Oral Presentations

Keynote address

K1

Genome interactions in hybrid *Solanum* species and RNA silencing

David Baulcombe

School of Biological Sciences, University of Cambridge, UK

dcb40@cam.ac.uk

Eukaryotes contain small (s)RNAs that have been referred to as the dark matter of genetics. They are typically 21 or 24 nucleotides long, associated with Argonaut or Piwi proteins. Some of these sRNAs guide the Argonaute (AGO)/Piwi protein to a complementary RNA and they are negative regulators of gene expression acting at the level of messenger RNA turnover or translation. Others participate in more complex regulatory systems affecting epigenetic modification of chromatin or acting as part of an RNA signal that moves between cells.

An atypical class of 22nt sRNAs initiate secondary sRNA production. They guide an AGO protein to a target RNA at which they may cause RNA cleavage or translational suppression like the 21 and 24nt species. However, unlike the typical size classes, these 22nt sRNAs cause an RNA dependent RNA polymerase to convert the targeted RNA into a double stranded form that is cleaved by a DICER nuclease into the secondary sRNAs that may be 21, 22 or 24nt in length and have the corresponding targeting activity.

In hybrid plants we predicted that 22nt sRNAs would target RNAs from their heterologous parent and that novel secondary sRNA would be produced. To test this idea we analysed *Solanum lycopersicon* and *S. pennelli*, their F1 and F2 hybrids and a series of introgression lines (ILs) in which small regions of the *S. pennelli* genome are carried in the background of *Solanum lycopersicon.*

The results confirm that there are hybrid-specific sRNAs and that they are associated with suppression of gene expression and epigenetic modification of the genic DNA. However, they were absent from the F1 hybrids and present only in F2 and ILs. These hybrid-specific sRNAs may influence transgressive phenotypes in hybrids, including those affecting agronomic characteristics.

Session 1: SOL Biodiversity and Evolution

OP1.1

Interactions between anther morphology and pollinator behaviour in *Solanum* and their implications for floral evolution

Mario Vallejo-Marin

School of Biological and Environmental Sciences, University of Stirling, UK

mv9@stir.ac.uk

Flowers in the large genus *Solanum* (Solanaceae) lack nectar and rely on pollen as the main or only reward to recruit pollinators. In these flowers, pollen has a dual function, acting both as food for pollinators and as a vehicle to transport male gametes. This dual fate of pollen mediates pollinator attraction and fertilization success in *Solanum* with potential consequences for the evolution of flower morphology. Most *Solanum* flowers have a stereotypical morphology in which the anthers form a cone along the central floral axis and pollen is released via small apical pores via vibrations exerted by pollen-seeking insects. However, some species display a marked dimorphism in the shape, colour and form of anthers that allows them to specialize into feeding and fertilizing functions. This type of anther dimorphism is known as heteranthery. In heterantherous species, one type of anthers is brightly coloured and easily accessible to pollinators (feeding anthers), while the other type consists of larger, cryptically coloured anthers, placed in a position corresponding to the location of the stigma (pollinating anthers). Here I address how the interaction between anther morphology and pollinator behaviour in heterantherous *Solanum* determines the fate of pollen and has the potential to drive the evolution of floral form.

OP1.2

Evolution of allopolyploids in the genus *Nicotiana*

Andrew R. Leitch[1], Simon Renny-Byfield[1], Laura J. Kelly[1,2], James J. Clarkson[2], Marie-Angele Grandbastien[3], Marc Deloger[3], Elizabeth McCarthy[1,2,4], Jiri Macas[5], Mark W. Chase[2], Sandra Knapp[4], Ales Kovarik[6]

[1] *Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London, E1 4NS, UK*

[2]*Jodrell Laboratory, Royal Botanic Gardens, Kew Richmond, Surrey. TW9 3AB, UK*

[3]*Laboratoire de Biologie Cellulaire, Institute Jean-Pierre Bourgin, INRA, 78026, Versailles, France*

[4]*Natural History Museum, London, SW7 5BD, UK*

[5]*Biology Centre ASCR, Institute of Plant Molecular Biology, Branišovská 31, České Budějovice, CZ-37005, Czech Republic*

[6]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, CZ-612 65 Brno.*

a.r.leitch@qmul.ac.uk

Recent genome studies have revealed angiosperm evolution proceeds in cycles of polyploidy and diploidisation. The genus *Nicotiana* includes *c*. 75 species, *c*. 35 of which are recent allopolyploids. Using dated phylogenetic trees of *Nicotiana* (calibrated using endemics on volcanic oceanic islands of known geological age), we can estimate ages of allopolyploid species formation. We describe here the cytogenomic organisations of polyploids of different ages (including synthetics) in comparison with their diploid progenitors. In natural tobacco (0.2 my old) non coding tandem repeats are inherited without material change from the parents. Genome diploidisation proceeds in older allopolyploids (~1 my, species of section *Polydicleae*) with loss of many rDNA loci, evolution of new satellites and parental genome homogenisation. After even longer periods of time (~5 my, species of section *Repandae*) there is considerable to near complete turnover of repeated elements within these polyploid genomes. We reveal how 454 high-throughput DNA sequencing provides for fast and rapid whole genome scans, enabling comparisons between polyploid tobacco and its diploid progenitors. We show evidence in tobacco that the genome is “downsizing” and apparently degrading, whilst the genomes of the two diploid progenitors are showing other evolutionary trajectories.

OP1.3

Pollen tube guidance in Solanaceous species: a highly species-specific interspecific breeding barrier

Edith Lafleur, Mohammed Sabar, Yang Liu, Daniel P. Matton

Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, Canada.

dp.matton@umontreal.ca

Pollen tube guidance is part of a complex breeding barrier system that starts from the earliest pollen-pistil interactions down to gamete delivery. One important step, micropylar guidance, relies on the production of ovule-derived chemoattractants and can be seen as the penultimate barrier before gamete fusion. As for other breeding barriers, like self-incompatibility, it is likely that plant families have recruited different signaling modules to guide pollen tubes towards the ovules. To decipher how Solanaceous species have solved this problem, we devised a semi *in vivo* guidance system and used species from the *Solanum* Petota section where natural interspecific hybridization is common among many species. A MAPKKK mutant in affected in megagametogenesis confirmed the involvement of the embryo sac cells as the source *Solanum chacoense* of attracting molecules. Species-specificity of the attraction was found to be robust even with very close species. Attraction competence was developmentally acquired but cross-talk between the growing pollen tubes and the ovary from a distance was shown to modulate the attraction response. The proteinaceous nature of the attractant was confirmed in our bioassay using various fractionated protein extracts. Recent proteomic and transcriptomic strategies, including deep transcriptomic sequencing, are currently used to isolate the Solanaceous pollen tube chemoattractant and will also be presented.

OP1.4

Genome admixture of *Solanum lycopersicum* var *cerasiforme* allows successful association mapping in tomato

Nicolas Ranc[1], Stephane Muños[1], Marie-Christine Le Paslier[2], Aurélie Chauveau[2], Rémi Bounon[2], Sophie Rolland[1], Jean-Paul Bouchet[1], Dominique Brunel[2], Mathilde Causse[1]

[1]*INRA, UR GAFL Génétique et amélioration des fruits et légumes, Domaine Saint-Maurice BP 94 84143 MONTFAVET CEDEX France*
[2]*INRA, UR1279 Étude du polymorphisme des génomes végétaux - Institut de Génomique - Centre National de Génotypage 2 rue Gaston Crémieux CP 5721 91057 EVRY CEDEX France*

mathilde.causse@avignon.inra.fr

Linkage disequilibrium mapping is an efficient tool to dissect molecular bases of interesting phenotypes but suffers severe limits when dealing with inbred crop like tomato. Cultivated tomato has low molecular polymorphism and high linkage disequilibrium extent, reducing mapping resolution. Cherry type tomato (*S. lycopersicum var. cerasiforme*) genome was described to be admixture between cultivated tomato and its wild ancestor. We wanted to harness admixture to increase resolution of association mapping. To assert this hypothesis, we sequenced 81 DNA fragments, spread over chromosome 2, on a tomato core collection (N=90) mainly composed by cherry type tomato that was also phenotyped for fruit weight, fruit locule number and fruit soluble solid content. We assessed the structure of molecular polymorphism and the extent of linkage disequilibrium over genetic and physical distance. A large set of polymorphisms (340 SNPs and Indels) was detected and *S. l. cerasiforme* showed a higher rate of polymorphism than the cultivated or wild groups. Linkage disequilibrium decreased under r^2=0.3 within 1 cM and minimal estimated values (r^2=0.13) were reached over 20Kb on physical regions studied. Associations of polymorphism with phenotypes were detected with structured association methods. We showed efficiency of genomic admixture to overcome the low-resolution limitation of association mapping for an inbred crop. We validated previously identified QTL and we found associations with new QTL and new candidate genes.

OP1.5

Chromosomal evolution in *Solanum* with cross-species FISH painting

Dora Szinay[1], Erik Wijnker[1], Ronald van den Berg[3], Yuling Bai[2], Richard Visser[2], Hans de Jong[1]

[1]*Wageningen University and Research Centre (WU), Laboratory of Genetics*
[2]*Wageningen University, Laboratory of Plant Breeding*
[3]*Wageningen University, Biosystematic Group, Centre for BioSystems Genomics*

dora.szinay@gmail.com

Solanum is one of the largest plant genera and contains several economically important species, including tomato, potato, pepper and eggplant. Its phylogenetic relations based on morphological traits and DNA sequences are complex and in few cases not always clear. In our study we focus on the occurrence of large chromosomal rearrangements between *Solanum* species as a new tool for phylogenetic relations. Most rearrangements reported so far are paracentric inversions[a,b,c] although pericentric inversions and trans-locations may occur as well[d]. Such events are considered rare evolutionary marks and may therefore be robust traits for phylogenetic analyses. We used cross-species BAC FISH to visualize large chromosomal rearrangements in *Solanum* species by hybridizing tomato and potato BACs as probes on spread pachytene complements of male meiocytes. We focused on nine chromosome arms that in previous genetic studies or our FISH studies were found to contain chromosomal rearrangements. Five evenly distributed BACs per chromosome arm were selected that resulted in certain patterns, which we compared in a matrix containing the rearrangements of all species under study. As the result we discovered six previously undescribed rearrangements. Moreover our study strongly corresponds to previous published phylogenetic trees on the *Solanum* section Lycopersicon[e]. Specific new insights for the cytogenetics analysis will be discussed.

References: a) Tang and Szinay et al. (2008) Genetics. b) Iovene et al. (2008) Genetics. c) van der Knaap (2004) Genetics. d) Anderson et al. (2010) Cytogenetic and Genome Research. e) Spooner et al. (2005) Taxon.

Session 2: Plant Growth and Development

OP2.1

The tomato Auxin Response Factor ARF8 is a central figure of the mechanism controlling fruit set initiation.

Mondher Bouzayen[1,2]

[1]*Université de Toulouse, INP-Toulouse, Génomique et Biotechnologie des Fruits, Avenue de l'Agrobiopole BP 32607, Castanet-Tolosan F-31326, France;*
[2]*INRA, Génomique et Biotechnologie des Fruits, Chemin de Borde Rouge, Castanet-Tolosan, F-31326, France*

bouzayen@ensat.fr

Fruit set is naturally initiated upon successful fertilization of the flower and it is well known that changes occurring during the flower-to-fruit transition are regulated by a complex network of hormone signaling. Auxin is one of the major players in this process and application of auxin can stimulate fruit set and development without requirement for pollination. Likewise, over-expression of genes either conferring higher auxin production or increased auxin sensitivity results in fertilization-independent fruit set in tomato. However, the molecular mediators by which auxin impacts the flower-to-fruit transition remains rather elusive. Transcriptional regulators act as essential mediators of hormones responses and are responsible for recruiting the target genes required for driving a given developmental process. We previously showed that down-regulation of IAA9, a tomato member of the *Aux/IAA* gene family, leads to precocious fruit development prior to flower fertilization giving rise to parthenocarpy. Aux/IAAs proteins regulate, in combination with ARFs (Auxin Response Factors), the expression of auxin-responsive genes. The present work shows that ARF8 is a central figure of a regulome that controls tomato fruit set and identifies IAA9 and MicroRNAs as key actors of this control mechanism. In the ovary tissue, the transcript levels of Sly-miR167 and ARF8 evolve in perfect opposite direction during the transition from anthesis to post-anthesis concomitant with the directed-cleavage of ARF8 transcripts by this microRNA. On the other hand, the experimental evidence for the interaction between ARF8 and IAA9 proteins provided in this work, strongly suggests the control of ARF8 activity by IAA9 at the post-translational level. Reverse genetics approaches revealed the role of both Sly-miR167 and its ARF8 target gene in controlling the flower-to-fruit transition. Both down-regulation of miR167 and up-regulation of ARF8 resulted in pollination-independent fruit set. In the emerging regulation model, fruit set is triggered by the up-regulation of ARF8 whose expression is post-transcriptonally regulated by sly-miR167 and post-translationally regulated through direct interaction with IAA9. The data allow defining a control mechanism of the fruit set process where the expression of the central player ARF8 is tuned by Aux/IAA9 and microRNAs.

OP2.2

EvoDevo meets Genomics – The Lycopersicon complex in tomato

Neelima Sinha[1], Dan Koenig[2], Dan Chitwood[1], Jose Jimenez Gomez[1], Lauren Headland[1], Seisuke Kimura[1], Ravi Kumar[1], Julin Maloof[1]

[1]*Department of Plant Biology, UC Davis, Davis, CA - 95616*
[2]*Max Planck Institute, Tubingen, Germany*

nrsinha@ucdavis.edu

Plants acquire the bulk of their energy from light capture by leaves, and leaf shape has direct consequences on the efficiency of light capture and photosynthetic carbon fixation. As a result, leaf shape must be optimized in response to variation in light quality. To understand the genetic programs controlling fundamental developmental processes, we must integrate genetic networks regulating both environmental response and morphological form. We have undertaken a genomics approach to understand natural variation in leaf morphology and light response, and to investigate the mechanism by which these two genetic networks are integrated to ensure optimal developmental pattern. These goals are being accomplished by characterizing near isogenic lines created from crosses between tomato species varying in both light response and leaf complexity. Characterization of these lines for transcriptional, genotypic, and phenotypic variation is being performed using transcriptome sequencing. Massively parallel sequencing has allowed us to acquire genome-wide mRNA sequence and SNP information in wild and domesticated tomato. The resulting data is being used for expression and genotyping analysis. The end goal is to construct genetic networks regulating leaf morphology and light development.

OP2.3

Developmental regulation of ripening in fleshy fruits

Graham B. Seymour

Division of Plant Sciences, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD, UK

Graham.Seymour@nottingham.ac.uk

Fruits are an immensely important part of the human diet. Low fruit and vegetable intake is recognised as a major factor for increased risk of heart disease and certain cancers. The beneficial effects of fruits are due to novel phytochemicals and almost certainly other tissue components such as pectins that act as dietary fibre. However, increased uptake of fruit products depends on attributes that will attract the consumer including colour, texture, taste and nutritional benefits. These factors are all intimately linked with the ripening process. Tomato is the model system for studying fleshy fruits. We are using an integrative approach involving non-ripening mutants, QTL cloning and Systems Biology approaches. The presentation will highlight progress on identification of genes underlying fruit texture QTL using the new tomato genome assembly and on the development of model to describe the molecular regulation of fruit ripening.

OP2.4

Regulation of tomato compound leaf development by cytokinin and auxin

Eilon Shani, Hadas Melnik, Ido Shwartz, Sharona Shleizer-Burko, Osnat Yanai, Yogev Burko, Naomi Ori

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel

ori@agri.huji.ac.il

Plant leaves show unique variability in size and form. The compound tomato (*Solanum lycopersicum*) leaf provides a sensitive system to explore mechanisms underlying variations in leaf shape, size and complexity. We investigated the role of the plant hormones cytokinin (CK) and auxin in compound leaf patterning in tomato. Manipulation of CK levels led to alterations in leaf complexity, and exposed a unique potential for prolonged growth and morphogenesis in tomato leaves. Genetic analysis implied that CK mediates the activity of KNOXI proteins in prolonging the morphogenetic activity of the leaf margin. The auxin response marker DR5 was found to mark the sites of leaflet initiation. Mutations in the *ENTIRE* (*E*) gene, encoding the putative auxin response repressor E/SlIAA9 (E) caused the expansion of DR5 expression to the entire leaf margin, and led to a simplified leaf shape, similar to auxin or NPA treatments. While *e* leaves still initiated leaflets, leaflet initiation was completely abolished in plants that lack the activities of both E and GOB, a transcription factor involved in leaflet initiation and separation. Genetic analysis showed that the effect of CK on leaf complexity depends on proper localization of auxin signaling, but is not mediated by auxin. These results imply that KNOXI proteins act via CK to maintain the developmental window of morphogenetic potential at the tomato leaf margin, and the spatial and temporal peaks of auxin and GOB activities act within this window to promote leaflet initiation and separation.

OP2.5

Developmental mutants in *Petunia*

T. Gerats, K. Heijmans, K. Vijverberg, M. Vandenbussche, J. Zethof

IWWR/Plant Genetics, Radboud University and CNRS, Lyon (MV)

t.gerats@science.ru.nl

Petunia is a well known model system for a variety of topics, amongst which research on for example pollination syndromes, speciation, fragrance, mycorrhizal interactions, plant development and not least flower development are presently well-noted. One of the basic features concerns the similarities and differences that can be found with other systems in comparative analyses. On the technical level, we will present the latest improvements on our transposon mutagenesis approaches, which will lead to the availability of a huge collection of Insertion Flanking Sequence Tags. We will present a summary of our findings in floral development.

OP2.6

A petunia R2R3-MYB transcription factor involved in the dynamic process of anthesis

Thomas A. Colquhoun, Bernardus C.J. Schimmel, Julian C. Verdonk, Michael Schwieterman, Danielle M. Marciniak, Ashlyn Wedde, Joo Young Kim, David G. Clark

Environmental Horticulture Dept., University of Florida, USA

geranium@ufl.edu

R2R3-MYB transcription factors are involved in many diverse aspects of plant biology from determining cell shape, phenylpropanoid metabolism, to hormonal mediation. We isolated a cDNA sequence from *Petunia x hybrida* cv "Mitchell Diploid" (MD) that is highly similar to an R2R3-MYB family member in *Arabidopsis* (AtMYB24), and named it PhMYB24. PhMYB24 transcripts accumulate to relatively high levels in the floral tissues of MD with maximum accumulation at anthesis. Utilizing the 3' sequence of the PhMYB24 cDNA as an RNAi inducing fragment, multiple transgenic RNAi lines (ir-PhMYB24) were generated for PhMYB24 in the MD genetic background. Compared to MD, ir-PhMYB24 floral buds elongated slowly and did not reach a maximal length or undergo anthesis before senescing prematurely. Detached ir-PhMYB24 floral buds supplemented with sucrose, trans-cinnamic acid, and gibberellic acid elongated farther and entered anthesis; however, complete petal limb expansion was not achieved through this complementation methodology. Comparative transcript accumulation assays demonstrated L-PHENYLALANINE AMMONIA-LYASE (PAL) transcript levels were reduced in the ir-PhMYB24 lines. Together, these data suggest PhMYB24 is involved in the developmental process of anthesis and directly or indirectly regulates multiple molecular events.

Session 3: SOL Genomes

OP3.1

The genome that makes tomatoes

Giovanni Giuliano, The Tomato Genome Sequencing Consortium

Italian National Agency for New Technologies, Energy and the Environment (ENEA), Casaccia Research Center, Rome, Italy

giovanni.giuliano@enea.it

Tomato is the second most important horticultural crop worldwide and a model system for biological processes such as plant and fruit development, photomorphogenesis, and carotenoid biosynthesis. It has a diploid genome of 950 Mb and extensive mutant and TILLING collections. It is an important dietary source of vitamin C, provitamin A and lycopene (implicated in the prevention of prostate cancer). It was domesticated in the Americas prior to European contact and introduced to Europe in the mid-16th century. Its genome was sequenced using a combination of BAC-by-BAC and WGS approaches. The latter effort was based on 22x coverage with 454 (shotgun and paired ends) and 3.5x Sanger (plasmid, fosmid and BAC paired ends). The resulting assembly is publicly accessible on the site www.solgenomics.net. The current release (v1.03) covers approx. 800 Mb in 3761 scaffolds, with contig and scaffold N50 sizes of 55 Kb and 4.5 Mb, respectively. The first release (v1.0) was validated and annotated. Over 95% of the tomato ESTs are represented in the assembled genome, and a comparison with genes sequenced with traditional methods has revealed a very low ($<10^{-4}$) error rate in coding regions. The annotation performed by iTAG (international Tomato Annotation Group) is done through a distributed network and uses SGN as a central exchange server. The structural gene prediction is done with EuGene and integrates EST/cDNA spliced alignments, protein homology, and *ab initio* runs of AUGUSTUS, GeneID, Glimmer and GM.hmm. The 33,926 predicted proteins are in agreement with previous estimates of tomato gene number. Functional annotation is performed with the Afawe-tool, which integrates Interpro-scan results, blastp to curated proteins, GO and phylogeny. An average gene density of ~23.4 Kb/gene reflects the occurrence of large “gene deserts”. Large scale genome duplication, synteny, and duplication history are currently being studied.

OP3.2

The Potato Genome Sequence

C. R. Buell, The Potato Genome Sequencing Consortium

Plant Biology Department, Michigan State University, USA

buell@msu.edu

Potato is the world's most important vegetable crop, and a key member of the Solanaceae. The 840 Mb genome of potato has been sequenced by the global Potato Genome Sequencing Consortium (PGSC). Initially, the sequencing effort employed a chromosome by chromosome, BAC by BAC sequencing strategy of the diploid 'RH89-039-16' (RH) clone. In addition to RH, a doubled monohaploid clone 'DM1-3 516R44' (DM) has been used to generate a high quality draft sequence using Next Generation Sequencing. Progress towards generation of draft sequences of the two genotypes has been rapid, with high genome coverage of both genotypes. Currently version 3.0 of the DM assembly is available (www.potatogenome.net). Resources developed include fosmid and BAC libraries, improved physical maps, and an anchored physical/genetic reference map, onto which more than 80% of the DM genome assembly has been mapped. We have annotated the genome and are currently analyzing the transcriptome and genes critical to potato biology. The timely release of the potato genome sequence provides the entire Solanaceae research community an opportunity to exploit the genome sequence for fundamental and applied biological studies, including plant breeding. The potato genome sequence will serve to accelerate potato improvement and help to meet the challenges facing food production in the 21st century.

OP3.3

Challenges of Tobacco Genome Sequencing and Assembly

N. Sierro, G. Bindler, MC. Peitsch, N. Ivanov

Philip Morris International, Research and Development Department, CH-2000, Neuchâtel, Switzerland

Nikolai.Ivanov@pmintl.com

Tobacco (*Nicotiana tabacum;* 2n=48) is an important agricultural crop and a model plant organism. It harbors ~4.5 gigabases of genomic DNA that likely emerged about 200'000 years ago from interspecific hybridization between *Nicotiana sylvestris* (2n = 24) and *Nicotiana tomentosiformis* (2n = 24). Similarly to many other plant genomes, the tobacco genome sequence is estimated to contain a significant proportion of repeats (70-90%) and a high number of gene duplications. The size, polyploidy and repeats are the major factors that complicate the sequencing and assembly of a high quality tobacco genome. We have considered a range of approaches used in the scope of numerous large-scale genome sequencing initiatives. The Tobacco Genome Initiative (TGI) project followed a methyl-filtration strategy and resulted in more than 1.4 million reads which upon assembly produced a highly fragmented partial genome sequence. Building on the experience gained from working with the TGI data as well as with assemblies of available genomes we decided to use a combination of BAC-by-BAC and Whole Genome Shotgun (WGS) approaches for our sequencing strategy. We have built a BAC library of ~450,000 clones with a genome coverage exceeding 10X and are currently in the process of ordering them into a physical map. In addition to being an invaluable resource for targeted BAC sequencing, the tobacco physical map served as a scaffold for the assembly of a high quality tobacco genome obtained through next generation technologies and TGI whole genome shotgun sequences. The genomic loci underlying important phenotypic traits can be identified through the linkage of physical and genetic maps. In conclusion, we are convinced that despite the enormous quantity of data next generation sequencing can produce, a successful high quality assembly of such a complex genome requires the use of a physical map to overcome the challenges of polyploidy and high repeat content. A similar strategy may be applicable to a growing number of large polyploid genomes especially in the plant kingdom.

OP3.4

Toward Completion of the Genome that Makes Hot Pepper

Minkyu Park[1], Jongsun Park[1], Seungil Kim[1], Byoung-Cheol Kang[2], Yonghwan Lee[1], Doil Choi[1,2]

[1]*Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea*
[2]*Department of Plant Science, College of Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea*

doil@snu.ac.kr

As an effort to make a reference genome sequence of pepper, we are sequencing a pepper wild race, *Capsicum annuum*, CM334. The genome size of CM334 is estimated at 2.7 Gb, which is three times larger than that of tomato. A total of 306 Gb of pepper raw sequences have been generated using Illumina/Solexa Genome Analyzer 2. To date 156 Gb of raw sequences corresponding to 57x coverage have been successfully assembled. The total contig length was 2.57 Gb and the total contig number was 1,876,612. N50 and average length were 3,890 bp and 1,370 bp, respectively. Our current effort is focused on the assembly of all the 306 Gb raw sequences. Along with the whole-genome shotgun sequencing, we also have sequenced pepper BAC clones. To pick out euchromatin-enriched BAC clones, we used labeled cDNAs of pepper or tomato mRNAs (tomato mRNA was used to avoid the screening by retrotransposons) as probes in the BAC library screening. As a result, a total of 2,382 BAC clones were selected and the sequences were determined using 454 or Solexa. In addition, a total of 435 BAC clones were selected using NBS-LRR genes as probes. Those BAC clones have been sequenced by BAC pooling using Roche/454 FLX or Illumina/Solexa Genome Analyzer 2. Recently we have assembled the 1,270 pepper BAC sequences generated by Roche/454 FLX, resulting in 34,743 contigs, with average contig size of 2,707 bp, and total length 0.94 Gb. The progress of our pepper genome sequencing will be presented.

OP3.5

Genomics solutions on developing reference genomes in BGI

Bicheng Yang

Beijing Genomics Institute, BGI-Shenzhen, China

yangbicheng@genomics.org.cn

Next-generation DNA sequencing technologies provide ultra-high throughput at a substantially lower cost; however, the data is presented in very short read-length sequences, making *de novo* assembly extremely challenging. BGI has developed a novel method for *de novo* assembly of large genomes from short-read sequences and successfully assembled the panda (2.7Gb), cucumber (367Mb), Chinese cabbage (500Mb), and potato (830Mb) genomes. The development of this *de novo* short-read assembly method creates new opportunities for building reference sequences and carrying out accurate analyses of unexplored genomes in a cost effective way.

Empowered by the capacities, BGI announced the "1000 Plant and Animal Reference Genomes Project" (ldl.genomics.org.cn) in January 2010 and calls for collaborations from all over the world with the aim to generate reference genomes of a thousand economical and scientific important plant/animal species in two years.

Unraveling whole genome sequences of a species will tremendously accelerate basic research, increase knowledge on the functions of important genes, and facilitate their applications and manipulations. Genetic variations and evolutionary process can be identified through comparative analysis of population and individuals, and finally lead to huge impacts on scientific discoveries and society development.

Session 4: Biotic Stress

OP4.1

Exploiting pathogen effectors in breeding and deployment of disease resistance

Sophien Kamoun

The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, UK

sophien.kamoun@tsl.ac.uk

Filamentous plant pathogens, such as fungi and oomycetes, secrete an arsenal of effector proteins that modulate plant innate immunity and enable parasitic colonization. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants became a driving paradigm in the field of fungal and oomycete pathology. This presentation will illustrate how effectors can be used in crop breeding and deployment of disease resistance with examples from the *Phytophthora infestans-Solanum* pathosystem. For instance, effectors have been used to identify disease resistance (R) proteins and classify their activities into discrete recognition specificities. Effector assays can accelerate the cloning of R genes and help to avoid redundant cloning and breeding efforts. The availability of effectors that match particular R genes is also useful in assisting with the deployment of R genes in agriculture. Evaluations of the distribution of effector variants across pathogen populations coupled with activity assays have been used to monitor pathogen populations for the potential occurrence or emergence of races that overcome the R genes. Lastly, mechanistic and population biology knowledge of effectors can be integrated to devise novel strategies for disease resistance, such as the engineering of synthetic R genes with extended spectrum of effector recognition and pathogen resistance.

OP4.2

Dissection of systemin/jasmonate-signaled defense responses in tomato

Chuanyou Li, Jiuhai Zhao, Liuhua Yan, Hongling Jiang, Bao Wang, Hongshuang Li, Minmin Du

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

cyli@genetics.ac.cn

It has been proposed that the wound-induced local and systemic expression of defense related genes are regulated by intercellular mobile signals. Wound-inducible defensive proteinase inhibitors (PIs) in tomato plants provide an attractive model system to identify the long-distance wound signals and to dissect the signal transduction pathway leading from injury to marker gene expression. Among the proposed signals for wound-induced *PI* gene expression are systemin, an 18-aa peptide derived from proteolytic cleavage of a larger precursor protein called prosystemin, and the fatty acid derived phytohormone jasmonic acid (JA). A wealth of genetic and biochemical evidence indicates that systemin and JA work together through a common signaling pathway to coordinate wound-induced systemic expression of PIs and other defense related genes. Reciprocal grafting experiments using tomato mutants defective in JA biosynthesis (*spr2*) or signaling (*jai1*) provides compelling evidence that JA acts as a transmissible signal for systemic defense. To further dissect the systemin/JA-signaled defense pathway using a genetic approach, we employed a transgenic tomato line (named *35S:PS*) over-expressing the *PROSYSTEMIN* gene and therefore, show constitutive activation of JA-mediated defense responses. A large genetic screen has identified 7 *spr* (for *s*uppressor of prosystemin-mediated response) mutants that block the constitutive expression of wound-responsive genes of the *35S:PS* plants. Our current efforts to characterize these *spr* mutants promise to shed new light on the understanding of systemin/JA-mediated defense signaling. On the other hand, we carried out a microarray analysis to compare the JA-induced gene expression profiles between *35S:PS* and the tomato *jai1* (*JA-insensitive 1*) mutant, which harbors a loss-of-function mutation of the tomato JA receptor gene. We are currently investigating the biological function of the identified genes showing differential expression between *35S:PS* and *jai1*.

OP4.3

A pepper hybrid proline-rich protein 1 (HyPRP1) is a negative regulator in defense response against pathogens but a positive regulator in cell death

Seon-In Yeom, Doil Choi

Department of Plant Science, Plant Genomics and Breeding Institute, Seoul National University, Seoul, 151-921, Republic of Korea

snusunin@hanmail.net

In plant, primary defense to pathogens is associated with cell wall modification and defense-related gene expressions such as secreted proteins. In a previous study, CaS203 (*Capsicum annuum* secreted protein 203) was isolated by yeast secretion trap. CaS203 is a member of HyPRP gene family containing signal peptide, proline-rich domain, and a conserved eight-cystein motif (8CM) in lipid transfer proteins. The transcript level of CaS203 decreased prior to the appearance of HR and cell death by pathogen. CaS203 was constitutively expressed in various organs, mostly in root.

Transient over-expressions (TOE) of CaS203 cause microscopic cell death through accumulation of ROS in chloroplast under white light illumination in *Nicotiana benthamiana*. These plants suppress the expression of defense-related and reactive oxygen species (ROS) scavenging genes. In addition, TOE of CaS203 accelerated HR and cell death induced by pathogen. TOE of the sub-domains of CaS203 revealed that the 8CM domain with signal peptide is essential for inducing cell death. Furthermore, identified *Arabidopsis* and *N. benthamiana* orthologs show cell death by ectopic expression of its 8CM domain in *N. benthamiana*.

On the other hand, silencing of CaS203 delays HR and pathogen-induced cell death. When monitored defense-related and ROS-scavenging genes in silenced plants, PR genes, catalase, and superoxide dismutase are increased as compared to that of the control plant.

These results provide a new insight into the conserved role of ubiquitous plant cell wall structural protein, HyPRP in regulating defense and cell death in plants.

OP4.4

Mapping of QTLs associated with resistance to a virus causing Tomato yellow leaf curl disease (TYLCD) in tomato

P. Kadirvel, R. de la Peña, S. Geethanjali, L. Kenyon, W-S. Tsai, P. Hanson

AVRDC: The World Vegetable Center, PO Box 42, Shanhua, Tainan-74199, Taiwan

kadirvel.palchamy@worldveg.org

Tomato yellow leaf curl disease (TYLCD) caused by diverse begomovirus species can devastate tomato production in the tropics and subtropics. Developing tomatoes with natural resistance is the most cost-efficient method to control the disease. Effective sources of resistance were identified in wild relatives of tomato and are currently used in breeding programs. There are five described genes conditioning resistance to different genotypes of begomovirus: *Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, and *Ty-5*. FLA456 is a tomato line from the University of Florida that has demonstrated high levels of resistance to TYLCD in multilocation trials. The resistance in FLA456 is derived from the wild species *Solanum chilense* (accession LA2779). Mapping resistance genes in FLA456 and designing markers for marker-assisted selection would facilitate breeding this resistance into elite varieties. Genetic analysis of an F_2 population (from a cross between FLA456 and CLN1621L-*Solanum lycopersicum*) indicated that resistance to *Tomato yellow leaf curl Thailand virus* (TYLCTHV-Taiwan strain) in FLA456 is recessive and possibly controlled by two quantitative trait loci (QTLs). The QTLs were mapped on chromosomes 4 (TyQTL4.1 with the marker interval of SNP0667-SSR43) and 6 (TyQTL6.1 with the marker interval of SLM6-23-SNP1236) and accounted for 16.4 and 18.5% of phenotypic variation, respectively. The resistance genes detected in FLA456 appear to be different from the previously mapped Ty genes. This provides an opportunity to diversify sources of resistance for breeding. Validation and further mapping of these QTLs are in progress using a recombinant inbred population.

