

VERONA 2015

Program

FRIDAY 5th JUNE

8-9	Registration
9-9.15	Mario Pezzotti and Toni Granell – Welcome
9.15	Session 1: Biotechnological Approaches, Chairperson: Susan McCouch
9.15-10.00	Cathie Martin (John Innes Centre, UK) – “Metabolic engineering of anthocyanins in tomato fruit allows for assessment of their dietary health benefits”
10.00-10.45	Toni Granell (CSIC, SP) - “Facilitating technologies to improve/engineer tomato fruit contents”
10.45-11.15	Coffee Break / Posters
11.15-12.00	Nicola Patron (The Sainsbury Lab, UK) "Plant Genome Engineering"
12.00-12.45	Julian Ma (St. George Univ. of London, UK) – “Molecular pharming, the production of recombinant pharmaceuticals using plant biotechnology – still a palatable prospect?”
12.45 – 2.30	Lunch – University cafeteria /Posters
2.30	Session 2: Overcoming limitations imposed by climate changes, Chairperson: Dani Zamir
2.30 -3.15	Mondher Bouzayen – “Auxin signalling is a central trigger of fruit set and parthenocarpy”

3.15-4.00	Andrea Mazzucato (Univ. Tuschia, IT) “Parthenocarpy, a means to set fruit in challenging environments”
4.00-4.30	Coffee Break / Posters
4.30-5.15	Pedro Rodriguez (IBMCP-CSIC, SP) - “Biotechnological approaches for enhancing plant drought resistance based on fundamental research in ABA signalling”
5.15-6.00	MiquelÀngel Conesa – (Univ. Illes Balears, SP) “Studies of ramellet tomato landraces may reveal a basis for extending fruit shelf-life and the significance of water availability during cultivation”
7.30	Workshop Dinner - Osteria da Ugo

SATURDAY 6th JUNE

9.00	Session 3: Genotype X Environment Interaction, Chairperson: Cathie Martin
9.00-9.45	Mario Pezzotti (Univ. Verona, IT) – “G X E interaction in grapevine: The genomic approach”
9.45-10.30	Antonio Monforte (IBMCP-CSIC, SP) - “Stability of QTL effects on tomato fruit quality traits in a new genomic library of introgression lines from <i>Solanum pimpinellifolium</i> ”
10.30-11.00	Coffee Break / Posters
11.00-11.45	Elise Albert – “Genetic and genomic control of response to water deficit in cultivated tomato”.
11.45-12.30	Michele Morgante (Univ. Udine, IT) – From one to the many genomes of

	Grapevine: the evolution of the grapevine Pan-genome
12.30-2.00	Lunch – University cafeteria / Posters
2.00	Session 4: Breeding For Staple Food, Chairperson: Mondher Bouzayen
2.00-2.45	Jorge Dubcovsky (UC Davis, USA) – “Exome sequencing of tetraploid and hexaploid wheat mutant populations reveals millions of novel mutations”
2.45-3.30	Susan McCouch (Cornell Univ., USA) – “Linking genome wide association studies (GWAS) and plant breeding in rice”
3.30-4.15	Dani Zamir (Hebrew Univ., IL) - “Where have all the fruit QTL gone?”
4.15	Concluding Remarks
4.30	END of Workshop / Refreshments

LIST OF CONFIRMED SPEAKERS



Elise Albert (INRA Avignon, FR)

