ABSTRACTS

OP1 8.35am - Monday 20 March

BioView – an enterprise bioinformatics system for automated analysis and annotation of non-genomic DNA sequence

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BioView represent an automated analysis and visualization platform developed over the last 6-7 years at HortResearch for the annotation of non genomic DNA. The system is deployed on Unix/Linux and implemented to utilize both a dedicated compute grid as well as transiently available company desktops. The system is primarily written in PERL and employs MySQL as a relational database management system for persisting data and job scheduling. The automated pipeline assembles sequences into tentative consensus sequences, and implements modules for performing BLAST comparisons, identifying simple sequence repeats and motifs, predicting single nucleotide polymorphisms and microRNA targets, designing micro-array oligonucleotides, compiling keyword dictionaries, and defining gene families. Visualizing relationships captured within the database is achieved via a web viewer. Administering the system, tracking and sharing mining efforts, initiating follow on sequencing and vector construction, performing virtual subtraction, and augmenting automated annotations by capturing end user knowledge is also undertaken via the web viewer.

The system has been used to annotate databases of sequences from fungal, insect, and plant origins but functionality primarily targets those from plants. An overview of BioView sequence annotation system will be presented in context of the annotation of a *Malus* sequence database comprised of all HortResearch *Malus* ESTs, full cDNAs, and PCR product libraries merged with all *Malus* sequences extracted from Genbank during Feb, 2006.

OP2 9.20am - Monday 20 March

Unigene Development and SNP Discovery from Rosaceae Family EST Resources

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Development of a family-wide unigene from ESTs across related species can be useful for comparative genomics, gene discovery and genomic scale research. However, the methods of assembling a unigene for an entire family with inherent sequence differences have not been well characterized. Current research for the Genome Database for Rosaceae has yielded three different methods for producing unigenes across families. Two rely on the CAP3 algorithm for EST assembly, and the third utilizes BLAST sequence similarity searching. To verify the different assembly methods, sequence similarity searches against various protein databases were performed. Specific gene families were examined to find levels of over- and under-assembly. The final Rosaceae family unigene was mined for markers including single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs). The Arabidopsis proteins were extensively compared to the Rosaceae unigene, highlighting the unique genes that are found in fruit trees. The Rosaceae unigene is available online for further comparative and genomic research and may be incorporated into a microarray chip to facilitate both within species and across species gene expression level studies. Assembly of ESTs from multiple species in a plant family was shown to be an effective tool for genomic research.

OP3 10.00am - Monday 20 March

In silico identification of apple rootstock specific transcripts

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There are a number of phenotypic traits conferred by apple rootstocks upon the scion and desirable rootstock-specific traits. In an attempt to identify genes which may be responsible for these traits, we have used the public expressed sequences (EST and cDNA) to identify genes expressed uniquely in apple rootstocks. 203,2211 ESTs and cDNA sequences from apple were downloaded, screened for vector, and separated into 9,228 from root and 193,993 non-root tissues. Each set of sequences was separately clustered (root: 1,868 contigs, 3247 singletons; non-root: 23,340 contigs, 10,668 singletons). 189 contigs and 955 singletons expressed in root tissue had no match among the non-root sequences (Blast E-value > 1E -20). These sequences have been annotated agains SwissProt and the Genbank NR databases. We are in the process of verifying the tissue-specific transcription of these sequences in the laboratory.

OP4 11.00am - Monday 20 March

A Resistance Gene Map for *Prunus*

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Breeding for disease resistance is a key component of most breeding programs. The identification of pathogen resistance loci in peach provides information about resistance loci, the organization of resistance genes throughout the genome, and permits comparison of resistance regions among other Rosaceae genomes. This information will facilitate the breeding of resistant species of *Prunus*. A candidate gene approach was used for locating resistance loci in the peach genome. Candidate genes representing analogs of major resistance genes, pathogen response (PR) proteins, and resistance-associated translation initiation factors were hybridized to a peach BAC library and mapped by using the genetically anchored, peach physical map/database and the Genome Database for Rosaceae (GDR). A resistance map for *Prunus* was generated and currently contains 42 map locations for putative resistance regions distributed among 7 of the 8 linkage groups.

OP5 11.20am - Monday 20 March

Genetic localisation of new major and minor pest and disease resistance factors in the apple genome

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Multi-resistant new apple cultivars are requested to promote environmentally-friendly production in commercial orchards. Breeding such cultivars can be speeded up thanks to the use of genetic markers located closely to pest and disease resistance genes in order to pyramid them. We report the genetic mapping of several new major and minor (i.e., QTL) resistance genes which should be useful in marker-assisted breeding. A new scab (*Venturia inaequalis*) resistance gene, called *Vdr1*, from the broad-spectrum scab resistant cultivar 'Dülmener Rosenapfel' has been identified and located on the top of LG6 of the apple genome (Freslon et al. 2006). *Vdr1* is a chlorotic-type resistance gene like *Vf*. A second, necrotic-type, scab resistance gene, called *Vdr2*, has been identified in the same cultivar, which location is underway. A third scab resistance gene, *Vfh* from *M. floribunda* #821 (Bénaouf and Parisi 2000), has been mapped on top of LG8, close to *Pl-w*, *Er1* and *Er3* (James and Evans 2004; Gardiner et al. 2006). Powdery-mildew (*Podosphaera leucotricha*) resistance QTLs have been located on 7 linkage groups (LG1, LG2, LG8, LG13, LG14, LG17) in the progeny 'Discovery x TN10-8' (Calenge et al. 2006): two QTLs (top-LG2 and middle-LG13) are stable over 4 years of field assessment, and one (top-LG8, colocalising with *Pl-w*) over 3 years. Finally, we also mapped a major resistance gene, *Dp-fl*, against rosy apple aphid (*Dysaphis plantaginea*; Lespinasse et al. 1985) on the bottom part of LG8 in the cultivar 'Florina'. These new results enlarge the knowledge on the apple genomic organisation of biotic resistance factors.

OP6 11.40am - Monday 20 March

The role of *Vfa* RGA's at the *Vf* locus in resistance to *Venturia inaequalis*

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Apple scab, caused by the ascomycete *Venturia inaequalis,* is the most important disease of the cultivated apple (*Malus X domestica* Borkh.). The genetic locus for resistance to *V. inaequalis* most frequently used in apple breeding is *Vf*, derived from *Malus floribunda* 821. A cluster of four resistance gene paralogs (*Vfa1, Vfa2, Vfa3,* and *Vfa4*) encoding proteins with leucine-rich repeats and transmembrane domains were isolated and cloned from the scab-resistant apple cultivar Goldrush. *Vfa3* was determined to be truncated, and was not used in this study. Intact candidate genomic genes (*Vfa1, Vfa2,* and *Vfa4*) were separately cloned into the binary vector pCAMBIA2301 to include the intact ORF, at least 2 kb of the native promoter of the gene, and the 3'-UTR region. Each of the three constructs was separately introduced into the scab-susceptible apple cultivars McIntosh and Galaxy via *Agrobacterium*-mediated transformation. Plants of transformed lines grown in a growth chamber were inoculated with a mixture of five races (1 to 5) of *V. inaequalis*. Disease symptoms were assessed on individual plants of each of the transgenic lines for type of resistance reaction and amount of sporulation. Transformed lines expressing the *Vfa4* gene were as, or more susceptible, than control (non-transgenic McIntosh and Galaxy) plants. In contrast, *Vfa1* and *Vfa2* increased resistance to *V. inaequalis* in transgenic lines when compared to controls. Fewer leaves were infected, and amount of sporulation was lower in *Vfa1* and *Vfa2* plants. Microscopic studies supported the macroscopic evidence of increased resistance in *Vfa1* and *Vfa2* plants. Although inoculations must be repeated, the results obtained so far clearly indicate that *Vfa1* and *Vfa2* are involved in apple scab resistance. Determination of levels of *Vfa* transgene expression is in progress using RT-PCR and western blot analysis on leaves inoculated or not inoculated with *V. inaequalis*.

OP7 12.00 noon - Monday 20 March

Phylogenetic analysis of a large database of apple resistance gene analogues identifies NBS clades widely conserved in the Rosids

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Phylogenetic analysis of our database of 378 sequenced PCR clones from resistance gene analogues was used to examine the diversity of the nucleotide binding site (NBS) leucine rich repeat family in apple. Based on the level of redundancy, the number of genes of this class is estimated to be over 400. Since this data covers a significant proportion of the predicted genes, it provides the most complete picture to date of apple resistance gene analogues in this gene family. By including public domain apple and *Arabidopsis* resistance gene analogues from the NBS region, the analysis highlights how a large database was used to identify clades that may have unusually highly conserved NBS domains between apple, *Arabidopsis* and (in some cases) a number of other Rosids. We present and discuss the possible significance of this data.

OP8 12.20pm - Monday 20 March

Genetic and genomic analyses of disease resistance genes in roses

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Rosa, NBS-LRR, BAC library, black spot, powdery mildew

Analysing the genetics of disease resistance genes in roses has importance for both fundamental and applied research. To date we characterised four genes against black spot (*Dilplocarpon rosae*) and two genes against powdery mildew (*Podosphaera pannosa*) genetically. For all of them map positions were obtained in mapping experiments utilising various molecular markers. For one gene, *RPP1* directed against the powdery mildew race 3, both qualitative and quantitative loci could be mapped. For *Rdr1*, another gene directed against black spot, candidate genes were identified within a BAC contig of about 400 kb. Within this highly conserved TIR-NBS-LRR gene family data on the genomic structure and expression in different rose tissues and under diverse stresses could be obtained. Furthermore, comparative analyses of this candidate gene family in various rose species of different ploidy levels and in other rosaceous species was started.

OP9 12.40pm - Monday 20 March

Biodiversity of the apple powdery mildew fungus (*Podosphaera leucotricha)* and interactions with its host

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Resistance to powdery mildew is one of the major aims in apple breeding. Prerequisite of successful resistance breeding is a good understanding of host-pathogen-relationships, including the population structures of fungal pathogens and the virulence behaviour of different races. Recent results of the work on *P. leucotricha* from the EU-INCO project SMADIA will be presented. As shown earlier a high level of diversity in terms of virulence do exist in apple powdery mildew, although the overall genetic diversity evaluated on a molecular level appeared to be low. Twelve new isolates were tested for their pathogenicity on a differential *Malus* set consisting of different resistance gene donors. The Chinese isolate F1-12 was the isolate which was able to infect the highest number of *Malus* genotypes. However, in comparison with earlier results for European isolates there was no clear tendency visible that the Asian isolates were different regarding the aggressiveness. The results also showed that there are no isolates in the tests included, which belong to the same virulence type, as all isolates show different reactions. The resistances of *Pl1* and *Pl2*-donor plants (A142/5 and *M. zumi* 274) seemed to be overcome. It is also shown that *Pl1-Pl2* gene combinations were resistant to isolates with virulence types that could overcome the single resistances. On the molecular level (analysed by AFLP markers) it was possible to distinguish between two separate major groups of isolates.

Regarding apple powdery mildew resistance gene identification and mapping we will give some new information about the map position of *Pl1* verified by SSR mapping and the tight linkage to a NBS-LRR type resistance gene analog (RGA) and further R genes.

OP10 2.30pm - Monday 20 March

Quality assessment of the DNA marker technology DArT using *Arabidopsis thaliana*, and application to apple

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DArT™ (Diversity Arrays Technology) is a high-throughput technique for analysis of DNA polymorphism. DArT is based on hybridisation and uses micro-arrays as a platform. We validated this technology, using *Arabidopsis thaliana* as a model. We digested genomic DNA of *A. thaliana* Landsberg *erecta* (L*er*) using three restriction enzymes. After attachment of adapters we amplified a specific set of restriction fragments. A subset of these fragments was individualised by means of cloning, and was spotted on slides. We hybridised these micro-arrays with DNA restriction fragments from L*er,* from *A. thaliana* Colombia (Col), and a segregating F_2 population of L*er* x Col. This revealed clones that could be used as molecular markers. The quality of these markers was evaluated. We made a genetic linkage map containing the DArT markers, using JoinMap. The polymorphic clones were sequenced and the sequences were aligned with the published Col DNA sequence. The genetic linkage map showed a perfect collinearity with the sequence map of *A. thaliana.* This underlines the quality of the DArT markers. We applied DArT to the fungal pathogen *Mycosphaerella graminicola* which provided over 2000 genetic markers. Further we used DArT for making a genetic linkage map for apple (*Malus*). Results will be discussed.

OP11 3.00pm - Monday 20 March

Molecular control of recurrent blooming in rose by genetic and genomic approach

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Date and mode of flowering are essential and variable traits in garden roses. For instance, recurrent roses are able to flower several times a year whereas non recurrent roses flower only once a year. In order to understand the molecular basis of flowering in rose, we have undertaken different approaches:

Genetic approach: a rose genetic map, based on AFLP and SSR markers has been developed. Important characters controlling flowering have been localised on the genetic map as recurrent blooming (*RB*, a monogenic recessive gene) or flowering time (as a QTL).

Gene candidate approach: genetic and physiological data in rose suggest that different pathways, as GA (gibberellic acid) or floral repressors are involved in the control of flowering. Homologous genes implicated in GA signalling or metabolism (*RGA, GAI, GA2OX, SLY, SPY*), floral repression (*TFL1, LHP1, EMF1, EMF2*) and floral key genes (*AP1, SOC1, LFY, CO*) have been isolated in rose. These genes were mapped using SSCP or CAPS markers. A *RGA* homologous (gene involved in GA signalling) was mapped in the vicinity of *RB*. Expression of the isolated genes was studied in different tissues during the floral process by RT-PCR. *GA2OX*, a gene involved in GA catabolism, is induced during floral initiation.

Differential approach: a 5k Affymetrix® oligo chip is under construction in order to perform differential experiments. The unigenes were developed from public ESTs (petals) and ESTs produced in the laboratory (from vegetative and floral buds). This chip will be used to compare transcript during the floral process: floral initiation and recurrent blooming.

All these molecular and previous physiological data indicate an important role of GA in the control of flowering in rose.

OP12 3.20pm - Monday 20 March

Characterisation of several traits affecting flower and fruit in peach (*Prunus persica* L. Batsch) and new genetic linkage map

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A F_2 population, issued from a cross between Ferjalou Jalousia® and Fantasia (JxF), was created in order to analyze QTLs controlling organoleptic and nutritional fruit quality in peach. Ferjalou Jalousia® is a flat low-acid clingstone peach, and Fantasia is a round, normally-acid freestone peach. Moreover, this population is segregating for six Mendelian characters: fruit acidity (*D*), pollen sterility (*ps*), peach or nectarine fruit (*g*), flat or round fruit (*S*), clingstone or freestone fruit (*F*) and a new character reported here for the first time: trees bearing aborting fruits, named '*af*'. These trees have flowers but fruits start to fall two months after blooming. This recessive character, was demonstrated to be linked to the flat shape (S) of the fruit. We build a second generation genetic linkage map using 207 individuals including additional SSR markers (82), EST-SSRs (10), and AFLP markers. Molecular markers linked to the six Mendelian characters were identified. One of them (SSR MA014a), cosegregating with the *S* and *af* genes, has already been used for marker assisted selection among new hybrids created for the cloning of the *D* gene.

OP13 3.40pm - Monday 20 March

The European project HiDRAS: An innovative multidisciplinary approach to breed High-quality Disease Resistant Apples for a Sustainable Agriculture

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HiDRAS (High-quality Disease Resistant Apples for a Sustainable Agriculture) is a collaborative effort of 15 European groups aimed at the identification of genetic factors controlling fruit quality. Innovative approaches based on the phenotypic and molecular characterization of almost 2000 related genotypes has been adopted to identify important genes controlling fruit quality and disease resistance related traits. One of the goals of the project is to provide breeders with new tools, such as molecular markers, linked to fruit quality and disease resistance, in order to improve “marker assisted selection” strategies. New software programs are under development to allow the full exploitation of genotypic, phenotypic and pedigree data. Consequently fruit quality QTLs could be identified and the transmission of their alleles along the pedigree followed. A large number of highly informative markers, mainly SSRs and markers derived from fruit-quality related genes, will be generated and employed to saturate the existing apple linkage maps, increasing its accuracy and suitability for QTL detection. To establish the quality parameters determining the success of new apple cultivars, consumer tests are going to be carried out in several Countries. In such occasions our scientific research will be presented, increasing the trust of European citizens towards disease resistant apples, thus fostering their diffusion. An apple data repository has been established to collect and share data among participants. Initially the database access will be limited but eventually public access will be granted to most of it. A HiDRAS website (http://www.hidras.unimi.it) has been created to allow a world-wide diffusion of the objectives of the project, the work progress and the participants involved. The original and well concerted multidisciplinary approach, including apple breeding, genetics, molecular biology, statistics and bioinformatics, will ensure the achievement of the project goals.

OP14 4.00pm - Monday 20 March

Characterization of wild *Malus* populations using genotypic and phenotypic traits

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Malus sieversii, a wild relative of domestic apple, represents an important source of genetic variation for several horticulturally important traits including fruit quality and disease resistance. Collections made by the USDA in Kazakhstan have now been analyzed to determine the extent of diversity and genetic structure. The diversity and differentiation of more than 1000 *M. sieversii* individuals collected from 10 sites have been determined based on data collected from seven unlinked microsatellite loci and 21 quantitative traits. Bayesian assignment analyses identified 10 genetic clusters that are variously admixed among sites. These data can be displayed as a minimum spanning network that enables us to correlate genetic diversity with geographic distances. We have also identified core subsets of individuals that can be used in QTL mapping, allele mining, and other comparative genomic studies.

OP15 8.35am - Tuesday 21 March

Self-compatibility in tetraploid sour cherry (*Prunus cerasus*) results from the accumulation of non-functional S-haplotypes.

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Sour cherry selections can either be self-incompatible (SI) or self-compatible (SC); however, any successful cultivar must be SC to avoid the cost and any inefficiencies associated with having to provide a pollinator. The specificity of the SI reaction in cherry is known to be controlled by a minimum of two genes located within the S-haplotype, the stylar S (S-RNase) and pollen S (SFB). Genetic studies determined that the genotype dependent control SI SC in tetraploid sour cherry is caused by the accumulation of non-functional S-haplotypes that have lost either stylar or pollen specificity function. Five functional and seven non-functional S-haplotypes have been identified. SC selections must have a minimum of two non-functional S-haplotypes. Unlike in the Solanaceae, a match of one-functional pollen S gene in the 2x pollen with the stylar S-RNase, is sufficient to render the pollen incompatible. To date, we have determined the structural changes resulting in five of the non-functional S-haplotypes. Our data indicates that mobile element insertion, point mutation, and deletion, are evolutionary forces resulting in loss of SI in *Prunus*.

