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Somatic genetic variation contributes to phenotypic variation in perennial crop species like grapevine, with many cultivars being vegetatively propagated for centuries. Phenotypic variation among lines and clones derived from the same original zygote results from somatic mutations affecting different cells and cell layers of the plant and that, in many cases, are stably maintained through vegetative propagation. Somatic variation has been described for many leaf, cluster and berry grapevine traits and has likely participated in the progressive improvement and diversification of domesticated genotypes. It provides genetic diversity for clonal selection, contributing to adaptation of elite wine cultivars to new production and quality requirements. Moreover, mutations in the L2 cell layer, which gives rise to gametes, can be transmitted to the next sexual generation.

Genome derived tools help understanding the molecular basis of somatic variation. In the last years, several reports have identified the causal mutations in several somatic variants from different cultivars. These mutations fall into three main groups: point mutations, transposon insertions and chromosomal reorganizations. Furthermore, most somatic variants analyzed to date share characteristic common features: i) they display dominant phenotypes caused by gain of function alleles in single genes; ii) independent somatic variants repeatedly appear in the same or in different genetic backgrounds as a result of mutations in the same genes and iii) phenotypes can show cell layer specificity. We will discuss the possibilities of this sort of genetic variation in cultivar improvement as well as in the discovery of new gene biological functions.
Interspecific Reproductive Barriers (IRBs) prevent hybridization between species, and in plants IRBs are often linked to mating system. Many wild species of tomato (Solanum sect. Lycopersicon) display S-RNase-based gametophytic self-incompatibility (SI), whereas others are self-compatible (SC). In crosses between all 13 tomato clade members, we found that pistils of SI species always reject pollen of SC species. This pattern held true for crosses between SI and SC populations collected from sympatric sites. We documented additional IRBs between sympatric species pairs including conspecific pollen precedence, lack of ovule targeting, and defective seed development. However, three of 19 crosses resulted in fertile F1 hybrid plants suggesting that interspecific hybridization may occur at low frequencies in nature. We investigated the relationship between mating system and pollen-pistil IRBs, in S. habrochaites – an SI species that contains recently evolved SC populations. We found that a low-activity S-RNase allele is associated with SC at the southern margin, but pistil IRBs are unaffected. At the northern range margin, at least two independent mutations have resulted in the loss of S-RNase expression and are associated with SC and weakened IRBs. We also identified pollen-side mutations that result in reproductive isolation between SC populations and central SI populations. Using transgenic approaches, we directly demonstrated that factors involved in SI also function in interspecific pollen rejection. However, it is clear that additional factors can also contribute to pollen-pistil IRBs in wild populations. We are currently using transcriptomic, genetic and transgenic approaches to identify these novel factors.
LECTURES AND SESSIONS

Monday, September 4

Session I BIODIVERSITY: GENOMES GALORE and CROP DIVERSITY I

WHY TAXONOMY MATTERS - THE AMAZING DIVERSITY OF EGGPLANT (SOLANUM MELOGENA) WILD RELATIVES

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After tomato, eggplant (aubergine; Solanum melongena L.) is the most widely consumed and most economically important Solanaceae vegetable. Its origins are in Asia, but the great diversity of related species is in Africa, and have long been a complete taxonomic tangle. Solanum melongena is a member of the hyperdiverse spiny solanums – the Leptostemonum clade, which comprises about 550 species worldwide. Our recent taxonomic work has focused on sorting out the identities and relationships of these species, and relating their evolution to the origins of the domesticated species. The species number of wild relatives has dramatically increased, by a combination of additional species recognition and the recognition of some species as closely related to eggplants that were previously not considered as such. We have also shown that these species are also at considerable conservation risk – thus impacting on their future use for crop improvement programmes. The ecological niches of these species encompass areas where drought, heat stress, extreme precipitation variability and fungal pathogens are common. Phylogenetic studies have revealed surprising patterns of relatedness, and are bringing up new questions of just how eggplants got to Asia. And paradoxically, on Madagascar, drought tolerant species have evolved to occupy mesic habitats. All this insight, however, begins with the basic framework of solid, well-evidenced taxonomy and distribution analysis. We will explore how we go about these kinds of studies, and how cooperation between taxonomists and plant breeders can be extremely fruitful.

THE EGGPLANT GENOME REVEALS PALEOPOLYPLOID ORIGIN OF FRUIT RIPENING

Giovanni Giuliano

The Eggplant Genome Consortium

A high quality genome sequence of eggplant (S. melongena L.) - a representative of the Leptostemonum subgenus with over 450 species - was produced and anchored to the 12 chromosomes using a Recombinant Inbred Line population. The eggplant genome contains 34,916 predicted genes.
Comparisons to pepper, tomato and potato, highlighted a rapid evolution of miRNA:mRNA regulatory pairs, and allowed a reconstruction of the ancestral Solanaceae, Solanum and Potatooe chromosome complements. Retrotransposon bursts occurred in the four genomes relatively recently (~3 mya to ~0.3 mya), and a positive correlation is observed between numbers of retrotransposons and chromosomal translocation rates. Species-specific tandem amplification of R genes provides a basis for the differential pathogen sensitivity of Solanaceae, and the absence of a glycoalkaloid gene cluster on chromosome 12 provides a basis for the absence of these secondary metabolites in pepper. Overexpression of TAGL1 in tomato and eggplant results in similar developmental phenotypes (sepal expansion), but in different biochemical ones (lycopene vs flavonol accumulation). Solanaceae genomes contain 10 to 15% of syntenic paralogs (ohnologs), generated by the four paleopolyploid events that occurred before the radiation of seed plants, angiosperms, eudicots and Asterid II plants. These ohnologs are enriched in transcription factor genes in Solanum, but not Capsicum, providing a possible explanation for the huge morphological differentiation in the former genus. The regulatory module comprising transcriptional regulators controlling fruit ripening is populated with ohnologous pairs dating back to the angiosperm paleopoliploidy event or even before, suggesting that the basic network controlling fruit ripening in Solanaceae was present in the common angiosperm ancestor.

RESEQUENCING OF SEVEN EGGPLANT (SOLANUM MELONGENA) AND ONE WILD RELATIVE (S. INCANUM) ACCESSIONS FOR GENETIC DIVERSITY EVALUATION AND USE IN BREEDING

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Common eggplant (Solanum melongena L., 2n = 2x = 24) lagged behind in genomic sequencing compared to other major crops, despite its economic importance. Here we report the results of the resequencing of seven common eggplants from different areas of Europe and Asia and one accession of the eggplant wild relative S. incanum collected in Israel. The eight accessions displayed a high phenotypic diversity in traits with agronomical interests such as color (black, purple, purple striped, green and white), shape (elongated, ovoid, flattened), size, prickliness, as well as phenolics content and tolerance to abiotic stresses. The genomes were reconstructed at a chromosomal scale using a mapping approach (20X) and interrogated for variants (around 15M) and variant effects on genes. The variants of each chromosome and genotype were also plotted and compared to search genomics regions with the same variants distribution, as well as missed regions in the reference genome. The genomes were also functionally and structurally annotated based on comparison with public databases. Undoubtedly, S. incanum was the most genetically distinct among the accessions, presented the lowest number of mapped reads (95% vs nearly 100% of the others) and the highest number of variants compared to the reference genome (11.7M, 7.6 to 10.6 fold more than the other genotypes). This preliminary study was a starting point to better understand the unexplored genetic diversity in the eggplant gene pool from the genomic point of view and how to use it for broadening the narrow genetic base using a wild relative.
HIGH QUALITY GENOME OF THE HIGHLY HOMEOLOGOUS ALLOTETRAPLOID
COFFEA ARABICA L.

Alexander Kochko

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*Coffea arabica*, the most widely cultivated and consumed *Coffea* species, is the sole representative of the genus resulting from a spontaneous hybridization event that resulted in the formation of this allotetraploid (2n=4x=44). The sub-genomes of the two parental species, *C. eugenioides* (?) and *C. canephora* (?) are highly similar (± 98%) but are believed to remain separated and not undergoing reciprocal recombination.

The international Arabica Coffee Genome Consortium (ACGC) has undertaken the sequencing of this genome with the aim to produce a high quality sequence where both sub-genomes result defined and anchored on 22 pseudomolecules. The most advanced nowadays technologies were used to reach this goal: long and short read sequencing (genomic and transcriptomic), optical mapping and chromosomal conformation capture (Hi-C). Finally the results were anchored thanks to genetic maps.

In order to survey the events that may have happened during, or after, the polyploidization event, both parental species genomes were also sequenced. In addition, for assessing the genetic diversity of the species, and eventual neo diversification introduced by man, a set of 35 *C. arabica* accessions, wild and cultivated were re-sequenced using short reads.

The obtained results will provide a considerable amount of new knowledge on *C. arabica* such as identification of genes involved in key agronomic issues (yield, tolerance to pests, resilience to climate change, quality of the coffee beverage…) and bring a large amount of genetic markers covering the entire genome allowing new breeding approaches (GWAS) for this highly economically important crop species.
**Session II BIODIVERSITY: GENOMES GALORE and CROP DIVERSITY II**

**GENOME EVOLUTION AND FUNCTION OF CUCUMBER AND TOMATO**

Sanwen Huang

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The genome sequences of cucumber and tomato paved the way for identification of genome-wide variation. We sequenced 115 cucumber lines and 610 tomato lines to build genome variation maps that consist of millions of SNPs and other types of variants. These big datasets facilitated the discovery of genomic features related to the domestication, divergence, and breeding of the two crops. We subsequently revealed the domestication of cucumber bitterness and develop a chemical genetic roadmap to improve tomato flavor. These knowledge can help to develop tastier varieties.

**DE NOVO ASSEMBLY OF THE ZUCCHINI GENOME REVEALS A WHOLE GENOME DUPLICATION ASSOCIATED WITH THE ORIGIN OF THE CUCURBITA GENUS**

José Blanca, Javier Montero-Pau, Aureliano Bombarely, Peio Ziarsolo, Cristina Esteras, Carlos Martí-Gómez, María Ferrol, Pedro Gómez, Manuel Jamilena, Lukas Mueller, Belén Picó & Joaquín Cañizares

We have assembled a high-quality draft of the zucchini (Cucurbita pepo) genome. The final assembly has a 1.8Mb N50 scaffold size, 34,240 gene models and 263 Mb. It is estimated to cover 93% of the genome and it includes 92% of the conserved BUSCO gene set. The genome is integrated with a genetic map of 7,718 SNPs and it is organized in 20 pseudomolecules. The genome shows clear evidences of a Whole Genome Duplication: the karyotype organization and the distribution of 4DTv gene distances within gene families. Additionally, 40 transcriptomes of 12 Cucurbita species were sequenced and assembled. Phylogenetic trees were inferred for the gene families and a consensus species build was build from them. The duplication was detected in all 12 Cucurbita species, but was not found in the more distant cucurbits belonging to the Cucumis and Citrullus genera.

**DOMESTICATION REWIRED GENE CO-EXPRESSION AND NUCLEOTIDE DIVERSITY PATTERNS IN TOMATO**

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Plant domestication has led to considerable phenotypic modifications from wild species to modern varieties. However, although changes in key traits have been well documented, less is known about the underlying molecular mechanisms, such as the reduction of molecular diversity or global changes in gene co-expression patterns. In this study, we used a combination of gene expression and population
genetics in wild and crop tomato to decipher the footprints of domestication. We found a set of 1,729 differentially expressed genes (DEG) between the two genetic groups, belonging to 17 clusters of co-expressed DEG, suggesting that domestication affected not only individual genes but also regulatory networks. Five co-expression clusters were enriched in functional terms involving carbohydrate metabolism or epigenetic regulation of gene expression. We detected significant differences in nucleotide diversity between the crop and wild groups specific to DEG. Our study provides an extensive profiling of the rewiring of gene co-expression induced by the domestication syndrome in one of the main crop species.
Session III GENETICAL GENOMICS/ MOLECULAR BREEDING

GENETICAL GENOMIC APPROACH OF THE TOMATO RESPONSE TO WATER DEFICIT

Elise Albert, Christopher Sauvage, Jean-Paul Bouchet, Frédérique Bitton, Matthieu Beukers, Renaud Duboscq, Yolande Carretero, Mathilde Causse

INRA – UR GAFL Genetics and Breeding of Fruit and Vegetables – Avignon - France

Tomato is an important vegetable adapted to Mediterranean conditions but requiring a large amount of water. Tomato fruit quality is characterized by a large number of components influenced by the genotypes and the environment, notably water status. In order to unravel the genetic control of these components, quantitative trait loci for transcript amount (eQTL), metabolic and phenotypic traits (phQTL) have been mapped under control and water deficit conditions. We used a RIL population derived from an intraspecific cross between a cherry tomato line and a large fruited line. The genomes of both parents were sequenced and differ by more than 2 million SNPs. RNAseq of parental lines revealed a number of functions affected by water stress, but also some major differences according to the parents. We harvested fruit and leaf samples of the RIL population and mapped eQTL for 270 differentially expressed genes using the fluidigm technology. Several eQTL were mapped, some with strong effects, a majority of which acting as cis eQTL, and clusters of eQTL and phQTL were demonstrated. Analysis of the transcriptome of the F1 hybrid allowed the quantification of allele specific expression and identification of almost 2000 cis-regulated genes and 1,450 trans regulated genes. Contrary to trans regulatory divergences, cis regulatory divergences were strongly conserved between organs and between watering regimes. Results will be discussed in regard to the gene functions and co-localisations with metabolite or phenotype phQTLs.

CAROTENOID METABOLIC FLUX REGULATION IN MELON FRUIT PROPOSES A NOVEL APPROACH FOR BETA-CAROTENE BIOFORTIFICATION OF FOOD CROPS

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Beta-carotene, the major pro-vitamin A in the human diet, determines the flesh color of many fruits including melon. Accumulation of β-carotene in melon fruit is governed by the ‘golden’ SNP of the Orange gene (CmOr) through an unknown mechanism. In Arabidopsis, AtOR posttranscriptionally regulates phytoene synthase (PSY), the first committed reaction in carotenogenesis. We characterized ‘low β’, a CmOr nonsense mutation (Cmor-lowβ), which lowered β-carotene levels by 30 folds, inhibited chromoplast biogenesis, reduced Or gene expression and abolished OR protein levels and dimerization capacity. Cmor-lowβ exerted a minimal effect on PSY transcripts but significantly decreased PSY protein levels and enzymatic activity, leading to reduced carotenoid metabolic flux and accumulation. Nonetheless, PSY protein level was not differentially affected by the ‘golden’ SNP indicating that posttranscriptional regulation of PSY protein is not the major mechanism underlying the CmOr-induced carotenoid accumulation in melon fruit. Double mutant analyses of developing melon fruits carrying a nonsense mutation in carotenoid-isomerase (CRTISO) validated the metabolic flux reduction caused by ‘low β’ but not by the ‘golden’ SNP. Our study shows that the ‘golden’ SNP increases β-carotene accumulation in orange melon fruit chromoplasts by arresting further metabolism of β-carotene and can
induce carotenoid accumulation independently of CmOr. The work on melon CmOr defines a precise site for DNA editing as a novel path toward β-carotene biofortification and suggests a metabolic flux analysis as a preceding step to choose potential varieties of biofortified crops.
FUNCTIONAL GENOMICS OF TOMATO FRUIT NUTRITIONAL QUALITY IN SOLANUM HABROCHAITES LA1777 INTROGRESSION LINES

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Key words: tomato, introgression lines (ILs), S. habrochaites LA1777, RNA-seq, carotenoids

Tomato fruit are a major dietary source of health promoting phytochemicals, and an excellent model system to investigate fleshy fruit development and ripening. A rich variation in health and sensory related compounds is found within germplasm of the tomato clade, and populations of introgression lines (ILs) originating from interspecific crosses provide powerful tools to harness this mostly untapped genetic potential for both fundamental biology studies and for crop improvement.

In order to gain further insights into the regulatory systems controlling synthesis of flavour and nutrition chemicals in tomato, a systems approach was pursued that explores natural variation harbored in a new set of COSII-anchored S. habrochaites (LA1777) ILs in the genetic background of the processing cv. E6203. Transcriptome sequencing using Illumina RNA-seq was performed on red ripe fruit pericarps of 88 field grown LA1777ILs, and of the recipient parent. Pericarp samples were also analyzed for carotenoids by HPLC. Variation in individual carotenoids within the population indicates both wide variation and presence of variation at numerous loci impacting carotenoid content within this population. RNA-Seq data were used for high-density IL mapping and gene expression profiling. High-density genotyping allowed precise definition of the boundaries of each IL facilitating use of this congenic resource also for breeding. Co-localization of S. habrochaites introgressions and known or putative genes provided candidate genes underlying carotenoid QTL for functional characterization.

MAPPING THE POTATO CYST NEMATODE RESISTANCE GENE H2 IN A TETRAPLOID POTATO

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Plant parasitic nematodes (PPN) of the genera Meloidogyne, Heterodera, and Globodera are the most economically important PPNs. The potato cyst nematodes Globodera rostochiensis and G. pallida encapsulate their fertilised eggs in a tough outer cyst which allows them to persist in the soil for decades, while remaining viable, making control by crop rotation a lengthy and not always successful process. Due to EU restrictions on nematicides (EU 99/414/EEC) the best method to combat these pests is through the breeding of resistance genes into commercial cultivars. Several PCN resistances have already been introgressed into cultivars, most notably the H1 gene from Solanum andigena CPC1673; active against G. rostochiensis. The control of G. rostochiensis in British fields led to a skew in population favourability which led to the rise of G. pallida populations. Unlike G. rostochiensis, there is no single gene which can control all populations of G. pallida present in Britain. Therefore, to tackle this pathogen several resistance genes active against distinct G. pallida pathotypes need to be bred into a single cultivar. The H2 gene from the wild potato species Solanum multissectum confers a high level of resistance to the Pa1 pathotype of G. pallida. A mapping population was generated through the breeding of a hetergenic resistant and homogenic susceptible (Rrrr x rrrr) plant. Targeted gene enrichment, followed by SNP (single nucleotide polymorphism) filtering, and the generation of allele specific markers
allowed a 4.7Mb region of chromosome V to be highlighted as the most probable location of the H2

EVALUATION OF A SEQUENCED TOMATO CORE COLLECTION FOR POST-HARVEST
SHELF-LIFE AND BIOCHEMICAL CHARACTERISATION OF SOME CONTRASTING
LINES

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Fruit shelf-life is an important quality trait in tomato and the economic consequences of postharvest fruit softening have brought considerable attention into this problem. In this study, we phenotyped a tomato core collection, consisting of 97 sequenced tomato accessions including old cultivars and land races, for several shelf-life parameters and characterised biochemical changes during the postharvest shelf-life of fruits from selected accessions. The core collection was grown in a controlled greenhouse, fruits were harvested at Breaker-turning stage and stored in a controlled climate chamber for 49 days. The shelf-life related parameters such as fruit firmness, weight loss and pigment production were measured once a week and evaluated over time. All three shelf-life related parameters varied markedly among genotypes, resulting in fruits with different shelf life. The most promising genotypes of the first screen were regrown and analysed to validate the initial results and 6 genotypes with contrasting shelf-life were selected for metabolite analysis. Fruits were harvested at Breaker stage and stored for 35 days at 18 °C. Samples were taken at weekly intervals and analysed for volatile compounds, primary metabolites and monomers of cell wall polysaccharides. Several metabolites showed considerable variation between genotypes and between ripening stages.

Key words: Tomato, Shelf-life, Firmness, post-harvest, Cell wall, Metabolite

This research is supported by a grant from the Dutch government (EZ-2012-19) and is carried out in collaboration with Bejo seeds, Semillas Fito and BHN seeds.
Session V  VEGETATIVE DEVELOPMENT AND TRANSITION TO REPRODUCTIVE

USING GENE NETWORKS TO ELUCIDATE DEVELOPMENTAL PROCESSES

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How morphological diversity has arisen is a key question in biology. Angiosperms exhibit a great diversity in leaf shape and leaf development has been characterized in several species, making leaves ideal targets to understand the mechanism behind morphological natural variation. Leaves are also functionally significant for generating biomass and leading to agricultural yield. Comparative transcriptomics between tomato and related species, as well as with leaf developmental mutants, was utilized in gene network construction to identify key network modules that play a role in leaf development. Super self-organizing map clustering identified major changes of gene expression patterns in leaf development. Our analyses suggest that not only massive differential gene expression but also changes in the system-level regulation of gene expression pattern differentiate leaf developmental stages. We have used differential correlation interactions (DiffCorr) between genes in the Gene Regulatory Networks (GRN) to detect GRN rewiring and identify genes that play a role in generating developmental differences during leaf morphogenesis.

TREHALOSE 6-PHOSPHATE AND NITRATE SIGNALING GATING DEVELOPMENTAL TRANSITIONS: TRANSFERRING KNOWLEDGE GAINED IN ARABIDOPSIS THALIANA TO SOLANUM TUBEROSUM

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Apart from environmental and other endogenous cues plants integrate their metabolic and energy status to ensure timely transitions between developmental stages and to regulate growth. How this is achieved is poorly understood. Trehalose 6-phosphate (T6P) has been implicated as a signal of sucrose status (1). We have found that it plays a central role in the induction of flowering, acting in the leaves via FLOWERING LOCUS T. T6P concentration rises in the shoot apical meristem over time, increasing significantly during the floral transition and initiates flowering via the age pathway (2).

Soil nitrate has a complex influence on many developmental processes including flowering time (3). We have made use of an almost natural soil-based nitrate-limited growth system that allows analysis of plants that have adapted to a small but sustained reduction of nitrate (4). We will present molecular details on how nitrate derived signals integrate into the canonical flowering time network. The research done in Arabidopsis thaliana provides a basis to understand the regulation of flowering and tuberization in Solanum tuberosum. We will present how this is achieved with emphasis on the T6P pathway and nitrate signaling.

(1) Lunn et al., Biochemical Journal, 397, 139 (2006)
(3) Marin et al., Planta, 233, 539 (2011)
(4) Tschoep et al., Plant Cell & Environment, 32, 300 (2009)
A MICROPROTEIN ENCODING GENE REGULATES THE FORMATION OF SHOOT APICAL MERISTEM AND FLOWER DEVELOPMENT IN TOMATO

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The architectures of inflorescences affect flower formation and crop yield. Different with the monopodial growth of Arabidopsis, tomato has sympodial growth habit, which new meristems called sympodial meristems (SYM) take over the growth after the transition of shoot apical meristems (SAM) to inflorescence meristems (IM). Although several genes have been shown to regulate SAM and/or IM formation in tomato, it still remains elusive how these meristems are initiated and maintained during plant development. Here, we identified and analyzed a tomato mutant fasciated flower and fruit (faf), showing defects in flower and fruit development. During vegetative phase, the faf mutant had flattened and fasciated stems and also affected axillary bud formation. After the transition of SAM to IM, the faf mutant produced long enlarged IM and flowers with increased floral organ numbers. The phenotypic observations suggest that FAF likely plays an important role in maintaining the fate of shoot apical meristems, and is also required for flower development in tomato. Map-based cloning revealed that FAF encodes a microprotein similar to Arabidopsis ZPR3. We also showed that FAF functions as a partner of several HD-ZIP III transcription factors including tomato REVOLUTA (SlREV) to regulate SAM development, and the faf mutation was found to weaken its binding ability to the transcription factors. Our results demonstrated that FAF plays an important role in regulating tomato SAM development.

GENETIC AND MOLECULAR REGULATION OF FLOWERING TIME IN PEPPER

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Pepper (Capsicum spp.) exhibits a sympodial shoot growth and insensitivity of flowering to photoperiod. In order to dissect the genetic control of flowering time in pepper, we selected EMS-induced late and early flowering mutants as well as employed QTL analysis for natural variation in flowering time. Two flowering repressors were identified, the first is FASCICULATE (FA), the ortholog of tomato SELF-PRUNING (SP) and the second is a member of the APETALA2/ERF gene family (CaAP2). By analyzing a segregating population from a cross of early-flowering cultivated inbred and a late-flowering wild C. annuum accession, we identified AP2 as a candidate for controlling late flowering in wild pepper and a likely factor controlling domestication syndrome in this species. Several flowering promoters were identified, among them is a homolog of tomato SINGLE FLOWER TRUSS (SFT), CaFT, that is a candidate for acting as the pepper florigen. An additional flowering promoter is the homolog of VERNALIZATION INSENSITIVE3 (VIN3) that controls vernalization response in Arabidopsis. Detailed phenotypic and molecular characterization of single and double mutants of these genes allowed to study their function in controlling transition to flowering and shoot architecture in pepper, the relationships among them and the extent of conservation of gene function compared to model plant species.
Session VI  REPRODUCTIVE DEVELOPMENT I

HIGH-RESOLUTION SPATIOTEMPORAL MAPPING OF TOMATO FRUIT DEVELOPMENT AND REGULATORY CONTROL MECHANISMS: EXPLORING A NEW FRONTIER IN FUNCTIONAL GENOMICS

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Fleshy fruits are organs with complex anatomies and functional structures consisting of distinct tissue and cell types. However, biochemical and molecular studies of fruits typically use a homogenized mixture of tissues as a source of target molecules. This severely limits insights into cell specialization, and lower abundance molecules are often diluted below the level of detection. This results in a critical 'information void' and an incomplete picture of many aspects of fruit biology. Our consortium has been using RNA sequencing (RNA-seq) coupled with laser microdissection (LM), which allows precise isolation of specific tissue/cell types, to generate comprehensive transcript profiles of tomato fruit development at an unprecedented level of resolution. This has uncovered numerous highly spatially defined regulatory and structural gene networks, including families of transcription factors and hormone synthesis or signaling pathways, as well as variations in the patterns of DNA methylation status associated with epigenetic control. These profiles have also revealed complex gradients of gene expression initiating in interior tissues then radiating outwards, and basipetally along a latitudinal axis. Gene co-expression network analyses have allowed us to define spatiotemporal expression modules, which have been used to investigate diverse aspects of tomato fruit development. Examples will be presented of the many new insights into commercially important traits, such as texture, flavor and aroma, color and sugar metabolism, as well as core questions related to the orchestration of transcriptional, hormonal and epigenetic regulatory mechanisms. The expression and co-expression data are available through an interactive online database, the Tomato Expression Atlas (TEA).

SURVEYING THE TOMATO GENOME AND GENETIC DIVERSITY FOR REGULATORS OF FRUIT RIPENING

James Giovannoni

USDA-ARS and Boyce Thompson Institute for Plant Research, USA

The cultivated tomato (Solanum lycopersicum) is a tractable and efficient model for fruit development, storage quality and nutrient accumulation, in addition to being a crop of established and expanding production, consumption and culinary importance the world over. Diverse, well characterized and freely available germplasm resources, combined with efficient transformation and a high-quality genome sequence have accelerated the pace of tomato biology with practical implications to crop improvement. Our lab explores the function of ripening transcription factors underlying fruit ripening mutations including those altered in the rin, nor, and u mutations defining fruit development roles for the MADS, NAC and GLK transcription factor families, respectively. Mining of these families provided additional genes affecting fruit development and ripening characterized in transgenic tomato plants. Additional regulators have been uncovered via examination of fruit quality QTLs and genes associated with ripening based on expression profiles. Genome enabled analysis of fruit development further indicates that transcriptional control intersects with changes in the epigenome. Exploration of additional crop genomes suggests that
some of the regulators identified in tomato are conserved through evolution. Discoveries made through identification of genes underlying mutations and QTLs, combined with analyses of ripening phenomena as they permeate the maturing fruit tissues, reveal a complex developmental process regulated by multiple factors with often conserved components that may serve as useful targets for crop shelf-life and quality improvement across a diverse spectrum of important fruit crop species.

A BZIP TRANSCRIPTION FACTOR IS INVOLVED IN THE REGULATION OF TOMATO FRUIT RIPENING, UPSTREAM OF MADS-RIN AND NAC-NOR RIPENING REGULATORS

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Tomato (Solanum lycopersicum) fruit development is a complex process involving early developmental phases (fruit set, cell division and cell expansion) followed by ripening. It has been shown that ripening is largely coordinated by ethylene and that MADS-RIN, NAC-NOR and SBP-CNR transcription factors play a crucial role in triggering fruit ripening. However, to date, the transition between early fruit development and the onset of ripening is very poorly understood.

In a previous work, we identified several regulatory hubs possibly involved in the transition between cell expansion and mature green (MG) stages (Mounet et al., Plant Physiol, 2009), among which a bZIP transcription factor of unknown function. To get further insights into its role relative to other known regulators of fruit ripening, we generated plants expressing a chimeric dominant repressor bZIP-SRDX specifically targeted to the cell expansion and MG stages using the fruit specific SlPPC2 promoter.

The bZIP-SRDX expression resulted in profound alterations of the ripening process in the transgenic fruits, including a delayed onset of ripening and a slowdown of the ripening process. Detailed developmental, metabolic and transcriptomic characterization of bZIP-SRDX fruits, together with the analysis of fruits from bZIP-SRDX x rin and x nor double mutants confirmed the crucial role of bZIP in the transition from IG to MG stage and further demonstrated that this bZIP acts upstream of NAC-NOR and MADS-RIN TFs. This bZIP TF therefore constitutes a new actor in the ripening regulatory network, which establishes a continuum between early fruit development and the onset of ripening.

MIR156/SPL/SBP PATHWAY INTERACTS WITH SLDELLA IN THE CONTROL OF TOMATO FRUIT DEVELOPMENT

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The development of fleshy fruits consists in an important process in terms of agronomic traits, and understanding the mechanisms that control this process is the basis for the development of various biotechnological tools. Non-coding RNAs regulate members of various gene families that control fruit development, from the initial steps of early carpel development to the latest steps of fruit ripening, consisting in regulatory networks where these RNAs play a central role. As an example, various members of the SQUAMOSA promoter binding protein-like (SPL/SBP) family are regulated post-
transcriptionally by the microRNA156 (miR156). Tomato plants overexpressing miR156 (miR156OE)
showed misregulated expression of genes related to meristem maintenance and organ boundary
formation during ovary development, such as the transcription factors GOBLET (GOB) and Tomato
Knotted 2 (TKN2), resulting in fruits with a higher number of locules and undetermined growth, with fruitlike structures in the style end of the fruit. The tomato SlDELLA loss-of-function mutant procera (pro) also
presents modified fruits with fruit-like structures in the style end. GOB expression increased by 3-fold in
early closed flower buds of this mutant and TKN2 levels remained unchanged. We crossed pro and
miR156OE to understand if these pathways interact to control of ovary development. Fruits of the F2
offspring are seedless, amorphous and extremely undetermined. Most of these fruits does not even form
locules. We also treated miR156OE plants with GA3, and treated plants also produced extremely
undetermined fruits. These modifications suggest an interaction between miR156/SPL/SBP and
gibberelling signal transduction pathways in the control of ovary development.

JASMONATES IN TOMATO OVULE DEVELOPMENT
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Jasmonic acid (JA) and its derivatives, collectively known as jasmonates, are lipid-derived signaling
molecules involved in defense against pathogens and in response to wounding by herbivores, in the
regulation of mutualistic interactions of plants with microorganisms, but also in plant growth and
development. Many of them alter gene expression positively or negatively in a regulatory network. Their
function in development of tomato flowers was concluded from a mutant defective in JA perception (jai11), being known to exhibit defects in female development leading to female sterility. Using isolated
ovules of three different stages of flower development, from both wild type and jai1-1 and showing
drastic differences in their JA content, we identified putative JA-dependent regulatory components.
Among differentially regulated genes were two genes encoding MYB transcription factors, which
orthologues in Arabidopsis are known to have a crucial role JA-regulated stamen development. To
analyze their function in tomato ovule development, respective TILLING mutants were identified and
CRISPR/Cas9 mutants created. The phenotypic characterization of these mutants confirmed a function
of the identified MYB transcription factors in ovule development of tomato. Comparative transcriptomics
using carpels of a CRISPR/Cas9-mutant line, jai1-1 and wild type will help to identify putative MYB
transcription factor target genes and to verify their role in JA-regulated tomato ovule development.

MOLECULAR ASPECTS OF DUAL REPRODUCTION IN POTATO
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3
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Potato (Solanum tuberosum L.) is a perennial plant that can undergo sexual reproduction and vegetative
propagation during the same growing season. The edible underground tubers represent the perennating
organs. Following a period of dormancy, new clonally propagated plants will develop from the axillary
buds of the tubers, allowing the plant to survive over consecutive years.
Potato tuber development has been extensively investigated. The timing of tuber induction is regulated
by several signals, one of them being photoperiod. Under short day (SD) conditions the SELF PRUNING
6A (StSP6A)protein, a homolog of Arabidopsis FLOWERING LOCUS T, is expressed in leaves and then
moves below ground to induce tuber formation. Above ground, the induction of flowering is promoted by
SELF PRUNING 3D (StSP3D), a close homolog of SP6A and orthologue of SINGLE FLORAL TRUSS

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(SFT) in tomato. At the transcriptional level, StSP3D and StSP6A are largely co-regulated, so that they are both induced under SD conditions. However, the onset of flowering occurs independently of photoperiod and irrespective of StSP3D transcript levels. Notably, under SD conditions or in genotypes strongly induced to tuberise, flowering is aborted at early stages, suggesting a competition between the two reproductive strategies.

We are exploring at which level flowering and tuberisation compete. While the presence of the tuber-sink alone does not seem to fully explain flower abortion, we found that StSP3D and StSP6A can form heterodimers as well as share additional partners of interaction. Elucidation of the biological function of these protein interactions is under current investigation.
Gamma-aminobutyric acid (GABA) is primarily metabolized via a short pathway called the GABA shunt in higher plants. The GABA shunt bypasses two steps (the oxidation of α-ketoglutarate to succinate) of the tricarboxylic acid (TCA) cycle via reactions catalyzed by three enzymes: glutamate decarboxylase, GABA transaminase, and succinic semialdehyde dehydrogenase. The GABA shunt plays a major role in primary carbon and nitrogen metabolism and is an integral part of the TCA cycle under stress and non-stress conditions. Tomato is one of the major crops that accumulate a high level of GABA in its fruit. The GABA levels in tomato fruits dramatically change during fruit development; the GABA levels increase from flowering to the mature green stage and then rapidly decrease during the ripening stage. Although GABA constitutes up to 50% of the total free amino acids at the mature green stage, the molecular mechanism of GABA accumulation and the physiological function of GABA during tomato fruit development remained unclear. Then we have elucidated GABA metabolism and developed technologies to regulate GABA contents in tomato fruits. And we have characterized tomato plants with higher or lower GABA contents for development. These results suggest a potential role of GABA in fruit development. In this presentation, we summarize recent studies of GABA metabolism in tomato fruits and discuss the potential biological roles of GABA in tomato fruit development.

The metabolic fate of imported photosynthate in the developing fruit sink is determined primarily by carbohydrate metabolism in the fruit itself. Natural genetic variability for patterns of carbohydrate metabolism and accumulation exists for fleshy fruit of both tomato and melon. Among the tomato germplasm there is genetic variability for multiple metabolically determined traits including sucrose, starch, fructose, glucose and organic acid accumulation in the developing fruit. This variability is due to differences in both enzymatic and transporter activities. Within the Cucumis melo germplasm, in contrast, variability is limited to a continuum of sucrose and organic acid levels in the developing fruit with no evidence yet for variability within the species for starch or fructose accumulation. The natural genetic variability is a promising source for improvement of fruit quality. While there are numerous similarities in the carbohydrate metabolic pathways of the two genera, some strategies of carbohydrate metabolism nevertheless differ between them, in part due to the primary photoassimilate transported, sucrose for Solanum and galactosyl-sucrose for Cucumis. Deciphering the underlying metabolic pathways leading to genera-specific patterns of carbohydrate accumulation can stimulate the
development of novel sugar accumulating genotypes in the non-related genera which lack the natural phenotypic variability for the trait.
TRANSCRIPTOME PROFILING OF SORTED ENDOREDUPLICATED NUCLEI FROM TOMATO FRUITS: HOW GLOBAL SHIFT IN EXPRESSION ASCRIBED TO DNA PLOIDY INFLUENCES RNA-SEQ DATA NORMALIZATION AND INTERPRETATION

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Most eukaryotic organisms, ranging from insects to mammals and plants, present variation in cell ploidy levels resulting from somatic endopolyploidy as part of their normal development. In higher plants, endoreduplication is the major source of endopolyploidy and is widespread in Angiosperms where it takes place in various tissues, such as in the fleshy pericarp tissue of developing tomato fruits.

The functional significance of endoreduplication during plant development is not fully understood. During tomato fruit development, endoreduplication contributes to establish a highly structured and integrated cellular system that acts as a morphogenetic factor supporting cell growth. Endoreduplication has also been suggested to lead to an increased metabolic activity that could originate from a global increase in transcription.

To investigate the potential influence that endoreduplication may confer on gene expression in tomato fruit and to further decipher the functional role of endoreduplication, we tested the feasibility of a RNA-Seq approach using total nuclear RNA extracted from purified populations of FACS-sorted nuclei based on their DNA content. We have shown that cell-based approaches to study expression profiles by RNA-Seq need to take into account the possible global shift in expression between samples for correct analysis of the data and therefore correct interpretation of the observed expression measurements. From the ploidy-specific expression profiles we also found that the activity of the cells is related to their ploidy level and tissue location showing that endoreduplication is likely to be an important determinant of cell identity.

GENETIC VALIDATION THAT JOINTLESS-2 PHENOTYPE IS CAUSED BY MUTATION IN A MADS-BOX TRANSCRIPTION FACTOR

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Abscission is the mechanism by which plants disconnect unfertilized flowers, ripe fruits, senescent or diseased organs from the plant. In tomato, pedicel abscission is an important agronomic factor that controls yield and post-harvest fruit quality. Two non-allelic mutations, jointless (J) and jointless-2 (j-2), controlling pedicel abscission zone formation have been documented but only j-2 has been extensively used in breeding. J was shown to encode a MADS-box protein. Using a combination of physical mapping and gene expression analysis we identified a positional candidate, Solyc12g038510, associated with j-2 phenotype. Targeted knockout of Solyc12g038510, using CRISPR/Cas9 system, validated our hypothesis. Solyc12g038510 encodes the MADS-box protein SIMBP21. Molecular analysis of j-2 natural variation revealed two independent loss-of-function mutants. The first results of an insertion of a Rider retrotransposon element. The second results of a stop codon mutation that leads to a truncated protein form. To bring new insights into the role of J and J-2 in abscission zone formation, we phenotyped the single and the double mutants and the engineered alleles. We showed that J is epistatic to J-2 and that the branched inflorescences and the leafy sepals observed in accessions harboring j-2 alleles are likely the consequences of linkage drags.
A CHEMICAL GENETIC ROADMAP TO IMPROVED TOMATO FLAVOR

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Modern fruits and vegetables have lost their flavor in large part due to extensive breeding for commercial traits. Extensive breeding for shelf-life, firmness, fruit uniformity and disease resistance has resulted in the loss of flavor. To understand and ultimately correct that deficiency, we quantified flavor-associated chemicals (sugars, acids and aroma volatiles) in 398 modern, heirloom and wild varieties. A subset of these varieties was evaluated in consumer panels to understand the contribution of each of these compounds to good tomato flavor. Modern commercial varieties contain significantly lower amounts of many of these important flavor chemicals than older varieties. Sequencing the genomes of 398 tomato varieties and genome wide association studies identified loci, and in some cases genes, responsible for desirable levels of these compounds. We can use this knowledge to develop molecular markers for easily breeding for improved tomato flavor.

MODULATING TOMATO FRUIT ASCORBATE CONTENT VIA THE IDENTIFICATION OF ASCORBATE-DEFICIENT AND ASCORBATE-ENRICHED GGP MUTANTS.

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The ascorbic acid (AsA) is an essential antioxidant in both plants and humans. Plant-derived AsA is the major source of vitamin C in the human diet. In addition to its effect on tomato nutritional value, increasing tomato AsA content would likely affect postharvest storage and resistance to pathogens of the fruit. To investigate the effect of AsA level on these trait, we screened EMS tomato mutant collections (cv. Micro-Tom) for both AsA-deficient (asa-) and AsA-enriched (asa+) fruit mutants using reverse and forward genetic approaches. TILLING allowed the identification of asa- mutants mutated on a GDP-L-Galactose Phosphorylase (GGP) gene which is a key regulator of ascorbate biosynthesis. In parallel, we combined direct genetic on a tomato EMS mutant population with a mapping-by-sequencing approach (Garcia et al., 2016) for identifying mutations underlying the AsA+ trait. A mutant line displaying up to 20-fold increase in fruit AsA content as well as parthenocarpy was identified. Mapping-by-sequencing revealed an amino acid change in a cis-acting open reading frame (uORF) upstream of the GGP gene which could be responsible for the AsA+ phenotype. This uORF likely represses the translation of GGP under high ascorbate concentration (Laing et al., 2015). The asa- and asa+ mutants provide precious genetic tools for deciphering the role of GGP in the regulation of ascorbate and for studying the effect of ascorbate on fruit quality traits.
USE OF A SELF-COMPATIBLE DIPLOID POTATO GENOME ASSEMBLY AND A MUTANT COLLECTION FOR FORWARD GENETIC STUDIES

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We have sequenced the genome of the self-compatible tuber-bearing wild Mexican diploid potato species Solanum verrucosum (VER54). The genome assembly is based on several sequencing and physical mapping technologies, such as Illumina short reads, PacBio long reads, BioNano Genomics optical maps and Dovetail artificial Hi-C data to allow accurate assembly, and to permit comparisons of genome assembly methods. The assembled genome was evaluated for completeness by checking the presence of CEGMA core eukaryotic genes and validated locally by the alignment of the 96 BAC-end sequences. Furthermore, Genotyping by Sequencing (GBS) of a backcross S. verrucosum population (VER54xVER3939) has been used to anchor the assembly into pseudomolecules. The sequenced genotype is also being used as a model for forward genetic studies. Mutant collections are being produced using ethyl methanesulfonate (EMS) and gamma irradiation. The objective is to combine the two elements of the project (i.e de novo assembly and a mutant population) together to study mutants carrying interesting traits that can be physically located by sequencing and mapping them in the genome assembly. The generation of a genome sequence for S. verrucosum and a mutant collection are highly valuable genetic resources. The VER54 inbred genotype can serve as a useful genetic model for further studies about comparative genome structure and adaptive within the Solanaceae. S. verrucosum is a likely genome donor to the wild Mexican polyploid species and it's genome is likely to be useful in further studies of this important species group.

THE REGULATION OF POTATO TUBER FORMATION BY MIR169

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Recent developments have led to an increase in our understanding of the regulatory mechanisms of potato tuber formation. However, the regulation of genes taking part in this process is poorly studied at the post-transcriptional level. In this study we focus on short, single stranded, non-coding RNAs called micro RNAs (miRNA) and how they can influence potato tuber formation. A previous genome-wide study revealed that miR169 shows strong expression change in Solanum tuberosum Group Andigena lines when they were grown under short and long day conditions, miR169 expression level being higher under short days. These lines are under strict photoperiod induced tuberization; they produce tubers under short days but do not tuberize under long day conditions. The expression level of miR169 was analysed in different potato lines (PHYB-silenced, GIi) and potential upstream regulators were identified. Additionally, target genes were predicted and validated for miR169 and a functional study was performed to analyse the role of miR169 in tuber formation.
UNRAVELLING SOLANACEAE SECONDARY METABOLISM THROUGH THE INTEGRATION OF HETEROGENEOUS AND SPATIAL DATA FROM METABOLOMICS, GENETICS AND INFORMATICS

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The regulation of metabolic pathways in plants is constantly tuned in order to suit the needs of development and fitness. Our main research objective is to unravel networks of genes and proteins which coordinate the activity of metabolic pathways, predominantly secondary metabolism, during plant development and stress response. An integrated investigation of several members of the Solanaceae family (mainly tomato, potato and eggplant), rather than studying a single plant, provided us with unprecedented insights to metabolic biology in these species. Most if not all processes characterized, impact to a certain degree key quality, nutritional and post-harvest traits of these crop plants. Integrating cutting-edge transcriptomics, proteomics and metabolomics tools together with genes co-expression assays were of great value in making several key discoveries. In a recent example, combined co-expression analysis and metabolic profiling in tomato and potato led to the discovery of the multi-step, core pathway leading to the formation of the renowned Solanum alkaloids including the biosynthesis of their precursor, cholesterol. This class of molecules represent important anti-nutritional compounds in these crop plants. In the presentation, I will highlight several advanced technologies and genetic research tools and the invaluable knowledge on core metabolic traits obtained through combining them in a single study. Most if not all could be applied in the coming years to the study of key traits in other, less studied plant species.

A HAIRY STORY: HOW IS TRICHOME DEVELOPMENT CONTROLLED IN TOMATO

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Trichomes are hairs that cover the surface of aerial plant tissues which protect them from biotic and abiotic stresses. Despite agricultural interest in trichomes, research on their development has been carried out mainly in Arabidopsis thaliana. Studies in tobacco and tomato have shown that the regulatory pathways identified in Arabidopsis are not shared by species outside the Rosid clade. Moreover, eight different types of trichomes have been described in Solanum, including both glandular and non-glandular trichomes, in contrast to Arabidopsis, where only one type of non-glandular trichome is present. The lack of a model to explain the initiation, development, identity and spatial distribution of trichomes in tomatolimits the full exploitation of trichome engineering for crop improvement.