OP4.5

Isolation of *N'*, one of the most famous plant virus resistance genes

Ken-Taro Sekine[1], Reiko Tomita[1], Shigeharu Takeuchi[2], Akinori Kiba[3], Yasufumi Hikichi[3] and Kappei Kobayashi[1]

[1]*Iwate Biotechnology Research Center, Kitakami 024-0003, Iwate, Japan*
[2]*Laboratory of Plant Pathology, Kochi Agricultural Research Center, Nankoku, Kochi 783-0023, Japan*
[3]*Laboratory of Plant Pathology & Biotechnology, Kochi University, Nankoku, Kochi 783-8502, Japan*

k-sekine@ibrc.or.jp

The *N'* gene of *Nicotiana sylvestris* and *L* genes of *Capsicum* plants confer hypersensitive resistance (HR) that is commonly elicited by tobamovirus coat proteins (CPs) but with different virus species specificities. Recently, we cloned L^3 by map-based methods and some other *L* genes using a homology-based method. Here, we report the identification of the *N'* gene. We first amplified and cloned an *N'* candidate by using PCR primers designed on the basis of *L* gene sequences. The *N'* candidate encoded a CC-NBS-LRR-type resistance protein, which shared amino acid sequence identity of 68.7% and similarity of 89.2% with the L^3 protein. Functional analysis revealed that the candidate induced HR in response to the co-expressed tobamovirus CPs with the identical specificity reported for the *N'* gene. Analysis of 3 each of *N'*-containing and tobamovirus-susceptible *Nicotiana tabacum* accessions confirmed that the candidate was the *N'* gene itself. Chimera analysis between *N'* and L^3 revealed that their LRR domains determine the spectrum of their tobamovirus CP recognition. Deletion analysis of *N'* and L^3 revealed that their conserved sequences in the 5'- and 3'-ends play important roles in the induction of HR. Although *N'* and L^3 share high sequence similarities, the chimera and deletion analyses collectively suggested that different LRR units contribute to the recognition of their common elicitors. Further comparative analysis of these genes would help in understanding the essential mechanisms underlying pathogen recognition by plants via gene-for-gene interactions.

Session 5: Abiotic Stress

OP5.1

Improving water use efficiency and water capture in tomato: transgenic and QTL approaches

Andrew J. Thompson[1], Sajjad Awan[1], Howard Hilton[1], John Andrews[1], Liz Harrison[1], Rachel Smeeton[2], Charlotte White[2], Ian B. Taylor[2]

[1]*Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK*
[2]*Department of Plant Sciences, University of Nottingham, Leicestershire, LE12 5RD, UK*

A.J.Thompson@warwick.ac.uk

Sustainable use of water is an essential component of food security. Tomato field crops are usually irrigated and there is a need to maximize the returns from irrigation. With a given amount of water, yield can be increased by either increasing water use efficiency (WUE, the biomass gained per unit of transpiration), or by increasing the use of the available water, e.g. by capturing more of the water stored in the soil profile through development of a more extensive root system.

Stomatal conductance (g_s) has a close relationship with yield but the relationship is non-linear such that under some conditions a limit to g_s can greatly reduce transpiration whist having a relatively small effect on CO_2 assimilation, thus leading to an increase in WUE. Reduced transpiration also delays onset of water deficit in the soil and crop, and prolongs the period of active growth. Abscisic acid (ABA) is a key modulator of plant responses to water deficit and the maintenance of a favorable plant water status. Over-expression of 9-*cis*-epoxycarotenoid dioxygenase (NCED), a key rate-limiting enzyme for ABA biosynthesis, causes ABA accumulation which leads to stomatal closure, improved plant water status, and greatly improved WUE. However, the amount and location of the enhanced ABA synthesis is critical in order to obtain the benefits of increased WUE without undesirable effects on growth and development. I will describe our attempts to optimize ABA synthesis levels to suit tomato plants growing with limited water availability through the use of transgenics and exploration of natural genetic variation. QTL mapping for tomato root traits such as rooting depth and ability to penetrate resistant materials will also be described.

OP5.2

The role of Solanaceae aquaporins in improving plant vigor, abiotic stress tolerance and yield production

Menachem Moshelion[1], Basia J. Vinocur[2], Arava Shatil[1], Ron Seligmann[1], Rony Wallach[1], Hagai Karchi[2], Nir Sade[1]

[1]*The Robert H. Smith Faculty of Agriculture, Food, and Environment, Hebrew University of Jerusalem, Rehovot 76100, Israel*
[2]*Evogene Ltd, 13 Gad Feinstein St., Rehovot 76121, Israel*

moshelio@agri.huji.ac.il

Crop plants' ability to conduct water is strongly correlated with growth rate and yield production. Abiotic stress conditions induce a rapid reduction in the plant's hydraulic conductance, which has a major impact on its growth and yield production. In this study we hypothesized that aquaporins take part in the molecular mechanism regulating plant hydraulic conductance. Using computational mining, two key stress-induced aquaporins were selected: the tonoplast intrinsic protein 2;2 from tomato (SlTIP2;2), and tobacco aquaporin 1 (NtAQP1). Transforming tomato (M82) plants with these aquaporins resulted in increased cell osmotic water permeability and whole-plant transpiration rate. Under salinity and some drought conditions, both plants transpired more and for longer periods, using more of the available soil water and resulting in significant increases in fruit yield, harvest index and plant mass relative to controls. Although both aquaporins led to improved plant performance, the physiological-molecular roles of the channels were distinct: SlTIP2;2 was involved in regulating the plant's relative water content via control of vacuolar water permeability, whereas NtAQP1 increased root hydraulic and mesophyll CO2 conductance, resulting in improved water-use efficiency. In conclusion, it is proposed that the regulation mechanism controlling cellular water and CO2 permeability might have a role in determining the whole-plant hydraulic conductance, and thus its abiotic stress tolerance

OP5.3

Genetic control of fruit quality traits in tomato plants under water deficiency

Antonio Di Matteo[1], Vasco Maria[1], Rosalba De Stefano[1], Concetta Lotti[2], Luigi Ricciardi[3], Amalia Barone[1]

[1]*Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)*
[2]*Department of Agro-Environmental, Chemistry and Crop Protection, University of Foggia,Via Napoli 25, 71100 Foggia, Italy*
[3]*Department of Biology and Chemistry Agro-Forestal and Environmental, Genetics and Plant, Breeding Unit, University of Bari, Via Amendola 165/A, 70125 Bari, Italy*

adimatte@unina.it

Understanding molecular mechanisms underlying tolerance to water deficit and cross-interaction between response to reduced water availability and fruit quality enhancement may help breeders to develop superior tomato genotypes for sustainable cropping systems. Water deficit tolerance is a quantitative trait and previously in our laboratories a QTL (Quantitative Trait Loci) for this trait was identified in the IL9-2-5, a *Solanum pennellii* introgression line.

The aim of this research was to identify gene networks controlling fruit quality under water deficiency in a susceptible (M82) and a tolerant tomato genotype (IL 9-2-5). Plants were grown according to a split-plot design with three replicates in semi-controlled conditions. Three different levels of water restitution were applied. As water restitution decreased, IL9-2-5 showed a lower yield loss than M82. Also, IL9-2-5 performed higher ascorbate concentration in red-ripe fruit at lower water restitution volume.

A comparative transcriptomic analysis of red-ripe fruit from IL9-2-5 and M82 at different water treatments was carried out on the Combimatrix TomatArray1.0. It allowed the identification of 160 differentially expressed transcripts between genotypes and 241 among treatments. A model network describing the interactions between the plant response to water deficit and the increased fruit quality was developed. It included genes involved in stress perception and transduction, ethylene biosynthesis and antioxidant metabolism. Currently, candidate transcripts are being validated by qPCR approach.

Functional characterization of candidate genes will prove their involvement in fruit quality control and provide additional genetic means for tomato quality enhancement in high sustainable cropping systems.

OP5.4

Genetic, physiological, and molecular approaches to improve drought tolerance in tropical tomato

Rachael C. Symonds[1], Palchamy Kadirvel[1], Joyce Yen[1], Jean Lin[1], Robert de la Peña[2]

[1]*AVRDC – The World Vegetable Center, P.O. Box 42, Shanhua, Tainan 74199 Taiwan*
[2]*Monsanto Singapore Co. (PTE) LTD*

rachaelsymonds@hotmail.com

Drought is a major abiotic stress and a serious constraint to tomato production in the tropics as many modern tomato (*Solanum lycopersicum*) varieties are sensitive to drought. A large source of genetic variation for drought tolerance existing in wild relatives of tomato remains relatively untapped. AVRDC – The World Vegetable Center maintains a collection of both cultivated and wild relatives of tomato with enhanced stress tolerance traits. The aim of this work was to identify tomato plants with drought tolerant characteristics from within the AVRDC germplasm collection through genetic, physiological, and molecular approaches. Wild relatives of tomato were screened for drought tolerance using several different screening methods. A drought pool protocol was developed to assess the comparative drought tolerance of different plant genotypes in comparable soil moisture conditions. A deficit irrigation assay was developed to assess water use efficiency under conditions of moderate drought stress. Drought tolerant and sensitive germplasm lines were identified and one drought tolerant line, LA1579 (*S. pimpinellifolium*) was selected for further characterization. A mapping population consisting of 96 backcross inbred lines (BC1F4) was produced from the cross between a drought sensitive cultivated tomato parent, CLN2498E, and the drought tolerant LA1579. The mapping population was evaluated for physiological and yield traits under drought stress and non-stress conditions. Quantitative trait loci (QTL) analysis and candidate gene approaches have been taken to identify genes associated with drought stress tolerance in tomato.

OP5.5

Ameliorating the impacts of salinity on crop yield by altering root-to-shoot hormonal signaling

Michel Edmond Ghanem[1,2], Alfonso Albacete[2], Ian C. Dodd[3], Stanley Lutts[4], Francisco Pérez-Alfocea[2]

[1]*Université Catholique de Louvain, Belgium*
[2]*CEBAS-CSIC, Spain*
[3]*Lancaster Environment Centre, UK*
[4]*Université Catholique de Louvain, Belgium*

michel.ghanem@uclouvain.be

Soil salinity is an increasing problem for agriculture around the world. While most attention has focused on the role of crop ion accumulation (especially in reproductive organs that contribute to yield), salinity also constitutes an osmotic stress that alters plant hormone relations immediately after stress imposition. Hormonal profiling following stress imposition revealed temporal correlations between leaf growth and senescence and concentrations of the plant hormone cytokinin (CK) in roots, xylem sap and leaves. By increasing root cytokinin production through two different approaches (inducible root CK production and grafting a onto a CK overproducing rootstock) we have doubled tomato vegetative growth and increased fruit yield under moderate salinity.

OP5.6

New insights into the biosynthesis, functions and architecture of the tomato fruit cuticle through tissue specific RNA-seq profiling and other 'omics' platforms.

Jocelyn K.C. Rose

Department of Plant Biology, Cornell University, Ithaca, NY 14850

jr286@cornell.edu

The plant cuticle, a lipophilic layer that covers all aerial plant organs, forms the outermost barrier between a plant and its environment. As such, it plays a critical role in restricting water loss, protecting the plant against a range of other abiotic stresses and providing the primary line of defense against pathogens. Moreover, the cuticle has been shown to be an important factor in regulating organ development and integrity. However, given its multifunctional nature, and compared with other macromolecular structures, remarkably little is known about cuticle biosynthesis, deposition, trafficking and restructuring, particularly in fruit. Tomato fruits offer an excellent system to investigate cuticle biosynthesis and function for several reasons. Unlike other model plants, such as Arabidopsis, whose cuticle is very thin, tomato fruits have a relatively thick cuticle which lack stomata, providing a pore-less uniform material that is easy to isolate and handle. Tomato also has various experimentally important genomic and genetic tools and represents an important crop for which water status, and hence resistance to desiccation, is crucial for determining quality.

We have been developing a multi-pronged strategy to investigate cuticle assembly, structure and functions, including: the use of cell type-specific transcriptome and proteome profiling, the development of new imaging tools and the characterization of cuticles from mutant tomato lines and wild tomato species. Data will be presented suggesting that cuticles make a perhaps unexpectedly important contribution to tomato fruit softening, both directly as a mechanical support and indirectly through the regulation of transpiration and water status, as well as resistance to microbial infection. The identification of new genes that play a role in cuticle biosynthesis and assembly will also be reported, together with an overview of cuticle diversity and the characterization of a previously uncharacterized cuticular layer.

Session 6: Translational Genomics and Molecular Breeding

OP6.1

Classification of tomato varieties based on germplasm class, fruit shape category, fruit shape genes, and genetic clusters

Gustavo Rodriguez, Brian McSpadden Gardener, David Francis, Esther van der Knaap

The Ohio State University, Wooster OH 44691, USA

vanderknaap.1@osu.edu

Phenotypic diversity within cultivated tomato is particularly evident for fruit shape and size. Several genes underlying the major quantitative trait loci (QTL) that control tomato fruit shape have been cloned. The elongated shape is controlled by SUN and OVATE, whereas fruit locule number and flat shape is controlled by FAS. The origin of the mutations, the distribution of alleles in the cultivated germplasm, and the contribution to shape in different genetic backgrounds had not been previously investigated. Here, we describe the effects of the major fruit shape genes in a collection of 368 tomato accessions classified based on morphological as well as geographical and historical diversity. Fruit were visually classified into eight shape categories that were supported by objective measurements obtained from image analysis using the Tomato Analyzer software application. We evaluated the distribution of the alleles of SUN, OVATE and FAS in all accessions and found a strong association between fruit shape classification and the presence of different fruit shape mutations. We genotyped 116 accessions, representative of the entire collection, with an additional 25 markers distributed evenly across the genome. The genotypes were analyzed through model-based clustering using the STRUCTURE program resulting in five genetic clusters. We demonstrated that shape categories, germplasm classes and the shape genes were non-randomly distributed among the genetic clusters at $p < 0.001$, implying that selection for fruit shape genes was critical to subpopulation differentiation within cultivated tomato. Our data also suggested that the FAS and SUN mutations arose in a similar lineage while the OVATE mutation arose in a separate lineage. Furthermore, the occurrence of the OVATE and FAS mutations in Latin American germplasm suggested that the fruit shape mutation might have arisen prior to domestication. The SUN mutation, on the other hand, appeared to be a post-domestication event arising in Europe.

OP6.2

SolCAP - developing SNP markers in elite germplasm for applied potato breeding

Walter De Jong[1], David Douches[2], David Francis[3], Allen Van Deynze[4], John Hamilton[2], Lukas Mueller[5], Robin Buell[2]

[1]*Cornell University, Ithaca, NY, USA*
[2]*Michigan State University, East Lansing, MI, USA*
[3]*Ohio State University, Wooster, Ohio, USA*
[4]*University of California-Davis, Davis, CA, USA*
[5]*Boyce Thompson Institute, Ithaca, NY, USA*

wsd2@cornell.edu

The USDA-funded SolCAP project aims to provide infrastructure to link sequence variation in genes with valuable traits in potato (and tomato). Our initial efforts have focused on the identification of SNPs within cultivated potato germplasm. Normalized cDNA libraries from three elite potato cultivars (Atlantic, Snowden and Premier Russet) were prepared from callus, tuber, leaf and flower tissue. Libraries were pooled and sequenced using Illumina GAII sequencing technology. The reads, single and paired end, were assembled using Velvet generating on average 38 Mb of transcriptome sequence. Due to the high quality and depth of sequence coverage, we were able to identify more than 150,000 high quality SNPs in potato. To identify the genomic context of the assemblies and permit higher level analyses, we aligned the potato transcriptome to the draft genome sequence of *Solanum phureja* DM1-3 516R44, released by the Potato Genome Sequencing Consortium. An Infinium 10,000 SNP array will be used for potato SNP detection. We identified about 60,000 high quality SNPs that meet Infinium design specifications and are single copy in the genome. Of these, we have selected 2769 SNPs in 500-plus candidate genes, 508 SNPs in genetic markers, and 6723 SNPs distributed throughout the genome for the array. We estimate that our SNPs cover about 650Mb of genome scaffolds. These markers will be used to genotype several hundred potato varieties and advanced breeding clones as well as a reference tetraploid mapping population; all are now being evaluated for agronomic traits at several locations across the USA. We anticipate that these SNP markers will simplify and markedly accelerate future genetic mapping in elite potato germplasm. To encourage American potato breeders to use these SNPs, over the next two years the SolCAP project will be genotyping well-characterized breeding populations for free.

OP6.3

Gene-containment system based on artificial-microRNA mediated inactivation of two general transcription factors in *S.melongena* L

L. Toppino[1], M. Kooiker[2,+], G. Grazioli[1], M.G. Tacconi[1], M. Lindner[2], M.M. Kater[2], G.L. Rotino[1]

[1]*CRA-ORL, Unità di Ricerca per l'Orticoltura, 26836, Montanaso Lombardo, LODI, Italy.*
[2]*DSBB, Department of Biomolecular Sciences and Biotechnology, University of Milano, 20133 Milan, Italy.*
[+]*Present address: CSIRO Plant Industry, 306 Carmody Rd, St Lucia, QLD, Australia.*

laura.toppino@entecra.it

Since decades plant male sterility is considered a powerful tool for biological containment to prevent unwanted self-pollination during the breeding process and for hybrid seed production. Furthermore it prevents pollen dispersal which could also answer to the raising concerns regarding the transgene flow via pollen from GM crops and the strong demand for implementing technologies that will allow co-existence of GM and non-GM agriculture. However, for crops that are not usually vegetatively propagated, an irreversible system is undesirable; therefore, strategies for making the male sterility trait reversible, yet tightly controllable, are needed. We developed a conditional system that prevents gene transmission via pollen in eggplant. The strategy for inducing male sterility is based on the anther-specific artificial microRNA-mediated silencing of two endogenous general transcription factors, TAF10 and TAF13, known as acting during pollen development. The amiRNA constructs target a specific sequence of TAF genes under control of TA29 and NTM19 promoters, expressed in the tapetum and microspore, respectively. The ability to complement potential eggplant male sterile TAF-amiRNA lines to restore fertility is based on ethanol-inducible expression of a RNAi-insensitive form of the TAF target retrieved from tomato. Transgenic eggplants are completely male sterile and fertility can be fully restored by short treatments with ethanol, confirming the efficiency but also the reliability of the system in view of open field cultivation. These features were transmitted to the T1 progenies. Moreover, by combining this system with induced parthenocarpy we provide a novel example of complete transgene containment in eggplant.

OP6.4

Natural variation in potato: Linking candidate gene variation to complex traits

Christiane Gebhardt[1], Claude Urbany[1], Li Li[1], Damaris Odeny[1], Astrid. Draffehn[1], Camila Nader-Nieto[1], Joao Paulo[2], Benjamin Stich[2]

[1]*Max-Planck Institute for Plant Breeding Research, Koln, Germany*
[2]*Biometris, Wageningen University, The Netherlands*

gebhardt@mpipz.mpg.de

Most phenotypic characters relevant for potato breeding are complex that is they are controlled by multiple genetic and environmental factors. Knowing the genes and their allelic variants underlying agronomic traits allows the development of molecular diagnostic tools for selecting improved potato cultivars. Diagnostic DNA-based markers are either derived directly from polymorphisms in genes causal for a trait of interest or are in linkage disequilibrium with those genes. They can be used to identify superior genotypes among parents and progeny in breeding programs (Precision Breeding). By association mapping using as markers functional and/or positional candidate genes we identified DNA variants, which explain significant portions of the natural variation of late blight resistance or tuber traits (starch content, yield, starch yield, bruising, chip color) in tetraploid breeding populations. Novel candidate genes were revealed by comparative proteomics studies. Epistatic interactions were found between allelic variants at candidate loci, which increase tuber starch content and starch yield. Natural variation of candidate genes associated with complex traits was studied on the DNA and functional level. Comparative sequencing of candidate gene alleles reveals an amazing degree of molecular diversity in potato, which results from the reproductive biology of this important crop species.

OP6.5

Variation in pigment content in pepper fruit is associated with plastid compartment size

Arnon Brand[1], Yelena Borovsky[1], Sagit Meir[2], Ilana Rogachev[2], Asaph Aharoni[2], Ilan Paran[1]

[1]*Institute of Plant Sciences, The Volcani Center, Bet Dagan, Israel*
[2]*Department of Plant Science, Weizmann Institute of Science, Rehovot, Israel.*

iparan@volcani.agri.gov.il

Fruit color is one of the most important quality traits of pepper. Color contributes to the visual attraction of the fruit and pigments such as chlorophyll, carotenoids and anthocyanins that are beneficial to the human diet. Studies on the genetic control of pigment content in pepper fruit focused mostly on monogenic mutations leading to change in fruit color. In addition to the qualitative variation in fruit color, quantitative variation in pigment content and color intensity exits in pepper giving rise to a range of color intensities from light green and red to dark green and red colors. However, the genetic basis of this variation has been very little studied, hindering the development of peppers that are rich in these beneficial compounds. We studied quantitative variation in pigment content in a cross of a dark-green and deep-red Poblano pepper with a light-green pepper. We identified two major pigment content QTLs, pc8.1 and pc10.1 that control chlorophyll, carotenoids and alpha-tocopherol content mostly at the mature green stage and to a lesser extent in the red ripe stage. Confocal and TEM histological analyses of green fruits of the parents and QTL-NILs indicated that plastid compartment size is a major factor associated with variation in pigment content. LC-MS metabolomic analyses are underway to obtain a more comprehensive metabolic characterization of the pepper lines. The map position of the QTLs indicates no overlap with currently known genes associated with pigment content variation in the Solanaceae. Therefore, identification of the genes controlling pigment content QTLs in pepper will provide new understanding of this important trait and new opportunities for breeding peppers and other Solanaceae species with enhanced nutritional value.

Session 7a: Informatics and Computational Biology

OP7A.1

Sequencing of the *S. pennellii* genome as an approach to investigate diversity in the tomato clade

Bjorn Usadel, The *S. pennellii* sequencing consortium

Max Planck Institute of Molecular Plant Physiology, Germany

usadel@mpimp-golm.mpg.de

Solanum pennellii is a wild tomato species showing a range of phenotypic differences to the domesticated tomato (*Solanum lycopersicon*). However, despite the overall phenotypic differences between *Solanum pennellii* and *Solanum lycopersicon*, the genome of these species is highly similar. Indeed, *Solanum pennellii* was chosen as the donor parent of the extensively phenotyped tomato introgression line (IL) population generated in the Zamir lab.

To further our understanding of the diversity in the tomato clade and to provide a resource for mining the IL population, we set out to sequence the *S. pennellii* genome using mostly paired end Solexa sequencing and generated additional coverage for the *Solanum lycopersicon* variety M82 which was used as the other parent for the IL population.

Here, we present the latest progress on the genomic structure of the *Solanum pennellii* genome, we will discuss how to best mine this resource and we will present tools that allow users to query this genome.

OP7A.2

A combined approach for tagging R-genes candidate loci in tomato genome

W. Sanseverino[1], S. Rombauts[2], Y. Van Der Peer[2], L. Frusciante[1], M.R. Ercolano[1]

[1]*Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples "Federico II", Via Università 100, 80055 Portici Italy*
[2]*Department of Plant Systems Biology, VIB, 9052 Gent Belgium*

ercolano@unina.it

In silico resources can be very useful to discover new R-genes and to mark out new hypothesis about defense mechanisms. A specific resource, the plant resistance gene database (PRGdb, www.prgdb.org), that collects R functional genes and all putative R-gene sequences retrieved by UNIGENE and NCBI nucleotide datasets, was developed. Two distinct prediction pipelines have been built up to perform predictions analysis based on these data. Merging literature data, genetic information, prediction data and molecular results a fast and efficient system for discovering new R-genes and for verifying their functionality was developed. Tomato genome sequence was used to test our prediction systems and to delineate a complete view of its immune system. A specific R-gene prediction tool based on HMM profiling and Interpro database scanning was used to indentify R gene in tomato genome. In total, 867 genes with the mean coding sequence of 2256 bp were predicted. Based on these results we proceeded to assign each sequence to one of the five already known R-genes classes as well as in new classes. The candidate R genes were located either at chromosomal regions previously shown to contain R genes or at positions not yet identified as R-genes regions. In addition, R genes prediction pipelines has been tested on other four sequenced genomes (*Arabidopsis*, Potato, Grapevine and Poplar) to perform comparative studies. To explore the germplasm potential and to validate *in silico* results DNA and RNA was isolated from cultivated tomato and from different wild tomato species and used to amplify putative R-genes retrieved. In order to test their functionality three candidate R genes were also cloned in an easy-vector system for further characterizations. The main purpose of this study was to build an efficient prediction system to being a powerful tool for identifying functional R-genes.

OP7A.3

Developing informatics resources for end users of the Potato Genome Sequence project data.

D.M.A. Martin[1], D.M. Bolser[1], S.K. Sharma[2], G.J. Bryan[2]

[1]*College of Life Sciences, University of Dundee, Dow Street, Dundee. DD1 5EH UK.*
[2]*Genetics Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA UK.*

d.m.a.martin@dundee.ac.uk

Over the course of a genome project many different data types are generated. From raw sequence of markers, through to assembled pseudomolecules; annotations for genes to QTL markers on a variety of different maps, data sources are numerous, in different formats and often difficult to integrate computationally. Typically solutions may be well engineered but are either not user friendly, do not incorporate all the data or do not meet the interrogative needs of the community beyond the immediate research group.

We have explored the data analysis needs for the community by developing the Annotation DB as a browseable archive for the data generated from the Potato Genome Sequencing Consortium. This brings together information at all levels, where possible allowing direct links, aggregation and visualisation. To avoid excessive duplication it links out to external sites where appropriate and uses data-specific external viewers such as Jalview for multiple alignments.

Annotation DB is a research tool for understanding the heterogenous data structure, the way that different data types need to be combined, and enabling the community to determine what the analysis workflows are that future developments should enable. The engineering decisions taken in developing the DB (django web toolkit and an oracle database) were geared towards an environment capable of rapid integration of new data types and views. Dialogue with end users to understand their needs and how they change in response to data availability is key to 'crowdsourcing' a specification for a future persistent resource.

OP7A.4

Single Nucleotide Polymorphism Identification from Potato and Tomato Short Read Transcriptome Sequences

John P. Hamilton[1], David M. Francis[2], Allen Van Deynze[3], Walter De Jong[4], Dave Douches[5], C. Robin Buell[1]

[1]*Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA*
[2]*Dept. of Horticulture and Crop Sciences, The Ohio State University, Wooster, OH 44691, USA*
[3]*Seed Biotechnology Center, University of California, Davis, CA 95616, USA*
[4]*Department of Plant Breeding, Cornell University, Ithaca, NY 14853, USA*
[5]*Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA*

jham@msu.edu

To assess the genetic diversity within cultivated U. S. potato and tomato germplasm, we constructed normalized cDNA libraries from three tetraploid potato cultivars, five inbred tomato varieties, and *Solanum pimpinellifolium*. Using Illumina GAII sequencing technology, we generated 2.82 Gb and >2.5 Gb of total sequence for each potato and tomato cultivar, respectively. The 60 bp reads, single and paired end, were assembled using Velvet generating 38 Mb of transcriptome sequence for potato and 32.5 Mb for tomato. For potato, a pipeline was developed using Maq, SAMtools, and custom perl scripts to call putative Single Nucleotide Polymorphism (SNPs) from reads aligned to the Velvet contigs and filter the SNPs further on quality. The doubled monoploid *S. phureja* v3 draft genome sequence generated by the Potato Genome Sequencing Consortium was used to identify putative SNPs, yielding a total of 69,011 putative SNPs anchored to the genomic sequence. Tomato SNPs were called by aligning uniquely mapping reads to the Tomato Genome Initiative draft genome Heinz 1706 sequence using Bowtie, calling SNPs with SAM tools and filtering with custom perl scripts. A total of 20,828 putative SNPs were identified, 66% of these were *S. pimpinellifolium* only SNPs. Characterization of the potato and tomato SNPs including distribution across the genome, synonymous/non-synonymous SNPs, gene function classes, and market classes will be presented.

OP7A.5

In silico approaches for anchoring the potato genome

Dan M. Bolser[1], Sanjeev Kumar Sharma[2], Jan. de Boer[3], T. Borm[3], G. Bryan[2], D.M.A. Martin[1]

[1]*The University of Dundee, Dow Street, Dundee, DD1 5EH, Scotland, UK.*
[2]*The Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK.*
[3]*Wageningen University, P.O. Box 9101, 6700 HB Wageningen, The Netherlands.*

dan.bolser@gmail.com

The potato genome has been assembled into 622 super-scaffolds larger than 250 kb that together constitute 90 percent the total assembled genome sequence (727.4 Mb). Positioning and orientating these super-scaffolds into their 12 linkage groups is an important next step for the assembly. A variety of genetic and sequence resources can be combined to achieve this goal. We have used the Ultra High Density potato genetic map and the associated BAC End Sequences from *S. tuberosum* and the tomato EXPEN 2000 genetic map to construct two *in silico* physical maps for the sequenced genotype. A genetic map developed for the sequenced clone 'DM' anchors approximately 50 percent of the genome (393 Mb in 336 super-scaffolds). This figure can be increased to 86 percent (623 Mb in 651 super-scaffolds) by combining data from the UHD and tomato *in silico* maps. In my talk I will briefly describe some of the underlying bioinformatics steps that were used obtain and combine these two *in silico* physical maps. This work has been done as part of the Potato Genome Sequencing Consortium.

Session 7b: Tools and Emerging Technologies

OP7B.1

Filling the fruit toolbox with new biotech tools: from transient expression to shared genetic pieces and beyond.

Diego Orzaez[1], Leandro Hueso-Estornell[1], Patricia Fernandez[1], Sara Torre[1], Vicente Moreno[1], Eugenio Butelli[2], Cathie Martin[2], Antonio Granell[1]

[1]*Fruit Quality and Biotechnology Lab, IBMCP (CSIC-UPV), Spain.*
[2]*John Innes Centre, Norwich, UK*

dorzaez@ibmcp.upv.es

Fruits are attractive sink organs for biotechnologists as they accumulate interesting biological compounds as e.g. nutrients, flavours and health-promoting substances. To assist in the biotechnology of fruit, we have developed a number of molecular tools aimed to facilitate gene function analysis and genetic engineering.

First, we developed tools for gene function analysis, primarily based in Agrobacterium-mediated transient expression (fruit agroinjection), which allows limited but fast transgene expression in fruit tissues. Agroinjection opened the way for in fruit delivery of viral infective clones and consequently to the development of fast Virus-Induced Gene Silencing methodologies for fruit reverse genetics (fruit-VIGS). An improved, colour-tracked fruit-VIGS strategy was developed based in anthocyanin accumulating fruits. Colour-guided fruit VIGS was shown very effective in assessing gene function in combination with metabolomic profiling.

Secondly, to facilitate fine-tuned genetic engineering in tomato, we isolated and characterized a number of promoter regions operating in the fruit. The analysis of promoter activity was brought to a higher resolution level by the transcriptional profiling of FACS-sorted fruit protoplasts, as exemplified with the analysis of PVIN1, a vacuolar invertase promoter. All the isolated fruit-operating promoters were incorporated in a combinatorial collection of multisite gateway entry vectors. The so-called pENFRUIT collection also incorporates additional genetic elements (e.g. tags, terminators, etc) and a set-up for fruit-directed hpRNA gene silencing. The pENFRUIT collection is a first step in an attempt to build a shared collection of genetic building blocks (solbricks) for genetic engineering in Solanaceous plants.

OP7B.2

RNA-seq analysis of the shade avoidance response in tomato and its wild relatives

Jose M Jimenez-Gomez[1,3], Dan Koenig[1], Ravi Kumar[1], Seisuke Kimura[1], Jie Peng[2], Neelima Sinha[1], Julin N Maloof[1]

[1]*Department of Plant Biology, University of California Davis, Davis, CA 95616, USA*
[2]*Department of Statistics, University of California Davis, Davis, CA 95616, USA*
[3]*Max Planck Institute for Plant Breeding Research, Cologne, Germany.*

jmjimenez@ucdavis.edu

Wild tomato species are native to diverse habitats in South America and show wide morphological and ecological diversity that has proven useful in breeding programs. Our preliminary studies show that tomato species vary in their ability to detect neighboring vegetation via changes in light quality and trigger a shade avoidance response. The study of this response is critical for tomato as a crop since it has significant effects on development (e.g. increased elongation), and resource allocation (shifts resources from fruits to stems).