GENETIC AND GENOMIC CONTROL OF RESPONSE TO WATER DEFICIT IN CULTIVATED TOMATO

In the next decade water will be increasingly limiting crop production, in particular in Mediterranean region. Improving plant water use efficiency (WUE) by studying genotype x water regime (G x WR) interactions is of main interest to improve plant adaptation to low water availability. At different degrees, plants can change their phenotypes (molecular, morphological and physiological levels) in response to environmental changes. These modifications relate to phenotypic plasticity. In Tomato (*Solanum lycopersicum* L.), extensively grown in Mediterranean region, first studies have shown genetic variability in the response to water deficit, but very few genes/QTLs have been identified and mostly in wild relative species. Studying water deficit in this fleshy fruit is of particular interest since a well mastered water deficit can stimulate secondary metabolism production, increasing plant defenses and concentration of compounds involved in tomato fruit quality at the same time. In our laboratory, we analyzed 119 recombinant inbred lines (RIL population) and 142 unrelated cherry tomato (*Solanum lycopersicum* L. *cerasiforme*) accessions (GWA population), grown in greenhouse under two watering regimes (WR), in two locations (Morocco and France). Plants were phenotyped for plant phenology, plant vigor and fruit quality traits. We assessed genetic variability and G x WR interactions, for the above traits in the two populations. QTL and GWA analysis were conducted to identify QTL x watering regime (QTL x WR) interactions. The presentation will give a short overview of the research methods available to study genotype by environment interactions in plant and to present the first results of our research project. The possible use of slight water deficit to improve tomato fruit quality in future breeding programs will be investigated.

Mondher Bouzayen (INRA, INP/ENSAT Toulouse, FR)

AUXIN SIGNALLING IS A CENTRAL TRIGGER OF FRUIT SET AND PARTHENOCLARPY

The making of a fruit is a developmental process unique to plants that involves a complex network of interacting genes and signalling pathways. It involves three main stages: (i) fruit set, (ii) fruit enlargement, and (iii) fruit ripening corresponding to transition steps associated with major metabolic reorientations and structural changes. Hormones play a central role in these developmental shifts but other clues are also involved in triggering of these processes. Indeed, successful pollination of the flower triggers the onset of ovary development into fruit, the so-called fruit set. However, the signaling components that drive the flower-to-fruit transition are not fully understood. Even though the role of auxin and GA is now well documented. The present study addresses the specific role of Auxin Response Factors (ARFs) and Aux/IAA in mediating auxin action at the onset of ovary development into fruit. We show that down-regulation of *SIIAA9*, a tomato member of the Aux/IAA gene

family, leads to fruit set independently from pollination giving rise to parthenocarpy. On the other hand, reverse genetics approaches revealed that up-regulation of *SIARF8* and down-regulation of Sly-miR167 also lead to pollination-independent fruit set and to the production of seedless fruit. Overall, the study uncovers a complete regulome module controlling the flower-to-fruit transition which involves *SIARF8* as a central figure and *SIIAA9* and Sly-miR167 as components of the same mechanism underlying the fruit set process. The outcome of the study provides important clues for designing new breeding strategies for improving fruit yield in harsh environmental conditions and for producing parthenocarpic fruit.