To gain insights into trichome development, and its relationship to the development of other epidermal cell types, we screened the Solanum pennellii introgression lines (IL) population for quantitative trait loci (QTLs). We characterised the trichome phenotypes in the ILs by scanning electron microscopy, which led to the identification of several genomic regions involved in determination of trichome density, identity and morphology. We have identified and characterised a transcription factor (TF) underlying a trichome patterning QTL using transient silencing, stable overexpression and CRISPR knock-out. Our results have shown that this TF acts as a negative regulator of trichome initiation and is responsible for trichome spacing in the tomato epidermis. We are confident that these findings will contribute to our understanding of trichome development in Solanum species, establishing the foundation for more resilient tomato varieties.
IDENTIFICATION OF GENES RELATED TO SKIN DEVELOPMENT IN POTATO

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Keywords: corky tissue, periderm, phellem, potato skin, suberin, Solanum tuberosum

Potato tuber skin is a protective tissue made of suberized phellem cells. The transcriptomes of skin and tuber-flesh were compared to identify genes that involve in skin formation. Several genes that were differentially expressed in the skin had not been previously identified in potato. These included the StKCS20-like, StFAR3, StCYP86A22 and StPOD72-like genes, that may be closely related to known suberin-related genes; the StHAP3 transcription factor that directs meristem-specific expression; and the StCASP1B2-like and StCASP1-like genes, which are two orthologs of a protein family that mediates the formation of Casparian strips in the suberized endodermis of Arabidopsis roots. GUS reporter assay in transgenic potato plants indicated expression in tuber skin, as well as in other corky/protective cells such as the root endodermis, the vascular cambium and the epidermis of the stem. Cis-regulatory elements within the respective promoter sequences support this gene-expression pattern.

Characterization of the potato skin may help to control costly skin disorders, and shade additional light on the development of corky tissues in planta, e.g., skin of underground organs, cork of woody plants, wounding-periderm and peel russetting of fruits and vegetables.

*Vulavala et al., Plant Molecular Biology doi: 10.1007/s11103-017-0619-3

IDENTIFYING THE ENZYMES THAT PRODUCE GERANYLGGERANYL DIPHOSPHATE FOR CAROTENOID BIOSYNTHESIS IN TOMATO

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Carotenoids are plastidial isoprenoids highly demanded as natural pigments and health-promoting nutrients (e.g. beta-carotene as pro-vitamin A). In plants, carotenoids are essential for photoprotection in leaves and contribute to animal-driven pollination and seed dispersal by coloring flowers and fruits. They are also precursors for the production of apocarotenoids such as the hormones abscisic acid and strigolactones. As most plastidial isoprenoids, carotenoids derive from geranylgeranyl diphosphate (GGPP) synthesized by GGPP synthase (GGPPS) enzymes encoded by gene families in plants. In Arabidopsis thaliana, 5 GGPPS isoforms specifically produce GGPP in plastids, endoplasmic reticulum and mitochondria. However, the production of carotenoids depends solely on the activity of GGPPS11, the only essential GGPPS in Arabidopsis. Little is known about GGPPS families in other plants, including those with interest as a source of dietary carotenoids. We are currently analyzing the GGPPS family in tomato (Solanum lycopersicum). Carotenoid levels increase dramatically during tomato fruit ripening, providing orange and red colors to ripe tomatoes. We speculate that this process most likely involves the activity of specific GGPPS paralogs which may be different from those producing GGPP for carotenoids involved in photoprotection (in leaves) or mycorrhizal associations (in roots). We found 9 putative GGPPS paralogs in the tomato genome and we experimentally confirmed that 4 of them are primarily localized in plastids. Identifying which of the GGPP-producing plastidial isoform(s) is responsible for carotenoid biosynthesis in particular organs will allow us to specifically improve the nutritional quality of fruits without interfering with photoprotection or tolerance to stress.
Most biological processes present diurnal or annual oscillations and are controlled by an internal timekeeper called the circadian clock. This molecular mechanism integrates light and temperature cues, among others, thus ensuring proper synchronization of the organism with the environment. We have recently found that circadian rhythms have been delayed in tomato during domestication. This occurred through positive selection of large effect mutations affecting two genes involved in light signaling. The wealth of re-sequencing data available for tomato nowadays allowed us to follow the evolution of these mutations during domestication. We hypothesize that these mutations allowed tomato to cope with the light conditions present outside its original range. We also show that both of these mutations are epistatic to PHYB, the main red light photoreceptor in tomato, and that their effect in circadian rhythms is light dependent. Recent reports show that mutations in circadian clock or light perception genes are not uncommon in crops as a tool to increase their range of cultivation. Tomato is a perfect model to study the interaction between circadian rhythms and agricultural traits because of the many genetic and genomic tools available.

COMPARATIVE GENOMICS OF STRESS RESISTANCE IN WILD TOMATOES.

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One of the greatest barriers to understanding and predicting plant responses to future environmental change is our poor understanding of the functional and genomic basis of resistance traits for ecological adaptation. Namely, how do stress response pathways interact to shape organismal fitness. To investigate the genomic basis of stress response pathway interaction and how this impacts organismal fitness, we capitalize on tomato, which has a wealth of important genomic/genetic tools available and is also a member of a small clade of closely related (inter-fertile) species. We characterize molecular differences among distinct genotypes using an experimental transcriptomics approach in the wild tomato, Solanum pimpinellifolium.

INDUCTOME: TRANSCRIPTOME ANALYSIS OF VALUABLE SECONDARY METABOLISM IN GREEN PLANT RESIDUALS FROM TOMATO AFTER ABIOTIC INDUCTION

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The capacity of plants to produce valuable secondary metabolites (SM) for extraction and utilization as pharmaceuticals or food additives is well known. Biosynthesis of such SM can be extensively induced in
response to abiotic stress increasing the extractable amount of valuable SM, a method, which has yet not been utilized in combination with green plant residuals from food production.

Here, we present a transcriptome analysis performed to identify induced SM pathways in the commercially used tomato (Solanum lycopersicum) cultivar Lyterno and the wild tomato Solanum pennellii. Different stress treatments (nitrogen depletion, elevated light, cold during night) and combinations thereof were applied to six week old plants of both species. Our analysis shows a significant induction of the flavonoid and phenylpropanoid biosynthesis as well as lignan biosynthesis pathways in the cultivar Lyterno, especially under triple stress conditions (nitrogen depletion, elevated light and cold during night). Solanum pennellii shows the same trend for the triple stress, but with a stronger increase of induction and more upregulated genes.

In addition, metabolite profiling, performed using LC-FTICR-MS, supports the transcriptional changes observed in Lyterno in different treatments.

Further analysis will identify valuable metabolites induced by stress treatments and combinations thereof in green tomato residuals. The induction of SM by abiotic stresses after tomato fruit harvest in green houses provides a complementation of the current value-chain of tomato production by adding the extraction of valuable metabolites from green plant residuals.

GENOME-WIDE IDENTIFICATION OF GENES INVOLVED IN THE POTATO RESPONSE TO DROUGHT INDICATES FUNCTIONAL EVOLUTIONARY CONSERVATION WITH ARABIDOPSIS PLANTS.

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Potato is one of the four most important food crop plants worldwide and is strongly affected by drought, which is the main climate threat causing a major decrease in tuber yield. The development of high-throughput RNA sequencing (RNA-seq) techniques has provided novel tools for qualitative and quantitative global analyses of gene expression. The following two pairs of potato cultivars, which are related in ancestry but show different drought tolerances, were chosen for comparative gene expression studies: Gwiazda/Oberon and Tajfun/Owacja. Comparative RNA-seq analyses of gene expression differences in the transcriptomes obtained from drought-tolerant versus drought-sensitive plants during water shortage conditions were performed. The twenty-three top-ranking genes were selected, 22 of which are described here as novel potato drought-responsive genes. Moreover, all but one of the potato genes selected have homologs in the Arabidopsis genome. Of the seven tested A. thaliana mutants with altered expression of the selected homologous genes, compared to the wild-type Arabidopsis plants, six showed an improved tolerance to drought. The evolutionary conservation of the functions of the selected genes in the plant response to drought confirms the importance of these identified potato genes in the ability of plants to cope with water shortage conditions. Knowledge regarding these gene functions can be used to generate potato cultivars that are resistant to unfavorable conditions. The approach used in this work and the obtained results allowed for the identification of new players in the plant response to drought.
TOO HOT TO HANDLE – HOW HIGH TEMPERATURE AFFECTS POLLEN DEVELOPMENT

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Heat wave-like temperature regimes negatively affect growth and fertility of plants and thus pose a severe risk to human food security. Given the expected further increase in global temperatures, a better understanding of these problems is urgently needed. I will show that production of pollen is a major limiting factor for fertility of tomato under long-term mild heat. I will present data on the molecular and physiological effects of high temperature and on the genetic determinants of pollen thermotolerance. Finally, implications for crop improvement are discussed.
PLANT INTERACTIONS WITH PATHOGENIC MICROORGANISMS

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Plants are sessile and cannot escape from interactions with other organisms, including beneficial and pathogenic microorganisms. To protect themselves against invading pathogens, plants have developed very sophisticated defence system, such as preformed passive barriers and the plant immune system. In the last decade, there have been major advances in our understanding of the ‘zigzag’ model, which encompasses two layers of the plant immune system triggered upon pathogen attack. In the first layer, receptors on the plant cell surface are able to recognize conserved pathogen molecules (PAMPs) and lead to PAMP-triggered immunity. The pathogen can secrete effectors to successfully overcome this first layer. Some effectors, previously known as Avr genes, can be recognized by receptors encoded by plant resistance (R) genes resulting in the second layer of the plant immune system. In addition to the ‘zigzag’ model, the other unifying concept that emerged in the last decade is that plant susceptibility (S) genes play an important role in plant interactions with pathogenic microorganisms. Plant genes are termed S genes when a pathogen takes advantage of them for its own benefit during the colonization of the plant. Pathogens are impeded from colonizing the plant when plant S genes become dysfunctional as a result of a recessive mutation or non-expression. In this talk, I will first present an overview of the intricate network of plant interactions with pathogenic organisms, and then focus on our recent results on plant S genes.

MELON- FUSARIUM AND MELON-POTYVIRUS CROSSTALK: MOLECULAR AND PROTEIN INTERACTIONS

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NBL(nucleotide binding site–leucine rich repeat) encoding genes are the prevalent class of plant resistance genes. Their products recognize pathogen effectors (Avr factors) and launch a defense response, providing plants with a dynamically evolving system to monitor pathogen invasion. Their mapping, Cloning and functional characterization in crop species provide important tools for plant protection. We have cloned the genomic locus for melon resistance towards two pathogens, Fusarium oxysporum f.sp. melonis (FOM) races 0 and 1, and papaya ring spot virus (PRSV). The two adjacent Prv and Fom-1 genes encode proteins of the TIR-NBL family. Prv carries an extra NB domain, and both genes have alternative, differentially expressed splice variants. Paired resistance genes represent a subset of R-genes found in head-to-head orientation in plant genomes. They were recently suggested to function together in a novel cooperative mechanism, and we are exploring such intriguing possibility for Prv and Fom-1. Here we provide an overview of such a model and present preliminary data that suggest such a possibility.

A SRNA CASCADE REGULATING THE IMMUNE SYSTEM OF TOMATO
Small interfering RNA species derived from protein-coding or noncoding loci may align to their long precursor RNA in 21-nucleotide (nt) "phased" intervals. The production of these "phasiRNAs" is generally triggered by a microRNA and they act in trans as suppressors of any complementary mRNAs. In many species there are phasiRNAs aligning with the mRNA for the NLR defense proteins (nucleotide binding site leucine rich repeat) and their trigger is miR482. In the Solanaceae, in addition to the miR482 phasiRNAs, we have identified a single long non coding RNA (TAS5) that has similar sequence to conserved regions of NLR mRNAs. We hypothesize that the effect of this miR2118-TAS5 system is to form a regulatory cascade in Solanaceae that adds to downregulation of NLRs by miR482.

To test the effect of this Solanaceae cascade we disrupted both the miR482-NLR and the miR2118b-TAS5 pathways with target decoys of the miRNA triggers. In both instances, there was reduced phasiRNA production at the target NLR and TAS5, and enhanced resistance to pathogen infections. We hypothesise that these systems reduce the expression of the NLR defense system in the absence of pathogens to reduce the cost of disease resistance to the host plant. Many pathogens including viruses and oomycetes produce suppressors of silencing so that, in infected plants, NLR mRNAs would be induced and defense would increase. Further testing will explore the possibility that manipulation of this miRNA-NLR cascades could be used to enhance the level of basal defense in crop plants.

SYMPLASMIC CONNECTION BETWEEN OROBANCHE AEGYPTIACA AND TOMATO

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Root parasitic plant Orobanche spp. invades haustorium and absorbs nutrient and water from the host. Since Orobanche causes damage to crops, understanding of parasitism mechanism is necessary to control them. In previous studies, various molecules, mRNAs and plant viruses, have been shown to move to parasitic plant from host plant. However, it is not clear whether these molecules moved cell-to-cell or via phloem, and how the pathway is formed between host and parasite. In this study, to clarify the way how symplasmic continuity in the haustorium is established, we observed the transport of symplasmically mobile GFP by using Orobanche aegyptiaca and transgenic Solanum lycopersicum that expresses AtSUC2::GFP. When O. aegyptiaca parasitized to S. lycopersicum, GFP from phloem of the tomato root moved to O. aegyptiaca via symplasmic pathway adjacent to O. aegyptiaca's xylem in the haustorium and reached the tip of tubercle. In the haustorium, this pathway consists of arrays of cells that contain nucleus. On the other hand, pathways in tubercle had callose-deposited sieve plate and looked like sieve element as in usual roots. This suggested that specific symplasmic pathway between S. lycopersicum and O. aegyptiaca is present in haustorium, and it allows transport of contents in tomato sieve element to the Orobanche.

MYC2 ORCHESTRATES A HIERARCHICAL TRANSCRIPTIONAL CASCADE THAT REGULATES JASMONATE-MEDIATED PLANT IMMUNITY IN TOMATO

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The hormone jasmonate (JA), which functions in plant immunity, regulates resistance to pathogen infection and insect attack through triggering genome-wide transcriptional reprogramming in plants. We show that the basic helix-loop-helix transcription factor (TF) MYC2 in tomato (*Solanum lycopersicum*) acts downstream of the JA receptor to orchestrate JA-mediated activation of both the wounding and pathogen responses. Using chromatin immunoprecipitation sequencing (ChIP-seq) coupled with RNA sequencing (RNA-seq) assays, we identified 655 MYC2-targeted JA-responsive genes. These genes are highly enriched in Gene Ontology categories related to TFs and the early response to JA, indicating that MYC2 functions at a high hierarchical level to regulate JA-mediated gene transcription. We also identified a group of MYC2-targeted TFs (MTFs) that directly regulate the JA-induced transcription of late defense genes. Our findings suggest that MYC2 and its downstream MTFs form a hierarchical transcriptional cascade during JA-mediated plant immunity that initiates and amplifies transcriptional output. As proof of concept, we showed that during plant resistance to the necrotrophic pathogen *Botrytis cinerea*, MYC2 and the MTF JA2-Like (JA2L) form a transcription module that preferentially regulates wounding-responsive genes, whereas MYC2 and the MTF ETHYLENE RESPONSE FACTOR.C3 (ERF.C3) form a transcription module that preferentially regulates pathogen-responsive genes.
HORMONE SIGNALING AND EPIGENETIC REGULATION ASSOCIATED WITH THE TRANSCRIPTOMIC REPROGRAMMING UNDERLYING FRUIT SETTING IN TOMATO

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The combination of world demographic growth and global warming threatens human society with the inability to meet food needs in the future. In higher plants, yield is a complex trait relying on the successful completion of flower pollination which can be impaired by environmental factors such as elevated temperature. Like other developmental shifts, the flower-to-fruit transition, so-called fruit set, is associated with major transcriptomic reprogramming guided by the convergent action of hormone signalling and epigenetic regulation. Histone marking and DNA methylation are the main operating modes of epigenetic regulation underlying genetic reprogramming. To better clarify their respective contribution to fruit setting, genome-wide transcriptomic profiling, ChIP-sequencing and DNA bisulfite sequencing were applied to tomato, a major economic crop as well as a model system for fleshy fruit.

The study highlights the prominent role of histone marking compared to DNA methylation in transcriptomic reprogramming associated with fruit setting and uncovers H3K9ac and H3K4me3 marks as primary players in this control mechanism. Consistently, the expression of fruit set-related genes involved in hormone metabolism, cell division, and embryo development correlated with the appropriate histone mark repositioning, but not with changes in DNA methylation. The study identified epigenetic modifiers genes that are most active in this developmental shift, opening new leads towards improving fruit setting and thus crop yield.

Keywords: fruit set, epigenetics, transcriptomic reprogramming, tomato

ANALYSIS OF RNA-BINDING PHLOEM PROTEINS POTENTIALLY INVOLVED IN THE LONG-DISTANCE TRAFFICKING OF RNAs IN CUCURBITS

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Long-distance RNA transport occurs in land plants through the phloem, a conducting tissue that integrates the wide range of signaling pathways required to regulate plant development and response to stress processes. How these RNA molecules translocate through the phloem is not well understood, but recent evidence indicates the presence of translocatable phloem proteins able to bind RNA can act as potential components of long-distance RNA transport system.

Cucurbits constitute a diverse family that has a high complexity at both structural and phylogenetic levels. This variability is evidenced in the protein complexity of the phloem exudate. It has been shown that diverse phloem-proteins are involved in the long-distance RNA transport as part of a complex systemic RNA signaling network that employ the phloem as a distribution pathway throughout the plant. In this work, we characterize the phloem-proteins accumulation patterns of 45 accessions of different geographic origins of the Cucurbitaceae family, in order to establish similarities and/or differences
between representative members of this complex taxonomic group. Additionally, the existence of RNA-binding proteins in their phloem exudate was also analyzed. Our results indicate that in the analyzed members of the Cucurbitacea family exists a great variety of phloem-proteins accumulation-patterns, mainly determined by the genus and not related to the geographical origin of the accessions. Interestingly, RNA-binding assays revealed the presence of at least one RNA-binding protein in all the analyzed inputs, which suggests that RNA transport through phloem seems to be a highly conserved feature within the Cucurbitaceae family.

DOES JASMONIC ACID (JA) ACT AS A LONG-DISTANCE SIGNAL OF DRYING SOIL?
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Tomato (Solanum lycopersicum) is an ideal species to investigate long-distance signalling mechanisms of drying soil, since sufficient xylem sap for multi-analyte phytohormone analysis can readily be collected, many phytohormone-related transgenics/mutants exist and it is easily grafted to evaluate the physiological significance of the root system. Imposing deficit irrigation (by automatically irrigating plants when soil water content decreased below a threshold value) increased xylem ABA concentration approximately 2-fold while xylem JA (jasmonic acid) concentrations increased 5-fold. To determine the physiological significance and sources of these changes in xylem phytohormone concentrations, near isogenic wild type (WT) tomato and the def1 mutant (which fails to accumulate JA during water stress) were self- and reciprocally-grafted. During soil drying, def1 scions maintained a higher leaf water potential (than WT scions) as their lower stomatal conductance (gₛ) was lower. Although soil drying increased OPDA (a JA-precursor and putative antitranspirant) concentrations in def1 scions, foliar JA accumulation was negligible and foliar ABA accumulation reduced compared to WT scions. Stomata of def1 self-grafts were relatively insensitive to soil drying, but normal sensitivity was restored by grafting onto WT rootstocks, coincident with increased drought-induced ABA and JA accumulation, but decreased OPDA accumulation. Xylem-borne jasmonates were biologically active, since supplying exogenous JA via the transpiration stream to detached shoots decreased transpiration of WT seedlings but had the opposite effect in def1. Thus normal stomatal response to drying soil requires WT levels of both ABA and JA.

MINI ZINC FINGER PROTEINS, A MISSING LINK IN THE REGULATION OF FLORAL MERISTEM TERMINATION IN ARABIDOPSIS AND TOMATO

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In most angiosperm species, the gynoecium is the last structure to develop within the flower, due to the determinate fate of the floral meristematic stem cells. The maintenance of the stem cell activity before its arrest at the stage called floral meristem termination, impacts directly the number of carpels. The necessary inhibition at this stage of WUSCHEL (WUS), the gene responsible for the maintenance of the stem cell activity within the floral meristem, involves a two-step mechanism: firstly through a direct repression mediated by the MADS domain transcription factor AGAMOUS, and next through an indirect repression requiring the C3H2 Zinc-finger KNUCKLES (KNU) protein, allowing complete termination of the floral stem cell maintenance. Here, using Arabidopsis and tomato as model plants, we report that the MIni zinc Finger 2 (MIF2) and Inhibitor of Meristem Activity (IMA) proteins act during the floral meristem
termination process as adaptor proteins, recruiting KNUCKLES and SiKNUCKLES respectively, in a transcriptional repressor complex together with TOPLESS (TPL) and HISTONE DEACETYLASE19 (HDA19). This complex binds to the WUS locus leading to WUS repression via a chromatin deacetylation mechanism. These data provide novel insights in the molecular mechanisms governing floral termination, and highlight the essential role of MIF2/IMA during this essential step of flower development that determines carpel number and therefore fruit size.

INFLUENCE OF THE ROOT GENOTYPE ON THE CHEMICAL COMPOSITION OF THE XYLEM SAP AND FRUIT OF GRAFTED TOMATO PLANTS

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Although recent studies on root-to-shoot communication have identified organic bioactive molecules, the direct effect of the root system on the chemical signature of xylem sap and fruit remains unknown. Therefore, a commercial tomato cultivar (Solanum lycopersicum cv. Boludo F1, Monsanto) was either self-grafted (L/L) or grafted onto the interspecific commercial tomato rootstock Maxifort (Monsanto) obtained from a cross between S. lycopersicum and S. habrochaites (L/H). Grafted plants were cultivated under commercial greenhouse conditions during the spring season in a tomato-growing area in Mazarrón (Spain). Xylem sap, collected by root pressure, and fruit extracts were injected into a U-HPLC-MS system (EXACTIVE, ThermoFisher Scientific). Venn diagram analysis revealed that several features were specific to L/L or L/H graft combinations in both xylem sap and fruit. From those metabolites that showed significant differences (P ≤ 0.01) between both graft combinations evaluated, 46 (40% of the total) were overproduced in the xylem sap and 33 (52% of the total) in fruit of L/H plants. Importantly, a significant correlation between the metabolites present in the root xylem sap and in the fruit was observed. Indeed, we have identified metabolites of the primary and secondary metabolism as well as some important bioactive compounds (i.e. nebularine, a purine nucleoside with antibiotic effects) which travel through the xylem and accumulate in the fruit of 'habrochaites' L/H plants. By using the activity network platform, biochemical connections among all compounds were established, being amino acids and sugar phosphates pathways predominant. We have thus shown that the root leaves a chemical mark in the fruit, with important scientific and socioeconomic impacts in agriculture and in food security.

Keywords: Habrochaites, rootstock, root-to-shoot communication, feature, bioactive compound
DISSECTING TOMATO METABOLIC NETWORKS TO PROVIDE NOVEL TOOLS FOR TRAIT ENGINEERING

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Plants are capable of producing an overwhelming variety of metabolites, both in terms of complexity and quantity. Primary metabolites that occur in every plant species, such as sugars and amino acids, support plant growth and development. Others, the so-called specialized metabolites, allow plants to cope with all kinds of stresses or other signals from the environment. Specialized metabolites are usually bioactive and more specific to a plant species or family, such as carotenoids and glycoalkaloids in tomato. In turn, phytohormones, such as auxin, jasmonate and ethylene, are involved in the monitoring of plant growth and defence programs and thereby steer plant metabolism to guarantee a proper response to ever-changing developmental and environmental signals. Yet, the impressive plant metabolic machinery is still only partially understood and therefore not fully exploited.

By using cutting-edge functional genomics tools, we aim to map the metabolism of crop, medicinal and model plants. We have created a technology platform that enables comprehensive investigations and large-scale gene discovery programs in plant metabolism. This platform, which we are presently expanding for research dedicated to the dissection of tomato metabolic networks, is built on the integration of genome, transcriptome, interactome, proteome and metabolome profiling data. Currently, we are focusing on the production of glycoalkaloid and other terpenoid molecules in different tomato organs in response to different developmental and environmental cues. Besides providing novel fundamental insights, our findings aim to foster the generation of tomato cultivars with superior traits, such as enhanced resistance to pathogens or improved nutritional value.

BIO-ENGINEERING OF TOMATO WITH NUTRITIONAL AND MEDICINAL BENEFICIAL COMPOUNDS

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L-3,4-dihydroxyphenylalanine (L-DOPA) is synthesised from tyrosine, through hydroxylation, in several plant species, such as velvet bean and beetroot. L-dopa has been shown to have allelopathic activity, together with antioxidant properties, in addition to its role as a precursor of Betalain pigments. However, L-DOPA has most significance in its role in prevention and treatment of Parkinson’s disease in humans. We introduced the recently identified beetroot gene, CYP76AD6, into tomato. Transgenic tomato fruit showed the ability to synthesise and accumulate L-DOPA. A further increase in the levels of L-DOPA was gained by overexpression of the AtMYB12 gene. However, L-DOPA is prone to oxidation, giving rise to the dark melanin compound. To prevent this oxidation in fruit we are attempting to increase antioxidant capacity in tomato fruit. Ascorbate (vitamin C) can prevent the oxidation of L-DOPA, therefore in a complimentary study, putative regulators of the ascorbate biosynthetic pathway were identified and studied through yeast 2 hybrid assays and genetic manipulation. Tomato is an important source of ascorbate in the human diet, because it is the most widely consumed fresh fruit, and ascorbate plays an important role as an essential vitamin and free-radical scavenger in all living cells. It plays many roles in
the human body, for example in the immune system and in collagen formation. Its deficiency can lead to scurvy and immunodeficiency problems. Through combining materials from these two studies, we aim to produce vitamin C-biofortified tomatoes, and L-DOPA engineered tomatoes for cultivation in developing countries, where these two compounds have restricted availability.

**TARGETED MODIFICATIONS OF ENDOGENOUS TOMATO GENES USING A GEMINIVIRAL REPLICON AND THE CRISPR/CAS9 SYSTEM**

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Current breeding and selection still rely mostly on random mutagenesis and recombination. However, with the availability of whole genome sequences and a better understanding of gene function, targeted genome editing is becoming an important tool for precise plant breeding. Using the clustered regulatory interspaced short palindromic repeats (CRISPR) system combined with a geminiviral rolling circle replicon we optimized a system for targeted mutations and gene replacement. The carotenoid isomerase (CRTISO) and phytoene synthase 1 (PSY1) genes from the carotenoid biosynthesis pathway were chosen as targets due to their easily detectable change of phenotype. We took advantage of the geminiviral replicon amplification as a mean to provide a large amount of donor template for the repair of a CRISPR-Cas-induced DNA double strand break (DSB) into the target gene, via homologous recombination. Mutagenesis experiments, performed in the Micro-Tom variety achieved precise modification of the CRTISO and PSY1 loci at an efficiency of up to 92%. In the gene targeting experiments, our target was a fast-neutron-induced crtiso allele that contained a 281bp deletion. This deletion was replaced by the wildtype sequence through homologous recombination between the CRISPR-Cas-induced DSB in the crtiso target and the amplified donor at a frequency of 22.9%. This is to our knowledge, the highest rate of unselected gene replacement reported so far in a higher plant.

**GENOME EDITING IN TOMATO USING THE CRISPR CAS9 GB3.0 TOOLKIT**

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Genome editing technologies enable the precise modification of selected traits in plant genomes aiming to obtain new varieties with desirable phenotypes. Among gene editing techniques, CRISPR (clustered regularly interspaced palindromic repeats)/Cas9 has emerged in recent years for its simplicity, versatility and efficiency in a variety of different organisms, including many crop species. We integrated the features of the CRISPR/Cas9 system into the GoldenBraid cloning standard to produce a series of molecular tools which support the practical and versatile design of a variety of constructs for genome editing. A series of molecular targets were selected to test the efficiency of the GoldenBraid CRISPR Cas9 tools in tomato (Solanum lycopersicum L.), along with different construct architectures. Our targets included the greenflesh1/sgr and the ty5 loci of tomato, and three different targets in the replicase and coat protein of TYLCV to provide the plant with an immunity system against viral infections. In the first case we identified a series of edited alleles at the target locus, which reproduce the naturally occurring green flesh phenotype, characterized by the lack of chlorophyll degradation in mature fruits and senescent
leaves. Ty5 mutants were also obtained using a single guide RNA, as spontaneous mutants for this gene, which is involved in ribosome turnover, have proved to be resistant to Geminivirus. Finally, we performed transient expression assays in N. benthamiana and transformed tomato accordingly using multiplex constructs with three guide RNAs arranged as a polycistronic transcript and designed against TYLCV Sardinia and Israel species.

GENOME EDITING VIRUS RESISTANCE

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Genome editing of elf4E and elfFiso4E exhibited variable potyvirus resistance in cucumber. Genome editing in plants has been boosted tremendously by the development of the CRISPR/Cas9 technology. This powerful tool allows substantial improvement of plant traits in addition to those of classical breeding. Here we demonstrate the development of virus resistance in cucumber (Cucumis sativus L.) by utilizing Cas9/sgRNA technology to disrupt the recessive elf4E and elfFiso4E gene function. Cas9/sgRNA constructs were targeted to elf4E and elfFiso4E genes. Small deletions and SNPs were observed in the elf4E gene targeted sites of T1 generation transformed cucumber plants, but not in putative off-target sites. Non-transgenic heterozygous elf4E mutant plants were selected for production of non-transgenic homozygous T3 generation plants. Homozygous progeny following Cas9/sgRNA that had been targeted to both elf4E and elfFiso4E sites exhibited variable resistance to different potyviruses. In contrast, heterozygous-mutant and non-mutant plants were highly susceptible. The deferential potyvirus resistance and breaking resistance will be discussed.
Parallel Session I CUCUMBER and MELON

DECIIPHERING BIOCHEMICAL AND GENETIC FACTORS THAT DETERMINE AROMA-QUALITY TRAITS IN MELON FRUIT

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Fruit quality in melon (Cucumis melo) and in other cucurbit species is primarily determined by sweetness, acidity, aroma, color and shelf-life. During ripening, the mesocarp (fruit flesh) generally softens due to degradation of cell walls, and accumulates soluble sugars, organic acids, volatiles and additional secondary metabolites. Flesh and rind undergo developmental changes, the most noticeable of which are changes in pigmentation and aroma formation. The large-scale data generated by next generation sequencing (NGS) technologies combined with powerful computational tools, have enabled a major technological leap from low-resolution to high-resolution QTL mapping and gene discovery. Recent studies on genes involved in the major metabolic pathways affecting the formation of volatile organic compounds that contribute to the unique aroma of melon fruit will be discussed. These studies include RNA-Seq-based QTL and eQTL analyses of an advanced RIL population, genotyping by sequencing of a biparental population, genome wide association study (GWAS) and bulked segregant analysis and were complemented with biochemical studies and functional analyses of key genes.

INDIAN GERMPLASM IS A CENTER OF MELON DIVERSIFICATION BASED ON GENOTYPING-BY-SEQUENCING ANALYSIS

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Previous SSR-based genetic diversity analysis supported that Oriental and Occidental melon varieties arose by divergent selection from Indian ancient varieties. Samples from interesting Indian germplasm collections, a broad range of Occidental, Oriental cultivars and some African accessions were analyzed by Genotyping-by-Sequencing. A total of 6169 informative SNPs were obtained, showing a high level of genetic diversity (n=0.32, He=0.33) in the worldwide melon germplasm. Tajima’s D was 2.63 which may reflect balancing selection due to the fixation of alternative alleles in different world regions. Linkage disequilibrium among SNPs was very low, within 1 kb, even in centromeric regions. PCA displayed a high genetic structure; groups including African accessions, conomon, dudaim, cantaloup, inodorus and Indian cultivars were clearly defined, only a few accessions did not clustered within these groups. Genetic variability among populations was 32% and 68 % within populations. Indian germplasm cluster was located in the center of the PCA plot, confirming the original hypothesis. Genomic regions involved in the genetic differentiation between Indian, conomon, inodorus and cantaloup groups were identified through the genome by Fst and Hs analysis. Interestingly, a few large haplotypes blocks were found to be specific of some groups, being also candidate regions for harboring genes selected by farmers to develop the present cultivated types.
MAPPING OF FRUIT TRAITS IN A CANTALOPE X INODORUS MELON RIL POPULATION

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Melon (Cucumis melo L.) shows a broad diversity in fruit morphology and quality. The existence of climacteric and non-climacteric varieties has led to propose melon as an alternative model to elucidate the genetic bases of the ripening process. A recombinant inbred line (RIL) population of 91 individuals was developed using as parental lines two commercial cultivars: “Védrantais”, from the cantaloupensis group, and “Piel de Sapo”, from the inodorus group. Both types show desirable quality traits for the market, but their fruits differ in many traits such as rind and flesh color, size, shape and ripening behavior. Five replicates of the RIL population were phenotyped for quality, morphology and ripening traits. We used a genotyping-by-sequencing (GBS) strategy to construct a dense genetic map of the population, and we detected 60 stable QTLs/major genes involved in fruit traits. Five major genes related with fruit morphology, quality and climacteric ripening were mapped in short genomic intervals. Several QTLs involved in soluble solid content, fruit weight and fruit shape showed LOD scores higher than six. The average confidence interval of the QTLs was around 1 Mb, suggesting that the high density of the genetic map allowed to increase the mapping resolution. Our work will contribute to understand the genetic basis of fruit traits and to apply this knowledge in breeding programs.

QUANTITATIVE TRAIT LOCUS ANALYSIS OF FRUIT TEXTURE TRAITS IN CUCUMBER

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3Full text

Fruit texture traits, e.g., firmness and crispness, are among the most important characteristics of the cucumber (Cucumis sativus L.) because they are directly related to its commercial value. Fruit texture traits other than firmness have been difficult to quantify objectively and their genetics remain largely unexplored. We report new results of quantitative trait locus (QTL) analyses associated with fruit texture traits. We used F2 populations from the non-crispy cucumber line CS-PMR1 and an inbred line from the crispy Japanese cucumber ‘Encore10’. The firmness of the fruit skin, placenta, and flesh, and the crispness of the flesh were evaluated quantitatively and objectively, based on puncture-test data (force–deformation curves). We constructed a genetic linkage map based on simple sequence repeat (SSR) markers, and performed QTL analysis for each texture trait by composite interval mapping. There was wide variation among F2 individuals for all texture traits, particularly flesh crispness. QTL analysis indicated that multiple genes contribute to most traits: two for skin firmness, three for placenta firmness, one for flesh firmness, and four for flesh crispness. Among these, QTLs located on chromosome 1 have comparatively large effects on flesh firmness and crispness. Polygenic inheritance makes it difficult to breed new cultivars with desirable firmness and crispness; therefore, the development of DNA markers tightly linked to genes controlling fruit texture traits is important for efficient breeding. The results of this study are a useful step toward developing practical DNA markers for fruit texture.
Melon (Cucumis melo L.) exhibits a wide variation in fruit phenotype such as ripening pattern (i.e. climacteric and non-climacteric ripening), and there are > 13 subgroups in this species. While its whole genome information has been published in the non-climacteric DHL92 line, it is currently unknown about the details of genetic variation beyond single nucleotide polymorphisms (SNPs) and short indels because short-read based resequencing is hard to resolve structural genetic variation including copy number variation (CNV). In Japan, some muskmelon cultivars are known to produce high-grade fruit and have an economic importance especially for farmers. To understand the molecular basis of genetic variation in our muskmelon, we conducted whole genome de novo assembly in the cultivar ‘Earl’s favorite Harukei-3’ (Harukei-3) which is used for breeding of high-grade melon. By combining > x40 Pacbio RS II long reads and > x190 Bionano single molecule data in addition to > x110 Illumina Hiseq data, we assembled Harukei-3 genomic scaffolds with N50 > 10 Mb. Some scaffolds span chromosome arm, indicating that Pacbio and Bionano technologies were effective to construct long scaffolds without reference. Bionano single molecule data also suggested the presence of highly repetitive 10.8 kb unit in the Harukei-3 genome. When compared with DHL92, Harukei-3 assembly exhibited profound intrachromosomal inversion and translocation in chromosome 10. Other chromosomes also exhibited local inversions, insertions, and deletions, while chromosome 9 structure seemed similar between the two plants. In this presentation, we’d like to also discuss about CNV between DHL92 and Harukei-3.
Parallel Session IV  TOMATO

EXPLOITATION OF SEMI-DOMESTICATED TOMATO FOR CROP IMPROVEMENT TRAITS AND GENE DISCOVERY

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As a widely consumed crop, the cultivated a research model organism. Crop improvement strategies for quality traits such as weight, flavor and taste, have often underutilized ancestral germplasm that arose at the onset of domestication of the tomato. Our aim is to target this unadapted germplasm to search for traits that could improve the modern tomato quality and novel gene discovery. Using a collection of 27 S. pimpinellifolium (SP, fully wild), 120 S. lycopersicum cerasiforme (SLC, semi domesticated) and 18 SLL, we conducted whole genome sequencing and performed extensive phenotyping on each accession. For this study, a total of 8,512,064 SNP variants were associated by genome-wide association studies (GWAS) to fruit weight and weight-related traits. The GWAS on the SLC resulted in several novel loci for the phenotype examined as well as loci that were in concordance with previous studies. The results presented here provide a platform for further genetic evaluations and characterizations to identify the molecular mechanisms of tomato improvement by harnessing the diversity within SLC. This project is funded by NSF IOS 1564366.

MORPHOLOGICAL CHARACTERIZATION OF NEW ETHYLENE RECEPTOR MUTANTS OF TOMATO ISOLATED FROM MICRO-TOM MUTANT LIBRARY BY TILLING

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1 Full text

Ethylene receptor is a key factor for ethylene signal transduction. In tomato, six ethylene receptor genes (SLETR1-6) have been identified. Mutation in different ethylene receptor gene results in different phenotypes and useful for elucidating a role of each ethylene receptor gene. In this study, we screened mutants for three ethylene receptor genes, SLETR4, SLETR5 and SLETR6 from Micro-Tom mutant library by TILLING. We identified three ethylene receptor mutants with altered phenotypes, and named as Sletr4-1, Sletr5-1 and Sletr6-1. Sletr4-1 has a mutation between transmembrane and GAF domains while Sletr5-1 and Sletr6-1 have a mutation within the GAF domain. Sletr4-1 showed increasing hypocotyl and root length longer under ethylene exposure, moreover the fruit shelf life was extended, suggesting a reduced ethylene sensitivity of the mutant. In contrast, in both Sletr5-1 and Sletr6-1, at the absence of exogenous ethylene, hypocotyl and root length were shorter than wild type, and the fruit shelf life was shorter than wild type, suggesting a increased ethylene sensitivity of the mutants. The gene expression analysis showed that SLETR3 was up regulated in Sletr5-1 and Sletr6-1 mutant alleles, in contrast down-regulated in Sletr4-1 mutant allele, suggesting that a functional role of three ethylene
receptors in ethylene signalling, and firstly demonstrating a function of GAF domain of ethylene receptors.
Keywords: ethylene receptor, mutant, tomato
SLPTI4 REGULATES FRUIT RIPENING, SEED GERMINATION AND DROUGHT RESISTANCE IN TOMATO (SOLANUM LYCOPERSICUM CV. MICRO-TOM)

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Although the role of tomato transcription factor SlPti4 in disease-resistant process has been elucidated in higher plant, whether SlPti4 plays a role in the regulation of fruit development and ripening remained unknown. Here, we demonstrate that a tomato ethylene-responsive factor (ERF) SlPti4, is involved in fruit ripening, seed germination and drought stress responses via regulating ABA metabolism and ABA signaling. SlPti4 expression is very low in the early stages of fruit development, but it increases rapidly during fruit ripening and can be induced by exogenous ABA, 1-aminocyclopropane 1-carboxylate (ACC) as well as drought stress. RNA interference-induced silencing of SlPti4 accelerated fruit ripening by 2-3 days, due to the advanced accumulation of ABA and the alteration of expression of ABA signaling genes, which causes the early ethylene release and early expressions of ripening-associated genes. SlPti4-RNAi transgenic seeds accumulate less ABA and SlPYLs but more SlPP2Cs, which causes an insensitivity to ABA treatment and higher seed germination rate. Furthermore, SlPti4-RNAi transgenic plants with low ABA levels were more sensitive to drought stress than wild type plants. Collectively, these results demonstrate that SlPti4 is an important regulator of tomato fruit ripening, seed germination and response to drought stress. This study provides new insights into the understanding of complicated interplays between ethylene and ABA signaling in tomato fruits.

ENGINEERING AND PROTECTING CHLOROPLASTS IN TOMATO FRUIT FOR INCREASED QUALITY/ADDED VALUE TRAITS

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Plastids are the cellular organelles that contribute to the visual, health and flavor-related metabolites and therefore are valuable for both consumers and breeders. During the last few years transcription factors (TFs) implicated in plastid biogenesis have been identified, among which GLKs and APRR2 have been shown to be important for fruit plastid development. Moreover, constitutive overexpression of those TFs was associated to increased organoleptic and nutritional quality in ripe fruits. Our strategy consisted of introducing SIGLKs and SIAPPR2 genes, separately and combined, in a Moneymaker background (uniform ripening /SlGLK2 deficient as most modern varieties) under the control of tomato spatio-temporal promoter which drives fruit specific expression early in development. Engineered plants show a range of chloroplast enhancement fruit phenotypes which were analyzed in detail at the structural, proteomics, metabolomics and molecular levels, highlighting a novel additive effect when both TFs were co-expressed. Ripe fruits are also affected in quality accumulating more sugars, carotenoids and specific volatiles than Moneymaker. Paradoxically, functional GLKs have been avoided in modern varieties, among other reasons because they are more prone to oxidative stress-related disorders such as yellow shoulder and fruit cracking, especially when exposed to high irradiance. In order to increase tolerance to oxidative stress in traditional varieties (SIGLK2 functional), the tomatoMYB-BHL-WD40 anthocyanin regulatory complex
was expressed under the control of a fruit epidermis specific promoter. Those plants accumulated high levels of anthocyanins in the fruit peel and showed other alterations in specialized metabolism.

WHERE IS FRUIT-SPECIFIC CAROTENOGENESIS BLOCKED IN THE GREEN-FRUITED SOLANUM HABROCHAITES?

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Tomato clade has S. lycopersicum as sole domesticated species and several wild relatives differing greatly in their fruit coloration. The wild species are predominantly green fruited except one red fruited and one orange fruited species. However, the introgression lines generated from the crosses between green-fruited S. pennellii and red-fruited M82 gave colored fruits. This prompted us to examine the nature of blocks that hinder fruit coloration in a representative species, S. habrochaites. To decipher the nature of blocks, we compared the carotenoid profiles, plastid ultrastructure, carotenogenic gene expression and proteome profiles of green-fruited S. habrochaites (SH), with cultivated tomato (S. lycopersicum; SL). Our analysis showed that though green-fruited SH has a full complement of chromoplast-specific carotenogenic genes, the coloration is blocked at multiple sites. The first block is the lack of disintegration of thylakoid membranes to a chromoplast-like structure. This is aided by retention of photosynthesis and failure to destabilize chlorophyll. The second block is at the level of key carotenogenic enzyme function. Green-fruited SH though showed normal expression of chromoplast-specific phytoene synthase1 (psy1) and lycopene b-cyclase (cycb) genes akin to orange/red-fruited species but failed to accumulate lycopene and b-carotene. While PSY1 is functionally active, chromoplast-specific CYCB is inactive in SH fruits. The third block is the dearth of key carotenogenic and carotenoid sequestration proteins in SH as revealed by proteomics studies. A combination of all these and hitherto undiscovered blocks collectively prevent the fruit coloration in SH.
Parallel Session VII NICOTIANA AND OTHER SOLANACEAE

GENOMIC INSIGHTS INTO THE EVOLUTION OF THE NICOTINE BIOSYNTHESIS PATHWAY IN TOBACCO

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In tobacco (Nicotiana tabacum), nicotine is the predominant alkaloid. It is produced in the roots and accumulated mainly in the leaves. Jasmonates play a central signaling role in damage-induced nicotine formation. The genome sequence of tobacco provides us an almost complete inventory of structural and regulatory genes involved in nicotine pathway. Phylogenetic and expression analyses revealed a series of structural genes of the nicotine pathway, forming a regulon, under the control of jasmonate-responsive ETHYLENE RESPONSE FACTOR (ERF) transcription factors. The duplication of NAD and polyamine metabolic pathways and the subsequent recruitment of duplicated primary metabolic genes into the nicotine biosynthesis regulon were suggested to be the drivers for pyridine and pyrrolidine ring formation steps early in the pathway. Transcriptional regulation by ERF and cooperatively acting MYC2 transcription factors are corroborated by the frequent occurrence of cognate cis-regulatory elements of the factors in the promoter regions of the downstream structural genes. The allotetraploid tobacco has homologous clusters of ERF genes on different chromosomes, which are possibly derived from two ancestral diploids and include either nicotine-controlling ERF189 or ERF199. A large chromosomal deletion was found within one allele of the nicotine-controlling NICOTINE2 locus, which is part of one of the ERF gene clusters, and which has been used to breed tobacco cultivars with a low-nicotine content.

THE NICOTIANA BENTHAMIANA GENOME 2.0: FROM GENES TO PSEUDOMOLECULES.

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Nicotiana benthamiana is one of the most popular model plants used in the study of plant-pathogen interactions, gene silencing, and protein expression. N. benthamiana is an herbaceous species that is native of Australia. It has a genome size of 3.2 Gb with 19 chromosomes, and is an allotetraploid that arose from a recent polyploidization event shared with other species of the Suaveolentes clade. Two draft versions of the genome of the common research strain of N. benthamiana were published in 2012 with neither version had the genomic scaffolds anchored to pseudomolecules. We will present an improved version of the N. benthamiana genome based on an assembly that included additional Illumina and PacBio sequence data. The total assembly size is 3.05 Gb distributed in 44,826 scaffolds (N90=3,000 sequences/L90=271Kb), and it is anchored to 19 pseudomolecules using a genetic linkage map and HiC. We will also present an updated annotation of this genome and some insights about the evolutionary origin of this species.
NICOTIANA BENTHAMIANA: GENOMES, TRANSCRIPTOMES OF WILD ACCESSIONS

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Nicotiana benthamiana is widely used as a model plant and has been instrumental in making revolutionary discoveries in plant biology. One transgenic line, 16c, expresses the Aequorea victoria green fluorescent protein (GFP), highly and constitutively, and has been a major resource for visualising the mobility and actions of small RNAs. Insights into the mechanisms studied at a molecular level in N. benthamiana 16c are likely to be deeper and more accurate with a greater knowledge of the GFP gene integration site. Therefore, using next generation sequencing, genome mapping and local alignment, we identified the location and characteristics of the integrated T-DNA.