To better understand shade avoidance variation and transcript evolution in tomato, we obtained mRNA samples from different tissues, developmental stages and light conditions from *S. lycopersicum*, *S. pennellii*, *S. habrochaites* and *S. pimpinellifolium*. We used RNAseq to generate 50x to 150x coverage of the transcriptome of each species. We will present the results from our analysis of these reads assembled *de novo* and mapped to the available tomato genome. We have used a combination of well-established and custom methods to search for transcriptome-wide polymorphisms and gene variants. In addition, we have characterized whole-genome expression profiles in each species, tissue and in response to shade. Finally, we perform functional and evolutionary analysis of the polymorphisms found, and we correlate these polymorphisms with the expression profiles obtained to identify the genetic networks affected by the shade avoidance response in an evolutionary context.

OP7B.3

Plant transformation using DNA minicircles without vector backbone sequences

Anthony J. Conner, Julie M. Pringle, Annemarie S. Lokerse, Sathiyamoorthy Meiyalaghan, Jeanne M.E. Jacobs.

New Zealand Institute for Plant & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand
tony.conner@plantandfood.co.nz

Minicircles are supercoiled DNA molecules devoid of plasmid backbone sequences, formed as a product of in vivo excision by site-specific recombination. They can be induced to form in bacterial systems, but due to the absence of bacterial replication origins and selectable marker genes, they do not persist. Minicircles offer an important tool for effective delivery of cisgenes, intragenes and transgenes through transformation without the inadvertent integration of vector backbone sequences, an important limitation of current technology. The induction of minicircles from T-DNA regions in *Agrobacterium* immediately prior to co-cultivation of tobacco and potato leaf tissues allowed transformation from T-strands limited to a simple, well-defined expression cassette. The careful assembly of minicircle regions using only petunia or potato sequences, including plant-derived sequences for site-specific recombination and/or a T-DNA border, provided a means to assure the absence of foreign DNA during intragenic transformation using direct DNA update or *Agrobacterium*-mediated gene transfer.

OP7B.4

Construction of an intra-specific linkage map in eggplant and SNPs identification by next generation sequencing of RAD tags

L. Barchi[1,2], S. Lanteri[1], E. Portis[1], A. Acquadro[1], G. Valè[3], L. Toppino[2], Gl. Rotino[2]

[1]*DIVAPRA Plant Genetics and Breeding, University of Torino, 10095 Grugliasco (Torino), Italy*
[2]*CRA-ORL Unità di ricerca per l'Orticoltura, 26836 Montanaso Lombardo (Lodi), Italy*
[3]*CRA-GPG Genomic Research Centre, 29017 Fiorenzuola d'Arda (Piacenza), Italy*

lorenzo.barchi@unito.it

An anther-derived doubled haploid (DH) and an F_2 intraspecific mapping populations were developed by crossing the eggplant lines '305E40' and '67/3', the former carrying the *Fusarium oxysporum* resistance locus *Rfo-sa1*. Initially, 28 AFLP primer combinations were applied to 93 randomly chosen individuals of both populations, resulting in 170 polymorphic AFLP markers. In the DH population 117 of these markers were distorted, while in the F_2 population, segregation distortion was restricted to just ten markers. A set of 141 F_2 individuals was chosen for map construction and genotyped with AFLPs, SSRs, tomato RFLPs and CAPS markers linked to *Rfo-sa1*. The framework map consisted of 238 loci spanning 718.7cM. In order to develop further markers for map saturation, SNPs were identified using a Restriction-site Associated DNA (RAD) approach. RAD tags on Illumina platform from genomic DNA of the map parental lines were generated and sequenced on Illumina platform. Globally, around 16.67 Mb of *de novo* eggplant sequences were obtained resulting in 10,284 SNPs and 1,661 InDels. A subset of 2,435 SNPs were found as suitable for genotyping assays adopting the Golden Gate Technology (Illumina). For sequence annotation, a non-redundant *S. melongena* dataset was established, including 44,219 sequences (33,396 common between parents; 5,527 singlets from '67/3'; 5,296 singlets from '305E40'). BLAST search against TAIR9 and Cornell Unigene databases identified 11,670 sequences, clustering with 6,902 *Arabidopsis* loci and 17,817 sequences, clustering with 14,356 SGN unigenes respectively, indicating a high proportion of putatively expressed sequences. All the 11,670 *S. melongena* sequences were finally categorized under Gene Ontology categories.

OP7B.5

Eco-TILLING in tomato to unravel the hidden gifts of nature

Vijee Mohan, Soni Gupta, Mickey Hanjabam, Sherinmol Thomas, Charakana Chaitanya , Suresh Kumar Gupta, Vineeta Singh Chauhan, Reddaiah Bodanapu, Kapil Sharma, Supriya Sarma, Nongmaithem Sapana Devi, Kilambi Himabindu Vasuki, Yellamaraju Sreelakshmi, Rameshwar Sharma

School of Life Sciences, University of Hyderabad, Hyderabad-500046, India

rameshwar.sharma@gmail.com

Nucleotide sequence diversity is a measure of the genetic variation that is present in a species and is important because it is key to most phenotypic variations and can reflect evolutionary history. Single nucleotide polymorphisms (SNPs) represent the most common variations across a genome. SNPs have several uses in genetics, for example to detect alleles responsible for a trait of interest, inferences of population history, fingerprinting and generation of genetic maps etc. Eco-TILLING is a high throughput, low cost technique for rapid discovery of polymorphisms in natural populations by heteroduplex analysis using a mismatch-specific endonuclease. A collection of tomato accessions obtained from different sources like NBPGR (India), IIVR (India) and TGRC (California, USA) was analyzed for the frequency of naturally occurring SNPs. A set of genes which play key roles in various biochemical pathways controlling the growth and development of tomato, was selected for screening of SNPs, which include ACS-2, PHYA, PHYB1, COP1, ddb1, ZBF1, PSY1 and BETA. A number of SNPs were detected for different genes but with a varying frequency. The morphological features during different developmental stages and chemotypic observations indicated wide variations among the accessions. Correlating these variations with the location of the SNPs may throw light into some promising alleles for crop development.

Session 8a: Parallel Sessions – Tomato

OP8A.1

Tomato trichomes as a model system for exploring diversity within specialized secondary metabolism

Eliana Gonzales-Vigil[1], Jeongwoon Kim[2], Anthony Schilmiller[3], A. Daniel Jones[3,4], Robert Last[2,3], Eran Pichersky[5], Cornelius Barry[1]

[1]*Department of Horticulture, Michigan State University, East Lansing, MI, USA*
[2]*Department of Plant Biology, Michigan State University, East Lansing, MI, USA*
[3]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI,USA*
[4]*Department of Chemistry, Michigan State University, East Lansing, MI, USA*
[5]*Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*

barrycs@msu.edu

Trichomes are epidermal appendages that are present on the surfaces of many plant species that provide both physical and chemical protection to a variety of biotic and abiotic stresses including herbivore attack, pathogen infection, extreme temperature, and excessive light. Trichomes often possess glands at their tips that synthesize specialized secondary metabolites and are therefore referred to as storage/secreting glandular trichomes (SGTs). SGTs are the site of synthesis for several important compounds that have value as pharmaceuticals, fragrances, food additives, and natural pesticides. The SGTs of cultivated tomato (*Solanum lycopersicum*) and related wild species are anatomically diverse and each type produces a distinct set of specialized metabolites including terpenes, methylketones, methylated or glycosylated flavonoids, and acylsugars. Metabolite profiling of trichomes from a set of chromosome substitution lines created from a cross between *S. lycopersicum* and the wild relative *S. pennellii*, together with extensive profiling of *S. habrochaites* accessions reveals considerable diversity in both acylsugar and terpene production. This chemical diversity makes the tomato trichome an excellent model system for elucidating the biosynthesis of these specialized metabolites. Currently, we are utilizing a combination of comparative and functional genomics, genetics, and biochemistry to identify these pathways and the overall pattern of their diversification.

OP8A.2

APETALA2 functions in fruit development and in a ripening regulatory network together with *CNR*

Rumyana Karlova[1], Jacqueline Busscher-Lange[2], Alisdair Fernie[3], Thi Phuc Do[3], Paul Fraser[4], Charles Baxter[5], Gerco Angenent[1,2], Ruud de Maagd[2]

[1]*Laboratory of Molecular Biology, Wageningen University, Wageningen 6700 AP, Netherlands*
[2]*Business Unit Bioscience, Plant Research International, Wageningen 6700 AA, Netherlands.*
[3]*Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany.*
[4]*School of Biological Sciences, Royal Holloway, University of London, Egham TW200EX, UK.*
[5]*Syngenta, UK*

rumyana.karlova@wur.nl

APETALA2 (*AP2*) has been implicated as a major player in proper floral organ development, primarily through the specification of sepal and petal identity, and more recently in seed development. In this study, by down-regulation of expression through RNA interference, we show that the tomato *AP2* ortholog is necessary for fruit ripening and development. Tomato fruits with reduced *AP2* protein accumulation displayed alterations in shape and size, orange colored fully ripe fruits and changes in carotenoid accumulation. Microarray analyses of the ripe *ap2* fruits showed altered expression of genes in different pathways, mainly the phenylpropanoid, and carotenoid pathways, hormonal regulation as well as gene expression of several transcription factors and other ripening-associated genes. Interestingly, in the pericarp of these fruits mRNA levels of another transcription factor involved in tomato fruit ripening, *CNR*, were elevated. Moreover, we found that *CNR* directly binds to the promoter of *AP2* and positively controls its expression in tomato fruits. The two proteins were also able to interact with each other in plant cells *in vivo*.

OP8A.3

Searching for the genes involved in the production and release of flavour-related tomato fruit volatiles

I. Romero de la Fuente, Y. Tikunov, A. van der Veer, J. Molthoff, A. van Houweling, R. de Vos, A. Bovy

Plant Research International, Wageningen, The Netherlands

irene.romero@wur.nl

The flavour of tomato fruit comprises sugars, organic acids, free amino acids and volatile compounds. Although over 400 volatiles have been identified in tomato fruits, only a limited number are considered essential to tomato flavour according to their concentration and odour threshold. We screened red-ripe tomato fruits for variation in their volatile profile using a collection of 94 tomato cultivars, representing the current diversity within the commercial germplasm. In that study, three phenylpropanoid (PhP) volatiles, methyl salicylate, guaiacol and eugenol, were found to be discriminatory within this germplasm collection and roughly divided the cultivars into two groups. Some genotypes are able to release PhP volatiles upon disruption of fruit tissue through cleavage of the corresponding glycoconjugates, identified putatively as hexose-pentosides. However, in certain genotypes, phenylpropanoid volatile emission was arrested due to the conversion of the corresponding hexose-pentoside precursors into glycoconjugate species of a higher complexity: dihexose-pentosides and malonyl-dihexose-pentosides. These results indicated that genes encoding glycosyl- and malonyltransferases play an important role in determining the release of these phenylpropanoid volatiles. By combining genetic, transcriptomics and genomics data, several potential candidate genes were identified. Our currents efforts focus on functional characterisation of these genes using reverse genetics approaches. In this way, candidate genes for several steps in the phenylpropanoid volatile conjugation pathway have been obtained.

OP8A.4

Phototropin1: A new player in regulating the shelf life of tomato fruits

Sulabha Sharma, Himabindu Vasuki, Eros Kharshiing, Rameshwar Sharma, Yellamaraju Sreelakshmi

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad-500 046, India

syellamaraju@gmail.com

More than 130 years ago, Darwin first described the directional movement of plants towards light, and current molecular evidence indicates that phototropins 1 and 2 mediate this phenomenon. Among the plant photoreceptors, both phytochrome and cryptochrome have been shown to regulate the pigmentation of tomato fruits. We now report that apart from mediating chloroplast movements, phototropism, leaf movements and stomatal opening, phototropin also regulates pigmentation and shelf life of tomato fruits. We isolated a tomato NPS1 (nonphototropic shoot) mutant, which harbours a lesion in the PHOT1 gene. Analysis of fruit development in *Nps1* mutant showed that though the fruits of the mutant were pale green at mature green stage, on ripening they turned dark red and exhibited significantly higher lycopene content than the wild type fruits. At the same time, no significant changes in the β-carotene content were observed in the mutant fruits. Apart from the delay in fruit color development, the fruits of *Nps1* mutant also retained their firmness for a longer period both on-vine as well as off-vine. Gene expression analysis revealed a differential expression of senescence related genes in the mutant fruits. Our results implicate a yet unreported role for phototropin in regulating fruit ripening in tomato and provide potential molecular target(s) for further genetic manipulation towards improving fruit quality and shelf life.

OP8A.5

The Tomato GAME1 Glycosyltransferase is Involved in the Metabolism of Steroidal Glycoalkaloids

Maxim Itkin[1], Ilana Rogachev[1], Noam Alkan[3,] Linda Zamariola[2], Sergey Malitsky[1], Tally Rosenberg[4], Laura Masini[2], Ric de-Vos[2], Dov Prusky[3], Saul Burdman[4], Jules Beekwilder[2] and Asaph Aharoni[1]

[1]*Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel*
[2]*Plant Research International, 6700 AA Wageningen, The Netherlands*
[3]*Agricultural Research Organization - the Volcani Center, Bet Dagan 50250, Israel*
[4]*Faculty of Agricultural, Food, and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.*

max.itkin@gmail.com

Glycoalkaloids, members of the triterpene class of compounds, typically posses a sterol skeleton of six nitrogen containing heterocyclic rings with one or more carbohydrate residues, attached to the 3-OH group of the aglycone. They play an important role in the plant defence against pathogens and herbivores, and are also known to have various pharmaceutical activities. Glycosylation of the glycoalkaloid molecule is known to be an important modification for their bioactivity against various plant pathogens. To identify genes associated with glycolalkaloid metabolism in the Solanaceae family we investigated the tomato *GAME1* gene that putatively encodes a glycoalkaloid-glycosyltransferases. Silencing of *GAME1* resulted in up to 50% reduction in the principal tomato glycoalkaloid, *α*-tomatine. Surprisingly, transgenic plants silenced for *GAME1* displayed a severe morphological phenotype, including growth retardation, abortion of fruit set, and development of necrotic regions on various plant parts. Silencing of *GAME1* also resulted in a dramatic change to morphology of the plastids which contained enlarged, abnormal plastoglobules. The same plants also exhibited a significant increase in levels of the *α*-tomatine aglycone, tomatidine. Since tomatidine was previously shown to interfere with sterol biosynthesis we profiled various intermediates of the sterol pathway. These experiments showed a clear alteration in the sterol profile of the *GAME1* silenced plants. Thus, our study points to the importance of glycosylation in avoiding toxicity to the plant cell on one hand while increasing toxicity to pathogens on the other. For metabolic engineering experiments, it provides a good example for the consequences of altering the glycosylation pattern of secondary metabolites that could result in accumulation of toxic, intermediate aglycones.

Session 8b: Parallel Sessions – Potato

OP8B.1

Potato genetic engineering: Adapting existing varieties to the emerging needs of the 21st century

Caius M Rommens

JR Simplot Company, Boise ID 83706, USA

Caius.Rommens@simplot.com

The spread in the production of potatoes from small regions in the Andean highlands to 19 million ha worldwide coincided with, and enabled, in part, the unprecedented population growth and urbanization of the 18th and 19th century. However, issues in traditional plant breeding have hampered efforts to meet evolving needs for agricultural sustainability, and currently available varieties lack many of the agronomically important traits needed to control farm input costs. Shifting consumer preferences, from high calorie diets to foods that are rich in phytonutrients, have also resulted in a decline in the consumption of potato. The easiest and fastest way of adapting existing varieties to the needs of the 21st century is offered by genetic engineering. Currently available input traits provide resistance against some of the most important pathogens, pests, and herbicides, as well as black spot bruise. Additional quality traits enhance the antioxidant power of potato while lowering their toxin-forming potential. In many cases, it is possible to improve existing varieties by transforming them with native DNA only. Consumer surveys indicate that consumers prefer such “intragenic” approaches over conventional transgenic methods that introduce foreign DNA into the food supply. Several genetically modified potato varieties are currently being evaluated for commercial food production, both in the United States and Europe. New biotechnology quality management systems have been developed to ensure that this regulated material remains segregated from traditionally-bred varieties. Subsequent implementation of additional Identity Preservation systems is needed to avoid the development of international trade issues after launch.

OP8B.2

Sequence tag based transcriptome analyses are providing valuable insight into the molecular responses underpinning differential yield as well as biotic and abiotic stress responses of different cultivars

Kåre L. Nielsen, Mads Sønderkær, Sanne Hedegaard, Sireesha Dommaraju, Kacper Kaminski, Hanne Grethe Kirk, Annabeth Høgh Petersen

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Aalborg, Denmark.

kln@bio.aau.dk

Potato is a space efficient crop with more than twice the energy yield per area unit compared to cereals and is therefore an important crop to consider when trying to meet the increasing demand for food and renewable precursors for chemicals, pharmaceuticals and energy production in the future. However, presently potato production is primarily limited by economic and environmental cost of pest management. Therefore in order to realize the cost-effective future production of potatoes, superior cultivars must be produced either by traditional breeding or by GMO. Rationalizing that because existing elite cultivars display phenotypic differences with respect to yield, disease and drought resistance there must be underlying genotypic differences that are reflected in the transcriptome. To identify such differences, we set up 14 cultivars in disease, drought yield trials and collected plant material in time series. RNA was isolated from the more than 1200 samples and subjected to DeepSAGE tag-based transcriptome analysis. Following extensive data analysis, novel candidate genes for disease resistance, drought/yield relationship and yield have been identified.

OP8B.3

An integrative -omics approach for studying potato tuber quality traits

Bjorn Kloosterman[1], Animesh Acharjee [1,2,4], Chris Maliepaard[1,4], Ric de Vos[3,4], Christian Bachem[1,4], Richard GF Visser[1,4]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Center, POBox 386, 6700 AJ Wageningen, The Netherlands
[2]Graduate School Experimental Plant Sciences;[3]Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands;[4] Centre for BioSystems Genomics, P.O. Box 98, 6700 AA, Wageningen, The Netherlands

bjorn.kloosterman@wur.nl

Utilization of the natural genetic variation in traditional breeding programs remains a major challenge in crop plants. The development of high throughput technologies has brought the potential of population wide data collection in fields like transcriptomics, metabolomics and proteomics. As a result, large amounts of data have become available which need to be tested for association with observed trait variation. We have screened a diploid potato population for gene-expression using a microarray approach, and secondary metabolite content using LC-MS. Variation within the data was treated as a quantitative trait and resulted in the identification of many expression and metabolite quantitative trait loci (eQTL and mQTL, respectively). Through the combined efforts of the PGSC the potato genome sequence has now become available and genes can be directly mapped on the different linkage groups which allows the analysis of cis- and trans-acting transcriptional control. In addition, we present approaches to study the various '~omics' datasets to allow the construction of networks integrating gene expression, metabolites and phenotypic data. We used univariate regression and modern regression methods to select subset of the metabolites and transcripts that showed association with potato tuber flesh colour. Network reconstruction after data integration can then be used to visualize a pathway of individual components associated with a trait of interest.

OP8B.4

Progress in our understanding of carotenoid accumulation in potato tubers

Raymond Campbell[1], Gavin Ramsay[2], Glenn Bryan[2], Mark Taylor[1]

[1]*Plant Products and Food Quality Programme, SCRI, Dundee, UK*
[2]*Genetics Programme, SCRI, Dundee, UK*

raymond.campbell@scri.ac.uk

Despite extensive studies characterising the biosynthetic genes involved in the carotenoid pathway little is known about the mechanisms regulating carotenoid accumulation in non-green tissues. The aim of this study is to identify and characterise the key genes and regulatory mechanisms affecting the accumulation of tuber carotenoids in two F_1 diploid populations segregating for tuber carotenoid content using a combined genetic and molecular approach.

Genetic maps have been constructed for both populations with two major QTL affecting the overall tuber carotenoid content identified on chromosomes 3 and 9 respectively. A β-carotene hydroxylase gene, crtR-b2, was found to underpin the QTL identified on chromosome 3 and the underlying mechanism characterised. To date, no known carotenoid biosynthetic gene has been mapped to chromosome 9. Major QTL affecting levels of individual carotenoids such as β-carotene were also identified.

Additional candidate genes for tuber carotenoid content have been identified in a series of microarray experiments, analysing samples bulked according to individual carotenoid traits. Transgenic potato plants have been developed silencing the best candidate gene, a carotenoid cleavage diooxygenase, (CCD4), resulting in increased levels of tuber carotenoids and unexpected effects on tuber morphology that mimic a heat sprouting phenotype.

OP8B.5

Construction and utilization of a dense genetic map to anchor and organize the potato genome

Gisella Orjeda, Potato Genome Sequencing Consortium

Universidad Peruana Cayetano Heredia

gisella.orjeda@upch.edu.pe

The potato genome of a doubled monoploid of *Solanum phureja* (DM) was sequenced by the PGSC using a mixture of WGS-NGS technologies and Sanger sequencing. In order to anchor and orient DM super- scaffolds, a genetic map was developed *de novo* using a population of 182 individuals derived from an F_1 of the cross DM and DI (*Solanum gonioacalyx*), backcrossed to DI. The map was built using several types of sequence-tagged-site (STS) markers: i) Microsatellites developed from scaffold sequences of the DM assembly, ii) DArT markers developed from several potato genotypes, including the parents; DArTs were further sequenced and located in the DM super¬scaffolds, iii) SNP markers were searched directly in the DM assembly, and iv) Markers and genes of previously known chromosome locations that were either screened in the backcross population or located *in silico* by computational methods. The fourth group included previously published microsatellites from various genetic maps and P450 superfamily gene members previously mapped to linkage groups. We have analysed a total of 4836 markers including 2174 DArTs, 358 SSRs and 2304 SNPs in the population. The data from 2603 polymorphic STS markers comprising 1881 DArTs, 393 SNPs and 329 SSR alleles was analysed using JoinMap4 and yielded the expected 12 potato linkage groups. The total map length is 923.3 cM. This map has been used to order and orient the sequence of the potato genome.

Session 8C: Parallel Sessions – Pepper, Tobacco, Eggplant, Petunia

OP8C.1

A Genetic Map for *Nicotiana tabacum* Based on an F2 from Red Russian X Hicks Broad Leaf

Gregor Bindler[1], Nicolas Bakaher[1], Paolo Donini[1,] Martin Ganal[2], Nikolai Ivanov[1], Jörg Plieske[2].

[1]*Philip Morris International, Research and Development Department, CH-2000, Neuchàtel, Switzerland.*
[2]*TraitGenetics GmbH, 06466 Gatersleben, Germany*

gregor.bindler@pmintl.com

Nicotiana tabacum L. is an amphidiploid species (2n = 48) with composed of the genomes S and T derived from a hybridization event between *Nicotiana sylvestris* Spegazzini and Comes, and *Nicotiana tomentosiformis* Goodspeed. Progress on the development of molecular markers and the construction of a genetic map has been slow in *Nicotiana tabacum,* which is holding back research on genome structure and the molecular basis of tobacco's agronomic and chemical properties. Currently, only a few incomplete linkage groups have been characterized, particularly those surrounding disease resistance genes of interest. We describe a genetic map for *Nicotiana tabacum* based on Simple Sequence Repeat markers (SSRs). Data generated as part of the Tobacco Genome Initiative were used to identify microsatellite-containing DNA sequences that might be useful for development of SSR markers and that might be polymorphic between tobacco genotypes. 5119 microsatellites were identified in both coding and non-coding sequence from EST and genomic sequence data. PCR primers were designed to amplify PCR fragment between 100 and 300 base pairs to allow for good separation of fragments on most sequencing platforms. Subsequently, the loci were tested for the presence of polymorphisms in a panel of 16 tobacco varieties, among which Red Russian (RR) and Hicks Broad Leaf (HBL, a Flue Cured tobacco type). 2317 SSR markers polymorphic between the two parents were subsequently mapped on 186 F2 individuals from a RR X HBL cross.

OP8C.2

The survey of Natural variations and EMS induced mutations in *Capsicum*

Heejin Jeong, Jin-Kyung Kwon, JeeNa Hwang, Hoang Ngoc Huy, JoongHwan Bae, Byoung-Cheorl Kang

Department of Plant Science, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

chungo30@snu.ac.kr

Allele mining is a method to find undiscovered natural variation or genetic materials in a plant. The application of allele mining in plant breeding has been increased for identification of genetic variation. High throughput methods are required to expedite the identification of novel alleles in large numbers of germplasm accessions. Here we describe high resolution melting (HRM) method to detect natural variation and EMS induced mutation in *Capsicum*. We have scanned single polymorphic mutation in a specific fragment of the pvr1 gene. Fifteen allelic variations in a total of 249 germplasm were obtained and pvr HRM13 allelic variation was confirmed to be resistant to TEV-HAT strain. In addition, five single polymorphic mutations induced by EMS were also found in the pvrHRM gene fragment using HRM. These results demonstrate that HRM allows the rapid identification of new allelic variants in both natural and artificial mutant population. Therefore, this strategy can be performed for the identification of molecular diversity of agriculturally important genes.

OP8C.3

Diversity of phenolic content in Asian eggplant landraces and near wild relatives

Rachel Meyer[1], Bruce Whitaker[2], Amy Litt[3]

[1]*City University of New York, Graduate Center / New York Botanical Garden, Biology, 365 Fifth Avenue, New York, NY, 10016, USA*
[2]*USDA Agricultural Research Service, Produce Quality and Safety, 10300 Baltimore Avenue, Bldg 002 Barc-West, Beltsville, MD, 20705, USA*
[3]*The New York Botanical Garden, 200th St and Southern Boulevard, Bronx, NY, 10458-5126, USA*

rmeyer@nybg.org

Phenolic compounds in fruits and vegetables are a prominent focus in food chemistry due to their perceived health-beneficial properties and flavor. In eggplant (*Solanum melongena*), a crop with diverse medicinal and culinary uses, considerable variation in phenolic content and composition has been observed among fruits of different varieties. Using a germplasm collection of over 200 Asian eggplant landraces and closely-related species (including the putative progenitors, *S. incanum* and *S. undatum*), 80 genetically and ethnobotanically diverse accessions were chosen for evaluation of phenolic constituents. We used high performance liquid chromatography to generate profiles of hydroxycinnamic acid (HCA) derivatives, the most abundant class of phenolics in eggplant, for fruits representing each of these chosen accessions. Results show greatest variation in phenolic profiles between species, followed by variation among accessions collected from different geographical regions of Asia. Several accessions were found to possess substantial amounts of certain potentially health-beneficial HCA derivatives, and several profiles included relatively high concentrations of one or more unknown HCA conjugates. Results of these phytochemical analyses are being correlated with ethnobotanical attributes of accessions as well as the phylogeographic relationships among accessions. We aim to address how regional selection for different eggplant landraces may have contributed to variation in the biosynthesis and accumulation of potentially desirable phenolic compounds.

OP8C.4

PhMYB4 Fine-Tunes the Floral Volatile Signature of Petunia x hybrida

Thomas A. Colquhoun, Joo Young Kim, Ashlyn E. Wedde, Laura A. Levin, Kyle C. Schmitt, David G. Clark

Department of Environmental Horticulture and Plant Molecular and Cellular Biology, University of Florida, University of Florida, USA

ucntcme1@ufl.edu

In *Petunia x hybrida* cv "Mitchell Diploid" (MD), floral volatile benzenoid/phenylpropanoid (FVBP) biosynthesis is thoroughly controlled spatially, developmentally, and daily at molecular, metabolic, and biochemical levels. Multiple genes have been shown to encode proteins that either directly catalyze a biochemical reaction yielding FVBP compounds, or are involved in metabolite flux prior to the formation of FVBP compounds. Since the transcription of these genes are coordinately regulated, and few transcription factors have been identified we hypothesized multiple transcription factors are involved in the precise regulation of all necessary genes ultimately resulting in the specific volatile signiture of MD flowers. After acquiring all available petunia transcript sequences with homology to *Arabidopsis thaliana* R2R3-MYB transcription factors, PhMYB4 (named for close identity to AtMYB4) was identified, cloned, and characterized. PhMYB4 transcripts accumulate to relatively high levels in floral tissues after anthesis and throughout open flower stages, which coincides with the spatial and developmental distribution of FVBP production and emission. Upon RNAi suppression of PhMYB4 (ir-PhMYB4) both petunia CINNAMATE-4-HYDROXYLASE (PhC4H1 and PhC4H2) gene transcript levels were significantly elavated, ultimately leading to elavated levels of "downstream" FVBPs (isoeugenol and eugenol emission) compared to MD. Together, these results indicate that PhMYB4 and AtMYB4 have a similar molecular function in repression of C4H transcription. However, AtMYB4 indirectly controls the accumulation of UV-B sunsreens in *Arabidopsis* leaf tissue, while PhMYB4 indirectly controls the balance of FVBP production in petunia floral tissue.

OP8C.5

Phylogenomic Analysis of Cultivated Tobacco

A. Bombarely Gomez[1], K.D. Edwards[2], S.A. Coates[2], L.A. Mueller[1].

[1]*Boyce Thompson Institute for Plant Research, Tower Rd, Ithaca, NY, USA 14853-1801*
[2]*Advanced Technologies (Cambridge) Ltd, 210 Cambridge Science Park, Cambridge, UK.*

ab782@cornell.edu

Polyploidization events are a major specialization mechanism, which is very common in the plant kingdom but not well understood. *Nicotiana tabacum* (2n=4x=48) is an allotetraploid species that originated from the hybridization of the ancestors of *N. sylvestris* (2n=2x=24, section Alatae as S-genome donor) and *N. tomentosiformis* (2n=2x=24, section Tomentosae as T-genome donor) around 0.2 Myr ago. In this project, the leaf transcriptomes of these three species were sequenced and assembled, producing three species specific assemblies with 25,290, 35,870 and 29,473 contigs, respectively, and a total of 41,221 contigs for an assembly combining the sequences of all three species. A phylogenetic analysis pipeline was developed to analyze the relationship of the *N. tabacum* genome to its two putative parental genomes. Alignments containing at least one representative of each species were used to calculate the phylogenetic trees for each set of putatively ortholog sequences. Around 11,100 contigs were analyzed, which were categorized into different groups. Analysis of the phylogenetic trees revealed that most *N. tabacum* sequences have close orthologs in both parental species. A group of highly conserved sequences was identified, in which the trees could not be resolved due to high sequence similarity. Another group of clusters were composed by contigs with a common branch for *N. sylvestris* and *N. tomentosiformis*, showing bigger differences between *N. tabacum* and its genome donors than between the parents closest relatives. The implications of these results for the evolution of tetraploids will be discussed.

Session 9: Metabolomics/Proteomics

OP9.1

The chemical composition underlying Brix

Sonia Osorio[1], Roni Tadmor[2], Dani Zamir[2], Alisdair R. Fernie[1]

[1]*Max-Planck-Institut fur Molekulare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Golm, Germany*
[2]*The Hebrew University, Faculty of Agriculture, Rehovot, Israel.*

osorio@mpimp-golm.mpg.de

Tomato constitutes the most produced and consumed vegetables worldwide and also represents an important source of fiber and nutrients in the human diet. Furthermore it is a central model for the study of fruit biology. In order to delimit the metabolic constituents exerting the greatest influence on Brix (the total soluble solid content) and to elucidate the genetic basis, underlying this trait we phenotyped 150 accessions from EU-SOL core collection representative from low to high Brix. These plants were grown in the open field as well as in the greenhouse. The accessions were analyzed on the basis of fruit weight, Brix and GC-MS-based metabolite profiling. Results and future strategies will be discussed.

OP9.2

TAGL1 and ORR Impinge on Ripening and Carotenoid Metabolism in Tomato Fruit

Asaph Aharoni

Department of Plant Sciences, The Weizmann Institute of Science, Rehovot, 76100, Israel

asaph.aharoni@weizmann.ac.il

Ripening of fleshy fruit is accompanied by tremendous metabolic changes, in both central as well as secondary/specialized metabolism. We are interested in understanding how these changes are controlled in space and time during fruit development. The induction of ripening through ethylene signaling and it's biosynthesis pathway are key elements in controlling fruit metabolism. Recently, a functional screen of tomato transcription factors showed that silencing of the *TOMATO AGAMOUS-LIKE 1* (*TAGL1*) MADS-box gene results in altered fruit pigmentation. Overexpressing *TAGL1* as a chimeric repressor suggested its role in controlling ripening, as transgenic tomato fruit showed reduced carotenoids and ethylene levels, suppressed chlorophyll breakdown and downregulation of ripening-associated genes. Moreover, fruits overexpressing *TAGL1* accumulated more lycopene, while their sepals were inflated, accumulated high levels of the yellow flavonoid naringenin chalcone and contained lycopene. Examination of TAGL1 transcript and its overexpression in the *rin* mutant background pointed to RIN-dependant and -independent processes that are regulated by TAGL1. The results added a new component to the current model of the regulatory network that controls fleshy fruit ripening and its association with the ethylene biosynthesis pathway. In a related study, we show by the characterization of Orr^{Ds}, a dominant transposon-tagged tomato mutant deficient in the NDH-M subunit, that the NADH dehydrogenase (Ndh) complex is also essential for the fruit ripening process. In higher plants the plastidial Ndh complex supports non-photochemical electron fluxes from stromal electron donors to plastoquinones. Alteration to the NDH complex in fruit changed the climacteric, ripening-associated metabolites and transcripts as well as fruit shelf life. Metabolic processes in chromoplasts of ripening tomato fruit were affected in Orr^{Ds} as mutant fruit were yellow-orange and accumulated substantially less total carotenoids, mainly *β*-carotene and lutein. The changes in carotenoids were largely influenced by environmental conditions and accompanied by modifications in levels of other fruit antioxidants, namely flavonoids and tocopherols. These studies therefore pave the way for further studies on the role of redox and the transcriptional control network in the regulation of fruit ripening and its associated metabolism.