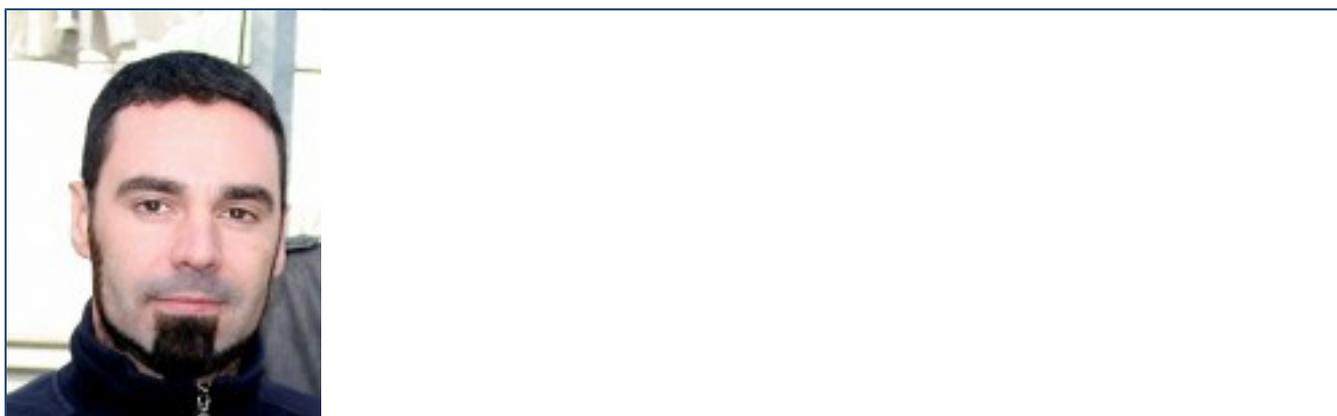


Jorge Dubcovsky (UC Davis, USA)

EXOME SEQUENCING OF TETRAPLOID AND HEXAPLOID WHEAT MUTANT POPULATIONS REVEALS MILLIONS OF NOVEL MUTATION

We have developed an exome capture platform for wheat including 82,511 genes selected from previously published transcriptome analysis in the tetraploid variety Kronos. The capture includes 286,800 exons (76% padded with 30 bp of genomic sequence). The Initial design was optimized based on 43 captures and a # design of 84 Mb was designed. On average, we capture and map 112 Mb with roughly 70% of the mapped reads on target. We are using this exon capture platform to sequence 1500 tetraploid Kronos and 1500 hexaploid EMS mutants. Based on the analysis of the first 1000 mutant lines we predict more than 3,000,000 mutations at >99% confidence in each species. We are fining an average of 2000-2500 mutants per line, which will result in a final mutation density of >40

mutations per kb in the two populations combined. A database searchable by BLAST has been created and access is provided on an individual basis on request. Sequencing has been completed and we expect to complete the bioinformatics analysis by the summer of 2015. This resource will facilitate the functional characterization of most wheat genes. As examples, I will describe our mutants for the starch branching enzyme genes SBEIIa and SBEIIb, which have resulted in a 700-900% increase in resistant starch in tetraploid wheat, and our dissection of the wheat flowering pathway.



Miquel Angel Conesa (University Illes Balears, SP)

STUDIES OF THE RAMELLET TOMATO LANDRACES MAY REVEAL A BASIS FOR EXTENDING FRUIT SHELF-LIFE AND THE SIGNIFICANCE OF WATER AVAILABILITY DURING CULTIVATION

Current improvement in horticultural crops is focused on increasing productivity, perdurability and nutritional content. However, projected climate changes will impose limitations with regard to crop water availability and so there is growing interest in the interaction between crop water regimes and fruit quality.

Several Mediterranean tomato landraces have two important traits for potential crop improvement: drought resistance and extended fruit shelf-life; however, little is known about the possible interactions between these factors the effect on fruit quality. Both traits are characteristic in the *Ramellet* tomato from the Balearic Islands (*TdR*), which have been selected over generations based on these traits. *TdR* plants have clearly adapted to allow to exhibit high water use efficiency. In addition, unlike many non-ripening mutants, *TdR* plants, as well as and the *dfd* (*delayed fruit deterioration*) mutant and the *Penjar* tomato, exhibit long shelf-life (LSL) phenotypes while retaining important ripening features. LSL

has been associated with the *alc* mutation, although in *TdR* lines homozygous for *alc*, the shelf-life of only some lines was found to be substantially reduced when cultivated without water deficit.

The aims of our current studies of *TdR* are: i) to reveal the possible biomechanical, physiological or composition-related determinants of fruit traits within *TdR* accessions; and ii) to compare such behaviors and determine the mechanistic basis of the response to water stress in other LSL tomato varieties.

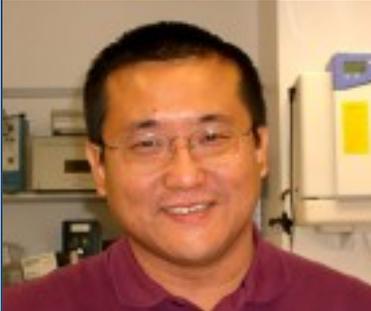
We expect that the outcomes of our studies will allow the detection of key traits that quantitatively or qualitatively underlie extended shelf-life in *TdR*, irrespective of the irrigation regime during cultivation. This may also determine, for other extended shelf-life tomatoes, the sensitivity of this trait to the cultivation method.