Nicotiana benthamiana is peerless in its susceptibility to viruses and its amenability in transiently expressing transgenes. These unparalleled characteristics have been associated both positively and negatively with a disruptive insertion in the RNA-dependent RNA polymerase 1 gene, Rdr14–6. For a plant so routinely used in research, the origin, diversity and evolution of the species, and the basis of its unusual abilities, have been relatively unexplored. We compared wild accessions from across the spectrum of the species' natural distribution, and show that the laboratory strain of N. benthamiana is an extremophile originating from a population that has retained a mutation in Rdr1 for ~0.8 Myr and thereby traded its defence capacity for early vigour and survival in the extreme habitat of central Australia. Our genome assembly and the transcriptomes of wild accessions (www.benthgenome.com) provide a resource to further characterise the adaptation needed to survive in Australia’s diverse environments.

COMBINING GENE EXPRESSION, METABOLOMICS, AND CONVENTIONAL BREEDING TO INCREASE THE NITROGEN USE EFFICIENCY OF BURLEY TOBACCO

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High rates of nitrogen fertilization are required currently for cultivation of Burley tobacco to achieve the desired leaf yields and qualities. However, Maryland type tobaccos are grown using a nitrogen fertilization rate that is ~60-70% lower than that used typically for Burley. Increased nitrogen use efficiency (NUE) can help increase productivity, reduce environmental impact and potentially limit undesired nitrogen containing constituents. The objective of this study was to understand the NUE potential of Maryland tobacco by identifying possible gene targets that differentiate nitrogen utilization of Maryland tobacco from Burley tobacco. Maryland and Burley tobacco plants were grown in the greenhouse using either 100ppm nitrogen or 25ppm nitrogen and were fed continuously in an ebb and flow system. Gene expression was analyzed at a whole genome level by RNA-seq. Metabolite profiling was performed using multiple approaches. Twenty seven diagnostic metabolites were found that discriminate between the Maryland and Burley phenotypes. The levels of these metabolites were used as fingerprints of NUE. Differentially expressed genes were found that correlated with the NUE fingerprint. Correlation analysis was used to determine which genes had expression levels that correlated
with 27 metabolites. Whole genome SNP analysis of breeding populations coupled with the gene expression correlations revealed genomic loci with concentrated numbers of differentially expressed genes. This study not only revealed possible gene targets that could be modified in the future to improve nitrogen utilization in Burley tobacco, but also identified genetic loci that could be used for selecting nitrogen use efficient lines in breeding programs.

CSPCAS9 VS. AS/LBCPF1: COMPARISON OF RNA-GUIDED ENDONUCLEASES IN NICOTIANA BENTHAMIANA

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RNA-programmable endonucleases are powerful plant biotechnology tools. Until very recently, the only CRISPR-associated site-directed nuclease available for plant breeding was SpCas9, but fortunately new enzymes with additional features are increasing the breeder’s toolbox. An interesting and very recent addition is Cpf1, a Class II nuclease recognizing a TTTN PAM site different from the NGG PAM site of SpCas9, a feature that could give access to loci that cannot be conveniently targeted by SpCas9, the induction of larger deletions and/or the reparation via homologous recombination, which need to be implemented in plants.

We have adapted to the GoldenBraid 3.0 cloning system all the elements to perform gene editing in plants using As and Lb orthologues of Cpf1, and have tested them in *Nicotiana benthamiana* using transient and stable transformation. Transient expression experiments were carried out to compare As/LbCpf1 and SpCas9 activities at several loci. In general, mutation frequency of Cpf1 was comparable to the SpCas9 or even higher at many loci, but As- and Lb- Cpf1 activities were highly dependent on the structure of the guide RNA. Stable transformation produced a high ratio of biallelic mutants most of them deletions in contrast with the small indels of SpCas9. These experiments will provide information about the structural determinants of the gRNA needed for a successful targeted mutagenesis.

Cpf1 is therefore suitable for targeted mutagenesis in Solanaceae and a complementary tool of SpCas9. We are happy to provide the plant scientific community with a new verified GB3.0 tool for plant gene editing.
SP5G encodes an FT-like protein proposed to function as a tuberization repressor. In andigena species that depend on short day for tuber formation, StSP5G is induced in leaves under LDs, but is not expressed in these organs in SDs. We showed that the potato StCOL1 factor suppresses tuberization by binding a conserved TGTGGT element in the StSP5G promoter and specifically activate expression of this gene in LDs. Consistent with a tuberization repressive function of the SP5G protein, potato plants down-regulated for StSP5G expression tuberize under non-inductive LD conditions. Nevertheless, these plans show much reduced levels of activation of the StSP6A tuberigen signal in leaves as compared to StCOL1-RNAi plants. Here, we analyzed whether StSP5G exerts its repressive effect in the leaves or stolons by generating grafts of StSP5G-RNAi, StSP5G-OX and wt plants. Notably, all graft combinations carrying the StSP5G-RNAi plants as donors tuberized in LDs, which correlated with strong activation of the StSP6A gene in underground stem and stolons of the stock plants. Moreover, similar StSP6A activation levels were observed in the wt, StSP5G-RNAi and StSP5G-OX stocks, demonstrating that the StSP5G repressor inhibits tuberization by acting in the leaves. Genome wide expression analyses showed that the StSP5G repressor mediates down-regulated expression of several isopentenyl phosphate transferases (IPT) and MADS-box genes in leaves. In Arabidopsis, the MADS-box transcription factors SVP, FUL and SOC1 are direct targets of the FT/14-3-3/FD floral activator complex (FAC), highlighting a function of this family of regulators in the control of tuberization. Furthermore, StSP5G is later expressed in tubers, where we show it plays a pivotal function in preserving shoot identity of the tuber sprouts.

MIRNA-MEDIATED REGULATION OF FLOWERING LOCUS T IS REQUIRED FOR COORDINATED SOURCE-SINK DEVELOPMENT IN POTATO

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Potato (Solanum tuberosum L.) is the third most important food crop on earth, mainly grown for its starch accumulating tubers. Tuberization is mainly controlled by the Flowering Locus T (FT) homolog SP6A. SP6A mRNA levels are developmentally regulated in potato. Prior to tuberization SP6A transcript levels are low and increase progressively during tuberization. So far regulation is thought to be mediated by CDF1/CONSTANS. In Brachypodium, however, the Pooidae-specific miR5200 has been identified, which potentially regulates transcript abundance of FT levels. Overexpression of a codon optimized SP6A<sup>opp</sup> variant in potato leads to early tuberization which is accompanied by an increased assimilate export, as indicated by starch and photosynthesis measurements. As a consequence, shoot development is impaired and the source-to-sink balance is shifted towards the sink. The described phenotype could be due to an altered posttranscriptional regulation of SP6A<sup>opp</sup> transcripts, i.e. by miRNAs. To identify regulatory miRNAs controlling tuberization, miRNAs and miRNAs present in source leaves of potato plants before and after tuberization were analyzed by NGS.
Using this approach a potential miRNA targeting SP6A could be identified. Expression profiles of the SP6A-specific miRNA and its target are negatively correlated during development and under elevated temperatures. In silico analysis revealed SP6A\textsuperscript{CSE} is an unlikely target for the miRNA which could be verified by co-expression experiments. Thus, modulation of the miRNA binding site in the SP6A gene opens up new chances for potato breeding.

THE GENETIC CONTROL OF TUBER DORMANCY AND SPROUT ELONGATION IN POTATO

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Tuber dormancy is one of the most commercially important traits in potato as their long-term storage is necessary to ensure year-round availability. Tubers undergo a phase of dormancy after their maturation and this inherent feature is one of the key determinants of tuber postharvest life. Premature dormancy release in potato tubers during storage is accompanied by significant deterioration in quality. Use of chemical inhibitors for controlling sprouting is under threat due to legislation based on environmental concerns. Tuber storage under temperatures lower than currently practised is also discouraged because of consequential increase in energy use and adverse impacts on the environment, as well as increased reducing sugar accumulation. Besides the need to develop new storage strategies for potato, breeding potato cultivars with extended dormancy is desirable. With this goal in mind we are dissecting the genetic components underlying tuber dormancy using a large diploid Phureja?Tuberosum mapping population genotyped using the potato 8K SNP array. Tubers were assessed for dormancy-release and sprout growth in storage at nine time-points over two consecutive growing seasons. Genetic analysis has revealed the presence of two major as well as other smaller effect QTLs on several potato chromosomes. Bulk-segregant-analysis using whole exome capture sequencing of low and high dormancy pools has been used for fine mapping of QTLs. Candidate genes located in the vicinity of these QTLs have been identified and transgenic-tester lines were developed. In addition, the identified genomic regions were examined for variants in key dormancy-related alleles to develop diagnostic tools for tuber dormancy.

VERTICILLIUM DAHLIAE DISEASE RESISTANCE AND THE REGULATORY PATHWAY FOR MATURITY AND TUBERIZATION IN POTATO

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Verticillium dahliae Kleb. is a pathogenic fungus causing wilting, chlorosis and early dying in potato. Genetic mapping of resistance to \textit{V. dahliae} was done using a diploid population of potato. A quantitative trait locus (QTL) on chromosome 9 co-localised with the \textit{Ve2} \textit{Verticillium} will resistance gene marker. Another major QTL for \textit{Verticillium} resistance was found on chromosome 5. The StCDF1 gene controlling maturity and tuberization was mapped within the interval. Epistasis analysis indicated that the
two loci on chromosomes 5 and 9 had a highly significant interaction, and that StCDF1 functioned downstream of Ve2. Expression QTL (eQTL) analysis was carried out to understand the relationship between gene expression and phenotype. Gene Ontology (GO) analysis was conducted and genes with eQTL at the StCDF1 and Ve2 loci were both found to have similar functions involving the chloroplast, including photosynthesis. However, differences were also noted. Among the GO terms that were specific to genes with eQTL at the Ve2, but not the StCDF1 locus, were those associated with fungal defense.

The expression of genes regulated by StCDF1 and functioning downstream in the pathway for regulation of maturity and tuberization were also genetically mapped. The mobile tuberigen, StISP6A, and the gene upstream of it, StISP5G, were found to have an eQTL on chromosome 5 at the same location as that found for V. dahliae resistance. Together these results suggest a connection between genes controlling maturity and tuberization and those involved V. dahliae defense.

DIPLOID POTATO HYBRIDS – QUALITY MEETS RESISTANCE

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The reason for the slow progress in the development of potato cultivars is the inefficient way of breeding in tetraploids. Solynta has developed a diploid hybrid breeding program for potato over the last decade that enables much faster, more efficient and more directed genetic progress. Hybrid cultivars can be released as true seeds which can be produced in a fast way and the seeds are devoid of almost every pathogen. Vigorous diploid potato inbred lines have been developed by many generations of selfings, crosses and selections. The first and second series of hybrid seeds were made and tested in replicated multi-location field trials in Europe to compare their performance to commercial controls. The hybrids were evaluated for tuber yield and quality traits. The best experimental diploid hybrid cultivars match the yield and quality requirements of commercial tetraploid cultivars. The next steps will be the introduction of a number of resistance genes. Many resistance genes against nematodes, viruses, wart disease and Phytophthora infestans have been described. Solynta is now developing parental lines with a combination of resistance genes. In each parental line a number of different resistance genes can be stacked. A nice example is the stacking of two to five different Phytophthora infestans resistance genes in single, high quality hybrids. We will show that combining (stacking) is possible in a limited period of only 2 years. A following step can be the introduction of genes involved in abiotic stresses like drought and heat.
Parallel Session VIII TRADITIONAL VARIETIES

THE TRADITOM PROJECT: DIGGING IN THE VARIABILITY POOL OF TRADITONAL EUROPEAN TOMATO

The TRADITOM partners as in traditom.eu (presented by A. Mazzucato)
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Increased interest towards traditional tomato varieties is fuelled by the need to rescue desirable organoleptic traits and to improve the quality of fresh and processed tomatoes in the market. In addition, the phenotypic and genetic variation preserved in tomato landraces represents a means to understand the genetic basis of traits related to agronomic, nutritional and organoleptic aspects and to breed them in modern varieties. In the frame of the EU-funded project TRADITOM we have started to characterize the genotypic and phenotypic variability of 1500 traditional tomato varieties collected throughout Europe. A total of 300 Gb of genotype information have been produced and approximately 2x the whole TRADITOM collection has been cultivated and phenotyped; a total of 19 qualitative and 117 quantitative traits have been measured and recorded. Genotyping of the TRADITOM collection has revealed that, despite the reduced variability, there is still a possibility to associate it with geographical and phenotypic characteristics. Differently, great variability has been found in phenotypic traits related to plant architecture and physiology and fruit characteristics, such as fruit size, shape, Brix and fruit ripening time. This contribution reports updated insights into the extensive phenotypic characterization of the collection, the efforts to select a core collection for more targeted analyses and the perspective to use it to develop GWAS approaches.

GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS OF PALESTINIAN SNAKE MELON (CUCUMIS MELO VAR. FLEXUOSUS) LANDRACES USING SNP AND DARTSEQ MARKERS

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Crop land races represent a source of useful genes endowing tolerance to biotic and abiotic stresses, and other agronomic traits including yield. Our study involved 88 Palestinian snakemelon, (Cucumis melo var. flexuosus) accessions [representing four Palestinian snakemelon landraces known with the folk names of Baladi-Abiadh, Baladi-Akhdar, Sahouri-Akhdar, and Sahouri-Abiadh] distributed in 9 districts in the West-Bank. We report the first successful application of genotyping by sequencing in snakemelon. A total of 4875 SNP and 7400 DArTseq genetic markers were used to evaluate genetic diversity and population structure of Palestinian snakemelon landraces. Clustering based on neighbor-joining-analysis, principle coordinate and Bayesian Model implemented in structure indicated that patterns of genetic diversity of snakemelons depend on their geographical origin and revealed the presence of two major groups (Sahouri, Baladi) with accessions from each local landrace clustering together. Significant correlation was observed between DArTseq and SNP markers in Mantel correlation test. We also detected significant relationship between genetic and geographic matrices (P<0.0001). Analysis of molecular variance indicated that majority of variation (90%) was due to the difference within accessions. Average pairwise genetic distance (PGD) among snakemelon accessions using SNP and DArTseq markers were 0.261 and 0.238, respectively, whereas av.PGD were 0.038, 0.033 among the four landraces, respectively. These Palestinian landraces seem to possess unique genes that might allow enrichment of global snakemelon gene pool and improvements in its production world-wide. Our next
objective is to identify genotypes with promising attributes and to conduct association mapping studies focusing on Fusarium-wilt resistance, yield, biotic and abiotic stresses.
VARIABILITY IN FRUIT RIPENING WITHIN THE EUROPEAN TRADITIONAL POOL OF TOMATO VARIETIES

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Fruit ripening is a highly coordinated and regulated process that involves changes in color, texture, metabolic composition etc. In general, traditional varieties show rapid ripening and shorter post-harvest life than modern hybrid varieties, most of them carrying the ripening-inhibitor (rin) mutation in heterozygosis in order to slow down ripening.

To evaluate postharvest ripening variability present the traditional European tomato pool, 220 varieties composing the Core Collection of European Traditional tomato, representative for the genotypic and phenotypic diversity available in over 1500 TRADITOM repository collection, were studied. Postharvest response was also analyzed in 13 inbreed lines and 27 commercial hybrids, most of them having rin allele in heterozygosis.

Analysis of firmness and color evolution during fruit ripening indicated that, despite the general trend is to be red and soft, differences in the response of some varieties: some remain green and firm, some green and soft and other red and firm. Indicating that color and firmness could proceed at different speeds according to the genotype. In addition, differences between traditional and commercial tomatoes were also observed.

To explore the molecular genetic basis, haplotypes associated to different ripening behavior groups were analyzed. In addition, expression levels of master ripening regulators and key genes involved in the different aspects of ripening were analyzed by using a mid-throughput Fluidigm RT-PCR platform.

GENOTYPING-BY-SEQUENCING IN CHILE PEPPERS FROM OAXACA, MEXICO YIELDS INSIGHTS INTO EVOLUTIONARY HISTORY OF DIFFERENT CHILE PEPPER USE-TYPES

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Studying genetic diversity among morphologically diverse named chile pepper types from near the C. annuum domestication center may elucidate fruit-trait loci, domestication, and comparisons with extensive fruit-trait and population structure research in tomato (Solanum lycopersicum). Our study objectives were 1) to determine whether various named Mexican chile pepper types, especially fresh- vs. dry-use-types, represented distinct genetic lineages 2) assess how these are related to chile peppers from around the world. 103 chile pepper seed accessions of 22 named types were collected in 2013 from 29 sites in southern Mexico. From these seedlots, 190 plants were grown and genotyped via GBS. In this population, we obtained 32,623 SNPs and built a phylogeny. We repeated SNP-calling and analysis after adding raw GBS reads from 41 C. annuum individuals from a global collection, sequenced at lower coverage. We performed in-silico genotyping using these SNPs on the published CM334, Zunla-1, and chilepin genomes. The largest fresh- and dry-use-types clustered into separate monophyletic clades, confirming them as distinct lineages. However, some phenotypically distinct named types were polyphyletic. CM334 Mexican landrace was most related to our elite landraces. Global accessions showed close relatedness to plants from the Yucatán peninsula, indicating C. annuum spread to Eurasia via the Yucatán, as did S. lycopersicum.
HIGH-QUALITY DE NOVO GENOME ASSEMBLY OF THE TOMATO GENOME USING THE LATEST SEQUENCING AND OPTICAL MAPPING TECHNOLOGIES

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Long sequencing technology offer the possibility of dramatically improving the contiguity of genome assemblies and able to extend paths into problematic or repetitive regions. In addition to Long sequencing reads approaches, recent technologies like optical mapping and linked reads from 10X Genomics are capable to bring additional scaffolding to achieve chromosome-level assemblies. In order to improve the actual tomato reference genome sequence, we generated 70X coverage of Pacific Biosciences (PacBio RSII) long reads, 100X of optical mapping using two different enzymes and 100X illumina HiSeq3000 2x150b paired-reads sequencing from Chromium linked 10X Genomics libraries. The integration of these three approaches allowed to reach a genome size of ~830 Mb with an N50 of 45Mb. The assembly contiguity reached chromosome-arm-levels. Interestingly, one full chromosome (Ch12) has been fully assembled in one scaffold. Some of the remaining scaffolds revealed large parts of some centromeric regions, even including some of the heterochromatic regions. We assessed the quality of contigs and scaffolds using illumina mate-pair libraries and genetic map information. The integration of the genetic map allowed to generate the 12 pseudomolecules corresponding to the 12 tomato chromosomes. Several regions corresponding to chromosome zero in the SL3.0 reference genome were included in the current assembly. We took advantage from the large RNA-Seq data of the TomExpress platform (http://gbf.toulouse.inra.fr/tomexpress/) and use them to annotate this new genome assembly. To assess the completeness of the genome and the transcriptome assembly, busco v3.0 software has been used and shown a high percent of gene coverage (> 95%).

THE VALUE OF LINKED OPEN DATA FOR SOLANACEAE RESEARCH

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With the evolution and widespread adaptation of contemporary information technologies, data has taken an increasingly central role in almost all areas of human activity. On the other hand, it is accompanied by a number of challenges. In the domain of Big Data, Linked Data and Semantic Web, common examples of these challenges include scale, performance, availability, security, diversity, complexity, semantics, manageability, and findability. We use semantic technologies to improve identification of candidate gene prediction using available genome annotations and interoperability with literature (unstructured data) and other databases (structured data). One challenge we specifically address is extracting QTL information from literature. However, QTL information is mostly captured as tables, in full-text or supplementary material of a scientific article. Traditional text-mining techniques that focus on extracting knowledge from unstructured
We will show an proof-of-concept of a tool that extract QTL information from tables and demonstrate how we combine this information with the genome annotation(s) utilizing Linked Open Data technologies. The outlook of such data integration technologies is that researchers can focus on addressing fundamental questions more effectively rather than spending time on combining information. This will lead to, for example, more efficient trait-lead discovery.

TRANSLATIONAL RESEARCH, FROM INTEGRATION OF OMICS DATA TO REVERSE GENETICS VALIDATION

Brahim Mania, Fabien Marcel, Marion Dalmais, Christelle Troade, Celine Camps, Vivien Sommand, Joseph Tran, David Latrasse, Moussa Benhamed, Adriane Boualem; Abdel Bendhmane

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Genetic improvement of crops is strategic for developing more productive and sustainable agricultures. In the post-genomic era, one would expect that the discovery and the optimization of the gene networks controlling key agronomic traits could contribute to this genetic improvement. This will requires fine characterization of agronomic traits, identification of the genes involved and engineering leader alleles and subsequent pyramiding to produce highly performing plant prototypes. Over the last five years we have concentrated our efforts on the development of tools to integrate omics data and focussed our effort on three agronomic traits: sex determination, carpel development and inflorescence organization. We have also strengthened the TRANSLATIONAL RESEARCH in two major plant families, the Cucurbitaceae and Solanaceae. We generated large mutant collections and developed tools for allele engineering. How to use the developed tools will be discussed.

FINE-TUNING GENE FUNCTIONS; LESSONS FROM TOMATO MUTANTS

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Breeding companies screen crop mutant populations to find new genetic variation in genes. For over a decade Targeting Induced Local Lesions in Genomes (TILLING) has been used as a method to identify nucleotide substitutions in genes with functions in physiological processes that are known to be part of important traits in crops. Scientific publications that describe the molecular processes under these processes are a major source of inspiration for the selection of the targeted genes. The phenotypes that are observed after identification and growing of the identified mutants often validate, but sometimes contradict, the published data. However, most observations done in companies are not made public and remain unknown for the scientific community. Here we present some results that were obtained by analysing tomato mutants with defects in genes involved in flowering and fruit quality traits. A mutation in FALSIFLORA (FA) increases flower number and inflorescence branching, while the known fa mutant does not produce flowers at all. New mutant alleles for RIPENING INHIBITOR (RIN) were crossed into elite tomato lines and fruit shelf life was determined at several geographical locations. The results
strongly suggest that the trait gain of the conventional and commercially applied rin allele is not just related to lower RIN levels but to specific properties of the residual mutant rin protein. In addition, some other mutants with surprising phenotypes will be presented.
Parallel Session III: WATERMELON PUMPKIN and OTHER CUCURBITACEAE

GENETIC MAPPING AND IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO FUSARIUM OXYSPORUM F. SP. NIVEUM RACES 1 AND 2 IN WATERMELON

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Fusarium wilt is a major disease of watermelon caused by the soil-borne fungus Fusarium oxysporum Schlechtend.:Fr. f. niveum (E.F. Sm.) W.C. Snyder & H.N. Hans (Fon). Fon race 1 is most prevalent throughout the USA while race 2 is more virulent. Our overall objective is to identify and utilize different germplasm sources and gene loci (alleles) associated with resistance to Fon races 1 and 2 and incorporate the resistance alleles into the genome of watermelon cultivars. Here, we present the use of genotyping-by-sequencing (GBS) technology to construct a single nucleotide polymorphism (SNP)-based genetic map with an F2:3 population derived from closely related parental lines of the cultivated type watermelon Citrullus lanatus and segregating for Fon race 1 resistance. We identified a major QTL with a 6.1 cM interval on chromosome 1 that explained 59.9% of the variation in resistance to Fon race 1 and six minor QTL. We also used GBS to generate the first Citrullus amarus intra-vary genetic map (previously known as C. lanatus var. citroides collected in southern Africa). Here we used an F2:3 population (N=173) segregating for Fon race 2 resistance. A total of 2,495 binned SNPs formed 11 linkage groups with an average distance of 0.7 centiMorgans (cM) between SNPs and a total map length of 1,798.9 cM. One major and four minor QTL explained 69.3% of the variation in resistance. The major QTL was delimited to a 1.2 Mb interval of chromosome 9, with one putative disease resistance gene. We have subsequently used a QTL-seq approach to increase QTL resolution and develop “kompetitive allele specific PCR (KASP)” markers useful in breeding programs to enhance resistance to Fusarium wilt in watermelon.

GENETIC MAPPING OF A MAJOR CO-DOMINANT QTL ASSOCIATED WITH?-CAROTENE ACCUMULATION IN WATERMELON

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The common flesh color of commercially grown watermelon is red due to the accumulation of lycopene. However, natural variation in carotenoid composition that exists among heirloom and exotic accessions, results in a wide spectrum of flesh colors. We previously identified a unique orange-flesh watermelon accession (NY0016) that accumulates mainly β-carotene and no lycopene. We hypothesized this unique accession could serve as a viable source for increasing provitamin A content in watermelon. Here we characterize the mode of inheritance and genetic architecture of this trait. Analysis of test crosses of NY0016 with yellow and red fruited lines indicated a co-dominant mode of action as F₁ fruits exhibited a combination of carotenoid profiles from both parents. We combined visual color phenotyping with genotyping-by-sequencing of an F23 population from a cross of NY0016 by a yellow fruited line, to map a
major locus on chromosome 1, associated with β-carotene accumulation in watermelon fruit. The QTL interval is approximately 20 cM on the genetic map and 2.4 Mb on the watermelon genome. This study is a step towards identification of a major gene involved in carotenoid biosynthesis and accumulation in watermelon. The co-dominant inheritance of β-carotene provides opportunities to develop, through marker-assisted breeding, β-carotene-enriched red watermelon hybrids.

LARGE-SCALE DEVELOPMENT OF EST-SSR MARKERS AND CONSTRUCTION OF GENETIC LINKAGE MAP IN SPONGE GOURD

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More than 59 million sequencing reads of sponge gourd were generated using Illumina paired-end sequencing technology. 77,858 unigenes with a mean length of 1,339 bp were obtained with de novo assembly. Of all unigenes, 52,113 (66.93%) had significant similarity to known proteins from NCBI non-redundant (Nr), Swiss-Prot, Clusters of Orthologous Group (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A total of 12,932 putative SSRs were identified in 10,716 unigenes, with a distribution density of one SSR per 8.06 kilo-bases. Based on these SSR-containing sequences, 8,523 high-quality SSR primer pairs were designed, of which 1041 EST-SSR primer pairs were screened for the validation among S1174 (L.acutangula Roxb.), 93075 (L.cylindrica Roem.) and their hybrid F1. The results showed that 814 (78.19%) exhibited successful amplification, of which 417 (39.87%) revealed polymorphism between S1174 and 93075. By using an F2 mapping population derived from a cross between S304 and 93075, we further constructed a genetic linkage map consisting of 177 EST-SSR markers distributed in 14 linkage groups. The map spans 1,436.12cM with a mean distance of 8.11cM between makers. These EST-SSR markers and genetic linkage map are very valuable for the discovery of novel gene and marker-assisted selection in sponge gourd.

EVALUATION OF AN EMS-INDUCED SQUASH LIBRARY USING PHENOMIC AND GENOMIC APPROACHES

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Chemical induced mutations not only enable functional analysis of genes, but also the exploitation of new traits in plant breeding programmes. An ethyl methanesulfonate (EMS)-induced collection, consisting of 3,800 M2 independent lines, was developed in Cucurbita pepo. In this study, we have integrated several fast phenotyping methods and new-generation sequencing (NGS) to assess the quality of the collection, estimating the density and distribution of EMS-induced mutations in the squash genome. By phenotyping seedlings of the whole library, and adult plants from a set of 300 lines, we detected more than 15% of morphological mutants, concluding that the phenotypic variation in the collection is high. Moreover, high throughput screenings are currently ongoing to identify specific variants for significant agronomic traits, including plant-hormone insensitivity and tolerance to biotic and abiotic stresses. So far we have detected more than 10 new promising mutants with a weak ethylene response or a higher tolerance to oxidative damage. Four samples of two independent mutagenized lines were sequenced by NGS, and candidate SNVs identified by mapping to the squash reference genome v3.2 (https://cucurbigene.upv.es/). The EMS induced transitions (C to T, and G to A) were filtered from false
positive variants. Using mutant allele coverage of at least 4 and GQ>20, DP=20-250, a mutation rate of 19.28 and 10.25 heterozygous mutations/Mb was found across 230 Mb sequence sufficiently covered in each one of the mutant lines. The distribution of these EMS mutations in different scaffolds, chromosomes and gene regions is discussed.
GLOBAL DISTINCTIVE FEATURES BETWEEN POLLINATION AND PARTHENOCARPIC FRUIT SET IN ZUCCHINI

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Fruit set is defined as the transition of an ovary to a growing young fruit, and depends on the successful completion of pollination and fertilization. In the case of zucchini, one of the most important morphotypes of Cucurbita pepo, harsh conditions as low/high temperature or inadequate humidity, that occurs especially in off-season crops, prevents fruit set causes economic losses due to low fruit yield. Parthenocarpy, fruit development in the absence of pollination/fertilization, has been recognized as an important trait to avoid this problem in different fruit crops. However, the understanding of the mechanisms underlying parthenocarpy is limited in zucchini. The aim of this work is to analyse, by RNAseq, the difference between fruit transcriptomes during the fruit set of the two cultivars of zucchini, a non-parthenocarpic cultivar and a parthenocarpic cultivar, in an attempt to identify genes that play important roles during pollination, fruit set and parthenocarpy. Differentially expressed genes (DEGs) of fruit set were compared against unpollinated fruit of the non-parthenocarpic cultivar and parthenocarpic cultivar. Distinctive features of parthenocarpic and pollinated fruits were revealed by combining the analysis of the transcriptome. These results shed insight into the gene regulation of developmental process relevant for fruit set in zucchini as cell division, sugar metabolism or hormones responses.
Parallel Session VI PEPPER and EGGPLANT

GENOME EVOLUTION PATTERN IN THE SOLANACEAE FAMILY AND GENETIC RELATIONSHIPS IN THE EGGPLANT COMPLEXES

Sergio Lanteri (1), Jaime Prohens (2), Alberto Acquadro (1), Pietro Gramazio (2), Ezio Portis (1), Santiago Vilanova (2), Cinzia Comino (1), Mariola Plazas (3) and the Italian Eggplant Genome Consortium

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The Eggplant Genome Consortium has obtained an high quality genome sequence of eggplant (Solanum melongena L.), which was anchored to the 12 chromosomes. The assembly covered 1.06 Gb, and the estimated eggplant genome size was 1.2 Gb. Of the 34,916 predicted genes, 3,234 were paralogs (ohnologs) generated by the whole genome T triplication event, which also occurred in tomato, potato and pepper. In the four species only a limited number of ohnologs belonged to the same gene family, suggesting that the triplication was followed by lineage-specific genome fractionation. The putative chromosomal complements of the key founders of the different Solanaceae clades as well as the chromosome complements of extant species were reconstructed.

S. melongena belongs to the largest Solanum lineage, the ‘spiny solanums’ (subgenus or clade Leptostemonum), which also includes the cultivated species S. aethiopicum and S. macrocarpon. A GBS approach was applied to evaluate the genetic relationships within and between the gene pools of the three cultivated species. The study also included accessions of their close wild relatives and more distant species of the tertiary gene pool, for a total of 76 entries. Reads were aligned to the reference genome sequence, and of the 75,399 polymorphisms identified, 12,859 were found in coding sequences. Data, analyzed with different approaches, highlighted a clear separation among species, although not between each of the domesticates and their respective wild ancestors. The gathered information resolved some classification controversies and may guide further exploration and exploitation of the diversity in the large Leptostemonum clade.

MULTIPLE REFERENCE GENOME SEQUENCES OF HOT PEPPER REVEAL THE MASSIVE EVOLUTION OF PLANT DISEASE-RESISTANCE GENE FAMILY BY RETRODUPlication

Doil Choi

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Transposable elements (TEs) provide major evolutionary forces leading to new genome structure and species diversification. However, the role of TEs in the expansion of disease resistance gene families has been unexplored in plants. Here, we report two high-quality de novo genomes (Capsicum baccatum and C. chinense) and an improved reference genome (C. annuum) for peppers. Dynamic genome rearrangements involving translocations among chromosomes 3, 5 and 9 were detected in comparison between C. baccatum and the two other peppers. The amplification of athila LTR-retrotransposons, members of the gypsy superfamily, led to genome expansion in C. baccatum. In-depth genome-wide comparison of genes and repeats unveiled that the copy numbers of NLRs were greatly increased by LTR-retrotransposon-mediated retroduplication. Moreover, retroduplicated NLRs exhibited great abundance across the angiosperms; most cases are lineage-specific and therefore recent. Our study
reveals that retroduplication has played key roles for the massive emergence of NLR genes including functional disease-resistance genes in plants.

STUDY OF SOLASONIN CONTENT AND SGT1 GENE EXPRESSION IN IRANIAN EGGPLANT (SOLANUM MELONGENA L.) GENOTYPES

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Eggplant (Solanum melongena L.) is one of the most consumed vegetables in the world. The eggplant glycoalkaloids (GAs) are toxic secondary metabolites that may have detrimental effects on human health, particularly if the magnitudes of GAs are higher than the recommended food safety level (200 mg per kg of fresh mass). In this study, the content of solasonine compound and the expression patterns of solasodine galactosyltransferase (SGT1) gene were assessed in different tissues (mature leaves, flower buds, young, mature, and aged fruits) of two Iranian eggplant genotypes (D1 and J10) under field conditions. Considering D1, the maximum mass fraction of solasonine was detected in flower buds (135.63 µg/g), followed by leaf (113.29 µg/g), aged fruit (74.74 µg/g), young fruit (61.33 µg/g), and mature fruit (21.55 µg/g). Comparing both genotypes, the genotype of bitter fruits (J10) contained higher mass fraction solasonine, as one of the main factors for producing bitter flavour in the plant. Regarding the expression profiles of SGT1, in both genotypes, the activity of the gene was increased nearly parallel with the concentration of solasonine. In the genotype J10, transcript level of the gene was significantly higher than the genotype of sweet fruits (D1). Although both D1 and J10 genotypes are possibly recommendable for human food consumption, D1 is more suitable for daily diet.

MAP-BASE CLONING OF THE TOMATO SPOTTED WILT VIRUS GENE TSW AND ITS RESISTANCE MECHANISM IN CAPSICUM SPP.

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Tomato spotted wilt virus (TSWV) is an important viral disease affecting pepper production worldwide. Tsw, a single dominant resistant gene against Tomato spotted wilt virus (TSWV), has been mapped to chromosome 10. To perform genome-based cloning of the Tsw gene, comparative mapping, pooled transcriptome analysis, and genome walking were performed. SNP molecular markers tightly linked to the Tsw gene were developed using tomato and pepper whole genome sequencing databases. Among them, four SNP markers showed no recombination in two segregating populations of F2 'Telmo' (210 individuals) and ‘SP’ (843 individuals). Three scaffold sequences from the C. annuum genome database and two BAC clones from the BAC library of C. annuum ‘CM334’ containing the four marker sequences were identified. By aligning of two BAC clone sequences and the pepper scaffold sequences, the Tsw gene was delimited within 149 kb encompassed by two flanking markers. A total of 22 predicted genes were annotated in the target region. Among them, five predicted genes encoding CC/TIR-NBS-LRR were selected as candidate genes of Tsw and test their function by coexpression of the effector gene of Tsw. In summary map-base cloning study revealed that Tsw encodes typical NLR and shows the genomic structures and coding sequences of the two genes are strikingly similar coding sequences to Pvr4 conferring resistance to Pepper mottle virus, despite the fact that these two genes recognize completely different viral effectors. Phylogenetic studies revealed that these two immune receptors diverged from a progenitor gene of a common ancestor.
SGN Update by Lukas Mueller

Prashant Hosmani, Mirella Flores, Guillaume Bauchet, Noe Fernandez, Naama Menda, Hartmut Foerster, Isaac Tecle, Adrian F. Powell, Alex Ogbonna, Nicolas Morales, Bryan Ellerbrock, Thomas L. Fisher-York, David A. Lyon, Suzy R. Strickler, Surya Saha, and Lukas A. Mueller

SGN (https://solgenomics.net/) is a database for the Solanaceae containing whole genome sequences, large scale genotype, genetic map, expression, and phenotype data. It provides access to data using in-house and externally developed and open source annotation, analysis and visualization tools. Data is highly integrated and cross-referenced to allow discovery of knowledge. Crops with rich datasets in the databases include tomato, pepper, tobacco, coffee, and petunia. Tools include the Jbrowse genome viewer, BLAST sequence analysis, the VIGS tool for designing viral induced gene silencing (VIGS) probes, the tomato expression atlas (TEA), a genetic map viewer, and the SolCyc biochemical pathways database. Over the last few years, functionality for managing breeding programs was added, including the management of germplasm and pedigrees, the ability to design different types of trials, support for digital data capture for phenotyping in the field using tablets, and storage of high density genotyping information. The SolGS tool draws on the genotyping and phenotyping information to generate genomic predictions of breeding values for genotyped lines.

CUCURBIT GENOMICS DATABASE WORKSHOP by Zhangjun Fei

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The Cucurbitaceae family (cucurbit) includes several economically important crops, such as melon, cucumber, watermelon, pumpkin, squash, and gourd. During the past several years, genomic and genetic data have been rapidly accumulated for cucurbits. We have been developing the Cucurbit Genomics Database (http://cucurbitgenomics.org/) to store, mine, analyze, and disseminate the large-scale cucurbit genomic and genetic datasets in an efficient way and to provide a center portal for the cucurbit research and breeding community. Recently, under the CucCAP project (https://cuccap.org/) funded by the USDA NIFA program, the database has been redesigned and re-implemented using the Tripal toolkit (http://tripal.info/). The database currently contains all available genome and EST sequences and genetic maps for cucurbit species, as well as sequence annotations and biochemical pathways. A set of analysis and visualization tools and user-friendly query interfaces have been implemented in the database. This workshop will review the available data sets in the database and provide demonstrations on how to use the tools in the database to answer various biological and research questions. Future development of the database will also be discussed.
OS01: BIODIVERSITY: GENOMES GALORE and THEIR EVOLUTION

P0116 DEVELOPMENT OF A GENETIC LINKAGE MAP IN PEPINO (SOLANUM MURICATUM) AND SYNTENY WITH TOMATO

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Full text
The pepino (Solanum muricatum, 2n = 2x = 12) is an Andean solanaceous domesticate cultivated for its edible, fleshy and aromatic fruits. Despite being a neglected crop for a long time, pepino has a high yield potential and its fruit has important bioactive properties, such as antidiuretic, antihypotensive, and antidiabetic activities. Very few genomic resources exist for pepino, and up to now, no genetic maps exist. Genotyping-by-sequencing (GBS) generates a large number of markers (SNPs) directly available for use in genetic analysis. In order to generate a mapping population in pepino, a cross was made using S. muricatum cv. ‘Sweet Long’ as the female parent and an accession one of the key wild relatives, S. caripense, as the male parent. One F1 plants was selfed and DNA was extracted from the F2 generation. GBS was applied to 95 plants of this population. As a result, we obtained over 500 SNPs markers of high quality. These SNPs were used for linkage map construction, resulting in 13 linkage groups, with a total length of 1369.2 cM -- an average density of 0.365 markers/cM. By comparing the SNP marker position with the reference genome of tomato, we found a high level of synteny between the two species, and in addition we infer that these variations are uniformly distributed in the pepino genome. This set of SNPs provides an effective tool for understanding the evolution of Andean Solanum domesticates, for quantitative trait loci (QTL) identification, and other important breeding applications.
OS02: CROP DIVERSITY AND ITS VALUE

P0035 ANALYSIS OF CAPSAICIN AND DIHYDROCAPSAICIN IN GERmplasm COLLECTION OF PEPPERS

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1 Full text

Genetic diversity in crop species is a key factor for breeders to improve the organoleptic as well as nutritional parameters of existing cultivars. Apart from morphological description, phytochemical composition of crops is important and helps to characterize respective accessions. Pepper is very popular and widespread all over the world. It is appreciated for its taste, color and aroma. A significant factor distinguishing individual varieties is pungency, caused by capsaicinoids. Analytical methods for characterization of germplasm collection of pepper varieties are of high importance. We present here a high-throughput ultra-high performance liquid chromatography method with UV detection, used to screen through the collections of hot peppers for pungency. Method development, optimization and validation are shown. Capsaicinoids, such as capsaicin and dihydrocapsaicin are quantitatively determined.
Capsicum annuum is one of the five domesticated Capsicum species and the world’s most economically important pepper. It was domesticated in Mexico and migrated worldwide in post-Columbian times. C. annuum was introduced into Europe at the end of the XVth century, being Spain the place of arrival. Therefore, the Iberian Peninsula constitutes a significant secondary diversification centre, where part of the original American pepper genepool might be preserved in the form of landraces.

The aim of this work was to investigate the genetic diversity and genomic structure in a collection of pepper landraces from Spain and Portugal, establishing a comparison with accessions from Mexico. The analyses were performed with a set of microsatellite markers (SSR) and molecular markers linked to disease resistance.

The output obtained with the software Structure revealed two groups; one comprising Spanish and Portuguese accessions, and the other containing Mexican peppers. Further analysis performed only within Iberian landraces suggested the existence of additional sub-structure. Cluster and principal coordinate analyses displayed similar results.

Phyto.5.2 (Phytophthora capsici) and PR-Bs3 (Xanthomonas campestris), were the most common resistant loci observed within the collection, Mexico being the country with the highest percentage of potential disease resistance. Regarding viral diseases, resistant alleles for Tospovirus and Potyvirus were detected in both the Iberian Peninsula and Mexico.

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P0178 INVESTIGATION ON CUCURBITACEAE AND SOLANACEAE SPECIES USED IN ROMANIA FOR MEDICINAL PURPOSES

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1 Full text
This study represents an evaluation of potential use as medicinal of Cucurbitaceae and Solanaceae species. The investigated germplasm envisaged a screening of cultivated and spontaneous resources and their traditional use. Starting from the interest on safety products for human consumption, a large range of crops have been identified to play an important role in different industries as follows: food, pharmaceutics, cosmetics, medicinal, etc. Species from above mentioned botanical families are considered to be valuable resources thanks to their nutritional profile. The chemical content of Cucurbitaceae and Solanaceae species allows usage of different organs (flowers, fruits, seeds, etc), as raw material for multiple purposes. The aim of paper was to present a part of Romanian Cucurbitaceae and Solanaceae genetic resources in order to highlight the enormous potential of species to be used mainly as medicinal. In our study following species were identified in wild flora and used as medicinal: Bryonia alba L., Ecballium elaterium (L.) A. Rich., Atropa bella-donna L., Datura stramonium L., Hyoscyamus niger L., Physalis alkekengi L., Scopolia carniolica Jacq., Solanum dulcamara L. The study also focuses on medicinal profile of species, mainly known as vegetables: cucumber, melon, tomatoes and other. For all investigated species information related to nutritional profile and yield potential of organs used as medicinal are presented. In our region many species are still underused, despite their huge potential for multiple uses as culinary, medicinal, cosmetics, pharmaceutics, decorative.
P0229 VARIABILITY OF TREE TOMATO (SOLANUM BETACEUM CAV.) AND NARANJILLA (SOLANUM QUITOENSE LAM.) PROGENY FOR BREEDING IN ECUADOR.

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Naranjilla (Solanum quitoense) and tree tomato (Solanum betaceum) are plant species of the Solanaceae family which are fruit crops of great economic importance in Ecuador, Colombia and Peru, but recently became more popular worldwide. The naranjilla species “quitoense” (without spines) and “septentrionale” (with spines) have been replaced in many cases by hybrids between S. sessiliflorum and S. quitoense such as Puyo and Palora in Ecuador; whereas hybrids between S. quitoense and S. hirtum are currently cultivated in Colombia. In the case of tree tomato, local cultivars (orange and red), which are the product of natural crosses, are grown in Ecuador.

The Fruit Research Program of the National Institute of Agricultural Research (INIAP) has evaluated hybrids from species of the Lasiocarpa section (naranjilla), and a breeding population from an interspecific cross between S. betaceum x S. unilobum, including backcrosses with S. betaceum, obtaining a progeny showing phenotypic variability, reflected in agronomic traits, fruit quality and productivity (yield). Cluster analysis using Ward's minimum distance was carried out to create groups of individuals with similar traits. Desirable characteristics in naranjilla were high yield, green pulp, thornless plants and aromatic fruit; while for tree tomato were high yield, orange pulp, and high soluble solids content. The obtained Naranjilla and Tree Tomato populations showed variability that can be used in new breeding programs.
The genebank of the Institute for the Conservation and Improvement of the Valencian Agrodiversity (COMAV) of the Universitat Politècnica de València holds a collection of cucurbits and solanaceous crops of about 3700 and 10000 accessions respectively. The cucurbits collection includes the most important crops, melon (1500 accession), watermelon (400), pumpkin (1500) and cucumber (270) and other minor cucurbits, like Cyclanthera, Luffa, Lagenaria and other species. Most part of the collection comes from Spain, being a 33 % from other countries, mainly from South America and Africa.

Regarding the solanaceous collection, the best represented crop is the tomato with more than 6000 accessions. Pepper and eggplant have also a good representation with 3000 and nearly 500 accessions respectively. These numbers include the cultivated species as well as their wild relatives. The number of species is 19 for the Solanum genus (14 for tomato and wild relatives and 5 for eggplant and wild relatives) and 8 for the Capsicum one. Although most part of the collection is of Spanish origin, there is a representative proportion of the collection from other countries, mainly from Latin America, the center of origin of tomato and pepper. Collecting expeditions have been carried out to other continents, including Africa and Asia.