OP9.3

apricot (at/at): A Novel Ripening Regulator Controlling Antioxidant Accumulation in *Solanum lycopersicum* (cv. Ailsa craig)

Ryan P. McQuinn[1], James J. Giovannoni[2], Jocelyn Rose[1], Alisdair Fernie[3], Dani Zamir[4], Johannes Rohrmann[3], Takayuki Tohge[3]

[1]*Cornell University - Department of Plant Biology*
[2]*USDA-ARS, Cornell University, Boyce Thompson Institute*
[3]*Max Planck Institute of Molecular Plant Physiology*
[4]*The Hebrew University of Jerusalem.*

rpm28@cornell.edu

Tomato undergoes a complex array of alterations in the flux of many metabolic pathways impacting color, texture, aroma, flavors and pathogen susceptibility. Many of these characteristics are important to seed dispersing organisms and consumers. Specific to nutritional quality are carotenoid, phenylpropanoid, and ascorbic acid biosynthetic pathways. Despite extensive research targeted to increase understanding of the regulation of these pathways during fruit ripening, many questions remain. We performed carotenoid accumulation assessment of 32 different fruit color mutants, with a focus on carotenoid profiles and expression of pathway genes. Our results suggest some mutants possess unique carotenoid profiles and may represent more complex ripening defects. The apricot mutant was selected for further investigation due to its intense orange color despite minimal carotenoid accumulation in the ripe fruit, as well as its epistatic effects on the accumulation of ascorbic acid in an apricot; highpigment-1 double mutant. Microarray results showed that this mutation not only caused an alteration in the carotenoid pathway, more specifically by the down-regulation of isopentyl pyrophosphate isomerase, but also had a wide range of effects on other antioxidant pathways (e.g. phenylpropanoid and ascorbic acid biosynthesis). The apricot mutation creates an altered flux in the phenylpropanoid pathway leading to an increased production of naringenin chalcone within the epidermis in ripening fruit. Our work provides evidence that apricot is not solely a carotenoid specific mutation, rather a mutation in a more general regulator of tomato fruit ripening.

OP9.4

Connecting the Solaneceae genome to the metabolic networks via SolCyc and MetaCyc

Anuradha Pujar, Ron Caspi, Naama Menda, Isaak Tecle, Aureliano Bombarely-Gomez, Peter Karp, Lukas Mueller

Boyce Thompson Institute, Cornell University, Ithaca, NY and Bioinformatics Research Group, SRI International, Menlo Park, CA

lam87@cornell.edu

The metabolic information of the Solanaceae family is rapidly increasing and large numbers of pathways are being characterized, simultaneously high throughput studies on their metabalome and transcriptome are also being done. The availability of the Tomato genome sequence provides leverage to connect disparate metabolic information to the gene sequence, genetic resources and phenotypes. At the Solanaceae Genomics Network (SGN) (www.solgenomics.net), an interactive platform was created that interfaces metabolic data with genomic and other kinds of information, based on Pathway Tools Software, MetaCyc, and annotated data from SGN. MetaCyc (www.metacyc.com) is a metabolic encyclopedia of experimentally validated biochemical pathways curated from the scientific literature. Although MetaCyc covers all organisms, there is an emphasis on plant and microbial pathways. SolCyc (www solgenomics.net/tools/solcyc/) is a collection of Pathway Genome Databases, developed for the clade oriented Solanceae Genomics Network database. It has predicted metabolic pathway databases of significant Solanaceae species and includes Lycocyc (tomato), SolaCyc (eggplant), NicotianaCyc (tobacco), PetuniaCyc (Petunia), CapCyc (Capsicum) and PotatoCyc (potato). Annotation of pathways includes enzymatic data such as substrate specificity, kinetic properties, activators, inhibitors, cofactor requirements, genes if cloned and links to external databases. In addition curators provide concise, review-level summaries and extensive literature citations. Newly curated metabolic pathways of Solanaceae include; flavonoid and wax metabolism of Tomato fruit surfaces, tropane alkaloid pathways of *Solanum* species, GABA shunt metabolism during fruit ripening, tomato steroidal glycoalkaloid pathway, and secondary metabolite biosynthesis in Solanaceae trichomes.

OP9.5

“Genetical proteomics” to tomato fruit quality

Mireille Faurobert, Rémy Aurand, Emad Konozy, David Page, Jean-Paul Bouchet, Mathilde Causse.

INRA, UR1052, Unité de Génétique et Amélioration des Fruits et Légumes, Domaine St Maurice BP 94 – 84143 Montfavet Cédex France, Tél : 04 32 72 27 00, Fax : 04 32 27 02

Mireille.Faurobert@avignon.inra.fr

The organoleptic quality of tomato fruit is a complex characteristic involving a set of components such as fruit size, flavour, aroma and texture. Many of these traits exhibit complex quantitative genetic basis and are dependent on environmental conditions during fruit growth but also during postharvest period. We are developping a proteomic analysis of parental and QTL near isogenic lines in order to identify candidate proteins involved in the genetic variation of fruit sugar and texture traits.

We will sum up the main results we obtained studying tomato fruit proteome especially during tomato fruit development and storage. Some candidate proteins for size and sugar QTL have been detected and their genetic polymorphism has been characterized within a tomato collection. The pericarp proteomes of contrasted QTL lines for firmness have been compared during cold storage and networks of proteins related to temperature stress resistance will be proposed. Finally, we explored the fruit cell wall compartment and identified the main soluble and loosely bound cell wall proteins for several texture contrasted genotypes. Our proteomic database (SOLstIS : http:///w3.avignon.inra.fr/solstis) that puts together our experiments, gels and protein mass spectrometry data in a web accessible manner, will be presented.

Session 10: Functional Genomics and Systems Biology

OP10.1

Elucidation of biosynthetic pathway of secondary metabolism for design of metabolomics-assisted breeding approaches

Takayuki Tohge[1], Pierre Frasse[2], Patrick Giavalisco[1], Adriano Nunes-Nesi[1], Lothar Willmitzer[1], Mondher Bouzayen[2], Alisdair R. Fernie[1].

[1]*Max-Planck-Institut fur Molekulare Pflanzenphysiologie, 14476 Potsdam/Golm, Germany*
[2]*Université de Toulouse, INP-Toulouse, Génomique et Biotechnologie des Fruits, Avenue de l'Agrobiopole BP 32607, Castanet-Tolosan F-31326, France*

tohge@mpimp-golm.mpg.de

The approach of metabolomics-assisted breeding is systematic strategy for introducing high quality traits on the basis on information of natural variance of metabolite accumulation. Recently, QTL analysis of tomato introgression lines has been successfully applied in the analysis of important traits associated with primary metabolism. Since *S. lycopersicum* can easily be crossed with many of its wild species relatives, such exotic germplasm represents a valuable source for the improvement of agriculturally important traits.

In higher plants, flavonoids and phenylpropanoids play important roles in many biological processes such as pigmentation of fruits and vegetables, plant-pathogen interactions, protection against high light, high salt condition and chilling. On the other hand, these polyphenolic compounds are an integral part of the diet and there are increasing reports that dietary polyphenols are likely candidates for the observed beneficial effects of a diet rich in fruits and vegetables on the prevention of cardiovascular diseases and some other chronic diseases. In order to investigate the possibilities of engineering phenolic compounds in tomato, we have focused on high antioxidant flavonoids which are novel for *S. lycopersicum*.

An LC-MS and GC-MS based global metabolite profiling and microarray analysis were combined to allow comparisons between the relative metabolic levels of leaves, stems, flowers and fruits of *S. lycopersicum* and seven wild species tomato that can be crossed with it. We will discuss similarities of differences between the levels of variance observed between the different metabolite classes for the purpose of metabolomics-assisted breeding.

OP10.2

A fruit specific vin1 gus::gfp fusion reveals interspersed tomato fruit cells with an activated carbohydrate metabolism program

L. Hueso L, E. O'Connor, D. Orzaez D, A. Granell

Fruit Quality and Biotechnology Lab, IBMCP (CSIC-UPV), Spain.

agranell@ibmcp.upv.es

The tomato is a model for fleshy fruit development and ripening. Here we report the identification of a novel unique pattern of expression that was detected in transgenic tomato lines carrying a GFP GUS driven by a fruit specific VIN1P. The VIN1 promoter sequence confers a biphasic pattern of expression with a second phase clearly associated to fruit ripening. At the tissue/cellular level however, and in contrast to other fruit ripening promoters, VIN1 drives a 'salt and pepper' pattern of expression resulting from individual cells exhibiting a range of expression levels surrounded by cells with no expression. Activity is detected across different cell types with a higher preference for vascular, subepidermal layer and the inner part of the fruit. At the single cell level expression shows neither association with increased ripening nor with cell size, and only the number of cells with active promoter increases with ripening. Expression analysis of FACS-sorted VIN1 promoter active cells indicates they correspond to a transcriptionally distinct subpopulation of cells defined by increased expression of genes related to sucrose metabolism, and decreased activity in protein synthesis and chromatin remodelling. These findings suggest that local micro-heterogeneity may underlie some aspects of the otherwise apparently more uniform ripening program.

OP10.3

Putting omics tools to work: Unraveling the complex trait of seed quality in tomato by genetical genomics

Wilco Ligterink, Rashid Kazmi, Noorullah Kahn, Leo Willems, Henk Hilhorst

Laboratory of Plant Physiology, WUR, Wageningen, Netherlands

wilco.ligterink@wur.nl

The yield and economic success of horticultural crops depend to a large degree on the quality of the seed used to grow these crops. Seed quality attributes include dormancy, germination, seed and seedling vigour, seedling weight, normal embryo- and seedling morphology, as well as the ability to develop into a normal plant. The molecular-genetic dissection of the processes that underlie these quality parameters and their relationship with seed and seedling phenotypes will identify the regulatory genes and signaling pathways involved and, thus, provide the means to predict and enhance seed and seedling quality.

Our aim is to elucidate the mechanisms involved in the acquisition of seed quality and to develop molecular markers to aid in marker assisted breeding. To reach this aim we make use of the natural variation found in tomato RIL and IL populations. Besides extensive phenotyping, the RILs will also be profiled by transcriptomics (eQTL study) and have already been used for an mQTL study (metabolomics). In this way we could identify interesting QTLs for a wide range of primary metabolites, some of which collocate with physiological QTLs. The combined use of physiology, genetics and several omics technologies, followed by advanced data analysis, will allow the construction of regulatory networks involved in the different aspects of seed quality. This will allow us to relatively quickly identify genes that are responsible for seed quality related traits. Subsequent analysis of the relevant genes by reverse genetics, using knock-out and overexpression mutants will be employed to unambiguously confirm their function.

OP10.4

The dynamic transcriptomic and epigenetic landscape during tomato fruit development

Silin Zhong, Zhangjun Fei, Jim Giovannoni

Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York, 14853, USA.

sz284@cornell.edu

One of the prime objectives of tomato functional genomics is to understand the signaling networks and molecular mechanisms regulating fruit development. Toward this goal, we have generated a comprehensive time-series transcriptome profile of tomato fruit by ultra-deep sequencing. The strand-specific RNA-Seq approach, developed on the Illumina platform, enabled us to distinguish and capture the dynamics of mRNAs, anti-sense RNAs and nascent non-coding RNAs in tomato fruit. We then sought to understand the complex regulatory network of fruit development by breaking it down into smaller pathways. This was achieved by comparing the transcription profile of mutants with defects in fruit development and by investigating gene expression in response to ethylene. In order to further illustrate how genes are differentially regulated at different developmental stages and in different tissues, we hypothesized that tissue specific epigenetic marks on the chromatin could result in such differences in gene expression. Hence, we mapped the repressive and active chromatin modifications in the tomato genome using ChIP-Seq (chromatin immunoprecipitation followed by deep sequencing). Indeed, we have observed that loci encoding key regulators of fruit development are subjected to tissue-specific histone (de)methylation. These data not only provide us with a glimpse of the tomato transcriptome and epigenome, but also reveal how their interplay could determine plant organ development.

OP10.5

Integration of short RNA, mRNA and degradome profiling during fruit development

Tamas Dalmay

University of East Anglia, Norwich, UK

t.dalmay@uea.ac.uk

Short non-coding RNAs are involved in one of the most recently discovered gene expression regulation mechanism, gene silencing. The short RNA pathways are particularly complex in plants due to diversification of genes playing roles in the biogenesis of short RNAs. Our aim is to decipher the role of short RNAs in fruit development and ripening. To achieve this we profiled short RNAs of MicroTom at ten time points between flowering bud and ripened fruit. Analysis of the profiles includes identifying known and new microRNAs (miRNAs) and other classes of short RNAs and selecting differentially expressed (DE) short RNAs. DE short RNAs with similar profile were also grouped into clusters. We also identified targets of miRNAs at a genomic scale using the "degradome" approach. Then we compared the expression profiles of miRNAs and their targets following mRNA expression analysis at the same ten time points using Afymmetrix arrays. Some miRNA/target pairs showed the expected negative correlation but surprisingly high percentage showed mixed or positive correlation. The implications of this correlations will be discussed.

Poster Abstracts

PP1

Traditional farming practices of sustainability in potato (*Solanum tuberosum*) municipalities of Boyacá - BOYACÁ.

Alvaro E. Alvarado Gaona, Luz Adriana Pita Morales

Universidad Pedagógica y Tecnológica de Colombia, Colombia

alvaro.alvarado@uptc.edu.co

Tuberous plants were an important part of the diet in native American cultures. Potato (*Solanum tuberosum*) was cultivated in cold places of the Andean Colombian region by the indigenous cultures as Panches, Guanos, Chitareros, Pastos, Quillacingas, Chibcha and Muiscas settled in Cundinamarca and Boyacá departments. It appears that there were two forms of indigenous land use, the first near to the places of residence, devoted to food crops, and the second one to intensive crops in more remote areas. There were also two different kinds of production systems one with exclusively one species and another with associated species. The dominant plant species were for example, potato with beans. Another important practice of crop rotation was to alternate several species in orchards cycle to cycle. Today it is well known that the practices that our ancestors performed as Association and crop rotation contribute to conserve environmental and natural resources; for this reason we evaluated in the municipality of Boyacá, Boyacá, Colombia, the current use of such practices. At the end of the study it was concluded that 33% of the analyzed population still use crop rotations as potato - arracacha, and 89% have their crops in associated systems, those contributing to ecosystem, diversity, food supply, pest and disease control, pollination, seed diversification, erode and nutrient loss control, soil enrichment and water and climate regulation.

PP2

The chemical composition of potato tubers: Profiling of quality parameters to evaluate genetic resources

Andre Schlichting[1], Klaus J. Dehmer[2], Robert Müller[3], Peter Leinweber[3]

[1]*Steinbeis-Transferzentrum Soil Biotechnology, An der Wohrte 19, D-18059 Huckstorf, Germany*
[2]*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gross Luesewitz Potato Collections (GLKS), Parkweg 3a, D-18190 Gross Luesewitz, Germany*
[3]*Institute for Land Use, University of Rostock, Justus-von-Liebig-Weg 6, D-18059 Rostock, Germany*

andre.schlichting@stw.de

The evaluation of potato genetic resources is essential for their intensified utilization in plant breeding programs as well as for the quality improvement concerning desired ingredients in cultivated potatoes. The aim of this study was to apply analytical methods able to discern relevant parameters and, therefore, potentially contribute to an improved selection process. Here, particularly pyrolysis-field ionisation mass spectrometry (Py-FIMS) is known to be a powerful tool for the screening of complex matrices.

In sum 224 GLKS accessions were grown in field or greenhouse according to good management practise, ranging from cultivated varieties and Andean cultivars to wild species. Lyophilised tuber samples were initially subjected to methods for the quantification of total extractable phenolics (TP), total calcium (Cat) and potassium (Kt) concentrations, antioxidant capacity (TEAC) on dry weight basis, colour values (L*a*b-scale) and acidity (pH). Selected samples (n=92) were subjected to Py-FIMS for a molecular-chemical characterisation of the lyophilisates.

The first evaluation step was supported by wide ranges of all investigated parameters, especially for concentrations of Cat (63.6 to 1996.4 mg kg-1), Kt (11.8 to 35.3 g kg-1) and TP (0.67 to 9.286 g kg-1), whereas TP was significantly correlated with TEAC ($P<0.05$). The “molecular-chemical fingerprinting” by Py-FIMS revealed mass-signals, mostly (about 90 %) assigned to known marker signals. The multivariate statistics of the Py-FIMS data evidenced, that discriminating power was improved by pre-selection and ranking. The statistical link-up of all methods used provided relationships, which were statistically proven. In consequence a sequential scheme for an efficient evaluation strategy was derived.

PP3

Wild potatoes and fluorescent SSRs: genetic diversity assessment in potato genetic resources

Klaus J. Dehmer

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gross Luesewitz Potato Collections (GLKS), Parkweg 3a, 18190 Gross Luesewitz, Germany

dehmer@ipk-gatersleben.de

Genetic resources of cultivated potato constitute an important gene reservoir for resistances to diseases or pests or valuable agronomic traits. In a project aiming at the development of molecular markers for alleles confering resistance to potato wart (*Synchytrium endobioticum* (Schilb.) Perc.), wild potato accessions from the IPK Genebank were examined in order to assess their degree of intra- and inter-accession variability, as well as their interspecific diversity (where applicable).

Because of earlier publications on their resistance to potato wart, twelve species from nine taxonomic series of tuber-bearing potatoes were selected. After tuber production from appr. 800 individual genotypes coming from 82 genebank accessions, resistance against *Synchytrium* race 18 was tested at JKI Kleinmachnow. Leaf material of all individuals was harvested and genomic DNA extracted. SSR analyses were conducted in order to elucidate the applicability and power of resolution of microsatellite markers from different sources of cultivated potato. 14 SSRs were employed in four multiplex PCRs and separated as two combined 6- or 8-plexes on an automated fragment analysis system using fluorescence labeling.

The evaluation of the generated banding patterns shed a light on the degree of diversity within accessions - e.g. mainly low levels in selfers like *S. acaule* or *S. demissum* vs. higher levels in outcrossers like *S. sparsipilum* or *S. trifidum* - and also within and between species. In combination with resistance data for the respective genotypes, this diversity assessment will provide the basis for the planned identification of novel resistance alleles against potato wart.

PP4

A multidisciplinary approach to study the effect of genome doubling in potato

R. Aversano[1], I. Caruso[1], C. Fasano[1], F. Dal Piaz[2], N. De Tommasi[2], A. Di Matteo[1], L. Lepore[2], L. Frusciante[1], D. Carputo[1]

[1]*Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy*

[2]*Department of Pharmaceutical Sciences, University of Salerno, Via Ponte don Melillo, 84084 Fisciano (SA), Italy*

raversan@unina.it

In the plant kingdom, polyploidy has long been recognized as a prominent force shaping the evolution. The genomic interactions that occur following genome doubling may result in a "genomic shock", which is essentially tolerated by cells through extensive rearrangements. Overall, such upheaval at nuclear and cellular level may result in a dramatic restructuring of the transcriptome, metabolome and proteome. In this work we aimed to study synthetic polyploids of *S. commersonii* from the biochemical and transcriptomic standpoint to shed light on the effect of autopolyploidization. As for metabolite profiles, the analysis of 4x genotypes showed that leaf content of total alkaloids was significantly lower in the 4x genotypes than in the 2x progenitor. By contrast, we observed an increase of some phenol compounds (e.s. caffeine and ferulic acids and rutin) in tetraploids. To investigate the molecular events associated to autopolyploidization, we performed a microarray analysis. Towards this goal we designed a Custom Array 90K Combimatrix which allows to analyse up to 30000 gene sequences using the 25000 potato TC present in the SolEST database. Moreover, we designed this array to study gene expression of 6000 genes with unknown orientation. We have analysed differences in gene expression between the parental and three different tetraploids. The results of annotation, clustering and mapping analyses will be presented.

PP6

Cytochrome P450 monoxygenase-mediated hydroxylation of carotenoids in tomato.

Giovanni Giorio, Lucia Adriana Stigliani, Caterina D'Ambrosio

Metapontum Agrobios, SS Jonica Km. 448.2, Metaponto (MT), ITALY

ggiorio@agrobios.it

The pathway of carotenoids starts with the synthesis of phytoene and proceeds along a single path up to lycopene which can be transformed to b-carotene by the sole action of the lycopene b-cyclase or to a-carotene through the sequential action of a e-cyclase and a b-cyclase. Lutein and zeaxanthin biosyntheses are performed by structurally unrelated hydroxylases. Biosynthesis of b,b-xanthophylls is performed by the non-heme di-iron hydroxylases while the biosynthesis of lutein requires two cytochrome P450 carotene hydroxylases. Two members of non-heme di-iron group, BCH1 and BCH2, with complete overlapping functions are present in *Arabidopsis*. Similarly, in tomato CrtR-b1 and CrtR-b2 genes show a tissue-specific subfunctionalization. Two P450 carotenoid hydroxylases, CYP97A3 and CYP97C1, have been characterized in Arabidopsis. CYP97A3 is highly active toward the b-ionone rings while CYP97C1 is specific for e-ring of a-carotene. Here we report on the isolation in tomato of genes CYP97A29 and CYP97C11, encoding the P450 carotene beta- and epsilon-hydroxylases. The genes have the same number of exons of their *Arabidopsis* orthologs. Expression analysis of eleven carotenoid biosynthetic genes was carried out across different tomato organs. Functional characterization of CYP97A29 and CYP97C11 was complemented by an in planta analysis through the use of transgenic plants. Altogether our results confirmed the *Arabidopsis*-based model of lutein biosynthesis. Functional analysis of target genes to test the enzyme specificity or the pathway regulation seems a necessary step to plan carotenoid-targeted improvement programs.

PP7

Role of endoreduplication in fruit growth

Frederic Gevaudant[1], Elodie Mathieu-Rivet[1], Mehdi Nafati[1], Michel Hernould[1], Christian Chevalier[2]

[1]*Université Victor Segalen Bordeaux 2- UMR 619 Biologie du Fruit*
[2]*INRA - UMR 619 Biologie du Fruit.*

frederic.gevaudant@bordeaux.inra.fr

Tomato fruit size results from the combination of cell number and cell size which are respectively determined by cell division and cell expansion processes. As fruit growth is mainly sustained by cell expansion, the development of fleshy pericarp tissue is characterized by the concomitant arrest of mitotic activity, inhibition of Cyclin Dependent Kinase (CDK) activity, and numerous rounds of endoreduplication inducing a spectacular increase in DNA ploidy (up to 512C) and mean cell size. Although a clear relationship exists between endoreduplication and cell growth in plants, the exact role of endoreduplication has not been clearly elucidated, since most experiments inducing changes in endoreduplication levels also affect the cell division process. We first studied the kinetics of pericarp development in tomato fruit at the morphological and cytological levels, and showed that endoreduplication is directly proportional to cell diameter and fruit diameter in the course of fruit development. We were able to establish a mathematical model for tissue growth according to the number of divisions and the number of endocycles. To decipher the molecular basis of the endoreduplication-associated cell growth in fruit, we investigated the putative involvement of two different cell cycle markers, namely the APC/C activator CCS52A and CDK inhibitor SlKRP1 from tomato. In Pro35S:Slccs52A antisense plants, fruit size was reduced, originating from the reduction in cell size within the pericarp, due to a severe impairment in endoreduplication. Although endoreduplication was impacted by the over-expression of SlKRP1 under the control of a fruit-specific promoter expressed during the phase of cell expansion of early fruit development, we could not observe any morphological, cytological or metabolic phenotypes, indicating that cell- and fruit size determination could be uncoupled from endoreduplication in these conditions.

PP8

Identification of target genes of LATERAL SUPPRESSOR using ultra-high-throughput sequencing

Susanne Roβmann, Gregor Schmitz, Klaus Theres

Max Planck Institut for Plant Breeding Research, Cologne, Germany

rossmann@mpipz.mpg.de

Axillary meristems (AMs) play a crucial role in the elaboration of the aerial architecture of seed plants. They are initiated in the boundary region between the shoot apical meristem and leaf primordia. One of the most important and most specific regulators of this process is the transcriptional regulator LATERAL SUPPRESSOR (Ls). In order to understand the mechanism of axillary meristem initation in tomato and to identify target genes of Ls, we compared transcript profiles of leaf axils between wild-type and ls mutant. Using the Illumina Genome Analyzer, mRNA from leaf axils of both genotypes was sequenced in three biological replicates. In total ~90 million 76bp reads were generated and mapped to the tomato genome.

Comparing the RPKM (reads per kilobase of exon model per million mapped reads) values of wild type and ls, we found that 95 genes were downregulated and 211 upregulated in ls with a minimum fold change of two at level of significance of $p<0.05$. Expression analysis of selected candidate genes using RNA in situ hybridization revealed two genes with a defined expression in leaf axils where later new AMs will be formed. These genes are 6.3 and 7.3 fold up downregulated in ls. To test whether they are potential downstream targets of Ls, we will test for complementation of ls mutant phenotype in transgenic plants expressing these two genes from the Ls promoter. Phenotyping of loss-of-function mutants (RNAi or tilling) will further help to reveal the function of these genes.

PP10

Tomato homologue of AtGEM (Glabra2-Expression Modulator) is implicated in flower and fruit development

N. Viron[1,2], C. Bres[1,2], A. Grimault[1,2], F. Mounet[1,2], A. Moise[1,2], J. Petit[1,2], V. Garcia[1,2], A. Moing[1,2], C. Rothan[1,2], M. Lemaire-Chamley[1,2]

[1]*INRA - UMR 619 Fruit Biology, Villenave d'Ornon, France*
[2]*Université de Bordeaux, Bordeaux, France*

nviron@bordeaux.inra.fr

Tomato (*Solanum lycopersicum*) is a model plant to study fleshy fruit development. Tomato fruit size is mainly determined during early fruit development, before the onset of ripening, through a first phase characterized by intense cell divisions followed by a second phase characterized by cell expansion (Gillaspy et al. 1993).

In order to identify new candidate genes potentially involve in the regulation of cell size during tomato fruit early development, we searched for correlations between cell size and regulatory gene expression level. For this, transcriptomic and cytological analyses of two fruit expanding tissues were performed (Mounet et al, 2009). A strong correlation was highlighted between mean cell size and transcript level of a tomato homologue of AtGEM (Glabra2-Expression Modulator). In *Arabidopsis thaliana* this protein plays a crucial role in cell division, fate and differentiation during root development (Caro et al., 2007). In silico analyses identify two GEM homologues in tomato genome subsequently named SlGEM1 and SlGEM2. Their respective expression profile in tomato organs and during fruit development has been studied by real-time RT-PCR. In addition, we investigated the role of SlGEM1 in tomato by generating transgenic lines (RNAi silencing and over-expression) and identifying EMS mutants by tilling. Phenotypic characterization of the different lines is underway with a special focus on flower and fruit development.

PP11

Genetic manipulation of self-incompatibility in diploid potato species

Daniel Dzidzienyo[1], Tim Robbins[1], Glenn Bryan[2]

[1]*Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*
[2]*Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK*

sbxdkd@nottingham.ac.uk

Cultivated potato, *Solanum tuberosum*, is a tetraploid (2n=4x=48), making it difficult to carry out genetic studies on the crop. However, many wild and some cultivated species of potato are true diploids (2n=2x=24) and more amenable for genetic studies. Unfortunately their use is complicated by self-incompatibility (SI) which is widespread in diploid *Solanum* species. The production of self-compatible (SC) diploid lines would benefit potato breeding.

SI in *Solanum* is gametophytic and pistil specificity is controlled by a polymorphic ribonuclease (S-RNase), typical of the Solanaceae. This project aims to manipulate the expression of S-RNases in diploid potatoes using the RNAi technique. This approach to engineering self-compatibility into SI species has already been successfully demonstrated in petunia.

To date relatively few S-RNase sequences are available for *Solanum* species. We have characterized S-RNases present in accessions of *Solanum stenotomum*, *S. phureja* and *S. okadae*. RT-PCR using degenerate primers was used to amplify S-RNase sequences from pistil RNA. PCR products were cloned and sequenced. Database searches revealed that several of these sequences are novel S-RNases.

RNAi constructs are currently being designed to down-regulate specific *Solanum* S-RNases. Allele-specific primers and qRT-PCR will be used to confirm the reduced expression of S-RNases of primary transformants. SI/SC phenotypes will be determined using controlled pollinations. Seeds from plants exhibiting SC will be used to initiate the development of inbred lines.

PP12

Harnessing novel parthenocarpic mutants in tomato by TILLING a hormone response-related gene

A. Mazzucato[1], F. Cellini[2], S. Minoia[2,3], A. Petrozza[2], E. Santangelo[1], L. Selleri[1], F. Carriero[2]

[1]*Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.C. de Lellis s.n.c., 01100 Viterbo, Italy*
[2]*Metapontum Agrobios, SS Jonica 106 Km 448,2, 75010 Metaponto - MT Italy*
[3]*Current address: ENEA, Casaccia Research Center, PO Box 2400 Roma 00100AD, Italy*

mazz@unitus.it

The parthenocarpic growth of the ovary into a seedless fruit is an attractive trait for the breeders and has been extensively studied in tomato, where natural, facultative parthenocarpy sources are known. The recent light shed on molecular mechanisms controlling fruit set in tomato paved the way to harness new mutations for parthenocarpy.

A member of the Aux/IAA family of transcription factors, *SlIAA9*, has been described as a major player in the network of growth repressors established in the ovary of mature flowers. Silencing of *IAA9* can release the autonomous development of the ovary, that results into parthenocarpy.

To identify tomato genotypes carrying mutations in the *SlIAA9* coding sequence, a TILLING approach has been undertaken using the Red Setter tomato mutant collection. The analysis of 5.200 M3 families yielded three lines carrying a genetic lesion in the coding sequence: two consisted in a point mutation leading to amino acidic substitution and the third in a single-base deletion leading to a frame-shift and a premature stop codon. Characterization of the former lines, showed some of the expected phenotypes, albeit with low penetrance and expressivity. Characterization of the latter put in evidence, in agreement with those expected, more severe vegetative phenotypes that mainly consisted in a loss of leaf compoundness. The reproductive behaviour of this mutant is under study.

The final characterization of these lines and the assessment of their value in breeding tomato parthenocarpic varieties will be finally assessed when their genetic load will be further reduced.

PP13

Genetic and molecular characterization of a leaky mutant of FALSIFLORA, the tomato ortholog of LEAFY

F. Ruiu, M. E. Picarella, P. Mosconi, A. Mazzucato

Dipartimento di Agrobiologia e Agrochimica, Universitá degli Studi della Tuscia, Via S.C. de Lellis, s.n.c., 01100 Viterbo, Italy

mazz@unitus.it

Like the gene *LEAFY* (*LFY*) in Arabidopsis, the tomato ortholog *FALSIFLORA* (*FA*) acts as a floral promoter regulating floral transition and the identity of the floral meristem and ultimately of each floral whorl.

A candidate gene approach and an allelism test confirmed that *pistillate* (*pi*) represents a leaky allele of *FA*, showing a *fa*-like phenotype, including delayed flowering, loss of inflorescence meristem identity and aberrant petals and stamens.

Expression analysis of transcription factors known as downstream targets of *LFY* in *pi* and *fa* 'flower buds' indicated that class B MADS-box (*LeAP3* and *LePI*) genes are down-regulated in the mutants compared to the controls. Differently, the *TAG1* class C MADS-box was down-regulated in *fa* but not in *pi*.

FA sequence in the *pi* mutant revealed a point mutation in the first exon leading to a methionine to threonine (M101T) substitution in the deduced protein. An *in silico* analysis predicted that such a substitution is not tolerated for the protein function, being responsible, as showed in the 3D model, of an altered protein folding resulting in a spatial contraction of binding sites. The M101 residue is highly conserved only in flowering plants. Intriguingly, the only angiosperm sequence lacking the M101 amino acid was found in the genus *Peperomia*, that includes species whose flowers lack the perianth.

Altogether the insights derived from the study of the *pi* mutation suggest that the N-terminal conserved region of *LFY*/*FA*, where M101 is located, hosts residues that could have been crucial for the evolution of the flower.

PP14

Antioxidant synthesis and accumulation in callus culture of tomato fruit

Maria Minutolo, Antonio Di Matteo, Pasquale Chiaiese, Elvira Lotti, Angela Errico.

Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy

adimatte@unina.it

Ascorbic acid (AsA) and polyphenols (PP) are secondary metabolites important for plant cell protection against oxidative stresses. In addition, food with high content of those compounds are useful for human health. Among vegetables, tomato is the most important specie due to its significant consumption at worldwide level. However, very little is known about antioxidant genetic regulation in this species. In order to provide additional biological systems for studying changes in gene expression associated to the antioxidant control in tomato we have obtained callus from *Solanum lycopersicum* cv. M82 and *S. pennellii* introgression lines (ILs) IL7-3 and IL12-4. IL 12-4 have been previously shown to express a QTL for increased fruit AsA content while IL7-3 showed higher fruit content of AsA and PP. Callus tissue offers the possibility to avoid effects due to the physiological status of the plant that can often masks single gene activity.

Callus cultures were successfully obtained from unpeeled pericarp explants of mature green (MG), turning red (TR) and red ripe (RR) fruit. Frequencies of callus, AsA and PP were explants and genotype dependent. High percentage of callus was observed in all explants of screened genotypes except for RR explant of IL12-4 fruits. After 60 days of in vitro culture AsA and PP were found to be higher than the differentiated tissues in all genotypes and explants assayed. In differentiated tissues and calli a reduced expression of genes involved in cell wall metabolism and ethylene biosynthesis was observed in both ILs than M82. Further gene expression analyses will elucidate genetic mechanisms controlling AsA and PP accumulation in tomato.

PP15

The role of auxin in tuber initiation

Efstathios Roumeliotis, Bjorn Kloosterman, Richard G. F Visser, Christian Bachem

Laboratory of Plant Breeding, Dept. of Plant Sciences, Wageningen-UR, Wageningen, The Netherlands

efstathios.roumeliotis@wur.nl

The induction of tuber organogenesis in potato plants is the result of a complex network of factors that begins with the perception of changes in day length and the transduction of a signal from the leaves to the stolon tips. It is known Gibberellins (GA) play an important role in the tuber signaling pathway. On the contrary, a regulatory role for another important hormone, auxin, has not yet been established. Support for the role of auxin in tuberisation comes from the large number of auxin related genes differentially expressed during the early stages of tuber formation. We designed an *in vitro* tuberisation system in which we can study the role of auxin, by applying IAA to the basal or apical part of the stolon after removal of the stolon apex. Explants with removed stolon apices tuberised more rapidly in comparison to the controls and when auxin was applied on these ablated tips the phenotype was reverted. In addition, preliminary data on auxin concentration measurements reveal differences in the auxin concentration between stolon and tuber tissues. The use of additional treatments, auxin measurements in stolons and tubers support the role of this hormone in the regulation of tuberization. Based on these results, we propose a model for directional movement of auxin in potato stolons, analogous to the polar auxin transport (PAT) in shoots, in which auxin is transported to the basal parts of the stolon, thereby inhibiting axillary bud outgrowth bellow the stolon apical meristem (StAM).

PP16

High-resolution mapping and identification of candidates for a fruit firmness quantitative trait locus (QTL)

Natalie H. Chapman[1], Charles Baxter[2], Laurent Grivet[3], Julien Bonnet[3], Ian Puddephat[4], Viven Taylor[1], Rebecca Smith[1], Guiping Sun[1], Graham B. Seymour[1].

[1]*Plant and Crop Sciences, School of Biosciences, University of Nottingham, UK.*
[2]*Syngenta Seeds Limited, Jealott's Hill International Research Centre, Bracknell, Berks, UK.*
[3]*Syngenta Seeds Limited, 12, chemin de l'Hobit, 31790 Saint Sauveur – France*
[4]*Syngenta Seeds B.V., Westeinde 62, 1601 BK Enkhuizen, Netherlands*

natalie.chapman@nottingham.ac.uk

Fruit firmness of tomato and other fruits is determined by a number of factors including cell wall structure, turgor and cuticle properties. Fruit texture is a complex polygenic quantitative trait involving the co-regulation of many genes. Quantitative trait loci (QTL) of fruit firmness were located on the tomato genome using genetic resources such as interspecific introgression (IL) lines. We have identified a number of major QTLs associated with fruit firmness located on chromosome 2 using a mapping population consisting of 7500 lines. These QTLs have a marked effect on probe penetration measurements, which is indicative of enhanced fruit firmness in both the outer and inner pericarp. Using a range of genetic markers each QTL was mapped to a physical location within the genome. In order to identify candidate genes a QRT-PCR platform is currently being used. One candidate gene was identified from a Microarray experiment, and was found to be up-regulated approximately 100 fold in an IL line compared to M82. Further, this candidate gene has been mapped to one of the texture QTL.

PP17

Abiotic and biotic stress responses in the *Solanum phureja* clone DM1-3 516R44 as measured through whole transcriptome sequencing

Alicia N Massa, Brieanne Vaillancourt, Brett Whitty, C. Robin Buell

Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

amassa@msu.edu

Potato is the most important vegetable crop in the world and the fourth most important crop, after wheat, rice, and maize. Efforts to characterize its genome include whole genome sequence of two potato clones generated by the Potato Genome Sequencing Consortium (PGSC). To complement the genome sequence for the purposes of improving genome annotation and to understand gene expression, a large set next generation transcript sequencing data was generated by the PGSC Transcriptome Group. Using the doubled monoploid *S. phureja* clone DM1-3 516R44 (DM), transcriptome sequences were generated using mRNA-Seq with the Illumina Genome Analyzer platform from plants grown under abiotic and biotic stress conditions. For biotic stress, leaves were challenged with *Phytophthora infestans* and the elicitors acibenzolar-s-methyl and DL--amino-n-butyric acid. For abiotic stress, plants were exposed to salt, drought, salinity, heat, and a panel of four hormones. Leaves were also wounded to mimic herbivory. mRNA-Seq reads from these 12 treatments were mapped to the corresponding DM potato genome and used to determine expression levels. We will present data on differentially expressed genes under abiotic/biotic stress conditions as well as the overlap of genes involved in these stress response pathways.

PP18

An update of tomato chromosome 3 sequencing

Jianfeng Ren, Liuhua Yan, Shengxiong Huang, Hongling Jiang, Chuanyou Li

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China.

renjianfeng@genetics.ac.cn

Tomato (*Solanum lycopersicum*) is considered as a model for studying fruit biology and plant defenses. As small genome and high synteny conservation with other Solanaceae, tomato genome will serve as the reference of Solanaceae. With further efforts, the draft genome sequences were obtained with the combination of the initial approach of BAC-by-BAC and the latter whole-genome shotgun.

Here we report the improved sequence of chromosome 3. According to the latest assembly version, thirteen scaffolds were anchored on chromosome 3. The assembly sequence is 64.64 Mb, accounting for 73.3% of the estimated chromosome length (88.20 Mb) and 8.3% of the total assembly length (781. 63 Mb).

To improve the draft sequence, different strategies were adopted to close the gaps. For inter-scaffold gaps, we extended each end of the scaffolds with IL mapping verified BACs and sequenced them. For the gap of intra-scaffold larger than 10 kb, the BAC containing the gap was sequenced. Remaining gaps were filled by PCR with the BACs as the template. Up to now, more than 50 BACs are sequencing and 300 PCR gaps were closed. In addition, 15 large scaffolds in total of 9.0 Mb on chromosome 0 will be mapped to specific chromosomes.

With the improved draft sequence, the transposable elements will be identified and the gene annotation will be refined again. Calculation of gene density and genetic recombination rate, and syntenic analysis will also performed based on the improved draft sequence.

PP19

Current sequencing progress of *Solanum lycopersicum* Chromosome 4

Rosa Lopez-Cobollo[1], Giulia Bonciani[1], Daniel Buchan[2], James Abbott[2], Rosalind Cutts[2], Ioannis Filippis[2], Sarah Butcher[2], Purnima Pachori[3], Mario Caccamo[3], Jane Rogers[3], Helen Beasley[4], Clare Riddle and Mapping Core Group[4], Karen McLaren and Finishing Team 464[4], Pre-finishing Team 584[4], Graham Seymour[5], Glenn Bryan[6], Heidrun Gundlach[7], Klaus Mayer[7], Stephen Stack[8], Dora Szinay[9], Hans de Jong[9], Shusei Sato[10], Satoshi Tabata[10], Gerard J. Bishop[1]

[1]*Division of Biology, Imperial College London, Exhibition Road, London SW7 2AZ, UK*
[2]*Bioinformatics Support Service, Imperial College London, Exhibition Road, London SW7 2AZ, UK*
[3]*The Genome Analysis Centre (TGAC). Norwich Research Park, Colney, Norwich, NR4 7UH, UK*
[4]*Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK*
[5]*University of Nottingham, School of Biosciences, Division of Plant Sciences, Sutton Bonington Campus, Loughborough, Leics LE12 5RD UK*
[6]*The Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK*
[7]*Institute for Bioinformatics (IBI) at National Research Centre for Environment and Health, Neuherberg, Germany*
[8]*Colorado State University, USA*
[9]*University and Research Centre, Wageningen, Netherlands*
[10]*Department of Plant Gene Research, Kazusa DNA Research Institute, JAPAN*

r.lopez-cobollo@imperial.ac.uk

Tomato (*Solanum lycopersicum*) chromosome 4 is being sequenced in the UK as part of the International Tomato Genome Sequencing Project. We have carried out BAC-by-BAC sequencing and contributed a ~7-8kb paired end read using SOLiD3 technology to the next generation sequencing initiative. We followed the strategy of minimum tiling path of overlapping BAC clones that have been selected using both fingerprint and BAC end sequence (BES) information. A basic set of seed BACs were confirmed by FISH technology. 64 sequenced BACs were assessed to be on Chr4 using IL mapping. A total of 22 BACs and 3 Fosmids were identified by 3D superpools screening; extension of BAC sequence contigs using the BES and Fosmid end sequences (FES).

We have also used PCR to assess the nature of the gaps between contigs in the scaffolds from the next generation sequencing initiative. Sixteen scaffolds from the assembly version 1.5 were mapped to Chr 4 with a total length of 63.09 Mb, with five scaffolds covering ~15.8Mb. We integrated the 134 Chr4 BAC sequences that identified a total of 508 gaps. PCR analysis of the gaps shows that they are smaller than expected. We will generate an AGP file to describe all the elements that were integrated in the final sequence for Chr4. The EMBL/Genbank accessions of the WGS contigs and PCR products will be used to identify the sequences in the AGP. These accession numbers will enable us to track the source of every fragment in the assembly in future assembly updates.

PP20

A rapid method for identifying sites of T-DNA integration in activation-tagged potato

Daniel Frank[1], Helen Tai[2], Jeremy Duguay[1], David De Koeyer[2], Vicki Gustafson[3], Sharon Regan[1]

[1]*Biology Department, Queen's University, Kingston, ON, Canada K7L 3N6.*
[2]*Potato Research Centre, Agriculture and Agri-Food Canada, P.O. Box 20280, Fredericton, NB, Canada E3B 4Z7.*
[3]*Solanum Genomics International Inc., 921 College Hill Road, Fredericton, NB, Canada E3B 6Z9.*

df@queensu.ca

Late blight is a major disease of potato caused by *Phytophthora infestans*, an oomycete pathogen which necrotizes leaf and tuber tissue. Emerging fungicide resistance of *P. infestans* demonstrates a need to leverage endogenous traits within potato to reduce pathogen susceptibility and reliance on fungicide control. The Canadian Potato Genome Project (CPGP) has generated over 6000 lines of activation tagged potato plants through T-DNA mediated integration of CaMV 35S enhancer elements within the potato genome. The inserted enhancers drive expression of nearby genes, creating dominant gain-of-function mutants. In ongoing screens of transgenic lines for late blight resistance, sites of T-DNA integration may reveal candidate genes involved in improved performance. Here, a rapid capture technique for identifying sites of T-DNA integration has been developed for activation-tagged potato. Pulldown of biotinylated probes annealing to T-DNA loci using streptaviden-conjugated paramagnetic beads provides an efficient method for targeted capture of genomic DNA flanking sites of T-DNA integration. Captured sequences can be readily located within the genome through the Potato Genome Sequencing Consortium database and candidate genes contributing to reduced pathogen susceptibility identified.

PP21

In silico integration of genetic and physical and sequence maps in potato using BAC sequence tags

J.M. de Boer[1], D. Bolser[2], S.K Sharma[3], G. J. Bryan[3], C.W.B. Bachem[1], R.G.F. Visser[1]

[1]*Wageningen University Plant Breeding, Netherlands*
[2]*University of Dundee, Dundee, UK*
[3]*Genetics Programme, SCRI, Dundee, UK*

jan.deboer@wur.nl

Whole Genome Profiling (WGP) is a recently developed novel approach for BAC library skim sequencing, which can be used for de novo physical map construction of genomes and whole genome sequence scaffolding (1, 2). We have applied WGP to 86016 BAC clones of the diploid potato genotype RH89-039-16 (RH), and we report on a novel application of these short (26 bp) sequence tags in genomics research. BLAST alignments with WGP tags from RH physical map BAC contigs against a pre-release of the *Solanum phureja* 'DM' genome sequence (Beijing Genomics Institute, Shenzhen, China) allowed a full integration of the RH physical and genetic maps with the available DM sequence and genetic maps. As such, approximately 1300 AFLP™ markers from genotype RH could be transferred to the DM genome, without the need for additional marker sequencing or progeny genotyping. In reverse, previously unanchored RH physical map BAC contigs can now be assigned to chromosome locations, from their WGP alignments to the DM genome. These two integrated potato genomes form a firm basis for future fundamental and applied research in potato.

(1) Van der Vossen et al. 2010, Whole genome profiling of the diploid potato clone RH89-039-16. Plant & Animal Genomes XVIII Conference, San Diego.

(2) http://www.keygene.com/services/services_molecular_WGP.php

PP22

Cytogenetic distribution of chromosome 5 BAC sequences from the diploid potato genotype RH

J.M. de Boer[1], X. Tang[1,2], E. Datema[1], T.J.A. Borm[1], E. Bakker[3], B. te Lintel-Hekkert[4], R.C.H.J. van Ham[4], H. de Jong[2], C.W.B. Bachem[1], R.G.F. Visser[1]

[1]*Wageningen UR Plant Breeding, The Netherlands*
[2]*Laboratory of Genetics, Wageningen University, The Netherlands*
[3]*Plant Sciences Group,Wageningen UR, The Netherlands*
[4]*Plant Research International, Wageningen, The Netherlands*

jan.deboer@wur.nl

Chromosome 5 of the heterozygous potato genotype RH89-039-16 (RH) has been sequenced to near completion using the BAC by BAC approach. A total of 97, usually linkage-phase specific, BAC tiling paths were sequenced, starting from 187 genetically anchored seed contigs in the RH BAC physical map. The overlapping BAC sequences were merged into scaffolds, comprising total length of 53 Mbp of non-redundant sequence. The cytogenetic distribution of the BAC sequences was examined in detail by multi-color BAC ladder FISH on pachytene chromosomes. 16 Mb of sequence were located in the euchromatic arms, while 37 Mb originated from the repeat-rich pericentrimeric heterochromatin. The cytogenetic map shows the relation between genetic and physical distances across chromosome 5, and three regions of suppressed recombination were identified. Clusters of resistance gene homologs (RGHs) were located both in the euchromatin, and in the border region with the central heterochromatin. The cytogenetic map has given ample confirmation of the AFLP-marker derived chromosome 5 anchor points in the RH BAC physical map, and allowed fine ordering of BAC sequences beyond the resolution of the genetic map. Our integrated sequence and cytogenetic map is a standard for future comparative Solanaceae genomics research on chromosome 5.

PP23

An integrated genome wide genetic map of sequenced NB-LRR disease resistance gene homologues (RGH) and resistance loci in potato

E. Bakker, T. Borm, P. Prins, E. van der Vossen, G. Uenk, G. Sabatino, M. Arens, J. de Boer, H. van Eck, J. Vossen, G. van der Linden, M. Muskens*, S. Allefs*, R. Visser, J. Bakker, A. Goverse

Plant Science Group, Wageningen University and Research Centre, Wageningen, The Netherlands
**Agrico Research BV, Bant, The Netherlands*

erin.bakker@wur.nl

Like all plants, potato has evolved a surveillance system consisting of a large array of genes encoding for immune receptors that confer resistance to pathogens and pests. These so-called resistance or R proteins are composed of functional modules involved in pathogen recognition and the activation of a defence response. The majority of resistance genes identified to date belongs to the class of genes encoding nucleotide binding (NBS) and leucine rich repeats (LRR) domains. To date, twelve functional resistance genes have been identified in potato. However, at more than twenty regions in the potato genome, one or more resistance loci have been mapped, but none of the underlying genes have been identified. Therefore, we screened the RH BAC library with NBS probes and a representative set of 288 unique BACs was selected for sequencing and mapping. This resulted in the identification of 767 NB-LRR genes and gene fragments, which could be grouped into forty-five discrete NB-LRR clusters that were mapped throughout the potato genome. Ten RGH clusters are syntenic to previously identified functional R genes, whereas thirty-five clusters are novel. Integration of functional resistance loci described in literature revealed that they often co-localize with the RGH clusters. Hence, this integrated whole genome RGH map provides a rich source to employ marker assisted selection, candidate gene approach for the identification of resistance genes in potato and comparative mapping with other Solanaceous species like for instance tomato.

PP24

Comparative genomics of the doubled monoploid potato genotype DM and the heterozygous diploid potato genotype RH

E. Datema[1], J.M. de Boer[2], T.J.A. Borm[2], B. te Lintel-Hekkert[1], R.C.H.J. van Ham[1], C.W.B. Bachem[2], R.G.F. Visser[2]

[1]*Plant Research International, Wageningen, The Netherlands*
[2]*Wageningen UR Plant Breeding, The Netherlands*

erwin.datema@wur.nl

The Potato Genome Sequencing Consortium has recently produced the draft genome sequence of the doubled monopolid *Solanum phureja* genotype DM1-3516R44 (DM). Previous sequencing efforts on another potato genome, the heterozygous diploid *S. tubersoum* genotype RH89-039-16 (RH), resulted in approximately 200 Mb of BAC linkage-phase specific tiling paths. Comparison of the RH BAC tiling paths to the DM draft genome sequence revealed an overal sequence identity of 97.5% between the two genomes, corresponding to 1 SNP every 40 bp and 1 indel every 394 bp on average. Gene sequences were more highly conserved (98.4%). Remarkably, the overal sequence identity between the two linkage phases of RH was slightly lower (96.5%), with 1 SNP every 29 bp and 1 indel every 286 bp between. A comparison of an 800 kb region on chromosome 5, for which sequence of all three alleles was available, revealed that the overal sequence colinearity is well preserved, but that several genes in this region are unique to one or two of the three alleles. Within this region, colinearity was apparently lost in a subregion of approximately 150 kb. Preliminary resuls suggest that this loss of colinearity can be attributed to differences in repeat organisation wthin this region.

PP25

The Potato Genome Sequencing Initiative

Sanjeev Kumar Sharma, The Potato Genome Sequencing Consortium

Genetics Programme, SCRI, Dundee, UK

sanjeev.sharma@scri.ac.uk

Potato is the world's number one non-cereal food crop, and a key member of the Solanaceae. The 840 Mb genome of potato has been sequenced by the global Potato Genome Sequencing Consortium (PGSC). Using a whole genome shotgun approach coupled with Next Generation Sequencing, the PGSC has generated a high quality draft sequence of a completely homozygous 'doubled monoploid' clone (DM1-3 516R44) of *S. tuberosum* group Phureja complementing their earlier efforts employing a chromosome by chromosome, BAC by BAC sequencing strategy of the diploid and heterozygous 'RH89-039-16' (RH) clone. Progress towards generation of draft sequences of the two genotypes has been rapid, with high genome coverage of both genotypes. Currently version 3.0 of the DM assembly is available (www.potatogenome.net). Resources developed include fosmid and BAC libraries, improved physical maps, and an anchored physical/genetic reference map, onto which more than 80% of the DM genome assembly has been mapped and anchored using a number of STS (sequence tagged sites) markers. We have annotated the genome and are currently analyzing the transcriptome and genes critical to potato biology. The timely release of the potato genome sequence provides the entire Solanaceae research community an opportunity to exploit the genome sequence for fundamental and applied biological studies and various other widespread applications in potato genetics. The potato genome sequence will serve to accelerate potato improvement and help to meet the challenges facing food production in the 21st century.

PP26

An integrated approach to reconstruct the tomato genome

Roeland van Ham, The International Tomato Sequencing Consortium

Plant Research International, Wageningen, The Netherlands

roeland.vanham@wur.nl

The tomato genome was sequenced with next generation sequencing technologies. The resulting 454, SOLiD, and Illumina data were used in combination with Sanger sequence data to reconstruct the tomato genome. The assembly process involved an initial *de novo* assembly of 454 and Sanger reads, error correction using the SOLiD and Illumina data, scaffolding with BAC- and fosmid-end sequences, integration of sequenced BACs, and gap closure using an alternative assembly. The current assembly (v2.10) consists of 781 Mb in 3433 scaffolds, and 95% of the assembled sequence is contained in the largest 73 scaffolds. We used a novel genetic map, a SNaPshot and a Whole Genome Profiling physical map, and a cytogenetic map to reconstruct the twelve tomato chromosomes. Here we present an automated approach for the integration of the various maps and the assembly. As a result of the method 90 scaffolds, which collectively contain 97% of the assembled data (759 Mb), could be reliably assigned to a chromosomal position, 50 of the scaffolds could also be oriented. Based on DNA content estimates of pachytene chromosomes, the completeness of the chromosome sequences ranges from 56% for chromosome 2 to 96% for chromosome 8 with an average of 80%.

Keygene N.V. owns patents and patent applications covering its Whole Genome Profiling technology.

PP27

Protein profiling of potato leaf tissues to determine mechanisms of late blight disease suppression

G. Wang-Pruski[1], S. Lim[1], D. Pinto[2], R.H. Coffin[3], S. Veenhuis-MacNeill[3], W. Hardy[3], R.D. Peters[4], H.W. Platt[4], I. Macdonald[4], K. Drake[4], K.I. Al-Mughrabi[5].

[1]*Nova Scotia Agricultural College, PO BOX 550, Truro, NS, B2N 5E3, Canada*
[2]*National Research Council-IMB, Halifax, NS, B3H 3Z1, Canada*
[3]*Cavendish Farms, Summerside, PE, C1N 5J5, Canada*
[4]*Agriculture and Agri-Food Canada, Charlottetown, PE, C1A 4N6, Canada*
[5]*New Brunswick Department of Agriculture and Aquaculture, Wicklow, NB, E7L 3S4, Canada*

gwangpruski@nsac.ca

Late blight, caused by *Phytophthora infestans*, is the most devastating disease of potatoes, resulting in global constraints in potato production. Proteomics is a powerful tool for investigating complex biological processes at the molecular level. ConfineTM, a phosphorous acid (PA)-based fungicide, is a relatively harmless chemical to human and environment. Field trials were conducted in Prince Edward Island, Canada for three years between 2007 and 2009 to evaluate the efficacy of PA in foliar late blight control. Evaluation of the infection rate revealed that plants treated with Confine suppressed late blight infection. To understand the biological responses occurred in treated leaves, treated and non-treated samples were collected and protein profiles were generated using two-dimensional liquid chromatography-tandem mass spectrometry. Since most molecules involved in host-pathogen interactions are in the cell wall and cytosol, protein profiles were completed in these subcellular locations. In total, 8.6% (102 proteins) of reproducible proteins were regulated by Confine treatment. Confine-induced proteins were classified into response to stress, proteolysis, metabolism, signaling, and translation according to their biological responses. More than 50% of up-regulated proteins are involved in defense mechanisms based on the literature search. Approximately 60% of down-regulated proteins play roles in metabolic processes. These preliminary results showed that Confine has an indirect mode of action to trigger leaf proteins involved in defense mechanisms in potatoes.

PP28

Mapping of new resistance genes against potato late blight originating from *Solanum michoacanum* and *S. ruiz-ceballosii*

Jadwiga Śliwka, Henryka Jakuczun, Marcin Chmielarz, Agnieszka Hara, Iga Tomczyńska, Andrzej Kilian, Ewa Zimnoch-Guzowska

Plant Breeding and Acclimatization Institute, Młochów Research Centre, Platanowa 19, 05-831 Młochów, Poland

j.sliwka@ihar.edu.pl

Two novel Rpi (Resistance to *Phytophthora infestans*) genes were identified and mapped using sequence specific markers, Diversity Array Technology (DArT) and JoinMap ® 4 (Van Ooijen 2006) software, within the presented work.

The first one, *Rpi-mch1* was identified in 1EBN species *Solanum michoacanum* that is a natural hybrid between *S. pinnatisectum* and *S. bulbocastanum*. The mapping population segregating for the resistance to *P. infestans* was developed from the intraspecific cross and the progeny (n=164) was tested by a laboratory detached leaf test with spray inoculation in two subsequent years. The *Rpi-mch1* gene was mapped to chromosome VII.

The second gene, *Rpi-rzc1* was identified in *S. ruiz-ceballosii* syn. *S. sparsipilum*, a 2EBN species that can be crossed with *S. tuberosum*. The mapping population (n=114) was an interspecific cross of *S. ruiz-ceballosii* and dihaploid potato cultivar Balbina. The population was tested for late blight resistance by standard laboratory detached leaflet and tuber slice tests with droplet inoculation in three seasons. The *Rpi-rzc1* gene was mapped to chromosome X.

Both genes can be exploited for breeding potato resistant to *P. infestans*, *Rpi-mch1* - by somatic hybridization or cloning and cisgenesis, while *Rpi-rzc1* – by direct introduction into conventional potato breeding programs *via* 4*x* – 2*x* crosses with use of marker-assisted selection.

PP29

Genetic study of the tomato lethal disease induced Cucumber mosaic virus and D satellite RNA

Ping Xu, Hua Wang, Jun-ying Ma, Frank Coker, Yuhong Tang, Mark Taylor, Marilyn Roossinck

Plant Biology Division, The S. R. Noble Foundation, Ardmore, OK 73401, USA

pxu@noble.org

Cucumber mosaic virus (CMV) in combination with D satellite RNA (satRNA) causes an epidemic disease in tomato (*Solanum lycopersicum*), which involves rapid development of lethal systemic necrosis. No resistance to this disease has been found in tomato, but a non-lethal response (NLR) was found in a wild relative of tomato, *Solanum habrochaites*. We developed a line of *S. habrochaites* with homogenous NLR to infection by CMV/D satRNA. D satRNA accumulated slightly less in *S. habrochaitis* than in tomato, and less vascular cells were infected in the vascular bundles of *S. habrochaitis*. This line of *S. habrochaitis* was crossed with tomato and F1 plants were generated. All the infected F1 plants survived the infection, indicating the NLR is a dominant trait. In addition, the NLR trait was successfully passed to the first generation of backcrosses (BC1). The phenotype and genotype segregation was analyzed in the BC1 population. The analyses indicate that the NLR trait is determined by quantitative trait loci. Four putative major QTLs associated with the NLR trait were mapped in chromosomes 5 and 12.

PP30

Identification of Novel Secreted Proteins using Yeast Secretion Trap in Pepper following *Phytophthora capsici* infection

Seon-In Yeom[1], Hyang-Ku Baek[1], Sang-Keun Oh[1], Won-Hee Kang[1], Sang Jik Lee[2], Eunyoung Seo[1], Jocelyn K.C Rose[2], Byung-Dong Kim[1]*, Doil Choi[1]*

*Equally contributed as corresponding author

[1]*Department of Plant Science, Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*
[2]*Department of Plant Biology, Cornell University, U.S.A*

snusunin@hanmail.net

In plants, primary defense to pathogen is mostly inducible and associated with cell wall modification and defense-related gene expressions such as secreted proteins. To study these secreted proteins, the yeast-based signal sequence trap screening was conducted with the RNA of *Phytophthora capsici*-inoculated root of *Capsicum annuum* "CM334". A total of 101 CaS (*C. annuum* Secretome) genes were isolated and identified. Out of 101 Cas clones, 91 CaS clones were predicted as have signal sequence at their N-terminal region that could be translocated outside host cells. To identify differently expressed CaS genes between resistant and susceptible pepper, reverse Northern blots and real-time RT-PCR were performed with RNA samples isolated at different time points following *P. capsici* inoculation. Expression of most of CaS mRNAs were induced upon inoculation. However, temporal difference in CaS gene transcript levels were observed between resistant and susceptible pepper. To address the role of assigning biological functions to *in silico* predicted CaS genes, we performed *in planta* knock-down assay using TRV-based gene silencing vector. Eight of CaS genes are found to suppress hypersensitive response (HR) in silenced plants following pathogen inoculation. Three of CaS genes did exhibit phenotypic abnormalities in silenced plants. One of them, CaS259-silenced plants showed not only HR suppression but also apparent phenotypic abnormalities compared with control plants. Thus, these observations provide evidence that CaS genes play an important role in pathogen defense as well as developmental process in plants.

PP31

Plant hormones signaling pathways are involved differently in qualitative and quantitative resistances to tomato powdery mildew

A. Seifi, Z. Zheng, S. Pavan, L. Faino, Fien Meijer-Dekens, Y. Bai.

Plant Breeding Laboratory, Wageningen University, The Netherlands

ali.seifi@wur.nl

A significant body of evidence suggests that plant hormone imbalance influences the plant's innate immunity. It is believed that salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling pathways are the backbone of phytohormone network in plant immune system, which interact with other hormonal signaling pathways. In tomato, resistance to tomato powdery mildew, *Oidium neolycopersici*, is governed by both qualitative resistance genes (including Ol-1, ol-2, Ol-4) and quantitative resistance loci (Ol-qtls). Microscopically, different mechanisms are associated with these Ol-genes and Ol-qtls: slow-HR (Ol-1), papilla formation (ol-2), fast-HR (Ol-4) and a combination of papilla formation HR (Ol-qtl). By crossing tomato mutants, epi (ET overproducer), Nr (ET-insensitive), def1 (JA-deficient), and not (ABA-deficient) with near-isogenic lines bearing each of mentioned Ol resistance loci, we evaluated the involvement of different hormonal signaling pathways in tomato resistance to *O. neolycopersici*. Our preliminary results suggest ET, JA and ABA signaling pathways involved differently in qualitative and quantitative resistances to tomato powdery mildew.

PP32

Deep profiling of the transcriptome of potato to identify late blight resistance gene networks in potato.

Sireesha Dommaraju[1], Hanne Grethe Kirk[2], Mads Sonderkarr[1], Kare Lehmann Nielsen[1].

[1]Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Aalborg, Denmark. [2]LFK-Vandel, Vandel, Denmark

sd@bio.aau.dk

Phytophthora infestans is an oomycete causing the devastating disease late blight in Solanaceae species such as potato. Different cultivars of potatoes are displaying differential degrees of resistance towards the disease, in general thought to be mediated by the presence of specific R-genes in the different cultivars. In this study we have analyzed three late blight infected potato cultivars Sarpo Mira (highly resistant), Kuras (medium resistance), Bintje (low resistance) using a sequence tag-based transcriptome platform. Using PCA analysis and multiple pair wise comparisons a set of candidate genes with gene regulation associated with infection was identified. Following hierarchical clustering of the genes a specific early up-regulation of several genes was identified in the highly resistant Sarpo Mira variety. Several MAP kinases and a WRKY transcription factor, important candidates for an R-gene mediated signaling pathway was identified; as well as a LRR-NBS-TIR which was cloned and sequenced from Sarpo Mira. We propose this gene is the candidate resistance gene of Sarpo Mira.

PP33

R3a resistance protein mutants with extended effector recognition

Maria Eugenia Segretin, Sophien Kamoun.

The Sainsbury Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UH, United Kingdom

maria.segretin@tsl.ac.uk

Phytophthora infestans is one of the most devastating pathogens affecting potato production worldwide. One strategy to generate resistant cultivars is the introduction of resistance genes that recognize *P. infestans* effector proteins with avirulence activities. R3a, a resistance protein discovered in potato, can trigger a hypersentive response upon the recognition of the avirulence effector AVR3aKI from *P. infestans* but cannot recognize AVR3aEM, the product of another allele that is predominant in pathogen populations. To date, all the characterized *P. infestans* strains in nature carry at least one of these AVR3a proteins. The objective of this work is to extend R3a recognition specificity to AVR3aEM. To accomplish this, we generated a library of R3a mutant variants obtained by random mutagenesis. We screened the mutant clones by co-agroinfiltration with AVR3aEM in *Nicotiana benthamiana* plants, and evaluated the presence of HR-like phenotypes. Of approximately 2200 evaluated clones, 20 triggered different degrees of HR-like responses in different infiltration experiments. In parallel, the candidate clones were co-infiltrated with AVR3aKI and with a negative control plasmid to determine maintenance of the original R3a recognition specificity and also to eliminate auto-active R3a mutants. In total, 17 clones were selected and sequenced, most of them harboring multiple mutations. To investigate the contribution of the different mutations to the observed phenotypes, single amino acid R3a mutants were generated and assayed. This led to the identification of individual mutations that enhance recognition of AVR3aEM. This work highlights how knowledge of pathogen effectors can be exploited for engineering novel resistance genes.