Toni Granell (CSIC, SP)

FACILITATING TECHNOLOGIES TO IMPROVE /ENGINEER TOMATO FRUIT CONTENTS

Tomatoes are a rich source of healthy nutrients for the population in a large number of countries. The tomato is also a model system for fruit development and ripening. The tomato genome was sequenced a few years ago and is facilitating the identification of genes important for the plant to develop a tasty nutritious fruit even in the face of a changing environment. More recently a large number of tomato cultivars, varieties and tomato related species have been re-sequenced paving the way for new approaches to associate variations in sequence at specific locations in the genome with variations in phenotype. Candidate genes resulting from a combination of different technologies often require validation which is often done by reverse engineering using transient or stable transformation. With new gene functions demonstrated it is possible to perform new and more complex engineering projects designed to obtain tomato fruits with enhanced or new properties. In this talk we will present some examples on how we are 1) developing

increasingly complex gene constructs for plant engineering, 2) identifying and testing genes to increase fruit quality or composition.



Julian K-C Ma (St. George's Hospital Medical School, London UK)

MOLECULAR PHARMING THE PRODUCTION OF RECOMBINANT PHARMACEUTICALS USING PLANT BIOTECHNOLOGY STILL A PALATABLE PROSPECT?

Most biopharmaceuticals are currently made at great expense in fermentation vats containing bacteria or mammalian cells. But the mass production of medicines in genetically modified plants, first proposed in 1989, coupled to early considerations of using edible crops for manufacture and delivery, could reduce costs and therefore make an important contribution to global health, particularly in developing countries.

Molecular Pharming has faced a number of challenges. There has been widespread scepticism that recombinant proteins could be manufactured in plants to the same standard and quality as current conventional systems. Developing a robust and reproducible manufacturing process for plants has therefore been an important priority for the field, an achievement that has recently been reached.

The concept of edible vaccines has required re-evaluation, to address immunological concerns, as well as those of regulators.

This talk will describe the rationale for developing GM plants for pharmaceutical production, the immunological constraints for oral delivery, and the key areas of concern for regulators in approving pharmaceutical manufacturing processes in plants, including the use of fruits and minimally processed plant preparations.



Susan Mc Couch (Cornell University, USA)

LINKING GENOME WIDE ASSOCIATION STUDIES (GWAS) AND PLANT BREEDING IN RICE

Understanding the relationship between genetic and phenotypic variation lies at the heart of the study of genetics and is also critically important to applications in plant and animal breeding. New sequencing technologies and the development of high-resolution SNP assays has made it possible to assess genotype-phenotype associations using genome wide association (GWA) studies in a wide range of species. Here we report on a GWA study based on genotyping a purified panel of diverse rice accessions with 700K SNPs and the evaluation of those accessions for a wide range of agronomic and physiological traits in diverse environments. Using cross-population-based mapping strategies, we identified numerous common variants influencing complex traits.

Significant heterogeneity was observed in the genetic architecture associated with subpopulation structure and response to environment. This work establishes an open-source translational research platform for genome-wide association studies in rice that directly links molecular variation in genes and metabolic pathways with the germplasm resources needed to accelerate varietal development and crop improvement for diverse environments.



Cathie Martin (John Innes Center, UK)

METABOLIC ENGINEERING OF ANTHOCYANINS IN TOMATO FRUIT ALLOWS FOR ASSESSMENT OF THEIR DIETARY HEALTH BENEFITS

Anthocyanins are pigments in plants that are increasingly being recognised as promoting human health when included in the diet. A combination of two transcription factors (Delia and Rosea 1) has been used to upregulate anthocyanin biosynthesis in tomato, resulting in purple tomatoes which extend the life span of cancer-prone p53 knock-out mice. Studies with breast cancer cell lines show that extracts of the purple tomatoes promote apoptosis compared to extracts from control, red tomatoes, indicating that the mechanism whereby dietary anthocyanins partially complement deficiencies in p53 activity may involve promotion of apoptosis in cancer cells.

