may be responsible for this adaptation a candidate gene approach was used. Looking for candidate genes representing analogs of twenty proteins known as anaerobic polypeptides (ANPs), such as enzymes involved in sucrose composition, glycolisis, ethanol fermentation and aerenchyma formation. Public expressed sequences (EST and cDNA) were utilized to identify genes expressed in *Prunus* rootstocks. ESTs and cDNA sequences from Genomic Rosaceae Database (GRD) and ESTree database were downloaded, after performing BLAST comparisons, screened for vector and separated into tentative consensus sequences by specific oligo design. Now we report the cloning and characterization of three ANPs: *ADH, PDC* and *XET,* differentially expressed under hypoxia and anoxia conditions, showing the different homologies with the corresponding genes from other species and with the gene expression pattern in *myrobalan* plum as compared to the reference a*mygdalus* rootstocks.

Keywords: adaptative traits, anoxia, hypoxia, ESTs, water-logging

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Genetic and Quantitative Analysis of Red Raspberry (Rubus idaeus) for Heat Tolerance and Longer Chilling Requirement

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Despite the proximity to major markets, there are no major producers of raspberries (Rubus idaeus) in the southeastern part of the United States due to lack of adapted cultivars. Well adapted cultivars in southern states like North Carolina, require tolerance to warm humid summers, and high chilling requirements to tolerate temperature fluctuations in the winter. The berry-breeding program at North Carolina State Univ. has generated a population that segregates for both traits by crossing a R. parvifolius x idaeus cv 'Tulameen' hybrid to a R. idaeus cv 'Qualicum'. The specific aims of this study is to develop a genetic linkage map of red raspberry based on this population, and locate significant QTL regions associated with these two main traits. Chilling requirements have been screened in this population for winter of 2006 and 2007. We discuss the preliminary results of the map and associated QTL regions to tolerance to fluctuating winter temperatures.

Keywords: red raspberry, QTL mapping, Rubus idaeus.

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DEVELOPING A GENOMIC LIBRARY OF NEAR ISOGENIC LINES (NILs) IN DIPLOID FRAGARIA

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The genomes of the cultivated (*Fragaria x ananassa*) and wild (*F. vesca*) strawberry show high macrosynteny and colinearity, suggesting high homology and recent polyploidisation. *F. vesca* has been proposed as a model for genome analysis in the genus, since it has several advantages: small genome size, ability for genetic transformation and regeneration, ability to be inter-crossed with a diverse array of diploid Fragaria species providing segregating populations that have a short intergeneration time (12 to 20 weeks). Furthermore, wild strawberries are characterized by much higher concentrations of plant volatiles than the cultivated ones, being interesting donors for strawberry breeding programs. Near Isogenic Lines (NILs) are potentially powerful tools for the study of quantitative trait loci involved in fruit quality and other important complex traits, besides the introduction of new genetic variability in modern cultivars from exotic sources. In order to develop a NIL collection in diploid Fragaria, we took as starting point the parents of the F2 population used for the construction of the diploid strawberry reference map: F. nubicola FDP601 (used as donor parent) and *F. vesca* FDP815 (used as recurrent parent).We estimate that each NIL will contain a unique introgression of approximately 30 cM (6%) from the donor genome, and an overlap of introgressed fragments between NILs of about 10 cM (33%), therefore each line will contain 20 unique centimorgans from the F. nubicola genome. The complete NIL set will contain about 25-40 lines.

Keywords: Near Isogenic Lines, Fragaria, population, phenotypic variation.