PP34

Monitoring avirulence effector genes from *Phytophthora infestans* to assist the deployment of *Solanum* resistance genes

Ricardo Oliva[1], Marco Thines[2], Sophien Kamoun[1]

[1]*The Sainsbury Laboratory, NR4 7UH, Norwich, UK*
[2]*University of Hohenheim, D 70593 Stuttgart, Germany*

Ricardo.Oliva@tsl.ac.uk

Stacking disease resistance (R) genes into commercial varieties is a valuable strategy to gain broad-spectrum resistance against the potato late blight pathogen *Phytophthora infestans*. Two such R genes, Rpi-blb1 and Rpi-blb2 from *Solanum bulbocastanum*, show promising potential for usefulness in agriculture based on preliminary transgenic potato trials. Rpiblb2 confers immunity by recognizing the *P. infestans* avirulence effector protein AVRblb2 when it is translocated inside the plant cell. This effector belongs to the RXLR effector family and is under strong positive selection. Structure-function analyses revealed a key polymorphic amino acid (position 69) in AVRblb2 that is critical for activation of Rpi-blb2. In this study, we reconstruct the evolutionary history of Avrblb2 and further characterized its genetic structure in worldwide populations. Our data suggest that Avrblb2 evolved as a single copy gene in a putative ancestral species of *P. infestans* and has recently expanded in the *Phytophthora* species that infects solanaceous hosts. As a consequence, at least four active variants of AVRblb2 arose in *P. infestans*. One of these variants, Phe69, evades recognition by the cognate resistance gene. Surprisingly, all Avrblb2 variants are maintained in pathogen populations suggesting a potential benefit in keeping duplicated versions of AVRblb2. Our findings are particularly timely because they enable monitoring the possible emergence of virulent *P. infestans* strains following Rpi-blb2 deployment.

PP35

Identification and the molecular phylogeny of the Mlo gene family in *Solanum lycopersicum*

Zheng Zheng, Robin P. Huibers, Yuling Bai

Plant Breeding Laboratory, Wageningen University, The Netherlands

zheng.zheng@wur.nl

The plant Mlo gene family encodes for heptahelical transmembrane proteins. The barley Mlo gene was discovered first and its presence found to be required for powdery mildew pathogenesis. Later it was shown that this requirement is conserved among plants as mutations in AtMlo2, AtMlo6 and AtMlo12 in *Arabidopsis* and SlMlo1 in Tomato result in powdery mildew resistance. SlMlo1 is the only Mlo gene family member so far identified in tomato or other Solanaceae plants. Using the pre-released tomato genome sequence we were able to identify 16 Mlo homologs. They were located on chromosomes 1, 2, 3, 4, 6, 8, 9, 10, with only one obvious clustering on chromosome 2. Full length cDNA sequences for the majority of identified SlMlo genes were obtained and sequenced. Phylogenetic analysis of Tomato, *Arabidopsis* and Barley MLO members showed that four Tomato homologues (including SlMLO1) are closely related to the AtMLO2, AtMLO6, AtMLO12 and barley MLO proteins. Expression studies upon mildew infection are on going to understand the role of these SlMlo genes in the defense modulation to powdery mildew fungus. We eventually aim to use a homology-based cloning strategy to isolate SOL Mlo orthologs that are required for powdery mildew pathogenesis.

PP36

Towards Understanding the Molecular Basis of Systemin/JA-Signaled Defense Response in Tomato--A Genetic Approach

Jiuhai Zhao, Liuhua Yan, Hongling Jiang, Chuanyou Li

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China.

jhzhao@genetics.ac.cn

The systemic defense response of tomato plant in response to insect attack and wounding is regulated by the 18 amino acid peptide systemin and the phytohormone jasmonic acid (JA). Recent genetic analyses based mainly on *spr* (suppressors of prosystemin-mediated responses) mutant screens have led to the hypothesis that systemin acts at, or near, the site of wounding to amplify the production of JA, which in turn functions as a mobile signal to promote the systemic defense response. In order to identify more components involved in the systemin/JA-signaled defense response, we carried out a larger scale screen for new *spr* mutants in tomato. In which, *spr6* is impaired in wound- and systemin-induced defense gene expression. Using a candidate gene approach based on genetic linkage, we demonstrate that *spr6* is allelic to *jai1-1*, which is a loss-of-function allele of the tomato homolog of CORONATINE-INSENSITIVE1 (*COI1*) in Arabidopsis. Besides, *spr7* is seriously affected in responses to wounding and herbivore attack, trichomes development, plant growth and fertility. The defect in defenses is caused by reduced level of JA. By map based cloning, *Spr7* was located to a small region in chromosome1. In the other hand, we use microarray gene transcription profiling of wild type, *jai1* mutant and 35S::prosystemin plant to examine the functions of systemin and JA on plant defenses. The data shows that systemin induces plant defense genes expression by regulating JA biosynthesis and signaling transduction.

PP37

Identifying key *Phytophthora infestans* effectors as targets for more durable late blight resistance

Ingo Hein[1], Paul R J Birch[1,2], Glenn J Bryan[1]

[1]*Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*
[2]*Division of Plant Sciences, College of Life Sciences, University of Dundee at SCRI, Invergowrie, Dundee DD2 5DA, UK*

Ingo.Hein@scri.ac.uk

Phytophthora infestans, the causal agent of potato and tomato late blight disease, is a pathogen with high "evolutionary potential" and changes in *P. infestans* populations via migration and mutation are well documented. A new strategy in the fight against potato late blight is to identify essential pathogen effector proteins likely to be secreted during infection and translocated into host cells to manipulate host metabolism and defence responses. Many of these effectors are targeted by host surveillance systems to trigger resistance that is effective and potentially durable.

We have identified *P. infestans* effectors that appear universally expressed and functionally non-redundant and have studied the allelic variation of avirulent effectors in isolates from around the world to reveal the selection pressures imposed on these genes in pathogen populations. Further, we have screened the Commonwealth Potato Collection (CPC) for resistant wild potato accessions that recognise known variances of these effectors and setup crossed to demonstrate co-segregation between effector recognition and late blight resistance in the future. In an independent experimental approach we have manipulated functional resistance genes *in vitro* to extend their effector recognition specificity.

PP38

Determination of drought tolerance traits in advanced potato clones.

S. Legay[1], I. Levevre[1], R. Schafleitner[2], D. Lamoureux[1], J.F. Hausman[1], L. Hoffmann[1], T. Bohn[1], D. Evers[1]

[1]Centre de Recherche Public-Gabriel Lippmann, Department "Environment and Agro-biotechnologies", 41, rue du Brill, L-4422 Belvaux, Luxembourg
[2]Germplasm Enhancement and Crop Improvement Division, International Potato Center, Avenida La Molina 1895, Apartado 1558, La Molina, Lima 12, Peru

legay@lippmann.lu

Potato is an important staple food for developing region. However, the majority of potato cultivars is drought-sensitive and cannot maintain acceptable yield under water limiting condition. The scientific knowledge of drought tolerance mechanisms has been well described in plant model species, while in non-model plants further investigations are needed. We have designed a long-term drought exposure experiment with two *Solanum tuberosum* L. advanced cultivars, 397077.16 (tolerant) and Canchan (susceptible), differing for physiological drought tolerance traits. After 21 days of drought exposure, gene expression was studied using cDNA microarrays. Results show that the tolerant cultivar (393770.16) displays more differentially expressed genes than the sensitive one, suggesting greater adaptation ability. Moreover, 397077.16 exhibits a large pool of up-regulated genes belonging to cell rescue and stress responsive genes. We also found a clone-dependent expression of a gene coding for galactinol synthase, the key enzyme for the synthesis of be raffinose family oligosaccharides (RFOs) synthesis, which are thought to be involved in tolerance to water deprivation. In order to validate the putative involvement of RFOs in the drought tolerance of 397077.16, we focused on gene expression of enzymes and metabolite content related to carbohydrate and polyols pathways after 32 days and 49 days of drought. Our findings displayed higher expression of UDP-4-glucose epimerase, galactinol synthase and raffinose synthase in 397077.16, which was correlated with higher galactinol, and raffinose content. In summary, this study provides important data for tolerance enhancement and yield maintenance under water limiting conditions.

PP39

Crosstalk between the chaperone network and heat stress transcription factors in plants

Alexander Hahn, Daniela Bublak, Enrico Schleiff, Klaus-Dieter Scharf

Goethe-University Frankfurt, Frankfurt am Main, Germany

scharf@bio.uni-frankfurt.de

In tomato (*Lycopersicon esculentum*), at least 20 heat stress transcription factors (Hsfs) are involved in adaptation of transcription to changes in environmental conditions. The function of Hsfs is controlled on the transcriptional level, but also by a tight crosstalk with the chaperone network of the cell. This interaction of the two functional distinct networks targeting either transcriptional regulation or protein folding is consistent with the accumulation of both, Hsfs and heat shock proteins (Hsps) in stressed cells.

The functional crosstalk between the two networks is manifested by physical interactions of the three major Hsfs, i.e. HsfA1a, HsfA2, and HsfB1, with Hsp70 and Hsp90. At the same time these interactions and the functional consequences are factor-specific. Hsp70 was found to repress the activity of HsfA1a including its binding to the target DNA. In contrast, HsfB1 is tightly controlled by both Hsp70 and Hsp90. The latter is involved in targeting HsfB1 for proteasomal degradation, but at the same time Hsp90 enforces the DNA-binding of this Hsf. In contrast to HsfA1a and HsfB1, which are both regulated at the protein level, the crosstalk between HsfA2 and Hsp90 influences the degradation of hsfA2 transcripts.

Summarizing these and other recent data it becomes evident that during repeated cycles of heat stress the activity and composition of the cellular Hsf network is dynamically controlled by factor-specific interactions with distinct chaperones from the Hsp70, Hsp90 and small Hsp families. Our findings implicate the existence of a versatile regulatory system based on mutual feedback mechanisms between the two central networks required for the efficient adaptation of protein homeostasis under permanently changing temperature conditions.

PP40

Effect of drought to the transcriptional changes of the drought tolerance TPS1 transgenic potato

Mihály Kondrák, Ferenc Marincs, Zsófia Bánfalvi

Agricultural Biotechnology Center, Gödöllő, Hungary, P.O. Box 411

kondrak@abc.hu

Drought stress severely reduces the yield of major crops world-wide. To decrease the effect of drought, a number of plants accumulate low molecular weight water-soluble compounds, such as betaines, polyols, sugars (mannitol, sorbitol, and trehalose) and proline at high concentrations to provide stress tolerance to their cells without disturbing cellular machinery. Expression of stress-related genes in crop species is one important means of modern molecular biology to engineer plants to be stress tolerant. Transgenic derivatives of the potato cultivar White Lady expressing the trehalose-6-phosphate synthase (*TPS1*) gene of yeast display drought tolerance, without accumulating trehalose. To understand the molecular basis of this phenomenon, we have analysed the transcriptomes of the leaves of wild-type and *TPS1*-transgenic plants under drought and no stress conditions, using a potato microarray containing 42,034 potato unigene probes. Comparing *TPS1*-transgenic and wild-type plants under drought condition, 322 differentially expressed genes, of which 153 were up- and 169 were down-regulated, were identified. Under drought versus no stress condition, 1143 (165 up- and 978 down-regulated) and 383 (115 up- and 268 down-regulated) genes, were identified in *TPS1*-transgenic and wild type plants, respectively. Using the MapMan software, the differentially expressed genes were assigned into functional groups. To verify the microarray results, we used RNA gel blot analysis to examine the expression of some genes.

Our results indicate that drought induces alterations in a number of biochemical and regulatory pathways in both *TPS1*-transgenic and wild-type plants, whose transcriptomes, however, display substantial differences under drought stress.

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PP41

Calvin cycle regulating protein CP12 is regulated during mild drought stress and is associated with differential yield in potato

Sanne Hedegaard[1], Hanne Grethe Kirk[2], Kare L. Nielsen[1]

[1]*Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Aalborg, Denmark.*
[2]*LKF-Vandel, Vandel, Denmark*

sh@bio.aau.dk

Potato is a space efficient crop with more than twice the energy yield per area unit compared to cereals and is therefore an important crop to consider when trying to meet the increasing demand for food and renewable precursors for chemicals, pharmaceuticals and energy production in the future. According to existing climate models, the global mean temperature will increase and longer periods without rain is predicted in the growing season in most of potato producing areas in the future, all increasing the risk of drought leading to decreased crop yield.

In the present study the transcriptional response to short term drought (up to 12 days) of 14 field grown potato cultivars was studied using tag-based gene expression profiling on potato leaves. One of the many interesting changes observed was a subtle but significant regulation of CP12. CP12 is a regulatory protein known to inhibit two key enzymes of the carbon assimilating Calvin cycle. The 14 cultivars can be divided into two groups according to their pattern of CP12 regulation very early (day 2) in the drought stress period of 12 days, much before traditional markers for root ABA elicitation is detected. Root ABA elicitation leads to well known water stress adaptations such as stomatal closure. Highly interestingly, these two potato groups display a significant difference of yield of 33 % during regular field trials suggesting that regulation of Calvin cycle activity by CP12 is an unrecognized important determinant of yield under field conditions.

PP42

Increasing water use efficiency in *Solanum* by enhancing abscisic acid biosynthesis

E.P. Harrison[1], I.B. Taylor [2], A.J. Thompson[1]

[1]*Warwick-HRI, University of Warwick, UK*
[2]*School of Biosciences, University of Nottingham, Sutton Bonington, Leicestershire, UK*

liz.p.harrison@warwick.ac.uk

Potato and tomato production often relies on supplementary irrigation to secure crop yield. With the predicted changes in our climate resulting in reduced summer rainfall and increases in evapotranspiration due to increased temperatures, the availability of water for crop irrigation is likely to be reduced. Minimising the amount of water required for crop production is vital if we are to maintain economic and environmentally sustainable production in the future. Developing new varieties that can produce equivalent yields with reduced water inputs (improved water use efficiency (WUE)) is an important step in maintaining crop production.

Abscisic acid (ABA) is an important regulatory hormone that controls plant responses to drought and the key rate limiting step in ABA biosynthesis is catalysed by the enzyme 9-cis-epoxycarotenoid dioxygenase (NCED). Previous work in tomato has shown that constitutive over expression of NCED leads to an increase in leaf ABA content and dramatically increases the efficiency with which plants use water through changes in stomatal conductance[1]. However, constitutive over expression of NCED also impacts on germination and early growth due to the high levels of ABA produced at this stage of development. To optimize the benefits in improved WUE we need to selectively regulate ABA biosynthesis during plant development through altering the timing, location and the level of expression of NCED within the plant. Two approaches are described here (i) the use of alternative promoters to drive NCED expression at different stages of development and (ii) the exploitation of natural allelic variation through the production of near isogenic lines (NILs) containing the NCED locus derived from 10 different wild relatives of *Solanum* lycopersicum. Preliminary data from both approaches will be presented.

[1]Thompson et al (2007) Plant Physiol, 143, 1905.

PP43

Water Use Efficiency in Potato

Ankush Prashar, Alison Roberts, Dale King, Lyn Jones, Gavin Ramsay, Paul Hallet, Timothy George, Pete Hedley, Jim McNicol, Finlay Dale, Philip White, Glenn Bryan.

Scottish Crop Research Institute, Dundee, UK

ankush.prashar@scri.ac.uk

Water is one of the key resources challenging the sustainability of modern agriculture. In developing countries, potato production is increasing because of its ability to provide nutritious food in a short season. However, the potato crop requires profuse irrigation. Yields of commercial potato varieties are often restricted by water availability. Their root systems are generally sparse and shallow, and they close their stomata, preventing photosynthetic carbon assimilation, whilst water is still available in the soil. To enable breeding of drought tolerant varieties, we are developing phenotypic screens that will allow us to explore the genetic basis of key traits for water use efficiency (WUE).

From field trials of a genetic mapping population, ten genotypes with contrasting transpiration efficiencies based on leaf $\delta^{13}C$ values were cultivated under controlled glasshouse conditions. After emergence, plants were grown for 30 days in soils watered to field capacity (30% volumetric content, -5 kPa water potential) before being divided into three groups irrigated to 30%, 20% (-300kPa, slight stress) and 12% (-1500kPa, wilting point) volumetric content. As WUE is a complex phenomenon, we evaluated a number of associated physiological and morphological traits. Tissue samples were also collected at different time points to determine differentially expressed genes at these moisture levels.

Response to water stress not only includes closing stomata but also reducing the density of stomata during leaf development. Preliminary data from our experiments shows that transpiration-efficient genotypes, as indicated by low leaf $\delta^{13}C$ values, have consistently lower stomatal conductance at 12% volumetric soil moisture than transpiration-inefficient genotypes. Thus transpiration-inefficient genotypes transpire more water at lower soil water content.

PP44

Optimization of Water Use Efficiency in Tomato by Transposition of an LeNCED1 Transgene

Sajjad Z. Awan[1], Ian B. Taylor[2], Andrew J. Thompson[1]

[1]*HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK*
[2]*Plant and Crop Sciences Division, School of Biosciences, Sutton Bonington Campus, University of Nottingham, LE12 5RD*

s.awan@warwick.ac.uk

Abscisic acid (ABA) mediates plant adaptation to abiotic stress and 9-cis-epoxycarotenoid dioxygenase (NCED) is a key regulatory enzyme in ABA biosynthesis. Over-expression of LeNCED1 from the Gelvin "superpromoter" increased ABA levels and water use efficiency [1], and, although germination and seedling establishment were delayed, long-term growth was not affected [1]. The use of rbcS as an alternative promoter gave even higher ABA levels but growth was greatly reduced [2].

This study aims to generate useful, novel variation in NCED expression by allowing LeNCED1, driven by the histone H2A promoter (pH2A), to transpose to new loci where the expression may be modified. We used the maize Ac/Ds system: a stabilized activator element (sAc) was combined with an engineered dissociation element (Ds1::pH2A::LeNCED1::Ds2) by cross-pollination and then transposition was observed in F1 plants. 270 F2 plants were then identified that contained Ds but that had lost sAc by segregation so that further transposition was prevented. These were screened for low stomatal conductance (gs) and 41 plants were selected. Three F_3 families were investigated further: they showed multiple stable Ds transpositions, heritable low gs, increased water use efficiency and a range of growth rates. The ABA contents, germination, growth and development are being studied in the F4 generation. The project will allow an assessment of the optimum level and distribution of ABA for improved crop performance in water limited environments.

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PP45

Identification of tomato flavour QTL

Piotr Jasionowicz, Rob Linforth, Graham Seymour, Andy Taylor

University of Nottingham, School of Biosciences, Plant Sciences Division, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, United Kingdom

sbxpj@nottingham.ac.uk

Tomato is one of the most important fruit crops due to the volume of product consumed in both a fresh and processed form and makes a major contribution to human diet and health. Tomato taste is a complex composition of sugars, acids and volatiles. There are over 400 volatiles in tomato, but only about 30 are considered to contribute to flavour. We want to identify genes responsible for regulating volatile production and improving fruit quality. A population of *Solanum pennellii* introgression lines is being used in this study. This is a population of 76 lines that contain regions of the wild-green fruited *S. pennellii* genome introgressed into the *S. lycopersicum* cv. M82 background. The introgressions are marker defined and divide the genome into 107 separate bins. The release of volatiles from tomato fruits is being monitored using APCI-MS (Atmospheric Pressure Chemical Ionisation - Mass Spectrometry) as a high throughput system, simulating the process of chewing the tomato fruit. During two screens of the tomato IL populations undertaken in 2007 and 2008, over 1500 fruits were tested at a specific stage of ripeness for the presence of several volatiles. Volatile QTLs have been identified and selected data were validated with use of GC-MS SPME. Some promising introgression lines have been chosen. Perception, likeness and interactions of tomato volatiles are now being tested in sensory assays based on a tomato juice system. Future work will include GeneChip experiments to identify candidate genes underlying QTL. Gene function will be tested in transient experiments.

PP46

Comprehensive Expression Analysis of Sugar Transporters and Aquaporins in MicroTom

Katsuhiro Shiratake[1], Tomohide Yasuda[1], Chiharu Mori [1], Toshiko Gamano [1], Junpei Hioki[1], Kenji Nashima[1], Koh Aoki[2], Daisuke Shibata[2],Shohei Yamaki[3]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan*
[2]*Kazusa DNA Research Institute, Kisarazu 292-0818, Japan*
[3]*Department of Environmental Biology, Chubu University, Kasugai 487-8501, Japan*

shira@agr.nagoya-u.ac.jp

Sweetness is one of the most important factors to decide fruit quality. Sugar transporters play indispensable roles in long distance sugar transport from source leaves to fruits and in sugar accumulation in fruit cells. Water status in plant is known to decide Brix of fruits. Aquaporin, which is also called water channel, regulates water status in plants. Therefore we focus our attention to sugar transporter and aquaporin of tomato. We searched to find all cDNAs encode sugar transporter and aquaporin in KafTom (Kazusa Full-length Tomato cDNA Database) and classified them into families. Twenty sugar transporter homologues, 3 sucrose transporters (SUT/SUC), 5 hexose transporters (STP/HXT), 1 polyol transporter (PLT), 1 myo-inositol transporter (ITR/MIT), 3 plastidic glucose transporters (pGlcT), 2 putative monosaccharide sensing proteins (AZT/MSSP), 3 sugar porter family proteins (SFP) and 2 vacuolar glucose transporters (VGT), were found. Twenty-six aquaporin homologues, 9 plasmamembrane intrinsic proteins (PIP), 8 tonoplast intrinsic proteins (TIP), 4 Nod-26 like intrinsic proteins (NIP), small intrinsic proteins (SIP) and 2 XIP, were found. XIP is unique aquaporin family of tomato and was not found in Arabidopsis and rice. We determined gene expression of the aquaporins and sugar transporters their expression in MicroTom by semi-quantative RT-PCR. We will present these data in this meeting.

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PP47

Marker assisted selection for functional male sterility in tomato

Mirosława Staniaszek[1], Katarzyna Szajko[2], Elżbieta Kozik[1], Marzena Nowakowska[1], Hanna Habdas[1], Waldemar Marczewski[2]

[1] *Research Institute of Vegetable Crops, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland*
[2] *Plant Breeding and Acclimatization Institute, Platanowa 19, 05-831 Młochów, Poland*

mstan@inwarz.skierniewice.pl

The gene *ps-2* confers functional male sterility in tomato. The use of breeding line with *ps-2* can decrease costs of the F_1 tomato hybrid seed production. This gene was mapped on tomato chromosome 4. The aim of our study was the identification of molecular markers useful for MAS.

Three tomato lines with *ps-2* and three fertile lines were received from the collection of The Department of Genetic, Breeding and Biotechnology in Institute of Vegetable Crops in Skierniewice. The F_1 hybrids and F_2 progeny was obtained. RAPD, ISSR and COSII methods were used to find informative markers. The CAPS marker designated as $C4\text{-}30_{1000,}$ was useful for selection of male sterile tomato plants.

PP48

Understanding potato quality traits important to consumers

Wayne Morris, Laurence Ducreux, Gordon McDougall, Heather Ross, Pete Hedley, Jenny Morris, Glenn Bryan, Mark Taylor

SCRI, Invergowrie, Dundee, DD2 5DA, Scotland, UK

Wayne.Morris@scri.ac.uk

Potato flavour and texture are important factors in consumer preference trials. In order to make improvements in germplasm it is important to increase our understanding of the molecular basis of these traits. These traits are however very difficult to assess quantitatively. Tubers from *Solanum tuberosum* group Phureja cultivars score consistently higher than *S. tuberosum* group Tuberosum cultivars in sensory evaluation by trained panels. The blend of volatile and non-volatile metabolites that impact on flavour attributes is not well defined. In this study, quantitative descriptive analysis of potato samples by a trained taste panel was undertaken, comparing tubers from *Solanum tuberosum* group Phureja with those from *Solanum tuberosum* group Tuberosum, both at harvest and following storage. Correlation and principal component analyses revealed differences between the potato cultivars and storage conditions and demonstrated associations of metabolites with the different sensory attributes.

Phureja tubers also exhibit a very different cooked texture compared with Tuberosum and cook in approximately half the time. Previous work has identified tuber pectin methyl-esterase activity (PME) as a potential factor influencing textural properties. Manipulation of the PME expression level altered total PME activity and resulted in changes in the pectin methylation status of tuber cell walls. Finally, the cooked potato textural properties were strongly influenced by the expression level of PME, reinforcing the role of pectin methylation in product quality.

PP49

Mass production of miraculin, a taste-modifying protein using transgenic tomato fruits and plant factory

T. Hirai[1], K. Hiwasa-Tanase[1], K. Kato[1], M. Yano[1], D. Narendra[1], N. Kikkawa[1], N. Kurokawa[1], T. Ichikawa[3], T. Mizoguchi[1], N. Fukuda[1], H. Kakuta[2], K. Takane[3], H. Ezura[1]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba*
[2]*Plant Ecochemicals Research Center*
[3]*Inplanta Innovations Inc.*

thirai@gene.tsukuba.ac.jp

Miraculin is a taste-modifying protein isolated from miracle fruit (*Richadella dulcifica*) in West Africa. Miraculin is able to turn a sour taste into a sweet taste. Therefore miraculin has a great potential as an alternative low-calorie sweetener for diabetic and dietetic purposes. Recently, we have succeeded in producing transgenic tomato (line 56B, upright type, cv. Moneymaker) expressing miraculin protein. In this study, we tried to further improve the transgenic tomato for massive miraculin production in plant factory.

Several useful transgene constructions, in which 3' non-coding genomic sequence of miraculin gene was used as a terminator, or selection maker for transgene selection was eliminated, were newly used in order to develop the transgenic lines accumulating more miraculin. We also developed a new cultivation system for tomato in plant factory in order to produce the recombinant miraculin stably. The fruit yield of line 56B using this system was 45 tFW/10a/ year and the miraculin content of line 56B was stable in plant factory. These results indicate plant factory is more suitable for miraculin production than in field condition. Moreover we engineered tomato architectures as to be suitable for plant factory using cross breeding. The line 56B was crossed with a dwarf tomato (cv. Micro-Tom). Two suitable lines were finally selected in F_7 generation, and the miraculin productions of them were higher than that of line 56B because of higher fruit yields and miraculin accumulations.

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PP50

The HSP terminator of *Arabidopsis thaliana* increases miraculin accumulation in tomato fruits

N. Kurokawa, Y-W. Kim, T. Hirai, K. Kato, K. Hiwasa-Tanase, H. Ezura

Graduate School of Life and Environmental Science, University of Tsukuba

natsuko81@hotmail.co.jp

Miraculin is a glycoprotein included in miracle fruit (*Richadella dulcifica*) native to South Africa. The miraculin has a taste-modifying activity and it changes sour taste to sweet taste. Therefore, it expected to be used as an alternative sweeter. However it is difficult to produce miraculin in large scale in Japan because of its tropical origin. We succeeded in developing transgenic tomato producing miraculin stably in fruits Miraculin accumulation in transgenic tomato fruits is much lower than that in miracle fruit. In order highly to accumulate miraculin in transgenic tomato fruit, we tested the heat shock protein 18.2 (HSP) terminator. The terminator, which was transected in *Arabidopsis* T87 protoplast, has strong ability of transcriptional termination and results in increasing expression level of a foreign gene than NOS terminator (Nagaya et al. 2010). In this study, we produced 20 lines transgenic tomato (cv. Micro-Tom) with miraculin gene terminated by the HSP terminator. The HSP terminator increased mRNA levels about 7.3-fold higher than NOS terminator. Moreover accumulation of miraculin increased about 6.5-fold more than control line with miraculin gene terminated by NOS terminator. Miraculin concentration in the transgenic tomato was 6.9% per total protein. In conclusion, the HSP terminator is effective for highly accumulating miraculin in transgenic tomato fruits.

These works were supported by the Ministry of Economy, Trade, and Industry of Japan (METI).

PP51

Characterization and mapping study of a single dominant gene controlling CMV resistance in peppers (*Capsicum annuum* L.)

Won-Hee Kang[1], Hoang Ngoc Huy[1], Hee-Bum Yang[1], Jin-Kyung Kwon[1], Sung-hwan Jo[2], Jang-Kyun Seo[3], Kook-Hyung Kim[3], Doil Choi[1], Byoung-Cheorl Kang[1]

[1]*Dept. of Plant Science, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea*
[2]*Bioinformatics Research Center, KRIBB, Daejeon, Korea*
[3]*Dept. of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea*

hui81@snu.ac.kr

Cucumber mosaic virus (CMV) is one of the most destructive viruses in Solanaceae family. *C. annuum* “Bukang” is a commercial cultivar known to contain a single dominant gene resistant to CMV, including CMVKorean and CMVFNY strains. We designated the name *Cmr1* (Cucumber mosaic resistance 1) in “Bukang” to this resistant gene. Analysis of cellular localization using CMV-GFP showed that “Bukang” inhibits systemic movement of virus from mesophyll cell layer to mesophyll cells. Mapping study and FISH analysis revealed the *Cmr1* gene is located at the upper end of LG2 near TG31A. A total of three markers were developed by comparative genetic mapping study. One intron based marker was developed using a pepper EST sequence homologous to *Tm-1*. We also developed two additional SNP markers using tomato BAC sequence which is located syntenic position to *Cmr1*. We expect that the SNP markers developed in this study will be useful for developing CMV-resistant cultivars and for fine mapping the *Cmr1* gene.

PP52

Development of molecular markers linked to the L locus with high specificity only to the L4 allele in pepper

Hee-Bum Yang, Won-Hee Kang, Byoung-Cheorl Kang

Dept. of Plant Science, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

yhbk0130@snu.ac.kr

Many resistance genes have been isolated from various plants. Nucleotide binding site (NBS) - leucine rich repeat (LRR) is a gene structure shared in most of resistance genes (R gene). Nucleotide sequences of R genes were conserved well in NBS domain, whereas LRR domains showed very high diversity. The L4segF&R marker was developed based on LRR sequence of candidate NBS LRR gene. The L4segF&R was tested in Special (L4/L0), Myoung-sung (L4/L1) and Cupra (L3/L0) F2 population. Co-segregation analysis was performed with molecular markers linked to the L locus on L4-segregating populations and breeding lines that contain the L4 gene. The L4segF&R was most closely linked to the L4 locus and it is located about 0.5cM far from the L locus. The L4segF&R is a L4 allele specific marker and can be very useful to isolate individuals that contain the L4 alleles from PMMoV resistant breeding lines from unknown PMMoV resistance population.

PP53

SNP Markers Developed by HRM Analysis and Inter-specific Linkage Map Construction in *Capsicum*

Soung-Woo Park, Jin-Kee Jung, Byoung-Cheorl Kang

Dept. of Plant Science, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

ssambak0417@naver.com

Single nucleotide polymorphism (SNP) is sequence variation of single nucleotides in the genome and the most abundant in genomes of animal and plant. For discovery of SNP markers, 440 conserved ortholog set II (COSII) markers were amplified and sequenced. Two hundred twenty COSII markers were found to have SNPs between *C. annuum* "RNaky" and *C. chinense* "PI159234". Intron-Based Polymorphic (IBP) markers were also developed using intron flanking sequence. To detect SNPs, high resolution melting analysis (HRM) was performed. Up to now, we developed 170 COSII and 351 IBP polymorphic markers using HRM analysis. These developed markers were placed in an interspecific pepper linkage map AC99. Finally, this map was validated through the comparison with other intrerspecific "Pepper-COSII" and map positions of common markers were syntenic.

PP54

***Solanum pennellii* 700 backcross inbred lines: a tool to study epistasis**

Itai Ofner, Dani Zamir

The Hebrew University of Jerusalem, Faculty of Agriculture, Institute of Plant Sciences, P.O. Box 12, Rehovot 76100, Israel

itai.ofner@mail.huji.ac.il

The advantage of the S. *pennellii* introgression lines (ILs) in resolving individual QTL is also a drawback - epistatic interactions between unlinked loci, which are a major component of the phenotypic variation, cannot be directly estimated. For this purpose, a new S. *pennellii* (LA716) based population of ~700 backcrossed inbred lines-BILs (BC_2 and BC_3 self 6) was constructed in the determinate M82 background. Each BIL genotype carries multiple wild species introgressions permitting phenotypes to be associated with specific epistatically interacting QTL. Once the BILs are phenotyped and mapped, individual ILs and sub-ILs can then be used to reconstruct any epistasis detected in the BILs and to study the genetic and developmental components underlying the specific interactions.

PP55

The origin of pepper CMS cytoplasm deduced by chloroplast DNA derived molecular marker

Yeong Duek Jo, Hee-Jin Jeong, Byoung-Cheorl Kang

Dept. of Plant Science, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

cho1414@snu.ac.kr

Cytoplasmic male sterility (CMS) is a maternally inherited inability to produce functional pollen, which may be caused by incompatibility between nuclear and mitochondrial genomes as the result of interspecific crosses. Although the CMS cytoplasm from a pepper accession "PI164835" has been widely used to produce F_1 hybrid seeds in pepper, the origin of this cytoplasm has not been determined. Because plastid genome is co-transmitted with mitochondrial genome and highly stable compared to mitochondrial genome, we used plastid barcode sequences to deduce the origin of the pepper CMS cytoplasm. Two plastid barcode sequences, *trnH-psbA* intergenic sequences and *rpl16-rpl18* intergenic sequence, were used to analyze cytoplasm types of pepper germplasms which include six *Capsicum* species. Plastid barcode analysis revealed that cytoplasm types can be divided into eight types and five types for *trnH-psbA* and *rpl16-rpl18*, respectively. The barcode sequences of CMS pepper lines were identical to the sequence of a cytoplasm type of a particular clade of *C. annuum*. Further investigation using a larger number of germplasms confirmed that the cytoplasm type of CMS is identical to the cytoplasm type of the particular *C. annuum* clade. These results suggest that the CMS cytoplasm of pepper may be originated from a cross between a female plant belonging to the *C. annuum* clade and other *Capsicum* species.