OP16 9.00am - Tuesday 21 March

Genetic Control of Plant Branching

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Plant architecture is greatly influenced by the production of lateral branches from axillary buds. In the model plant *Petunia hybrida*, a group of three *decreased apical dominance (dad)* mutants showing an increase in branching have been identified. The genes responsible for the *dad1* and *dad3* mutations have been isolated and belong to a family of carotenoid cleavage dioxygenase (CCD) enzymes which includes the *RMS1*gene isolated from the branching mutant *ramosus1* of pea and the *MAX4* (*AtCCD8*) and the *MAX3* (*AtCCD7*) genes from the *more axillary growth* (*max*) mutants of Arabidopsis.

Grafting experiments in petunia have shown that the increased branching phenotype of both *dad1* and *dad3* can be reverted to near wild type by grafting the mutant scions onto wild type root stocks. This indicates that DAD1 and DAD3 genes control the levels of a graft transmissible substance that affects branching.

To determine if the branching control mechanism observed in a model plant is conserved in woody perennials the DAD1 and DAD3 genes have been isolated from apple and the expression patterns of Dad1 has been investigated. Knockout vectors have been constructed to alter the expression level of Dad1 in apple. We will present our latest results examining the role of DAD1 in apple architecture.

OP17 9.25am - Tuesday 21 March

Wide range QTL analysis for complex architectural traits in a one-year-old apple progeny

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Tree architecture and shoot morphology are putative target traits in apple breeding programmes, with the aim to select new cultivars less demanding for intrants, and with controlled vegetative development and yield regularity. The present study aimed at investigating the genetic determinism of such traits in a 1-year-old apple F1 progeny deriving from a 'Starkrimson' x 'Granny Smith' cross. A precise description of phenotypes taking into account both tree topology (growth and branching) and geometry was performed on 125 offsprings. Using both parental and consensus genetic maps, QTL were investigated for a wide range of traits. Several loci controlling tree geometry were identified: (i) integrated traits such as tree surface or volume on LG3, (ii) traits related to the form of long sylleptic axillary shoots (LSAS) such as bending (LG2, LG8) or basis angle (LG10), and (iii) traits of finer components such as internode length either on trunk (LG3, LG7) or LSAS (LG3, LG10, LG16). Regarding tree topology, the genetic determinism of sylleptic branching was detailed by investigating QTL for short, medium and long shoots (LG3, LG13, LG16). A QTL controlling the length of upper part of the trunk that remain un-branched was also mapped on LG11. With the consensus map, LOD scores and R^2 values of the detected QTLs ranged from 3.8 to 7.3 and from 7% to 30%, respectively. Discussion will focus on particular genomic regions in which several QTL co-localise and on the relevance of traits in relation with their possible implication in further tree development.

OP18 9.50am - Tuesday 21 March

Fruit size QTL in sweet cherry: Cell number is under stronger genetic control than cell size

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Maximizing fruit size is critical for profitable sweet cherry (*Prunus avium* L.) production, yet little is known about cellular differences among and within cultivars that contribute to fruit size differences. Fruit size varies widely between sweet cherry cultivars, and significant variation exists among genetically identical fruit due to cultural and environmental differences. The relative contributions of flesh cell number and cell size to final fruit size in sweet cherry were determined by analyzing equatorial sections of several sweet cherry cultivars at maturity. Cells intersecting a transverse line were counted and the average cell length was calculated. The average cell numbers of 'New York 54' (1.4 g/fruit), 'Emperor Francis' (6.5 g/fruit) and 'Selah' (10.5 g/fruit) were significantly different (P<0.05) (29, 41, and 79, respectively), while average cell size was only significantly different for 'Emperor Francis' (P<0.05). To determine the components of cellular morphology that account for differences in fruit size within a cultivar, fruit from 'Bing', 'Regina' and 'Selah' trees exhibiting a range of fruit size were measured. Within each cultivar, average cell number was not significantly different (P=0.6, P=0.9, P=0.8, respectively), while average cell size was significantly different for the range of fruit sizes within 'Bing' and 'Regina' (P<0.05) Therefore, fruit flesh cell number is genetically controlled and the potential exists to improve this trait through breeding efforts. We are currently mapping QTL for this trait, as well as additional important sweet cherry fruit quality traits, utilizing a population developed from a cross between 'New York 54' and 'Emperor Francis'.

OP19 10.10am - Tuesday 21 March

Genetic mapping of *Dw1*, a locus required for dwarfing of apple scions by 'M.9' rootstock

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The use of dwarfing rootstocks in apple (*Malus x domestica* Borkh.) results in a reduction in the vegetative growth of the grafted scion. Although this phenomenon has been exploited by horticulturalists for hundreds of years, the molecular and genetic basis of this effect is not well understood. The breeding of dwarfing apple rootstocks is difficult and time consuming due to the time required to assess their phenotypes. Techniques such as Marker Assisted Selection (MAS) would help accelerate future rootstock breeding programmes.

Using bulked segregant analysis (BSA) of a population derived from a cross between 'Malling 9' ('M.9') (dwarfing rootstock) and 'Robusta 5' ('R5') (non-dwarfing), a locus influencing dwarfing and designated as *Dw1* was identified and mapped in a 2.5 cM interval. Microsatellite markers covering the apple genome were then screened over phenotypic bulks enabling *Dw1* to be positioned on linkage group 5. However, further analysis demonstrated that *Dw1* alone is not sufficient to fully explain the dwarfing phenotype, suggesting more complex, multigenic control of this character.

SSR, SCAR, SNP and RAPD markers are being utilized to construct a linkage map of the 'M.9' apple rootstock (Celton *et al*. 2006 Plant and Animal Genome XIV) in preparation for QTL analysis that will enable a fuller understanding of the genetic basis of dwarfing, and ultimately lead to the development of genetic markers, that will be directly applicable to apple rootstock breeding.

OP20 11.00am - Tuesday 21 March

Microarray-based gene expression studies of dormancy phase transition in raspberry (*Rubus idaeus* L.) buds

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The prevention of bud break through a temperature-sensing mechanism is a key ecological factor in temperate perennial plant survival. Failure to receive the required amount of chilling results in poor lateral bud break in the spring and reduced yields, whereas the breaking of buds after minimal amounts of chilling could leave the developing buds liable to subsequent frost damage. This project aimed to characterise bud dormancy in woody perennial plants at the molecular level. To resource this, a total of 5,300 cDNAs were generated from endodormant (true dormancy) and paradormant (apical dominance) raspberry meristematic bud tissue. Expression patterns of these cDNAs during the endodormancy – paradormancy transition were determined using microarrays, comprising spotted PCR products. Furthermore, the effects of ethylene treatment on gene expression during the chilling process, which resulted in increased consistency of bud burst and enhanced rates of development when canes were returned to glasshouse or field conditions, were profiled. Approximately 400 cDNAs exhibited significant differential expression patterns and included several transcription factors, hormone-responsive proteins, and genes associated with cell expansion and oxidative stress response. Two differentially expressed MADS box genes, which belong to a family of transcription factors that control multiple developmental processes in flowering plants, were utilised to screen a raspberry BAC library and positively identified approximately 60 BAC clones, which are currently being fingerprinted and mapped. The potential roles of these differentially expressed genes in dormancy regulation and ethylene-response will be discussed.

OP21 11.40am - Tuesday 21 March

Transcriptomics of ripening in apple as a tool to improve apple quality traits

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Apple breeding is a slow and work-intensive process. Screening for interesting genotypes at the seedling level by using molecular markers could represent a major breakthrough. Most fruit quality characteristics, such as texture quality, are the result of complex processes involving the activity of multiple genes. As a consequence, the development of good markers for such traits in apple is not yet a reality and more knowledge of the underlying genetics is required.

In an attempt to find candidate genes involved in maturation and ripening, we performed a transcriptional screening. This was done by cDNA-AFLP expression analysis on apple skin and flesh tissue sampled during a period of 3 months. It yielded an inventory of around 600 differential expression profiles. By means of clustering algorithms different groups were discriminated of which some contained profiles with expression peaking around the climacterium. At present, we have characterized the sequences of more than 300 of these gene fragments. After functional annotation, we have identified a number of interesting gene homologues which justify further investigation.

It is our goal to map the outcome of this transcriptional study, as well as publically-available apple gene sequences or ESTs involved in fruit quality, on an available genetic linkage map. On this map, QTL-regions for different aspects of fruit quality have been recently localised. The combination of these results will allow us to develop promising markers for the breeding of superior new apple cultivars.

OP22 12.10pm - Tuesday 21 March

Microarray analysis of ripening in Apple (cultivar Royal Gala)

Robert Schaffer, Ellen Friel, Edwige Souleyre, Bart Janssen, Kate Thodey, Rebecca Bishop, Marcus Davy, Jia-Long Yao, Dan Cohen, Richard Newcomb.

HortResearch. Private Bag 92169, Auckland, New Zealand.

Ripening in apples is characterised by an increase in volatiles, sweetening and softening of the flesh and a reduction in acidity. We have been investigating the regulation of gene expression across fruit ripening using an in-house 16K oligo apple microarray. A two-pronged approach has been taken. Fruit from Royal Gala trees containing an antisense construct for ACC oxidase, which produce no detectable ethylene, were induced with ethylene and the volatiles analysed. Skin and cortex tissue from these apples were harvested and changes in the RNA content were measured on the microarrays. Secondly, RNA from the ripening stages of a non-transgenic Royal Gala cultivar was also measured using microarrays. These results identified classes of genes that were involved with ethylene ripening, ethylene induced transcription not related to ripening, and genes that were involved with ripening that were ethylene independent. In addition to identifying candidates that are involved in the ripening traits mentioned above, we have identified candidate pathways that lead to production of volatiles in apples.

OP23 12.35pm - Tuesday 21 March

Systems approach to the functional analysis of apple fruit quality

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Rosaceae crops and apple in particular have been recognized as an important resource of phytonutrients essential for a balanced diet and for disease prevention. Preserving fruit quality is key to the delivery of vital health enhancing phytonutrients as well as for sustaining the economic viability of these crops. Our systems based approach involves integrating various platform technologies like functional genomics, plant transformation and RNAi with transcriptome and metabolome analysis to both discover and validate the relationship between genes/pathways that determine fruit quality. In apple we have investigated various important quality traits via the transgenic suppression of the ethylene and sorbitol biosynthetic pathways. In transgenic fruit obtained from these trees we are analyzing specifically the impact on various quality traits that include volatile flavor, sugar-acid composition and texture. We are also carrying out microarray and metablome analysis to study the impact of these specific perturbations on the general expression patterns in fruit tissues. We are investigating parthenocarpy via the tissue specific up regulation of auxin genes to improve fruit set and to eliminate seed formation in fruit, thereby improving quality and productivity.

OP24 2.30pm - Tuesday 21 March

Fruit softening in *Prunus*: progress and prospects of the candidate gene approach

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Understanding the genetic control of fruit softening and texture in peach (*Prunus persica* [L.] Batsch), the model fruit tree species, is of considerable consequence in many fruit crops for not only optimizing pre- and postharvest fruit handling to increase consumer satisfaction but also long-term genetic improvement. Using the candidate gene approach, a gene for endoPG from the Clemson peach EST library was found to control the major softening/texture traits of *Melting flesh* and *Freestone* in peach and nectarine. EndoPG has three functional alleles, with complete co-segregation between endoPG genotype and *F-M* phenotype in almost 500 segregating progeny and over 150 cultivars and breeding lines. The PCR test developed is proving valuable for unambiguously determining fruit type when it may be confounded by environmental factors, fruit under-development in early maturing varieties, or interacting alleles of other firmness loci such as *Stony hard*. This endoPG PCR test is also applicable for marker-assisted selection to differentiate *F-M* fruit type. Allelic diversity of endoPG in *Prunus* was explored using a microsatellite attached to the gene. Ten alleles of endoPG were observed in peach and nectarine, each placed in one of the three functional categories but potentially having quantitative effects. Surveying unimproved germplasm and closely-related species revealed many additional alleles with potential value for introducing novel softening and stone adhesion characteristics. EndoPG polymorphism was also observed in apricot, plum, and sour cherry (but not sweet cherry), and the locus may control *F-M*-like traits in these other *Prunus* fruit crops. Additional candidate genes are under investigation, and a “softening gene map” was constructed to facilitate discovery of useful gene-trait associations.

OP25 2.50pm - Tuesday 21 March

In vitro flowering of transgenic pears (*Pyrus communis* L.) expressing *CiFT,* a *Citrus* ortholog of the *Arabidopsis FT* gene.

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Juvenility in fruit trees hinders from analyzing the function of genes, expressing in reproductive tissues, via transformation methods. We aimed to develop a new tool for the gene functional analysis in reproductive tissues through transformation of floral induction genes. In this study, we investigated whether transformation of floral induction gene induces an early flowering in pears.

The two pear cultivars, 'La France' and 'Ballade', were transformed with a Citrus ortholog of *Arabidopsis FT* gene (*CiFT*) by *Agrobacterium tumefaciens.* Using axillary shoot meristems of 'La France' or leaflets of 'Ballade' as explants, the transformation efficiency was 1.0% and 0.9%, respectively. As a result, total 16 kanamycin resistance shoots of both cultivars were identified. DNA blot analysis indicated the presence of 1 to 5 copies of *CiFT* in these resistance shoots. Interestingly, 13 lines of transformants showed *in vitro* flowering on growth medium. No correlation was observed between *CiFT* copy number and frequency of *in vitro* flowering. However, RNA blot analysis indicated high correlation between *CiFT* mRNA expression level and frequency of *in vitro* flowering. Therefore, we confirmed that the expression of *CiFT* caused *in vitro* flowering in pears. *In vitro* flowers formed sepals, petals, stamens and pistils, although the organ number of *in vitro* flowers usually differed from that of wild type flowers. Also, we could rarely observe an enlargement of receptacle on *in vitro* flowers.

This study suggests the possibility of functional analysis for the genes, at least expressing in flowers, using *in vitro* flowering.

OP26 3.10pm - Tuesday 21 March

Explaining natural variation in apple fruit size in terms of cell production and cell cycle gene expression

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Five apple cultivars, exhibiting considerable natural variation in fruit size, were studied to investigate the role of the cell division cycle in determining fruit size. The selected cultivars differed three-fold in terms of fruit diameter and six-fold in terms of cortical distance. Small fruit size of 'Hopa' crabapple resulted from lower relative growth rate early in the season, both in terms of peak rate and duration. This was due to a much lower cell relative production rate (CPR) throughout early fruit development. 'Golden Delicious', the largest fruited cultivar in the study, had high peak CPR and also a long duration of cell production. Conversely, cell production in 'Hopa' crabapple had a low peak rate and a short duration. 'Gala' and 'Pixie Crunch' both bore medium sized apples, but achieved their fruit size by different mechanisms. "Gala' had a lower intensity of CPR but a long duration of cell production while 'Pixie Crunch' had high peak CPR but only for a short duration.

Expression patterns of 3 core cell cycle genes, *Md;CDKB1*, *Md;CycB2* and *Md;CycD3* were closely related with cell production dynamics, with higher gene expression during times of high CPR. It appears likely that these genes may play a role in regulating cell production by alteration of both the CPR and cell production duration.

OP27 3.30pm - Tuesday 21 March

The MYB transcription factors of Apple: a family of genes involved in controlling a wide range of plant responses

Andrew C. Allan, Karen Bolitho, Richard V. Espley, Karryn Grafton, Roger P. Hellens, Kui Lin-Wang, Sakuntala Karunairetnam, Andrew P. Gleave, and William Laing

HortResearch, Mount Albert Research Centre, Auckland, New Zealand

Sequence analysis of HortResearch's apple EST database has allowed us to identify, and then full length sequence, 37 apple MYB transcription factors. All the members of this gene family have been over expressed in *Arabidopsis*, with 7 producing strong phenotypes. Transient experiments against promoters of interest, transformation into apple, and qPCR analysis of expression profiles in apple, have led to the assigning of putative function for several genes. Apple responses for which this transcription factor family appear to influence include anthocyanin biosynthesis, response to abiotic stress, pathogen response, temperature tolerance, dwarfing and branching.

OP28 3.50pm - Tuesday 21 March

Reduction of juvenile phase in apple by transgenic approaches

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Studies on the mechanism of floral induction in the crops are very important because the process is linked directly to the production and the quality of the crops. In tree fruits, the mechanism of maintenance of juvenility and floral induction has remained to be cleared. Thus, we have studied the apple (*Malus* x *domestica*) genes putatively involved in the regulation of the process to clear the function of the genes and to apply them to the reduction of the juvenile phase in fruit trees such as apple. Expression and function analysis of *MdAP1* (*APETALA1* ortholog of apple) and *MdTFL1* (*TERMINAL FLOWER1* ortholog of apple) demonstrated that *MdAP1* accelerates the flowering and that *MdTFL1* represses the flowering in transgenic *Arabidopsis* ectopically expressing *MdAP1* or *MdTFL1*, respectively. *MdAP1* starts to be expressed 2-3 month after flower bud differentiation in apple, suggesting that it is not involved directly in flower induction. On the other hand, *MdTFL1* is expressed transiently before and after flower bud differentiation in the SAM (Shoot Apical Meristem). Transgenic 'Orin' apples expressing *MdTFL1* antisense RNA first flowered 8-22 months after the transfer to the greenhouse, whereas non-transgenic control 'Orin' apples flowered 69 months after the transfer to the greenhouse. The expression of endogenous *MdTFL1* was suppressed in the antisense lines, but not completely suppressed. On the other hand, transgenic apples with *35S*::*MdTFL1* sense also showed precocious flowering, possibly due to co-suppression. These works in apple suggest that *MdTFL1* functions as one of the regulators of the switch from vegetative to reproductive phase.