We have extended our preclinical studies to examine the effects of high-anthocyanin diets on the ApoE mouse model of atherosclerosis. High anthocyanin diets reduced the formation of plaque and modified lipid metabolism, so reducing markers for the disease.

Given these positive results from studies of the protective effects of diets enriched in anthocyanins we have undertaken pilot cultivation of processing tomatoes with the high anthocyanin trait, prepared juice and we are initiating human intervention studies with subjects at risk of cardiovascular disease. We have also started notification of the FDA in the US, as the first step towards a commercial product available for human consumption.



Andrea Mazzucato (University Tuschia, IT)

PARTHENOCARPY, A MEANS TO SET FRUITS IN CHALLENGING ENVIRONMENTS

Abiotic stresses occurring during reproduction are considered one of the major constraints of plant adaptation to changing environments. Thus, the increasing threat of climate change is predicted to have a deleterious impact on the productivity of crops producing edible fruits or seeds. Parthenocarpy, the autonomous growth of the ovary into a seedless fruit without pollination and/or fertilisation, is a very attractive trait for breeders because it enables fruit set under environmental conditions that are suboptimal for plant reproduction. The action of genes conferring parthenocarpic attitudes may represent an adaptive value in wild species, allowing the setting of fruits with very few seeds when pollination becomes a limiting factor. In crops, seedless fruits in addition represent a valuable commodity for consumers. To exploit parthenocarpy using mutants or engineered plants, an important breeding target in many species in the last years, requires to understand the genetic mechanisms that positively or negatively control ovary growth at anthesis. Several studies on parthenocarpic mutants and reverse genetics approaches started to shed light on a process that until recently was largely unknown. The tomato has been a species of choice in this field. We focused our researches on the *parthenocarpic fruit (pat)* genotype, a mutation that associates a strong competence for parthenocarpy with homeotic transformation of anthers and aberrancy of ovules. This approach allows to address the mechanisms for parthenocarpy in the frame of the purported interaction of the ovary with the surrounding floral organs. Our results, together with those from other research groups working in the field, offer novel insights into the framework of transcription factors underlying fruit set and parthenocarpy thus improving the possibility to harness this fruitful character in future crops.



Michele Morgante (University Udine, IT)

FROM ONE TO THE MANY GENOMES OF GRAPEVINE: THE EVOLUTION OF THE GRAPEVINE PAN-GENOME

The analysis of variation in plants has revealed that their genomes are characterised by high levels of structural variation, consisting of both smaller insertion/deletions, mostly due to recent insertions of transposable elements, and of larger insertion/deletion similar to those termed in humans Copy Number Variants (CNVs). These observations indicate that a single genome sequence might not reflect the entire genomic complement of a species, and prompted us to introduce the concept of the plant pan-genome, including core genomic features common to all individuals and a Dispensable Genome (DG) composed of partially shared and/or non shared DNA sequences. The very active transposable element systems present in many plant genomes may account for a large fraction of the DG. The mechanisms by which the CNV-like variants are generated and the direction of the mutational events are still unknown. Uncovering the intriguing nature of the DG, i.e. its composition, origin and function, represents a step forward towards an understanding of the processes generating genetic diversity and phenotypic variation. Additionally, since the DG clearly appears to be for the most part the youngest and most dynamic component of the pan genome, it is of great interest to understand whether it is a major contributor to the creation of new genetic variation in plant evolution as well as in the artificial selection processes of plant breeding. We have resequenced to high coverage more than 50 grapevine accessions and used a variety of approaches to detect structural variants, including de novo assembly of a selected set of genotypes. We will discuss the extent and composition of the pan genome in grapevine, the different mechanisms that generate and maintain the dispensable portion, the epigenetic and phenotypic effects of the DG and the rates and modes of creation of new genetic variation due to DG components.