PP56

Advanced backcross QTL analysis of a *Solanum lycopersicum — Solanum chilense* cross

Pasquale Termolino[1], Theresa Fulton[2], Olga Perez[3], Nancy Eannetta[4], Yimin Xu[4], Steven D. Tanksley[4], Silvana Grandillo[1]

[1]*CNR-IGV, Institute of Plant Genetics, Portici, Italy*
[2]*Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853 USA*
[3]*Scuola Superiore Santâ Anna, International Doctoral Programme on Agrobiodiversity, ENEA-Cr. Casaccia, Rome, Italy*
[4]*Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853, USA*

termolin@unina.it

The objective of this work was to extend the Advanced Backcross QTL (AB-QTL) strategy to another wild tomato relative, *Solanum chilense*, in order to explore the potentials of this wild species as a source of useful QTL alleles for yield-related and fruit quality traits. For this purpose, a population of 237 BC_2 plants, derived from a *Solanum lycopersicum* (TA3011) x *S. chilense* (acc. LA1932) cross, was genotyped with 104 PCR-based markers (77 CAPSs and 27 SSRs), and approximately 200 BC_2F_1 families were grown and phenotyped for 18 agronomic traits, in two locations in California. Significant QTL were identified for all traits, for a total of 144 QTL, ranging from 5 to 11 QTL detected for each trait. A good portion (59%) of these QTL were conserved across locations. Consistent with previous QTL mapping studies, numerous clusters of QTL were observed. For the 15 traits for which allelic effects could be deemed as favorable or unfavorable, 21 loci (17%), corresponding to six traits, had trait-improving alleles originating from the wild parent, most of which might be new positive QTL alleles found in *S. chilense*. To our knowledge, this represents the first report of an extensive QTL mapping study conducted on an interspecific tomato cross involving the wild species *S. chilense*. Results from this study demonstrate that, despite its inferior horticultural characteristics, *S. chilense* contains alleles capable of improving traits of economic importance for processing tomatoes, which could be targeted for marker-assisted breeding.

PP57

QTL analysis in backcross inbred lines of *Solanum neorickii* (LA2133)

Pasquale Tripodi[1], Michi Brog[2], Francesco Di Dato[1], Dani Zamir[2], Silvana Grandillo[1]

[1]*CNR-IGV, Institute of Plant Genetics, Via Universita 133, 80055 Portici, Italy*
[2]*The Hebrew University of Jerusalem, Faculty of Agriculture, Institute of Plant Sciences, P.O. Box 12, Rehovot 76100, Israel*

ptripodi@unina.it

A population of 142 backcross inbred lines (BILs) (BC_2F_7) was developed from the interspecific cross between *Solanum lycopersicum* (cv. E6203) and *Solanum neorickii* (acc. LA2133). Within the framework of the EUSOL project (http://www.eusol. net/), the BILs were genotyped for 123 marker loci covering the entire tomato genome, including four SSRs, two CAPSs and a common set of 117 COSIIs. During the summers of 2008 and 2009, the BIL population was evaluated in Akko (Israel) for several agronomic traits. A preliminary QTL analysis, performed for total yield, fruit weight, soluble-solids content (brix) and brix x yield, allowed the identification of 75 significant QTL, the majority of which (64%) were detected in both years. The number of significant QTL identified for each trait ranged from a minimum of 13 QTL for brix to a maximum of 24 QTL for fruit weight. For 16 (21%) of the significant QTL the wild allele was associated with a favorable effect. Wild alleles that could be targeted for further marker-assisted introgression into cultivated tomato were identified.

PP58

A genetic platform of tomato multi-species introgression lines: present and future

Pasquale Tripodi[1], Francesco Di Dato[1], Sandra Maurer[2], Saleh Seekh[3], Michi Brog[4], Mark van Haaren[5], Luigi Frusciante[6], Ayed Mohammad[3], Steven D Tanksley[7], Dani Zamir[4], Christiane Gebhardt[2], Silvana Grandillo[1]

[1]*CNR-IGV, Institute of Plant Genetics, Portici, Via Universita, 133, 80055 Portici, Italy*
[2]*Max-Planck Institute for Plant Breeding Research Carl von Linne Weg 10 50829 Cologne, Germany*
[3]*Hebron University, Faculty of Agriculture, P.O. Box 40, Hebron, Palestine*
[4]*The Hebrew University of Jerusalem, Faculty of Agriculture, Institute of Plant Sciences, P.O. Box 12, Rehovot 76100, Israel*
[5]*Keygene N.V. P.O. Box 216, 6700 AE Wageningen, The Netherlands*
[6]*Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Via Universita 100, 80055 Portici, Italy*
[7]*Department of Plant Breeding & Genetics, Cornell University, Ithaca, NY 14853, USA*

ptripodi@unina.it

"Exotic libraries", which consist of sets of introgression lines (ILs), have proven to be powerful tools for plant breeders allowing a more efficient exploration and exploitation of wild species diversity. To enhance the rate of progress of introgression breeding, we have undertaken the development, and further refinement, of a multi-species IL platform, derived from a diverse selection of tomato wild accessions (including *S. pennelii* LA0716, *S. habrochaites* LA1777, *S. chmielewskii* LA1840, *S. neorickii* LA2133). Multi-species ILs are highly divergent in phenotypes providing abundant segregation for whole genome naturally selected variation affecting yield, morphological and biochemical traits, and allow multiallelic effects to be captured. In order to facilitate marker-assisted breeding based on these wild species resources, and to facilitate comparisons between function maps of tomato and potato, the tomato mapping populations have been anchored to the potato genome using a common set of conserved ortholog set II (COSII) markers. This work has been conducted within the framework of the EU-SOL project (http://www.eu-sol.net/), and, in collaboration with other partners, the new genetic platform is being evaluated for numerous traits, allowing the identification of QTL and genes of potential interest for tomato improvement. Recently, sequence information has become available for the parents of a *S. lycopersicum* (cv. E6203) x *S. pimpinellifolium* (LA1589) BIL population (http://solgenomics.net/). This information will greatly enhance the possibility of identifying new polymorphic markers, thus allowing to add the BIL population to the COSII-anchored genetic platform, and to better explore the full potential of this valuable wild accession.

PP59

Hagfish or: How I learned to stop worrying and love the coverage plot

Mark Fiers

The New Zealand Institute for Plant & Food Research Limited

mark.fiers@plantandfood.co.nz

One of the principal quality measures in a genome (re-)sequencing project is a coverage plot. Such plots document the number of reads covering each base in the reference sequence. In a *de novo* sequencing project, a coverage plot is an indication of the quality of the assembly. In a re-sequencing project a coverage plot can indicate the extent to which the source and reference sequences are similar, depending on the relationship between source and reference,

Hagfish is a tool kit that creatively employs coverage plots to gain quick insights into the results of a (re-) sequencing project. This poster describes two applications of Hagfish. The first application is an approach to visualize assembly errors based on the correct alignment of read pairs against the reference sequence. The second application employs comparative coverage plots from two different sources aligned against a single reference genome to get an indication of structural variation between the two sources. The second application could be applied to determine the structural variation between two cultivars or closely related species.

The software can be acquired from the author.

PP60

KaFTom: A Database for Full-length cDNA and Genome Structures in Tomato

Shingo Kawamura[1], Kazuki Hamada[1], Ayako Suzuki[1], Hirofumi Otsuka[1], Koji Yokoyama[1], Naoki Yamamoto[1], Kunihiro Suda[2], Atsushi Kurabayashi[2], Tatsuya Suzuki[2], Kazuhide Ooga[2], Takanori Narita[3], Tadasu Shin-i[3], Yuji Kohara[3], Daisuke Shibata[2], Koh Aoki[2], Kentaro Yano[1]

[1]*Bioinformatics Lab., Meiji Univ., Japan*
[2]*Kazusa DNA Res. Inst., Chiba, Japan*
[3]*Center for Genetic Resource Information, National Institute of Genetics, Japan*

kyano@isc.meiji.ac.jp

We have determined sequences of 115,062 ESTs and 13,227 full-length cDNAs (HTCs) from a miniature tomato cultivar Micro-Tom. With sequence analysis, we have constructed tomato unigenes with other publicly available tomato ESTs and predicted DNA polymorphisms among cultivars, functional annotations (including GO terms, metabolic pathways) of genes, functional domains of proteins and genome structures. The information has been provided from databases MiBASE (http://www.pgb.kazusa.or.jp/mibase/) and KaFTom (http://www.pgb.kazusa.or.jp/kaftom/). With the recent progress in genome sequencing of a cultivar 'Heinz 1706', the genome annotations was also updated by comparisons betwen HTCs and genome sequences from the SOL Genomics Network (SGN).

The HTCs were sequenced from 22,900 representative clones, which were selected from sequence clustering of 5' ESTs. By the clone-by-clone primer walking and assembling method, 13,227 HTCs were obtained. HTCs were mapped onto the prerelease of tomato genome shotgun sequence (S_lycopersicum_scaffolds_ 20091201) using BLASTN (threshold E-value, 1e-50). Each pair of scaffold and HTC was then submitted to exon and intron prediction using est2genome. From exon-intron structures predicted by est2genome, exons in the genome sequences were collected and used to predict coding sequences (CDSs) and translated amino acid sequences, where CDSs were defined as a sequence region coding the longest amino acid sequence. The amino acid sequences were also used to detect GO terms and functional domains by InterProScan. The current version of KaFTom provides information of these annotations and the clone request. All of the full-length cDNA clones (89,872 clones) are available from the National Bioresource Project Tomato (http://tomato.nbrp.jp).

PP61

Solanaceae Genomics Resource

Brett R Whitty, C. Robin Buell

Michigan State University, Plant Biology, East Lansing, MI, 48824, USA

whitty@msu.edu

Ongoing projects in the Solanaceae are increasingly depositing a wealth of genomic and transcriptomic data to the public sequence databases, including forthcoming data from whole genome sequencing projects in Potato and Tomato. With funding from the National Research Initiative (NRI) Plant Genome Program of the USDA National Institute of Food and Agriculture (NIFA), we have developed the Solanaceae Genomics Resource: http://solanaceae.plantbiology.msu.edu, a web-accessible suite of databases and tools that provides a centralized repository of Solanaceae sequence data, and the results of value-added bioinformatic analyses on this data, to enable breeding and research.

For draft genomic sequences in the clade, we provide a consistent set of novel gene predictions and sequence analyses to supplement any existing public annotation data. Using transcript sequences and predicted gene models we have identified putative orthologs, paralogs, SNPs, and lineage-specific genes within the Solanaceae allowing intra- and inter-species comparisons. As well, we have identified homologs of Solanaceae species within a number of model dicot species allowing users to leverage the resources from these model species and apply them to studies in Solanaceae. Our analysis pipelines are run on all publicly available sequence data from Solanaceae species, including all varieties of transcript-derived and genomic sequence. Our results are accessible through the open source Generic Model Organism Database (GMOD) Gbrowse genome viewer, and through custom views and displays. Overall, we provide a robust and integrated comparative genomics resource permitting data-mining of Solanaceae sequences by the community, publicly accessible through a unified, user-friendly web portal.

PP62

P4: Towards Public Precision Phenotyping of Potato (P4)

Merideth Bonierbale, Walter Amoros, Reinhard Simon

International Potato Center, Lima, Peru

m.bonierbale@cgiar.org

High precision phenotyping data will translate sequence information into effective tools to accelerate breeding progress. Further acceleration can be achieved through public release of phenotyping data on key genomic materials, community review and participation. We announce an interactive tool for translational genomics in potato built on a key genetic resource, the DM/DI//DI mapping population. The tool uses published standards for morphological characterization, standard tools for querying and integrating diverse data and best practices for germplasm documentation. It includes a) high resolution images optimized for online 'zooming' and biometric analysis, b) SSR marker profiles, c) sample qualitative and quantitative trait data and respective evaluation protocols, d) passport data, e) mapping information, f) sequence information and g) gene and pathway information, delivered online for interactive exploration and download. The biomart database is integrated with CIPs online germplasm ordering system. Qualitative annotations and summaries can be contributed through a wiki. As a result, tools and guidelines are available for the community to allow contribution of high quality documented phenotyping and genomic datasets following best practices for international public research. The P4-initiative is expected to facilitate and increase the scope of collaboration on potato research and breeding. This is work in progress and we invite critical review and contributions. The database and wiki are accessible via CIPs corporate website at: http://www.cipotato.org.

PP63

The SGN database (http://solgenomics.net/) - what's new?

Aureliano Bombarely, Naama Menda, Isaak Tecle, Robert Buels, Jonathan Leto, Susan Strickler, Anuradha Pujar, Joyce van Eck, Lukas A. Mueller

Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY 14853

lam87@cornell.edu

The SOL Genomics Network (SGN, http://solgenomics.net/) is a clade-oriented database (COD) providing a scalable, comparative framework for genetic, genomic, phenotypic, and taxonomic information of the Solanaceae and closely related Asterids. Currently, major sequencing efforts are underway for several Solanaceae species, three of which are already available from SGN, including the tomato reference genome. Unigene sets have been updated and newly added, such as for *Coffea arabica*. Notably, a new expression module has been implemented, containing an expression atlas of *Nicotiana tabacum*, and expression data of more species are being added. A main focus of SGN is the development of a comprehensive database for Solanaceae phenotypic variation, linked with the extensive Solanaceae genotype data, including the development of a Solanaceae-specific phenotype ontology in collaboration with breeders and other scientists. Extensive phenotyping experiments conducted by the SolCAP project have recently been incorporated into the database, which will enable new features in our breeder's toolbox. In addition, SGN supports wide-ranging community curation features, in which members from the community can obtain locus editor privileges for annotating genes and phenotypes using simple online interfaces, creating a rich and continuously updated resource. SGN is supported by the NSF and the USDA.

PP64

Development of Micro-Tom TILLING platform

Yoshihiro Okabe, Erika Asamizu, Takeshi Saito, Tohru Ariizumi, Tsuyoshi Mizoguchi, Chiaki Matsukura, Hiroshi Ezura

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

yoshihiro-13@hotmail.co.jp

Now entire tomato genome sequence information has become available, and many gene sequences can be obtained from the database. We are developing a saturated Micro-Tom mutant population with an aim of establishing functional genomic tools for tomato (Ariizumi et al., See PP88). To enable to reverse genetic approach using this mutant population, we developed Micro-Tom TILLING platform. TILLING allows identification of allelic series of mutants with a range of modified functions for desired target genes. We made DNA pools from 3000 EMS mutant lines with different EMS mutagenesis conditions at 0.5 % and 1.0 % (Watanabe et al., 2007), and the mutation density was determined by screening 6 and 2 genes, respectively. It was estimated that 0.5 % EMS population had a mild mutation frequency of one mutation per 1180 kb, whereas one mutation per 470 kb was estimated in the 1.0 % EMS population, which is suitable for screening a mutant. One to 6 mutated allele(s) (average 3.5 alleles) were obtained for a gene screened. We are currently screening more various genes, and developing additional TILLING pool from new 1.0 % EMS mutant population.

Reference:

Watanabe et al., (2007): Ethylmethanesulfonate (EMS) mutagenesis of *Solanum* lycopersicum cv. Micro-Tom for large-scale mutant screens. Plant biotechnology

PP65

Dilemmas of re-sequencing a heterozygous tetraploid plant

SJ Thomson, MWEJ Fiers, JME Jacobs

The New Zealand Institute for Plant & Food Research Limited

susan.thomson@plantandfood.co.nz

Plant & Food Research New Zealand (PFR) is an active member of the international Potato Genome Sequencing Consortium (PGSC), which has recently released an advanced draft of the potato genome sequence. The cultivar used to generate the draft sequence is a homozygous doubled monoploid (DM1-3 516 R44), chosen for ease of sequencing and assembly using Next Generation Sequencing technologies but with little genetic or commercial relevance.

PFR has since embarked on the genomic re-sequencing of four commercially relevant potato lines with a view to expanding our knowledge and understanding of the gene alleles involved in traits of interest and to further develop marker assisted selection tools. These potato lines are highly heterozygous autotetraploids that have so far yielded over 20 billion nucleotides of genomic sequence data using Illumina technology. A major issue surrounding re-sequencing is manipulating and making sense of this data, including: mapping back to the reference genome, quality assurance, identifying genetic variation and knowing how much data is enough.

We present some initial results derived from our re-sequencing efforts so far, detailing quality and quantity of data. We will also describe pipelines for determining genetic variation, including common and unique SNP variations across genomes that target annotated gene-rich regions.

Additionally, application of an in-house developed method for quickly gaining insight into small-scale variation of re-sequencing data will be presented (see poster PP59 from Mark Fiers for more detail on this pipeline).

PP66

SolaR80: A Framework for Comparative Study of *Solanum* R-gene Families

Edmund A. Quirin[1], Harpartap Mann [1], Alessandra Traini[2], Maria Luisa Chiusano[2], James M. Bradeen[1]

[1]*Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108 USA*
[2]*Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Portici, NA 80055 Italy*

jbradeen@umn.edu

Candidate gene resources and cross-species comparative genomics facilitated mapping and cloning of several *Solanum* disease resistance (R) genes. Most (~75%) R-genes belong to the nucleotide binding site-leucine rich repeat (NBS-LRR) class. PCR primers targeting conserved NBS motifs yield candidate R-gene fragments and enable survey of the R-gene space of plant genomes. Candidate R-gene libraries have been created for tomato, potato, and several wild *Solanum* species. We created a candidate R-gene library of 92 sequences for the disease resistant *S. bulbocastanum*. Comparing sequences of all *Solanum* candidate and cloned R-genes, we defined R-gene "families" based on 80% DNA sequence similarity a definition that reflects DNA cross-hybridization results. We refer to these families as SolaR80 ("*Solanum* R-genes at 80% sequence similarity") lineages. NBS-LRR gene families can undergo birth/death, diversification, or expansion/contraction as a result of plant speciation. We explored SolaR80 lineage distribution throughout *Solanum* using sequence- and hybridization-based analyses. Our results document examples of R-gene family expansion/contraction and diversification/loss associated with speciation. Our research also suggests that the SolaR80 framework will be useful for marker development for R-gene mapping and plant improvement. The SolaR80 framework for defining and comparing R-gene families across *Solanum* species is amendable and expandable. Recent improvements in DNA sequencing technologies are likely to yield sequences of both whole genomes and R-gene spaces from multiple *Solanum* species in the near future. We offer our rudimentary framework to the Solanaceae community as a starting point for pursuit of detailed, cross-species comparisons of R-gene lineages.

PP67

Adaptation of tomato and other dicotyledonous plants to day and night rhythms

M. Ryngajllo, A. Nunes-Nesi, F.M. Giorgi, A. Bolger, M. Lohse, K. Koehl, I. Balbo, R. Sulpice, A.R. Fernie, B. Usadel

Max Planck Institute of Molecular Plant Physiology, Germany

ryngajllo@mpimp-golm.mpg.de

Diurnal changes and concomitant fluctuations in the availability of carbon are thought to have a great influence on plants, regulating not only gene expression but also influencing many metabolic and physiological processes. Thus it is not surprising that studies in *Arabidopsis* have revealed that many thousands of genes oscillate in diurnal and circadian experiments and that hundreds of genes react to sugar starvation induced by a phase of prolonged darkness. Although some progress has been made in cis-Element detection, a profound understanding of the regulatory programs underlying these responses is still lacking. To further investigate these regulatory programs in Solanaceous plants, we studied the changes during a diurnal cycle and an extended night in tomato (*Solanum lycopersicum* cv. Money Maker) using a combination of microarrays, deep sequencing and GC-MS.

We present a comparison of the observed transcriptomic and metabolic responses in tomato to that in public datasets covering a diverse set of plants, including *Arabidopsis*. We explore the biological context of the diurnal and sugar starvation induced changes using the Mapman pathway tools. Furthermore, we will present first results of the analysis using the same experiments across species to identify conserved cis-Elements which should greatly increase the confidence in their prediction. Moreover, making use of the dense time-course in our data set, we will show how potentially causal links between metabolites and transcripts can be extracted and verify the extracted links by comparison to bona-fide metabolite-transcript pathways in tomato and *Arabidopsis*.

PP68

The Identification of Umami Taste QTL in tomato using Taste Receptor Cells

Bee Lynn Chew, Graham. B. Seymour, Kerstin Wieland, Jacquie de Silva

University of Nottingham, UK

sbxblc@nottingham.ac.uk

The taste of foods is perceived as one or a combination of five sensations which are sweet, sour, bitter, salty and umami. The umami taste is best described as a savoury sensation (Ikeda, 2002) and it is of central importance in food flavour. This project aims to identify genes controlling the development of umami in plant products using tomato as a model system.

The umami sensation is known to be detected in mammals by receptors in the tongue. The mammalian taste receptors T1Rs form two heteromeric G-protein-coupled receptor complexes and taste receptors T1R1 and T1R3 function together as an umami taste detector on the tongue (Zhao et al., 2003). It is already well established that monosodium glutamate and L-aspartate evoke the umami sensation and are highly detected by these taste receptors (Li et al., 2002).

We want to identify regions of the tomato genome contributing to known and novel umami components. The strategy we have adopted is to use the well characterized *Solanum pennellii* introgression lines (ILs) to pin-point genomic regions of interest. The 76 *Solanum pennellii* ILs each contains a single marker defined region of the wild *Solanum lycopersicum* background (Eshed & Zamir, 1994). Crude extracts of the ripe fruits from each IL have been prepared. The aim of the first part of the project is to challenge T1Rs expressed in an in vivo reporter system with the IL extracts.

A functional calcium assay based on G-protein coupled receptor signaling was used to test pure compounds of monosodium glutamate (MSG), inosine-5'-monophosphate (IMP) and the crude tomato IL extracts. Results proved that this calcium assay is very sensitive towards the detection of pure MSG and IMP samples but is not suitable for the detection of umami compounds in crude tomato extracts.

Future work will involve the optimization of the newly developed umami assay where crude tomato extracts from all of the introgression lines can be screened for compounds which induce the umami response other than just glutamate.

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PP69

Mapping of the *AOX1a* and *β-amyI* genes on potato chromosome VIII

Dominika Krusiewicz, Henryka Jakuczun, Iwona Wasilewicz-Flis, Danuta Strzelczyk-Żyta, Waldemar Marczewski

Plant Breeding and Acclimatization Institute- National Research Institute, Platanowa 19, 05831 Młochów

d.czyzewska@ihar.edu.pl

Alternative oxydaze (AOX) catalyses the O_2-dependent oxidation of ubiquinol and the reduction of oxygen to water. The alternative pathway of the electron flow is not coupled to ATP production. β-amylase (β-Amy) is the starch-degrading enzyme, which provides maltose by hydrolytic degradation of polyglucan chains. Maltose can be further metabolized to glucose. The genes *AOX1a* and *β-amyI* have been mapped on potato chromosome VIII. We found for the first time an allelic variation in the loci *AOX1a* and *β-AmyI*, which was significantly correlated with reducing sugar content in potato. Their marker alleles explained the R^2 values ranging between 8.2 and 17.5% of the variability for chip color and reducing sugar content in diploid potato tubers at harvest and after cold storage.

PP70

Transcriptome analysis of tuber carbon metabolism reveals great plasticity of gene expression in cultivars of contrasting yield

Kare Lehmann Nielsen, Annabeth Høgh Petersen, Kacper Piotr Kaminski

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Denmark

ahp@bio.aau.dk

Potato is the third most important food crop in the world, with overall production of 323,5 thousand tons in 2007 [1]. Potatoes are energy rich and space efficient, which is of utmost importance for future optimization of agriculture land usage in context of growing world population [2].

The major constituent of potato tubers, starch, is synthesized in plastids by two parallel pathways, by direct integration of glucose-1-phosphate (G1P) into starch by starch phosphorylase (SP), or via ADP-Glucose pyrophosphorylase (AGPase) and starch synthase (SS). DeepSAGE transcriptome analysis [3] of Jutlandia (low), Desiree (medium), and Kuras (high yield) revealed rather little gene expression differences in tuber starch metabolism. One exception, however, was plastidial phosphoglucomutase (pPGM). pPGM catalyzes reversible conversion of glucose-6-phosphate (G6P) and G1P which are both imported into plastids [4,5]. Due to a low pPGM gene expression in Kuras, plastidial concentration of G1P is likely to be increased in Kuras favoring starch synthesis via SP. In contrast, in the medium yielding cultivar Desiree, pPGM expression is high and the excess starch content compared to Jutlandia seems to be caused by high expression of SS (2.05 and 2.65 fold higher than Kuras and Jutlandia respectively). Hence, in contrast to Kuras, the starch synthesis in Desiree might be limited by plastidial availability of ATP required for this starch synthesis route.

This great plasticity of the gene expression in starch synthesis pathways provides a possible explanation for the confusing results obtained by direct manipulation of the expression of individual genes such as pPGM [5,6,7].

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PP71

Genetic analysis of alkaloid composition and translocation in wild *Nicotiana* species

Phattharaporn Pakdeechanuan, Tsubasa Shoji, Takashi Hashimoto

Graduate School of Biological Sciences, Nara Institute of Science and Technology

t-shouji@bs.naist.jp

Nicotine and related alkaloids in *Nicotiana* species are predominantly produced in the root and translocated through xylem to the aerial parts of the plants. Closely related *Nicotiana* species, *N. alata* and *N. langsdorffii*, differ in the translocation ability and alkaloid composition. *N. langsdorffii* produces predominantly nicotine in root, and accumulates almost exclusively nicotine in the leaf. In contrast, *N. alata* produce mainly nicotine and minor amounts of anatabine and nornicotine in the root, but does not accumulate tobacco alkaloids in the leaf. Reciprocal grafting showed that shoot-scions of either species could accumulate tobacco alkaloids when grafted on root-stocks of *N. langsdorffii* but not *N. alata*. Moreover, the tobacco alkaloids were detected in the xylem sap of *N. langsdorffii* but not of *N. alata*. Overall, these results indicate that *N. alata* is deficient in translocation of tobacco alkaloids to the aerial parts.

For genetic analysis, interspecific crossing between *N. alata* and *N. langsdorffii* was performed and alkaloid profiles in F_2 plants were analyzed. The *N. alata* x *N. langsdorffii* F_1 hybrid showed non-translocating and high-nornicotine phenotypes similar to *N. alata*, indicating a dominant trait that causes a defective in long distance translocation. Backcrossing of F_1 and *N. alata* produced only the non-translocating plants, confirming the genetic control of translocation-deficient trait by an *N. alata*-derived dominant allele. Moreover, both translocating and non-translocating plants were found in backcrossing progenies of F_1 and *N. langsdorffii*, consistent with the above results.

PP72

Tomato cultivar tolerant to ToLCNDV infection induces virus-specific siRNA accumulation & defense associated hostgene expression

Pranav Pankaj Sahu[1], Neeraj K Rai[1], Supriya Chakraborty[2], Major Singh[3], Debasis Chattopadhyay[1], Manoj Prasad[1]

[1]*National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, INDIA*
[2]*School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA*
[3]*Indian Institute of Vegetable Research, Gandhinagar, Varanasi-221005, INDIA*

manoj_prasad@nipgr.res.in

Tomato leaf curl New Delhi virus (ToLCNDV) infection causes significant yield loss in tomato. To understand the molecular mechanism of virus tolerance in tomato, the abundance of viral genomic replicative intermediate molecules and virus-directed short interfering viral RNAs (siRNAs) by host plant in a naturally tolerant cultivar H-88-78-1 and a susceptible cultivar Punjab Chhuhara at different time points after agroinfection were studied. We report here that less abundance of viral replicative intermediate in tolerant cultivar may have a co-relation with a relatively higher accumulation of virus-specific siRNAs. To study defense-related host genes expression in response to ToLCNDV infection, suppression subtractive hybridization technique was used. A library was made from tolerant cultivar H-88-78-1 between ToLCNDV-inoculated and Agrobacterium mock inoculated plants of this cultivar at 21 day post-inoculation (dpi). A total of 106 non-redundant transcripts were identified and classified into 12 different categories according to their putative functions. By reverse northern analysis and qRT-PCR, we identified differential expression pattern of 106 transcripts, out of which 34 transcripts were up-regulated (>2.5 fold induction). qRT-PCR analysis was carried out to obtain a comparative expression profiling of these 8 transcripts between Punjab Chhuhara and H-88-78-1, upon ToLCNDV infection. The expression patterns of these transcripts showed a significant increase in differential expression in the tolerant cultivar mostly at 14 dpi and 21 dpi in comparison to that in the susceptible cultivar as analyzed by qRT-PCR. Our study reveals that changes in host gene expression that occurred during ToLCNDV interaction were associated with tolerant characteristics of cultivar H-88-78-1. A strong correlation of siRNA accumulation with ToLCNDV tolerance was also observed in cultivar H-88-78-1. The probable direct and indirect relationship of siRNA accumulation and up-regulated transcripts with ToLCNDV tolerance mechanism is discussed.

PP73

Near isogenic lines of tomato for texture contrasted QTL showed altered pericarp histology and cell wall chemistry

Marc Lahaye[1], Bernard Quemener[1], Marie Francoise Devaux[1], Mervin Poole[2], Mathilde Causse[3], Graham Seymour[2]

[1]*INRA, Biopolymers, Interactions, Assemblies, BP 71627, 44316 Nantes, France*
[2]*University of Nottingham, School of Biosciences, Plant Sciences Division, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, United Kingdom*
[3]*INRA, Research Unit for Genetic Improvement of Fruit and Vegetables, Domaine Saint Maurice, BP 94, 84143, Montfavet, France*

lahaye@nantes.inra.fr

Pericarp tissue from near-isogenic lines of Levovil (L), VilB (V), Moneyberg (B) and M82 (M) tomato fruits were characterized by mechanical, histological and cell wall chemistry methods to identify the impact of QTL on key texture determinants. Chromosomes 2, 5, 9, 4 or a combination of chromosomes 3 and 4 (3.4) and 2, 5, 9 (2.5.9) were affected. L9 fruits, V9 and M3.4 were firmer while V9 and M2.5.9 were softer than their parent. L4 and B5 had larger cells while B2 and B9 had smaller cells than their parent. L9 red fruits were richer in galactose compared to Levovil. The fine structure of cell wall polysaccharides demonstrated a remarkable variability originating from both the genetic and ripening status of the fruit. In particular, L9 red fruits were affected in the hemicellulose glucomannan and the pectic galactan side chains while L4 fruits were affected in the hemicellulose xylan.

Correlations between the different measures show a dependency of mechanical parameters on cell size distribution and on the xyloglucan and xylan fine structure. Pectic galactan in the Levovil QTL group was positively correlated with mechanical stress while glucomannan fine structures were negatively correlated in the M82 QTL lines. Thus, the texture contrast in the QTL lines of tomato involves different key cell wall determinants according to the genetic background and construction. These results emphasize the importance of histological characteristics and cell wall hemicelluloses fine structures on tomato pericarp tissue mechanical characteristics.

PP74

Novel function of eukaryotic translation elongation factor 1B related with virus infection in plants

JeeNa Hwang, Byoung-Cheorl Kang

Department of Plant Science, Seoul National University, Seoul 151-921, Korea

gooodscientist@hanmail.net

Viruses are completely dependent on the host cellular environment for their invasion. Host factors play important roles in most steps of virus infection, and identifying such host factors involved in virus replication and movement provide an important source of virus resistance. To identify new host factors involved in viral pathogenicity, we selected candidate host genes including eukaryotic translation elongation factors (eEFs) in pepper by comparison with *Arabidopsis* host gene information. In this study, we confirmed that the accumulation of Tobacco mosaic virus (TMV), Turnip mosaic virus (TuMV) and Cucumber mosaic virus (CMV) was dependent on both eEF1A and eEF1B. Indeed, based on our understanding, this is the first report that eEF1B is closely related with these virus multiplication in plants. In addition, the viral proteins VPg and RNA-dependant RNA polymerase (RdRp) were interacted with both *Capsicum* eEF1A and eEF1B proteins. These results suggest that not only eEF1A but also eEF1B plays essential roles in the replication and movement of viral infection in plants.

PP75

Evaluation of molecular markers for application towards potato molecular breeding within Australia

L. Schultz[1], N.O.I. Cogan[1], J.W. Forster[1], B.C. Rodoni[2], M. Milinkovic[2], A.T. Slater[2]

[1]*Victorian AgriBiosciences Centre, Victorian Department of Primary Industries, Biosciences Research Division, Park Drive, Latrobe University Research and Development Park, Bundoora, Victoria 3083, Australia*
[2]*Knoxfield Centre, 621 Burwood Highway, Knoxfield, Victoria 3180, Australia*

lee.schultz@dpi.vic.gov.au

Commercial cultivar development within Australia currently uses a conventional potato breeding strategy, relying on outcrossing and screening of a large number of derived lines to identify improved cultivars. Implementation of marker-assisted selection (MAS) is highly desirable so as to increase efficiency in identification of improved cultivars. Over five hundred Australian parental accessions have been genotyped with a suite of 12 simple sequence repeat markers (SSRs) to identify genetically divergent parents, in order that future crosses exhibit increased hybrid vigour and minimal inbreeding. Development of cultivars resistant to potato cyst nematode (PCN) *G. rostochiensis Ro1* is a high priority for Australia. Parental germplasm has been phenotypically screened for PCN resistance and in parallel, screened with the TG689 molecular marker linked to the *H1* resistance gene. There was 98% congruence between phenotype and genotype data, suggesting that provided pedigree is taken into consideration, TG689 is an effective marker for predicting PCN resistance. Potato virus Y (PVY) incidence has dramatically increased in Australia over the past decade, requiring urgent development of PVY resistant cultivars. Parental germplasm is currently being phenotypically screened for PVY resistance, and in parallel, two molecular markers linked with resistance genes Ry_{adg} and Ry_{sto}, are being evaluated. Molecular markers for PVX and PVS resistance will also be evaluated over the next year. Overall, molecular markers for several disease resistance traits will be evaluated and optimised, ideally for trait stacking.