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The RCA2 Marker does not Segregate with Anthracnose Resistance in Florida Strawberry Cultivars or Core Wild Octoploids

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The disease spectrum induced by Colletotrichum species leads to large-scale crop loss and substantial decreases in production worldwide. In many regions Colletotrichum acutatum is the causal agent of anthracnose fruit rot. Symptoms are exacerbated by wet conditions and manifest as black lesions on runners and petioles, but also cause brown sunken lesions on fruit. Many cultivars maintain inherent resistance to C. acutatum and a previous study has demonstrated that a single dominant SCAR marker segregates well with anthracnose resistance in a substantial cross section of strawberry genotypes from different geographical regions (Lerceteau-Kohler et al, 2005). Anthracnose is a particularly deleterious problem in Florida, USA, so the presence of the RCA2 markers (RCA240 and RCA417) was assessed in regional cultivars. The markers were also applied to the wild octoploid core collection. Unlike in the previous study, the results show no correlation between the presence of the SCAR marker and anthracnose resistance in Florida cultivars and the core collection. In fact, the most sensitive cultivars maintain the dominant marker that segregates with anthracnose resistance in other populations. The results indicate that the marker is not useful for prediction of anthracnose sensitivity in Florida cultivars. This finding may be due to other genetic sources of resistance and susceptibility in this germplasm or possibly local variants C. acutatum that remain active in the presence of the marker.

Keywords: strawberry, Fragaria, anthracnose, Collectotrichum acutatum, molecular marker.

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Evaluation of Strawberry Cystatin Gene Family Members as Sting Nematode Antifeedants

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Studies in the Diaz lab (ESTI, Madrid, Spain) have characterized Cyf1, a strawberry gene encoding a plant cystatin. Cystatins are cysteine protease inhibitors naturally produced in plants and animals that function in a variety of developmental and physiological contexts. In Florida, USA, post methyl-bromide strawberry cultivation is threatened by the sting nematode, Belonolaimus longicaudatus, a soil pest that may cause crop loss approaching 100% in some cases. The sting nematode utilizes a cysteine protease as its primary means of digestion, and efficient feeding precedes rapid reproduction. To test the hypothesis that strawberry cystatins may be used to slow nematode colonization, Cyf1 and several other members of the strawberry cystatin family were isolated from cDNA libraries and introduced back into plants under constitutive overexpression. The family contains at least 8 distinct members, several that are differentially expressed in various tissues. The inhibitory activity of four members was tested in vitro using substrate competition assays. Various members were transformed into octoploid and diploid strawberry for evaluation in greenhouse trials. Additional trials will test multimerized c-termini, as well as multimerized inhibitory loops for enhanced efficacy toward sting nematode feeding. The successful identification and deployment of plant cystatins may be a useful tool in engineering nematode resistant plants, and also may substantially curb use of volatile fumigants, decreasing environmental impacts of strawberry farming in Florida.

Keywords: Strawberry, cystatins, gene resistance

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Gene Pair Detective: a Computational Tool to Identify Microcolinearity in Unsequenced Gene Space

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The era of plant genomics has brought advances throughout agriculture. However, the very basis of genomics-level analyses relies on capture of sequence information. While some plants have been completely sequenced and the information is available in public repositories, other species have been barely addressed. In particular, a large number of horticulturally relevant and economically important crop plants have negligible treatment with respect to total sequence coverage. The existing sequence collections, if any, contain predominantly ESTs, with little genomic sequence. Examples of this class include strawberry, sweet cherry and peanut. In these cases most of the sequence that is available arises from a relatively small set of ESTs, sequences derived from expressed genes. While informative, EST sequences frequently do not contain complete coding sequence for the gene they represent. The coding regions of genes present in ESTs also represent the least variable portions of genic sequences, making them less applicable to their use in marker assisted breeding, as such efforts exploit variability between closely related species or cultivars. A computationally-aided method has been devised to specifically capture and characterize discrete intergenic sequences in sequence-poor species. The approach leverages the known genomic sequence of the *Arabidopsis thaliana* to order ESTs from a query species, and identify cases where ESTs likely represent consecutive genes in the genome. The prediction is based on the conservation of microcolinearity to a reference genome, in this case either *Arabidopsis thaliana* or Populus deltoides. Tests of ESTs collections from completely sequenced genomes validate the approach in silico, and predictions from strawberry EST sets were tested and verified in the laboratory. This program allows researchers with small EST sets to find collinear gene relationships, allowing characterization of intergenic genomic sequences.