Toni Monforte (IBMCP-CSIC, SP)

STABILITY OF QTL EFFECTS ON TOMATO FRUIT QUALITY TRAITS IN A NEW GENOMIC LIBRARY OF INTROGRESSION LINES FROM *Solanum pimpinellifolium*

A new genomic library of introgression lines (ILs) from the *Solanum pimpinellifolium* L accession TO-937 was developed in the 'Moneymaker' (MM) genetic background. The process of IL development was accelerated thanks to the implementation of high-throughput single-nucleotide polymorphism (SNP) genotyping during the molecular breeding program. The definitive library consisted of 53 IL covering 94% of TO-937 genome. The ILs showed a high level genetic background isogenicity, most of the ILs had no additional introgressions, and in those with such additional introgressions, they were in general very small (< 2 Mb). The genomic library of ILs was agronomically characterized in three locations (Alginet, Orihuela and Málaga, in the Mediterranean coast of Spain) for several fruit quality traits: fruit morphology, organoleptic characteristics, external and internal fruit color. Genetic and environmental effects varied among traits, being the genetic component more important in fruit morphology traits and soluble solid concentration (SSC) than for fruit acidity and color.

Concomitantly, QTL effects for the fruit morphology and SSC were more consistent among trials than for fruit acidity and color. For example, three ILs showed significant effects on fruit shape in at least 2 trials, while other four showed effects only in one trial, whereas only one IL showed significant effects on fruit acidity in three three locations but 10 ILs showed significant effects in only one trial. A total of 67 QTLs related to fruit quality with consistent effects in at least 2 trials were defined. Additionally, volatile profile was assessed in a selected number of ILs in order to verify previously mapped QTLs. These results demonstrate that the current IL collection is an appropriate tool to study the genetic control of fruit quality traits and to assess the effect of the environment on QTL effects

Nicola Patron (The Sainsbury Lab, UK)

PLANT GENOME ENGINEERING

Plant synthetic biology offers the means and opportunity to engineer plants and algae for new roles in our environment, to produce therapeutic compounds and to address global problems such as food insecurity and the contamination of ecosystems with agrochemicals and macronutrients. Such complex engineering requires standardized, automatable methods for assembling DNA as well as techniques for manipulating nuclear genomes. Random mutagenesis and gene-insertion methods are being superseded by genome editing technologies that enable precise manipulation of specific sequences. We are developing easily programmable molecular tools based on re-engineered CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) prokaryotic adaptive immune systems. Using these tools we can induce single and double stranded DNA breaks at pre-determined loci, thus activating the endogenous mechanisms of DNA repair.



Mario Pezzotti (University of Verona, IT)

GENOTYPE X ENVIRONMENT INTERACTION IN GRAPEVINE: THE GENOMIC APPROACH

The phenotype (P) is the result of the interaction between the genetic composition of the plant (G) and the environment (E). To produce suitable genotypes for multiple environments the interaction GXE should be accounted and assessed for each plant

species. Plants are sessile, they cannot migrate when challenged by fluctuation in environmental conditions therefore adaptation strategies should be activated to cope with environmental heterogeneity. A fundamental strategy is to change the phenotypic expression, this phenomenon is called “phenotypic plasticity”. Phenotypic plasticity refers to the range of phenotypes a single genotype can express as a function of its environment. When phenotypic plasticity differs between genotypes, this is described as GXE. Although phenotypic plasticity is an important ecological phenomenon, the underlying genetic and molecular mechanisms remain still poorly characterized. The availability of high-throughput expression profiling technologies now makes it possible to analyze gene expression on a global scale, so that transcriptome plasticity can be investigated directly. We investigated the extent to which phenotypic plasticity in grape berries reflects underlying changes in the transcriptome by using microarray technology to study global gene expression profiles of: 1) a single clone of *Vitis vinifera* cv Corvina cultivated in different vineyards and harvested at different developmental stages over 3 consecutive years; 2) two grapevine varieties, in three environments during four developmental stages of berry development over 2 consecutive years. We studied the relationships among differential gene expression profiles, growing conditions and ripening parameters and identified several putative candidate genes for the definition of berry quality traits.