The genebank participates in some projects funded by H2020, the National Science Foundation of USA, and National Programmes, promoting the utilization of the conserved seeds. The rate of utilization of the solanaceous and cucurbits collections in the last 5 years has been a 75 % and 72 % respectively.
Valencian tomato traditional varieties are raising an increased interested as they are locally associated to better organoleptic properties. In particular the ‘Valenciana d’El Perelló’ tomato which grown in sandy soils close to the sea in the Albufera Natural Park has had a dramatic increase in demand in the last years. This tomato has different morphological (pointed and fleshy fruits), agronomic and quality traits that make it attractive to the farmer and the consumer. The materials used by the farmers, sometimes presents problems of uniformity and irregular production. In addition, this variety has a high susceptibility to diseases such as caused by the Tomato mosaic virus (ToMV), which is mechanically transmitted with high efficiency. Thus, introduction of resistance to ToMV is a breeding objective in this variety. Using a participatory approach we selected 15 materials that had a good agronomic performance and had a fruit morphology and quality fitting with the ‘Valenciana d’El Perelló’ ideotype. A backcross breeding program has been undertaken to introgress resistance to ToMV, conferred by the $Tm^2$ resistance gene from a hybrid into the genetic background of these selections. Marker assisted selection for $Tm^2$ using SNIP specific marker has been used to select resistant plants in the backcross generations. A rapid recovery of the ‘Valenciana d’El Perelló’ traits has been observed in the backcross generations. The new selections resistant to ToMV will represent a material with better uniformity and yield than the materials used by the farmers and resistant to the main phytopathological problem of this variety.
Increased consumer complaints about a reduction in flavour quality of fresh tomato varieties have urged sensory quality to become a major breeding objective. In order to improve tomato fruit sensory quality and to diversify this product, a clearer understanding of the factors influencing tomato flavour and consumer preferences is required. Towards this aim, 17 traditional and modern fresh market tomato varieties (belonging to three typologies “Oxheart”, “Marmande” and “Round”) were selected based on their reputation of good flavour, and different partially overlapping sets were cultivated and evaluated in France, Italy and Spain in the framework of the European TRADITOM project (http://traditom.eu/). Two different growing conditions were tested in France (soilless under greenhouse vs. soil under plastic tunnel) and in Spain (open air vs. soil under greenhouse), while in Italy the products were evaluated at two different ripening stages. In each country fruit quality was assessed at three levels using consensus protocols: objective description of sensory properties by trained panels, consumer preference tests and physico-chemical measurements. For the hedonic tests, after the overall liking (OL), consumers were asked to answer a Check-All-That-Apply (CATA) survey. The results obtained so far highlight a strong genotype effect on consumer OL; while the effect of different growing conditions on OL was either not significant (Spain), or was variety dependent (France). In general, consumer preferences were oriented towards a “juicy”, “sweet” and “aromatic” product, while “mealy texture” was the descriptor that negatively impacted the assessment of products.
Tomato yellow leaf curl virus (TYLCV), transmitted by white fly, is a major disease that causes severe yield loss in tomato production worldwide. Five resistance loci (Ty-1 to Ty-5) against this disease have been previously identified from wild species including Solanum chilense. In the present study, we investigated sequence variations of the Ty-3 gene to develop a molecular tool for improving TYLCV resistance in cultivated tomato (S. lycopersicum). The predicted genomic sequence of Ty-3 (4,242 bp) on chromosome 6 was obtained from the tomato reference genome assembly (v2.50) at the Sol Genome Network database. With this sequence, three sets of primers were designed to produce 669-813 bp amplicons representing all coding sequences of the Ty-3 locus. Sequence analysis was conducted using a cultivated tomato collection consisting of the TYLCV resistant (TB38 and TB45) and susceptible (TB61 and TB70) varieties. We detected a total of 31 single nucleotide polymorphisms (SNPs) and four insertion and deletions (InDels) that separated resistant from susceptible varieties in the Ty-3 locus. The 12 SNPs and one InDel were distributed across five predicted exons and seven SNP were non-synonymous. For these non-synonymous SNPs and InDels, five cleaved amplified polymorphic sequence (CAPs) markers were developed based on four SNPs and one InDel, respectively. These gene-based markers will be useful molecular tools to develop elite cultivars with TYLCV resistance in tomato breeding programs.
P0057 EXPLORING NEW ALLELES INVOLVED IN TOMATO FRUIT QUALITY IN AN INTROGRESSION LINE LIBRARY OF SOLANUM PIMPINELLIFOLIUM

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1 Full text

We have studied a genomic library of introgression lines (IL) from the Solanum pimpinellifolium TO-937 accession into the genetic background of the “Moneymaker” cultivar in order to evaluate the accession’s breeding potential. We identified chromosomal regions associated with both vegetative (plant vigor, trichome density) and fruit-related (morphology, organoleptic quality, color) traits. A trichome-density locus was mapped on chromosome 10; two loci for plant vigor were defined on chromosome 2 and 3, a locus for anthocyanin accumulation was found on chromosome 5. A large number of Quantitative Trait Loci (QTL) were identified for fruit weight, most of them decreasing the trait, except one in chromosome 12 that increased it. We were able to identify QTL for shoulder height, in spite of their low genetic effects, thus demonstrating the power of the current population for detecting minor QTL. Regarding organoleptic traits, consistent QTL were detected for SSC. Interestingly, QTL on chromosomes 2 and 9 increased SSC but did not affect fruit weight, making them quite promising for introduction in modern cultivars. ILs with introgressions on chromosomes 1, 2, and 10 increased the internal fruit color, making them candidates for increasing the color of modern cultivars.
P0063 USING GENOMIC VARIANTS TO PREDICT FRY COLOUR IN POTATO

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1 Full text

Like many countries, Ireland produces potatoes to satisfy its indigenous crisping and chipping industries. When potatoes for processing are stored below 8°C glucose can accumulate leading to dark fry colours and potential acrylamide build-up. Sprouting occurs above 8°C and reduces quality. This demands the use of sprout suppressants such as chlorpropham, the use of which is set to be phased out due to health concerns. Ideally we would be able to develop potatoes that can be stored below 8°C without suffering from low temperature sweetening (LTS).

We are accumulating data to build up a population on which we can train models that enable us to use genomic information to predict phenotypes. Entries were phenotyped for dry matter and fry colour at various times points during storage at 8°C and 4°C. Genotypes were collected on each entry using a genotyping-by-sequencing approach. We used rrBLUP for genomic prediction and evaluated our predictive accuracy with cross-fold evaluation.

To date we have accumulated genotypes and phenotypes on 256 entries, and already can demonstrate, through cross-validation, that high predictive accuracies can be achieved for traits such as resistance to LTS. Furthermore, these models have been validated in an independent collection of entries. Initial work suggests that feature selection can be used to reduce the dimensionality of the data without significantly reducing predictive ability, opening the possibility for the development of low cost, high throughput genotyping assays based on hundreds of loci that are presumably in linkage disequilibrium with genes controlling the trait of interest.
P0078 ANTHOCYANIN-RELATED GENES ENLIGHTEN THE PURPLE PEEL COLOR IN TOMATO ADVANCED LINES

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1 Full text

Tomato (Solanum lycopersicum L.) is one of the most important and popular fruity vegetables worldwide. In tomato, fruit color is the key quality trait welcomed by consumers. Presently, a wide range of tomato cultivars is available in respect of fruit color including white, red, pink, orange, yellow, green, brown and black/purple. Lycopene colors tomato fruit red. The purple peel color of tomato fruit is due to the accumulation anthocyanin pigments. Anthocyanins are beneficial for both plant and human. Surprisingly, cultivated tomato fruits normally do not make anthocyanin. There are some wild relatives of cultivated tomato like Lycopersicon chilense, L. hirsutum, L. cheesmanii, and Solanum lycopersicoides produce anthocyanins in their fruits. In this study, the purple fruit color was obtained through crossing between ‘OSU blue’ (blue fruit) and ‘Purple mimi’ (brown fruit) and subsequent self-pollination. Anthocyanins are produced via phenylpropanoid biosynthetic pathway and are regulated by a transcriptional complex MYB, bHLH and WD40 repeats. We determined expression profiles of genes related to anthocyanins biosynthesis in tomato genotypes with distinct fruit colors by qRT-PCR. Both the early and late biosynthetic genes of anthocyanins were up-regulated in peel of purple fruited tomato except 5GT. Moreover, expression of regulatory genes ANT1, AN1, AN11 was dramatically increased in purple peel. Besides, the expression of ANT1 was positively correlated with anthocyanin biosynthetic genes. This result indicates that the peel color of purple fruited tomato is due to up-regulation of anthocyanin regulatory and biosynthetic genes. (This research was supported by Golden Seed Project, Grant no. 213007-05-1-CG100)
P0080 INDEL SEQUENCE VARIATION IN ACETYLTRANSFERASE GENE REVEALS HIGH AND LOW SUGAR CONTENT IN TOMATO (SOLANUM Lycopersicum) GENOTYPES

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1 Full text

Consumer’s preference to have sweet taste in tomato fruits within certain acid range. Therefore, sweetness and enhanced sugar contents are desirable traits. Previous research conducted to find the candidate genes involved in sugar metabolism or signaling in tomato. One of these genes, acetyltransferase, involve in acylsugars production in tomato. Acylsugars consist of aliphatic acids of different chain lengths esterified to sucrose. Hence, this particular gene is assumed as candidate gene for variation in sugar content of tomato fruits. A previously annotated tomato acyltransferases (SlAT1: Solyc01g105550) gene sequence was taken and their paralogous genes were searched in tomato genome using BLAST tool. Several primers were designed covering 3′-UTR (untranslated region) to 5′-UTR for each gene. The primers were tested for polymorphism in PCR product of DNAs collected from two distinctly different inbred line 863 (with high sugar content 220-230 µmol/g FW) and line 589 (with low sugar content 110-120 µmol/g FW). Out of twelve, two genes showed clear InDel variation between two lines. Cloning and sequencing of InDel variation revealed 165 bp deletion of Solyc01g105560 gene might be responsible for higher sugar content. InDel variation of cloned data was used to redesign the primers and will be confirmed and reported by using different F₂ segregants of crosses high and low sugar lines and also in cultivars. This candidate gene based molecular marker might be contributed in Marker Assisted Back crossing for developing high sugar content tomato cultivars.

(This research was supported by the Golden Seed Project, Grant no. 213007-05-1-CG100)
P0081 IDENTIFICATION AND DEVELOPMENT OF POWDERY MILDEW RACE-SPECIFIC SNP MARKERS IN MELON

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Melon (Cucumis melo L.) is highly valued for its fruit quality. However, this fruit crop is severely affected by powdery mildew (PM) fungus Podosphaera xanthii and occurs year round. Exploration of PM race-specific resistant molecular markers would be effective to develop race-specific resistance cultivars. Young leaves from four melon cultigens, susceptible SCNU1154, and race-specific resistant Edisto47, PMR5, and MR-1 were sequenced by whole-genome re-sequencing (WGR). A total of 591 (Race1-specific), 372 (Race5-specific) and 1183 (RaceN5-specific) single nucleotide polymorphisms (SNPs) was found on chromosomes 2, 5, and 12 from WGR data. Total 31, 34, and 62 putative R-gene SNPs were identified based on resistance and susceptibility spectra of the cultigens against three PM races; Race1, Race5, and RaceN5, respectively comparing reference R-gene. Physical maps were constructed using putative SNPs which located on chromosome 2, chromosome 5, and chromosome 12. Two common putative R-gene SNPs of Race5 and RaceN5-specific were identified from chromosome 12 that are tightly linked to reference Race5 SSR marker CMBR150. Using derived cleaved amplified polymorphic sequence (dCAPS) we detected allele-specific PCR-gel-based polymorphisms which were phenotypically matched with Race5-specific susceptible (SCNU1154, PMR45, WMR29, and Edisto47); and resistant (PI414723, PMR5, and MR-1) cultigens. Therefore, we named them as C. melo PM Race5-1 and Race5-2 markers. These candidate markers might be useful for PM race-specific marker assisted breeding program in melon. (213007-05-1-CG100, 312065-05-5-HD030)

Keywords SNP markers, Physical map, Powdery mildew races, Whole-genome re-sequencing, Melon
Gummy stem blight (GSB) is one of the destructive diseases of melon caused by the ascomycete fungus, *Didymella bryoniae* (Auersw.) Rehm., that causes significant economic losses. In Korea, GSB resistant genotypes identification and their genetic detection have rarely been studied. The aim of the present study was to assess melon (*Cucumis melo* L.) germplasm for resistance to *D. bryoniae* both genotypically and phenotypically. We screened 16 lines and 44 cultivars of melon by *D. bryoniae* fungus collected from USA and Korea. Among 16 lines, the following five lines with PI 482398, PI482399, PI140471, PI136170 and PI420145 showed high level of resistance whereas PI157082, MR1, PMR5, PMR45, WMR29, Edisto47, PI414723 and SCNU1154 showed susceptible disease reaction. In contrast, majority of the cultivars were found as susceptible except Asia Papaya and Supra. We believe that the phenotypic observations of resistance and susceptibility could be influenced by environmental variations. We therefore, further assessed all genotypes using 20 polymorphic SSR markers obtained. The SSR marker CMCT505 linked to Gsb-1(gummy stem blight resistant gene located in chromosome 1) perfectly grouped resistant and susceptible lines as like as phenotypic data. Thus, the resistant PIs 482398, 482399, 140471, 136170 and 420145 and the cultivars Asia Papaya and Supra, those were found resistant in both phenotypic and genotypic assay, could be recommended for further melon breeding program. Furthermore, the SSR marker CMCT505 which is tightly linked with Gsb-1 can be used for screening melon germplasm. (213007-05-1-CG100, 312065-05-5-HD030)

**Key words:** *Didymella bryoniae*, Gummy stem blight, melon, SSR markers and Screening
P0085 TOWARDS THE DEVELOPMENT OF A SOLCUC AFFYMETRIX GENOTYPING ARRAY

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Besides genome re-sequencing for SNP analysis, the genotyping of lines with an array containing a set of known SNP markers is a potentially cost-saving alternative. We have started to develop a multispecies genotyping array that contains markers from tomato (Solanum lycopersicum), melon (Cucumis melo), cucumber (Cucumis sativus) and watermelon (Citrullus lanatus). For this purpose, we have collected available genome sequencing information in public database for these four species (tomato – 360 accessions, melon – 8 accessions, cucumber – 115 accessions, watermelon – 20 accessions) and extracted the observed SNPs. Based on a number of criteria including suitability for SNP assays on the Illumina and Affymetrix array platforms, polymorphism in the germplasm, allele frequency, distribution along the physical genome and the genetic map, informative marker subsets were selected. For validation and improvement of our marker selection prior to the array design, we have tested subsets of 48 markers as KASP markers for each species on a collection of lines and varieties representing current breeding material provided by a number of vegetable breeding companies. The results show that our marker selection consists mostly of markers that are polymorphic. In the Cucurbitaceae species, it was observed that a considerable proportion of the cucumber and melon SNPs were also useful in the other species. These results can now be used for the development of a 200K Affymetrix array that will contain approximately 25000 markers for melon, 50000 markers each for cucumber and watermelon, and 75000 markers for tomato.
P0122 SUPER-RESOLUTION RIBO-SEQ IDENTIFIED NOVEL SMALL PEPTIDES IN TOMATO

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1 Full text

Peptides encoded by small open reading frames (sORFs; usually less than 100 codons) are crucial in plant development and environmental responses. Despite their importance on diverse functions, it is challenging to identify these peptides. First, genes encoding small peptides are less likely to be targeted by common mutagenesis methods, presumably due to their small size. Second, computational genome annotation typically excludes proteins that are less than 100 amino acids. Finally, although comprehensive bioinformatic searches indicate thousands of potential sORFs are present in plant genomes, it remains difficult to identify sORFs are translated from non-translated ones. We have exploited Ribo-seq (deep sequencing of ribosome footprints) and transcriptome assembly to identify actively translated open reading frames in Solanum lycopersicum (cv. Heinz-1706') root and shoot. Taking advantage of the 3-nt periodicity displayed by actively translating ribosomes in Ribo-seq and the novel transcript information derived from RNA-seq, we uncovered hundreds of new peptides outside of the current tomato genome annotation. In addition, our study revealed over 1500 translated upstream ORFs in the 5' untranslated regions of protein-coding transcripts. Several studies have shown that uORFs provide translational regulation on their downstream main ORFs involved in specific developmental and metabolic processes. By experimentally identifying novel translated regions in the genome, including sORFs and uORFs, our work provides a starting point to study new signaling peptides and untapped regulatory mechanisms in gene expression.
P0123 PHENOTYPIC PLASTICITY AND QTL MAPPING IN A MULTI-PARENTAL TOMATO POPULATION GROWN UNDER CONTROL, WATER DEFICIT AND SALINITY STRESS ENVIRONMENTS

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1 Full text

Drought and salinity are among the major abiotic stresses that limit crop productivity in many species, but may be favorable to organoleptic quality of fruit and vegetables. Quality involves several polygenic traits, which often exhibit variable levels of genotype x environment (GxE) interaction.

In order to assess the impact of water deficit and salinity on tomato fruit quality and productivity, we have evaluated a multi-parental advanced generation intercross (MAGIC) population in contrasted conditions over two years in greenhouse, one year in control vs water deficit experiment (EXP.1) and the other in control vs high salt experiment (EXP.2). A set of 241 and 253 lines from the MAGIC population was used to identify QTL for fruit quality traits (fruit weight, fruit number, Soluble Solid Content, firmness), phenology (time to flower and ripe) and plant vigor (leaf length) in the two experiments.

All the traits showed a large genotype variation and high heritability whatever the year or treatment, and most of them were highly correlated. Significant GxE interactions were detected in both experiments.

QTL were mapped using 1354 SNP markers. A total of 54 main effects QTL were detected while 15 QTL revealed GxE interactions. Some regions were identified carrying co-localizations of several QTL suggesting pleiotropic regulation. We then defined a strategy for candidate gene identification based on the high mapping resolution and the allelic effect of each parental line at the QTL.
P0135 IDENTIFICATION OF QTLS CONTROLLING FLAVONOID ACCUMULATION IN TOMATO FRUIT CUTICLE

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\textbf{1 Full text}

Tomato fruit cuticle accumulates the flavonoid chalconaringenin during ripening, a yellow-orange colored phenolic that contributes to the ripe fruit color. Chalconaringenin additionally provides mechanical resistance to the cuticle and participates in the regulation of cell size.

Our study aims to identify candidate genes involved in the biosynthesis and genetic regulation of flavonoids and therefore genes implicated in the control of fruit color and size, a possible additional function of flavonoids that has not been demonstrated yet. As first step for this issue, a QTL analysis was conducted using a RIL population developed from the interspecific cross between \textit{Solanum lycopersicum} 'Moneymaker' and \textit{Solanum pimpinellifolium} 'TO-937' for which a saturated linkage map of 4500 SNP markers was available. Five QTLs for the percentage of phenolic compounds were located on chromosomes 1, 4, 7, 8, and 12. QTL validation was performed on a population of 52 introgression lines (IL) developed from the same parental cross.

ILs carrying the QTLs identified for % of phenolics together with newly-created ILs by pyramidization of QTLs developed from the former lines showed significant changes in the percentage of phenolics, amount of cuticle and fruit size and color. Particular loci combinations representing interacting QTLs were identified by several software programs, e.g. for the % of phenolics. Significant QTL interactions were observed for 2-loci combinations: (1;7) and (1;8) and for 3-loci combinations: (1;7;8), (1;4;7), (1;4;8). Candidate gene identification is currently ongoing.

This work has been funded by the AGL2015-65246-R project of the Spanish National R+D Plan by MINECO.
Potato cyst nematodes (PCN) pose an increasing threat to the potato industry. Finding new sources of resistance and introgressing them into commercial potato cultivars is urgently required to secure current production levels and to address food security issues. The diploid potato wild species *S. spegazzini* shows resistance to *G. pallida*. It is likely that one or more resistance (R) genes are responsible for this resistance. A subset of progeny (250 clones) from a larger population of approximately 1000 clones, derived from *S. tuberosum* group Phureja cultivar Mayan Gold, were screened for resistance to *G. pallida* population Lindley Pa2/3. We identified resistant and sensitive bulks of 20 plants and performed a RenSeq (R gene enrichment and sequencing) assay. The sequence reads of resistant and susceptible plants were compared and candidate single nucleotide polymorphisms (SNPs) linked to the resistance were identified. Our data suggest the presence of a resistance gene on chromosome 11. Our current goal is to develop markers flanking the gene, to allow fine mapping on the larger population and to facilitate map based cloning of the resistance gene.
Steroidal glycoalkaloids (SGAs) are toxic specialized metabolites that are found in Solanaceae. Potato (Solanum tuberosum) contains the SGAs a-solanine and a-chaconine, which are biosynthesized from cholesterol. Several biosynthetic genes including SSR2 and two CYP genes (CYP72A188 and CYP72A208) have been identified, and the transgenic potato plants silencing these biosynthetic genes showed SGA-reduced phenotypes (1) (2). Here we demonstrate our recent strategy towards metabolic engineering of potatoes accumulating pharmaceutically useful compounds by genome editing.

CYP88B1, which is involved in a later step of the SGA biosynthetic pathway with unknown catalytic function, is co-ordinately expressed with the known SGA biosynthetic genes. We applied CRISPR/Cas9 system to knockout potato CYP88B1. The CYP88B1-knockout potatoes showed a SGA-free phenotype, and furthermore accumulated the corresponding amounts of useful steroidal saponins. These steroidal saponins are useful steroidal saponins equivalent to saponins from the tubers of Yam (Dioscorea spp.), which are valuable starting materials for synthesis of pharmaceutical steroidal drugs such as anti-inflammatory, androgenic, estrogenic, and contraceptive drugs.

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Due to anthocyanins health benefits the genes determining anthocyanin synthesis (AS) in crop species are of importance. Anthocyanin 1 is a gene encoding the main transcripational factor for specific activation of the genes encoding AS enzymes. Comparative analysis of its allelic variants in different Solanaceae crops was performed in the current study. The known tomato Anthocyanin 1 specific CAPS-marker was used to identify orthologues in Capsicum annuum and Solanum melongena. Comparison of the sequences obtained with those of different tomato species (Ant1L (Solanum lycopersicum), Ant1C (S. chilense), AntP (S. pennellii)) allowed identifying the presence of the Ant1L and Ant1C alleles within sweet pepper lines with high accumulation of anthocyanins. Ant1L differed from Ant1C by a number of single nucleotide substitutions. Within eggplant lines, Ant1L and AntP were detected. The latter differed from Ant1L by a 165 bp deletion. In potato, the Anthocyanin 1 gene is presented by the copies StAN1, StMYBA1 and StMYB113. StAN1 allelic functionality depends on a number of repeated r-motives in exon3. This polymorphism was used to develop PCR-markers for detection of functional StAN1 allele. This marker as well as the specific markers developed for StMYBA1 and StMYB113 were used for amplification of cDNA of potato samples contrasting for the pigment. Specific presence of amplicons of cDNA in colored tissues was shown for StAN1 only. The part of the study on potato genes was supported by the RSF (16-16-04073), on other crops – by the BRFFR (B 15 SO-051).
Most Solanaceae plants contain much cholesterol, but the reason is still unknown. Steroidal glycoalkaloids (SGAs) are harmful specialized metabolites that widely occur in Solanum. Potato contains the SGAs solanine and chaconine, which are accumulated in green tubers and cause distaste. SGAs are biosynthesized from cholesterol. Now we focused on sterol D24 reductase gene, SSR2 encoding a key enzyme in cholesterol and SGA production[1]. Potato has another D24 reductase gene, SSR1 homologous to SSR2, which is essential for C-24 alkylsterols production.

To disrupt only SSR2 and to decrease SGAs in potato, we designed a TAL effector nuclease (TALEN), and transformed tetraploid potatoes (Sassy, Saikai35) and a self-compatible diploid (97H32-6) with a vector with expression cassette of the TALEN. Most lines of TALEN-expressed potato transgenic plants had completely disrupted-SSR2 alleles in the genome, and no off-target was shown in SSR1 alleles. The lines were observed to demonstrate less SGAs as expected. It revealed that genome editing should be a very good way in alteration of genome in polyploidy plants.

This study was supported in part by Cross-ministerial Strategic Innovation Promotion Program (SIP).

Among the vegetables grown in Piedmont (North-West Italy), pepper (*Capsicum annuum* L.) plays a key role. Valuable ecotypes (landraces) are present in cultivation, which are morphologically recognizable and possess a certain genetic identity. They are the result of secular selections for adaptation to specific ecological niches and provide a product with organoleptic and sensorial qualities particularly appreciated by consumers. The recent availability of the *Capsicum annuum* cv. CM334 genome sequence makes possible the re-sequencing of genotypes of the species and the identification, at high precision, of allelic and structural variants.

We report on the Illumina resequencing (paired-end, 2 x 150 bp), at a coverage of ∼35X, of four previously developed breeding lines, which are representative of the main ecotypes (i.e. 'Cuneo', 'Quadrato', 'Corno' and 'Tumaticot') in cultivation in the Piedmont Region (North-West Italy). Reads were aligned to the reference genome using standard pipelines. Overall, ∼19 M SNP/Indel were shared: 16.65 M in 'Cuneo', 18.01 M in 'Quadrato', 18.07 M in 'Corno', 16.33 M in Tumaticot. The heterozygosity ranged from ∼0.2% in 'Corno' to ∼0.1% in Tumaticot. The reconstruction of the four genomic sequences at a chromosomal scale, and their structural/functional annotation is ongoing. The identified genetic variants will represent key tools for the development of diagnostic markers and to dissect the path from sequence variation to phenotype.
Solanum insanum, considered the wild progenitor of the eggplant (Solanum melongena), is an annual weed able to grow on a wide range of climatic and soil conditions including poor soils and dry areas. Biochemical analysis has also shown that S. insanum has higher total levels of bioactive phenolic metabolites of interest for human health than S. melongena. Despite all these features, S. insanum has remained largely underutilized in S. melongena breeding. The development of introgression lines (ILs) using wild species could be of great interest to eggplant breeders. For these reasons, we started the construction of a S. insanum ILs using the most recent molecular tools available. An F1 plant, derived from a cross between S. melongena and S. insanum, was backcrossed to obtain the BC1. In order to accelerate the ILs construction process, 196 BC1 plants were genotyped with 40 SNPs using a Sequenom platform. These SNPs were distributed across all the chromosomes according to the genome information. Based on the best S. melongena background recovery and good distribution of the S. insanum segments, 52 BC1 plants were selected and backcrossed again. In the BC2, 96 plants were selected and genotyped with 90 SNPs. Thirty-seven selected BC2 plants will be backcrossed in order to obtain the BC3. Following this methodology and based on the actual results we expect to start selfing selected plants in the BC3 generation, bringing forward the time frame usually needed to obtain ILs by 2 to 3 years.
P0238 DEVELOPMENT OF EGGPLANT MATERIALS WITH INTROGRESSIONS FROM SOLANUM INCANUM AND IDENTIFICATION OF CANDIDATE GENES FOR DROUGHT TOLERANCE

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1 Full text

Solanum incanum is a wild relative of eggplant (S. melongena) that grows in desertic and semi-desertic areas. Introgression materials of S. incanum in the genetic background of S. melongena can be useful for genetics and genomics studies of drought tolerance and domestication, as well as contribute to breeding new cultivars in this crop. Using a marker-assisted-selection backcross scheme, we have developed a set of advanced backcrosses (ABs) and fixed introgression lines (ILs) in eggplant. The ABs materials (from BC2 to BC5) cover 99% of the S. incanum genome, while the set of fixed ILs consists of 45 lines, each carrying a single introgressed fragment in homozygosis, covering altogether 71.7% of the S. incanum genome. The introgressed size fragment in the ILs contains between 0.1% and 10.9% of the S. incanum genome, with an average value of 3.4%. A preliminary screening for candidate genes for drought tolerance has been made to identify the most promising materials. A total of 68 candidate genomic regions containing candidate genes for drought tolerance introgressed from S. incanum have been identified in the ILs set. Currently, a subset of the ILs is being grown in two different environments, and is being phenotyped for several plant, flower and fruit traits in order to detect genes and QTLs involved to these traits. The introgression materials obtained will be extremely useful for the genetic dissection of traits of interest for eggplant breeding and will be readily incorporable into the breeding pipelines for developing new improved eggplant cultivars.
How the gender of a flower or plant is determined has practical applications in agriculture and plant breeding. Cucurbit flowers have bisexual floral primordia. Sex determination occurs by arrest of the inappropriate sexual organ growth, leading to unisexual flowers. In melon, sex determination is governed by CmACS11, CmWIP1 and CmACS-7 genes. The interplay of alleles of these genes results in a range of sexual types. The goal of this project is to unravel the global gene expression dynamics during early carpel and stamina development in hermaphrodite and unisexual flowers. Laser microdissection of carpel or stamen primordia in gynoecious (GPC, GPE) hermaphrodite (HPC, HPE) and androecious (APC, APE) flowers at bisexual stage (<800 µm) were performed with an optimized paraffin embedding protocol allowing the recovery of good quality RNA (RIN>7) and the production of illumina sequencing libraries from ultra-low RNA input. The MDL-libraries samples were validated by checking Q-PCR expression of the sex determination genes, CmWIP1 and CmACS-7. On average, 30 million reads per sample were generated and 70 % were unequivocally assigned to the Cucumis Melo 3.5.1 reference genome. Descriptive analyses, sample by sample clustering and PCA, discriminate samples by organ type (carpel or stamen) and developmental status (developing or inhibited organ). Statistical analysis using generalized linear model (GLM) comparisons highlighted: (i) 146 genes following the expression pattern of the carpel inhibition gene, CmWIP1, (ii) 108 genes expressed specifically in the developing carpel of gynoecious and hermaphrodite flower buds, (iii) 877 expressed in the inhibited stamens of the gynoecious buds and (iv) 220 genes expressed in the developing stamens of the hermaphrodite and male flower buds. Functional validation of some candidate genes, using the TILLING approach, and data mining of different “omics” data will led to the identification of new hubs in the sex determination network. We propose the use of the identified genes and the engineered alleles, as new means to improve productivity in melon, and in Cucurbitaceae in general.
P0248 GENOMIC PREDICTION FOR DRY MATTER AND CHIPPING QUALITY IN TETRAPLOID POTATO

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1 Full text

Potato is one of the most space-efficient food crops and is of vital importance for global food security. The traditional “mate and phenotype” breeding approach is costly and time-consuming; however, genomic selection using genome-wide molecular markers is becoming increasingly applicable to crops and is thus an attractive breeding alternative.

Genotyping-by-sequencing was used to genotype a large number of individuals from three panels. The main panel, the MASPOT population, consisted of 762 individuals derived from a larger population generated from biparental crosses of 18 tetraploid parents. Additionally, two test panels were established, one panel consisting of breeding clones from the same breeding station as the MASPOT population, named Test panel DK, and one panel from a breeding station in the UK, named Test panel UK.

Genomic prediction models were generated for dry matter and chipping quality. High cross-validated prediction accuracies of 0.72-0.82 were obtained for dry matter within each population, while prediction accuracies for chipping quality varied from 0.17 to 0.79. Similar prediction accuracies were obtained when combining the panels in one large training population, while across-population predictions were low or moderate.

Overall, the results suggest that genomic prediction and hence selection of breeding material can be obtained with good accuracies within tetraploid potato. Although the most optimal prediction accuracies were obtained when predicting within the same population, the results from combining training populations with genotypes from different populations suggest a promising approach for establishing a broad-application prediction model for the implementation of genomic selection in tetraploid potato breeding programmes.
P0256 INCREASE OF GAMMA-AMINOBUTYRIC ACID (GABA) CONTENT IN TOMATO RED-RIPE FRUITS BY CRISPR/CAS9

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1 Full text

Gamma-aminobutyric acid (GABA) is well known as non-proteinogenic amino acid which lowers blood pressure effectively. Tomato (Solanum lycopersicum, L) is one of the most produced vegetables in the world and widely consumed in daily diet. In addition, tomato contains relatively higher levels of GABA than other major crops. By developing a new genetic resource with even higher GABA content, further health promoting effect would be expected. Glutamate decarboxylase (GAD) is the key enzyme of GABA biosynthesis. The C-terminal region of GAD contains an autoinhibitory domain responsible for the regulation of enzyme activity. Previous studies showed that deletion of this domain increases the GAD enzyme activity. Tomato contains the five GADs (SlGAD1, SlGAD2, SlGAD3, SlGAD4, and SlGAD5). Two of five SlGADs, SlGAD2 and SlGAD3, are expressed during tomato fruit development.

To increase GABA content in tomato, we attempted to delete the autoinhibitory domain of these SlGADs with Clustered, Regularly Interspaced, Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology, which enables us to introduce the mutation on specific site of genome. Induction of stop codon just before autoinhibitory domain in the SlGADs was detected in several tomato lines. Those mutation-induced lines showed 7 to 15 times higher GABA accumulation than wild type in red-ripe fruit, and this level would be sufficient for the health-promoting in daily diet. We demonstrated that CRISPR/Cas9 is an effective tool for increases GABA content in tomato.
P0276 TOMATO BHLH TRANSCRIPTION FACTOR JA5 NEGATIVELY REGULATES JA SIGNALING BY REPRESSING MYC2-ACTIVATED GENE TRANSCRIPTION

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1 Full text

It is believed that the activation of jasmonate (JA)-signaled wounding response is required for resistance against herbivorous insects. Recently, tomato bHLH transcription factor MYC2 has been demonstrated to play a critical role in activating the JA-signaled wounding responsive gene expression. Here, we reported a negative role of another bHLH transcription factor JA5 in regulating JA-signaled wounding responsive gene expression. We found JA5 is a direct target gene of MYC2 and wounding induced the expression JA5 in a COI1- and SlMYC2-dependent manner. Detailed genetic experiments demonstrated that, JA5 is a negative regulator of JA-mediated wounding-responsive gene expression and plant defense against insect attack. Furthermore, combining sequence analysis and biochemical experiments, we conclude that JA5 can compete with MYC2 for binding to the G-box DNA motif in the promoters of their shared target gene (e.g. early wounding responsive genes), thereby inhibiting MYC2-directed activation of these genes. Together, our results support that plants have evolved to provide a fine feedback regulatory mechanism for avoiding exhausted and harmful excess JA responses upon mechanical wounding or insect attack.
C0279 EVOLUTION AND KARYOTYPE STABILITY OF ALLOTETRAPLOID CUCURBITA GENOMES

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1 Full text

The Cucurbita genus in the Cucurbitaceae family contains several economically important species. We report high-quality draft genome sequences of C. maxima and C. moschata, and provide evidence supporting an allotetraploidization event in Cucurbita. Although the diploid genome donors have not been sampled and may be extinct, we are able to partition the genome into two homoeologous subgenomes, marked by different genetic distances to the species in the Benincaseae clade, including melon, cucumber, and watermelon. We estimate that the two diploid progenitors successively diverged from Benincaseae around 30.6-32.5 and 25.9-27.4 Mya, and the hybridization between the progenitors happened earlier than 3.1-4.0 Mya, when C. maxima diverged from C. moschata. The subgenomes have presumably well maintained the chromosome structures of their diploid progenitors. Such karyotype stability after polyploidization is in contrast with many other plant polyploids, whose genomes experienced considerable rearrangements. The two subgenomes have evolved to lose similar numbers of genes with neither subgenome being globally dominant in gene expression. About 70% of the duplicated genes due to the genome merger were retained in two homoeologous copies, most of which exhibit different expression patterns. The genome sequences of C. maxima and C. moschata allowed us to perform an allele-specific gene expression analysis in the interspecific F1 hybrid widely used as rootstock, to parse the genome-wide expression differences between C. maxima and C. moschata into cis- and trans- regulatory effects, and detect transgressive gene expression changes in the hybrid correlated with heterosis in important agronomic traits.
P0308 CONSTRUCTION OF HIGH-DENSITY GENETIC MAP AND QTL ANALYSIS FOR SEED SIZE IN WATERMELON

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1 Full text

Watermelon is one of important species in cucurbit crops in the world. Commonly most people prefer seedless watermelon fruit, but the fruit bearing very small seeds can lessen the consumer’s eating inconvenience. In this study we have tried to determine QTL for seed size trait from F2 progeny of C. lanatus ssp. citroides (PI 189225) and C. lanatus ssp. lanatus (TS, Korean cultigen) producing very large and tiny in seed length, respectively. With the purpose of development high-density genetic linkage map, 165 of F2 progenies were analyzed by GBS method. Surely convinced markers were selected using optimized filtering condition, and total of 2,309 SNP loci were confirmed as co-dominant markers. When the markers were mapped to 165 F2 progenies using JoinMap4.1 software, the resulting map finely generates 11 linkage groups spanning 1,394cM with average marker interval 1.2cM. QTLs for the trait of seed size were determined using QTL Cartographer2.5 that each of two loci in chromosome 2 and 6 were supposed to be responsible for producing small and large seeds. To develop linked markers for selecting lines ranges very tiny to normal seed size in length, near-isogenic lines representing four classes of very tiny, tiny, small, and normal seed sizes were resequenced and candidate genomic regions were validated by HRM method. Further validation using development of locus-specific markers would help to breed a watermelon cultivar having target seed sizes.
In the era of the genome sequencing projects, gene functional assignment is a complex task still unfinished. Mutational analysis is one of the most efficient methods to isolate and understand gene functions. In particular, T-DNA collections allow for an easy and straightforward identification of the tagged gene, serving as the basis of both forward and reverse genetic strategies. In this work, we report the phenotypic and molecular characterization of an enhancer trap T-DNA collection generated from two commercial cultivars backgrounds of tomato (Solanum lycopersicum L.), i.e. Moneymaker and P73. More than 7800 T0 lines were generated using the pD991 binary vector. A total of 4189 T0 lines and 1858 T1 families were screened under greenhouse conditions, allowing the isolation of 522 mutants (205 dominants, 274 recessives and 43 that do not follow strict Mendelian inheritance), which were affected in plant growth and/or reproductive development. Southern blot analysis showed an average number of 2.01 ± 0.9 T-DNA insertions per T-DNA mutant. Cloning of T-DNA flanking sequences revealed that 37.7% of insertions were located in either the coding or the promoter region of annotated genes. As a proof of concept, we provide a novel gene encoding for a UTP-glucose-1-phosphate uridylyltransferase involved in programmed cell death and leaf development. In addition, new T-DNA alleles of known genes as LYRATE and MACROCALIX were also isolated, which may lead to further insights into their functions during plant development. Together, results support that our T-DNA mutant collection is a valuable resource for functional genomics in tomato.
1 Full text

Breeding tomato cultivars having both high-sugar content and high-yield quantity is still challenging. Understanding molecular mechanisms for the traits is a possible way to achieve such breeding. However, although great efforts have been made on it, few reports have reached a basic resolution so far. It may be one of the possibilities for the reason that the traits are controlled by multiple genes with minor effects on phenotypes, which is difficult to be detected by conventional genetic analysis. To overcome this situation, we employed an integrated approach of transcriptome-wide association study and expression quantitative trait locus analysis as well as genome-wide association study. For the analysis, genotyping data based on restriction-site associated DNA sequencing and low-coverage whole genome resequencing, transcriptome data by RNA sequencing, and phenotyping data including sugar contents in fruits measured with a high performance liquid chromatography were collected from recombinant-inbred lines derived from a cross between a high-sugar content Japanese F1 tomato and a high-yield quantity European hybrid. The integrated analysis would be effective for genomic dissection of agronomic traits not only in tomato but also in other plant species.
A demand for increasingly affordable tomatoes has led to breeders selecting foremost and with great success for yield. A knock-on effect is a general decrease in tomato flavor. There is now demand to improve flavor within the affordable tomato segments. Yet it remains a struggle for breeders to uncouple the negative correlation between yield and flavor using the diversity present in domesticated varieties. One approach taken over the last two decades to break this trend is to leverage natural diversity held within wild relatives through interspecific introgressions. Here we will present on the opportunities and difficulties of these approaches taken within Monsanto. Within this approach we target flavor QTLs that work in many tomato segments grown under a wide range of environmental conditions. While enabling our breeders with sources for improved flavor and MAS, we also work around the hindrances associated with the introduction of wild tomato DNA into elite material. Such pre-breeding efforts remain crucial to be able to deliver on a global scale high-yielding varieties to our customers and flavorful products to our consumers.
P0343 IDENTIFICATION OF EST-SSR MARKERS IN SNAKE MELON (CUCUMIS MELO VAR. FLEXUOSUS)

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1 Full text

The snake melon (Cucumis melo var. flexuosus), is a member of Cucurbitaceae and considered as one of the ancient horticultural crops in many parts of the world including Iran. Different accessions of snake melon are traditionally cultivated in several parts of Iran and are an important horticultural crop. The study of genetic variation leads to the production of reliable markers for the selection of varieties with better characteristics. SSR markers which derived from ESTs are more useful than genomic markers for tagging of traits, because they are the representative of the transcript and thus, directly related to the functional genes. The aim of this study was the EST-SSR identification of genes involved in cucurbitacine biosynthesis pathway with the emphasis on 1-deoxyxylulose-5-phosphate synthase, Hydroxymethylglutaryl-CoA synthase, Isopentenyldiphosphate isomerase, Mevalonate kinase, Phosphomevalonate kinase and Cucurbitadienol synthase. For this purpose, we used RNA-seq data which were available in the NCBI database and 6 EST database was examined. Generally, 9 EST-SSR markers were identified in these genes. Among them Isopentenyldiphosphate isomerase and Cucurbitadienol synthase, each had 2 EST-SSRs with repeat length 3 and 2 EST-SSRs with repeat length 4 were identified in Mevalonate kinase. Hydroxymethylglutaryl-CoA synthase has an Est-SRR with repeat length one and 1-deoxyxylulose-5-phosphate synthase and Phosphomevalonate kinase, each had an EST-SRR with repeat length 3. These predicted markers can be used for marker assisted selection and genetic analysis study in snake melon. It is also provides a valuable source for genetic analysis of some genera of Cucurbitaceae.
P0373 BIOCONTROL ACTIVITY OF NONPATHOGENIC MUTANTS OF FUSARIUM OXYSPORUM AGAINST THE PARENTAL WILD-TYPE STRAIN

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1 Full text

Fusarium oxysporum is a soil-borne fungal pathogen that causes vascular wilts in Solanaceae and Cucurbitaceae plants. Preinoculation of nonpathogenic F. oxysporum strains often protect plants against Fusarium wilt disease, however, the genetic and physiological characteristics of effective strains remain largely undefined. We assessed the biocontrol activities of nonpathogenic mutants of F. oxysporum f. sp. melonis (melon pathogen) generated by disruption of known pathogenicity genes. The FOW2 gene encodes a fungal-specific Zn(II)2Cys6-type transcriptional regulator, which is required for the invasion of the root tissue, but is dispensable for vegetative growth and the use of carbon sources. Hyphae of the ΔFOW2 mutant were developed and colonized on the root surface as same as the wild-type strain. Melon plants preinoculated with the ΔFOW2 mutant markedly reduced the development of symptoms caused by its parental strain in an inoculum concentration–dependent manner. Conidial germination and hyphal elongation of the wild-type strain on the root surface were significantly inhibited when the ΔFOW2 mutant was colonized nearby. Competition for carbon sources in soil, as represented by a reduction in vegetative growth of pathogen in the presence of the nonpathogenic F. oxysporum, was observed to be an important mechanism of action for ΔFOW2 mutant. It also showed similar biocontrol effects against other soil-borne pathogens. ΔFOW2 mutant derived from F. oxysporum f. sp. lycopersici (tomato pathogen) demonstrated control of Fusarium wilt disease in tomato plants. These results suggested that the FOW2-deficient mutant competed with the wild-type strain for nutrients and niches.
The control of developmental transitions is important for plants to ensure reproductive success. Understanding the regulatory networks is crucial for further improving the agricultural productivity of crop species. The knowledge gained from research in Arabidopsis thaliana serves as a basis to investigate pathways and regulators controlling developmental transitions in other species such as potato (Solanum tuberosum). The trehalose 6-phosphate (T6P) pathway has been shown to regulate flowering time at two sites of signal perception in Arabidopsis. In the leaves it is required to induce FLOWERING LOCUS T, whereas at the shoot apical meristem it affects the age pathway by reducing miRNA156 levels and by inducing SQUAMOSA-PROMOTER BINDING LIKE PROTEINS 3, 4 and 5 expression. T6P is a small sugar phosphate shown to reflect the sucrose status in plants. In Arabidopsis T6P is synthesized by Trehalose Phosphate Synthase 1 (AtTPS1). The potato genome encodes two TPS1 orthologues - StTPS1A and StTPS1B. StTPS1A expression exceeds that of StTPS1B and its transcript is present throughout floral transition and tuberization. T6P levels are highest in young developing leaves and significantly increase during the floral transition and tuberization. Overexpression of AtTPS1 in potato leads to a reduction in tuber yield, early flowering and more complex leaves. This phenotype corresponds with reduced levels of miR156. Our data supports, that the T6P pathway regulates the transition from the juvenile to the adult phase in potato and acts upstream of the age pathway to regulate flowering and tuberization.
Optimal timing of the floral transition is one of the major determinants of crop productivity and yield. Flowering regulation integrates diverse environmental and internal factors. Common regulators have been found to also control the stolon-to-tuber developmental transition in Solanum tuberosum ssp. andigena. In both developmental processes a balanced nitrogen (N) status of the plant is crucial. Interestingly, nitrate, the major source of N in soil not only serves as an important macronutrient but also acts as a signalling molecule affecting plant's developmental transitions. Here, we studied flowering time and tuber induction of five different S. tuberosum varieties grown using a previously described soil-based N-limited system. By comparing flowering time, tuber yield and quality traits of plants grown in N-limited and optimal N conditions the varieties were classified in N-sensitive and N-insensitive. These potato cultivars are interesting candidates to determine molecular traits predicting performance under N-limited conditions. We aim at determining predictive traits for potato productivity and yield under N-limited conditions by performing whole genome transcriptome analyses and metabolite profiling using these opposite N-responsive varieties. Furthermore, using the knowledge from the model plant Arabidopsis, specific N-dependent regulators are characterized in more detail during potato development.
The spatio-temporal regulation of leaf formation from the shoot apical meristem (SAM) is a crucial factor to determine plant architecture and crop yield in vegetable species as tomato (Solanum lycopersicum L.). In this work, we describe the recessive tomato T-DNA mutant defective shoot apical meristem 1 (dam1) that displayed abnormalities in vegetative development. Homozygous dam1 plants germinated but their growth was early arrested after developing one to two pairs of small leaves with fewer leaflets, which leads to a significant reduction in plant height and leaf area. Furthermore, dam1 mutants exhibited alterations in the typical spiral phyllotaxis displayed by tomato wild-type plants; thus, dam1 leaves were arranged following an opposite pattern instead of a spiral one. Analysis of the genomic sequences flanking the T-DNA insertion shown that it was located in the second intron of a gene coding for a WRKY-type transcription factor. A co-segregation analysis was performed using allele-specific primers designed from both the T-DNA and the genomic regions flanking T-DNA sequences, which revealed that the T-DNA insertion co-segregated with the dam1 mutant phenotype. Functional analyses, currently in progress, will demonstrate whether phenotypic alterations detected in dam1 plants are caused by the loss-of-function of the DAM1 gene, and will help to understand the role of this WRKY transcription factor during SAM development and the establishment of the leaf phyllotaxis in tomato.
P0312 THE TOMATO 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE (DXS1) IS REQUIRED FOR PROPER PLANT DEVELOPMENT AND GROWTH

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1 Full text

The tomato (Solanum lycopersicum L.) 1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS1) catalyses the first step of the 2-C-methyl-D-erythritol-4-Phosphate (MEP) pathway playing a fundamental role during fruit carotenoid biosynthesis. With the aim to elucidate the functional role of DXS1 during plant development, this study reports the isolation and molecular characterization of the tomato white lethal seedling-2297 (wls-2297), a T-DNA mutan affected in DXS1 gene. After seed germination, seedlings of the wls-2297 mutant expanded albino cotyledons, which were not able to develop true leaves resulting in premature lethality. Cloning of DNA genomic fragments flanking the T-DNA sequences followed by a co-segregation analysis showed that T-DNA integration caused a 38.6 kb-deletion, which affected the DXS1 and three PEROXIDASE (POX) genes. Functional analyses of DXS1 and POX RNAi-silencing lines, as well as both DXS1 overexpression on the wls-2297 mutant and in vivo complementation assays with 1-Deoxy-D-xylulose-5-phosphate (DXP), demonstrated that the wls-2297 mutant phenotype is due to a knock-out mutation of the DXS1 gene. Further characterization of genes involved in the MEP pathway revealed different expression profiles in the DXS1 silencing lines compared to wild-type plants, which indicated a role for DXS1 in transcriptional regulation of the first steps of the MEP pathway. Overall, these results support that DXS1 plays other important roles besides that proposed during fruit carotenoid biosynthesis; thus, DXS1 is required at early developmental stages for the growth and survival of tomato plants.
OS06: REPRODUCTIVE DEVELOPMENT I

P0170 HORMONAL AND TRANSCRIPTOME PROFILING OF TOMATO FLOWER AND EARLY FRUIT DEVELOPMENT

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1 Full text

Hormones play crucial regulatory role in the majority of developmental processes in plants. In our research for the first time the transcriptome profiling for hormone-related genes identification was combined with hormonal metabolome analysis in order to reveal, which components of hormonal system are involved into natural processes of tomato cv. M82 reproductive organs development and to determine the possible interactions between these components. For the transcriptome analysis the Illumina mRNA sequencing was performed. For hormonal profiling physiologically active hormones as well as their precursors and catabolites were purified and fractionated by SPE and detected by UPLC-ESI-MS/MS. The measurements were conducted at different stages of flower (3, 5, 7 and 10 mm of flower bud length and anthesis) and early fruit (5, 7, 10 and 15 DPA) development and covered flower organs (sepals, petals, stamen, pollen, carpels) and fruit ones – (ovary, pericarp+peel, jelly, placenta, seeds), respectively. To strengthen the evidences of hormone interactions the different hormones deficient tomato mutants were included into analysis. Both RNAseq and hormone content data revealed spatially and temporally differential and complex hormonal expression throughout flower and early fruit development in tomato. The obtained results enrich the knowledge about the role of specific hormones across the developmental process of tomato reproductive organs. Based on the received data the hormone homeostasis can be modified in tomato plants using transgenic approach aiming to improve plant tolerance to abiotic stress. The first step in this direction was performed creating tomato overexpressing lines and mutants on auxin and jasmonate conjugating GH3 genes.
P0184 POTENTIAL ROLE OF RECEPTOR PHOSPHORYLATION IN ETHYLENE SIGNALING IN TOMATO FRUIT

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1 Full text

The plant hormone ethylene is perceived by a membrane-associated receptor family which is similar to the bacterial two-component histidine kinase receptors. Since ethylene receptors negatively regulate the signaling, the suppression is canceled upon ethylene binding, permitting responses including fruit ripening. Although receptors have autophosphorylation activity, the mechanism whereby signal transduction occurs has not been fully elucidated. We demonstrate that SlETR4, an important receptor for tomato (Solanum lycopersicum) fruit ripening, is multiply phosphorylated in vivo and the phosphorylation level is dependent on ripening stage and ethylene action. Although only phosphorylated isotypes were detected in immature and mature green fruits, the non-phosphorylated isotype appeared after ripening started. Furthermore, treatment of preclimacteric fruits with ethylene resulted in accumulation of SlETR4 with reduced phosphorylation while treatments of ripening fruits with ethylene antagonists, 1-methylcyclopropene and 2,5-norbornadiene, induced accumulation of the phosphorylated isotypes. A similar phosphorylation pattern was also found for Never ripe (SlETR3), another ripening-related receptor. Alteration in the phosphorylation state of receptors is likely to be an initial response upon ethylene binding since treatments with ethylene and 1-methylcyclopropene rapidly influenced the phosphorylation state. The SlETR4 phosphorylation state was closely related to ripening progress, suggesting that the phosphorylation state of receptors is implicated in the ethylene signal output in the fruits. This receptor phosphorylation potentially play a role as a regulator for the interaction with downstream components in ethylene signal transduction.
Gametogenesis is an important process during reproductive development in plants. After the formation of the gametes, the pollination and the successful fertilization of the ovules are a requirement for the formation of most types of fruits. However, in tomato plants the formation of fruits can be uncoupled from the fertilization process and some male sterile genotypes are able to develop seedless fruits. In previous work from the lab we isolated the tomato hydra mutant, a sterile mutant that produces seedless fruits (Rojas-Gracia et al. 2017). HYDRA gene (the ortholog of the SPOROCYTELESS/NOOZLE gene from Arabidopsis) is involved in the initiation of the sporogenesis and the control of fruit set in tomato. Quantification of endogenous hormones in hydra plants showed little changes compared to wild-type plants. However expression analyses showed changes in the expression pattern of genes involved in auxin biosynthesis and transport. To further analyze the role of HYD/SlSPL in the control of fruit initiation we generated tomato plants that overexpressed this gene. The analysis of the overexpressing plants provides information on the hormonal control of reproductive development in this crop.