Keywords: *Arabidopsis,* ESTs, Marker assisted

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Investigation of the Strawberry Acute Cold Response through Transcriptome Sampling

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Cultivated strawberry (Fragaria xananassa) is a valuable perennial crop, yet in most growing regions cold temperature stress can dramatically impinge on fresh fruit production. In the interest of long-term crop improvement it is important to understand the molecular response of strawberry to cold, and the underlying basic biology of cold response in the species. Although the molecular mechanisms that contribute to responses to cold are well defined in the model system *Arabidopsis thaliana*, little is known about these processes in strawberry. To better understand the response to cold, and simultaneously enrich the relatively sparse Fragaria EST collection, a cDNA library was sequenced and characterized from cold-stressed *F. vesca* seedlings. *F. vesca*, the woodland strawberry, is diploid and a highly amenable system for initial investigations into strawberry stress responses. Over 10,000 sequences were produced from which 9,936 high quality ESTs were obtained. Analysis of these ESTs resulted in 5,937 unique sequences, many recognizable as being involved in cold responses in other plants, but 23 of which are completely novel to *F. vesca*. The frequency at which abundant transcripts occurred exclusively in the cold library was analyzed and the cold induction of some of these transcripts was tested. The results show that a specific set of transcripts, including several not normally thought of as cold regulated, are strongly induced within 30 minutes of cold treatment in the mature plant. In the current study, the frequency of a given transcript can be used to infer relevance to this important biological process. This study also describes how the well defined molecular response to cold in the model system translates to a valued horticultural crop.

Keywords: strawberry, cold response, expression analysis

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Differential Phenotypic Expression Of Self-Compatibility In Almond

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Self-incompatibility in almond (*Prunus amygdalus* Batsch) is of the gametophytic type, characteristic of many plants in the Rosaceae family. Incompatibility occurs when the pollen S-allele matches with one of the two stylar S-alleles. The *S* locus in the genus *Prunus* encodes a stylar *S*-RNase and the pollen-specific expressed F-box genes. These F-box genes have been reported to be linked to the *S*-RNase. The observation of pollen tube growth through fluorescence microscopy during several years after self-pollination of 90 seedlings from the cross 'Vivot' ($S_{23}S_f$) × 'Blanquerna' (S_fS_f) showed an unexpected SI behavior in most seedlings, as well in the 'Vivot' parent. However, genotype determination and S-allele identification by non equilibrium pH gradient electrofocusing (NEpHGE) and by PCR has allowed to distinguish the individuals with SI and SC genotype, suggesting that the expression of the S_f allele is not the same in all cases, as shown by the SI phenotype of 'Vivot' and many seedlings with a SC genotype. This SI behavior may be caused by a presence of a functional S_f-RNase in the pistil arresting the S_f-pollen tube growth. This S_f-RNase activity may be due to a functional gene expression contrary to what happens in the self-compatible cultivars, where a deficient expression is supposed, as no RNase activity is detected. SC in almond could also be produced by a mutation in the S_f-pollen haplotype losing its functionality, such as it happens in other *Prunus* species. Further approaches are being undertaken to study the role of the S-RNases and SFBs codified by the *S*-locus and their expression, in order to clarify the SC and SI interactions in almond.