Pedro Rodriguez (IBMCP-CSIC, SP)

“BIOTECHNOLOGICAL APPROACHES FOR ENHANCING PLANT DROUGHT RESISTANCE BASED ON FUNDAMENTAL RESEARCHES IN ABA SIGNALING”

Plant stress biology faces several key challenges, particularly the generation of new crop varieties with improved water use efficiency and drought resistance. ABA receptors engineered to enhance ABA-dependent inhibition of protein phosphatases type 2C do have biotechnological potential to enhance plant drought resistance (Pizzio et al., 2013). In order to demonstrate the efficacy of mutant ABA receptors in crop plants, we generated barley transgenic plants that over-express mutant versions of *Arabidopsis* PYL4 receptors. As a result, we found that barley transformed plants that express PYL4^{A194T} or PYL4^{H82R V97A} showed enhanced drought tolerance and therefore, we describe a novel technology to cope with water stress. We have also identified the full set of tomato ABA receptors, which were functional in *Arabidopsis* plant cells and over-expression of two monomeric-type receptors conferred enhanced drought resistance. Additionally, chemical approaches based on recently identified ABA-agonists, such as quinabactin, or orthogonal ligand-receptor activation of ABA signaling might have biotechnological use in crops.

Auxiliary proteins that regulate half-life, trafficking or membrane targeting of ABA receptors also represent potential sources of candidate genes to modulate ABA signaling. We have identified a gene family, named RSL1/RFA, of single subunit E3 ubiquitin ligases that act as E3 ligases of ABA receptors and therefore control their ubiquitylation and half-life (Bueso et al., 2014). Targeting of ABA receptors by different RSL1/RFA E3s occurs at different cell compartments, which illustrates a novel mechanism to promote the turnover of ABA receptors -and attenuate ABA perception- in plasma membrane, cytosol and nucleus. We have also discovered that calcium-dependent interaction of ABA receptors with membranes is mediated through a new gene family of C2-domain ABA-related (CAR) proteins (Rodriguez et al., 2014). The function of ABA receptors in plasma membrane is eventually modulated through the RSL1 E3 ligase, which targets ABA receptors there and promotes their degradation.

Dani Zamir (Hebrew University of Jerusalem, IL)

WHERE HAVE ALL THE FRUIT QTL GONE ?

Phenotyping is a rate-limiting activity in genomic research. Plant geneticists and breeders have generated over the past decade numerous Mendelian populations that segregate for genetically mapped quantitative trait loci (QTL). Information about the map positions of QTL is included in more than 5000 publications but only a small

fraction of raw data finds its way into existing genomic databases. As opposed to sequence and expression data that need to be deposited in appropriate databases upon publication, the raw data of replicated phenotypic measurements are not deposited in any public repository and are usually lost. A major bioinformatic challenge facing the research community is to develop web-based resources to display the details of complex phenotypes to uncover hidden biological knowledge. Phenom Networks < <http://www.phenome-networks.com/>> provides such a platform where researchers can share data and compare their phenotypes and analyses to those that have already been deposited in the database to identify wider pleiotropic links. Uniting data from multiple syntenic crops on a common framework will enable the identification of common and unique bottlenecks for crop productivity and the formulation of rational strategies for genomic assisted breeding. The future sharing of phenomic data is the key for continuity of collaborative projects both in academic labs and the seed industry, and this vision will be demonstrated using results from our laboratory. What we eat are phenotypes - in view of the need to achieve global food security it is high time that we find efficient ways to link traits with genomes.