OP29 8.35am - Wednesday 22 March

The peach physical/genetic map database: a tool for Rosaceae genomics

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Development of peach as a model genome for Rosaceae combines structural and functional genomics efforts that focus on constructing a complete physical map and the development of a candidate gene database. An integrated physical/genetic map provides a foundation for positional cloning of genetically mapped genes and is essential for eventual sequencing of part or all of the peach genome. Here, we report the initial physical framework for peach. This physical framework is based on the BAC DNA fingerprinting on sequencing polyacrylamide gels and two large-insert BAC libraries developed for the Nemared (HindIII library) and the Lovell (Sau3A1 library) peach cultivars. We randomly fingerprinted BACs equivalent to 3x the peach genome and added selectively the entire EST positive clones from both libraries. Due to selective fingerprinting, the database is biased to the transcribed regions of the peach genome. The current physical framework is composed of 1,384 contigs and 11% of these (152 contigs) are placed on the *Prunus* map. According to a conservative estimate, contigs cover 60% of the peach genome and at least 30% of BACs in the FPC database have hybridization hits (i.e. genetic markers, ESTs, cDNAs and overgo probes). We developed a strategy to fill the gaps in the current physical framework using our new BAC library resource (Bsty1 Lovell library) and the high information-content fingerprinting (HICF). To saturate the physical/genetic map database with additional anchor points, we exploit the mapped EST-SSR dataset to design the overlapping oligoprobes for overgo hybridization. The peach physical/genetic database is publicly available at www.rosacea.org.

OP 30 9.15am - Wednesday 22 March

Microarray analysis of fruit development in apple

Bart Janssen, Robert Schaffer, Kate Thodey, Rebecca Bishop, Shavindra Bajaj, Kim Snowden, Ross Crowhurst, Judith Bowen, Susan Ledger, Marcus Davy, Daya Dayatilake, Shayna Ward, Steve McCartney, Jens Wunsche

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Apple fruit develop over a period of 150 days from pollination to full tree ripeness. Cell division occurs early during development and the basic pattern of the apple fruit is established before any significant cell expansion occurs. The fused base of the petals and sepals is expanded to become the cortex, vascular traces are established and the ovary tissue divides to become the core of the apple. At about 24 days after pollination cell expansion starts. As the fruit expands during development starch is built up and then as the fruit finally ripens sugars increase and the flavour components develop.

In order to begin to understand the molecular events that control and define this developmental process we have collected samples at 8 stages through apple development during the season, RNA from those samples has been used to probe a 16500 oligo array representing approximately 13000 unigenes. 1835 genes were identified whose expression changed significantly during development and cluster analysis revealed groups of genes associated with specific stages during development. Quantitative PCR has been used to confirm gene expression patterns for some of these genes. Genes involved in control of the cell cycle and genes involved in starch metabolism have been examined and correlated with developmental and physiological changes in fruit development. Results from these experiments will be presented and discussed.

OP31 9.55am - Wednesday 22 March

Insertional Mutagenesis as a Functional Genomics Tool in Diploid Strawberry (*Fragaria vesca*)

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We have developed a high-throughput platform for reverse and forward genetics in the diploid strawberry *F. vesca* based on insertional mutagenesis, gene identification and phenotype profiling. Diploid strawberry (*Fragaria vesca*) is an attractive model for developing insertional mutagenesis in Rosaceae due to its small genome size, short reproductive cycle, efficient transformation, and facile vegetative and seed propagation.

To facilitate the development of insertional mutagenesis we devised a new and highly efficient transformation protocol that can be used for systematic production of T-DNA tagged insertional mutants. Our transformation procedure is based on using explants of specific developmental stage, co-cultivation with *Agrobacterium* strain GV3101 and stringent selection on hygromycin (Oosumi et al. 2005).

We established a high-throughput method for amplification of T-DNA flanking genomic sequences in *F. vesca* by optimizing the thermal asymmetric interlaced (TAIL) PCR method. We obtained a 93% success rate on 43 transgenic plants tested using three arbitrary degenerate (AD) primers (AD1, AD2, and AD3) previously designed for *Arabidopsis thaliana*. Secondary TAIL-PCR products were sequenced directly using a T-DNA-specific primer. Twenty three percent of T-DNA tagged sequences analyzed had significant similarity to genes or proteins in other plants.

To validate our T-DNA tagging system we screened a subset of T-DNA insertional mutant lines for obvious morphological mutant phenotypes. Four lines with altered leaf shapes were found, one of which was further characterized by genetic and molecular analysis. Segregation analysis based on GFP expression, phenotype, and PCR analysis with the primers specific to T-DNA flanking genomic DNA indicated that plants with putative mutant phenotype were homozygous T-DNA insertional mutants. We amplified cDNA of this putative T-DNA tagged gene by rapid amplification of cDNA ends (RACE) and determined entire cDNA sequence. BLAST search revealed that the gene had high homology to unknown mRNAs of Arabidopsis and rice.

The generation of a T-DNA mutant collection of strawberry will provide important resource for the Rosaceae community and will impact functional genomics research and gene discovery in Rosaceae and other fruit crops.

Development of a genome-wide physical map of apple genome

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We are well underway with the construction of a genome-wide physical map of apple. To achieve this goal a complementary BAC library was recently constructed for cultivar Goldrush, using *Hin*dIII. The BAC library together with the previously constructed *Bam*HI BAC library contains 9,8304 clones with an average insert size of over 115 kb, representing 14.3 apple haploid genome equivalents. The BACs are being fingerprinted by the improved high-throughput fingerprint gel-based restriction method (The International Human Genome Mapping Consortium, *Nature* 409:934-941). Meanwhile, we have exploited 160,719 apple EST sequences, and found more than 2,000 simple sequence repeats. Primers have already been successfully designed for 1,050 simple sequence repeats to develop EST-SSR marker. Physical map contigs will be constructed, using two independent approaches. Firstly, BAC fingerprints were subjected to overlap analysis using the computer program FPC 4.7 (Soderlund et al., *Genome Res*. 10: 1772-1787). A drift of the physical map based on this analysis will be presented. Secondly, contigs will be identified using new developed EST-SSR markers and previous reported SSR markers by PCR-based BAC library screening strategy. Using this approach, an extensive integration of the physical map and the genetic map of apple will be made. This integrated map will serve as a source for map-based cloning of genes underlining important traits.

Workshop 4: Maloideae
OP33 11.15am - Wednesday 22 March

Pedigree Genotyping: Pedigree-based approaches to multiple crosses allow allele mining and detection of epistasis among QTLs

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To date, molecular markers have been linked to many economically important traits. Unfortunately, lack of knowledge of their allelic variation hampers full exploitation in commercial breeding programs. Usually these markers have been identified in a single cross. Consequently, only one or two favorable alleles of the related QTL are identified, whereas a breeding program may involve several alleles. Selection for just a few alleles means that many favourable genotypes are ignored. This decreases efficiency and leads to genetic erosion. Another consequence of the use of a single cross is that QTLs remain undetected in case of epistasis among genes of an oligogenic or polygenic trait.

A new approach, called pedigree genotyping, allows the identification and exploitation of the majority of alleles present in an ongoing breeding program. This is achieved by including breeding material itself in QTL detection, so covering multiple generations and linking many crosses through their common ancestors in the pedigree. The principle of Identity by Descent (IBD) is utilised to express the identity of an allele of a modern selection in terms of alleles of founding cultivars. These founder alleles are used as factors in statistical analysis.

This paper uses simulated data to demonstrate the power of this approach in the presence of complementary gene action. An integrated QTL analysis is performed using data from multiple populations with known ancestral pedigree relationships. The emphasis will be on the detection of QTL and estimation of effects of the various combinations of alleles. IBD propabilities were estimated by the FlexQTL^{TM} software.

Genetic transformation of apple without use of a selectable marker

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Selectable marker genes are widely used for the efficient transformation of crop plants. In most cases, selection is based on antibiotic or herbicide resistance. These marker genes are preferred because they tend to be most efficient (e.g. in apple up to 80% transformation). Due mainly to consumer and grower concerns, considerable effort is being put into developing a suite of strategies (site-specific recombination, homologous recombination, transposition and co-transformation) to eliminate the marker gene from the nuclear or chloroplast genome after selection. Current efforts concentrate on systems where the marker genes are eliminated efficiently soon after transformation. However, these methods are laborious and of doubtful reliability. For the commercialization of transgenic plants, use of a completely marker-free technology would be greatly preferable, since there would be no involvement of antibiotic resistance genes or other marker genes with negative connotations. With this goal in mind, we have now developed a technique for apple transformation without any selectable marker. Transformation of the apple rootstock 'M.26' with the constructs *pwiAtt35Sgusintron* and *pinMpNPR1* without the kanamycin resistance gene has been achieved. 1500 regenerants were harvested from leaf-piece transformation plates for each transformation. Between 250 and 300 were chosen randomly and tested by PCR for the presence of the transgenes (attacin, *GUS, NPR1*, or the pin2 promoter). Depending on the experiment, 22.0 to 25.4% of these regenerants showed integration of the transgene. Southern analysis will also be done for added confirmation of transformation. Some of these transgenic lines have been propagated and will be used to determine the uniformity of transformed tissues in the plantlets. A second genotype of apple, 'Galaxy', was also transformed with these two constructs. The preliminary results show 12% of the 'Galaxy' regenerants have integrated transgenes.

Genome Mapping in Pear

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Genetic linkage maps of the European pear (*Pyrus communis* L.) cultivars 'Bartlett' and 'La France', and the Japanese pear (*P. pyrifolia* Nakai) cultivar 'Housui' were constructed by using double pseudo-testcross F1 or three-way backcross population. The genetic linkage map of 'Bartlett' consisted of 481 loci including 322 AFLPs, 157 SSRs (84 pear, 68 apple, 5 *Prunus* SSRs), 1 isozyme and a self-incompatibility locus on 17 linkage groups over a total length of 1,020 cM. That of 'La France' consisted of 456 loci including 279 AFLPs and 176 SSRs (97 pear, 79 apple) on 17 groups encompassing a genetic distance of 1,177 cM. Since all pear linkage groups could be aligned to the apple consensus map and the number of linkage groups is compatible with the basic chromosome number 17, it was considered that our pear maps were sufficiently saturated to cover almost whole the genome. The map of 'Housui' contained 327 loci including 214 AFLPs and 112 SSRs on 15 linkage groups with a genetic distance of 1,165 cM, in which linkage groups 5 and 12 were not found. All tested SSR markers in the two groups showed homozygous genotypes for 'Housui', suggesting that these genomic regions were homozygous presumably due to inbreeding through several breeding generations. Disease resistance genes (pear scab, black spot) and important phenotypic traits are under investigation to identify their positions in pear genetic linkage maps.

Workshop 5:Prunoideae
OP36 11.00am - Wednesday 22 March

The chilean peach functional genomics innitiative, a progress report

Orellana,A.[1], Baeza, R.[3], Cambiazo, V.[2], Campos, R.[4], Defilippi, B.[4], Gonzalez, M.[2], Meisel, L.[1], Retamales, J.[4], Silva, H.[1]

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The Chilean consortium for functional genomics in peaches and nectarines has been focus on postharvest disorders, specially mealiness. In order to identify at the molecular level the changes that take place during this disorder we took a functional genomics approach. First we sequenced around 50,000 ESTs from four different libraries. These sequences were assembled and annotated. Around 9,000 unigenes were identified. A set of ESTs was used to print membranes for macroarray analysis. The results indicated that a number of transcripts change their abundance during postharvest. On the other hand, proteins were extracted from fruit exposed to different postharvest conditions and comparative proteomic was performed. A quantitative analysis was made using DIGE (differential gel electrophoresis) and changes in the profile and the content of proteins were observed. Some of the changes in transcript abundance observed by macroarrays coincided with the changes in protein abundance observed by proteomics however, this was not always the case.
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Microsatellite transportability across Rosaceae crops

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A total of 150 microsatellite primer pairs, 25 each of almond genomic, almond EST, peach genomic, peach EST, Japanese plum and apricot genomic, all of them known to produce single-locus and polymorphic SSRs in the species where they were developed, were studied in a set of eight cultivars from nine Rosaceae species (almond, peach, apricot, Japanese plum, European plum, cherry, apple, pear and strawberry). All microsatellites analysed were obtained in *Prunus* species and most of them (~90%) amplified bands of the expected size range in other species of this genus. Polymorphism was also very high in *Prunus* (~80%) for the primer pairs that amplified, with Japanese plum and almond as the most polymorphic among the diploid species (all but European plum) and peach the least polymorphic. Overall, approximately 2/3 of the primer pairs used produced bands that were polymorphic with no obvious differences between microsatellites of EST or genomic origin. Thirty-four microsatellites of all origins amplified and were polymorphic in all *Prunus* species studied. In contrast with the high frequency of transportability within *Prunus*, these microsatellites were much less useful in other Rosaceae subfamilies, with approximately 25% of them amplifying and being polymorphic in apple and pear and less than 10% in strawberry.

Workshop 5:Prunoideae
OP38 11.50am - Wednesday 22 March

Self-compatible peach has mutant versions of the *S* locus genes

Ryutaro Tao, Akiko Watari, Tsuyoshi Habu, and Hisayo Yamane

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Most of the fruit tree species in *Prunus* show the S-RNase based gametophytic self-incompatibility system. *Prunus* is a relatively large plant genus and contains both self-incompatible (SI) and self-compatible (SC) species, as usually observed with the genus that contains SI species. One of the good examples can be seen in peach (*P. persica*) and almond (*P. dulcis*). They are both diploid and classified into the same subgenus Amygdalus of the five subgenera in *Prunus*; however, peach is an SC species and almond is an SI one. In this study, we characterized and cloned *S* locus genes, *S-RNase* and *SFB*, in SC peach and found mutant versions of *S-RNase* and *SFB*, that are found in SI *Prunus* species. Furthermore, structural modifications of *S* locus as revealed by the physical distance between *S-RNase* and *SFB* were also found in the peach *S* locus region. Base on the nature of mutations, evolution and the possible molecular basis of SC in peach will be discussed.

Workshop 5:Prunoideae
OP39 12.10pm - Wednesday 22 March

Loss of pollen function analysis in two self-compatible selections of apricot (*Prunus armeniaca* L.). Evidence of a new pollen component involved in the SI mechanism.

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Apricots (*Prunus armeniaca* L.) are partly self-incompatible, nevertheless most early Spanish cultivars, genetically closely related, are self-pollinated. Two of these apricot self-compatible mutants, 'Currot' ($S_C S_C$) and 'Canino' ($S_2 S_C$), have been analyzed in this work. Loss of pollen-*S* function in the natural occurring S_C-haplotype has been associated with a 358 bp insertion in the S_C-haplotype specific F-box allele (SFB_C). This insertion produces a premature stop codon in the transcript that encodes a putative truncated protein lacking the last 75 aminoacid residues of the C-terminal half including the HVa and HVb domains. This result support previous evidence that point out *SFB* as the pollen-*S* gene in gametophytic self-incompatibility in *Prunus*. On the other hand, the *S*-allele segregation analysis of the 'Goldrich'($S_1 S_2$) x 'Canino'($S_2 S_C$) progeny showed that 'Canino' S_2-carrying pollen grains are able to grow on the incompatible 'Goldrich' ($S_1 S_2$) styles. We have demonstrated that the haplotype-specific SFB_2 gene from 'Canino' is intact and expresses correctly. In addition, no evidence of genetic duplication have been found in 'Canino'. On the basis of these results, and considering the *S*-genotype segregation ratios of the analysed populations, we suggested that the loss of function of an additional factor not linked to the *S*-locus is also involved in the breakdown of self-incompatibility mechanism in 'Canino'.

Workshop 6:Rosoideae
OP40 11.00am - Wednesday 22 March

Sequence samples and gene pair haplotypes in strawberry

Thomas M. Davis[1], M. Shields[1], Qian Zhang[1], Jeffrey L. Bennetzen[2], Ana Clara Pontaroli[2], Phillip SanMiguel3, Denise C.M. Tombolato[4], Kevin M. Folta[4]

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Genomic sequence samples from a diploid strawberry (*Fragaria vesca* ssp. *americana*) fosmid library have detected an average gene density of about 1 gene/ 6 kb, only slightly greater than that of *Arabidopsis thaliana*. SSR (simple sequence repeat) loci occur at about 1 per 5.5 kb. Intervals between many adjacent genes (gene pairs) were short enough (<2.5 kb) to permit conventional PCR amplification of these intergenic regions, using PCR primers located in conserved exon sequences of the flanking genes. These intergenic regions are rich in polymorphisms that can be useful for marker development, and can be used to define genome-specific, "gene pair haplotypes" (GPHs) in the cultivated and wild octoploid species, the putative genome composition of which is AAA'A'BBB'B'. Once defined, GPH markers can be used to assess genome-specific inheritance patterns, to assign particular alleles to particular genomes, and to resolve questions about disomic versus polysomic inheritance in the octoploid species. The genomic library itself was constructed using the Epicentre CopyControl™ pCC1FOS™ fosmid vector system, and consists of 33,295 clones with an average insert size of about 35 kb. Chloroplast and mitochondrial inserts account for 4% and 2.1%, respectively, of the library. Nuclear genome coverage is approximately 6x, assuming a 200 Mb nuclear genome size for *F. vesca*. Under current funding, sequencing of fifty targeted and randomly selected fosmids will be completed. Target genes are those involved in flowering, fruit quality, disease resistance, and other traits of economic interest. Initial results reveal varying degrees of microsynteny between *Fragaria* and other plant species.

Metabolomics screening of the biochemical diversity in strawberry fruit

Ric de Vos[1], Bert Meulenbroek[1], Jan Schaart[1], Robert Hall[1], Eleonora D'Amico[2], Gaetano Perrotta[2] and Arnaud Bovy[1]

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Flavonoids are generally considered as beneficial for human health. In particular proanthocyanidins (PA's), or tannins, show promising effects in the prevention of cardiovascular diseases.

Strawberry fruits are a rich source of flavonoids. The most abundant flavonoids in ripe fruits are the coloured anthocyanins, but proanthocyanidins have been detected in strawberry fruits as well. As part of the EU project FLAVO we aim to get insight into the varietal, developmental and environmental factors influencing the levels of proanthocyanidins and other flavonoids in strawberry fruit.

Using a metabolomics approach we screened the biochemical diversity in a collection of 100 strawberry varieties, derived from three different locations: Wageningen (NL), Cesena (I) and Metaponto (I). Red ripe fruits were harvested and analysed using HPLC-QTOF-PDA-MSMS and the output was subjected to multivariate statistical analyses. An up to 14-fold difference in PA content was observed between varieties, suggesting that there are good opportunities for selection and breeding of high PA varieties. The main proportion of PA polymers was present as dimers and the levels of the two major PA dimers correlated strongly with the levels of their direct precursor catechin. Hierarchical cluster analysis of phenolic compounds revealed the presence of specific types of anthocyanin (yet to be identified) in addition to the major strawberry anthocyanin pelargonidin-3-glucoside. These specific anthocyanins were present in a subset of the germplasm only and suggest the presence of specific modifying enzymes, such as e.g. glycosyltransferases, in those varieties.