Keywords: Almond, Self-incompatibility, RNase

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Extensive conservation of gene order and content between *Prunus* and *Populus* but not between *Prunus* and *Arabidopsis*

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We analyzed the degree of synteny conservation between *Prunus* and the two completely sequenced model species, *Arabidopsis* and *Populus*, using various *Prunus* sequences and their *Arabidopsis* and *Populus* homologs. The *Prunus* sequences include seven *Prunus* BAC sequences, 1093 *Prunus* genetic map-anchored sequences, and 2140 peach physical map-anchored sequences. We found a well conserved synteny across the *Prunus* species - peach, plum, and apricot – and the *Populus* genome using a set of homologus *Prunus* BACs. Conversely, we could not detect any conserved syntenic regions in *Arabidopsis* in this region. Other peach BACs also showed extensive synteny with the *Populus* genome. The syntenic regions detected were up to 477 kb in the *Populus* genome and 133 kb in the peach BACs. Two syntenic regions between *Arabidopsis* and these BACs were much shorter, around 10 kb, with only four or five gene pairs. We also found syntenic regions that are conserved between the *Prunus* BACs and the partially sequenced genome of *Medicago*, which belong to Rosid I with *Prunus* and *Populus*. The analysis using genetic or physical map-anchored *Prunus* sequences suggested that there is no significant macrosynteny between *Prunus* and the two completely sequenced model species. We did detect longer microsyntenic blocks, which span mega base pair levels, even though the gene order is often not conserved in those syntenic regions. The level of gene order conservation seemed much higher, though, in the *Prunus*-*Populus* comparison than in the *Prunus*-*Arabidopsis* comparison.

Keywords: Prunus , populus, Arabidopsis, Synteny

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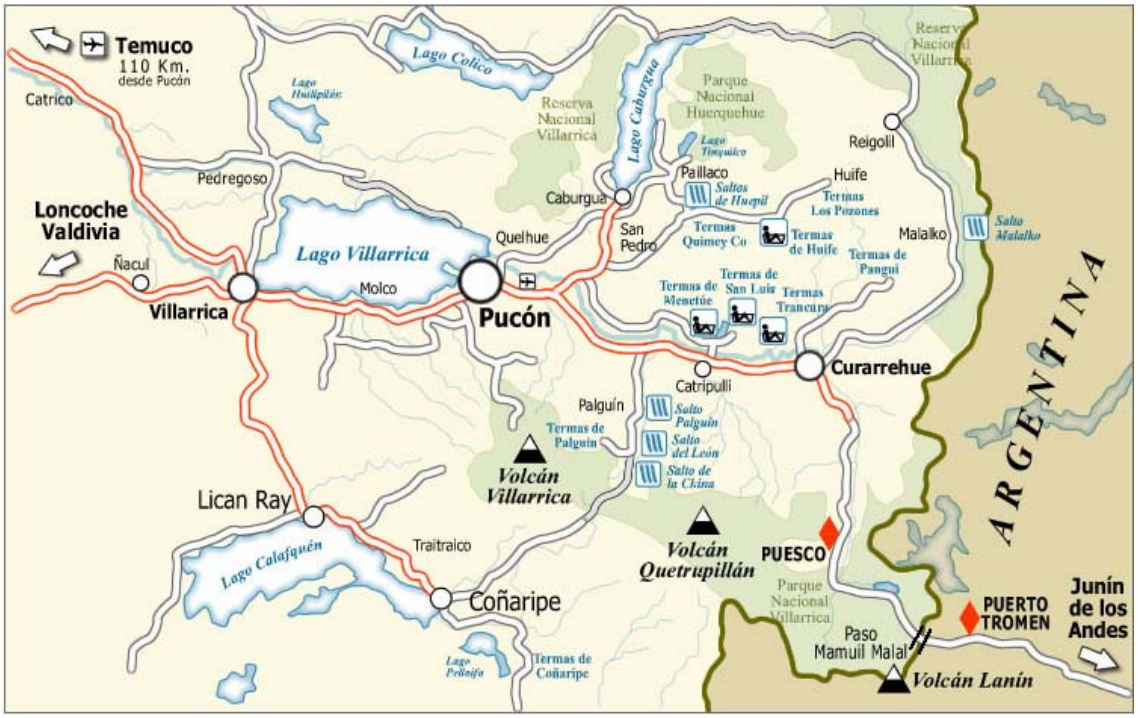
Map of City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Reigolil
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Saltos de Huepil
Termas Los Pozones
Caburgua
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