Rojas-Gracia et al. 2007. New Phytologist 214: 1198-1212
Jasmonates are important signalling compounds in plants. The female sterile phenotype of the tomato jasmonate-insensitive (jai1-1) mutant demonstrates their function in flower development. Among differentially expressed genes in ovules, a gene encoding a MYB-transcription factor was found showing high transcript levels in wild type and almost no expression in jai1-1. It is orthologous to AtMYB21, which is known to be involved in jasmonate-regulated stamen development in Arabidopsis. To test whether the tomato MYB21 is a functional orthologue of AtMYB21, SlMYB21 was expressed in Arabidopsis myb21-5 plants under control of the AtMYB21 promoter. Molecular characterization of SlMYB21 was done by analysis of its subcellular localization using GFP fusions expressed in tobacco protoplasts. SlMYB21 showed transcriptional activity in yeast. Moreover, its putative interaction with 14 tomato JAZ proteins was tested using Yeast-two-Hybrid approach and bimolecular fluorescence complementation in planta. For functional characterization of SlMYB21 in tomato, a knock-out of the encoding gene in wild type should test whether SlMYB21 does function on ovule development. Indeed, Slmyb21 knock-out mutants created by a CRISPR/Cas9 approach exhibited defects in flower opening and did not set seeds. SlMYB21 might function in driving down-stream genes, which are important for proper development of tomato ovules. An RNAseq approach comparing the transcriptome of myb21, jai1-1 and wild type carpels is envisaged to identify putative MYB21 target genes.
P0318 UNDERSTANDING THE GENETIC BASIS OF FRUIT ABSCISSION IN THE TOMATO FRD MUTANT

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Early fruit abscission prior to harvesting is of agronomic concern because it reduces crop yield, yet the presence of an abscission zone is also a requirement for harvesting and marketing of tomatoes for the fresh market. The identification of \textit{JOINTLESS} (j), \textit{MACROCALYX} (MC), \textit{JOINTLESS-2} (j-2), \textit{LATERAL SUPPRESSOR} (LS) and \textit{SEPELLATA} (SEP; SIMBP21) genes in tomato has facilitated our understanding of the regulation of AZ formation. To date, various hormone signalling genes, including \textit{Sl-IAA3}, Aux/IAA1 and 2, \textit{ARF1,2,7} and 19, \textit{SIERF52}, \textit{ETR1} and 2, and \textit{EIN3} have been reported to contribute to the activation of abscission process; however, the key gene(s) regulating this process are still not defined. Previous studies have been based on mutants with delayed abscission or those that lacked AZ, but we have discovered a novel tomato mutant that shows an earlier and more severe abscission. This mutant, named \textit{fruit-drop} (frd), exhibits an increased rate of separation at the AZ during fruit ripening and a brown septum colouration and calyx dehydration. The mutation was spontaneous in the Ailsa Craig genetic background during a transgenic experiment. Further backcross with Micro-Tom (MT) and its genetic characterisation indicates that \textit{frd} is a single recessive gene. The aim of this project is to identify the \textit{frd} gene by genetic mapping using bulk segregant NGS and transgenesis of candidate genes. We will also develop methodology to measure and understand the genetic variation in the degree of abscission that is present in the germplasm used in tomato breeding programs.
P0324 INSIGHTS ON PARTHENOCARPY: A POSSIBLE “ADAPTIVE” ROLE OF
SEEDLESS FRUITS AND HYBRIDITY AS A FORCE DRIVING DEPARTURES FROM
NORMAL SEXUAL PLANT REPRODUCTION

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1 Full text

Seedless fruits represent an apparent biological paradox because they do not contribute to offspring production. Their occurrence in Angiosperms was investigated by a bibliographic survey distinguishing monospermic from plurispermic species and wild from cultivated taxa. Out of 96 “parthenocarpic” taxa, 63% belonged to the plurispermic fruit category. Of these, cultivated species were about six-fold the number of wild species, suggesting a selective pressure for parthenocarpy during their domestication and breeding. In monospermic taxa, the number of wild and cultivated species was approximately the same.

The occurrence of parthenocarpy in wild species suggests that seedlessness may have an adaptive role. In monospermic species, empty fruits are claimed to reduce the probability of seed predation through deceptive mechanisms. In multispermic fruit species, parthenocarpy may exert an adaptive advantage under suboptimal pollination regimes, when flowers set very few embryos, possibly not enough to support fruit growth. Parthenocarpy will then offer the opportunity to accomplish the production and dispersal of few seeds, thus representing a selective advantage.

About 20 sources of seedlessness has been described in tomato in the early and recent literature. Excluding the parthenocarpic fruit (pat) mutation, obtained by EMS, all the other sources emerged after wide (interspecific) hybridization schemes. Following a theory postulated for apomictic species, wide hybridization may be the cause of disruption of synchrony in time and space of reproductive events, from sporogenesis to fruit development, driving the autonomous development of the ovary before fertilization has taken place.
P0340 A LOSS-OF-FUNCTION MUTATION AT THE TOMATO AGAMOUS1 (TAG1) GENE PROVIDES NOVEL INSIGHTS INTO THE FUNCTIONAL ROLE OF TAG1 DURING FLORAL ONTOGENY

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1 Full text

TOMATO AGAMOUS1 (TAG1) and ARLEQUIN/TOMATO AGAMOUS LIKE1 (TAGL1) are members of the tomato (Solanum lycopersicum L.) AGAMOUS clade of MADS-box encoding transcription factors. TAG1 play essential functions during flower development and TAGL1 is required for fruit ripening. TAG1 has been proposed as a functional orthologous of Arabidopsis AGAMOUS gene, controlling stamen and carpel identity, as well as floral determinacy. However, the lack of knock-out mutants has hindered so far a detailed understanding of the TAG1 function. In this study, we report the characterization of the first stable loss-of-function tag1 mutant, which represent a great chance to go in depth into the comprehensive analysis of the functional and genetic interactions of TAG1 during floral ontogeny. As expected, tag1 knock-out mutants exhibited homeotic transformations of the third and fourth floral whorls which are partially converted in petals and sepals, respectively. Furthermore, petal organs were observed inside fruit-like structures developed by tag1 mutant plants indicating the loss of floral meristem determinacy. Molecular characterization of tag1 mutant plants revealed a single splice site mutation, which results in alternative splicing event leading to a non-functional TAG1 protein. In addition, synergistic effects on both floral determinacy and stamen and carpel identities were found in double mutant plants affected in TAG1 and TAGL1 functions. Further characterization of double mutant plants provide a better understanding of TAG1 and TAGL1 functional redundancy and diversification.
OS07: REPRODUCTIVE DEVELOPMENT II

P0241 DOWN-REGULATION OF HISTONE DEACETYLASE SLHDA19 AFFECTS FRUIT RIPENING AND SEED DEVELOPMENT IN TOMATO

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1 Full text

Histone post-translational modifications (HPTMs) are recognized as playing crucial roles in the regulation of plant reproduction. In particular, a RPD3/HDA1-class histone deacetylase Athda19 mutant was reported to induce several defects in Arabidopsis such as reduced fertility, shorter siliques, and aborted seeds. The main goal of our work is to functionally characterize SlHDA19 in tomato (Solanum lycopersicum), an important crop and model species for fruit biology. Artificial miRNA approach was used to down-regulate SlHDA19 (Solyc09g091440) in tomato cv. Ailsa Craig. Remarkably, two independent transgenic lines, Slhda19-2 and Slhda19-6, show an early ripening phenotype associated with an increase in the ethylene production. Moreover, these lines display smaller fruits and an early accumulation of the principal carotenoids. Fruit from both lines display a predominance of undeveloped seeds, as has been shown in Arabidopsis siliques, indicated that seed set is also compromised. Currently, cyto-histological analysis of ovules/seeds at different stages post-anthesis to characterize embryo development in Slhda19 is ongoing.
Fruit development is regulated by coordinated changes in gene expression that can be strongly influenced by environmental stresses. In particular, drought can have a profound effect on many aspects of fruit biology and substantially reduce crop yield by suppressing reproductive development. However, most molecular studies of plant responses to drought have focused on vegetative organs, such as roots and leaves, and far less is known about the molecular bases of drought responses in fruit. Although some studies have suggested that fleshy fruits undergo changes in gene expression and metabolite profiles in response to water stress, such observations have yet to be supported by comprehensive transcriptomic analyses. More importantly, as fruits are complex organs comprised of various tissue types, tissue-specific transcriptome responses to water limitation need to be examined in order to obtain a full picture of the molecular mechanisms governing fruit development under drought conditions.

We have performed a tissue-specific transcriptome analysis of tomato (Solanum lycopersicum) fruit growth and ripening under different water stress treatments. A comparison of transcriptome changes in response to water stress between fruit and vegetative tissues allowed us to identify fruit specific responses to drought. The tissue-specific data sets have been used to construct a map of tissue-specific transcriptome responses to drought stress with emphasis in transcription factors, hormone biosynthesis and signaling, and primary and secondary metabolite pathways. We are using this spatially resolved fruit transcriptome data to highlight gene sets uniquely expressed in response to water stress and to predict gene regulatory networks controlling fruit development under water limitation.
The SUN gene, responsible for an elongated fruit shape in tomato, belongs to the IQ domain (IQD) gene family, which is involved in the growth and development of plants. In the present study, IQD gene homologs were evaluated for their roles in determining the fruit shape in cucurbit crops. A total of 151 IQD homologs and their chromosomal locations in Arabidopsis, tomato, and three cucurbit species—watermelon, melon, and cucumber—were searched based on their genomic information. A phylogenetic dendrogram of these IQD homologs showed that previously reported candidate fruit shape IQD genes in watermelon (ClSUN8), melon (CmSUN14), and cucumber (CsSUN2) were clustered under the same node with a high similarity coefficient. The comparison of the physical locations of IQD homologs with the fruit shape QTLs in genetic maps indicated that ClSUN8, CmSUN14, and CsSUN2 were co-localized with the major QTLs for the fruit shape index (FSI). Histological analysis of immature fruits indicated that elongated or round fruit shape is determined at the early stage of ovary formation, at least before −4 days after fertilization (DAF) and fruit elongation is due to increased cell division in the longitudinal direction. Quantitative RT-PCR showed that the highest gene expression of ClSUN8 occurred at −4 DAF and gradually decreased; however, there was no direct relationship between the gene expression level and fruit shape. A non-synonymous SNP was revealed between the ClSUN8 alleles of elongated and round watermelon accessions using RACE-PCR, suggesting that the SNP might be a causative mutation affecting the fruit shape variation.
P0211 THE MOLECULAR NATURE OF YELLOW-FLESH ALLELE R2997 AND ITS ASSOCIATION WITH THE EPISTASIS OF TANGERINE OVER YELLOW-FLESH IN TOMATO FRUIT

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1 Full text

During tomato fruit ripening, transcription of the gene Phytoene synthase 1 (Psy1), which codes for the first enzyme in the carotenoid biosynthesis pathway, increases 10-20 folds, leading to lycopene accumulation in the ripe fruit. Previous work in our laboratory has shown that the recessive mutation yellow-flesh LA2997 (r²⁹⁹⁷) eliminates the transcription of Psy1 in fruits. In the recessive tomato mutation tangerine, fruit color changes from red to orange due to the accumulation of tetra-cis lycopene (‘prolycopene’) as a result of a mutation in the carotene isomerase gene CrtISO. It was established over 60 years ago that tangerine is epistatic to r²⁹⁹⁷. Such epistasis is not trivial since PSY1 functions upstream to CRTISO in the biosynthesis pathway and it is expected that a block in PSY1 would be epistatic to a mutation in CRTISO. We have discovered that in a genetic background of tangerine (alleles t³⁰⁰² or t³⁴⁰⁶), transcription of PSY1 in r²⁹⁹⁷ was partially restored (Kachanovsky et al. PNAS, 2012). However, the cause of this phenomenon was unexplained since the molecular nature of the r²⁹⁹⁷ mutation has remained elusive. Here we show that the mutation in r²⁹⁹⁷ is caused by an insertion of 5 kb DNA sequence highly similar to a retrotransposon in the 5’ region of the coding sequence of PSY1. Consequently, a short non-functional transcript is produced in the mutant. Our results indicate that alternative splicing that occurs in the double mutant r²⁹⁹⁷/tangerine leads to the synthesis of a shorter yet functional PSY1, thus partially recovering phytoene synthesis.
In plant breeding of fleshy fruit crops, quality traits are a high priority. In particular, in melon, long shelf life (LSL) is a major component that strongly segmented the market. Melon fruit ripening behavior can be characterized by two main mechanisms: climacteric and non-climacteric genotypes, respectively depending on the occurrence or not of an autocatalytic ethylene synthesis increase. While the physiological basis of ethylene production in melon has been addressed in several publications, the genetic determinism of climacteric or non-climacteric fruit development is poorly understood. Indeed, this trait was mainly described by a complex inheritance involving several QTLs.

Recently, a new flower sex type, never observed in melon, has been obtained silencing the expression of the CmACS11 gene: a unique plant producing only male flower (Boualem et al., 2015). ACS genes are known as involved in the ethylene biosynthesis and therefore the regulation of ACS genes expression plays a major role in fruit ripening. However, there was no clear evidence about how CmACS gene can affect melon fruit shelf life.

In this context, our study presents how we succeeded in using the melon androecious plants for a synchronized production of long shelf life melon fruits. This is the first report of the simple genetic control by the CmACS11 gene of LSL fruit development.
A WIDE VARIATION IN FRUIT QUALITY AND SHELF LIFE TRAITS IDENTIFIED IN THE POOL OF TRADITIONAL VARIETIES FROM GREECE

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1 Full text

Among the objectives of the EU project TRADITOM was to assess the available genotypic and phenotypic variability present in the tomato genetic resources in the participating counties. Within this context, the phenotypic, agronomic and postharvest characteristics of 133 Greek tomato accessions were monitored in a greenhouse located in the Southern town of Tympaki. The first round of evaluation resulted in the selection of 15 short shelf life (SSL) and 15 long shelf life (LSL) accessions varying in color, shape and size. Next, these 30 accessions were assessed for postharvest performance and ethylene production rates and upon analysis of the data by PCA and Heat Map protocols, three LSL genotypes showed very good performance for two of the three traits (fruit decay, firmness, weight loss). Among SSL, one genotype show excellent performance (slow ripening rate) and six good performance. In terms of the effect of moderate salinity treatments on selected genotypes, the results indicated that the stressed accessions exhibited lower fruit weight but higher soluble solids content and firmness. Finally, the application of 1-MCP on two LSL accessions at the breaker stage was evaluated in storage at two temperatures (12 and 20°C). All parameters studied (color, weight, firmness, chemical composition, respiration and ethylene production rates) suggested that 1-MCP extended the time to reach red stage by 25-70%. (This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634561-TRADITOM).
OS09: SPECIALIZED CELLS and THEIR METABOLISM

P0368 JASMONATE MEDIATES STEROIDAL ALKALOIDS METABOLISM IN TOMATO

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1 Full text

Steroidal alkaloids and their glycosylated forms (SGAs) are specialized metabolites produced predominantly by members of the Solanaceae and Liliaceae plant families, serving as a chemical defense against a broad range of pests and pathogens. In previous work, we revealed that the control of SGAs biosynthesis in tomato is associated with SlMYC2 expression, a key component of the jasmonate (JA) signaling pathway (Cardenas et al., 2016). In this study, we observed that Methyl Jasmonate (MeJA) application significantly enhanced the level of α-tomatine, the main SGA in tomato. Notably, such response was not triggered in the jai mutant background, corresponding to COI, a known activator of JA signaling. Additionally, leaves of the jai1 (jasmonic acid-insensitive1) mutants lines exhibited downregulated expression of several SGA biosynthetic genes. In parallel, we used a transactivation assay and found that SlMYC2 was capable to activate the promoter of JASMONATE ZIM-DOMAIN (JAZ) genes (SlJAZ1 and SlJAZ2), the core component(s) of JA signaling. SlJAZ1 overexpression significantly reduced SGAs accumulation, while its downregulation resulted in increased SGAs abundance. Finally, we found that pathogen infection reduced the transcript level of sterol and alkaloid biosynthetic genes, which might be due to specific fungal effector(s). The results presented here provide the mechanistic insights to association between SGA biosynthesis and JA signaling. We are currently attempting to decipher the interaction between the tomato COI1-JAZ-MYC2 JA pathway proteins and discovering the direct link between JA signaling (and biosynthesis) and SGA accumulation.
OS10: PLANT-ENVIRONMENT INTERACTION

P0034 REGULATORY DIVERGENCE IN WOUND-RESPONSIVE GENE EXPRESSION IN DOMESTICATED AND WILD TOMATO

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1 Full text

The evolution of cis- and trans-regulatory components of transcription is central to how stress response and tolerance differ across species. However, it remains largely unknown how divergence in TF binding specificity and cis-regulatory sites contribute to the divergence of stress-responsive gene expression between wild and domesticated species. Using tomato as model, we analyzed the transcriptional profile of wound-responsive genes in wild Solanum pennellii and domesticated S. lycopersicum. We found that extensive expression divergence of wound-responsive genes is associated with speciation. To assess the degree of trans-regulatory divergence between these two species, 342 and 267 putative cis-regulatory elements (pCREs) in S. lycopersicum and S. pennellii, respectively, were identified that were predictive of wound-induced gene expression. We found that 35-66% of pCREs were conserved across species, suggesting that the remaining proportion (34-65%) of pCREs are species specific. This finding indicates a substantially higher degree of trans-regulatory divergence between these two plant species, which diverged ~3-7 million years ago, compared to that observed in mouse and human, which diverged ~100 million years ago. In addition, differences in pCRE sites were significantly associated with differences in wound-responsive gene expression between wild and domesticated tomato orthologs, suggesting the presence of substantial cis-regulatory divergence. Our study provides new insights into the mechanistic basis of how the transcriptional response to wounding is regulated and, importantly, the contribution of cis- and trans-regulatory components to variation in wound-responsive gene expression during species domestication.
P0106 NATURAL VARIATION OF REPRODUCTIVE ORGAN MORPHOLOGY IN 3 PETUNIA SPECIES

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1 Full text

Many angiosperms depend on animal-mediated pollination for their reproduction. Thus, flowers evolved to adapt to specific pollinator guilds in order to optimize pollination success, promoting reproductive isolation and eventually speciation. Flower color, scent and reproductive organ morphology are floral traits under strong selective pressure due to floral visitors. While advertising traits such as scent and color are now quite well-characterized, there is relatively few knowledge about the genes involved in the natural variation of floral morphology which may be important for pollinator attraction but also pollination efficiency.

Petunia genus present a great diversity of flower morphology which is associated with specific pollinators. We are using 3 different Petunia species with singular type of flower to identify genes underlying morphological differences. Petunia exserta has an exserted stigma, a star-like shape and a bright red corolla and therefore displays a typical hummingbird syndrome. P. axillaris axillaris and P. axillaris parodi display inserted stigma and white flowers but differ mainly by the length of their tube (as well as the reproductive organs).

The fine characterization of bud growth in those species as well as in the 3 reciprocal F1s led us to target informative developmental stages of tube and pistil for RNAseq experiments. My presentation will discuss the common and specific transcriptional features set up during pistil development. The combination of this transcriptional profiling with quantitative genetics approaches and DNAseq information allow the identification of a few candidate genes which may underlie morphological differences between species.
Salinity is a major environmental stress limiting tomato production in arid and semi-arid conditions due to irrigation. Although cultivated tomato is quite sensitive to salinity, several of its wild relatives exhibit halophyte properties. *Solanum chilense* is spontaneously present in salt-affected areas of North Chile but the physiological basis of its salt-resistance has received only minor attention so far. Comparing the behaviour of the cultivated glycophyte *Solanum lycopersicum* with its wild-relative halophyte *Solanum chilense* will help to unravel the strategies of plant response to salt stress. A holistic approach was used to compare the hormonal profile of *Solanum lycopersicum* and *Solanum chilense* plants subjected to 125 mM NaCl for 7 days. *Solanum chilense* displayed a contrasting behaviour comparatively to *Solanum lycopersicum*, not only for plant growth, mineral nutrition, osmotic adjustment but also regarding phytohormonal profiling. Salinity improved plant growth in *Solanum chilense*. This species behaved as an includer species accumulating higher concentration of Na+ in the shoot than *Solanum lycopersicum*, which exhibited an excluder behaviour. Moreover, the capacity to use inorganic ions as osmotica improved salt resistance in *Solanum chilense* and phytohormones could be involved in this process. Most hormonal compounds were higher in *Solanum chilense* than in *Solanum lycopersicum*. Interestingly, salicylic acid, ethylene and cytokinins were positively correlated with osmotic potential in *Solanum chilense* under salinity while these hormones were negatively correlated with osmotic adjustment in *Solanum lycopersicum*. This study revealed that both species respond differently to salinity and that involvement of phytohormones in this resistance was species specific.
P0125 DIFFERENCES IN LEAF TRICHOME DENSITY DETERMINED THE CHANGES OF WATER-USE EFFICIENCY IN SOLANUM PENNELLII INTROGRESSION LINES

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1 Full text

A role for trichomes has been suggested to fulfil a series of physiological functions, including resistance to biotic and abiotic stresses, and especially in terms of tolerance to drought stress. Nevertheless, further experiments are needed to confirm the relationship between trichomes and drought tolerance. The aim of this work was to study the effect of differences in trichome density on the response to water stress in a population of S. lycopersicum x S. pennellii introgression lines (ILs) under field conditions. For this purpose, three selected ILs (4-1, 10-2, 11-3) and their cultivated parental line Solanum lycopersicum cv. M82 were grown under two different water regimes, well-watered (WW, soil water content maintained to 100% field capacity) and water-stress (WS, soil water content reduced to 50% field capacity). Leaf trichome and stomatal density, plant morphology, photosynthetic traits and biomass measurements were performed in all plants. Differences were found in trichome density among the different ILs in WW conditions, and differences increased when cultivated under WS. Also, differences in the trichome-stomata ratio were found among ILs, either cultivated under WW or WS conditions. Negative correlation between stomatal density and trichome density was observed when cultivated under water-stress conditions. Thus, the ILs with higher trichome density had higher water-use efficiency, estimated as intrinsic water-use efficiency (WUEi, as $A_n/g_s$) or biomass water-use efficiency (WUEB, as biomass/water consumed). Hence, preliminary results indicate a possible adaptation in trichome density in some ILs under water-stress that led to a higher WUE.
Global changes in climate are having an important effect on horticultural crops. High temperature is one of the critical factors affecting tomato production and yield. For that reason, the search of new genetic resources with good performance under harsh temperature conditions and the identification of regions involved in tolerance to high temperatures in this species are the main objectives addressed in this work.

For the screening of genetic resources, a set of 143 accessions from *Solanum lycopersicon* including traditional, modern and cerasiforme varieties, 84 accessions of *S. pimpinellifolium* and two mapping populations derived from the cross between a *S. lycopersicum* cv. MoneyMaker x *S. pimpinellifolium* acc. To-937 (168 RILs and 53 ILs) were grown in a glasshouse under three temperature regimens. Plants grew sequentially at 25/20°C; 30/25°C and 35/30°C (day/night) during 4 weeks for each temperature regimen and were phenotyped for traits related with flower number, fruit set and pollen functionality among others. Based on their superior performance under high temperatures, genotypes from the different genetic groups analyzed were selected and tagged as promising genetic resource to be included in breeding programs. Moreover, the QTL analysis in mapping populations allowed the identification of genomic regions involved in the tolerance to high temperatures in terms of capacity of set fruits and pollen viability.
Wild tomatoes (Solanum sect. Lycopersicon, sect. Lycopersicoides, sect. Juglandifolia) have been an important source for biotic resistances to improve tomato crop. Further, adaptation of some of such species to stressful habitats has provided abiotic stress tolerance to the tomato crop, especially related to fruit quality improvement (e.g., salinity tolerance). Contrary to many biotic resistance traits, controlled by one or a few genes, targeting the genetic basis controlling abiotic stress tolerance traits is generally difficult. An alternative is to disentangle mechanistic, morpho-physiological determinants of such tolerance, allowing the use of particular traits as “markers” in future breeding programs to improve tomato plant tolerance. In last decades, there is growing interest in improving tomato plant efficiency in water use (“more crop per drop”). Our study of the tomato wild relatives has highlighted an array of particular plant adaptations related to relative growth rate, biomass allocation, leaf hydraulics and venation, and photosynthetic traits, providing a framework of plant traits to consider in future breeding programs focused in improving plant adaptation to more water-restrictive cultivation practices in tomato crop.

MiRNAs are a class of small (20-24 nts in length) non-coding RNAs widely distributed in Eukariota. Functionally, miRNAs regulate gene expression at the post-transcriptional level mediating cleavage or translational repression of their mRNA target. In plants, only a few annotated MIRNA gene families are highly conserved from mosses to higher flowering plants, while the majorities are family- and/or specie-specific. Currently, the general believe is that both miRNAs type, may coordinately contribute to the regulation of the stress response in plants. Stress-responsive miRNAs have been described in plants exposed to both biotic stress and abiotic stress. However, studies conducting systematic analyses of stress-responsive miRNAs during controlled exposure to various adverse conditions at identical plant-developmental stages are lacking. Conducting such studies is, however, key to infer robust regulatory networks of response to global stress modulated by miRNAs. Here, we analyze by high-throughput sequencing the miRNAs response profiles in melon (Cucumis melo L.) plants exposed to diverse abiotic (cold, drought, salinity and short-day) and biotic (viroid infection, Agrobacterium infiltration and Ascomycete root infection) stress conditions. The comparison of the miRNA response networks under the different conditions made it possible infer a network of stress-associated miRNA with potential to regulate a global stress response in melon plants. Remarkably, we found similar network architectures in stress-responsive miRNAs from different phylogenetically unrelated plants, such as maize and rice, suggesting that our network model for miRNAs response in melon may constitute a general phenomenon common to monocotyledonous and dicotyledonous species.
P0169 A RELATIONSHIP BETWEEN WATER DEFICIENCY AND FRUIT CUTICLE STRUCTURE, TRANSPRATIONAL LOSS AND FIRMNESS IN DIVERSE TOMATO GENOTYPES

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1 Full text

The softening of fleshy fruits, such as tomato (Solanum lycopersicum), is a complex process involving substantial cell wall metabolism and intercellular separation. However, transpirational water loss and changes in cellular water status are also important and, in this regard, a role for the fruit cuticle in determining fruit firmness was also suggested by studies of the delayed fruit deterioration (DFD) tomato genotype. DFD fruit exhibit remarkably extended shelf life and maintain internal and external quality for many months after ripening. One study has suggested that the long shelf life trait of DFD is promoted by growing the plants under limited water availability (water stress growing conditions; WSGC). However, the relationship between watering regime, cuticle properties, and effects on fruit quality is unclear. We observed that WSGC resulted in increased fruit firmness, enhanced cuticle thickness, higher cutin and wax load, and the up-regulation of cuticle biosynthetic genes in fruit of the normally softening tomato cultivar, Ailsa Craig. In addition, cuticle permeability and fruit transpiration rate were substantially reduced, as was the frequency of microbial infection. DFD fruit did not show such changes in cuticle properties or composition in response to WSGC, but we observed that DFD cuticles were 'pre-adapted' to minimize fruit deterioration under those conditions. We conclude that limiting water availability during tomato cultivation affects fruit cuticle biosynthesis and metabolism, thereby reducing cuticle permeability, diminishing transpirational water loss and extending shelf life. This study provides insights into the adaptation of tomato genotypes in environments where water is scarce.
P0172 HYPOXIA STRESS MEMORY IN TOMATO (SOLANUM LYPERSICON L.):  
WHOLE-GENOME TRANSCRIPTOME ANALYSIS  
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1 Full text  
Plants require water for growth but excessive water negatively affects their productivity. Due to limited diffusion of gas under water, low oxygen (hypoxia) environment in the root area is created. Some plants show improved tolerance to limited oxygen conditions when were subjected to preliminary period of acclimation to hypoxia. In the studies the hypothesis was evaluated: Plants that experience a period of hypoxia stress and then recover, during second exposure to hypoxia, plants ‘remember’ past hypoxia experience, allowing them to get better resistance. For that, the transcriptome respond of tomato roots to waterlogging were examined by RNA-Seq. The root hypoxia of two tomato genotypes cultivated in the greenhouse condition was introduced. The duration of hypoxia was 7 days and then plants were recovered for 14 days and another 7 days hypoxia treatment were established. Analysis of differentially expressed genes (DEGs) in tolerant and sensitive to hypoxia genotypes presented different transcript modes. After first hypoxia treatment, a total of 1978 and 1094 DEGs were detected in the hypoxia-sensitive and the hypoxia-tolerant genotypes, respectively. Repeatedly stressed plants displayed increase about 27% and 69% of DEGs in the hypoxia-sensitive and the hypoxia-tolerant genotypes. Pathway and gene ontology term enrichment analysis highlighted DEGs exclusive to each of genotypes and present after single or double hypoxia treatments. Results help to understand molecular mechanism of hypoxia tolerance in tomato and effect of hypoxic pre-treatment on improving tolerance to prolong hypoxia.  
Research was financed by the Ministry of Agriculture and Rural Development of the Republic of Poland
Glandular trichomes are structures involved in the production of many food-, medicine-, and agriculture-relevant chemicals. In some wild species related to the cultivated tomato (Solanum lycopersicum) the presence of type-IV glandular trichomes is associated with high levels of the natural insecticide acyl-sugar and resistance to whitefly (Bemisia tabaci). Recently, our group discovered that type-IV trichomes, previously described as absent in the cultivated tomato, are actually present on embryonic (cotyledons) and juvenile leaves of several tomato cultivars. This indicates that type-IV trichome formation is a marker of juvenility and that genes controlling the presence of this trichome in wild species might be related to heterochrony (evolutionary alterations in the timing of developmental processes). An analysis of potential genes involved in these structures was carried out using lines of the wild species S. galapagense introgressed into S. lycopersicum cv. Micro-Tom (MT). The MT near-isogenic line harboring type-IV trichomes in adult leaves was denominated Galapagos enhanced trichomes (MT-Get). MT-Get plants are smaller and more branched when compared to MT. The Get locus is being mapped using the mapping-by-sequencing approach, which consists of genetic mapping combined with whole-genome sequencing in order to accelerate identification of mutation frequencies in phenotypical groups. Ultimately, the identification of the gene(s) related to this trait will increase our understanding of the molecular basis of glandular trichome development, besides being a useful tool for obtaining new resistant varieties with reduced use of pesticides.
P0174 LOSS-OF-FUNCTION RBOHF TRANSGENIC LINES PRODUCTION FOR SALT TOLERANCE ANALYSIS IN TOMATO (SOLANUM LYCOPERSICUM CV. MICRO-TOM)

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1 Full text

Soil salinity affects crop production worldwide. This soil condition can become even more severe due to the combination of global warming (meaning more salt concentration by increased evapotranspiration) and intensification of land use, and irrigation practices, in the upcoming years. Salt stress affects yield by both slowing vegetative growth and impairing reproductive development. The protein RbohF is a NADPH oxidase essential for Casparian strip formation in roots and for establishing salt tolerance. The model tomato plant cultivar Micro-Tom (MT), has a small size, a short life cycle and has been successfully used in studies involving the production of transgenic lines for physiological and genetic analysis. This work aimed to produce loss-of-function transgenic MT lines to unravel the contribution of the protein RbohF in tomato salt stress tolerance. Transgenic plants were produced in vitro using the *Agrobacterium tumefaciens* system. Cotyledons used as explant were placed with the abaxial side down onto solid Root Inducing Medium (RIM - 0.4 µM NAA). Two drops of *Agrobacterium* suspension in liquid MS (containing the vectors) were applied per explant. After two days of cultivation in the dark, explants were transferred to Shoot Inducing Medium (SIM - 5 µM BAP), supplemented with kanamycin for in vitro selection of transgenic lines. Well-developed shoots were transferred to flasks to elongate and to form roots. Rooted T0 plantlets were transferred to the greenhouse for acclimatization and their T1 seeds were harvested. The phenotypes observed in hemizygous and homozygous T1 lines will be discussed.
P0215 DIFFERENTIAL RESPONSE OF GRAFTED PEPPERS DEPENDING ON PHOSPHORUS CONCENTRATION AND GENOTYPE

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1 Full text

Agricultural practices are provoking a fast reduction of natural resources (i.e. available mineral nutrients in soils). Phosphorus is one of the most important nutrients for plant growth and cannot be substituted by other alternative resources. As consequence, an improved acquisition and use of phosphorus by plants is of relevant importance. A possible way to improve this situation is by grafting commercial plants onto efficient rootstocks to phosphorus acquisition. In this experiment we evaluated the physiological behavior of two accessions of Capsicum annuum (R1 and R2) under two P concentrations (0.5 and 1.5 mM, named LP and HP respectively), used as rootstocks, and compared with an ungrafted commercial variety of pepper (A). Physiological parameters (biomass, photosynthesis and phosphorus concentration) were measured as sensitive parameters in seedling plants, 28 days after the beginning of the treatments. Considering the results, A/R2 had a high efficiency in P uptake by roots under HP; nevertheless, P concentration in leaves did not show significant differences between plants combinations. Large quantities of P could be retained in roots in A/R2, expressed with the highest root dry weight but not in aerial dry weight or CO₂ assimilation. Under LP, P in roots and leaves did not show significant differences, even A/R1 which could maintain root biomass and photosynthesis following A plants. Consequently, we could conclude that R1 was more adapted, being a suitable tolerant rootstock to be used in future experiments of productivity and quality of pepper fruits. This assay was framed in the INIA project RTA2013-00022-C02.
Utilization of transcription factors (TF) as a tool to generate improved crops with specific traits is an effective strategy that relays on the potential of TFs to regulate the expression of multiple genes involved in a particular pathway. Recently, Cycling Dof Factor 3 (CDF3) has been reported in Arabidopsis to participate in the regulation of flowering time and abiotic stress responses. Here we compare the effects of CDF3 genes in Arabidopsis and tomato on growth and nitrogen and carbon metabolism under different abiotic stress conditions.

AtCDF3 and SlCDF3 overexpression in Arabidopsis enhances tolerance of transgenic plants to drought, cold and osmotic stress and promotes late flowering, whereas AtCDF3/SlCDF3 overexpression in tomato enhances tolerance of transgenic plants to salt stress and biomass production and yield. Transcriptome analysis of Arabidopsis and tomato lines revealed that CDF3 regulates a similar set of genes involved in redox homeostasis, nitrogen assimilation, photosynthesis performance and primary metabolism. Consistently, metabolomic profiling showed that CDF3 induced changes in primary metabolism, triggering enhanced nitrogen assimilation, and disclosed that the amount of some protective metabolites including sucrose, GABA and asparagine were higher in vegetative tissues of tomato and Arabidopsis CDF3 overexpressing plants. Interestingly the overexpression of AtCDF3/SlCDF3 genes in tomato modified organic acid and sugar content in fruits, improving variables related to flavor perception and fruit quality. Overall, our results highlight the utility of CDF transcription factors in engineering C/N metabolism in plants and their use to improve plant yield under unfavorable environmental conditions.
Broomrape or broom-rape (*Orobanche*) is a parasitic plant, annual and herbaceous flowering. It has no leaf and Chlorophyll, therefore it can not produce required nutrients for itself. It takes them from the host plant root. Several species of it belong to Orobanche genus and the family of Orobancheae. So far 30 species have been identified in Iran and one of them is *Orobanche aegyptica* that has many hosts in the most regions of north eastern of Iran (north khorasan) such as potato, tomato, eggplant, melon and cucumber. In order to evaluate the amount of damages caused and managing and controlling the ways of broomrape in the fields of cucumber and tomato in North Khorasan province, this study was conducted during 2011-2014. Treatments(ways) included: physical and mechanical removal, soil solarization, booby stimulus plants, tolerant cultivars, planting date. The most important factors for determining the control methods were contamination, type of crop plant, farm Fertility and the size of farm. Although late planting was useful for reducing of the Orobanche population, but yield had high decrease. The result showed that physical and mechanical removal of parasitism and soil solarization were the best way for reducing of the Orobanche seed in the small farms. In the poor soil, using of organic fertilizer was suggested. Booby stimulus plant such as sorghum and sunflower was the best methods in big farms.
P0304 ENZYMES INVOLVED IN THE BIOSYNTHESIS OF STERYL ESTERS IN SOLANUM LYCOPERSICUM
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1 Full text

Phytosterols are integral components of plant membranes that modulate membrane fluidity and permeability, and play essential roles in modulating growth and development and the response to different stresses. Phytosterols are found in free form (FS) and conjugated as esters (SE), glycosides (SG) and acylglycosides (ASG). Tomato and other Solanaceae show an atypical content of conjugated sterols. However, the biological significance of this peculiar sterol metabolism is unknown and the knowledge about the enzymes involved in the synthesis of conjugated sterols is still very limited. Among these the acyltransferases are responsible for the synthesis of SE, which play a crucial role regulating the concentration of FS in the membranes. The only plant genes involved in SE synthesis characterized so far are the Arabidopsis genes encoding an acyl-CoA:sterol acyltransferase (ASAT1) and a phospholipid:sterol acyltransferase (PSAT1). In tomato (S. lycopersicum cv. Micro-Tom), we have identified a single gene coding for PSAT (SlPSAT) and 8 candidate genes coding for a putative ASATs (SlASAT1-8). These genes are differentially expressed in tomato organs and during fruit development and ripening, as well as in response to different exogenous stimuli (abscisic acid, salicylic acid, methyl jasmonate and flagellin) and stresses (osmotic, salt, cold, wounding). Functional complementation of the A. thaliana null mutants psat1-1 and asat1-1 has revealed that SlPSAT and SlASAT1 are the tomato orthologues of AtPSAT1 and AtASAT1, respectively. Studies aimed at understanding the specific role of SlPSAT and SlASAT1 in tomato plant growth and development, fruit ripening and their response to stress are currently under way.
Sterol glycosyltransferases (SGTs) catalyze the glycosylation of the free hydroxyl group at C-3 position of sterols to produce glycosylated sterols, which are primarily located in cell membranes where in combination with other membrane-bound lipids play a key role in modulating their properties and functioning. In contrast to most plant species, those of the genus Solanum contain high levels of glycosylated sterols, which in the case of tomato may account for more than 85% of the total sterol content. We report the identification and functional characterization of the four members of the tomato (Solanum lycopersicum cv. Micro-Tom) SGT gene family. Expression of recombinant SlSGT proteins in E. coli cells and N. benthamiana leaves demonstrated the ability of the four enzymes to glycosylate different sterol species including cholesterol, brassicasterol, campesterol, stigmasterol, and β-sitosterol. Subcellular localization studies based on fluorescence recovery after photobleaching and cell fractionation analyses revealed that the four tomato SGTs, localize into the cytosol and the PM, although there are clear differences in their relative distribution between these two cell fractions. The SlSGT genes have specialized but still largely overlapping expression patterns in different organs of tomato plants and throughout the different stages of fruit development. Moreover, they are differentially regulated in response to biotic and abiotic stress. This study contributes to broaden the current knowledge on plant SGTs and provides support to the view that tomato SGTs play overlapping but not completely redundant biological functions involved in mediating developmental and stress responses.
A collection of tomato T-DNA insertional mutants generated by using an enhancer trap T-DNA system was screened under salt and water stress conditions in order to identify novel genes involved in the plant responses to these abiotic stress responses. In these screenings the recessive res (restored cell structure by salinity) mutant showed morphological alterations and cellular disorganization under control conditions, but all these alterations were restored when res mutant plants were exposed to salt stress. Additionally, chlorosis of mutant leaves also disappeared when mutant plants grew at high temperatures and low relative humidity. Genetic analyses proved that res mutation was not due to a T-DNA insertion but it was of somaclonal origin. The RES gene has been cloned by a mapping-by-sequencing approach, which allowed us to demonstrate that the res phenotype is caused by an hypomorphic allele of a tomato gene coding for a DEAD-box RNA helicase. Silencing of the DEAD-box RNA helicase gene phenocopied the res mutant phenotype, whereas its overexpression is able to complement the mutation, demonstrating that the RES gene is responsible for the mutant phenotype. The molecular function of the RES DEAD-box protein has been investigated and a possible molecular mechanism to explain the recovery of the res mutant phenotype under abiotic stress conditions is discussed.
WANTED: DEAD OR ALIVE. A STUDY OF EXOCARP FORMATION IN KIWIFRUIT

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1 Full text

The berry fruit of Actinidia species (kiwifruit) is covered by an exocarp or ‘skin’. There are two distinct exocarp phenotypes found within Actinidia those that have epidermal exocarp ‘live skin’ and those that have a peridermal exocarp ‘dead skin’. The morphological development of the two exocarp types have been previously reported1, however little is known about the molecular factors involved in exocarp development. This project aims to characterise the exocarp phenotypes at the genetic and transcriptomic level. An RNA sequencing study is being undertaken to identify patterns of gene expression involved in periderm formation by comparing kiwifruit with an epidermal exocarp to those with a peridermal exocarp aligned to exocarp developmental changes. Gene changes will be compared to other plants that produce periderms such as potato tuber skin and russet pipfruit. In order to identify the control points of exocarp formation a genotyping by sequencing (GBS) study on a population that segregates for exocarp type is planned. Preliminary results suggest periderm formation in kiwifruit is a natural component of development with a cork meristem forming beneath the epidermis. This meristem produces thin walled cells that are suberized and compressed cells as the fruit matures. The difference between periderms formed by wounding or development will be further characterised in kiwifruit, with analysis of intermediate phenotyped russeted fruit found in the segregating population.