Non-targeted metabolomic analysis of the Italian varieties revealed a clear distinction in metabolic composition between varieties typical for and grown in Southern Italy (Metaponto) as compared to Northern Italy (Cesena). A set of 6 varieties was grown at two locations. For 4 varieties, the environmental effect exceeded the genetic differences, whereas 2 varieties were not much influenced by the environment.

This research was carried out as part of the EU-funded project FLAVO (Food CT-2004-513960).

Molecular breeding for root rot resistant raspberries suitable for low input growing systems

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The UK raspberry industry is faced with new challenges in a rapidly-evolving market; with the shift of production to low input systems, under cover, and with the use of fewer pesticides. However, there is a lack of cultivars resistant to some of the most damaging pathogens, notably to raspberry root rot caused by *Phytophthora fragariae* var. *rubi*, which is one of the most devastating diseases of raspberry.

Disease resistance is increasing in importance, but breeders have limited resources and is traditionally time consuming. Breeding can be more precise and rapid with the use of a genetic linkage map and the development and utilisation of diagnostic markers associated with genes that control complex QTLs. A genetic linkage map has been constructed from two phenotypically different cultivars; a susceptible European cv. Glen Moy and a resistant North American cv. Latham. Marker assisted selection and breeding would make possible the introduction of resistance into selected germplasm and breeding lines

The mapping population has been replicated and tested under both glasshouse and field conditions to assess the progeny for resistance status. The data has been analysed and regions on two linkage groups have been identified as important for resistance.

A1 Topic: Bioinformatics and databases
4.30 – 5.30pm Monday 20 March

"CHILLPEACH" a functional genomics database to understand peach chilling injury

Antonio Granell[2], Carlos H. Crisosto[1], Cristina Marti Ibañez[2], Thomas M. Gradziel[1], Javier Forment[2] and Cameron P. Peace[1]

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The application of new genomic technologies provides new tools to reveal in a more comprehensive manner how fruit cells interact with the environment and how this may result in specific peach chilling injury expression. In our previous work, we demonstrated that peach chilling injury has a molecular genetics basis that can be detected at the transcript level. Our next approach is to develop a number of genomics tools which are being deposited at the CHILLPEACH database. This database includes a large collection of sequenced full-length enriched cDNAs from peach mesocarp from sensitive and tolerant selections, and the 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, among others, and 3) sequence features, such as presence of SNPs or SSRs. In addition to being over 70% full-length sequences, 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. In addition to a general unbiased normalized library from peach mesocarp we developed a substractive hybridization library (SSH-MOS) enriched in cDNAs for messages regulated during cold storage.

A "chillpeach" microarray is being produced which contains the whole collection of unigenes developed in this project and will be used to identify transcriptome changes associated to chilling injury.

A2 Topic: Bioinformatics and databases
4.30 – 5.30pm Monday 20 March

Creation of a new versatile database for linking molecular and phenotypic information of apple (*Malus x domestica* Borkh.) : the HiDRAS *'AppleBreed Database'*

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The AppleBreed database stores and easily gives access to huge numbers of genotypic and phenotypic data coming from multiple pedigreed plant populations (progenies, cultivars used as progenitors, breeding selections). It insures a high traceability of the data flow over generations and years, thus being adapted to perennial plants with a long economical life time. It hereby supports geneticists and breeders in elucidating the genetics of economic important traits, in identifying associations between these traits and molecular markers (QTL) and in allele mining. It also supports the choosing of the best parental cultivars to cross with.

AppleBreed is a relational database which can answer to needs raised by geneticists and breeders who work with perennial plants. The aims are to link molecular information to phenotype assessment results and to guarantee the link between cultivars and crosses (families). In order to achieve these objectives the core of the database model is based on individual trees and individual DNA-samples which are together the structure 'genotype' that makes the link between all the available information. This structure allows multi annual observations to be stored individually by genotype even if the nature of these observations is very different (e.g., molecular data, physical-chemical measurements of fruit quality traits, evaluation of disease susceptibility etc.). Over and above that, phenotypic data, molecular marker data and links to the individual trees and DNA samples from which these data were derived, pedigrees, marker descriptions, primer sequences, molecular marker linkage maps and synonyms of the cultivar names are allowed due to the relational structure of the database. It also included validation procedures for phenotypic and marker data, and presents basic statistical overviews on the data.

AppleBreed was constructed within the European project (HiDRAS - High-quality Disease Resistance in Apples for Sustainable Agriculture - QLK5-CT-2002-01492).

A3 Topic: Bioinformatics and databases
4.30 – 5.30pm Monday 20 March

Computational Analysis of Putative Resistance Gene Analogs in Raspberry

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Plant R genes are known to confer resistance to a variety of pathogens in a gene-for-gene mode. Seventy-six putative resistance gene analogs (RGAs) containing conserved domains were amplified from red raspberry *Rubus idaeus* "Latham" using degenerate primers based on RGAs identified in other Rosaceae species. These sequences were clustered using a variety of methods such as Cap3 and Phrap. The consensus sequences of the resulting contigs and singletons were aligned and compared in a phylogenetic context with RGAs identified from other Rosaceae species. This work is part of a larger project to map RGAs in a red raspberry population segregating for resistance to *Phytophthora*.

A4 Topic: Bioinformatics and databases
4.30 – 5.30pm Monday 20 March

Bioinformatic advances in the chilean nectarine functional Genomics consortium

Meisel, L., Vizoso, P., Latorre, M., Saba, J., Loira, N., Tittarelli, A., Martinez, V., Vargas, C., Maldonado, J., Caroca, R., Bugueño, M., Segovia, S., Morales, A. and Silva, H.

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As part of the Chilean Nectarine Functional Genomics Consortium, we have sequenced and analyzed bioinformatically over 50,000 ESTs from *Prunus persica* fruits at different post-harvest stages. Towards determining the global changes in gene expression that may occur in *Prunus persica* under different postharvest conditions, we constructed cDNA libraries from four different post-harvest stages: (1) non-ripe fruits post-harvest; (2) ripened fruits; (3) non-ripe cold-treated fruits; and (4) ripened cold-treated fruits. The cDNA clones from these four libraries were sequenced at the 5' end. The sequence information from these clones (ESTs) were filtered to eliminate low quality sequence (P<20), cloning artifacts as well as contaminations. ESTs were subsequently assembled into contigs or singletons with the assembly parameters CAP3 –p 95 – o 60. The consensus sequences of these unigenes were analyzed by Eugene 'Home to identify full length cDNA sequences. Digital expression analysis was performed on the contigs via Winflat analyses. EST sequences and Unigenes were annotated based upon Blast analyses at the amino acid and nucleotide levels as well as InterproScan analyses. Results of these analyses were processed locally to assign an annotation based upon homology, InterproScan and Gene Ontology. Results of the sequence analyses and annotation can be easily viewed and searched using the locally developed application, JUICE.

Acknowledgments: supported by FDI G02P1001, ASOEX, FDF and Fundación Chile.

A5 Topic: Bioinformatics and databases
4.30 – 5.30pm Monday 20 March

GDR: A Database Resource for Comparative Genomics in Rosaceae

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Rosaceae is an economically important family which includes fruits and ornamental plants such as peach, almond, strawberry, apple, pear, blackberry, cherry and rose. The Genome Database for Rosaceae (GDR) is a curated and integrated web-based relational database, initiated for the effective utilization of the Rosaceae genomics and genetics data. GDR contains comprehensive data of the genetically anchored peach physical map, Rosaceae maps and markers and an annotated EST database of peach, almond, strawberry, and all publicly available Rosaceae sequences. Our integrated map viewer provides a graphical interface to the genetic, transcriptome and physical mapping information. ESTs, BACs and markers can be queried by various categories and the search result sites are linked to the integrated map viewer or to the WebFPC physical map sites. CMap, the comparative map viewer, allows users to compare various Rosaceae genetic maps and the transcriptome map. In addition to browsing and querying the database, users can compare their sequences with the annotated GDR sequences via a dedicated sequence similarity server running either the BLAST or FASTA algorithm. GDR can be accessed at http://www.rosaceae.org. To demonstrate the utility of the data available from GDR, we also report our analysis of the extent of conserved synteny between the Prunus and Arabidopsis genome.

A6 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Development of resistance to bacterial and fungal diseases by over-expressing the apple gene *MpNPR1* in apple

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The *NPR1* gene is thought to be pivotal in the defense cascade caused by systemic acquired resistance and by R gene resistance in plants. Its over-expression in Arabidopsis and rice has resulted in increased disease resistance and elevated PR-gene expression. An *NPR1* homolog, *MpNPR1*, was cloned from *Malus* X *domestica* and over-expressed in two apple cultivars, Galaxy and M26. Leaf pieces were transformed with *MpNPR1* cDNA under the control of the inducible *Pin2* or the constitutive *CaMV35S* promoter using *Agrobacterium tumefaciens* strain EHA105. PCR analysis confirmed the transgene integration in multiple events for each construct in each cultivar. Over-expression of *MpNPR1* mRNA was shown by RT-PCR. Activation of some PR proteins (PR2, PR5, PR8) was also demonstrated. Resistance to the serious bacterial disease, fire blight, was evaluated in the growth chamber by inoculation of the shoot tips of 30-cm tall, own-rooted potted plants with the virulent strain Ea273 of *Erwinia amylovora*. In the initial test, Galaxy clones with an additional copy of *MpNPR1* had shoot length infected of 32 to 40% compared with 80% in the control Galaxy plants. M26 clones with an additional copy of *MpNPR1* under the control of the CaMV35S promoter also showed a significant reduction of symptoms compared to the control M26. Preliminary tests indicate that some *MpNPR1*-over-expressing Galaxy clones may also have increased resistance to two important fungal diseases of apple caused by an ascomycete and a basidiomycete. Selected clones have been propagated for field trials for disease resistance and quality. Transgenic cultivars with resistance due to over-expression of a native apple gene are likely to be more acceptable to regulators, growers, and consumers than transgenics with heterologous genes.

B1 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Increasing resistance to Erwinia amylovora in apple by silencing apple *DIPM* genes

E.E. Borejsza-Wysocka[1], M. Malnoy[1], X. Meng[2], J.M. Bonasera[2], S.V. Beer[2] and H.S. Aldwinckle[1]

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The disease specific gene, DspE (=DspA) of the bacterium *Erwinia amylovora* encodes a pathogenicity effector of 198kDa that is essential for development of fire blight disease in apple plants. Yeast two-hybrid and in vitro protein-pull-down assays demonstrated that DspE interacts physically and specifically with four similar leucine-rich-repeat (LRR), receptor-like serine/threonine kinases from apple. Genes encoding the four DspE-interacting proteins of *Malus* (*DIPM* genes) are conserved in all hosts of *E. amylovora* tested, but not in tested non-host plants. The interaction between the *DIPM*'s and DspE is thought to be involved in disease development. Ca. 400bp sense sequences from non-conserved regions of each gene, with homology among each other of <50% were used to make constructs to be transformed into fire blight susceptible apple cultivars with the aim of silencing the *DIPM* genes and preventing interaction with DspE. In addition, three constructs containing the four 400bp sequences in tandem, a full length sense sequence of one gene, and an RNAi sequence of that gene were made. All seven silencing sequences were transferred into Galaxy apple cultivar by *Agrobacterium* inoculation of leaf pieces. Transgenic lines with all constructs were recovered. Assays of the transgenic plants for net expression of the target *DIPM*'s by RT-PCR for their mRNA's have shown evidence of silencing in some lines. Evaluation for resistance to fire blight by inoculation of shoots with *E. amylovora* strain Ea273 indicated that some lines with silencing had increased resistance. Resistance due to silencing of a native apple gene(s) is likely to be more acceptable to regulators, growers and consumers than the addition of heterologous genes.

B2 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Identification of SSRs linked to sharka resistance on apricot by mapping approach. Application on marker assisted selection.

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Most of the apricot breeding programs in progress are aimed at introgression of resistance to sharka or plum pox virus (PPV). Marker assisted selection (MAS) for the trait is one of the main objectives in molecular studies on apricot. The genetic control of the trait is not understood yet, some studies suggest the trait is under polygenic control. However, in our experimental conditions, it behaves as a binary response variable (resistant versus susceptible) and therefore does not follow a normal distribution. In this paper we mapped the PPV resistance trait as a QTL using the non-parametric method based on Kruskal-Wallis rank sum test. This approach allows the mapping of a QTL when the normality assumption cannot be made. Two mapping populations segregating for PPV resistance were used to study the marker-trait association. A F_2 progeny from self-pollination of the resistant cultivar 'Lito' obtained from a cross between SEO resistant to PPV and 'Tyrintos' susceptible to PPV and a F_1 progeny from a cross between 'Goldrich' resistant to PPV and 'Currot' susceptible to PPV. Results showed that all markers with high significance levels of the Kruskal-Wallis test are located in the upper part of the G1 linkage group of both maps, suggesting the presence of a major QTL controlling PPV resistance. We selected five SSRs (simple sequence repeats) putatively linked to the PPV resistance. They were screened in the resistant and susceptible genitors used in the breeding program, in selected resistant seedlings and in two additional segregating populations to validate their usefulness in MAS.

B3 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Map-based cloning of the avirulence gene *avrVg* of *Venturia inaequalis*

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Apple scab, caused by the ascomycete *Venturia inaequalis (Cke.)*, is the most important fungal disease of cultivated apples in temperate climates where cool and wet springs occur. It has been demonstrated that the interaction between *Venturia inaequalis* races and resistant apple cultivars undergoes a gene-for-gene relationship. The anthropogenic apple production system consisting in monocultures induced a constant selection pressure. The virulence alleles necessary to overcome the correspondent resistance genes present in these commercial cultivars become fixed (or at high frequency) in the pathogen population. The ways used by the fungus to circumvent the recognition by the host plant have not yet been elucidated. At present only one AVR protein has been isolated from *Venturia inaequalis* race 5 (avirulent on *Vm* resistant plants).

Here we present the status of the map-based cloning of *avrVg*, the counterpart of the resistance gene *Vg* first found in 'Golden Delicious'. The project started from the genetic map of the *avrVg* developed by INRA Angers. A BAC library has been constructed from an avirulent isolate. The library consists of 7680 clones with an average insert size of about 100kb. Using markers that co-segregate with the *avrVg* locus as starting point, a first group of clones was isolated and a chromosome walking was undertaken extending the contig outwards until the BAC-end-derived markers segregated from the *avrVg* locus itself. The contig spanning the *avrVg* locus will be presented.

B4 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Cloning and Characterization of Resistance Gene Analogs from Roses

Anja Hattendorf, Thomas Debener

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We cloned NBS-LRR-RGAs of the TIR and non-TIR type from roses and established an extensive RGA library comprising about 7000 clones. We characterised the RGA library in a combined process of sequencing and colony-hybridization to assess the sequence diversity as well as the number and size of RGA families isolated. We found 40 different RGA families of variable size and analysed them regarding their phylogenetic relationships among each other and to R genes and RGAs from other plants. Our results indicate the existence of at least four different starting points during the evolution of rose R genes and we assume that distinct duplication mechanisms acted upon R gene dispersion.

We then investigated the genomic copy number of selected RGAs in different rose genotypes and in *Prunus cérasus*. Most of the analysed RGAs were multicopy loci while no conservation of the rose RGAs could be detected in cherry.

For some RGAs we performed expression studies in rose flowers and leaves before and after inoculation with a black spot isolate and identified several TIR-RGAs with enhanced transcription after black spot attack. These most probably are directly involved in pathogen defense and therefore represent interesting candidates for black spot R genes.

Finally, we mapped the RGA loci in two different rose populations to find candidates for the already characterised rose R genes *Rdr1* against black spot and *Rpp1* against powdery mildew as well as hotspot regions for new R genes. Although we did not get direct candidates for the mentioned R genes we detected several RGAs in regions with QTL for powdery mildew resistance and some interesting RGA clusters indicating potential genomic positions containing R genes.

B5 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Molecular research for breeding disease resistant apple cultivars with high fruit quality

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At the Institute of Fruit Breeding breeding research on *Malus* cultivars and species is aimed to both biodiversity research and practical fruit breeding. Regarding apple scab the background of our research is, that new races of the fungus occurred in German apple growing areas which are able to overcome the *Vf* resistance. A scab resistance gene from the Russian seedling R12740-7A (called *Vr1*) was identified and mapped in several populations on top of the apple linkage group LG 2. Research is also focused on scab resistance genes from *M. sieversii* accessions. Race-specific scab tests are applied to mapping populations and the genetics of the resistance are studied. Several putative major genes have been identified, at least two of them being effective against race 7. A saturated linkage map has been created for the apple progeny C3 (Discovery x Prima). This map has been used for QTL mapping (scab, mildew, fruit quality parameters) as well as for mapping HcrVf- and NBS-LRR RGA genes. Some known major genes as i.e. *Vf, Vg, Pld* and *Pl1* were found to be tighly linked with RGAs located on several linkage groups. Resistance to powdery mildew is one of the other major aims in apple breeding. We are using several marker techniques such as RAPD, SSR, AFLP and SCAR markers to evaluate the genetic diversity of pure isolates as well as field samples of European and Asian populations of apple powdery mildew. Regarding apple powdery mildew resistance currently new resistance sources are being searched in wild species that can extend the genetic basis for apple resistance breeding. The major part of the work on fruit quality traits is presently running within the EU-project HiDRAS. Information about the contributions of the Institute of Fruit Breeding will be given.

B6 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Diversity and characterization in USDA *Malus* and tetraploid cherry germplasm in Geneva, NY

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The *Malus* and tetraploid cherry (*Prunus cerasus and P. fruticosa*) collections of the USDA, Geneva, New York are the World's most diverse collections for these species. The *Malus* collection includes 2400 clones grafted to EMLA 7 rootstock and represents a broad spectrum of *M.* ×*domestica*, hybrids, and 36 *Malus* species. A core collection of 250 accessions within this group has been identified and characterized. To greatly expand genetic diversity in the *Malus* collection, 1600 seedlots from wild habitats have been collected from sites in: 1) North America (4 species); 2) China (7 species); 3) Central Asia, the main center of origin for commercial apples (*M. sieversii*); 4) Caucasus region (*M. orientalis*); and 5) Germany (*M. sylvestris*). Over 5000 seedlings from 350 of the seedlots were grown and evaluated for 30 morphological descriptors and are being screened for resistance to apple scab (*Venturia inaequalis*), fire blight (*Erwinia amylovora*) and cedar apple rust (*Gymnosporangium juniperi-virginianae*). In addition, extent of genetic diversity and genetic structure in these populations have been characterized with molecular tools. Disease resistant individuals are being used in scion and rootstock breeding. The tetraploid cherry collection contains ca. 100 accessions with recent addition of germplasm of Russian origin. Some of these new accessions are resistant to cherry leaf spot (*Blumeriella jaapii*) and are being used as parents in the sour cherry breeding program at Michigan State University.