PP76

Evaluation of linkage disequilibrium in a tobacco core collection

Alessandra Stella[1], Agostino Fricano[1], Nicolas Bakaher[2], Pietro Piffanelli[1], Carlo Pozzi[2]

[1]*Parco Tecnologico Padano, Italy*
[2]*Philip Morris International, Research and Development Department, CH-2000, Neuchâtel, Switzerland*

carlo.pozzi@pmintl.com

This work is aimed at determining the population genetic structure and estimating the extent of linkage disequilibrium (LD) in a tobacco core collection. A collection of 312 accessions was assembled and characterized with 50 simple sequence repeats (SSR) markers evenly distributed on the genome. The genetic distance calculation revealed the existence of four/five genetic clusters, well in agreement with the known subdivision in types. Results of a model-based cluster analysis evidenced the presence of a structured diversity in the population. STRUCTURE analysis clusters followed more closely the “type” than the geographical distribution (K=4). PCoA analysis was also conducted and about 28% of the observed molecular variation was explained by the first component, while less than 10% of the observed variation was explained by geographical clustering.

Based on results from model-based and model-free analyses, an algorithm was developed to select a core collection (i.e., minimum number of accessions that capture most genetic variation of the whole population) which was constituted by 89 accessions. The core collection was screened with 425 SSRs located in seven selected genomic regions, and r^2 and D were calculated for each region and for the entire dataset. Considering the germplasm as a whole, moderate levels of linkage disequilibrium (average LD extent: 0.12) were found at distances <30 cM, with more than 82% locus pairs significant at $p< 0.01$. The extent of LD in tobacco is homogenous between types possibly due to the recent common breeding history of the species. "

PP77

Analyses of genomic regions in support of synteny studies and breeding programs in *Solanum* and *Capsicum* crops.

Sander A. Peters[1], Dora Szinay[2], Yuling Bai[3], Hans De Jong[2].

[1]*Business Unit of Bioscience, cluster Applied Bioinformatics, Plant Research International.*
[2] *Laboratory of Plant Breeding, Plant Sciences Group, Wageningen University.*
[3] *Laboratory of Genetics, Wageningen University and Research Centre. Building 107 Droevendaalsesteeg 1, 6708 PB Wageningen*

sander.peters@wur.nl

A high-density BAC synteny map reveals a comprehensive genome synteny of *Solanum* crops and focuses on comparative genomics of economically important traits. It sheds light on the macro- and micro rearrangement history in the Solanaceae genus, which provides imperative knowledge on the existence of small deletions, duplications, inversions and translocations in the region of interest that is of direct importance for introgressive hybridization programs. The overall physical organization of tomato BACs is used to identify chromosomal regions across *Solanum* and *Capsicum* species showing deviation in the genetic mapping of markers and quantitative trait loci. Furthermore, this macro synteny study provides the basis for a genome-wide micro synteny study through a comparative sequence analysis in order to identify conserved homoeologous segments in Solanaceae. We will use bioinformatics to gain insight in the conservation of gene content, order and structures and identify sequence elements which are related to the genes of interest. We will focus on discrepancies between genetic and BAC-maps to pinpoint the putative chromosome breakpoints. This information will be combined with available sequence data and introgression data and will allow breeders to shift from a classical, time consuming and costly breeding programs to a more directed approach.

PP78

Role of flavonoids on the mechanical properties of the cuticle in tomato fruits transiently modified by VIGS agro-injection

Laura España, Rafael Fernández-Muñoz, Antonio Heredia, Eva Domínguez

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", IHSM-UMA-CSIC, & Dpto. de Mejora de Hortícolas, Estación Experimental La Mayora, Algarrobo-Costa, Málaga 29760, Spain

espana_laura@uma.es

Aerial parts of higher plants are covered by a continuous extra-cellular layer, the cuticle or cuticular membrane. The cuticle is a complex composite biopolymer basically composed of a cutin matrix, waxes, hydrolysable polysaccharides and phenolics. Flavonoids are the main phenolics present in tomato fruit cuticle which accumulate during fruit ripening giving a characteristic orange-red colour to the cuticle. With the aid of the transgenic ReadyVIGS plants and fruit-VIGS agro-injection technique we have silenced Chalcone Synthase, the first committed gene involved in flavonoid biosynthesis. Thus, silenced and non-silenced regions were obtained and compared within the same fruit. Silencing could be detected at red ripe stage and affected pericarp tissue, including the epidermis, which exhibited a pink colour and a colourless cuticle. Transition regions, with intermediate amounts of flavonoids, could easily be detected in the cuticle but not in the pericarp. Cuticles of silenced and non-silenced regions displayed a similar amount of cuticle components except for flavonoids. The biomechanical properties of the isolated cuticles showed that flavonoids give stiffness to the cuticle since the Young (elastic) modulus was significantly lower in the silenced regions. Results suggest that agroinjection is a useful tool for rapid screening of genes potentially involved in cuticle biosynthesis.

PP79

WRKY transcription factors in potato genome

Hannele Lindqvist-Kreuze

International Potato Center, Lima, Peru

h.kreuze@cgiar.org

WRKY type transcription factors have been shown to function during plant pathogen interaction as regulators of defence responses acting as positive or negative regulators. Some WRKY TFs have defence suppression related functions and the inactivation of those proteins is essential to trigger strong immune responses. On the other hand it has been shown that the same WRKY TF is required for the basal defence and full function of an R-gene in Arabidopsis. Additionally different allelic forms of the same WRKY gene can have the opposite roles in resistance. WRKY transcription factors contain at least one conserved DNA binding region comprising the highly conserved WRKYGQK peptide sequence and a zinc finger motif. This conserved domain was used to search the potato genome sequence to identify WRKY homologs with the help of profile hidden Markov models. Gene expression profiles of the potato WRKY genes in different tissues and stress treatments will be shown.

PP80

Regulatory mechanisms of carotenoid biosynthesis in potato tubers

Stefania Pasare[1], Alison Roberts[1], Paul Fraser[2], Peter Bramley[2], Mark Taylor[1]

[1]*SCRI, Invergowrie, Dundee, UK*
[2]*RHUL, University of London, UK*

stefania.pasare@scri.ac.uk

Isoprenoids are vital compounds for all living organisms, fulfilling many functions as hormones (abscisic acid, strigolactones), carotenoids, sterols and terpenes. There are two known pathways by which the precursor of all these products, isopentenyl diphosphate (IPP) is synthesized: the mevalonate pathway, in the cytosol, and the non-mevalonate pathway, that starts with the 1-deoxy-D-xylulose 5-phosphate (DXP), in the plastids. Even though carotenoids have been extensively studied due to their importance in the light harvesting complex and nutrition, a clear understanding concerning the regulation of their accumulation and localisation has not yet emerged. Considering the value of these compounds in nutritionally improving crops, information on such aspects becomes evidently necessary.

The present study proposes two main strategies to investigate the regulation of isoprenoid accumulation in potato tubers. Firstly, aiming to detect the types of plastids involved in isoprenoid biosynthesis, GFP and RFP tagged carotenoid synthesis related enzymes will be directed to the plastids. These proteins will then be localised by confocal microscopy, and thus provide a conclusion on (a) the type of plastids involved in carotenoid biosynthesis and (b) the possibility of plastid import as a bottleneck for isoprenoid production. Secondly, the degradation of carotenoids will be addressed as a potential regulator of accumulation. This originates from the recent discovery of a carotenoid cleavage diooxygenase 4 (*ccd4*), present in white-fleshed potato tubers and responsible for lowered levels of carotenoids. Using a range of approaches, the aim will be to determine the roles of the different members of the potato *ccd* family.

PP81

Plastid study of tomato fruits using the proteomic techniques

Miho Suzuki[1], Sachiko Takahashi[1], Kensuke Kaneko[1], Hideo Dohra[2], Yoshikazu Kiriiwa[1], Masayuki Fujiwara[3], Youichirou Fukao[3], Noriko Nagata[4], Erika Asamizu[5], Reiko Motohashi[1]

[1]*Faculty of Agriculture, Shizuoka University, Japan*
[2]*Institute for Genetic Research and Biotechnology, Shizuoka University, Japan*
[3]*Graduate School of Biological Science, Nara Institute of Science and Technology, Japan*
[4]*Faculty of Science, Japan Women's University, Japan*
[5]*Gene Research Center, Tsukuba University, Japan*

motohasi@agr.shizuoka.ac.jp

We are analyzing the differentiation mechanism of plastids in tomato using the proteomic techniques as follows.

First, to analyze the comprehensive proteome involved in chloroplast to chromoplast differentiation, we established methods to isolate and identify chromoplast proteins of Micro-Tom fruits at four developmental stages (mature green, yellow, orange and red). We identified approximately 440 plastid proteins using LC-MS/MS. When we compared the chromoplast proteome data in Micro-Tom with the data in a bell pepper, we found 33 common proteins including carotenoid-related, isoprenoid-related, Calvin cycle, chaperon, amino acid metabolism, glycolysis, and chlorophyll breakdown proteins.

Second, we are trying to collect natural variations and mutants that have various colors of fruits such as white, black and orange, and then stop their ripening at the intermediate stage. We compare the chromoplast proteome in these fruits with that of Micro-Tom fruits at four developmental stages using two-dimensional gel electrophoresis, and find specific proteins related to chromoplast development, ripening, and the fruits color.

At this conference we will discuss about proteins important to differentiation into chromoplast.

PP83

Metabolic phenotyping in Tomato

Guillaume Menard, Stephanie Doumouya, Christophe Rothan, Yves Gibon

INRA UMR 619, Bordeaux, France

guillaume.menard@bordeaux.inra.fr

Tomato is the first non-cereal crop worldwide. Given the very large genetic resources available for research and the recent achievement of the genomic sequencing, it is an ideal model species for fruit development, physiology (Causse et al 2004) and metabolite composition (Friedman et al 2004) studies. Soluble sugars, organic acids and amino acids are major determinants of fruit nutritional quality and agronomical performance and metabolic phenotypes resulting from alterations in enzyme properties have been highlighted recently. Thus, Friedman et al. (2004) have shown that the introgression of an apoplastic invertase with a lower Km for sucrose, from the related species *Solanum pennellii*, results in tomatoes with higher brix (higher sugar content). One hypothesis is that alterations in the properties of given enzymes from the primary metabolism could lead to further interesting fruit phenotypes. Our project is to screen a large number of Tomato genotypes (related species that have been crossed with the cultivated tomato, varieties, mutants) to search for such alterations, to uncover their genetic bases and ultimately to find associations with fruit quality traits.

For this, we have established a robotised platform allowing the determination of multiple enzyme activities and properties (Vmax, Km, responses to T°C). We will present the first results obtained by screening related wild tomato species.

PP84

Comparative proteome profiling of processing potato cultivars

M. Fischer, T. Colby, J. Schmidt, C. Gebhardt

Max-Planck-Institute for Plant Breeding Research, Cologne, Germany

fischer@mpiz-koeln.mpg.de

To extend the period of marketability and prevent potato tubers from sprouting without the application of dormancy-prolonging chemicals storage at low temperatures is favoured. The negative aspect of cold storage is the accumulation of reducing sugars due to starch degradation in tubers (cold sweetening). High amounts of sugars can negatively affect the quality of tuber derived food products like French fries or potato chips.

To indentify proteins which correlate with processing quality we performed a comparative proteome analysis of tubers from 40 potato cultivars. The varieties were classified into two populations according to chip quality and the amount of accumulated sugars during twelve weeks of storage at 4°C. Prior to and during cold storage tuber proteins were extracted and separated on 2D SDS polyacrylamid gels (2D-PAGE) with different conditions in the first and in the second dimension to resolve proteins of any size. 2D-PAGE protein profiles from representatives of the two populations were analysed and proteins were identified by mass spectrometry.

We could identify several enzymes and patatin isoforms individual or differential expressed in the two populations. Diversity was also found in proteins categorized as protease inhibitors. A superior expressed protein in varieties exhibiting good chip quality was identified as a Kunitz-type inhibitor with potentially regulatory functions on the activity of invertases, which catalyse the final conversion of sucrose to reducing sugars.

PP85

Metabolite profiling of leaves from Colorado Potato Beetle-resistant *Solanum* species

Helen Tai[1], Yvan Pelletier[1], Kraig Worrall[1], Larry Calhoun[2], David De Koeyer[1]

[1]*Agriculture and Agri-Food Canada, Potato Research Centre, P.O. Box 20280, 850 Lincoln Rd., Fredericton, N. B. E3B 4Z7*
[2]*Department of Chemistry, University of New Brunswick, P.O.Box 4400, Fredericton, N. B. E3B 5A3*

Helen.Tai@agr.gc.ca

Colorado Potato Beetle (CPB) is a defoliator that causes serious damage to potato crops. Both adults and larvae feed on foliage of the cultivated *Solanum tuberosum*. A number of wild *Solanum* species closely related to *S. tuberosum* have resistance to CPB. These include *S. okadae*, *S. paucissectum*, *S. tarijense*, *S. chomatophilum*, *S. oplocense*, *S. piurae*, and *S. acroglossum*. In this study, LC-MS QToF was used to perform untargeted metabolite profiling of foliage extracts from wild *Solanum* and *S. tuberosum* sp. Shepody. Principle Component Analysis was used to group the species together based on metabolite profiles. *S. okadae*, *S. paucissectum*, *S. chomatophilum*, and *S. oplocense* could be placed together in one group and *S. piurae* is closely related. *S. tarijense* and *S. acroglossum* are more distant from the other wild *Solanum* and *S. tuberosum*, but show some similarity with each other. *S. tuberosum* could not be grouped with any of the other species. The mechanism of resistance to CPB shown by the wild species may be due to the production of anti-feedant compounds. Therefore, metabolite profiles for each wild *Solanum* species were compared with *S. tuberosum* using orthogonal partial least squares analysis. Compounds with higher concentration in each of the wild *Solanum* were identified. A compound with exact mass of 560.3935 was identified in both *S. paucissectum* and *S. oplocense*. Another compound of exact mass of 1046.5571 was identified *S. paucissectum*, *S. oplocense* and *S. chomatophilum*. Other compounds identified were enriched in a single wild *Solanum* species. Cross reference with chemical databases has identified the glycoalkaloid, dehydrocommersonine, as the compound with the mass 1046.5571.

PP86

Chloroplast-chromoplast transition in tomato fruit: proteomic and RNAi approaches

Matteo Ballottari[1], Pasquale Termolino[2], Linda Bianco[3], Alessandro Alboresi[1], Maria Cammareri[2], Gaetano Perrotta[3], Giovanni Giuliano[4], Silvana Grandillo[2], Roberto Bassi[1]

[1] *Dipartimento di Biotecnologie Universita di Verona, 37134, Italy*
[2] *Institute of Plant Genetics - CNR, Research Division Portici, Portici, 80055, Italy*
[3] *ENEA, Trisaia Research Center, S.S. 106 Jonica, Rotondella, Matera, Italy.*
[4] *Italian National Agency for New Technologies, Energy and the Environment (ENEA), Casaccia Research Center, Rome, Italy.*

termolin@unina.it

Plastid differentiation from chloroplast to chromoplast has been studied in *Solanum lycopersicum* fruits, applying a combined proteomic, biochemical and physiological approach. In particular MudPIT LC/MS proteomic analysis performed on plastids isolated from leaf tissues and from four different fruit ripening stages, allowed the identification of 1218 tomato proteins, corresponding to 869 *Arabidopsis thaliana* genes. Our results elucidate the distribution during fruit ripening of gene products involved in different metabolic functions including sugar, lipid, amino acid, protein, nitrogen and sulphur metabolism, tetrapyrroles and carotenoids biosynthesis, photosynthesis and abiotic stress. In particular, we detected a strong decrease of photosynthetic proteins during fruit maturation, while heat shock proteins (HSP), chaperonins and plastid lipid associated proteins (PAP) increased. A subset of the most interesting HSP, PAP and protease differentially expressed proteins was selected for a functional study by RNA interference (RNAi) using hairpin RNAi (hpRNAi) vectors based on the Gateway recombinational cloning. For some of the constructs To lines have been obtained and the phenotypic analysis is in progress.

PP87

Expression of novel taxanes in heterologous plant systems

Nathalie Narraidoo, Graham Seymour, Rupert Fray

Plant and Crop Sciences Division, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD.

Sbxnudn@nottingham.ac.uk

The taxanes are a group of polycyclic diterpenes produced by a variety of species of yew. The potent anticancer drug paclitaxel (Taxol™) is the commercially most important taxane and is a highly effective anticancer drug widely used in the treatment of various carcinomas, melanomas, and sarcomas. Its anticancer activity is due to its ability to arrest the cell cycle by stabilising polymerised microtubules. Taxol is presently derived largely by semisythesis from the advanced taxoid 10-deacetylbaccatin III which is present in yew tree needles and which can be converted to paclitaxel *in vitro* (Miller and Ojima, 2000). The first step of the pathway involves the cyclisation of geranylgeranyl diphosphate to taxadiene catalysed by the plastidial enzyme taxadiene synthase followed by the first oxidation step catalysed by the cytochrome P450 taxadiene-5α-hydroxylase, a cytochrome P450 monooxygenase.

A taxadiene 5α-hydroxylase gene was engineered into wild type *Nicotiana tabaccum* to give transgenic plants expressing Taxadiene 5α-hydroxylase mRNA, which were then crossed with taxadiene producing transgenic tobacco plants. An RT-PCR and a northern were performed on the progenies from the crosses to identify plants that expressed both transgenes. These plants will be tested by GC-MS for the presence of the downstream product taxadiene-5α-ol. The third step in the Taxol biosynthetic pathway is catalysed by taxadien-5α-ol-*O*-acetyl transferase. Transgenic tobacco plants were made that express the taxadien-5α-ol-*O*-acetyl transferase mRNA. These plants were crossed with those currently being assayed for taxadiene-5α-ol, the aim of this is to produce the downstream product taxadiene-5α-yl acetate. The sub-cellular localisation of Taxadiene synthase, taxadiene-5α-hydroxylase and taxadien-5α-ol-*O*-acetyl transferase enzymes were investigated by fusing each of the respective coding sequences to fluorescent protein tags at their carboxyl termini. The proteins were found to be expressed in the plastids, spatially distributed between the outer membrane of the chloroplasts and in ER respectively.

The central importance of cytochrome P450-mediated oxygenation reactions in Taxol biosynthesis and the uncertainties surrounding the precise order of oxidisation and hydroxylation of the taxane core, prompted the development of this approach for the production of taxadiene-5α-ol and the localisation of the above mentioned enzymes. The localisation data suggest that the Taxadiene 5α-hydroxylase may link the initial plastid- and later endoplasmic reticulum-located steps of the taxol biosynthesis pathway.

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PP88

Development of saturated Micro-Tom mutant populations

Takashi Saito[1], Tohru Ariizumi[1], Yoshihiro Okabe[1], Erika Asamizu[1], Tsuyoshi Mizoguchi[1], Naoya Fukuda[1], Chiaki Matsukura[1], Yukiko Yamazaki[2], Hiroshi Ezura[1]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan*
[2]*National Institute of Genetics, Japan*

ariizumi@gene.tsukuba.ac.jp

Tomato is a great model plant for studying for Solanaceae species, and is also a benefit crop material for scientists who want to characterize its unique feature such as fruit development. As a part of National BioResourse Project (NBRP) being progressed in Japan, 10,793 M_2 mutagenesis lines of dwarf variety Micro-Tom, consisting of 4,371 and 6,422 lines were generated by EMS-mutagenesis and gamma-ray irradiation, respectively. The M3 seeds propagated are now available for scientists upon request to NBRP (http://tomato.nbrp.jp/indexEn.html). From our mutagenesis lines, a number of different varieties of mutants defective in organ development have been isolated, categorized based on the plant ontology, and registered in mutant database called TOMATOMA (http://tomatoma.nbrp.jp/index.jsp). These mutant recourses are available upon request through TOMATOMA. From our mutant population, mutants showing known visible phenotypes were recovered, and several allelic mutants were genetically confirmed, suggesting that our mutants were nearly saturated. We also provide evidence that our mutant resources are genetically high level in qualitative aspect.

PP89

Coexpression analysis of tomato genes: sense and antisense transcripts

Koh Aoki[1], Soichi Ozaki[1],Yoshiyuki Ogata[1], Nozomu Sakurai[1], Daisuke Shibata[1], Kazuki Hamada[2], Kentaro Yano[2]

[1]*Kazusa DNA Research Institute, Japan*
[2]*Meiji University, Japan*

kaoki@kazusa.or.jp

Coexpression analysis is a powerful way to infer function of an unknown gene. We collected a gene-expression dataset from various tissues of *Solanum lycopersicum* cv. Micro-Tom by 71 hybridization experiments using Affymetrix Tomato GeneChip array. We attempted to extract biologically relevant coexpression clusters using a novel algorithm based on the strength of correlation and density of connectivity. On the basis of the gene-to-gene correlation coefficient calculated from 67 microarray hybridization data points, we performed a network-based analysis. This facilitated the identification of 199 coexpression modules. A gene ontology annotation search revealed that 75 out of the 199 modules are enriched with genes associated with common functional categories. The list of gene-to-gene correlation coefficient values was loaded onto KaPPA-View4 to visualize coexpression relationships between metabolism-related genes. Affymetrix Tomato GeneChip allowed the analysis of 681 sense-antisense transcript pairs in which translational direction can be determined. Comparing to randomly chosen pairs, sense-antisense pairs showed higher correlation coefficients in terms of expression levels. To confirm this, we also performed the analysis based on the mutual rank of correlation, and obtained consistent result. These results demonstrated that sense and antisense transcripts from given genes likely have similar expression profile during the development of Micro-Tom plant. By mapping sense- and antisense-transcripts onto the coexpression network graph, possible role of antisense-transcripts in gene regulation is also discussed.

PP90

Functional analysis of vitamin E biosynthesis in tomato fruits by a gfp-based VIGS system

Leandro Quadrana[1], Ramon Asis[3], Mariana Lopez[1], Juliana Almeida[2], Alisdair R. Fernie[4], Magdalena Rossi[2], Fernando Carrari[1].

[1]Instituto de Biotecnologia, Instituto Nacional de Tecnologia Agropecuaria (IB-INTA), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), PO Box 25, B1712WAA Castelar, Argentina (partner group of the Max Planck Institute for Molecular Plant Physiology, Potsdam-Golm, Germany).
[2]Departamento de Botanica-IB-USP, 277, 05508-900, São Paulo, SP, Brazil.
[3]CIBICI, Facultad de Ciencias Quimicas Universidad Nacional de Cordoba, CC 5000, Cordoba, Argentina.
[4]Max Planck Institute for Molecular Plant Physiology, Golm, Germany

lquadrana@cnia.inta.gov.ar

Vitamin E comprises a family of amphiphilic antioxidants incluiding tocopherols and tocotrienols, which are exclusively synthesized by photosynthetic organisms. Four isomers: alfa, beta, gamma and delta tocopherol are produced in tomato. Alfa- tocopherol is the most abundant in fruits and has the mayor vitamin E related activity. Even when most of the enzymes of Vitamin E biosynthesis were characterised in *Arabidopis*, the understanding of this biosynthetic pathway in tomato is scarce.

By screening the *Solanum pennellii* introgression line (IL) population we found that ripe fruits from IL 8-2, 8-2-1, 9-2 and 9-2-6 present significant alterations in their tocopherol profiles. Moreover, genomic analyses allowed identification of slvte1, slvte4 and slvte3, orthologs to the corresponding *Arabidopsis* genes onto the regions spanning the mentioned introgressions.

In order to study the function of these genes in tomato, we designed a suitable platform for functional studies based on viral induced gene silencing (VIGS). To overcome the well known limitations imposed by this system (ie. patchy phenotypes, pleiotropic effects of the tracking marker), we first analysed the impact on fruit metabolism of two reporter genes, the phytoene desaturase (pds) and gfp in WT and transgenic GFP plants respectively. GC-MS metabolite profiles demonstrated that pds silencing produces dramatic alterations on pigments contents (carotenoids and tocopherols) in both mature green and ripe tomato fruits. By contrast, gfp silencing produces alterations in neither primary metabolism nor pigments accumulation, making it a suitable system for tocopherol metabolism studies. Results obtained by silencing tocopherol genes in fruits will be discussed.

PP91

Functional characterization of a lysine decarboxylase associated with changes in amino acids content in tomato fruit.

F. De Godoy[1], L. Bermudez[1], F. Carrari[2], M. Rossi[1]

[1]*Departamento de Botanica, Instituto de Biociencias, USP, Brasil.*
[2]*Instituto de Biotecnologia, INTA, Argentina.*

fabidegodoy@hotmail.com

Tomato (*Solanum lycopersicum*) is the second most cultivated vegetable worldwide The genome of the cultivated tomato has a limited sequence variation due to bottlenecks during domestication and breeding. Numerous wild related species have demonstrated to be untapped sources of valuable genetic variability including pathogen resistance genes, nutritional and industrial quality traits. Schauer et al. (2006) identified 889 fruit metabolic loci in a *Solanum pennellii* introgression line population. It was reported QTL for amino acids content mapped on chromosome 7 (segment 7H) that co-localize with a lysine decarboxylase encoding gene (Slld). The protein product of this gene acts in amino acid metabolism and particularly in polyamine biosynthesis, converting lysine into cadaverine. By means of a comprehensive and comparative approach, in this work we present a functional characterization of the S. *pennellii* and *S. lycopersicum* alleles for this gene. Sequence analyses highlighted the presence of polymorphisms along both, the promoters and the coding regions. Alleles showed differential expression profiles explained by promoter differential activities, as demonstrated by transient expression assays. Sub-cellular localization experiments showed that this protein is exported to chloroplast. Results presented here reinforce the candidature of Slld to be involved in the regulation of the fruit amino acids contents. Moreover, *in planta* silencing experiments are in progress.

PP92

Gene expression analysis of starch metabolism using mRNAseq and the potato genome sequence

Mads Sønderkær[1], Bjorn Kloosterman[2], Christian WB Bachem[2], Kare Lehmann Nielsen[1]

[1]*Aalborg University Department of Biotechnology, Chemistry and Environmental Engineering, 9000 Aalborg, Denmark*
[2]*Wageningen UR Plant Breeding, Wageningen University and Research Centre, PO Box 386, 6700 AJ Wageningen, the Netherlands*

mson@bio.aau.dk

Accumulation of carbohydrates in the form of starch in potato tubers is the result of both anabolic and catabolic processes. These processes are highly redundant in terms of gene isoforms and separate genetic pathways. Synthesis of starch can take place by direct via incorporation of glucose-1-phosphate into starch catalyzed by starch phosphorylase or via UDP-Glucose catalyzed by AGPase and starch synthase , while starch breakdown can occur via phosphorylytic or hydrolytic reactions. In potato, starch synthesis takes place not only in tubers but also in leaves in the form of transient starch during the day, which is consumed in the absence of photosynthesis during the night.

Many genes of the starch metabolic pathway have been cloned and analyzed in potato but the completion of the draft genome sequence has enabled the possibility of getting a more complete overview of all gene isoforms participating in starch metabolism in potato. Using mRNA sequencing data, the expression of individual gene isoforms of a doubled monoploid DM, and the diploid breeding hybrid RH, the latter being a closer representative of cultivated potato, have been analyzed and mapped to the metabolic model of starch metabolism. Interestingly a high degree of tissue specificity is observed, indicating that to a great extent contrasting isoforms and metabolic routes are utilized in the tubers and leaves. Furthermore, while the hydrolytic starch catabolic pathway is transcriptionally active in the landrace DM, this is virtually non-existent in RH, possible reflecting the selection for high starch yield in European breeding programs.

PP93

Identification of Putative Rpiblb2 Gene Family Encoding Coiled-coil NBS-LRR Domain from Various Chili Pepper

Hyun-Ah Lee, Sang-Keun Oh, Saet-Byul Kim, Doil Choi

Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea

leehyunah1219@gmail.com

Late blight caused by *Phytophthora infestans* is the most serious potato and tomato disease in the world. Recently, several late blight resistance genes have been isolated. The Rpiblb2, late blight resistance gene of *Solanum bulbocastum*, confers broad-resistance to potato late blight. To date, there is no study on *Phytophthora* blight resistance genes such as Rpiblb2 analogs in chili pepper which is not a host of *P. infestans*. We examined *in planta* interaction between 104 accessions of *Capsicum annuum* and Avrblb2 effectors of *P. infestans* by Agrobacterium-mediated toothpick inoculation. As a result, hypersensitive cell death was induced in 39 accessions. This result suggests that chili pepper might have Rpiblb2 homologues. To isolate Rpiblb2 homologues from chili pepper, PCR primers were designed from comparative sequence analysis of Rpiblb2 homologues of Solanaceous plants. We cloned 62 Rpiblb2 homologues using genomic DNA PCR from various accessions of chili pepper. The Rpiblb2 homologues belong to the class of plant resistance gene that has a coiled-coil motif, a putative nucleotide binding and a leucine-rich repeat domain. All of them are about 3900bp long with two exons and one 120 bp-length intron, showing approximately 70% identification with potato Rpiblb2. The study on comparative analysis and in planta interaction of Rpiblb2 homologues with RxLR effectors from *P. infestans* will be presented in relation to the nonhost resistance of plants.

PP94

Genomic analysis of vitamin E QTL in tomato fruit

J. Almeida[1], L. Quadrana[2], R. Asís[3], F. de Godoy[1], F. Carrari[2], M. Rossi[1].

[1]Departamento de Botânica-IB-USP, 277, 05508-900, São Paulo, SP, Brazil.
[2]Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaría (IB-INTA), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), PO Box 25, B1712WAA Castelar, Argentina (partner group of the Max Planck Institute for Molecular Plant Physiology, Potsdam-Golm, Germany).
[3]CIBICI, Facultad de Ciencias Químicas Universidad Nacional de Córdoba, CC 5000, Córdoba, Argentina.

juliana_abs@yahoo.com.br

Vitamin E, which is naturally found in tomato (*S. lycopersicum*), plays an essential role as antioxidant for scavenging ROS generated from cellular processes. Understanding the mechanisms underlying synthesis, transport and accumulation of vitamin E in crops are of great interest because of its implications for human health. In the present work, a fruit tocopherol profile was evaluated in *S. pennellii* introgression lines that either presented vitamin E QTL previously described, or that their introgression fragment spanned genes for the biosynthesis core pathway.

Several quantitative trait loci (QTL) for vitamin E were found, some of those showed to be stable over different harvest years when compared against previously published data. A candidate gene approach allowed the identification of twelve genes co-locating with these QTL. The cDNA from the wild alleles were cloned and sequenced and the comparative analysis revealed polymorphisms in nucleotides and deduced amino acids sequences between *S. lycopersicum* and *S. pennellii* alleles. These results represent an important step for understanding the genetic determinants of vitamin E natural variation in tomato fruit.

PP95

Examining the conditions for high efficient mutagenesis in tomato induced by heavy ion-beam irradiation

S. Imanishi[1], A. Noguchi[1], N. Yokotani[1], Y. Kazama[2], T. Hirano[2], Y. Hayashi[2], I. Honda[1], T. Abe[2]

[1]*Nat. Inst. of Vegetable and Tea Science (NIVTS), NARO, Japan*
[2]*Nishina Center, RIKEN, Japan*

phpabstract_10@yahoo.co.jp

Mutant plants are useful tools, not only as parents of new cultivars, but also as materials for clarifying physiological mechanisms. We have induced mutations in the tomato cultivar "Micro-Tom" by irradiation with accelerated heavy ions. It is reported that irradiation with accelerated heavy ions is more effective per dose in creating mutations in plants than irradiation with other sources and is suggested that mutagenesis by accelerated heavy ions could be uniquely used for both forward and reverse genetics in plants.

To date, we have visually phenotyped 4,642 M_2 families (5-8 sibs per line) derived from seeds, which were irradiated with ion beams at various dose range, in the field and found 1,640 candidate plants differing from the wild type in one or more characteristics, such as the plant size, leaf color or flower color. To obtain detailed information on the molecular mechanism, we designed an oligonucleotide-based microarray from whole set of tomato unigenes. We monitored the differences of gene expression level in the various stages of organs between mutagenized lines and wild type with the microarray consisted over 41,000 probe sets.

Furthermore, in attempt to find the conditions for high efficient mutagenesis, we start to investigate the dose-, the ion species- and the linear energy transfer (LET)-dependence of survival rate and the appearance of mutants.