References
miRNAs are small, non-protein-coding RNAs that regulate gene expression post-transcriptionally. Strigolactones (SL) are phytohormones acting as ecological and developmental communicators, and involved in the responses to abiotic stress. In tomato (Solanum lycopersicum L.), the current model places a drought-triggered decrease of SL synthesis in roots upstream of a reduced SL flow shootward and of increased SL synthesis in above-ground organs. Perception of shoot-produced SL leads in turn to higher ABA sensitivity and to lower transpiration, thus increasing drought tolerance. A SL-miRNA cross-talk under drought is suggested, among other lines of evidence, by such role of SL in drought and by the drought inducibility of several miRNAs, among which miR156. We therefore investigated a SL-miR156 relationship in tomato. The results obtained so far indicate that exogenous SL are sufficient for the accumulation of mature miR156 in unstressed leaves. The comparison of WT and SL-depleted plants, both self- and hetero-grafted, shows that SL synthesis in the shoot, but not in the root, is needed for the accumulation of mature miR156 in both organs under drought; while SL synthesis in the roots is needed for drought-induced pri-miR156 transcript accumulation in the shoot. Additionally, shoot-synthesized SL seem to inhibit pri-miR156 transcription in the absence of stress; and under all conditions, to promote miR156 maturation - a process whose efficiency towards specific miRNAs is sensitive to cellular context and thus, possibly, to hormones. Plants overexpressing miR156 are being used to investigate whether miR156 is a mediator of SL-dependent water stress responses in tomato.
Cuscuta spp (dodders) use their vine-like stems to attach to aboveground parts of host plants, develop haustoria, invade and extend inside the host stem. High-throughput RNAseq data identified Cuscuta genes expressing highly in haustorial tissues, was compared to the high expressed genes in Striga haustoria tissues, and used to identify genes that have been co-opted to regulate haustorium formation across clades. Similar genes were found to play key roles in the Striga and Cuscuta parasitism processes. We generated transgenic tomato with hairpin RNAs that target key C. pentagona genes upon initial haustorial attachment. C. pentagona growing on these transgenic tomatoes transited to flowering earlier than C. pentagona growing on wild type, suggesting growth under stress due to insufficient nutrient acquisition from the host.

At the host end, RNAseq was also used to identify tomato genes involved in the response to attachment by C. pentagona in resistant and susceptible tomato varieties. We found upregulation of signaling proteins, transcription factors (TFs), and lignin biosynthetic genes. VIGS (virus induced gene silencing) of selected genes in resistant lines causes loss of resistance. Over-expression of the signaling and TF genes in susceptible tomato led to upregulation of lignin biosynthetic genes and lignin accumulation in stem cortex. Future experiments will fully characterize both the key genes in the parasite using HIGS and the host resistance mechanism using whole genome sequencing of resistant cultivars coupled with stable transgenic modulation of key host genes.
OS11: PLANT INTERACTION WITH OTHER ORGANISMS

P0018 TOLERANCE TO VIRUS INFECTION IN PLANTS RESEMBLES MUTUALISM AND IS CONDITIONED BY CHANGES IN PLANT SMALL RNA AND HORMONAL SIGNALING PATHWAYS

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1 Full text

Potato virus Y (PVY) is the most economically important potato viral pathogen worldwide. We aimed at unraveling the roles of small RNAs (sRNAs) in the complex immune signaling network controlling the establishment of tolerant response of potato cv. Désirée to the virus. We identified and quantified the endogenous miRNAs and phasiRNAs as well as virus-derived sRNAs. We constructed a sRNA regulatory network connecting sRNAs and their targets identified to link sRNA level responses to physiological processes. We discovered an interesting novel sRNAs – gibberellin (GA) regulatory circuit being activated as early as 3 days post inoculation, before viral multiplication can be detected. Increased levels of miR167 and phasiRNA931 were reflected in decreased levels of transcripts involved in GA biosynthesis. Moreover, decreased concentration of GA confirmed this regulation. The functional relation between lower activity of GA signaling and reduced disease severity was previously confirmed in Arabidopsis – CMV interaction using knockout mutants. We have additionally showed that this regulation is salicylic acid dependent as the response of sRNA network was attenuated in salicylic acid-depleted transgenic counterpart NahG-Désirée expressing severe disease symptoms. Moreover, we found that differentially expressed miR6022, which targets leucine-rich-repeat receptor-like kinases, likely links GA signaling with immune responses. Besides downregulation of GA signaling, regulation of several other parts of sRNA network in tolerant Désirée revealed striking similarities to responses observed in mutualistic symbiotic interactions. The intertwining of different regulatory networks revealed here shows how developmental signaling, symptomology and stress signaling are balanced.
Plants show sequential immune responses after sensing of pathogen patterns and effectors to induce pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively. MAPK signaling is known to play central roles in both immunities, facilitating robust MAPK activity during ETI accompanied by cell death. A spatiotemporal understanding of activation profiles of MAPK during PTI and ETI remains largely unknown due to the difficulty in analysis of MAPK activities in living cells. To observe the MAPK activity at the single cell and organelle levels, we established live-imaging system of MAPK activity by producing fluorescence resonance energy transfer (FRET)-based biosensor (MAPK sensor). The MAPK sensor was highly responsive to SIPK, a tobacco defense-related MAPK, in vivo. We confirmed that SIPK translocated to nucleus from cytoplasm in response to pathogen signals using GFP-tagged SIPK driven by the own promoter. Therefore, MAPK sensors were located in cytoplasm or nucleus to monitor SIPK activities in different subcellular compartments using subcellular localization signals. These two MAPK sensors were transiently expressed in Nicotiana benthamiana leaves, and ratio images of the FRET were observed under the microscope. During PTI signaling, FRET-based fluorescence was transiently seen in cytoplasm and nucleus. On the other hand, ETI signals induced the fluorescence intensely in cytoplasm and nucleus.
Clavibacter michiganensis subsp. michiganensis (Cmm) is considered the most severe bacterial pathogen affecting tomato. The major cause of outbreak is mainly through infected seeds. As for that, it is a quarantine organism in Europe and in many countries. The major challenges of performing disease screenings of Cmm in Europe are the strict regulations. This results in inoculations in small and expensive quarantine compartments. This process is not efficient for large scale disease screening, thus we developed a new protocol to do the disease screening in vitro. Inoculation was done on the susceptible and tolerant genotypes, the Solanum lycopersicum cv. Moneymaker and S. arcanum LA2157, respectively. Different variables were used, the plant age and inoculation methods, to determine the best approach of doing the in vitro inoculation. A new phenotyping approach using the PathoScreen™ system to determine the GFP-tagged Cmm in planta and to quantify the pathogen based on the percentage of corrected GFP (cGFP%) was also used. The system is highly sensitive in detecting the GFP-signal in the shoots compared to the microscopy technique. The best in vitro inoculation procedure was further tested on other tomato wild relatives to determine the correlation between symptom score in vitro and in the greenhouse. The correlation was moderate and seems to be genotype specific, but the protocol worked well in differentiating the two parents that we use in our breeding program. In conclusion, the in vitro inoculation procedure can be efficiently used for disease screening in a large scale replacing the greenhouse screening.
P0086 CHARACTERIZATION OF RESISTANCE TO CMV AGGRESSIVE STRAIN FNY IN MELON

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1 Full text

Cucumber mosaic virus (CMV), belonging to the genus Cucumovirus, can infect and cause significant loss in crop production in more than 1000 plant species including Solanaceae, Cucurbitaceae and Cruciferae. CMV can be divided into two subgroups, subgroup I and subgroup II. The melon accession PI161375, cultivar "Songhan Charmi" was reported to be resistant to most of CMV strains. The major gene, cmv1, confers total resistance to CMV subgroup II strains by preventing viral transport from the bundle sheath cells, surrounding the vein, to the phloem. Two other QTLs (qwcmv3.1 and qwcmv10.1) have been detected that cooperate with cmv1 conferring resistance against some strains in subgroup I, like M6. However, other strains of the same subgroup, like FNY, can still overcome the resistance, which means that there is still unknown QTL(s) playing important roles in resistance.

Using a set of introgression lines with different QTL combinations we found that the resistance is acting at the level of transport rather than replication. Melon lines with different QTL combinations showed a delay in symptoms appearance compared to the susceptible accession ‘Piel de Sapo’. Furthermore, our results indicate that the QTLs confer resistance to FNY by cooperating with cmv1 in restricting the transport of the virus from the bundle sheath cells to the phloem, rather than being involved in virus transport within the phloem.

We are also searching for the determinant of virulence of CMV responsible for overcoming the resistance conferred by three QTLs (cmv1, qwcmv3.1 and qwcmv10.1).
P0094 MORE THAN JUST PRICKLES: INVESTIGATING THE ANTI-PATHOGEN RESPONSES OF THE SPINY SOLANUM, S. SISYMBRIIFOLIUM

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1 Full text

Solanum sisymbriifolium, also known as “Litchi Tomato” or “Sticky Nightshade,” is an undomesticated plant species related to potato and tomato. This plant has been under investigation in both Europe and N. America as a trap-crop that can induce hatching of eggs of the cyst nematode, Globodera pallida, but then, by a yet unknown process, restricts further development after the larva have invaded plant roots. An initial estimation of genome size of S. sisymbriifolium has been calculated to be 4.7 pg, which is approximately the same size as a tetraploid potato. To reduce the complexity of future hunts for useful genes within this assembly, we elected to construct a high quality de novo transcriptome using single molecule real time (SMRT) sequencing, performing an in-silico combination and deredundification of individually sequenced bud, root, stem and leaf RNAs. The analysis of this transcriptome, specifically the RUBISCO small and large subunit genes, supports a previous study that indicated that the chloroplast and nuclear genomes have different evolutionary origins. In addition, a single-nucleotide polymorphism (SNP) analysis indicated that this species has undergone a recent genome duplication and re-diploidization. These past events may have contributed to this plant’s ability to mount a robust defense response against pathogens, varying from Agrobacterium tumefaciens to cyst-forming nematodes. We are currently conducting gene expression studies using in-silico and ex-silico methods using plants treated with pathogens, wounding, and plant signaling molecules. Understanding the components of this response could vastly increase the amount of available resources for genetic defenses in agricultural crops.
P0120 ANALYSES OF GENE EXPRESSION CHANGES IN CUCUMIS SATIVUS L. PLANTS IN RESPONSE TO PHYTOHORMONES AND FEEDING DAMAGE BY THRIPS PALMI.

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Full text

Melon thrips (*Thrips palmi*) is one of the important herbivores that causes serious damages on many agricultural plants including Cucumber (*Cucumis sativus* L.) and transmits Tospoviruses, such as Melon yellow spot virus (MYSV). Therefore, both feeding damage and viral disease are serious problems in many countries including Japan. Previous study showed that Jasmonic acid (JA) is essential roles in plant defense against thrips in the interaction between Crucifers and western flower thrips (*Frankliniella occidentalis*). However, it is still unknown whether this mechanism is common in the other interaction such as Cucurbitaceous plant and *T. palmi*. Although the draft genome sequence of the cucumber has been reported in 2009, transcriptome profiles about plant response against microorganism and harbivore were poorly understood. In this meeting, we introduce the results of gene expression analysis of cucumber against phytohormones (Ethylene, JA and Salicylic acid) using RNA-sequence analysis. We also introduce gene expression changes in *C. sativus* against feeding damage by melon thrips.
P0176 EXPLOITING WILD TOMATO GENETIC DIVERSITY FOR RESISTANCE AGAINST TOMATO YELLOW LEAF CURL DISEASE

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1 Full text

Tomato yellow leaf curl disease (TYLCD) is a viral disease caused by a cluster of Tomato yellow leaf curl virus (TYLCV) like viral species. This disease has become a worldwide constraint for tomato (Solanum lycopersicum) production. Using of the host resistance is the most effective method for controlling TYLCD. So far, a limited number of resistance genes (Ty-1 to Ty-6) has been identified in wild tomato relatives including S. chilense, S. habrochaites, and S. peruvianum. The diversity of the gene pool in wild tomato species allows us to identify and characterise novel resistance sources against TYLCV. Our objective was to screen a large collection of wild tomato species for their phenotypic responses to TYLCV infection. We screened germplasm collections including S. arcanum, S. cheesmaniae, S. chilense, S. chmielewskii, S. corneliomulleri, S. habrochaites, S. huaylasense, S. lycopersicoides, S. neorickii, S. pennelli, S. peruvianum, and S. pimpinellifolium. Various TYLCV resistant accessions corresponding to different wild tomato species have been identified that stay symptom-free throughout the testing period. They include mainly S. arcanum, S. chilense, S. corneliomulleri, and S. peruvianum accessions. Understanding the genetic basis of the newly identified resistant accessions is in progress. Mapping populations derived from some resistant accessions have been obtained that will be used to map and identify the underlying genes.
P0183 IDENTIFICATION AND CHARACTERIZATION OF A TOMATO SALICYLATE HYDROXYLASE INVOLVED IN SENESCENCE AND PLANT DEFENCE

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1 Full text

The plant defensive response involves significant changes in levels of plant hormones, among which salicylic acid (SA; 2-hydroxybenzoic acid) is considered as the signal molecule involved in avirulent infections. Although SA biosynthesis is well known, the pathways by which SA is catabolized remain unclear. Our laboratory identified gentisic acid (GA; 2,5-dihydroxybenzoic acid), a metabolic derivative of SA, as a signal molecule complementary to SA in virulent infection. SA conversion to GA should be mediated by a salicylate hydroxylase (SH) enzyme to catalyze the 5-hydroxylation of the aromatic ring.

In Arabidopsis thaliana, the S3H enzyme converts SA to both 2,3-dihydroxybenzoic acid (2,3-DHBA) and GA in vitro. Arabidopsis s3h mutants fail to produce 2,3-DHBA sugar conjugates, accumulate very high levels of SA and its sugar conjugates, and exhibit a precocious senescence phenotype.

In this work, we have identified the putative S3H tomato orthologous (Sl_Sh; Solanum lycopersicum salicylate hydroxylase). The study of the in vivo activity by overexpressing Sl_Sh in Nicotiana benthamina plants showed an outstanding reduction of SA levels, thus confirming its salicylate hydroxylase activity. RNAi transgenic tomato plants silencing Sl_Sh have been generated. Similar to Arabidopsis, these tomato plants displayed an accelerated senescence phenotype. We have infected the tomato Sl_Sh RNAi plants with the bacteria Pseudomonas syringae pv. tomato, and their levels of phenolics as well as the pathogen content have been analyzed, confirming the role of Sl_Sh in plant defence.
Plant viruses selectively use their host factors, such as eukaryotic translation initiation factors (eIFs), for their life-cycle. A loss-of-function of eIFs confers resistance to viral diseases. Cultivated tobacco (Nicotiana tabacum) is an amphidiploid plant with a complicated genome derived from two ancestors: N. sylvestris and N. tomentosiformis. In tobacco, N. sylvestris-derived eIF4E2 (eIF4E2-S) is a susceptibility factor for potato virus Y (PVY, the genus Potyvirus). Deficient eIF4E2-S confers resistance to PVY. This study investigated the relation between several tobacco eIFs and plant pathogenic viruses. We first generated RNAi tobacco plants for eIF4E1 and eIF(iso)4E. A virus inoculation test showed that eIF(iso)4E-down-regulated tobacco showed resistance (reduced susceptibility) to both a PVY strain that breaks eIF4E2-based resistance (PVY-RB) and tobacco bushy top virus (TBTV; genus Umbravirus). To identify either or both homeologous eIF(iso)4E genes involved in resistance, we selected tobacco plants with a non-sense mutation for either eIF(iso)4E-S (derived from N. sylvestris) or eIF(iso)4E-T (derived from N. tomentosiformis) genes and those for both homeologous genes using DNA markers we developed. Inoculation assay revealed that eIF(iso)4E-S and eIF(iso)4E-T-double mutant showed resistance to both PVY-RB and TBTV, whereas eIF(iso)4E-S mutant and eIF(iso)4E-T mutant respectively showed resistance to TBTV and PVY-RB. Results suggest that PVY-RB uses eIF(iso)4E-T, whereas TBTV uses eIF(iso)4E-S in their infection. This report is the first of a demonstration that homeologous genes for plant eukaryotic translation initiation factor are involved separately in susceptibility to viruses belonging to different genera.
P0204 PLANT-MEDIATED EFFECTS OF WATER DEFICIT ON THE PERFORMANCE OF TETRANYHUS EVANSI ON TOMATO DROUGHT-ADAPTED ACCESIONS

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1 Full text

The increase of drought periods expected by climate change is increasing the interest on studying the response to water deficit of drought-adapted tomato varieties. Furthermore, climate change is also expected to increase the performance and dispersal of some invasive species like Tetranychus evansi, which has been reported to take advantage of the nutritional changes induced by water-shortage on the tomato cultivar Moneymaker. We have examined the implications for mite’s biology of four accessions of the drought-adapted tomatoes, ‘Tomàtiga de Ramellet’ (TR), under moderate drought stress. Mite performance was enhanced by drought in two accessions (TR61 and TR154) though at lower levels that those found in Moneymaker, but not in the other two accessions (TR58 and TR126). Analysis of the plant nutritional parameters of sugars and amino acids show that on TR154 and Moneymaker plants, the essential amino acids were significantly induced whereas they were not altered in TR126. Moreover, gene expression and biochemical analysis of plant defense response indicate that the ability of T. evansi to suppress tomato plant defense response was reduced on the two TR accessions analyzed (TR126 and TR154), which might partially explain the lower performance of T. evansi on these plants, as compared with Moneymaker.

These results provide insights for the adoption of drought-adapted tomato varieties and emphasize the importance of studying plant biotic and abiotic stress factors in combination.
P0257 ESTERS OF (Z)-3-HEXENOL AS NEW CROP SHIELDS

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1 Full text
In response to stress signals, plants synthesize defence proteins and chemical compounds of diverse nature. Among others, volatile organic compounds (VOCs) belong to this defensive compounds group.

Using a non-targeted GC-MS metabolomics approach, we have identified the VOC profiles that are associated to the differential immune response of “Rio Grande” tomato leaves infected either with virulent or avirulent strains of Pseudomonas syringae DC3000 pv. tomato. The VOC profile of tomato leaves infected with the avirulent bacteria includes esters of (Z)-3-hexenol with acetic, propionic, isobutyric or butyric acid, defining the profile of an immunized plant response.

To confirm the defensive role of these esters, their direct defensive properties (antibacterial and/or antioxidants) were determined. Besides, exogenous treatments of tomato plants with these VOCs were performed to study the activation of the plant defence, by analyzing different aspects such as the modification of the resistance or susceptibility, the accumulation of signaling and defensive compounds or the induction of defensive proteins.

Our studies reinforce the importance of VOCs as components of the plant defensive response against pathogens. As a possible biotechnological application, transgenic plants emitting these compounds in a constitutive manner, may be used as inducers of resistance for their neighboring plants. Alternatively, these VOCs could be directly sprayed on the plants in order to confer resistance. In fact, a patent application for the (Z)-3-hexenyl-butyrate has been presented in the Spanish Patent and Trademark Office (P201730685).
P0294 AMMONIUM NUTRITION CHANGES C/N METABOLISM AND INDUCES RESISTANCE AGAINST PSEUDOMONAS SYRINGAE

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Plants take up inorganic nitrogen (N) available in the soil or nutrient solution in form of NO3- or NH4+. NH4+ assimilation requires less energy than NO3- assimilation, however, only a few species grow well when NH4+ is the only N source (Marschner, 2012). Elevated abundance of this cation is toxic for plants but sublethal concentrations can lead to mild stress potentially enhancing defense responses against another stress. Previous studies have shown that NH4+ nutrition induces resistance in tomato and citrus plants against Pseudomonas syringae pv tomato DC3000 and tolerance against salt stress, respectively (Fernandez-Crespo et al., 2012 and Fernandez-Crespo et al., 2015).

To study the influence of NH4+ nutrition on primary metabolism, we have analyzed N- and C-derived compounds, particularly amino acids and organic acids, as some of these have been shown to participate in stress responses. The results show that primary metabolism is altered under ammonium nutrition and we suppose that some of these amino acids might have an important action in induced resistance in tomato plants against the pathogen Pseudomonas syringae. Conversely, organic acids do not seem to be involved in biotic stress resistance although they change under ammonium nutrition.

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P0311 DEEP SEQUENCING REVEALS A COMPLEX SMALL RNA-MEDIATED ANTIVIRAL DEFENSE IN SOLANUM LYCOPERSICUM - POTATO VIRUS Y INTERACTIONS

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1 Full text

An aggressive isolate of Potato virus Y (PVY), named PVY(C)-to, induced on infected Solanum lycopersicum (tomato cv. UC82) plants a stunted phenotype with leaf distortions. We investigated by deep sequencing the nature and relative levels of endogenous small RNAs (siRNAs) at two different timepoints, 21 and 30 days post-infection (dpi).

Relative abundance of host siRNAs, and their distribution in different size classes, was severely altered at both 21 and 30 dpi, as compared to healthy plants. MicroRNAs (miRNAs) accounted for a 2.8% of total reads mapping to the tomato genome in mock-inoculated plants, and this figure raised up to 8.5% in infected plants at 21 dpi. Fifty-seven miRNA species showed at least a two-fold increased accumulation and 56 at least a two-fold decrease in infected vs. healthy plants at 21 dpi, and most of them were similarly altered at both timepoints. MiRNA target genes, whose expression was shown or predicted to be differentially modulated in infected plants, belong to specific functional categories involving transcription factors, kinases and genes with oxidoreductase activity, which may partially explain the disease symptoms induced by the virus. Abundant secondary siRNAs (e.g. phasiRNAs), depending on an upstream small RNA trigger and subsequent RDR and DCL activities, were induced by virus infection and shown to be biologically active by driving cleavage of pathogen-responsive genes, such as receptor-like kinases (RLKs).

Thus, miRNAs regulate plant defense responses, and their synergistic effects with secondary siRNAs fine-tune the antiviral immunity system, by targeting for instance RLKs and other genes.
Recessive resistance to Watermelon mosaic virus (WMV) in melon has previously been reported in the African accession TGR-1551. Using a population of recombinant inbred lines (RILs), derived from a cross between TGR-1551 and the susceptible Spanish cultivar ‘Bola de Oro’ (BO), a major quantitative trait loci (QTL) controlling the resistance was previously mapped to a region of 10 cM in linkage group (LG) XI. A genotyping by sequencing (GBS) analysis of the RILs population has provided new information that confirmed the position of this major QTL in LG XI and three minor QTLs in LGs IV, V and VI. Generations derived from the RILs population were subsequently used to fine map the resistance derived from TGR-1551. Two selfing progenies obtained from the second backcross to ‘Bola de Oro’ of two of the resistant RILs were phenotyped for resistance to WMV. Selfing progenies from 22 of these plants were selected, phenotyped for resistance and genotyped with a set of SNPs corresponding to LGs IV, V, VI and XI, with a Sequenom iPLEX® Gold MassARRAY. Selfing progenies from some selected plants recombinant in the candidate region were also phenotyped for resistance and genotyped with this set of SNP and with another set derived from a new GBS analysis of parentals and resistant/susceptible DNA bulks. The results obtained have allowed the narrowing of the candidate gene interval on LG XI and the confirmation of a minor QTL in LG V.
Compressive forces exerted on the soil surface decrease soil aggregation and pore space, thus reducing soil transport and holding capacity of water, air and nutrients, diminishing crop growth and plant resource capture. The mechanisms by which soil compaction decrease growth, in the presence or absence of soil drying, are unclear. Compact soil decreases shoot growth without changing plant water status, suggesting the action of chemical or hormonal signals. Abscisic acid (ABA) regulates stomatal aperture in response to multiple abiotic stresses (notably drought), but no consistent evidence has emerged that ABA regulates physiological responses to compaction stress. Multi-hormone analyses allow multiple signalling candidates to be measured from a single tissue or xylem sap sample, and may elucidate the roles of hormonal signals.

*Solanum lycopersicum* L. (cv. Ailsa Craig) was grown in 1.4 g cm\(^{-3}\) and 1.74 g cm\(^{-3}\) bulk density soil, under both well-watered and water-deficit conditions, to investigate physiological and growth responses to combined drought and compaction stress. Multi-hormone analyses of leaf tissues and root-sourced xylem sap (collected at *in vivo* transpiration rates) revealed that soil drying increased xylem ABA and salicylic acid (SA) concentrations independent of soil bulk density. Xylem jasmonic acid (JA) concentrations increased in compacted soil, but foliar JA concentrations did not change. Preliminary data indicate that soil compaction decreased gibberellin concentrations in expanding leaves, suggesting a possible role in shoot growth regulation. Further work is required to clarify roles of these hormones in growth regulation.
Double haploid line (LDH) protocols allow the production of genetically uniform lines within one generation. One of the strategies to obtain them is through reprogramming of microspores, which are cells committed to form pollen, from the gametophytic to the sporophytic pathway. After reprogramming, the microspore begins to divide symmetrically giving rise to embryos that will later generate double-haploid plants. At present, for *Solanum lycopersicum* there is no effective protocol to obtain LDHs. In contrast, for *Solanum melongena*, there is an efficient method through anthers culture *in vitro*. There are several factors that can affect the embryogenic response of microspores, as the genotype, the physiological state of the plant, the developmental stage of the microspores at the time of induction, the stress treatment or the regeneration media, among others. Most importantly, there may be genes or non-coding RNAs, such as miRNAs or sRNAs, which could be influencing the responsiveness to embryogenesis of the microspore. Our goal is to detect any differentially expressed genes or sRNAs that may be involved in the regulation of the induction process towards the production of LDHs. We have compared the changes that occur during the heat induction process in the two species. For this, we have performed a characterization of the first stages of development looking at the first cellular divisions that occur during induction. We selected four time points to study these cellular divisions in tomato and eggplant: day 0 (before induction), and three, five and seven days after heat induction at 35°C.
The understanding of protein interaction networks provides crucial insights into the molecular mechanisms of signal transduction, stress responses and developmental processes. Applied to plant science, this open up the way to help understand host-pathogen interactions. We have used a domain-based strategy to construct highly complex, random primed cDNA libraries from different tissues of 13 different species of plants. To ensure reproducible and exhaustive Y2H results, these libraries are screened to saturation using an optimized mating procedure. These libraries have been integrated into our high-throughput yeast two-hybrid platform and are available for screening on a fee-for-service basis. This strategy has been shown to be very successful with over 600 screens performed on libraries from model plants and >30 publications in high impact journals.

Antibodies represent central tools in most biological studies to analyze protein localization and function. One of the remaining limitations is the challenge to make them work inside a living cell. So far, the access to intrabodies was limited to highly trained lab specialists in this field. We have therefore set up a new platform for intrabody screening and designed for this purpose a fully synthetic humanized naive llama VHH library containing 3x10^9 antibodies, based on a unique scaffold with random complementary determining regions (CDRs). We use a combination of phage display and subsequent yeast two-hybrid (Y2H) screening to identify antibodies against native antigens and eventually intrabodies. The VHH clones are directly accessible and the recombinant antibodies can be produced as fusions to different Fc domain (human, mouse, etc.).
P0270 CRISPR/CAS9–MEDIATED KNOCK-OUT OF POLYPHENOL OXIDASE GENES IN EGGPLANT (SOLANUM MELONGENA L.)

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1 Full text

Eggplant fruits possess high antioxidant properties, due to their content in phenols and flavonoids. After cutting, the phenolics become available to polyphenol oxidase enzymes (PPOs), which catalyze their oxidation giving rise to browning of the fruit flesh to detriment of berry quality for both fresh consumption and industrial transformation. On the other hand, the selection of commercial varieties with low flesh browning has resulted in the indirect selection of genotypes with low concentrations in phenolics, and thus reduced nutraceutical properties.

Ten PPO genes (named from PPO1 to PPO10) have been isolated and characterized, also thanks to the recent availability of a high quality and annotated eggplant genome sequence. Their qPCR expression profiles were assessed in the berry flesh and peel of three eggplant commercial varieties, immediately and 30 min after cutting. Increases of transcripts at 30 min after cutting were spotted in PPO1, PPO3, PPO4 and PPO5 genes.

With the goal to ‘knock out’ the latter, and assess its effects in lowering berry flesh browning, we developed a GoldenBraid CRISPR Cas9 tool which contains 2 guide RNAs: one targeting PPO1 and 3, while one targeting PPO 4,5 as well as PPO6, due to the high homology between these gene family members. On the basis of both polyphenol content and in vitro regeneration potential, three eggplant varieties were selected for Agrobacterium-mediated transformation. The genomic DNA was extracted from in vitro regenerated shoots and mutations at the target sites as well as off-targets effects were assessed.
CRISPR/Cas9 system has become the most efficient and widely used tool for plant genome engineering, allowing to obtain heritable changes in most transformable crop species and providing a versatile and powerful implement for agricultural sciences. This system is composed of two main components: The Cas9 endonuclease and the sgRNA (crRNA + tracrRNA). The Cas9 protein is guided by sgRNA and produces double strand breaks on target DNA sequences. To set up the CRISPR/Cas9 technology in tomato we reproduced the work by Brooks et al. (Brooks et al., 2014, Plant Phys. 166:1292-1297) choosing as target *SlAGO7*, a gene involved in post-transcriptional silencing of auxine response factors; *SlAGO7* loss-of-function mutation results in a distinct and immediately recognizable phenotype. Two different vectors with one and two sgRNAs were designed to obtain mutated plants. Using our vectors, mutation efficiencies were 38.9% and 78.6% with one and two sgRNAs, respectively. DNA fragments encompassing the sgRNA targets were PCR-amplified and several clones were sequenced per mutated line; significant differences were identified in the nature of the mutations induced for each of the two sgRNAs used in this study. All together, our results show that ABIOPEP has successfully implemented the CRISPR/Cas9 technology in its laboratory, which could be used as a powerful tool in new breeding strategies.
OP1: CUCUMBER

P0023 GENETIC MAPPING OF ANGULAR LEAF SPOT RESISTANCE IN CUCUMBER (CUCUMIS SATIVUS L.)

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Full text

One of the important diseases of cucumber is angular leaf spot (ALS) caused by Pseudomonas syringae pv. lachrymans. Increased occurrence of this disease in open-field cucumber production caused significant losses over the last few years. ALS symptoms may vary depending on the virulence of bacteria strain, the host, and the environmental conditions. The aim of this study was to map major gene(s)/quantitative trait loci (QTLs) controlling ALS resistance in pickling cucumber. Previous studies confirmed that cucumber line Gy14 shows resistance and line B10 susceptibility to ALS. Recombinant inbred lines (RILs) mapping population was developed from the cross Gy14xB10. RILs were tested under phytotron conditions for type of ALS symptoms (presence/absence of chlorotic halo) and resistance (disease severity index). Based on SSR analysis 92 RILs were used for DArT-seq genotyping and linkage map construction. Developed map contained 546 markers in seven linkage groups, spanning 707.1 cM with 1.3 cM on average between adjacent markers. Monogenic inheritance of chlorotic halo, which is ALS symptom differentiating parental lines, has been confirmed and the locus was located on linkage group 5. Major QTL associated with ALS resistance was also located on linkage group 5. This study will facilitate the development of molecular markers and cloning of ALS resistance genes in cucumber.
P0074 FINE MAPPING OF TRICHOME-LESS GENE GL-2 IN CUCUMBER (CUCUMIS SATIVUS L.)

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1 Full text

Trichomes-less mutants are common in higher plants that an important tool in understanding regulatory mechanisms in protecting plants from environmental stresses such as heat, low temperature, high UV, and insect herbivory. Previous studies have reported three glabrous mutant plants containing the genes gl1, gl2 and gl3. In the present study, we conducted phenotypic characterization and genetic mapping of the cucumber trichomes-less mutant NCG042 conferred by the gl2 locus. Genetic analysis indicated that the glabrous phenotype was inherited as a single recessive gene, gl2. Fine genetic mapping allowed for the assignment of the gl2 locus to a 157.3kb genomic DNA region in chromosome 2 with two flanking markers that were 0.9 and 1.6 cM away from gl2, respectively. There were 9 candidate genes in this region. This study will facilitate marker-assisted selection (MAS) of the smooth plant trait in cucumber breeding and provide for future cloning of gl2.
P0159 PHYSIOLOGICAL RESPONSES OF CUCUMBER PLANTS TO WATER DEFICIT

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1 Full text

Drought is one of the most common environmental factors reducing plant growth and development in many regions of the world. The objective of this study was to investigate the effect of drought on physiological parameters of eight cucumber cultigens grown in a greenhouse at seedling and flowering stages. We examined transpiration and photosynthetic rate, stomatal conductance, chlorophyll fluorescence parameter $F_{v}/F_{m}$, and relative amount of chlorophyll at two irrigation treatments: optimal (-5 kPa) and reduced irrigation level (-40 kPa). Our results showed that all cultigens presented the significant decrease in the majority of tested physiological parameters in the flowering stage in comparison to seedlings stage. It could indicate on higher sensitivity of cucumber plants to drought stress during flowering stage. Among tested accessions, SU 1 and SU 2 appeared to be the most drought tolerant in both developmental stages, as indicated by both the lowest reduction in physiological parameters and no wilting symptoms. In summary, based on the data obtained, we concluded that the three parameters: transpiration and photosynthetic rate, stomatal conductance are good indicators of drought tolerance/sensitivity in cucumber.
Cucumber is a plant having a large demand for water. This is caused by a shallow root system, large leaf area and fruit having a high water content. We studied the effects of drought on morphological parameters (total root length and weight, plant height, leaf area, fresh weight) in ten cucumber cultigens grown in rhizoboxes with reduced water conditions (-40 kPa) compared to a control (-5 kPa) in a greenhouse. Plants were measured once a week to determine the root growth. Cucumber accessions differed in their response to water deficiency. Drought stress increased total root length in SU1, SU2 and PW1, respectively by 54, 40, and 34%, while the root development in other cultigens was retarded by 8-40%. Significant differences between control and stressed plants were also observed in the remaining morphological parameters (plant height, leaf area, fresh weight and root weight) for most of the cultigens. The highest average reduction have been found for root weight (64%), leaf area (60%), fresh weight (55%) and the lowest for plant height (32%). Among examined cultigens, SU1 and SU2 appeared to be the most drought tolerant, as indicated by both growth root stimulation in stress conditions and the lowest reduction in morphological parameters.
Sex differentiation of flower buds directly affects fruit yield of cucumber (Cucumis sativas L.). To investigate key genes involved in molecular regulatory networks of cucumber sex determination, we performed a genome-wide high-throughput RNA sequencing for young apical buds of B36 (gynoecious cucumber) and S6 (weak cucumber) inbred lines at three growth stages (one-leaf one-bud, three-leaf one-bud, five-leaf one-bud). B36 and S6 plants at the three growth stages were respectively named as B1, B2, B3 and S1, S2, S3. Seven comparisons (B1 vs B2, B2 vs B3, S1 vs S2, S2 vs S3, S1 vs B1, S2 vs B2, S3 vs B3) were made and their differentially expressed genes were analyzed and the results showed that compared with differentially expressed genes in S6 (weak cucumber), more genes were upregulated at the stage of one-leaf one-bud, and downregulated at the stage of three-leaf one-bud in B36 (gynoecious cucumber). In addition, there were differences in gene expression trends enriched significantly between B36 and S6, such as four kinds of gene expression trends (0, 1, 6, 7) enriched significantly in B36, and only two kinds of gene expression trends (5, 6) enriched significantly in S6. Together with the data of analysis of GO, pathway, gene expression trends and qPCR, 14 genes were identified and considered as candidate regulators that may be involved in sex differentiation regulation in cucumber. These genes included auxin-related genes such as Cs-MCM6, Cs-ACT3, Cs-XRCC4, Cs-SSPB, Cs-MCM2, Cs-BRCA2B, Cs-CDC45, Cs-Dpri, Cs-H2B, Cs-APC2, Cs-AHP4, Cs-AUX1, Cs-CDC20 and Cs-CNGC1.
P0202 GENETIC ANALYSIS AND IDENTIFICATION ON THE CANDIDATE GENE CONTROLLING IN VITRO COTYLEDON REGENERATION ABILITY IN CUCUMBER

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1 Full text

The regeneration ability, which plays a critical role in high efficiency genetic transformation, of the tissues of cucumber (Cucumis sativus L.) varies considerably across accessions and the underlying genetic mechanism remains unknown. In this study, 148 recombinant inbred lines (RILs) and a core collection were examined to identify candidate genes involved in regeneration.

1) 6QTLs for cotyledon regeneration were identified which located on Chromosomes 1, 3, 6 and explaining 9.7-16.6% of the phenotypic variation. The loci Fcr1.1 and Fcr1.1+ have been consistently detected under two different medium systems, shared the same marker interval between marker C1B266 and marker C1B267.

2) By genome-wide associations study (GWAS), 18 SNPs were significantly associated with cotyledon regeneration and located within the confidence intervals of C1B266-C1B267. The result of QTL and GWAS reduced the number of candidate genes to three. RT-PCR analyses revealed that the expression level of one candidate gene was significantly higher in high cotyledon regeneration genotypes than in low regeneration genotypes.

3) The native promoter-driven CDS of the candidate gene was transformed into 9110Gt, and molecular analyses showed that the TDNA construct was integrated into the genome of 8.6% of regenerated plantlets. Two thirds of the T0 seeds showed a significantly higher level of regeneration frequency compared to control.

These results demonstrate that the candidate gene may play a major role in regeneration, and provides a selectable marker for cotyledon regeneration.
P0255 A SURVEY OF THE CUCUMBER TRANSCRIPTOME USING SINGLE-MOLECULE LONG READS

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1 Full text

Today, the published plant gene annotation which were assembled from short reads were reported incomplete. Cucumber gene V2 annotation acquired from the same method were also limitedly demonstrated genes. Single molecule long, real-time sequencing technologies could eliminate the constraint by directly sequence the full length isoforms, avoid the genes reconstruction. Here we sequenced the cucumber 9930 transcriptome using single-molecule long reads with three SMRT cells. We produced 19,790 non-redundant isoforms captured 42.6% of the genes annotated in cucumber V2 genome. A large proportion of transcripts (40%) represent novel isoforms of known genes. Among them, 1222 isoforms were absent from cucumber genome, 471 isoforms were present in cucumber genome without annotation, 1645 isoforms covered more than one annotated V2 genes. Our result could validate V2 genes and improved the existing gene models. To validate the accuracy of isoforms, we found 98.6% splice junctions were supported by short reads with STAR. In addition, we also detected 273 fusion transcripts which is a hybrid gene formed from two previously separate genes, and may formed new functions. Our result show that cucumber 9930 transcriptome are somewhat incomplete, and that need to improve in the future.
P0330 RATIONALIZATION OF THE CUCUMBER COLLECTION OF COMAV, POLYTECHNIC UNIVERSITY OF VALENCIA, SPAIN

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Full text

The Genebank of the Institute for the Conservation and Improvement of the Valencian Agrobiodiversity (COMAV) holds 198 Spanish cucumber (Cucumis sativus L.) landraces. With the aim of rationalizing this collection, 195 Spanish cucumber accessions were characterized, using 17 qualitative and nine quantitative descriptors, eight of them referred to plant traits and 18 to fruit. The accessions were classified in five groups: ‘White’, ‘Short’, ‘French’, ‘Long’ and ‘Very long’, according to their morphological characteristics. Principal component analysis showed that, with few exceptions, the accessions grouped according to their phenotypic similarity.

A representative subset of 131 Spanish accessions was selected for molecular genotyping using 23 SSRs (Simple Sequence Repeats). Eighteen of them were polymorphic; the mean number of alleles, mean observed heterozygosity and mean polymorphic information content were 3.2, 0.065 and 0.229, respectively. Around 60% of the alleles showed a frequency higher than 0.05, and only one allele of the SSR31399 showed a frequency lower than 0.01. In addition, three accession-specific alleles were identified. A cluster analysis did not show any relation between genetic variation and morphological types or geographical origin. These results demonstrated that molecular diversity of the cucumber did not resemble its phenotypic variability.