C1 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Evaluation of horticulturally elite *Malus sieversii* germplasm for apple scab resistance genes using phenotypic and marker-based screening

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Apple scab (*Venturia inaequalis*) resistance was assessed for 39 horticulturally elite *Malus sieversii* accessions collected in Kazakhstan. Based on inoculation with a mixture of North American races 1-5 of *V. inaequalis*, eight of the 39 accessions were resistant. DNA was extracted from each elite accession and screened for presence of markers that have been reported to be linked to specific known scab resistance genes based on previous research with other germplasm. None of the elite accessions were positive for SCAR markers linked with *Vm* (LG 17) or *Vf* (LG 1). Numerous accessions, including both resistant and susceptible phenotypes, had positive reactions with SCAR markers linked to a group of several reported resistance genes on LG 2 including *Vh2*, *Vh4*, and *Vh8*, a recently described resistance gene that was identified in other *M. sieversii* germplasm. Of the accessions that had resistant phenotypes when screened, those testing positive for several markers associated with *Vh2* and *Vh8* included PIs 613987, 613991, 613998, and 613951. Resistant accessions testing positive for markers associated with the *Vh4* or *Vr2* region include PIs 613992, 613951, and 613957. Eleven accessions exhibited positive reactions with a less reliable (RAPD) marker linked to *Vb* on LG 1. Seven of the elite accessions were crossed with the susceptible cultivar Royal Gala and seedlings were screened with *V. inaequalis* races 1 and 2. The proportion of resistant progeny, combined over both races, ranged from nine to 67%. Four crosses gave significantly different proportions of resistant progeny in screens with race 1 compared with race 2. DNA was extracted from seedlings from 6 of these families and screened with up to eight markers linked to R genes on LG 2. Correlation of the markers with phenotype indicated some *M. sieversii* parents were likely to carry known resistance genes but, in some cases, exhibited segregation patterns suggesting they also contained novel resistance loci.

C2 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Cloning and linkage mapping of new NBS-LRR resistance gene candidates and identification of markers associated to susceptibility to *Fusicoccum* canker in almond

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In this work we have targeted resistance gene candidate (RGC) sequences and also looked for markers related to tolerance and susceptibility to *Fusicoccum* canker in almond (*Prunus dulcis* Mill.). RGC sequences were isolated from genomic DNA of one almond cultivar, 'Primorskyi', and two wild ecotypes of *Prunus webbii*. Using a PCR-mediated approach with degenerated primers, designed based on conserved motifs of the nucleotide-binding site (NBS) domain, 56 sequences sharing homology with known resistance gene candidates were cloned. Phylogenetic analysis showed that these sequences clustered together in 5 different groups, suggesting a high similarity of the genetic backgrounds from both species, in what concerns their resistances.

Regarding *Fusicoccum* canker, we have identified 3 putative RAPD markers related to tolerance and susceptibility to this disease in almond. Specific SCAR primers were designed for these markers which, however, resulted in a loss of the initial polymorphism. Based on the nucleotide sequences of those SCAR fragments, CAPS markers were then developed on the basis of *Dra*III and *Ear*I restriction sites that were present in susceptible cultivars and absent in tolerant ones. In 15 cultivars analyzed, these CAPS markers, named D19S-1F1R$_{EarI}$, D19S-2F2R$_{EarI}$ e D19S-2F2R$_{DraIII}$, showed a high percentage of agreement with the phenotype (tolerance or susceptibility). Co-segregation analysis on a F1 population (tolerant x susceptible), is underway.

Using the 'Texas' x 'Earlygold' *Prunus* reference map, mapping analysis of the almond RGC sequences and CAPS markers is underway to look for possible associations with already mapped loci related to resistance.

C3 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Resistance to Prune dwarf virus can result from RNA mediated gene silencing

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Prune dwarf virus is an *Ilarvirus* systemically infecting almond trees among other *Prunus* species. Resistance to plant viruses can be achieved through genetic engineering using the coat protein (cp) gene. We have investigated the possibility of applying this strategy to achieve resistance to PDV and used an herbaceous host, *Nicotiana benthamiana*, to study the resistance mechanisms. Several constructs carrying the cpPDV sequence were prepared. The cpPDV sequences in sense and antisense orientation, mutated or deleted were placed under the control of 35CaMV promoter, inserted in pGREEN vectors (John Innes Center), with *npt*II (kanamycin resistance) as selection marker. Using *Agrobacterium*-mediated transformation we regenerated transgenic *N. benthamiana* plants with distinct transgene integration patterns (detected by Northern blotting). Studies of transgene expression were conducted in regenerated transgenic plants and in their progenies. Transgenic plants with one transgene insertion that accumulated either cp RNA (confirmed by Northern blotting of RT-PCR products) or protein (confirmed by DAS-ELISA) were used in challenging assays. These were performed under controlled conditions, using virus infected leaf extracts, to evaluate resistance levels. Although our results indicate that resistance to PDV can be achieved in some plants expressing a mutated form of the coat protein gene (substituting one amino-acid involved in the activation of virus replication), our data show that resistance to PDV is mediated by RNA and probably occurring through a mechanism of gene silencing. It also shows that this strategy can be used to achieve resistance to distinct virus serotypes with cp sequence homologies above 91%.

C4 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Efforts to elucidate mechanisms and genetics of fire blight resistance in apple

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Five fire blight resistant apple genotypes of *Malus* x *atrosanguinea, M. baccata, M. fusca, M.* x *floribunda M.* x *prunifolia* and *M.* x *robusta*, were crossed to the susceptible cultivar 'Idared'. Resulting seedlings of four combinations were tested via artificial shoot inoculation for the segregation of the resistant trait.

Progenies of the cross 'Idared' x *M.* x *robusta* were screened for susceptibility to fire blight using four to 12 graftings / progeny. The severity of necrosis of progenies reached from 0.0 to 98.7 % with a mean of 35.4 %.

The transcription level of genes of the flavonoid pathway were estimated by real time PCR for the resistant wild species *M. baccata, M. fusca, M.* x *floribunda* and *M.* x *robusta* and the susceptible cultivars 'Idared' and 'Pinova' with and without fire blight inoculation. Different mechanisms of fire blight resistance can be assumed regarding different transcription patterns.

78 microsatellites were tested for polymorphisms at the progeny 'Idared' x *M.* x *robusta*. Multiplex PCR with up to eight different microsatellites / reaction were performed with the 62 polymorphic microsatellites at 150 progenies of the cross. A first linkage map, containing 15 linkage groups, could be established. The alignment of markers on the linkage groups in general is in common with the order on published linkage maps.

These map and a map of population 'Idared' by *M. fusca* (in progress) will be used to map fire blight resistance and to compare both maps especially regarding loci contributing to resistance to fire blight.

C5 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Studies on apple disease resistance genes

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The NBS-type (nucleotide binding site) resistance gene analogs (RGA) represent a very large gene family, which are involved in many examples of disease resistance in plants. Using PCR primers designed against conserved sequence motifs in the NBS-RGA family, a mixture of DNA fragments were amplified in 'Golden Delicious' and 'Anna' apple genomes, and these were then cloned and sequenced. 136 sequences obtained this way matched RGAs from other plant species. These were then analysed against *Arabidopsis* and cloned disease resistance genes from rice resulting in classification into two subgroups with 74 being assigned to the CNL class and 62 TNL. More RGA sequencing work was done using DNA from 'Anna'. All the clones with sequenced RGAs were further PCR amplified and products were spotted on nitrocellulose paper. Hybridisation to isolated total RNA from apple plants infected with *Venturia inequalis* was done to generate expression profiles for candidate disease resistance genes from the sequenced RGAs. The analysis of variation in expression levels of the different RGA genes will provide an important tool for the mapping of candidate genes that control disease resistance. These will then be of value in the application of genetic selection methods in the apple breeding program for the development of novel durable resistant selections with reduced requirements for fungicide use in production.

C6 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Genetic analysis of resistance to apple scab (*Venturia inaequalis*) in apple, (*Malus x domestica* Borkh)

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Apple scab, caused by the fungus *Venturia inaequalis*, is a major problem in the apple industry, with lesions developing on both fruit and leaves. Control of scab is either through extensive fungicide use, or by the use of scab resistant cultivars. V*f* is the major gene responsible for apple scab resistance and is found in cultivars like Prima and Priscilla. However, from analysis of scab resistance in mapping populations containing the *Vf* gene, it is clear that additional genes control the resistant phenotype. The incorporation of other scab resistance genes in addition to V*f* would provide greater and more durable scab resistance in new cultivars. In this work, a mapping population from a 'Lady William's' x 'Prima' cross, consisting of 192 individuals, has been categorized into four classes of resistance/susceptibility to apple scab. Microsatellite (simple sequence repeats) markers are used in this study and a linkage map is being constructed for this population, which will allow the minor (quantitative trait loci) QTLs for scab resistance (in addition to *Vf*) to be identified. With the availability of more QTLs combined with V*f*, it will be possible to use markers linked to each of the QTLs to select for more desirable cultivars resistant to apple scab in a high throughput marker assisted selection program. This will lead to the selection of seedlings highly resistant to apple scab for future evaluation work. In turn, this will lead to the production of new cultivars with enhanced disease resistance, leading to reduced chemical inputs in production.

D1 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Genetic analysis of resistance to powdery mildew (*Podosphaera leuchotricha*) in apple (*Malus x domestica* Borkh.)

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Apple powdery mildew, caused by *Podosphaera leuchtricha*, is one of the major diseases of the cultivated apple in the Western Cape. The damage caused by this pathogen causes losses in fruit quality and yield. Furthermore, the sector is affected as costs are incurred on the management of this disease. The application of fungicides is one of the strategies mostly used to control the spread of the pathogen. Since consumers demand high quality fruit with no harmful chemical residues, alternative approaches that minimize the reliance on chemicals for the control of this disease must be developed. Marker-assisted breeding for the development of new varieties that exhibit durable resistance to the fungus is the best and safest way to reduce damage caused by powdery mildew. In this study co-dominant microsatellite markers are used to construct a framework genetic map for a population of 104 individuals resulting from 'African Carmine' x 'Simpson', which has segregating resistance to *P. leuchtricha.* In this work the generation of a preliminary genetic map will be described, using microsatellite markers. From this map the location of genes contributing to resistance to *Podosphaera leuchtricha* will be analysed, and markers linked to these genes will be of importance in future selections for mildew resistance in the ARC Breeding program, which in turn will lead to the development of mildew resistant cultivars in the future.

D2 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Genetic analysis for resistance to Woolly Apple Aphid in apple rootstock breeding populations

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The woolly apple aphid (*Eriosoma lanigerum* Hausmn.) is a major pest in apple production in the Western Cape Province, South Africa. Breeding resistant rootstocks might provide a necessary and environmentally friendly method of control. This study aims to map genes for woolly apple aphid (WAA) resistance using microsatellite markers. A mapping population of 96 individuals from a 'Northern Spy' x 'Cox Orange Pippin' cross was generated. 52 seedlings were multiplied by *in vitro* propagation, planted in replicates of three blocks in a greenhouse (20-25 ^{0}C) and evaluated for susceptibility and resistance to WAA. A linkage map is being constructed using microsatellite markers to analyse the complete mapping population. Fluorescently labelled microsatellite markers were used in linkage map construction, and these were multiplexed by PCR for high throughput data generation. The computer programs Genotyper^{TM} and JoinMap^{TM} were used in linkage map construction. The current linkage map and analysis of quantitave trait loci (QTLs) for resistance to WAA will be presented. The identification of QTLs for WAA resistance will provide the tools required for the marker assisted selection of disease resistant genotypes in future rootstock breeding, providing the basis for the development of new disease resistant rootstocks that have reduced requirements for pesticide or fungicide treatment.

D3 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Identification and genetic characterisation of *Vdr1*, a new major scab resistance gene from the apple cultivar 'Dülmener Rosenapfel'

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Durable scab resistance aiming at reducing the use of fungicides in commercial orchards is one of the major objectives in apple breeding. In the European D.A.R.E. project (Lespinasse *et al.*, 2000), a few broad-spectrum scab resistant cultivars have been identified such as the German cultivar 'Dülmener Rosenapfel' which showed resistance to more than ten inocula of the fungus *Venturia inaequalis* under different environmental conditions (Laurens *et al.*, 2004). The genetic determinism of this stable resistance was investigated in a progeny derived from 'Dülmener Rosenapfel' crossed with the susceptible cultivar 'Gala'. This progeny (277 seedlings) was inoculated with a mixture of five strains (races 1, 6, 7 and 6+7) of *V. inaequalis*. A 1:1 segregation for a chlorotic-type symptom was observed, which result underlines the presence of a major gene, which we named *Vdr1*. With the aid of microsatellite markers, the gene was mapped at the top of LG-6 of the apple genome. The closest microsatellite marker is HB09TC (5 cM). HB09TC has been derived from a BAC clone identified by screening the 'Florina' BAC library with a probe derived from *HcrVf2* (Belfanti *et al.*, 2004). Because of the allopolyploid origin (duplication of genome) of apple and the chlorotic-type symptom conditioned by *Vdr1*, this genomic region located at the top of LG-6 may be homeologous to the LG-1 region carrying the *Vf* gene. The study of the resistance spectrum of *Vdr1* is underway by inoculating a selected number of seedlings of this progeny with individual strains of *V. inaequalis*.

D4 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Apple homologues of the tobacco *hsr203j* gene

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The *hsr203j* gene has been reported as being a gene that is specifically induced during the hypersensitive response (HR) in tobacco by Pontier *et al.* (1994). Its up-regulation is a useful indicator for incompatible plant/pathogen interactions and has also been demonstrated to have similar expression patterns in tomato, capsicum, pea and sunflower. Unfortunately recent results from pear (Malnoy *et al.* 2003) suggest that the heterologous *hsr203j* promoter from tobacco may not be active in the Rosaceae. For these reasons we have tried to identify homologues of the *hsr203j* gene in apple in order to test its up-regulation and identify its promoter. Database mining and PCR using degenerate primers have yielded four homologues of this gene in apple. Quantitative RT-PCR was used to demonstrate that one of these genes is induced in an incompatible reaction to the apple pathogen *Venturia inaequalis* and genome-walking revealed that the promoter of this homologue contains the TAAAAT motif, which has been shown in tobacco to be essential to the function of the promoter of HR-specific genes. Although *hsr203j* is a member of the large carboxyl esterase family of genes this is the first instance where several closely related homologues of *hsr203j* have been found in a single plant genome. We will present information on the cloning of *hsr203j* candidate genes and their promoters in apple, and their expression patterns.

D5 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Mapping of the apple scab resistance gene *Vb* from Hansen's baccata #2

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The apple scab resistance gene *Vb*, derived from the Siberian crab apple Hansen's baccata #2, is one of the six "historical" major apple scab resistance genes (*Vf*, *Va*, *Vr*, *Vbj*, *Vm* and *Vb*). In testcross experiments performed in the sixties, *Vb* was found to segregate independently from *Vbj*, *Vr* and *Vf*. Nevertheless recently, *Vb* and *Vf* were both mapped on linkage group 1 (LG1), a result clearly in conflict with the findings of the "old" testcross experiments.

In this study, simple sequence repeat markers (SSR markers) and a cross between Golden Delicious (*vbvb*) and Hansen's baccata #2 (*Vbvb*) were used to determine the precise position of *Vb.* The method we used is the genome scanning approach (GSA). The method consists in determining the inheritance frequency of SSR alleles of the resistant parent within a small subset of progeny plants (all resistant or all susceptible). The SSRs are chosen to be evenly distributed on the apple genome. Zones with a deviation from the expected ratio (1:1) are candidates to carry the resistance gene. Detailed analysis of the progeny plants of our cross with SSRs mapped on LG1 confirmed that *Vb* does not map on this LG, but clearly shows that the *Vb* resistance gene maps on the distal end of LG12. Therefore *Vb* and *Vf* map on two different LGs. This result is consequently in agreement with the results of the testcrosses. The identification of the LG carrying *Vb* by GSA and the mapping procedure used will be presented.

D6 Topic: Disease resistance
4.30 – 5.30pm Monday 20 March

Molecular Markers Linked to Disease Resistance Genes in Pear

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Pear scab (caused by *Venturia nashicola*) and black spot (*Alternaria kikuchiana*) are two major diseases often inflicting severe damage in the cultivation of Japanese pear (*Pyrus pyrifolia* L.). In order to conduct efficient MAS (marker assisted selection) of the resistant or susceptible seedlings to these diseases, it is necessary to identify their positions in genetic linkage maps and to get tightly linked molecular markers. The pear scab resistance gene *Vnk* of the Japanese pear 'Kinchaku' was identified at the middle of linkage group 1, indicating that *Vnk* was located in the same homologous linkage group 1 as *Vf* (apple scab resistance gene) but in different genomic regions. Pear scab resistance of European pear (*P. communis* L.) cultivar 'La France' was also analyzed, suggesting that its resistance was not controlled by a single gene but regulated by two or more loci. According to QTL analysis, one major QTL was identified in linkage group 2, and another QTL was located in linkage group 14. The susceptibility genes to black spot of the Japanese pear 'Osa-Nijisseiki' and 'Nansui' were mapped at the top of linkage group 11, and their positions were very close to the susceptibility gene to *Alternaria mali* in apple.

E1 Topic: Molecular markers
4.30 – 5.30pm Monday 20 March

Genotyping and identification of sweet cherry cultivars using SSR markers

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Recently, the importance in regard to the identification of economic crop varieties has increased by an illegal spread of a new variety and an indication of untrue variety name. In sweet cherry (*Prunus avium* L.), it turned into a grave issue that there is an outflow of major cultivars that are economically valuable from Japan. Therefore, in this research, we try to apply the SSR (Simple Sequence Repeats) DNA markers for the genotyping and identification of sweet cherry cultivars. SSR analysis was performed on sweet cherry cultivars and breeding varieties in Yamagata General Agricultural Research Center using ABI310 DNA Sequencer. More than 90 varieties were tested with 49 SSR primer pairs designed from peach (*P. persica* L.), sour cherry (*P.cerasus* L.) and sweet cherry. The parent-child relationships among cultivars were estimated. The genetic similarity values were calculated, and UPGMA (Un-weighted pair-group method analysis) cluster analysis was performed to generate a dendrogram. The cherry varieties showed high levels of polymorphism with 2 to 13 different alleles amplified per SSR primer pair. We selected some suitable SSR primer pairs for identification of sweet cherry cultivars. DNA purification from a cherry fruit was done using some DNA extraction kits. Using the selected primer sets, we can identify the variety from a cherry fruit.

E2 Topic: Molecular markers
4.30 – 5.30pm Monday 20 March

Construction of kiwifruit BAC contig maps by overgo hybridization and their use for the targeted region around the sex locus.

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The foundation of any map-based cloning project is a solid association between the genetic and the physical maps. We aim to locate the syntenic regions between *Arabidopsis thaliana* and *Actinidia chinensis* to obtain a scaffold of the physical map and incorporate the mapped ESTs and other markers. We characterised the *A. chinensis* BAC library regarding its coverage, cloned insert size, and contamination by organellar DNA. Using the *A. thaliana* genome as reference and our kiwifruit EST database as query we designed 1006 overlapping oligonucleotide (overgo) probes from *A. chinensis* to screen the library. 11,808 BAC clones were identified by non-radioactive methods. The secondary screening is in progress and aims to locate the BAC clones needed for constructing the contigs around the overgo probes.

To construct a BAC-contig around the sex locus in *A. chinensis* we screened the library with a non-dominant PCR probe derived from the SMX SCAR marker. Two BAC clones containing the SMX region were isolated as well as ten neighbouring clones flanking their 5'- and 3'-ends. Another 14 BACs containing either one of the two genetic markers flanking the sexflower marker, ke225 or udkac096, were isolated. One clone hybridised with an overgo from a primary pool. The contig assembled around this overgo will aid in the construction of the contig around the sex locus. We aim to associate the genetic distance from the linkage map around the sex locus with physical distances in the BAC contig, and develop new markers able to predict sex across species boundaries.

E3 Topic: Molecular markers
4.30 – 5.30pm Monday 20 March

Genetic diversity of apple rootstocks combining EST-based and anonymous nuclear microsatellite markers

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Using published bio-informatics methods we generated a set of two-hundred apple EST clusters containing perfect microsatellite repeats, from which sixteen were selected for preliminary testing. Fifteen of sixteen microsatellite markers amplified, and 11 displayed multiple alleles in the initial screening of test accessions. We performed annealing temperature gradient PCR on all SSR primer pairs using Malling 9 and Robusta 5 DNAs, and successfully defined optimal annealing temperatures. We tested these EST derived microsatellite markers as well as 21 anonymous nuclear microsatellite markers on an array of 96 commercial apple rootstocks and test accessions. We report the results of the EST derived microsatellite markers comparison to the anonymous nuclear microsatellites.

E4 Topic: Molecular markers
4.30 – 5.30pm Monday 20 March

Microsatellite markers for genomic linkage map in *Actinidia* species (Kiwifrut)

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The genus *Actinidia* consists of over 70 species which form a polyploid series from diploid to octaploid, with some species known to contain ploidy races. The species have diverse fruit and vine characteristics which are useful in the development of new cultivars. *A. deliciosa* and *A. chinensis*, the green and gold kiwifruits, are successful types in fresh fruit markets. Molecular markers have shown a high level of genetic similarity between these two species. As molecular technologies are being utilised for efficiency and accuracy in breeding programmes, transferable markers are desirable. A genetic map is being constructed in a diploid family of *A. chinensis* with EST-derived microsatellite markers. We evaluated the cross-species amplification of 20 of these markers which were fully informative in the mapping population across 21 species available in New Zealand. All 20 markers showed some level of cross-species amplification, with 25% of markers amplifying in all of the species used. Polymorphism information content (PIC) values were high, with 14 of 17 markers recording values of 0.90 and above. Sequence homology was observed especially in the translated region of the EST from which the marker was derived. These results confirm that EST-derived microsatellite markers from *Actinidia* species show cross-species amplification, which can be potentially useful in breeding programmes.

E5 Topic: Molecular markers
4.30 – 5.30pm Monday 20 March

Prunus Genetic Resources and Research at the Davis California National Clonal Germplasm Repository

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The USDA Germplasm Repository at Davis houses most Mediterranean-adapted fruits and nuts, including *Prunus*. The NCGR is part of the USDA National Plant Germplasm System (NPGS). Our missions are to acquire, preserve, characterize and distribute germplasm of designated crops (http://www.ars.usda.gov/pwa/davis/npgs). NPGS policy is to distribute plant material, free of charge, to research interests around the world. The NCGR *Prunus* collection includes >1300 accessions: 327 peaches, 228 cherries (192 sweet and 36 tart), 227 apricots, 117 almonds, and 418 plums. Accessions include: 514 named cultivars, 342 wild-collected relatives, and 190 land-races. An AFLP-based analysis of 113 NCGR accessions revealed genetic variability and differentiation within and among seven cultivated and seven wild *Prunus* species. The four well-supported clusters corresponded to the described *Prunus* sections of *Amygdalus, Armeniaca, Cerasus* and *Prunophora*. The molecular variation distribution pattern indicated that 32% of total variance was accounted for by the within-species variance component. The remaining 68% of variation found among species was hierarchically structured within and among sections (17 and 51%, respectively) or within and among subgenera (30 and 39%, respectively). Although cluster and principal components analyses indicate that the gene pools corresponding to the four sections were distinct, partitioning of molecular variation suggested considerable differentiation among the taxa within sections. Recently, 27 microsatellite markers were screened for reliability and polymorphism among diverse *Prunus* species. Of these, 16 markers were chosen to conduct fingerprinting on the entire NCGR *Prunus* collection. To date, 150 apricot and 350 plum NCGR accessions have been fingerprinted.

E6 Topic: Genetic Mapping
4.30 – 5.30pm Monday 20 March

Development of Marker Assisted Selection Technology for Apple and Pear Breeding in South Africa

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The development of new cultivars is important for the future competitiveness of the South African deciduous fruit industry. Previously, breeding has used traditional approaches with selection based on fruit quality only. However, in the last decade the approach has changed to incorporate the quantitative analysis of traits of importance to the producer, including the selection for disease resistance at early stages of the breeding program. The recent development of a framework genetic map for apples, together with the release of 200 000 EST sequences, provides the basis for the development of new genetic markers that can be used to create higher resolution genetic maps for QTL mapping and marker assisted breeding. At present the total number of markers in development and multiplex analysis is approximately 600, which will provide the basis for a portable framework map for a wide range of genetic mapping projects in apple and pear. The genetic mapping projects in progress cover traits such as disease resistance (apple scab, powdery mildew, woolly apple aphid), dormancy and bud-break, and fruit quality, using populations of seedlings or mature trees as appropriate. These will be used to identify markers linked to QTL, which can then be used in marker assisted selection in the breeding program. We are developing a high throughput marker assisted selection system, in order to perform the selection for multiple markers linked to the desired traits in a single analysis. This involves the use of automated and semi-automated steps to ensure the integrity of sample identity, and the multiplexing of reactions, in order to achieve high throughput, low cost analysis.

F1 Topic: Genetic Mapping
4.30 – 5.30pm Monday 20 March

SSRs genetic linkage map of sweet cherry (*Prunus avium*)

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Sweet cherry (*Prunus avium*) is one of the most popular temperate fruit crops. However, compared to other temperate fruits, such as apple and peach, the progress concerning breeding improvement for sweet cherry is rather slow. The long generation time and the large plant size of cherry trees severely limit the classical breeding method. Consequently, the integration of molecular markers in breeding programs should be a powerful tool. Inheritance and linkage studies were conducted with microsatellites in a F_1 progeny including 133 individuals of a cross between sweet cherry (*Prunus avium* L.) cultivars 'Regina' and 'Lapins'. These cultivars were chosen for their distinct agronomic characters: blooming and maturity dates, peduncle length, fruit color, weight, firmness, titratable acidity, refractive index and fruit cracking resistance which is the main limiting factor in sweet cherry production in Europe. 'Regina' is resistant and 'Lapins' is susceptible to fruit cracking. The evaluation for this character, directly on the tree by watering the fruits in greenhouse, or after harvesting with experimental process in laboratory, is in progress. For mapping, 427 *Prunus* microsatellites were tested for polymorphism: 340 give amplification, 86 were mapped in 'Regina', 67 in 'Lapins', 45 among them were mapped in both parents. The self-incompatibility controlled by the *S* gene was mapped in linkage group 6 ('Lapins' (S1S4') is self-compatible and 'Regina' (S1S3) is self-incompatible), in the same region as in apricot, in agreement with the high level of synteny observed within the *Prunus* genus. These sweet cherry maps will be used for QTLs detection.

F2 Topic: Genetic Mapping
4.30 – 5.30pm Monday 20 March

The construction of an almond linkage map using morphological and microsatellite markers on 'Nonpariel' x 'Lauranne' population.

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A set of 110 microsatellite primers has recently developed from almond, peach and apricot enriched genomic libraries was used to saturate a previously developed linkage map derived from "Lauranne X Nonpareil " F1 progeny. Forty-six microsatellites showed polymorphic patterns, seven have been mapped and detected nine, ten and eight linkage groups for *Nonpareil*, Lauranne and the integrated map, respectively. Other polymorphic markers will be screened on entire population to achieve eight linkage groups. A number of morphological traits such as shell, testa and kernel characteristics and percentage of doubles were measured and analyzed. Most of these traits will be mapped as quantitative trait loci (QTL). Bacterial spot assay have been carried out and preliminary results show remarkable differences within the segregating population.

F3 Topic: Genetic Mapping
4.30 – 5.30pm Monday 20 March

EST mapping of apple

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The EST information of apple (*Malus* X *domestica*) was applied to develop a linkage map with Cleaved Amplified Polymorphic Sequences (CAPS) markers. We designed STS primers based on the sequence of genes, ESTs of cDNA libraries and *Malus* UniGene database (NCBI; Build #13). The fragment patterns of STS digested with 40 restriction enzymes produced CAPS markers among 3 *Malus* cultivars, Ralls Janet, Delicious and Mitsuba Kaido (*Malus sieboldii*); the CAPS markers were applied to construct linkage maps. In addition, we applied to RAPD, AFLP, and SSR markers. SSR markers referred to apple consensus map (Liebhard et al. 2003). These markers were utilized for linkage analysis on 83 progenies of 'Ralls Janet' X Mitsuba Kaido (RxM) and 72 progenies of 'Delicious' X Mitsuba Kaido (DXM) (JoinMap ver. 3.0, LOD>5.0). The map of RXM consisted of 384 markers including 37 CAPSs, 68 SSRs, 96 RAPDs and 178 AFLPs. These markers were mapped into 17 linkage groups covering 1,126cM. The map of DXM consisted of 349 markers including 42 CAPSs, 68 SSRs, 103 RAPDs and 126 AFLPs. These markers were mapped into 17 linkage groups covering 1,077cM. These apple linkage maps could be successfully correspondingly to the apple consensus map.

F4 Topic: Genetic Mapping
4.30 – 5.30pm Monday 20 March

Alignment of sweet cherry linkage groups with the *Prunus* reference map

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A sweet cherry (*Prunus avium* L.) linkage map was constructed from 190 progeny from F_1 reciprocal crosses between a sweet cherry cultivar and a wild forest sweet cherry as a means of identifying QTL for fruit quality traits altered during domestication. The two parents are the cvs. Emperor Francis (EF), a large-fruited ~ 6 g, sub-acid, yellow/pink cultivated cherry, and New York 54 (NY54), a small-fruited ~ 2 g, acidic, dark-red colored wild forest cherry. The cross is fully compatible (EF=S_3S_4, NY54=S_2S_6) permitting linkage analysis of the *S*-locus region. Over 600 progeny from these reciprocal crosses are planted at the Michigan State University research farm and available for future fine mapping of QTL regions. A set of 190 progeny was used to construct an initial framework map. Simple sequence repeat (SSR) markers previously placed on other *Prunus* linkage maps were used extensively to permit comparative mapping. Amplified fragment length polymorphism (AFLP) markers were added to increase marker density. All markers were scored as single-dose restriction fragments (SDRFs) and map construction was done using JoinMap 3.0 software. Currently, there are a total of 123 total markers placed on 7 of the 8 linkage groups for EF and 5 of the 8 LG for NY54.

G1 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

SNP discovery in apple genes: application for red color

David Chagne *et al*

We are developing and mapping molecular markers linked to red flesh in apple, with the objective of providing HortResearch pipfruit breeders with fast and accurate tools to select high quality red flesh apple seedlings from large breeding populations. An approach based on the mapping of candidate genes was employed to identify the gene(s) that control(s) the phenotypic variation in red apple flesh. We chose a set of 15 candidate genes from the HortResearch apple EST database according to their functional annotation and their expression pattern in apple assessed by microarray and quantitative PCR, as well as following transformation into model plant systems such as *Arabidopsis* or tobacco. Two mapping populations were chosen based on their clear segregation for red flesh and foliage. We performed an initial screening of these candidate genes using RFLP analysis of extreme phenotypes from the two pedigrees. The candidate genes that exhibited a putative co-segregation between red colour and marker data were subsequently converted into PCR-based markers. We PCR-amplified and sequenced these candidate genes using DNA from the parents of both pedigrees. PCR-based markers linked to red flesh colour phenotypes were developed from identified between the parental DNA sequences and screened over individuals in one of the populations. The success of this candidate gene-based approach using SNPs shows its potential for developing molecular markers linked to other fruit and tree characteristics.

G2 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Genetic Analysis Of Temperate Fruit Quality And Health In The New European Project ISAFRUIT.

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ISAFRUIT is an "Integrated Project" within the 6th Framework Project of the European Union with the strategic objective of increasing fruit consumption. This project has been launched at the beginning of 2006 and includes research groups from 39 institutes from the EU, one from both New Zealand and the USA, and 22 private companies The project includes the evaluation of consumer's needs and satisfaction, human health and fruit consumption, new processed fruit products, pre- and post-harvest chain management, development of tools for the construction of marker free GMOs and genetics of fruit quality. The latter subject will be addressed by the research teams of this poster taking apple and *Prunus* (peach and apricot) as the crops of study. Map positions of major genes, candidate genes and QTLs for a range of fruit quality and health characters will be established. Allelic variability for quality-related genes will be assessed using phenotyped collections of germplasm. Special emphasis will be placed on fruit texture that will be studied using innovative approaches. The genetic basis of allergenicity will be studied, evaluating both map positions and allelic diversity of various allergen genes with regard to their effect on patients. Low allergenic cultivars will also be identified. Finally, the information of this and previous European projects will be utilized for marker- assisted breeding for disease resistance and quality in segregating populations.

G3 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

The Development of a Linkage Genetic Map for Chilling Requirement in Peach

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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 in nature. We are developing a genetic linkage map for chilling requirement in peach. Two populations have been developed for mapping chilling requirement. F_2 progenies from individually selfed F_1 trees have been developed from two pairs of high and low chilling requirement parents. The first cross is Contender × Fla92-2c and consists of 378 F_2 trees. The second cross is Hakuho ×UF Gold and consists of 57 F_2 trees. The initial data for flower bud flush date of F_2 individuals was obtained in spring of 2005 and will be repeated in 2006. 243 pairs of primers were used to screen for the polymorphic microsatellite (Simple Sequence Repeat, SSR) markers in two populations. 68 SSR markers are polymorphic in the Contender × Fla92-2c population, and 106 are polymorphic in the Hakuho ×UF Gold population. In both populations, polymorphic SSR markers are densely distributed in linkage group 1.

G4 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

MAS: development of AFLP markers in Prunus persica *diagnostic for response to the Peach Tree Short Life syndrome*

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Peach tree short life (PTSL) is a complex disease syndrome caused by multiple factors, the genetics of which is unknown. Amplified fragment length polymorphism (AFLP) technology and bulked segregant analysis (BSA) were used to identify diagnostic markers in peach rootstocks for induced scion susceptibility to the PTSL syndrome. Forty-four AFLPs were selected as potential PTSL-response associated markers based on the combined results of BSA screening and screening 11 PTSL tolerant, 2 intermediately susceptible and 2 highly susceptible genotypes with the polymorphic *Eco*RI/*Mse*I primer combinations. The results suggested a strong association of the AFLP marker, called EAC/MCCC1-96, with PTSL susceptibility. In addition, 43 other candidate markers that were diagnostic for the PTSL response were selected for further study.

G5 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Marker assisted selection (MAS) for fruit shape in peach (*Prunus persica*): flat or round.

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The flat fruit character, originated from China, was reported to be controlled by a dominant gene, named *S* for 'Saucer-shaped'. Most flat peach varieties have excellent flavour with sweet taste, low titratable acidity and high sugar content. They were also reported to have flowers with pistil lower than stamen and, for most of them, fertile pollen. The segregation of the *S* character was analyzed in an intraspecific F_2 population including 207 individuals issued from a cross between Ferjalou Jalousia® a flat low-acid clingstone peach, and Fantasia a round, normally-acid freestone peach. The *S* gene was located in linkage group G6 and co-segregated with another character, fruit aborting before maturity, named *af* for 'aborting fruit', that segregated as a recessive Mendelian character. Fruits from genotypes homozygous for *af* stopped growing one month after bloom and started to fall two months after bloom. The codominant microsatellite marker, MA014a, was found to co-segregate with the *S* and *af* characters. Our results showed that individuals homozygous for the Flat allele (*SS*) also produced fruits aborting before maturity. We developed an efficient SAM by using the Ma014a marker, analyzed on agarose gel, to screen 1339 individuals used for positional cloning project of the *D* gene controlling the acidity of the fruit in order to eliminate the plants which show the genotype *SS*.