Considering phenotypic traits, origin and molecular data, 47 accessions were chosen, being all types represented: ‘French’ (6 accessions), ‘Long’ (15), ‘Short’ (24), ‘Very long’ (1) and ‘White’ (1). The analysis of the set of selected accessions confirmed that it conserved the morphological and molecular variability found in the complete collection.
The new single-molecule sequencing technologies can generate reads with the average lengths of more than 10,000bp, thus providing the opportunity to improve the complicated reference genomes. Here, the improved cucumber genome (226.4 Mb) was assembled de novo into 879 contigs with an N50 length of 2.8 Mb from 50X single-molecular sequencing data. The error rate for each base was estimated to be 0.0001 using ~100X Illumina reads. These contigs were linked into 703 scaffolds with an N50 length of 11.6 Mb combining with the mate pair reads from different insert size libraries, fosmid ends and BAC ends. Using about 2000 genetic markers from three maps, 205.5 Mb were anchored onto the seven chromosomes. The genetic and physical positions show a high consistency, suggesting the high-quality accuracy of the contiguity. Compared with the previous assembly, 29.2 Mb novel sequences, which include a lot of repetitive sequences and novel genic sequences, were generated. This high-quality genome assembly will serve as a valuable resource for comprehensive analysis of genomic organization in cucumber as well as plant comparative genomics.
P0376 EPIGENETIC REGULATION OF CUCUMBER FRUIT SIZE

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1 Full text

Cucurbits are present all over the world and are among the largest and most diverse plant families that supply human with edible products. Cucurbit fruit develops from an inferior ovary, and becomes extremely larger in modern cultivars than the wild species. However, the genetic basis of cucurbit fruit morphology and domestication is still largely unknown. By EMS mutagenesis sequencing and map-based cloning, we found a cucumber mutant bearing short fruits (sf2), and the causative gene encoding a protein subunit of histone deacetylase complex, which expresses preferentially in early fruit cells. Compare to wild type fruit, the cell division of sf2 is repressed. CsSF2 encodes a conserved nuclear localized protein in eukaryotes. The specific polyclonal antibody to CsSF2 was generated. We demonstrated the CsSF2 forms a complex with histone deacetylase 1, histone deacetylase 2 and two SIN3-like repressors through coimmunoprecipitation and Mass Spectra. We identified the their interaction in vitro and in vivo. Moreover, we found histone H3 lysine 18 acetylation (H3K18ac) and histone H3 lysine 23 acetylation (H3K23ac) were significantly increased in sf2 mutant fruit, indicating an important role of SF2 in histone deacetylase regulation. Finally, RNA-seq and ChiP-seq analysis further revealed the directly repression of jasmonic acid synthesis and signal transduction in sf2, and finally the inhibition of quick cell division in fruit cell. Our results showed the important roles of epigenetic regulation in cucumber fruit size.
OP02: MELON

P0048 EFFECT OF EXOGENOUS SPERmidINE ON THE GROWTH AND ANTIOXIDANT SYSTEM OF MELON SEEDLINGS UNDER LOW TEMPERATURE STRESS

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1 Full text

Using substrate culture, we investigated the effects of 0.1~2 mmol·L⁻¹ exogenous spermidine (Spd) on the growth and reactive oxygen metabolism under low temperature stress (day (12±1) ℃/night (6±1) ℃) and recovery in climate chambers with melon variety ‘GL-1’ . The results showed that low temperature stress had significant effects on the growth and indexes of reactive oxygen metabolism. The suitable concentration of Spd treatment could alleviate the damage of low temperature stress in various degree, the best effect was observed in the treatment of 1 mmol·L⁻¹ Spd, which significantly increased the seedlings growth and the activities of SOD, POD, CAT, APX, DHAR and GR, and increased the contents of AsA, GSH, soluble protein and proline, and decreased the accumulation of H₂O₂ and MDA, production rate of superoxide anion radical (O₂⁻). The above results indicated that dosage effect of Spd existed on the alleviation of low temperature stress in melon seedlings, and the best alleviating effect was 1 mmol·L⁻¹ Spd, which was favorable for the seedlings to maintain the stability of AsA-GSH circulation system, decrease the accumulation of H₂O₂ and MDA, production rate of O₂⁻ and protect the stability of cell membrane structure by promoting antioxidant enzyme activity and antioxidant content, and thereby reduce the damage of the active oxygen to the melon leaf and enhance the cold resistance.
Melon (Cucumis melo L.) is a eudicot diploid plant of interest for its biological properties and economic importance. Oriental melon (Cucumis melo L. var. makuwa) is also one of the most important cultivated cucurbits grown widely in Korea, Japan, and northern China. It is cultivated because its fruit has a sweet aromatic flavor and is rich in soluble sugars, organic acids, minerals, and vitamins. Although genome of melon was unveiled, the evolutionary history of melon or oriental melon were remained to be elucidated. Furthermore, previous reference genome was constructed from hybrid between oriental melon (Korean landrace PI161375) and melon (Piel de Sapo). Thus, individual reference genomes are required to perform comparative and evolutionary analyses between oriental melons and melons. Here we report multiple reference of three oriental melons and three melons. The scaffold N50 of six melons were ranged from 1.1 Mb to 1.4 Mb and predicted genes were ranged from 32,000 to 36,000. The further comparative analyses of Cucurbitaceae family including previous melon reference and Cucumis sativus will be represented.
To accommodate the increased production and quality of crops in the next decades the efforts of plant science will be directed to improve traits having a great potential for yield, fruit quality and resilience towards stresses. Melon (Cucumis melo L.) is one of the most important horticultural crops, ranking in the ninth position of the world production of vegetables, with more than 27 million tons in 2012. Huge amounts of diverse data were until now generated for this species, including high-throughput genomic, transcriptomic and phenotypic data as well as wide collections of genetic resources. Moreover, a recent update of the melon genome was built and a new annotation, integrating a genome-guided transcriptome assembly based on a wide collection of RNA-Seq data, has been recently obtained (v4.0). The new annotation, including 29,980 genes, constitutes a new reference for the integration of different omics layers and will allow increasing the breath and coverage of the biological network underlying genetic important traits. As a proof of concept, by exploiting a cantaloupe x inodorus RIL population and available omics data, the candidate genes underlying two already known traits, the fruit flesh color $gf$ gene and a fruit rind color $CmKFB$ gene involved in flavonoid accumulation, were confirmed. Other important traits segregating in the RIL population will be explored and this will lead to get novel insight into the mechanisms underlying agronomical important traits and will support the development of new strategies for improving sustainable production for melon and other species belonging to the Cucurbitaceae family.
Melons (Cucumis melo L.) are challenged by a wide range of diseases and pests. It is common for growers to observe pathogenic viruses, oomycetes, ascomycetes and insects on a crop, which results in a significant negative impact on production and quality. The use of disease resistant varieties is a common practice that has been proven to be efficient. Breeding for varieties that combine multiple resistances is challenging as it requires to stack resistance loci in commercially relevant hybrids and lines. We review here the workflow designed to develop and use molecular markers for pre-breeding and breeding of multiple-virus resistant melons. With the aim to create molecular markers predictive for resistance to CYSDV, WMV, ZYMV, MNSV, PRSV and CMV, (1) QTLs involved in resistance have been precisely located on the melon genetic map via linkage mapping approaches and (2) publicly disclosed candidate genes or loci linked to resistance have been validated. The use of these molecular markers has been integrated in routine marker assisted-selection (MAS) breeding workflows. In addition, pre-breeding lines carrying the resistant alleles for target QTLs dissociated from deleterious traits have been created. This enables breeding for WMV, ZYMV and/or CMV resistance in the absence of undesired sex determination and fruit morphology traits such as male flowers and pentamerous fruits.
Fusarium wilt caused by *Fusarium oxysporum* f.sp.*melonis* is the most destructive diseases that cause severe losses in melon crops worldwide. FOMs are isolates classified into four physiological races (0, 1, 2, 1.2) based on host resistance genes. Two dominant resistance genes (Fom1, Fom2) control resistance to races (0 and 2), and (0 and 1), respectively. We aimed to evaluate the resistance of Palestinian snakemelon to Fom races, to search for a new resources of resistance to FOM’s, especially FOM1,2. A total number of 348 snakemelon accessions collected from 10 districts in the West-Bank were screened. The pathogenicity test was carried out by dipping the seedlings in prepared spore suspension and incubation at 25°C under 12h day-night light. Differential lines (Charentais-T, Charentais-Fom2, Vedrantais, Margot, and Isabelle) were used as controls for different FOMs. Area Under Disease Progress Curve (AUDPC) and rAUDPC were calculated to estimate accessions resistance on a scale of 0-9.

All snakemelon accessions screened have shown resistance to FOM0 and FOM2, while susceptible to FOM1 and FOM1,2. 28 seedlings belonging to 6 accessions have shown to be resistant for either FOM1, or FOM1,2. Inheritance studies will be carried out to demonstrate if resistant in all accessions to FOM 0 and FOM 2 is allelic to the well-known Fom1 gene. Distribution of tested accession on scale from 0-9 for FOM1, and FOM 1,2 were 53% and 89% very susceptible located between 6-9 range, while 47%, and 11% of the accessions were moderately susceptible (3-6) for FOMs, respectively.
P0370 ANALYSIS OF AROMA VOLATILE COMPOUNDS FROM A MAKUWA INTROGRESSION LINE COLLECTION ONTO VEDRANTAIS BACKGROUND.

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Volatile organic compounds (VOCs) are an important aspect of melon (Cucumis melo L.) fruit quality. The volatile profile of twenty-seven introgression lines (IL), developed from the Japanese cultivar “Ginsen makuwa” (subsp. agrestis var. makuwa, MAK) and the climacteric cultivar “Vedrantais” (subsp. melo var. cantalupensis, VED), as donor and recurrent parent respectively, was characterized in two environments. Fifty-eight VOCs (15 alcohols, 15 aldehydes, 25 esters and 3 ketones) were detected using purge and trap extraction followed by chromatography-mass spectrometry. Also the IL collection was genotyped with 2146 SNP evenly distributed throughout the melon genome.

VED fruits produced higher volatile amounts than MAK (2857.5 versus 1699.6 ng g⁻¹). MAK fruits had 40, 40, 70 and 95% less alcohols, esters, aldehydes and ketones, although they had higher amounts of some specific compounds. Significant genotype x environment interactions were detected for some compounds. Other effects were stable across environments. ILs MAK2-1 and MAK9-2 that share a MAK introgression in LG IX (carrier of the major flesh color gene wf), produced light green-fleshed fruits. The lack of carotenoids resulted in a decrease of ketones derived from the metabolism of apocarotenoids, compared with the orange-fleshed VED parental. In contrast, these lines displayed increased alcohol and ester levels. Other introgressions, such as that of MAK7-2, that shows an intermediated climacteric ripening, had reduced contents of most VOCs, but an increased level of carotenoid-derived ketones. This is a valuable population to study aroma biosynthesis in melon and to modify the aromatic profile of cantaloupe melons.
The long-vein type varieties are majority in pumpkin *Cucurbita maxima* which are consumed more land and labor cost. Previous studies have shown the yield can be improved in short-vein type varieties but the mechanism of vein growth was not clearly. In this project, a near isogenic line of short-vein type (*SHORT1*) had been constructed named *short1/NIL* displayed a long-vein phenotype and accumulated more GA levels. Genetic analysis showed a single recessive gene *short1* was involved in the vein growth regulation. Based on a 6 000 samples-line mapping combined with BSA sequencing strategy, 14 candidate genes involved in GA response were identified preliminary in a 300 kb physical distance. The downstream regulation pathway of *SHORT1* was confirmed by vein specific transcriptome sequencing and related molecular technique. Some genes involved in the lignin synthesis and vascular bundle differentiation were screened in this transcriptome sequencing. This study was thought to provide molecular evidence to understand the regulation of main vein morphogenesis. Meanwhile, the application of *SHORT1* gene will improve the efficiency of short vein type variety breeding in *Cucurbita maxima*.
P0103 A HIGH-DENSITY LINKAGE MAP AND QTL MAPPING OF FRUIT-RELATED
TRAITS IN PUMPKIN (CUCURBITA MOSCHATA DUCH.)

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1 Full text

Pumpkin (Cucurbita moschata) is an economically worldwide crop. However, there were almost no quantitative trait loci (QTLs) reported ever due to the lack of genomic and genetic resources. In this study, a high-density linkage map of C. moschata was structured by double-digest restriction site-associated DNA sequencing, using 200 F2 populations of CMO-1×CMO-97. By filtering 74,899 SNPs, a total of 3,470 high quality SNP markers were assigned to the genetic map spanning a total genetic distance of 3087.03 cM on 20 linkage groups (LGs) with an average genetic distance of 0.89cM. Based on this map, pericarp color and strip were both fined mapped to the same single locus on LG8 in a region of 0.31 cM with phenotypic variance explained (PVE) of 93.6% and 90.2%, respectively. QTL analysis was performed on carotenoids, sugars, tuberculate fruit, fruit diameter, thickness and chamber width with total of 12 traits. 29 QTLs distributed in 9 LGs were detected with PVE from 9.6% to 28.6%. It was the first high-density linkage SNP map for C. moschata which was proved to be a valuable tool for gene or QTL mapping and it will serve as significant basement for map-based gene cloning and draft genome assembling.
Calabash (Lagenaria siceraria) is one of the first cultivated plants. It is seldom consumed as food, but mainly used as a container to store liquids, as a toy, or a music instrument due to its lignified gourd. Although calabash utilisation is well-established in Africa and Latin-America, little is known about the detailed cell wall composition and resulting material properties of different calabash cultivars. Here we show a detailed analysis of the lignification in fruits from the cultivars Midi Bottle, Birdhouse and Cannonball. Via microscopic analysis we could show that the lignin deposition starts immediately with the transition from floral to mesenchymal meristem. A chemical analysis of the cell wall composition showed that cortex tissue comprises 50-60 % crystalline cellulose content and 20-30 % lignin content, the latter being comparable to hardwood.

Beside those properties, gourds have a lower density (0.2-0.3 g/cm$^3$) than timber (0.4-1.0 g/cm$^3$) which makes them of interest as a sustainable alternative material for technical applications. Therefore, we investigated the material characteristics of the above mentioned cultivars. Our results open up new possibilities for applications of calabash, for example in the core layer of sandwich structures used in lightweight constructions, addressing society’s increasing demand for sustainable materials.

With this approach we will highlight the relevance of further investigations to understand the cell wall metabolism of cucurbitaceae. A detailed knowledge of how the lignification process in cucurbitaceae is controlled will offer the opportunity for breeding calabash cultivars with ideal material properties for construction materials.
P0345 PHENOTYPING FOR OXIDATIVE STRESS TOLERANCE IN ZUCCHINI SQUASH (CUCURBITA PEPO)

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1 Full text

Abiotic stress induces the production of reactive oxygen species (ROS) causing oxidative damage in plants. A fast phenotyping method has been optimized for assessing the response to oxidative stress during seed germination. The method has been initially tested in different commercial cultivars of zucchini squash, and then in an EMS mutant collection. Seed of each cultivar was soaked in 1% H₂O₂ and 0.05 mM of the catalase inhibitor aminotriazole for 12 h at 4 °C. Water soaked seed of the same cultivar was used as control. Seed was then germinated and grown between moistened filter papers in the dark at 24 °C and 80% RH. The percentage of germination and the length of the radicle were assessed every 4 hours using digital image processing. Several parameters regarding germination and radicle growth were used to compare treated and untreated seed of each cultivar, and to assess the tolerance of each genotype to oxidative stress. The cultivars were statistically classified and grouped by their differential response to the treatment, demonstrating the existence of genetic variability for oxidative stress tolerance among zucchini squash genotypes. This phenotyping approach is currently being used to screen more than 35,000 plants from our zucchini EMS mutant collection. After testing 1.500 M² families (15,000 plants), several mutants have been detected showing higher and lower tolerance to oxidative stress than WT. The incorporation of these mutations to zucchini breeding programs would also require them to confer tolerance to the abiotic stresses to which this crop is usually exposed.
P0354 ILLUMINA-BASED DE NOVO TRANSCRIPTOME SEQUENCING AND IDENTIFICATION SNP AND SSR MARKERS IN PUMPKIN (CUCURBITA MAXIMA)

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1 Full text

Pumpkin (Cucurbita maxima) is a world-wide consumptional crop. Although the genomic information of its relative crop in Cucurbitaceae (cucumber, watermelon and melon) has been published, there was little known about the genomic information in Cucurbita maxima, which prevented the molecular mark assisted breeding of pumpkin. In this study, an illumina paired-end sequencing technology was used to obtain the genomic information of pumpkin with the purpose of obtaining SNP and SSR markers. More than 36 G high quality cDNA sequence reads were obtained and 55 163 unigenes were assembled. A total of 4 876 EST-SSRs were identified as potential molecular markers, with mononucleotide A/T repeats being the most abundant motif class and G/C repeats being rare. A total of 80 SSR loci were randomly selected for validation by PCR amplification as EST-SSR markers. Of these, 27 marker primer pairs produced reproducible amplicons that were polymorphic among 75 pumpkin accessions selected from diverse geographical locations. The large number of SNP and SSR-containing sequences found in this study will be valuable for the construction of a high-resolution genetic linkage maps, association or comparative mapping and genetic analyses in various Cucurbita species.
P0012 THE EFFECT OF THE LEAF EPIDERMAL STRUCTURE ON TOMATO YELLOW LEAF CURL VIRUS DISEASE RESISTANCE

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ABSTRACT: Tomato yellow leaf curl virus (TYLCV) was vectored by whiteflies. TYLCV caused significant crop losses in tomatoes worldwide. After infection, the plants had retarded growth, erect shoots, yellowing and curling of the leaves, and misshaped and markedly smaller leaflets. Two TYLCV-susceptible varieties ‘DiOuHongGuan’, ‘FenKaWo’ and two highly TYLCV-resistant varieties, ‘Mao T’, ‘FenLanDi’ were used to describe some of the microscopic features of the disease distinction between TYLCV-resistant and TYLCV-susceptible tomato plants after infection. The result showed that the resistant varieties had more villi and root hair, more stronger root hair, than susceptible varieties. ‘Mao T’ had the most villi, and 2-3 bifurcation on each villi. After inoculation, the mesophyll cells in the susceptible plants became large and irregular shape. There was no deformation in the resistant plants while the veins of the infected leaves were deformed in the susceptible plants as well as the companion cells adjacent to the sieve element. Therefore, the number of villi and root hairs of resistant varieties were more than susceptible varieties, and the root hair was more stronger than susceptible varieties ;The cell had markedly change in susceptible region, and performance is normal in not susceptible parts.

This work was supported in part by innovation project of Chongqing animal and plant varieties - Innovation of high quality, disease resistant and high yield vegetable varieties.
Steroidal glycoalkaloids (SGAs) are specialized metabolites of the Solanum species that protect them against a broad range of pathogens, while also contributing to the nutritional value of their consumed parts. For instance, the Solanum lycopersicum SGA α-tomatine functions as a phytonutrient that among other things lowers cholesterol levels. A biosynthetic pathway for these cholesterol-derived metabolites was recently proposed. However, how the tomato plant manages to regulate the diversity of steroid compositions in distinct organs and developmental stages is still largely unknown. Here we provide results that indicate that a tomato AP2/ERF transcription factor (TF) shuts down SGA biosynthesis during fruit ripening. Transient expression assays in tobacco BY-2 cells showed that this TF could trans-repress the promoter of the gene coding for the key SGA biosynthetic enzyme GAME4. The AP2/ERF TF possesses an EAR repressor domain through which it can interact with homologs of the Arabidopsis co-repressor TOPLESS (TPL), as shown in a yeast two-hybrid assay. Moreover, a transient expression assay demonstrated that an EAR-lacking mutated version of the AP2/ERF TF was no longer able to repress the promoter of GAME4. Identification of the cis-regulatory element(s) bound by this TF will allow us to subtly alter the transcriptional regulation of SGA biosynthesis through CRISPR/Cas9 genome editing. This strategy may provide a means to achieve higher tomato nutrient levels while bringing about minimal pleiotropic effects.
This project will establish an interactomics platform to unravel the regulatory mechanisms acting in the regulation of defense, metabolic, growth and developmental processes in the important crop tomato. For instance, the tomato crazy root disease is spreading and affecting tomato greenhouse production in Europe. Such a disease is characterized by an extensive development of the root system that partially suppresses the water uptake of the plant leading to withering and yield loses. Pathogenic Agrobacterium biovar 1 spp are the causative agent of crazy root disease. To date no effective treatments have been developed to fight against biovar 1 strains. Here, we will establish a tomato yeast-two-hybrid-sequencing (Y2H-seq) platform to analyze the molecular crosstalk between Agrobacterium biovar 1 and tomato proteins during the establishment and development of crazy root disease. Interestingly, several tomato Y2H libraries will be created using different tissues and conditions. This experimental approach will identify the tomato protein targets and eventually the molecular mechanisms that are twisted by Agrobacterium biovar 1 strains. In addition, we will apply Y2H-seq to get new insights in general stress and developmental responses in tomato. The knowledge gained within this project will establish the foundations for future research programs that will aim to help combating plant infections and to increase crop yield or high-value specialized metabolite production, hence promoting healthier and more sustainable agriculture practices.
Abscisic acid (ABA) signaling plays a crucial role in the fruit development and ripening; however, supporting molecular evidence has been lacking to date. Therefore, we investigate tomato (Solanum lycopersicum) type 2C protein phosphatase SlPP2Cs, which are core components of the ABA signaling. Three SlPP2Cs (SlPP2C1, SlPP2C3, and SlPP2C5) among fourteen tomato group A PP2C members, have been identified that are the negatively regulators in ABA signaling and fruit ripening. Three SlPP2Cs share the highest degree of similarity with AtAHG3 or AtHAI1 of Arabidopsis, respectively, and they interact physically with different ABA receptors SlPYLs in an ABA-dependent manner or not. The expression of three SlPP2Cs is clearly observed in all tomato tissues throughout development, particularly in the flower and fruit. RNA interference of SlPP2C1/3/5 significantly accelerated fruit ripening through enhancing ABA signaling that induced early release of ethylene. While the opposite phenotypes are observed in the overexpression lines. The expression of SlPP2C1/3/5-mediated ripening-related genes are advanced in RNAi fruits reverse the expression of these genes was delayed in OE fruits. Furthermore, SlPP2C1/3/5-RNAi plants show typical ABA hypersensitive phenotype in seed dormancy and germination, primary root growth and response to drought. Reverse SlPP2C1/3/5-OE transgenic plants showed the opposite phenotypes. These results demonstrate that SlPP2C1/3/5 plays a crucial role in the ABA-mediated fruit ripening, seed germination, and drought responses in tomato.
Abscisic acid (ABA) glucose conjugation mediated by uridine diphosphate glucosyltransferases (UGTs) is an important pathway in regulating ABA homeostasis. In the present study, we investigated three tomato SiUGTs that are highly expressed in fruit during ripening, and these SiUGTs were localized to the cytoplasm and cell nucleus. Among these three UGTs, SiUGT75C1 catalyzes the glucosylation of both ABA and IAA in vitro; SiUGT76E1 can only catalyze the conjugation of ABA; and SiUGT73C4 cannot glycosylate either ABA or IAA. Therefore, SiUGT75C1 was selected for further investigation. SiUGT75C1 RNA interference significantly upregulated the expression level of SiCYP707A2, which encodes an ABA 8'-hydroxylase but did not affect the expression of SiNCED1, which encodes a key enzyme in ABA biosynthesis. Suppression of SiUGT75C1 significantly accelerated fruit ripening by enhancing ABA levels and promoting the early release of ethylene. SiUGT75C1-RNAi altered the expression of fruit ripening genes (genes involved in ethylene release and cell wall catabolism). SiUGT75C1-RNAi seeds showed delayed germination and root growth compared with wild type as well as increased sensitivity to exogenous ABA. SiUGT75C1-RNAi plants were also more resistant to drought stress. These results demonstrated that SiUGT75C1 plays a crucial role in ABA-mediated fruit ripening, seed germination, and drought responses in tomato.
Steroidal alkaloids are specialized metabolites present in the Solanaceae family, where they play an important role in plant defense against pathogens and predators. When consumed, high concentrations of steroidal glycoalkaloids in food are associated with bitter taste and burning sensation in the throat. α-tomatine is the main steroidal glycoalkaloid present in tomato plants, accumulating predominantly in early stage green fruit, leaves and flower buds. However, during the ripening process α-tomatine levels dramatically decrease and its entire pool is converted to hydroxylated, glycosylated and acylated forms termed esculeosides and leucoperosides. Nevertheless, both wild accessions as well as commercial tomato varieties exist, that display high levels of α-tomatine in fully ripe fruits. The aim of the present study is to reveal the genetic and biochemical mechanisms that mediate α-tomatine accumulation in red, ripe fruit of a dozen tomato genotypes. Preliminary results indicate that all the accessions harbour a 612 bp deletion in a putative transporter which likely results in the halt of α-tomatine metabolism during the ripening and bitter taste of red stage fruit. The deletion starts in the coding region of the putative transporter gene continues in its 3' UTR. Notably, the transcripts of the gene harbouring the mutation of interest are accumulated during ripening. We propose that the ‘bitter’ fruit mutation results in modified α-tomatine subcellular localization which in-turn prevents its conversion to esculeosides and leucoperosides in the course of tomato fruit ripening.
P0043 TISSUE-SPECIFIC TRANSCRIPTOME PROFILING OF POLLINATION- AND DELLA-DEPENDENT TOMATO FRUIT SET

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Fruit set, a term used for the developmental differentiation of an ovary into a fruit, is induced by pollination and fertilization of the ovule, and the subsequent metabolism and signaling of phytohormones, such as auxin and gibberellin (GA). GA responses are mediated by degradation of DELLA proteins that suppress GA signaling pathways. The loss of function procera mutant of the single endogenous tomato DELLA/PROCERA gene thus shows constitutive GA responses, including the production of parthenocarpic fruit independent of pollination. To elucidate the transcriptional regulation of fruit set mediated by pollination and GA responses, we generated transcriptome profiles of the pericarp, columella, placenta and ovule in the ovaries of tomato wild-type and the procera mutant during fruit set, at a tissue level of resolution by coupling RNA sequencing (RNA-seq) with laser microdissection (LMD). The expression profiling revealed highly tissue-specific responses of auxin- and GA-related genes to pollination. A small number of auxin signaling associated ARF and Aux/IAA genes showed similar expression patterns in pollinated and parthenocarpic fruit tissues, suggesting their involvement in DELLA-dependent signaling crosstalk between GA and auxin in specific tissues. In addition, genes associated with ethylene, a potential suppressor of fruit set, showed reduced expression in all tissues in pollinated and parthenocarpic fruits, suggesting that DELLA-dependent GA response suppresses ethylene biosynthesis and signaling to promote ovary-to-fruit transition. Gene co-expression network analysis identified co-expressed gene modules associated with single or multiple tissue-types during fruit set, and provided potential evidence of the regulatory modules associated with pollination- and DELLA-dependent fruit set.
Vitamin E (VTE), or tocopherols and tocotrienols, describe a group of compounds, also known as tocopherols and tocotrienols. Tocochromanols are plastidial, lipophilic antioxidants, which are synthesised only in photosynthetic organisms. Within the human diet, tocopherols are the most abundant type of VTE, and are comprised of 4 forms: alpha (α), beta (β), gamma (γ), delta (δ). As α-tocopherol is the most bioactive form in humans, studies have correlated its increased dietary intake with a plethora of health benefits, including: anti-inflammatory responses, reduced cardio-vascular risk in patients with diabetes and an additional role in improved plasma membrane repair. Thus, increased consumption of VTE is beneficial in preventing diet-related diseases.

We are using tomato (Solanum lycopersicum) as a model to increase total VTE. Previous studies in other species have failed to improve VTE levels, and rather, have used VTE pathway gene knockouts to skew VTE composition to less bioactive forms. Therefore, we are using a novel approach to understand transcriptional regulation of the VTE pathway. Using the RNA sequencing from the S. lycopersicum x S. pennellii introgression line population, we have identified expression quantitative trait loci (eQTLs) that contain putative regulators of VTE synthesis. Within these eQTL regions, we have identified candidate genes, which have been tested using viral induced gene silencing (VIGS) and in stable transformations to produce nutritionally enriched tomatoes.
Due to the high consumption and biochemical composition, tomato fruit is an important source of antioxidant compounds for human diet, such as carotenoids, ascorbic acid and tocopherols (vitamin E, VTE). Besides its nutritional value, tocopherols are essential part of the photosynthetic machinery, affecting plant adaptability to light conditions and tomato fruit productivity. Tocopherols are synthesized in the plastids and light has a strong role in regulating chloroplast maintenance and activity; therefore, this environmental cue is expected to affect the accumulation of plastid-derived nutraceutical compounds, as already demonstrated for carotenoids. To understand the interplay between light and VTE accumulation, we investigated whether light regulates VTE biosynthesis. First, we found that tocopherol levels are altered in fruits from mutants impaired in light perception, as well as in fruits ripened under different light conditions. Moreover, the transcript profile of key genes of VTE biosynthesis, such as GGDR, VTE2, VTE3, VTE5 and VTE6, suggested that the light influence on fruit tocopherol content relies on the transcriptional regulation of these genes. As PHYTOCHROME INTERACTING FACTORS (PIFs) and HYPOCOTYL ELONGATED 5 (HY5) are the main transcription factors (TFs) involved in light-mediated signaling, we performed a de novo search for cis-motifs on promoter regions of VTE biosynthetic genes. Motifs recognized by both TFs were found in all the promoters analyzed. Thus, we investigated the TF-promoter interactions by transactivation assays and ChIP-qPCR analysis. Overall, our results suggest that TFs involved in light-signaling transduction regulate VTE biosynthesis by direct interaction with the promoters of VTE biosynthetic enzyme-encoding genes.
In order to investigate the role of hormone classes in salt response, we employed mutants and transgenic tomato plants affecting aspects of hormonal status/signaling in the same genetic background (cultivar Micro-Tom). The genotypes anti sense Chloroplastic carotenoid cleavage dioxygenase 7-35S::asCCD7 (CCD7), Salicylate hydroxylase-35S::nahG (nahG), diageotropica (dgt), entire (e), epinastic (epi), procera (pro), notabilis (not) and Never ripe (Nr) were used. All genotypes presented predictable salinity responses such as reduced growth, increased root:shoot ratio, increased endogenous levels of abscisic and ethylene, decreased auxin and cytokinins content and augmented Na:K ratio. In the multivariate analysis of genotypes four kinds of salt response among the genotypes were observed: i) High shoot growth in spite of high Na:K ratio presented by the strigolactone deficient CCD7 transgene; ii) High shoot growth and reduced accumulation of Na in tissues (probably due to dilution) presented by the auxin constitutive response e mutant; iii) The opposite response observed in “ii” presented by the low auxin sensitivity mutant dgt and iv) growth inhibition combined with reduced levels of Na and higher accumulation of K presented by the not, ABA deficient mutant. Such specific behaviors point for novel levels of salt response regulation, suggesting a role for auxin in Na dilution in tissues and specific mechanisms of ABA and strigolactones in the control of growth under salinity. Nevertheless, the lack of the DELLA repressor in the gibberellin constitutive pro mutant and ethylene insensitivity in the Nr mutant had less effect in the growth under salinity.
The recessive positional sterile (ps) mutation, which occurred spontaneously in tomato (Solanum lycopersicum L.), is characterized by connate petals, normal viable pollen grains, but non-dehiscent anthers because of persistent stomium. Based on phenotypical similarity of floral organs of ps mutant with the slcer6 wax mutant of tomato, defective in very-long-chain fatty acid elongation, the relationship between these two mutations was analyzed across four different ps lines and four fertile lines. We performed the quantitative reverse transcription (qRT)-PCR analysis to examine the expression profile of SlCER6 among flower organs, immature green fruits, peel of red fruits, leaves, and stems. The results showed significant differences in the levels of SlCER6 transcripts between the fertile and ps lines. The SlCER6 transcripts were detected in all fertile lines at different levels as per organs isolated, with the highest levels in the anthers. Comparatively, SlCER6 expression was negligible at most tested ps lines regardless of sampled organs, and did not exceed 0.9% of the expression level in the anther of fertile lines. The exception was ps line 2-303, where relative SlCER6 expression was slightly higher and reached up to 27% of the expression level in the anther of fertile lines, but it was still lower compared to fertile lines. The reduced expression of SlCER6 in the ps lines may suggest that both mutations slcer6 and ps are identical. Nevertheless, because of differences in the SlCER6 expression pattern between 2-303 and the other ps lines, more detailed studies need to be done.
P0091 CELLULAR AND BIOCHEMICAL MECHANISMS OF TOMATO RESISTANCE AGAINST PHYTOPTHORA INFESTANS

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Plant–pathogen interactions are regulated by a complex network of molecular and cytological signals that determinate level of plant susceptibility or resistance. Oomycete Phytophthora infestans is the causal agent of late blight, a devastating disease generating significant yearly losses of tomato in Poland and worldwide. This study aimed to examine the important biochemical and subcellular events underlying the tomato reaction to P. infestans linked to host plant’s age. The production of reactive oxygen species (ROS), the activities of antioxidant enzymes, and the contents of defensive compounds were studied in leaves of four- and eight-week-old plants of two resistant (L3708, WV700) and one susceptible (‘Rumba’) tomato genotypes inoculated with an aggressive local pathogen isolate. Leaf samples were collected at different time intervals (0, 6, 12, 18, 24, 48, 72, and 96 hpi) to gain insights into the dynamics of parameters analyzed. Our observations indicate that lines undergoing testing differ in their biochemical and subcellular responses in accordance with their macroscopically assessed resistance to P. infestans and plant age. Several of the tested biochemical parameters are present at both developmental stages indicating their universality in response to P. infestans infection and others show clear age-dependent trends.
A large variety of fresh market and processing tomatoes (*Solanum lycopersicum*) is grown and consumed worldwide. Post-harvest losses can account for up to 50% of the harvest and it is therefore important to improve tomato shelf life. Tomato shelf life depends upon the rate of fruit softening which accompanies fruit ripening and exacerbates damage during transport and handling. In addition, the susceptibility of tomatoes to fruit pathogens such as the necrotrophic fungus *Botrytis cinerea* (also known as grey mould) is critical for fruit quality and storage. Strategies to extend shelf life involve exploiting natural variation or transgenic approaches. In the past, genetically engineered Purple and Indigo tomato fruits containing high levels of delphinidin-type anthocyanins have been shown to exhibit enhanced shelf life and reduced susceptibility to *B. cinerea* (Zhang et al., 2013, 2015). To analyse the effects of different types of anthocyanins, i.e., delphinidin-, cyanidin- and pelargonidin-types on post-harvest properties of tomato fruits, we have compared the storage quality and susceptibility to *B. cinerea* of fruits from wild-type, red tomato fruits with high flavonol, Orange (*AtMYB12*), high delphinidin, Purple (*Del/Ros1*), high flavonol and high delphinidin, Indigo (*Del/Ros1;AtMYB12*), high pelargonidin, Crimson (*Del/Ros1;DFR;f3'5'h/-/) and high flavonol and high cyanidin, Magenta (*Del/Ros1;AtMYB12;DFR;f3'5'h/-*) tomatoes (Butelli et al., Solcuc2017) in two genetic backgrounds (MicroTom with small fruit and MoneyMaker with large fruit). Data on fungal lesion size on fruits (3 days post wound inoculation) as well as fruit firmness, weight and tissue collapse (2+8 weeks after breaker) across the different genotype combinations will be presented.
Ascorbate (AsA) is an antioxidant that can scavenge the reactive oxygen species (ROS) produced when plants encounter stressful conditions. The biosynthesis pathway of AsA is elucidated while the regulation mechanism remains largely unknown. Here, it was revealed by a yeast one-hybrid assay that a tomato (Solanum lycopersicum) HD-Zip I family transcription factor, SlHZ24, binds to the promoter of an AsA biosynthetic gene encoding GDP-D-mannose pyrophosphorylase 3 (SlGMP3). Both the transient expression system and the electrophoretic mobility shift assay (EMSA) showed that SlHZ24 binds to a regulatory cis-element in the SlGMP3 promoter, and further overexpression of SlHZ24 in transgenic tomato lines resulted in increased AsA levels, on the contrast, suppressing expression of the gene using RNA interference (RNAi) had the opposite effect. These data suggest that SlHZ24 can positively regulate AsA accumulation, and in support of this it was shown that SIGMP3 expression increased in the SlHZ24-overexpressing lines and declined in SlHZ24-RNAi lines. SlHZ24 also affected the expression of other genes in the D-mannose/L-galactose (D-Man/L-Gal) pathway, such as genes encoding GDP-Man-3',5'-epimerase 2 (SlGME2), GDP-L-Gal phosphorylase (SlGGP) and SIGMP4. The EMSA indicated that SlHZ24 bound to the promoters of SIGME2 and SIGGP, indicating a multi-targeted regulation of AsA biosynthesis. Finally, SlHZ24-overexpressing plants showed less sensitivity to oxidative stress and so we conclude that SlHZ24 promotes AsA biosynthesis, which in turn enhances oxidative stress tolerance.
Tomato (Solanum lycopersicum L.) is one of economically valuable vegetable crops worldwide. We have developed comprehensive tomato mutant populations generated by gamma ray irradiation and EMS mutagenesis in cv. Micro-Tom genetic background as well as its TILLING platform to screen mutants of gene of interests. A mutant that derived from EMS mutagenesis and mutated on F-box gene has been identified as a parthenocarpic mutant and has higher Brix value compared to its wild type. Additional F-box gene mutants, which were discovered by TILLING of target F-Box gene, have also shown higher Brix value by preliminary evaluation of fruits, but have not been evaluated for their potential of parthenocarpy trait. In this research, the characterization including parthenocarpy was carried out in three F-box gene mutants of tomato. The objectives of this study were: (1) to investigate the stability of parthenocarpy trait and (2) to characterize the mutants for their morphology, phenology and agronomic traits particularly in relation with parthenocarpy trait and fruit quality. The results showed that the percentage of parthenocarpic fruit formation varied among the mutants and significantly different between summer and autumn cultivation. It suggested that the parthenocarpic fruit formation in the F-box tomato mutants affected by environmental condition, especially the humidity. Lower humidity in autumn is less favorable for parthenocarpic trait expression. Allelism test among the genes, gene expression in NILs of commercial lines, and major metabolites profile will be further investigated to evaluate those F-box tomato mutants as breeding material for higher sugar content and parthenocarpy traits.
P0137 CAN TOMATOES TOUGHEN SKIN? EXPLORING STILBENES IN HEALTHY AGING OF HUMAN SKIN?

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1 Full text

The skin is the body’s largest organ, and is constantly exposed to many environmental stresses, which are involved in the pathogenesis of many skin diseases. Therefore, research into novel approaches to prevent and protect against the many environmental stresses that act on skin has become very important.

The stilbene resveratrol and its derivative pterostilbene are secondary metabolites found in grapes, peanuts and spruce-pine where they protect against environmental stresses. In humans, resveratrol and pterostilbene have been shown to have multiple pharmacological properties including anti-inflammatory, anti-aging and anti-cancer activities. Therefore, these compounds have promising potential to be used in protective or preventive skin care treatments.

Tomatoes represent excellent bio factories for the production of secondary metabolites. However, tomatoes lack the enzymes required to synthesise stilbenes. We aim to engineer resveratrol and pterostilbene in tomatoes, utilising grapevine stilbene synthase (vvStSy) under the E8 fruit specific promoter. For production of pterostilbene the grapevine resveratrol-o-methyltransferase (VvROMT) will be utilised under the control of the E8 promoter in addition to the VvStSy gene. The Arabidopsis transcription factor AtMyb12 will also be utilised in both tomato lines to enhance resveratrol and pterostilbene production. From the transformed tomatoes, juice extracts will be obtained, and analysed for skin health promoting functionalities.

Using a full thickness human skin platform the action of stilbenes and tomato juice on skin aging and inflammation will be analysed, by observing changes in skin morphology, and expression of genes known to be involved in skin inflammation and aging.
Phytochrome-Interacting Factors (PIFs) are a family of basic helix-loop-helix (bHLH) transcription factors that play different roles during plant development. In Arabidopsis thaliana, PIFs are involved in seedling deetiolation, shade avoidance, flowering, chloroplast development, elongation growth, or leaf senescence, among other light-regulated processes. However, little is known about the function of PIFs in other plants. Previous work in our lab showed that one member of the PIF family in tomato, PIF1a, is involved in the regulation of carotenoid accumulation during tomato fruit ripening. A careful examination of transgenic lines with a constitutively silenced PIF1a gene further suggested that this transcription factor might be involved in senescence. First, tomato fruit with reduced PIF1a activity did not show senescence symptoms when incubated in the dark. Second, transient expression experiments in Nicotiana benthamiana leaves confirmed that the PIF1a protein (but not other tomato PIF homologues) is able to promote senescence. In contrast with these results, the Arabidopsis pif1 mutant shows no senescence-associated phenotypes, whereas mutants for PIF3, PIF4, and PIF5 showed a delayed senescence of dark-incubated leaves. Experiments to address the molecular basis of such differences between Arabidopsis and tomato PIF homologues are in progress.
P0145 THE IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF NOVEL CLV3 ALLELES FROM MICRO-TOM MUTANT COLLECTION

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1 Full text

Shoot Apical Meristem (SAM) maintains the undifferentiated cells in Organaizing center (OC), while providing the margin cells for organic differentiation. To balance between maintenance and differentiation of the cells, plants require for the function of CLAVATA3 (CLV3) and WUSCHEL (WUS). CLV3 encodes a small peptide protein with the signal peptide consisting of 12 amino acids and has two hydroxyproline and is known to be expressed in outer layer of SAM. Both organ differentiation and accumulation of undifferentiated cells within SAM are suppressed when CLV3 function is disrupted, consequently, the SAM structure became hypertrophy dome formation due to accumulation undifferentiated cells. In contrast, WUS is expressed in OC and known to play a role as a repressor of CLV3, whilst it active CLV3, showing a feedback mechanism to balance the maintenance and differentiation of the cells in SAM.

This research aims to gain further insight into the molecular function of CLV3. For this purpose, we screened for CLV3 mutants by Target Induced Local Lesions IN Genomes (TILLING) comprising of 9,000 Micro-Tom mutant populations. As a result, three mutant lines (6012, 7412, 7523) having missense mutations were screened. line 6012 homozygotes showed an increase in the number of calyx, petal and carpel. line 7412 homozygotes showed an increase in the number of calyx, petal and carpel, and organic conversion of anther to carpel was observed. Besides, line 7523 homozygotes produced increased number of calyx and petal and carpel, which results in production of largely fascinated fruit-like structure.
Tomato (Solanum lycopersicum) is a major crop plant. It is ideal for research and breeding because of the rich genetic resources and introgression lines within wild and crop varieties, the fully sequenced genome and the relative ease by which it can be transformed. Reactive Oxygen Species (ROS) and Ca\(^{2+}\) are second messengers that are part of many signaling cascades involved in developmental processes and stress responses in plants. They often act in the same signaling pathways, but details about the interaction mechanisms are only beginning to emerge. ROS and Ca\(^{2+}\) play a central role in responses to many abiotic stresses including salt (Na\(^{+}\)) stress. Respiratory Burst Oxidase Homologs proteins (RBOHs) are NADPH oxidases that produce ROS. They are regulated by phosphorylation and Ca\(^{2+}\) binding. Thereby, they form an interconnection of Ca\(^{2+}\) and ROS signaling. Arabidopsis RBOHF is involved in different signaling pathways such as cold response, drought or salt tolerance. It is expressed in endodermal root cells in the Caspian strip domain. Under external high Na\(^{+}\) concentrations, rbohF loss-of function mutants displayed enhanced Na\(^{+}\) uptake into the stele compared to wild type plants.
High mobility group AT-hook protein (HMGA) is a class of non-histone nuclear proteins. The characteristic of HMGA protein is the presence of ‘AT’ hook which preferentially binds to the minor groove of AT rich DNA sequences. HMGA proteins have the ability to unwind, bend and introduce supercoils in DNA substrates which causes them to interact specifically with transcription factors. The Solanum lycopersicum genome contains three genes encoding HMGA proteins and their function remains largely unknown in transcriptional regulation of fruit ripening. To understand the function of HMGA proteins, we first examined their expression in different fruit ripening stages of wild type (WT) and rin mutant. The expression of SlHMGA1 was significantly upregulated at red ripe stage (RR) as compared to mature green stage (MG) whereas the expression level SlHMGA2 and SlHMGA3 remained relatively same at both stages. In rin mutant at RR, the expression SlHMGA1 was significantly downregulated compared to WT, whereas the expression of SlHMGA2 and SlHMGA3 remains unaffected. Virus induced gene silencing of SlHMGA1 in tomato Del/Ros1 fruits resulted in the downregulation of lycopene and β-carotene contents. Taken together, these results provide a novel functional role of SlHMGA1 protein in tomato fruit ripening.
P0156 NGS-BASED IDENTIFICATION OF INDUCED MUTATIONS IN A DOUBLY MUTAGENIZED TOMATO (SOLANUM LYCOPERSICUM) POPULATION

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1 Full text

The identification of mutations in targeted genes has been significantly simplified by advent of TILLING, speeding the functional genomic analysis of animals and plants. The NGS-based mutation detection is gradually replacing classical TILLING, as it allows analysis of a large number of amplicons in short durations. The NGS-approach was used to identify mutations in a doubly EMS-mutagenized population of tomato (Solanum lycopersicum). Twenty-five genes belonging to carotenoids and folate metabolism were PCR-amplified and screened to identify potentially beneficial alleles. To augment the efficiency, the 600 bp amplicons were directly sequenced in a non-overlapping manner in Illumina MiSeq obviating the need for a fragmentation step prior to library preparation. A comparison of the different pooling depths revealed that heterozygous mutations could be clearly identified up to 128-fold pooling. Evaluation of six different software; GATK, CRISP, LoFreq, CAMBa, VipR and SNVer revealed that no software was robust enough to predict mutations with high fidelity. Among these, CRISP and CAMBa predicted mutations with lower false discovery rates. The false positives were largely eliminated by considering only mutations commonly predicted by two different software. The screening of 23.47 Mb of tomato genome yielded 75 predicted mutations, of which 64 were confirmed by Sanger sequencing with an average mutation density of 1/367 Kb. Our results indicate that NGS combined with multiple variant detection tools can reduce false positives and significantly speed up mutation discovery rate.
Micro-Tom is a model tomato cultivar which exhibits dwarfism and rapid life cycle with fruit maturity occurring 70–90 d after sowing, allowing for large-scale growth at a high density which is ideal for indoor cultivation in most plant biology laboratories. Further, Micro-Tom is capable of both intraspecific and interspecific cross-pollination with cultivated tomatoes and wild relatives, allowing for the production of mapping populations as well as for the transfer of mutations into commercially available varieties. As a part of the National BioResource Project (NBRP) funded by the Japan Agency Medical Research and Development (AMED), Japan, families of over 20,000 M₂ mutagenized lines of Micro-Tom, consisting of 15,000 and 6,422 lines were generated by EMS mutagenesis and γ-ray irradiation, respectively by May 2017. From the mutagenized populations, over 2,400 individual mutants were isolated and these visible phenotyping data and other associated data such as carotenoid content and Brix values, as well as seed request are available through the database ‘TOMATOMA’ (http://tomatoma.nbrp.jp/). In addition to the mutant resources, as DNA resources, the sequence information of Micro-Tom full-length cDNA and EST is available from database ‘KaFTom’ (http://www.pgb.kazusa.or.jp/kaftom/) and EST database ‘MiBASE’ (http://www.pgb.kazusa.or.jp/mibase/), respectively. Information on genome structural annotations between Micro-Tom and Heinz 1706 is accessible through the genome browser in ‘TOMATOMICS’ (http://bioinf.mind.meiji.ac.jp/tomatomics/). Our bioresources of Micro-Tom will support further acceleration of tomato researches.
P0189 PHYLOGENETIC ANALYSES AND MOLECULAR CHARACTERIZATION OF THE CYCLING DOF TRANSCRIPTION FACTORS FROM TOMATO SPECIES AND ANALYSIS OF THEIR ROLE IN ABIOTIC STRESS RESPONSES

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1 Full text

Tomato is one of the horticultural crops of major economic importance in Spain. However, its production is being affected by adverse environmental conditions such as salinity, drought and extreme temperatures. Recent studies have shown that the use of regulatory genes such as transcription factors (TFs) represents a power tool to obtain new tomato varieties with greater tolerance to abiotic stresses. The DOF (DNA binding with One Finger) proteins form a family of plant-specific TFs that are involved in the regulation of particular plant processes but their precise roles in abiotic stress tolerance are almost unknown.