G6 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Identification of DNA markers closely linked to Pale green lethal in apple

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We investigated the genotypes of apple SSR markers of 257 apple cultivars and selected varieties. We found that the frequency of homozygote of 104-allele (104 shows the length of amplified DNA) in CH05c06 marker mapped on linkage group (LG) 16 was lower compared with the expected frequency. The genotype of CH05c06 was analyzed in F_1 segregates in which 104-homozygotes had been expected to be present. But we detected no 104-homozygote in F_1 population. Then we assumed that pale green lethal might be related to this genotype bias. Lethal individuals have pale colored cotyledon and die at cotyledon stage. To confirm our assumption, we analyzed other F_1 segregates in which pale green lethal were observed. The genotype of CH05c06 in living F_1 seedlings and dead F_1 seedlings were investigated. The 104-homozygotes were observed only in dead seedlings. From segregation analysis of some markers in LG16, it was shown that pale green lethal closely links to CH05c06 and *Ma* (fruits acidity) gene.

H1 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Genetic map and markers for Prunus associated with response to the Peach Tree Short Life syndrome

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Peach Tree Short Life (PTSL) is a devastating disease syndrome of peach (*Prunus persica*) caused by multiple factors, of which the genetics and molecular biology are unknown. *Prunus* rootstocks that confer PTSL tolerance are in high demand by peach growers. The difficulty of studying PTSL is that the actual phenotype (survival or death) is not obvious until 3-5 years after planting when the symptoms of PTSL first appear. Tolerance to PTSL was unknown in peach until the tolerant rootstock Guardian® was introduced into the commercial trade in 1994. To study the genetics of the response to PTSL, a controlled cross was made between Guardian® 'BY520-9' selection 3-17-7 (PTSL-tolerant) and Nemaguard (PTSL-susceptible). One hundred fifty-one AFLPs and 21 SSRs, including anchor loci from the *Prunus* reference genetic map, were used to construct a molecular genetic map based on a family of 100 F_2 seedlings. This map covers a genetic distance of 737 cM with an average marker spacing of 4.7 cM and is anchored to the *Prunus* reference map. Molecular markers associated with the PTSL response were identified by both bulked segregant analysis and screening the tolerant and susceptible parents from the aforementioned cross and additional susceptible plus tolerant genotypes. The distribution of the 38 AFLP markers associated with the PTSL-response on the constructed genetic map was compared with the *Prunus* resistance map. The location of twenty-three of these AFLP markers was determined in the corresponding regions of the resistance map using commonly mapped SSRs as anchor points.

H2 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Breeding functional apples; identification of QTL's for mean vitamin C contents of fruit skin and flesh.

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Traditionally, apple breeding has focussed on readily–identifiable targets such as colour, taste, storage ability etc. Technological advances however mean that it is now possible to consider more ambitious targets including for example nutritional enhancement. Vitamin C (L-ascorbate), is the most abundant and powerful low-molecular weight antioxidant present in plant tissues, and in addition to having wide-ranging and essential functions in plant metabolism, it is an essential dietary component that has also been implicated in the prevention of oxidative-stress related diseases and disorders in humans (Davey et al 2000). Here we present results from the analysis of the mean vitamin C contents of the skin and flesh from apples obtained from 140 individuals of a F1-mapping population derived from a cross between the varieties "Telamon" and "Braeburn", and for which we already have genetic linkage maps (Kenis and Keulemans 2005). Using the MapQTL, v4.0 software package, we identified 2 major QTL's (variance > 20%, LOD > 3.0), and one minor QTL, explaining in total between 61 – 71% of the observed variance in apple flesh mean vitamin C and total vitamin C levels, in both parents. Only one QTL was detected for mean skin vitamin C levels. Results are discussed in the context of breeding for enhanced nutritional contents and apples with improved storage qualities.

H3 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Marker-assisted selection tools for internal breakdown resistance in peach

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Internal breakdown (IB) is a significant problem to the stone fruit industry. The major symptoms in peach include mealiness, browning and bleeding, which commonly occur after cold storage and ripening of fruit. Genetic analysis of IB resistance, involving two segregating populations obtained from 'Dr. Davis' and 'Georgia Belle' peach cultivars, indicated that a few major genes control each symptom. A genetic linkage map was constructed for the first population and used to localize quantitative trait loci (QTLs) controlling IB. One major QTL and additive or compensating minor QTLs were detected for each symptom. Mealiness and bleeding were strongly influenced by a major QTL at the *Freestone-Melting flesh* (*F-M*) locus. Fruit of clingstone non-melting flesh (CNMF) progeny never developed mealiness. In contrast, freestone melting flesh (FMF) progeny had very low bleeding incidence. The *F-M* locus co-segregated with a gene encoding endopolygalacturonase (*endoPG*). A PCR test developed for *endoPG* distinguished between FMF and CNMF progeny in both populations. Further QTL analyses were conducted within the FMF (for mealiness) and CNMF (for bleeding) subsets of the population, yielding an additional four mealiness QTLs and two bleeding QTLs. A strategy involving bulked segregant analysis (BSA) was designed to saturate QTL regions other than *endoPG* with more markers. The second population and ~20 peach and nectarine cultivars were screened for QTL validation using linked markers. Markers are being converted to forms readily usable in a breeding program. Interactions among QTLs, including those for other quality traits, and a proposed marker-assisted selection scheme will be discussed.

H4 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Genetic mapping of fruit quality traits in apples (*Malus x domestica* Borkh.)

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Apple fruit quality is of utmost importance to apple farmers and breeders. This is reflected in major international markets, where failure to meet specifications can result in shipment rejections, reduced returns to growers and a damaged reputation as a supplier of top quality fruit. Fruit size, colour, texture, firmness and taste are all traits that affect the quality of fruit. In this study the genetic contribution of these traits is being evaluated in order to generate the genetic markers required for the application of marker assisted selection in fruit quality breeding. Two mapping populations, 'Prima' x 'Anna' and 'Golden Delicious' (GD) x 'Priscilla', consisting of 95 seedlings and 120 seedlings respectively, were used in the study. Fruit samples were analysed using visual and sensory techniques. This data will be correlated using statistical analysis. DNA was extracted from the progeny, and microsatellites were amplified by PCR using published and predicted primers. Scoring of these marker alleles was performed using Genotyper™, and then JoinMap™ was used to construct the genetic map. The location of quantitative trail loci (QTL) will be analysed using MapQTL™. Comparative genome analysis and the role of various genes on the outcome of fruit quality can then be investigated, using the integrated genetic map, and the QTLs identified can be used for marker assisted selection, to increase the speed and efficiency of the apple breeding program.

H5 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Genetic Analysis of Red Pigmentation in Bon Rouge Pears.

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Pome fruit cultivation and production is of major economic importance in the Western Cape region of South Africa. 'Bon Rouge' is a red pear cultivar that was derived from a rare, spontaneous bud mutation of the green pear cultivar William's Bon Chretien (Bartlett). In this study we aim to map the genetic location of this event, with a long-term view to cloning and characterising the gene involved. A closed cross between 'Bon Rouge' and 'Packham's Triumph' pears generated an F1 population of 800 seedlings with a 1:1 segregation of the red:green phenotype, indicating a simple Mendelian inheritance of this trait. We are currently mapping apple and pear microsatellites on 192 seedlings from the F1 progeny. Both apple and pear microsatellite markers were used in this study because of the high level of between-species cross-reaction. Microsatellites that show a high level of polymorphism are being used to construct a linkage map with the aim of identifying those markers that are tightly linked to the gene controlling the red/green trait. This will provide the basis for future genetic mapping in other pear mapping populations. To saturate our map in the region of the “red” locus, Amplified Fragment Length Polymorphisms (AFLPs) will be used to obtain a high density map in the region of this locus, which will provide the information required for a chromosome-walking strategy. The project will increase our understanding of the molecular mechanisms of colour development in pears, which will be of importance in the future for both breeding and production of pears with the desired colouration.

H6 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

An Investigation of the Molecular Mechanism Underlying the Production of Anthocyanin in 'Bon Rouge' (*Pyrus communis*, L) pear trees and their green reverted sports

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The production and stability of fruit skin colour in red and blush pears are desirable traits in many pear cultivars. To investigate the underlying molecular mechanism controlling the production, stability and loss of anthocyanin, we aim to characterise the differences found between red leaved 'Bon Rouge' (*Pyrus communis*, L.) pear trees and their green sports, using a number of molecular tools. The production of red and green skinned fruit from the same genetic background presents a system in which to study the control of red and green colour development under the same set of environmental conditions or variables. Differences in gene expression between the two phenotypic variants were measured by differential display and confirmed by quantitative RT-PCR. Seven of the differentially induced cDNA clones showed significant similarity to genes in database and include those associated with light stress, pathogenesis responses, and protein synthesis. We compared the proteome of the two phenotypic states (red and green leaves) during colour development using 2-dimensional gel electrophoresis. Our preliminary proteomics analysis shows substantial changes in the patterns of protein expression that was further characterised by mass spectrometry. HPLC analysis was performed using extracts from red and green plant tissues and individual peaks characterised by 1- and 2-dimensional ^{1}H and ^{14}C NMR. One compound was fully characterised as a natural anti-oxidant previously identified in sour cherries. Our preliminary analysis suggests that many stress response genes are involved in anthocyanin production in 'Bon Rouge' and we aim to assess whether the underlying mechanisms of colour development in this cultivar can be understood and in the longer term, manipulated.

I1 Topic: Trait Mapping
4.30 – 5.30pm Tuesday 21 March

Functional mapping and genomics approaches for the study of aroma determination in peach (*Prunus persica*)

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We report the development and mapping of genetic markers based upon expressed sequence tags (ESTs) polymorphisms and the positioning of ESTs in a physical framework map for peach genome. Based on ESTree DB (a collection of 20924 cDNA sequences), contigs and EST were selected as candidate genes (CGs) based on sequence similarity with genes relevant for fruit quality.

The ESTs generated from six different peach genotypes were aligned by AutoSNP and 1863 *in silico* SNPs (isSNP) were detected. A subset of 67 isSNPs was amplified and polymorphic fragments from parentals of a set of mapping populations were sequenced. Confirmed SNPs were genotyped in selected individuals of the segregating populations.

Approximately 200 ESTs were selected to be mapped on a physical framework map and 17 out of 46 ESTs which hybridized to the filters containing the BACs clones were localized on physically mapped contigs.

Esters, alcohols, aldehydes, terpens, lactones, C6 compounds are the main volatile components of the peach aroma. In ripe peach fruit shikimic acid derivates (eugenol, isoeugenol, chavicol, methyl benzoate) are present in a high concentration. An enzyme involved in eugenol biosynthesis belongs to the family of O-methyl transferase (OMT). 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. Here we report the cloning of genes coding for OMT, ATT and their expression patterns. On the other hand a different approach using microarray has been conducted across two different peach varieties.

I2 Topic: Comparative genomics
4.30 – 5.30pm Tuesday 21 March

Comparing the Linkage Maps of Two Levels of Ploidy in *Fragaria*

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Comparative genome mapping is a powerful tool for analysing genome evolution such as determining the nature of chromosomal rearrangements between cross-incompatible species. We conducted a study to investigate the relatedness between *Fragaria* diploid and octoploid genomes by comparing the collinearity between linkage groups (LGs). We used an approach of comparative mapping using microsatellites.

The diploid map was developed from an inter-specific cross between two diploid species (Sargent et al., 2003) using microsatellites. We developed a linkage map from a cross between two genotypes of the octoploid cultivated strawberry (*F.* x *ananassa*, 8x=56), Capitola and CF1116. We mapped 63 microsatellites in addition to the AFLPs previously mapped (Lerceteau-Köhler et al., 2003). The map was constructed using Mapmaker and analyses of couplage and repulsion phase markers were successively performed. Then, we obtained an integrated map using Joinmap with fixed order.

LGs of octoploid *Fragaria* were assigned as homoeologous on the basis of common microsatellites. In addition, homoeologous LGs of the octoploid *Fragaria* were considered homologous to one diploid LG when they shared microsatellite markers.

Results showed that the majority of the octoploid *Fragaria* homoeologous LGs series are strongly associated with a single diploid *Fragaria* LG. Seven assemblages of homoeologous linkage groups were assigned homologous to the seven LGs of the diploid map. Conservation of collinearity, within homoeologous LGs of the octoploïd species and between homologous LGs of the diploid and octoploïde species, indicated that polyploidy was not accompanied by extensive chromosomal rearrangement.

I3 Topic: Comparative genomics
4.30 – 5.30pm Tuesday 21 March

Comparative mapping in the *Rosoideae* tribe: *Rosa* and *Fragaria*

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A comparative mapping study has been done among two closely related genera in the *Rosoideae* tribe. Our objective was to use a common set of markers to show macrosynteny and macrocolinearity across the two genera *Fragaria* (strawberry: *2n = 2x =* 14) and *Rosa* (rose: *2n = 2x* = 14). We used anonymous SSRs derived from a genomic library (55 primer pairs from *F. x ananassa*) and from Expressed Sequence Tag (70 new primer pairs derived from *Rosa* (30)*, F.vesca* (18) and *F.x ananassa* (22) ESTs). In addition, we have tested 24 primer pairs derived from gene sequences involved in *Rosa* floral initiation. The anonymous SSRs and the SSR-EST were tested on denaturating polyacrylamide gel with silver staining. On the total of 125 primer pairs, only twelve (10%) were polymorphic in both populations. In order to reveal more polymorphism, we tested all primers pairs derived from ESTs with the Single-Strainded Conformation Polymorphism approach. Using this technique, more polymorphic SSRs were detected. Furthermore, primer pairs derived from floral initiation gene sequences in *Rosa* were tested on SSCP and 25 % were polymorphic in both populations. At this time, 24 markers will provide anchor points to link the 7 homologous linkage groups. In order to have a true comparison of the homologous linkage groups, we shall verify the orthology of alleles by sequencing them. This comparative mapping study will provide useful information regarding the evolution of the two genomes.

I4 Topic: Comparative genomics
4.30 – 5.30pm Tuesday 21 March

Molecular Characterization of the US Apple Germplasm Collection.

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The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) coordinates a system of about 30 germplasm repositories that each focus on specific crop collections. The collection of apple (*Malus* ×*domestica*) germplasm is maintained at the repository in Geneva, NY, on the satellite campus of Cornell University. This collection presently consists of 4179 accessions of *Malus* spanning at least 50 species. Our core mission for this collection includes the acquisition, maintenance, characterization and distribution of this diversity of *Malus*. Until now, characterization of accessions in the collection has been performed using as many as 154 categories of descriptors which include pomological, pathological, anatomical, phenological, and similar categories. For these types of descriptors there are presently over 95,000 independent observations recorded in the Germplasm Resources Information Network (GRIN: www.ars-grin.gov) database, making this a very well characterized collection. Nonetheless, we are presently beginning to extend our characterization efforts for this collection into the level of molecular genetics and comparative genomics. For this work, we will, at least initially, focus on using a core set of microsatellite markers. The specific selection of markers to be used is presently under consideration, and input is solicited from this viewer audience on this matter. Key criteria for desirable markers are that they amplify stably and widely across diverse *Malus* germplasm and that their localization in the *Malus* genome is not clustered. Beyond that, it would be desirable for the markers to be associated with traits of interest, and that is where viewer input for marker recommendations is requested. The initial scope of this effort is to describe all accessions with eight markers, with the likely extension toward twenty markers per accession. Deposition of the results will be in the GRIN database.

I5 Topic: Rosaceae Biology
4.30 – 5.30pm Tuesday 21 March

Using controlled environments to accelerate flowering of *Malus* seedlings

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Controlled environment (CE) conditions have been used to accelerate growth of *Malus* seedling families, to develop an "optimal" greenhouse-oriented regime for reliable, high frequency flowering within one year of sowing.

In 2004-5, two HortResearch seedling families were grown in CE rooms delivering continuously warm, humid conditions (26°C day-night, 85% RH), with high-irradiance, long days (>1000 µmol $m^{-2}s^{-1}$ 18 hr photoperiod), high R:FR (i.e., 4.0, vs 1.2 for natural sun), and high CO_2 (up to 1800 ppm). Plants were grown with limited root volumes (PB1.5 to PB3) but non-limiting irrigation and nutrition, with 80 to 200 ppm prohexadione-Ca applied regularly. End-of-day far-red (EoDFR) was applied to induce late-season bud-set and floral differentiation. In 2005-6, similar but warmer early-season conditions (29°C) have been applied to six families, with EoDFR trialed for control of lateral branching, prior to transfer to the greenhouse for later season growth.

In spring 2005, over half of plants in a vigorous family with a crab parent flowered, but not in a less vigorous family. Prohexadione-Ca kept internode lengths ~2cm, and maintained high leaf health. High levels of branching were observed on both families, requiring continuous lateral shoot removal during early-season growth, but degree of branching was not a good indicator of floral potential. Late-season EoDFR did not appear to affect frequency of flowering. However, in 2005-6, early-season EoDFR applied in an attempt to stimulate a phytochrome-based shade-effect suggested terminal buds are responsive to FR, except more so than lateral buds.

I6 Topic: Rosaceae Biology
4.30 – 5.30pm Tuesday 21 March

Analysis of QTLs affecting dormancy release in apple (*Malus* x *domestica* Borkh.)

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Winter temperatures in the Western Cape region of South Africa are not cold enough for normal dormancy release in apple trees during spring. Initial vegetative budbreak has been shown to be associated with prolonged dormancy symptoms experienced by local farmers. Marker assisted selection (MAS) of apple cultivars with a lower chilling requirement will be beneficial for local production and will ensure that South Africa remains one of the biggest competitors on the global export market. In this study 5 mapping populations have been analysed for time of initial vegetative budbreak, and these provide the basis for the analysis of the genetic control of this phenotype. The first step towards MAS is the generation of a genetic linkage map. We describe the development of 310 new SSR markers, using apple and pear sequences, containing di-, tri- and tetranucleotide repeats. The newly developed markers, together with 193 published markers, are being used to screen the progeny of the five controlled crosses made in order to study and identify quantitative trait loci (QTL) affecting dormancy release after winter. The prediction, optimisation, multiplexing and mapping of the complete set of markers is an ongoing process, and the current status of the maps will be described. The identification of genetic markers linked to QTLs controlling the time of bud break will allow the selection of the desirable genotypes in future marker assisted selection in the apple breeding program.

J1 Topic: Rosaceae Biology
4.30 – 5.30pm Tuesday 21 March

The *S* locus in self-compatible 'Cristobalina' sweet cherry

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Gametophytic self-incompatibility in sweet cherry is determined by genetic factors encoded at the *S* locus, that control the *S* specificity of the pollen and the style (*S-RNase* and *SFB*). We have investigated the molecular basis of self-compatibility using a self-compatible Spanish sweet cherry cultivar, 'Cristobalina'. The research carried out included the search for *S* duplications that may explain self-compatibility, the cloning and sequencing of the *S-RNases* and *SFBs* and their comparison with the sequences identified in self-incompatible genotypes with the same *S* alleles, the analysis of the expression of the *S-RNases* and *SFBs* by reverse transcription, and a quantitative analysis of the transcription levels of *SFB* in Cristobalina and a self-incompatible genotype with the same *S* alleles by using real time PCR. The results obtained showed no differences at the molecular level that could explain self-compatibility in this genotype. These results confirm previous classical genetic observations and suggest that additional factor(s) located outside the *S* locus may be the cause of self-compatiblitity in this genotype by affecting its pollen *S* function. Work is underway to identify such factor(s) using different molecular approaches.

J2 Topic: Functional genomics

4.30 – 5.30pm Tuesday 21 March

Identification of Genes with Modulated Expression During Fruit Development in *Malus x domestica* Borkh

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In 2003 a European project named HiDRAS (High-Quality Disease Resistant Apples for a Sustainable Agriculture) aimed at the identification of the genetic factors controlling apple fruit quality has started. HiDRAS involves 15 European groups and the present study is part of the project. Fruit development and ripening are fundamental processes determining fruit quality, therefore the knowledge of the genes involved allows the deployment of targeted, thus more efficient, selection methods leading to the production of apples better meeting consumer's expectations. In order to detect genes that are specifically expressed during fruit development and maturation, cDNA microarrays were constructed using two subtractive cDNA libraries. The first library was generated performing a selective subtraction of leaf cDNA vs. fruit cDNA, to enrich for fruit specific genes. The second library was produced using fruit cDNA, using Clontech PCR-Select cDNA Subtraction kit to normalize the sample, thus reducing sequence redundancy. About 1600 clones, derived from the two libraries have been printed in duplicate on the glass slides. Ninety-six clones per library were sequenced as a quality check. 80% and 97% of the sequences from fruit vs. leaf and fruit vs. fruit libraries appeared to be unique sequences, respectively. Microarray hybridizations were performed always comparing mRNA from fruits of the cultivar Prima, collected in May, with different mRNA samples extracted from the same plant at later developmental stages (June, July, August and September). The analysis of the expression profiles suggests that about 10% of the genes present on the slides show a modulated expression during fruit development. As expected, the number of genes differentially expressed increases from the June to September. Those genes have been sequenced. Their sequence identity and putative function will be presented and discussed. Finally, we are currently generating molecular markers from the most interesting genes, in order to place those genes on the apple reference map (Fiesta x Discovery) and to correlate their allelic state with QTLs controlling fruit quality traits.

J3 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Endopolygalacturonase Marker-Assisted Selection for Novel Fruit Types in Peach

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Endopolygalacturonase genotype has been shown to be strongly associated with final fruit texture in fresh market and processing peach. Three major functional alleles, F, f, and f1, have now been characterized in peach with a DNA marker assay that allows the prediction of melting/nonmelting and freestone/clingstone phenotype in breeding populations. A number of related and often unique alleles have also been identified in closely related peach and almond species as well as the more distantly related fruit species apricot, plum, and cherry. Interspecific hybridization and subsequent gene introgression have resulted in peach breeding lines showing a range of endopolygalacturonase genotypes and fruit phenotypes. Novel fruit phenotypes, including freestone-nonmelting fruit, and fruit in which the typical softening associated with overripe fruit mesocarp is suppressed, have also been characterized. The endopolygalacturonase DNA marker test has proven an effective predictor of fruit phenotype in a majority of breeding lines and has become an important tool for improving breeding efficiency. In breeding efforts to develop novel peach fruit types, knowledge of endopolygalacturonase genotype has been crucial for the dissection and characterization of other components of the endocarp-mesocarp interface and mesocarp texture. Vascular bundle ontogeny and ramification within the developing endocarp-mesocarp tissue appear to be an important determinant of endopolygalacturonase-associated phenotypes though control on the molecular and anatomical levels remain poorly understood.

J4 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Analysis and functional annotation of an expressed sequence tag (EST) collection of apple (*Malus* × *domestica*)

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A total of 27 cDNA libraries, primary and normalized, from different tissues of several apple (*Malus* × *domestica* Borkh.) cultivars (GoldRush, Jonagold, Granny Smith, Fuji, Braeburn, and Suncrisp) and rootstocks (M.9, M.111, Geneva 3041, 'Geneva 3041 x *Malus siversii'*) were constructed, and ESTs were obtained. Up to now, ESTs from nine cDNA libraries, 5 primary and 4 normalized, obtained from different developmental stages of leaf, bud, shoot, flower, and fruit tissues of cv. GoldRush were analyzed. From these nine libraries, 109,824 clones were partially sequenced from the 5' end, and 84,891 high quality ESTs were obtained. These ESTs coalesced into 13,538 contigs and 15,896 singletons. Detailed analysis of contig composition revealed that 40% of unique sequences consisted of ESTs derived from floral or reproductive tissues, while 21% consisted of ESTs originated from vegetative tissues. Sequence analyses assigned putative functional category for 82.5% of sequences, while 17.5% did not have any significant similarity with other unigenes and proteins present in the database and therefore could be considered as apple specific genes. Gene ontology classification assigned 38.5% sequences to biological process, 42.7% to cellular component, and 33.3% to molecular function. This collection of single-pass sequences targets a highly diverse set of apple genes involved in vegetative and reproductive development and constitutes an important new resource for the genomics of apple and other *Rosaceae* species.

J5 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Improving the knowledge of apple quality by functional genomics approaches. Perspectives at INRA Angers

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Fruit consumption should be an important part of human diet which is not always the case. In order to increase the demand, growers have to offer products of good quality (nutritional and organoleptic). This quality depends on environmental and genetic factors.

To improve the knowledge of genes involved in apple quality, genomic approaches are developed in Angers. We are currently designing several hundreds of oligonucleotides (70 mers) specific of genes involved in metabolisms acting on fruit quality (cell wall, sugar, regulation…). We will use them in microarray experiments in order to study gene expression: a) during fruit development and ripening and b) between two bulks of a segregating population differing for the character under study (mealiness or firmness for instance).

Then a differential library will be constructed between the two bulks to generate new EST sequences. Oligonucleotides designed from those sequences will be added to our microarray.

As plant material, we will use segregants of populations produced at Angers already described and phenotyped in the frame of the European project HiDRAS.

Variations at transcriptional level detected on microarrays will be corroborated by quantitative RT-PCR. The final validation will be performed either by enzymatic assays (if possible) or by transgenic validation in tomato plants.

We then will be able to colocalize candidate genes with the QTLs already described for fruit quality. The generated data will be helpful for future breeding programs.

J6 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Functional genomics in Prunus persica: proteomic analysis during postharvest and different varieties.

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Peach and nectarine varieties exhibit a number of agronomical and physiological differences. In addition, their postharvest life is also quite distinct being some of them more susceptible to physiological disorders. As an approach to identify their differences at the molecular level, we have taken a proteomic approach such that we may identify changes in protein abundance that may account for the varietal differences. In this work we presented a comparative proteomic analysis of 4 varieties (O´Henry, Elegant Lady, Angelus and July Red (nectarine)). Proteins extracted from fruit that was physiologically mature but firm was compared to proteins obtained from ripe fruit. Quantitative analysis showed several differences in the content and amount of proteins between the firm and the ripe fruit. In addition, many differences were observed when the protein profiles of different varieties were compared. As expected, the nectarine July Red exhibited the largest number of differences. Some of these proteins are currently being sequenced and their identity will help us to get a better understanding of the ripening process as well as the differences among varieties in peaches and nectarines.
Supported by FDI G02 P1001

K1 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

The use of zinc transporter genes to improve mineral absorption by apple rootstocks

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This work elucidates the feasibility of increasing the uptake and transport of mineral nutrients to the aboveground plant parts by constitutively over-expressing Zn transporter genes in M26 apple rootstock. Gene constructs containing the coding regions of Zn transporter genes ZNT1 or ZIP4 and a dual 35S promoter were introduced into *in vitro* M26 apple explants using *Agrobacterium*. The ability of three transgenic lines, T4 (ZNT1), T5 (Vector Control), T6 (ZIP4) and untransformed M26, to absorb mineral nutrients were evaluated in a greenhouse, using DTPA chelator-buffered nutrient solutions containing low (2 µM) or optimal (24 µM) Zn concentrations. ZIP4 and ZNT1 transporter genes had very limited and no effect on the plants' Zn nutritional status, respectively. The over-expression of ZIP4, but not the ZNT1, consistently increased the concentration of Ca and Cu in all roots and aboveground tissues. RT-PCR indicated that ZIP4 was expressed to a greater extent than ZNT1 corroborating the increased tissue nutrient concentrations of the T6 (ZIP4) line. Data will also be presented from a separate study where T4, T5, T6, T7 (ZIP4) and M26 plants, were exposed to four Zn levels in the rhizosphere: 4 µM (low), 8 µM, 16 µM and 24 µM (optimal).

K2 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Functional and Applied Genomic Research on Peach and Apple

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In the past several years, it has been recognized that a considerable effort in genomic research in the Rosaceae could provide valuable tools and information in addressing critical needs of the fruit industry. In collaboration with the fruit industry, universities, and federal funding program representatives, workshops have been held to form a consolidated Rosaceae community and identify priorities for research. These efforts are ongoing. Within our facility, several functional and applied programs of research on peach and apple genomics are being pursued. Subtractive hybridization approaches have been utilized to identify genes regulated by low temperature and short photoperiod. Proteomic analyses using DiGE technology of the same tissues used in the subtractive hybridization studies have also been conducted. The isolation of full-length clones of specific genes responding to abiotic stress and detailed characterization of their response have been conducted. Functional studies of some of these genes using over expression and silencing approaches are also in progress. Research on the functional role of zinc transporter genes is also being conducted. Subtractive hybridization approaches, cDNA-AFLP, and silencing technologies are also being used to better understand the genetic regulation of resistance and susceptibility to fire blight. Significant effort has been placed in characterizing genes responsible for peach fruit quality and using transgenic approaches to develop resistance to plum pox virus. These research efforts have involved collaboration with numerous university partners and government research agencies within the U.S. and around the world. Highlights of these efforts will be illustrated.

K3 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Identification of *anthocyanidin synthase* gene promoters in apples

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The accumulation of anthocyanins in apple fruit skin is genetically determined and influenced by environmental factors including temperature and light. We have shown that the expression level of the anthocyanin biosynthetic genes in 'Akane' (a well-colored cultivar) fruit skin was higher than that in 'Tsugaru' (a poorly-colored cultivar) fruit skin at the stage of ripening, and that the expression increased under low temperature and UV-B conditions in both cultivars (in submission). Especially, the expression of *anthocyanidin synthase* (*ANS*) gene was remarkably induced under these conditions. Southern blot analysis indicated the polymorphism in the genomic structure of *ANS* genes in 'Akane' and 'Tsugaru'. We isolated the five clones containing *ANS* gene from the 'Tsugaru' genomic library. The nucleotide sequences of the coding region of the five clones were almost identical, while those of the promoter region of the five clones were different from each other. To compare the nucleotide sequences of *ANS* gene between these two apple cultivars, the 'Akane' genomic library were screened and three independent *ANS* clones were isolated. The sequencing analysis revealed that one clone from 'Akane' was identical to one of the five *ANS* clones from 'Tsugaru', and the other two clones were different from any of these *ANS* clones. The results indicate the possibility that the expression of apple *ANS* gene is individually regulated by a promoter with specific nucleotide sequences.

K4 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Identification of RFLP markers for ethylene production in ripening fruits of Asian pear

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Ethylene production by cultivated Asian pear fruits varied from 0.1nl/g. f.w./hr to 300nl/g. f.w./hr during ripening. This data indicate that there are both climacteric and non-climacteric cultivars of Asian pear. Climacteric-type fruits exhibit a rapid increase in ethylene production and have a low storage potential, while non-climacteric fruits show no detectable ethylene production and fruit quality is maintained for over a month in storage. To elucidate the reason for the large differences in ethylene production among cultivars, we have cloned three 1-aminocyclopropane-1-carboxylate (ACC) synthase genes (*PPACS1, PPACS2* and *PPACS3*) and studied their expression during fruit ripening. *PPACS1* is specifically expressed in cultivars with high ethylene production, while *PPACS2* is specifically expressed in cultivars exhibiting moderate ethylene production. Moreover, we have identified two RFLP markers tightly linked to the locus conferring the ethylene production of ripening fruit, using RFLP analysis with two ACC synthase genes (*PPACS1* and *PPACS2*). RFLPs were designated as marker A for *PPACS1*, linked to high levels of ethylene and marker B for *PPACS2*, linked to moderate levels of ethylene. The absence of these two markers enabled the identification of low ethylene producers. Using these markers, we have identified ethylene genotypes for Asian pear cultivars that are commercially important and used in breeding programs. This information is very useful for the marker assisted selection (MAS) of Asian pear seedlings with good shelf-life.

K5 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Genomic cloning of a gene coding for an *O*-methyltransferase from apple

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Plant *O*-methyltransferases (OMTs) play important roles in secondary metabolism. Some OMTs are involved in ligning biosynthesis; while others, such as flavonoid OMTs, are involved in the biosynthesis of pigments and phytoalexins. There are few reports on the genomic cloning of *O*-methyltransferase (OMT) genes although cDNAs encoding of OMTs have been isolated from various plant species. Here, we report on the genomic DNA isolation of an *Aomt* gene from apple based on an EST sequence from a flower cDNA library. The gene consists of four exons and three introns, distributed over a length of ~3.9 kb. The putative protein encoded by *Aomt* contains 365 amino acid residues. Southern blot analysis suggests that there are several genes for OMT in the genome of apple, and BAC DNA fingerprinting analysis further indicates that some of the copies are clustered together. The coding nucleotide sequence of *Aomt* shows 85% homology to cDNA clones of *RcOMT1* and *RcOMT2* from *Rosa chinensis* involved in the biosynthesis of major scent components of rose flowers, and *PdOMT* from *Prunus dulcis*. The predicated amino acid sequence exhibits 88% similarity with RcOMT1, RcOMT2, and PdOMT. Further studies are needed to clarify the exact function of this *Aomt* gene in apple.

K6 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

The application of precocious flowering apple to functional genomics

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The length of juvenile phase is inversely related to the breeding efficiency of woody perennials such as fruit trees. Also, the genetic and molecular studies in fruit trees fall behind the herbaceous model plants such as *Arabidopsis*, rice and tomato because of their long juvenile phase. In apple, gain-of-function and loss-of-function experiments became possible since the transformation system was first developed in 1989 by James et al. However, the long juvenile period has hindered the early analysis of gene functions in reproductive tissues of the transgenic apples for many years. The transgenic approach to suppress endogenous *TFL1*-like genes will hasten the flowering and fruiting of the transgenic apple and enable us to analyze the function of the genes or the activity of the gene promoters in reproductive organs within a few years after regeneration. To facilitate the function analysis of genes expressed in apple fruits, co-expression system and re-introduction system have been postulated. In the co-expression system, both a flowering-promoting gene cassette and a target gene cassette reside on the same vector. In the re-introduction system, a target gene is introduced into precocious flowering apples such as line 705. Using these systems, we could analyze the gene function in fruits within one or two years after producing transgenic plants. The future use of these techniques should be of advantage in breeding, crop production, and basic research such as molecular studies on woody plants, including fruit trees.

L1 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

A molecular approach to the adventitious regeneration system and an improved genetic transformation system for functional studies in almond (*Prunus dulcis*)

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Regeneration of transformed shoots is still difficult in *Prunoideae* mainly because of low adventitious regeneration efficiencies. Aiming to identify key genes in the almond adventitious regeneration process, we have approached this system using genomic tools. In a first strategy, a candidate gene approach was used targeting almond Knotted-1 (*PdKn1*) and CDKA:1 (*PdCdc2a*), described as putative markers of organogenesis events. The *PdKn1* was found to have a differential expression being mostly linked to the second half of the shoot induction period. In a second strategy we have looked for new genes putatively involved in dedifferentiation/cell division competence (early organogenesis) and in cell determination and subsequent morphogenesis (late organogenesis). In this strategy, we have performed micro-array technology for transcript profiling, using two Suppression Subtractive Hybridisation (SSH) libraries constructed from two defined time frames of organogenesis. After micro-array validation by *Quantitative* RT-PCR, candidate genes putatively involved in critical events of the adventitious organogenesis were assessed in a daily basis during the shoot induction period. Some genes were pointed out as putatively involved in distinct events of the almond regeneration process, namely: an auxin down-regulated (ADR) gene, a 1,3 β-glucanase gene and a Gibberelic Acid Stimulated protein (GAST) gene. Using an improved transformation/regeneration system that we now have available for almond, with the transformation success increased from 0.1% to 12%, gene function studies in this fruit species are already possible. Besides studies in almond, we also aim to test the isolated genes in heterologous systems to assess their effect in adventitious regeneration.

L3 Topic: Functional genomics
4.30 – 5.30pm Tuesday 21 March

Fragaria Genomics at the University of Florida

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We are using a unique set of tools to study functional, structural and translational genomics in *Fragaria*. Primarily, we have developed the LF9 genetic line for studies of gene function in strawberry and the *Rosaceae*. This fast cycling, transformable octoploid genotype makes it possible to obtain many independent transgenic lines in under 8 weeks from a small amount of tissue. Using this system we have installed genes relevant to traits of agricultural interest (such as photoperiod pathway genes) to test paradigms established in the model systems. We have also developed several stage/tissue specific EST libraries from cultivated strawberry and are actively testing gene function by overexpression in LF9 and via complementation of *Arabidopsis* mutants. Transgenic tools relevant to agriculture are being tested, such as several lines expressing a stress-responsive transcription factor CBF driven from a stress-inducible promoter. The LF9 line is also host for a growing activation tagged population. LF9 is being employed to establish plastid transformation in *Fragaria* and plastid transformation vectors have been constructed. In collaboration with Dr. Tom Davis, structural genomics studies develop linkage relationships using complex novel markers in cultivated strawberry. We have developed a high-throughput method called “ASAP” that is being used to pursue comparative chloroplast structural genomics in *Fragaria* and *Rosaceae* in general. The method provides sequence snapshot of finite regions of a chloroplast genome, and inform deep level phylogenetic relationships across a large number of species. All of these activities contribute to understanding gene form and function in this valuable crop species.