Using the complete set of DOF genes from Solanum lycopersicum, we have searched for DOF genes in different tomato species. Sequence and phylogenetic analyses allowed us to classify them into four groups A-D, similar to the situation in Arabidopsis. In group D, we have identified DOF genes that show similar characteristics to the Cycling Dof Factors (CDFs) of Arabidopsis. These genes were considered orthologous to the Arabidopsis CDF1-5 named Solanum lycopersicum CDFs or SlCDFs. The expression analysis of the Solanum lycopersicum CDF1-5 genes showed distinct diurnal expression patterns. In addition, the SlCDF1-5 genes were differentially induced in response to osmotic, salt and low and high temperature stresses. Overall, our data provided new ideas of the evolution of DOF gene family in tomato and their roles during plant development and in response to different abiotic stress conditions.
P0191 ANALYSIS OF TRANSCRIPTION FACTORS WHICH REGULATE EXPRESSION OF GENES RELATED TO GABA ACCUMULATION IN TOMATO FRUITS

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Full text

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that exists in bacteria, animals and plants. In vertebrates, GABA is a major inhibitory neurotransmitter. It has been recognized as a functional component having roles in reducing blood pressure, neutral fat and stress. GABA is primarily metabolized via a short pathway, which is a bypass of the tricarboxylic acid (TCA), called the GABA shunt. In this pathway, GABA is synthesized from glutamate by the enzyme glutamate decarboxylase (GAD) and subsequently catabolized to succinate by GABA transaminase (GABA-T). In tomato, three SlGAD genes have been isolated and previously named as SlGAD1, SlGAD2 and SlGAD3. SlGAD2 and SlGAD3 play a predominant role in GABA synthesis in fruits, whereas, it remains yet question how these gene expressions are regulated. This research aimed to examine whether the down regulation of transcription factor, which had been isolated as candidate to control these gene expressions; SlbHLH (Solyc01g096370.2), was involved in GABA metabolism using RNAi strategy. Furthermore, we tried to reveal the function of SlbHLH. In the transgenic line in which SlbHLH expression was suppressed, SlGAD3 level was decreased in the immature green stage and mature green stage fruits. And the breaker stage was delayed a little in the SlbHLH-suppressed lines. These results suggested that SlbHLH is a transcription factor which positively regulates SlGAD3 expression. Alternatively, it is likely that SlbHLH related to the regulation of ripening. Additional experiment will clarify the mechanism how SlGAD gene expressions are regulated and the function of SlbHLH.
P0194 EVALUATION ON HEAT AND WATERLOGGING TOLERANCE OF TOMATO SEEDLINGS FOR BREEDING OF ELITE CULTIVARS
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1 Full text
Tomatoes are cultivated all year round in the facility, but stable production in the year is difficult due to poor growth in the hot summer season. In this study, we constructed a core group of 198 lines and investigated the growth characteristics of tomato seedlings in relation to heat and waterlogging tolerance. At the high temperature treatment of tomato seedling stage, the underground (root) fresh weight was significantly lower than that at the above ground level. When the fresh weight of the control (glasshouse, maximum temperature of 30.1°C / minimum temperature of 15.6°C) is 100, the fresh weight of the upper part of the high temperature treatment (growth phase, maximum temperature 42.0°C / minimum temperature 26.9°C, high temperature treatment period 2 weeks) was less than 50 in 84.9% and 50~100 in 15.1%. The fresh weight of the tomato at the high temperature treatment was higher than that of the control. When the fresh weight of the control was 100, the fresh weight of ground water in the waterlogging treatment was 67.2% in 50~100 and 32.8% in 100 or more. The underground fresh weight was 0.6% below 50 and 28.3% in 50~100, and 71.2% in 100 or more. It is considered that the tomato survives in the submerged water or partially regains the growth due to the occurrence of the adventitious root. The development of adventitious roots when tomatoes are submerged has an important effect on the recovery of stem growth after immersion.
Low calcium content in tomato fruits is one of the factors known to induce BER symptoms. However, reactive oxygen species (ROS), which can damage plant tissues, have also been proposed as inducing BER appearance in tomato. Ascorbate, the major antioxidant in tomato fruit, is generally lower during green fruit development, which corresponds to the stage of BER appearance. Accordingly, one hypothesis is that tomato cultivars with a lower susceptibility to BER under salt stress would have higher ascorbate contents and thus better control ROS levels. In this study, to clarify the relationship between BER incidence and oxidative stress, two BER resistant cultivars, ‘Managua RZ’ and ‘House Momotaro’ and one BER susceptible cultivar ‘Reiyoh’, were cultivated under salinity or control conditions. Total hydro-soluble antioxidants and ascorbate contents in the distal pericarp were measured, 1 to 2 days prior to symptom appearance and during symptom appearance, in healthy and affected fruits obtained in both cultivation conditions. When salt stress was applied, BER resistant cultivars showed significantly higher total antioxidant and ascorbate contents exclusively prior to BER appearance, as compared with their levels under control condition. Thus, total antioxidants and ascorbate were found to respectively increase by 44.6% and 63.6% in ‘House Momotaro’, 55.9% and 46% in ‘Managua RZ’, and only 27% and 0.06% in ‘Reiyoh’. The ability to increase the antioxidant capacity under salt stress condition might explain the resistance to BER development, in highly resistant cultivars, probably by the avoidance of oxidative induced cell necrosis.
P0199 MUTANTS GENERATED BY TARGET-AID TECHNOLOGY DEMONSTRATES THE FUNCTIONAL ROLE OF SLETR2 IN FRUIT RIPENING

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1 Full text

Several fruit-ripening mutants have been reported in tomato and demonstrated that the causative genes act as upstream regulators of the ethylene signaling network. Tomato has seven ethylene receptor genes in its genome, and its function has confirmed in some of the genes. However, function of SlETR2 is not still demonstrated. Therefore, in our previous study, we tried to isolate the SlETR2 mutants with amino acid substitution in its transmembrane domain from Micro-Tom mutant libraries using our TILLING platform. However, we could not isolate such mutants from mutant library over 9,000 EMS mutant lines, indicating the difficulty of mutant isolation using this technology. In this study, we aimed to generate mutants that have amino acid substitution in second transmembrane domains of SIETR1 and SIETR2 by Target-AID (target-activation-induced cytidine deaminase) technology. Several induced-mutants with amino acid substitutions in the target region were isolated. In triple response assay that investigating the ethylene responsiveness, any mutants did not show clear ethylene insensitive or reduced sensitivity phenotype. However, in the fruits ripening stage, both of SIETR1 and SIETR2 targeted mutants showed weak prolonged shelf life. And the double mutants showed strong delayed ripening and prolonged fruit shelf-life phenotype. These results suggest Target-AID is suitable technology to produce mutants with amino acid substitution, and firstly demonstrate the function of SIETR2 in ethylene signaling network. This work was supported by Cabinet Office, Government of Japan, Cross-ministerial Strategic Innovation Promotion Program (SIP), “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).
P0207 GLANDULAR TRICHOME IV INHERITANCE IN AN F2 POPULATION OF SOLANUM GALAPAGENSE × SOLANUM CHEESMANIAE

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1 Full text

Glandular trichomes of plants are bio factories for the production, storage and secretion of specialized metabolites. Among tomato wild relatives the presence of glandular trichome type IV is associated with pest resistance, including whiteflies. Production of glandular trichome IV is expected to be controlled by only two loci in S. galapagense PRI95004. However introducing glandular trichome IV to cultivated tomato may be more complicated. The accession S. galapagense contains high density of glandular trichome type IV like S. galapagense PRI95004. In contrast S. cheesmaniae is highly populated by non-glandular trichome type V. Phylogenetic studies showed that S. galapagense and S. cheesmaniae are very closely related species and close relatives of the cultivated tomato. This high level of genome synteny together with an extreme difference for glandular trichome IV production makes it interesting to study the inheritance of glandular trichome IV in a S. galapagense × S. cheesmaniae population. The genome similarity of this S. galapagense and S. cheesmaniae is around 12 times more than S.galapagense PRI95004 and S. cheesmaniae. This study aimed to estimate the association between small genetic differences present in these two extremes and glandular trichome IV presence/density on the F2 population derived from S. galapagense × S. cheesmaniae.
P0213 HIGH-THROUGHPUT MIRNA PROFILING OF TOMATO PLANTS UNDERGOING HERBIVOROUS INSECT ATTACK

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1 Full text

The coleopteran insect Leptinotarsa decemlineata (Colorado potato beetle, CPB) is a devastating pest of Solanaceae species such as potato, tomato and eggplant. Plants respond to herbivore attack through a variety of molecular mechanisms to counteract the detrimental effects on the plant. To characterize the response of tomato plants to CPB larvae damage, high-throughput sequencing analysis of microRNAs (miRNAs) was performed. miRNAs are a large class of small non-coding RNAs molecules of 19-25 nucleotides. They are involved in gene expression regulation by cleaving or inhibiting the translation of target gene transcripts. In this study, we used miRNA-Seq to investigate the miRNA differential expression between tomato plants infested or not with CPB larvae. A total of 10 550 413 raw reads were generated by high-throughput sequencing from 6 small RNA libraries obtained from control plants (3 biological replicates) and CPB-infested plants (3 biological replicates). A total of 64 known miRNAs and 12 novel miRNAs were identified. Six miRNAs exhibited a statistically significant ≥ 2-fold change in expression (Padj value ≤0.1) in plants infested with CPB (2 down-regulated miRNAs and 4 up-regulated miRNAs). Two miRNAs were randomly selected and validated by RT-qPCR. Putative miRNA target prediction was performed using miRNAconsTarget and psRNATarget software servers.
P0218 INFERRING THE GENETIC DETERMINANTS OF FRUIT COLORS IN TOMATO BY CAROTENOID PROFILING

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1 Full text

Carotenoids are essential for plant and animal nutrition, and are important factors in the variation of pigmentation in fruits, leaves, and flowers. Tomato is a model crop for studying the biology and biotechnology of fleshy fruits, particularly for understanding carotenoid biosynthesis. In commercial tomato cultivars and germplasms, visual phenotyping of the colors of ripe fruits can be done easily. However, subsequent analysis of metabolic profiling is necessary for hypothesizing genetic factors prior to performing time-consuming genetic analysis. We used high performance liquid chromatography (HPLC), employing a C30 reverse-phase column, to efficiently resolve nine carotenoids and isomers of several carotenoids in yellow, orange, and red colored ripe tomatoes. High content of lycopene was detected in red tomatoes. The orange tomatoes contained three dominant carotenoids, namely δ-carotene, β-carotene, and prolycopene. The yellow tomatoes showed low levels of carotenoids compared to red or orange tomatoes. Subsequent genetic analysis using DNA markers for segregating population and germplasms were conducted to distinguish previously identified mutants. This study establishes the usefulness of metabolic profiling for inferring the genetic determinants of fruit color and selecting unidentified mutants.
Anthocyanins are common phenolic pigments responsible for most of the red, purple and blue colours of flowers, fruit and vegetables. The different colours are largely determined by the degree of B-ring hydroxylation, with increasing hydroxylation causing a shift from the red end of the visible spectrum to the blue.

Besides colour, this hydroxylation pattern could also affect other properties, including the ability to confer resistance to plant pathogens or protection against human diseases by dietary anthocyanins. To assess these intriguing possibilities, we have developed different tomato lines that accumulate the three classes of anthocyanins: pelargonidin (one hydroxyl on the B-ring, as found in strawberry), cyanidin (two hydroxyls, as in raspberry) or delphinidin (three hydroxyls, as in blueberry).

The combinations of fruit-specific expression of regulatory and biosynthetic genes with the availability of a natural mutant resulted in fruit with striking and distinctive colours. UHPLC MS/MS analysis of the metabolically engineered tomatoes confirmed the positive outcome of our strategy and identified individual compounds with the predicted pattern of B-ring hydroxylation.
P0223 IDENTIFICATION AND CHARACTERIZATION OF A TOMATO INSERTIONAL MUTANT WITH AN ALTERED HYPERSENSITIVE RESPONSE

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1 Full text

The hypersensitive response is an essential process in the development of multicellular organisms, as well as to generate mechanisms of defense against certain external agents or the attack of some pathogens. The imm (lesion mimic mutants) mutants have been very useful as they have altered the mechanisms of stress response and appear to activate the hypersensitive response induced by pathogens, even though they are not being attacked. However, similar mutants should be identified that allow a deeper understanding of these mechanisms in crop species.

After the scrutiny of a collection of T-DNA lines we have identified a tomato mutant with an alteration in the hypersensitive response. The initial development of the mutant was similar to that of WT plants. However after a few weeks under control conditions the activation of the hypersensitive response could be observed both in vitro and in vivo. Mutant plants grown in the greenhouse for several months not only showed necrotic spots on leaves and stems, but also in the reproductive organs. In spite of this, they were able to produce fruits with viable seeds. The genetic analysis indicated that the phenotype was due to a recessive mutation and that this line carried a single T-DNA insert with a functional nptII gene. In addition, our results indicated that the mutant phenotype co-segregated with the T-DNA insert. At present we are tackling the identification of the tagged gene.

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This work was supported by grant AGL2015-64991-C3-3 and AGL2015-64991-C3-1 from the Spanish Ministry of Economy and Competitiveness
P0226 STUDY OF QUALITY TRAITS IN A SET OF TOMATO INTROGRESSION LINES FROM SOLANUM PIMPINELLIFOLIUM

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1 Full text

We have studied the soluble solids content and the titratable acidity in a set of 14 introgression lines (ILs) from Solanum pimpinellifolium into the genetic background of the Moneymaker, plus the cultivar Moneymaker. Plants were grown in a mesh greenhouse, in the spring-summer 2015 crop cycle in Orihuela (South-East Spain). The values of soluble solids were similar to those obtained in previous studies (2013). The IL with the highest content of soluble solids consistent in the two years was IL 9-1. This result suggests that the fragment of S. pimpinellifolium containing IL 9-1 has genes that increase the soluble solids content of the fruit. The titratable acidity values obtained were slightly higher than those of 2013 in all the studied ILs. In the correlations study, the high correlation value was obtained between the soluble solids content and the titratable acidity, as well as not significant correlations were obtained with the productive characters.
We have studied the effect of the introgression of Tm-2a and Ty-1 genes, which confer resistance to ToMV and TYLCV respectively, on leaves and shoots presence in the first three inflorescences of Muchamiel and De la pera tomato plants. Recombinants within the introgressions carrying those genes were previously obtained in order to check if linkage drag effects could be avoided and distinguish between pleiotropy and linkage. Recombinant and not recombinant progenies were grown in a mesh greenhouse, in the spring-summer 2017 crop cycle in Orihuela (South-East Spain). We studied the presence of leaves and shoots in the first three inflorescences in each plant. In De la pera, differences between the progenies were obtained, but were not associated with the size of the recombinant introgression region containing the Ty-1 gene. However, there was a clear association in two of the three Muchamiel families: recombinant lines had the lowest number of stems and leaves in the inflorescences. For the region containing Tm-2a gene, no differences on inflorescence typology were found within the two Muchamiel families. These results suggest that the chromosome region containing Ty-1 gene affects the inflorescence typology.
P0234 IMPACT OF HEAT STRESS ON TOMATO FRUIT QUALITY

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1 Full text

In tomato, the accumulation of carotenoids and other health-promoting compounds during fruit ripening is not only controlled by developmental program but also influenced by environmental signals and abiotic stresses. Although higher temperatures (a climate change-driven stress) have been demonstrated as one of the main factors controlling the fruit set and consequently yield in tomato, little is known about its consequences on fruit quality. In order to elucidate the cellular and molecular mechanisms underlying the response to elevated temperatures, transgenic tomato lines engineered with distinct carotenogenic-related genes (OE-PSY1, constitutively overexpressing tomato PHYTOENE SYNTHASE1; OE-CrtI, constitutively overexpressing bacterial phytoene desaturase CrtI; RNAi-DET1, with fruit-specific suppression of the transcription factor DE-ETIOLATED1, a component of the light signal transduction pathway), and producing different qualitative and quantitative carotenoid profiles, were exposed to short-term moderate heat stress at mature green fruit stage in greenhouse. The results obtained from the comprehensive phenotyping including analyses of fruit subplastidial fractions, metabolic and transcriptional profiling performed will be discussed.
P0288 OPTIMIZING SHELF-LIFE CONDITIONS FOR ANTHOCYANIN-RICH TOMATOES

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1 Full text

Shelf life is the length of time a product can be stored without becoming unfit for use. This is one of the most important quality traits in the tomato cultivation and it is usually shortened due to fast over-ripening caused by several different factors including changes in temperature, respiration and pathogen exposure. Genetically modified anthocyanin-rich tomatoes have already been shown to possess a significantly extended shelf life by delayed over-ripening and reduction of the susceptibility to certain pathogens.

In the present work, we compared different conditions of postharvest storage of anthocyanin-rich tomato fruits derived from conventional breeding. We took into consideration different light and temperature conditions and the combination of two. We analyzed several quality traits related to the fruit ripening including sugar level, pH, carotenoid content and anthocyanin accumulation during three weeks of the fruit storage. The aim of this study was to establish a protocol for optimal postharvest storage of these fruits, especially looking at extended shelf life with possible prolonged anthocyanin accumulation.

From our results we identified the most suitable light and temperature parameters for the fruit storage and postharvest anthocyanin accumulation among all the conditions tested. Under these parameters tomato fruits resulted with prolonged anthocyanin accumulation and unchanged flavor-related features up to three weeks after harvesting. These results also confirm that the presence of anthocyanins in naturally derived anthocyanin-rich tomatoes can have a significant impact on the shelf life extension.
Cold storage at around 12°C is recommended to reduce tomato decay and maintain tomato fruit quality while avoiding a reduction of tomato flavour that can occur when fruit are stored below the recommended temperature. Numerous reports have demonstrated that tomato storage at 5°C (household refrigerator temperature) affects the production of important tomato volatiles and a few of them showed that low temperature also affected the sensory evaluation or the consumer liking. However, the number of genotypes tested was very limited. In this work, the individual effects of storage time and low temperature on tomato flavour were evaluated using several tomato genotypes that contrast in their organoleptic quality. Fruits stored during one and two weeks at chilling (5°C) and non-chilling (15°C) temperature were compared with fresh harvested fruits using a consumer and/or an expert panel. Changes in fruit quality parameters (texture, soluble solids content, and acidity), primary metabolites, and volatile organic compounds (VOCs) were also measured. Our results indicate that although cold storage did impact the metabolic profile in all genotypes, these metabolic changes were only translated in a significant effect on tomato flavor in genotypes with low flavor quality (low levels of sugars, acids and VOCs). Moreover, the sensory data showed that in some cases the effect of storage was larger than the effect of low temperature. We hypothesized that the original organoleptic quality of the genotype is an important determinant of the effect of storage and low temperature on tomato flavor.
P0322 FORMATION AND SUBSEQUENT DEVELOPMENT OF AXILLARY BUD AFFECTED BY DOMINANT-NEGATIVE MUTANT ALLELE IN MICRO-TOM

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1 Full text

The axillary bud develops the axil of leaf and has the potential to form shoot or floral cluster. The growth of axillary meristems is known to be inhibited by the primary shoot or primary inflorescence. Pruning treatment can increase the size and quality of fruits in tomato cultivation, however, removing the growth point accompanies with depressing axillary bud growth which require much workload to take care the branch formation. An axillary bud suppression mutant (abs1) was found from a comprehensive Micro-Tom EMS-mutant population. The abs1 produced either vegetative shoots (stems and leaves) or reproductive shoots (flowers) with no observable abnormalities in specification of floral organs. The phenotype of significantly repressed axillary bud in the abs1 mutant and F1 hybrid, compared to the wild type, implies that the abs1 has a dominant-negative mutant allele of casual gene. Repression of axillary buds potentially utilized for facilitation of tomato cultivation, and a dominant-negative mutant allele can easily reveal its impact in hybrid breeding that improves working efficiency. Extensive genomic information has encouraged investigation on genetics of current mutant that insurance cloning genes underlying prominent phenotypes.
P0332 MORPHOLOGICAL, AGRONOMIC AND QUALITY CHARACTERIZATION OF ‘DE PENJAR’ TRADITIONAL TOMATO VARIETIES IN DIFFERENT GROWING SYSTEMS

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1 Full text

The so-called ‘De Penjar’ tomato is a traditional, long shelf-life landrace, well known in the Mediterranean region. This varietal type is highly appreciated for its ability to be stored for months without refrigeration and for its organoleptic characteristics. In the Spanish Mediterranean region it is mostly used for rubbing it on to bread slices. However, there is a lack of studies on its agronomic performance and quality characteristics depending on the growing system. In order to shed light on this, we performed a morphological, agronomic and quality characterization of three local Valencian varieties of ‘De Penjar’ tomato in two systems of cultivation (open field and greenhouse). The results obtained indicate that the three local varieties, despite presenting morphological differences, showed a good performance in both cropping systems, with few productive differences among them (4 – 4.5 kg/plant). Likewise, the quality attributes that are obtained are also very promising in both the open field and greenhouse systems. These three varieties have a good Brix degrees level. Especially interesting are the high levels of vitamin C and antioxidants. These results are very promising for the use of these varieties in protected cultivation (greenhouse) and to have this high quality product widely demanded by consumers during most of the year.
P0344 BEMISIA TABACI RESISTANCE IN A SOLANUM GALAPAGENSE RIL POPULATION

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1 Full text

_Bemisia tabaci_ is a phloem-feeding insect (whitefly) with many hosts. The main danger of _B. tabaci_ is that it can vector various viruses that cause large yield losses. Cultivated tomato is susceptible to this whitefly. Glandular trichomes are specialized tissues on the epidermis of leaves/plants that are associated with whitefly resistance and present in many wild relatives of tomato. Glandular trichomes can synthesize, store and emit a variety of specific specialized metabolites that play a role in pest resistance and in the interaction with the environment. _Solanum galapagense_ is one of the wild tomato species which is resistant to whitefly. This species is well covered by glandular trichomes (type IV) where different acyl sugar compounds are produced. To study the genetics of glandular trichome IV formation and acyl sugar production in _S. galapagense_ we carried out a QTL analysis in an F7 RIL population. A major QTL for trichome formation and acyl sugar production was found at the bottom of Chromosome 2, together with a few minor QTL located on other regions of the genome. These results suggest that development of trichome IV and production of some acyl sugars is regulated by a major gene(s) at the bottom of Chromosome 2 that play a role in whitefly survival on _S. galapagense_.

P0348 IMPROVING THE TOMATO REFERENCE GENOME AND ANNOTATION USING FULL-LENGTH BACS AND DIVERSE EXPRESSION RESOURCES

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1 Full text

We describe here our efforts to improve the tomato reference genome and the corresponding annotation. We have integrated 1,069 full-length phase hts3 BACs into the tomato genome to cover gap regions and replace shorter whole genome shotgun contigs which removed 11.7Mb of contig gaps. BioNano optical maps generated for heinz 1706 largely confirmed the reference genome assembly and excluding some minor structural and homopolymer corrections.

We also present an improved annotation (ITAG 3.2) for the new tomato reference genome. The annotation pipeline involved updating ITAG2.40 gene models, preserving locus identifiers (30,868) from the previous ITAG2.40 annotation and identifying new gene models using newly trained ab-initio gene predictors. We have incorporated expression data from various sources in our pipeline including tissue and treatment specific RNAseq data, 5' and 3' UTR enriched RNAseq data, RENseq for NBS-LRR genes and Pacbio RNA Iso-seq data which were all kindly provided by Solanaceae community. The de novo prediction of genes in the MAKER pipeline has identified 4,900 novel genes well-supported by expression evidence. Annotation of all the genes has associated quality metrics for structure and functional characterization. The increased continuity of the SL3.0 build and availability of diverse and comprehensive expression data has resulted in a significantly improved tomato annotation (ITAG3.2).

All data are available through the SOL Genomics Network website (SGN, https://solgenomics.net) and FTP site (ftp://ftp.solgenomics.net/tomato_genome/).
Improvement of the yield and quality in tomato has been a driving force behind breeding efforts and research in recent years. Much of this work has gone to improving flowering and fruit traits, largely ignoring vegetative factors such as leaves. In other systems, photosynthetic improvement has been identified as a way to improve yield. However, our early work has shown that there is a link between leaflet shape, leaf complexity, and the total quality of the fruit (yield and BRIX). A common understanding in the general populace is that heirloom tomatoes taste better and have better overall quality, with a subset of heirlooms called potato leaf types being the best. To explore this correlation, a population of twenty potato leaf heirloom tomatoes were grown in the field. Measurements of photosynthesis, stomatal conductance, leaflet shape, leaf complexity, and other factors were measured over 14 weeks of the growing season. The data was used to construct a Partial Least Squares-Path Model to identify important pathways leading to fruit quality. Our data shows that leaflet shape and size is a strong determining factor in final fruit quality, with rounder leaflets positively correlated to improved yield and BRIX. Our data also shows that photosynthesis is negatively correlated with fruit quality, improving vegetative biomass but reducing fruit traits. These results not only show the complicated interconnected relationships within a plant, but highlight two novel findings: Leaf shape is an important driver of fruit quality, and photosynthesis negatively influences fruit quality in tomato.
Towards the Identification and Characterization of Novel Regulators of Fruit Tissue Morphology in Tomato

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1 Full text

Tissue morphogenesis and overall fruit growth depend on complex cellular and molecular interactions that affect the balance between cell division and expansion. The molecular control of fruit tissue morphogenesis and growth remain largely underexplored and only few regulators have already been identified. Forward genetics appears as the most powerful approach to decipher these processes and their regulation, because natural and artificially-induced genetic variability offers invaluable resources for discovering new phenotypes. Thanks to the availability of tomato genomic sequence and WGS technologies, linking genotypic variations to associated phenotypic changes is now more accessible for fleshy fruit.

The phenotypic characterization of twelve EMS mutants revealed two additional modules controlling fruit growth that coordinate either the growth of the whole fruit or that of pericarp, through either isotropic or anisotropic cell expansion. Whole-genome-sequencing of representative mutants allowed us to demonstrate that these modules were not related to genes and processes previously described. We recently developed the mapping-by-sequencing approach (Garcia et al, 2016) that permitted the identification of a novel regulator encoded by the Guanylate Binding Protein (GBP) gene. Mutant alterations suggest a role for this protein in cytoskeletal organization and cell wall modifications with impact on tissue morphology and fruit growth.

References
Large scale phenotyping of tomato remains a daunting task when multiple traits or quantitative differences are measured. In addition, assessing the effect of different environmental conditions on various traits such as fruit yield or resistance to high temperatures usually necessitates multiple experimental trials. These are often carried out in different conditions (e.g. glasshouses or plastic greenhouses) and countries and at different periods of the year. This greatly complicates the data comparison between experiments. Novel approaches are required to screen the existing tomato germplasm for identifying genetic variations underlying important agronomic traits and explore the mechanisms they control.

We recently generated an EMS mutant collection in the miniature cv. Micro-Tom (Just et al., 2013; Garcia et al., 2016). We show here that the high-throughput phenotyping platform from INRA Dijon (https://www6.dijon.inra.fr/umragroecologie/Plateformes/Serres-PPHD) is well adapted to the automated phenotyping of Micro-Tom for major traits, including the fruit yield and root architecture traits. In a first study, we showed that standard segmentation analysis of images automatically captured along the whole developmental cycle of tomato plants already permits to precisely follow the phenology of the plants, including fruit developmental stages, and to give a very good estimate of fruit and plant biomass. In a second study, we showed that the RhizoTube™/RhizoCab™ system allows dynamic tracking of root development by image analysis with existing algorithms. Further processes specifically developed for tomato will enable more precise phenotyping of the various traits studied.
P0360 FUNCTIONAL CHARACTERIZATION OF SLGRAS10: UNVEILING ITS ROLE IN CLIMACTERIC FRUIT RIPENING.

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1 Full text

GRAS transcription factors integrate plant growth and developmental signals and were named after the first three proteins identified in Arabidopsis thaliana, GIIBBERELLIC ACID INSENSITIVE (GAI), REPRESSOR OF GA1 (RGA), and SCARECROW (SCR). More than 50 GRAS proteins have been identified in tomato but their participation in fruit ripening is still unknown. Genome-wide analysis of the GRAS gene family in Solanum lycopersicum and Vitis vinifera suggested an involvement of some members in fruit ripening, with SGRAS10 as a candidate to control some aspects of climacteric fruit ripening. The aim of this study is to disclose the role of SGRAS10 in tomato ripening using reverse genetics. To this end two single guide RNAs were designed to target different sites of the SGRAS10 using the CRISPR/Cas9 genome editing system in a GB cloning framework. Tomato (var. MoneyMaker) stable transformation was performed, shoots obtained and the effect of CIRSPR/Cas9 on SGRAS10 analyzed by PCR and sequencing. Off-target sites predicted using Cas-OFFinder algorithm will be analysed in the same tomato lines. Additionally, tomato plants overexpressing the SGRAS10 under control of 35S and E8 promoters, are being developed to study the effect of upregulation of SGRAS10 on fruit during ripening. In silico analysis of SGRAS10 promoter revealed several regulatory motifs associated with light regulation, and ethylene and salicylic acid signaling, as well as transcription factors associated with developmental processes (e.g., MADS box, Myb, and bHLH). This work will characterize for the first time the role of a GRAS transcription factor in fruit ripening.
The challenge is to breed tomato varieties that have a reasonable postharvest shelf life while maintaining excellent eating quality. Cell wall remodelling occurs during the process of tomato fruit softening and is a consequence of the combined action of multiple gene products. More than 50 cell wall modifying genes are expressed during fruit development and ripening (Tomato Genome Consortium, 2012). In this project, we are focusing on several of the most highly expressed ripening-related cell wall genes including POLYGALACTURONASE (PG2a), PECTATE LYASE (PL), BETA-GALACTANASE (TBG4), and xyloglucan endotransglucosylase-hydrolase (XTH5) to test if knocking them out by DNA editing can influence the progress of tomato fruit softening and extend shelf life.

The CRISPR/Cas9 system was efficient in tomato for targeted gene editing. Gene expression and enzyme activity were significantly reduced in CRISPR transgenic lines compared with the control azygous wild-type line. We evaluated the physicochemical characteristics in a range of CRISPR transgenic fruit and azygous wild type controls. Only the PL CRISPR lines showed substantial changes in fruit firmness. CRISPR edited PG2a, TBG4 and XTH5 produced fruits that showed a similar degree of softening to wild type controls. Experiments to investigate the effect of the CRISPR mutations on processing quality indicated that genes modifying pectin metabolism especially polyuronide chain length, such as PG2a and PL had a greater impact on paste viscosity than those that acting on pectin side chains or hemicelluloses. Immunocytochemistry is underway to further investigate the effects of the CRISPR induced mutations on tomato cell wall structure.

Reference


Uluisik et al., 2016. Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnology. 34(9), doi:10.1038/nbt.3602
P0372 GENETIC ANALYSIS OF ROOTSTOCK-MEDIATED NITROGEN UPTAKE AND ROOT-TO-SHOOT SIGNALLING AT CONTRASTING NITROGEN AVAILABILITIES IN TOMATO

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1 Full text

Selecting rootstocks for high nitrogen (N) acquisition ability may allow decreased N fertilizer application without reducing tomato yields, minimizing environmental nitrate pollution. A commercial hybrid tomato variety was grafted on a genotyped population of 130 recombinant inbred lines (RILs) derived from Solanum pimpinellifolium, and compared with self- and non-grafted controls under contrasting nitrate availabilities (13.8 vs 1.0 mM) in the nutrient solution.

Grafting itself altered xylem sap composition under N-sufficient conditions, particularly Na⁺ (8.75-fold increase) concentration. N deprivation decreased shoot dry weight by 72.7% across the grafted RIL population, and one RIL rootstock allowed higher total leaf N content than the best of controls, suggesting more effective N uptake.

Sixty-two significant QTLs were detected by multiple QTL mapping procedure for leaf N concentration (LNC), vegetative growth, and the xylem sap concentrations of Mn and four phytohormone groups (cytokinins, gibberellins, salicylic acid and jasmonic acid). Only three LNC QTLs could be common between nitrogen treatments. Clustering of rootstock QTLs controlling LNC, leaf dry weight and xylem sap salicylic acid concentration in chromosome 9 suggests a genetic relationship between this rootstock phytohormone and N uptake efficiency. Some candidate genes were found within 2 Mbp intervals of LNC and hormone QTLs that deserve future functional analyses.
OP05: POTATO

P0012 THE EFFECT OF THE LEAF EPIDERMAL STRUCTURE ON TOMATO YELLOW LEAF CURL VIRUS DISEASE RESISTANCE

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ABSTRACT: Tomato yellow leaf curl virus (TYLCV) was vectored by whiteflies. TYLCV caused significant crop losses in tomatoes worldwide. After infection, the plants had retarded growth, erect shoots, yellowing and curling of the leaves, and misshaped and markedly smaller leaflets. Two TYLCV-susceptible varieties ‘DiOuHongGuan’, ‘FenKaWo’ and two highly TYLCV-resistant varieties ‘Mao T’, ‘FenLanDi’ were used to describe some of the microscopic feature of the disease distinction between TYLCV-resistant and TYLCV-susceptible tomato plants after infection. The result showed that the resistant varieties had more villi and root hair, more stronger root hair, than susceptible varieties. ‘Mao T’ had the most villi, and 2-3 bifurcation on each villi. After inoculation, the mesophyll cells in the susceptible plants became large and irregular shape. There was no deformation in the resistant plants while the veins of the infected leaves were deformed in the susceptible plants as well as the companion cells adjacent to the sieve element. Therefore, the number of villi and root hairs of resistant varieties were more than susceptible varieties, and the root hair was more stronger than susceptible varieties. The cell had markedly change in susceptible region, and performance is normal in not susceptible parts.

This work was supported in part by innovation project of Chongqing animal and plant varieties - Innovation of high quality, disease resistant and high yield vegetable varieties
P0021 APPLICATION OF MOLECULAR MARKERS IN TETRAPLOID POTATO FOR EXTREME RESISTANCE GENES TO POTATO VIRUS X (PVX)

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1 Full text

Potato virus X (PVX) is one of the main potato viruses infecting potatoes worldwide. Utilization of resistant cultivars is the most cost-effective and reliable approach to control potato viruses. Two dominant genes, Rx1 and Rx2, confer extreme resistance to PVX in potato. This study was performed to screen 35 commercial cultivars and 24 advanced potato clones for Rx1 and Rx2 genes using phenotypic and molecular marker experiments. The reaction of genotypes to PVX was evaluated based on CIP standard method by mechanical and graft inoculation in the greenhouse condition. The result of both experiments showed that 12 potato commercial cultivars and 11 advanced clones are extremely resistance to PVX. Other genotypes showed different level of infection to PVX and considered as susceptible. For Rx1 gene, the published specific molecular marker, 5Rx1, amplified the expected 186 bp fragment in cultivar Cara, as control, and resistant genotypes in phenotypic test such as Agria, Fontane, Maranel, Opal, Lady Rosetta and Labadia; and further eight advanced clones. Application of published specific primer pairs, 106Rx2, for amplification of 543bp fragment in Rx2 gene revealed that resistant cultivars including Elodie, Lorrete, White Lady and Luca and further four advanced clones carry Rx2 gene. In the PVX susceptible genotypes, no PCR product could be detected which was completely matched with the result of phenotypic test in the greenhouse. It was concluded that specific molecular markers, 5RX1 and 106RX2, are reliable and promising for identification of Rx1 and Rx2 genes in breeding programs.
Solanesol is a 45-carbon, all-trans-nonaprenol, first isolated from flue-cured tobacco. Solanesol itself has useful medicinal properties and is an intermediate in the semisynthetic production of coenzyme Q10, a medicine used in the treatment of cardiovascular disease, cancer and atherosclerosis. Solanesol levels of up to 3.6% dry weight have been reported in tobacco leaves, the current industrial source. Solanesol is also present in other plants from the Solanaceae although a detailed survey of levels in these species is lacking as is comprehensive understanding of the biosynthetic pathway and its regulation. An alternative, economically viable source of solanesol may be developed from the foliage of Solanaceous crops other than tobacco that are normally discarded as waste. In view of the commercial potential of solanesol as a co-product in potato production, the aim of this study was to establish genetic and environmental factors that impact on solanesol accumulation. Transient over-expression of genes from the methylerythritol 4-phosphate (MEP) and mevalonic acid (MVA) pathways resulted in enhanced accumulation of solanesol in leaves of Nicotiana benthamiana. Expression of a potato solanesyl diphosphate synthase (SDS) gene, which underlies a major QTL for potato leaf solanesol content, produced the largest change in solanesol levels. We also demonstrate that in potato, leaf solanesol content is increased by up to six-fold on exposure to moderately elevated temperature and further enhanced when plants are subjected to drought conditions. Combined genetic and environmental approaches offer new insights into solanesol accumulation and strategies for developing a bio-refinery approach to potato production.
Synchytrium endobioticum is a soil borne obligate biotrophic fungus responsible for the formation of warts on the tubers of potato (Solanum tuberosum). The potato wart disease causes important yield losses and has a worldwide quarantine status. In Europe, more than 30 different pathotypes have been recorded, among which pathotype 1 is the most common. Identification of pathotype specific resistance genes and development of closely linked markers is essential for breeding resistant potato varieties. A Genome Wide Association Study was performed in a panel of potato varieties representative of the European germplasm. SNP markers, significantly associated with pathotype 1 resistance, confirmed the position of the Sen1 locus within the well-known subtelomeric R gene cluster on the north arm of chromosome 11 (Hehl et al., 1999). Most SNPs could be validated in biparental offspring, and were used for high resolution mapping. Nevertheless, a worrying number of false positive or false negative marker predictions were found in a large panel of potato varieties. We could solve some ambiguities by re-phenotyping of varieties. Other ambiguities were solved upon analysis of the haplotype specificity of SNP alleles. This allowed us to show that multiple founders have contributed alleles conferring resistance. A complicated haplotype structure was solved here for a qualitative monogenic trait. This approach shows a way forward to solve the missing heritability for complex quantitative traits.
Anthocyanins are the universal plant colorants, responsible for the red, purple, and blue hues evident in many fruits, vegetables, cereal grains, and flowers. In an endeavor to identify the active health-promoting ingredients, many researchers have focused on these compounds, because their antioxidant properties. It has been reported a high correlation between the color intensity, the anthocyanins concentration, and the antioxidant level. We identified and quantified the anthocyanins present in boiled tubers of 109 accessions belong to the Potato Work Collection of the Breeding Program at the National University of Colombia, using High Performance Liquid Chromatography. Five anthocyanins were identified, delphinidin, cyanidin, petunidin, pelargonidin and peonidin, they present a large variation in their concentration among genotypes, rang from 0 to 189.13 mg/g. The accessions are grouping in six clusters according to the type and concentration of anthocyanins. In the varieties with skin and purple flesh, the cyanidin and petunidin were predominant in comparison with red varieties that show higher content of pelargonidin. A Genome Wide Association Study for this trait was conducted with 3,791 SNPs obtained by GBS, using an analysis with Compression Mixed Linear Model by means of GAPIT program. The association analysis identified significant marker-trait associations for each anthocyanin. These results contribute to understand the natural allelic variation present for anthocyanins in potato, and identify genotypes that can be use in breeding programs to develop varieties with high concentration of anthocyanin that could contribute as protectors of human health.
P0206 HOW DO FLOWERING AND TUBERISATION IN POTATO PLANTS COMPETE?
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1 Full text
Potato plants (Solanum tuberosum L.) can reproduce both sexually through flowering and asexually through tuberisation. While tuber formation has been thoroughly studied, little research has been done on potato flowering. Short-day conditions induce tuberisation; however, in some genotypes short days impair flower bud development. This impaired development could indicate that day-length is regulating flowering oppositely to tuberisation, or that tuberisation and flowering are influencing each other. To understand how external factors like day-length regulate potato flowering, we must determine whether there is internal regulation of potato flowering by competition with tuberisation. To determine whether the absence of tubers improved flowering we prevented tuberisation in three different ways: (1) by grafting potato scions onto wild potato rootstocks, that were unable to form tubers; (2) by removing stolons, the underground structures on which tubers form; (3) by using transgenic plants that were silenced in the tuberisation signal StSP6A. The absence of a tuber-sink did not accelerate flower bud development or enable more plants to reach anthesis. However, reduced StSP6A expression enabled plants to properly develop their flower buds in short days. These experiments suggest that the tuberisation signal StSP6A plays a role in repressing flower bud development in potato, independently of the tuber-sink.
Plant shoot systems give rise to characteristic above-ground plant architectures. Shoots are formed from axillary meristems and buds, whose growth and development is modulated by systemic and local signals. These cues convey information about nutrient and water availability, light quality, sink/source organ activity and other variables that determine the timeliness and competence to maintain the development of new shoots. The information is then translated into a local response, in meristems and buds, of growth or latency. We are investigating the genetic pathways controlling systemically and locally the developmental decision of buds to grow or to remain dormant.

In Solanaceae we have found that like in other angiosperms, the TCP factors BRC1a and BRC1b participate in this process. Now we are investigating the degree of conservation of other components of the network controlling shoot branching, upstream and downstream of BRC1a and BRC1b, in particular hormonal signalling pathways, other master regulators and transcriptional gene regulatory networks. Our most recent findings will be presented.
P0287 GENOME-WIDE ANALYSIS OF SOLANUM COMMersonII SMALL NONCODING RNAs AND EXPLORATION OF THEIR ROLE IN THE RESPONSE TO COLD STRESS

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1 Full text

Recent studies have shown that stress induces aberrant expression of many small noncoding RNA (sncRNA) in several model and crop species. However, little is known about their global regulation under abiotic stress conditions in the cultivated potato as well as in its relatives. S. commersonii (cmm) is the potato species possessing genotypes displaying the highest tolerance to low temperatures; it is also the first potato relative whose genome sequence has been deciphered. With the aim to understand how sncRNAs are affected by cold stress, two genotypes of cmm contrasting in their cold response were used and theirsncRNAome has been analyzed through RNAseq strategy. A prevalence of 21- and 24-nt sncRNAs divided in three classes of mature miRNAs (273), ta-siRNA (5,737) and other smallRNA (134,868) were annotated in the genome of cmm. Among the miRNAs, 44 were conserved with high similarity with other plant species, and 229 were new or cmm-specific (not reported in any database). Targets of these novel miRNAs were determined by in silico prediction and several genes encoding transcription factors were identified as putative players in cold stress response. Among them, wrky, myb and gras TFs were the most abundant. Differential analysis provided evidence that several miRNAs showed significant changes during stress conditions, and that they were negatively correlated with their target ($r > -0.9$). Our results reveal possible roles of sncRNA in the regulatory networks associated with tolerance to low temperatures and provide useful information for a more strategic use of genomic resources in potato breeding efforts.
The cultivated potato (Solanum tuberosum) has a complex genetic structure as it is an autotetraploid, highly heterozygous species. To understand the genetic makeup of the cultivated potato, we constructed a gynogenic dihaploid (2n = 2x = 24) population from S. tuberosum Group Tuberosum cv. Superior. Between 2014-2016, multiple traits were scored to study fitness and agronomic performance of this population (total tuber yield, average tuber weight, number of tubers per plant, plant height, vine vigor, number of inflorescences per plant, specific gravity, and tuber shape). The dihaploid population showed extreme phenotypic variation, including a high rate of reduced male fertility. A high-density genetic map was built using single nucleotide polymorphic (SNP) markers from whole genome re-sequencing. Common QTL were identified for tuber yield traits, vigor and height on chromosomes 2, 4, 7 and 10, while specific QTL for number of inflorescences per plant, specific gravity and tuber shape occurred on chromosomes 4, 6, 10 and 11. Major allelic effects on specific homologous chromosomes in homozygous or heterozygous states were identified. Presence of nonfunctional alleles and genes associated with hormonal and sucrose metabolism could be associated with the QTL regions. A second dihaploid population from cv. Atlantic is being analyzed. Our efforts to study the genetic complexities of potato through chromosome reduction will serve as a model system for other vegetatively propagated, highly heterozygous crops.
P0321 PHENOTYPIC CHARACTERIZATION OF PHYTOPHTHORA INFESTANS ISOLATES INFECTION SOLANUM TUBEROSUM IN THE NETHERLAND

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1 Full text

Potato (Solanum tuberosum L.) is nowadays the most important non-cereal food crop in the world. It is prone to huge annual losses due to late blight, the disease caused by the oomycete pathogen Phytophthora infestans (Mont.) de Bary. Late blight can be controlled by weekly application of enormous amounts of chemicals. However, there are strategies to control late blight more environmentally safe as the introduction of durable Rpi-genes. In order to breed for more durable late blight resistance, virulence knowledge of current P. infestans populations is a prerequisite. It allows the selection of the most durable Rpi-genes or the best combination of them. In an attempt to characterize part of the AGRICO Research P. infestans collection and consolidate a new reliable differential set, a subset of 35 isolates coming from The Netherland were tested with the partial differential set described by Black and Mastenbroek (1953) and on genotypes which include most of the available new Rpi-genes. When using the Black differential set a wide range of complexity within the P. infestans collections was shown. From 33 P. infestans isolates tested, eight different physiological races were identified. On the other hand, the use of a set of genotypes with different combinations of Rpi-genes, allowed the differentiation of the set studied in 32 different races. Overall, the results indicate that extended differential set can be greatly informative for the purpose of distinguish variations among isolates. Also, pyramiding of Rpi-gene is a valid strategy to confer high and durable resistance to late blight.
Impact of Deficit Irrigation on Yield, Fruit Quality and Water Use Efficiency of Pepper (Capsicum Annuum L.)

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Irrigation water is an essential element for food production in the Mediterranean area. Furthermore, agriculture consumes more than two thirds of the total freshwater of the planet. The irrigation water use efficiency could be improved by an adequate irrigation management. For this reason, the productive response and the irrigation water use efficiency in response to three different irrigation managements (D1, D2 and D3) have been studied, apporting respectively 50, 75 and 100% of the water needs (ETc). The ETc was determined from the reference evapotranspiration, calculated from the evaporation pan class A, with a unique crop coefficient, adapting the duration of each phase to the growing cycle. The irrigation water applied was 408, 596 and 787 mm in D1, D2 and D3 respectively. The highest marketable yield was observed in D3 (4.56 kg m⁻²) and D2 (3.49 kg m⁻²), which were significantly higher than that obtained in D1 (0.73 kg m⁻², p≤0.01). Furthermore, D1 had a significantly higher appearance of fruits with blossom end rot (4.10 kg m⁻², p≤0.01), followed by D2 (1.90 kg m⁻²) and D3 (0.94 kg m⁻²). Fruit quality parameters as firmness, color index (a*, b*, L*), vitamin C, polyphenols and acidity were not altered by the deficit irrigation strategies, while °Brix increased with the decrease of irrigation doses. D2 leaded to the highest irrigation water use efficiency (6.25 kg m⁻³) of pepper cultivated in open field.

Keywords: Evapotranspiration; irrigation doses; open field; blossom end rot; quality parameters
The chili pepper, belonging to the capsicum family, is one of the most widely cultivated vegetable crops. The understanding of genetic diversity at the molecular level is important for the development of new species with higher productivity or resistance to disease. MicroRNAs (miRNAs) are non-coding small molecules that play an important regulatory role in diverse biological processes. In this study, we aim to analyze miRNA profiles of six different tissues in three different Capsicum species (C. annuum, C. baccatum and C. chinense) using high-throughput sequencing technology. We identify many differentially expressed miRNAs that are involved in many biological processes, including plant developments, disease resistance and fruit ripening. Consequently, the corresponding target mRNAs are predicted and validated, and the potential functions of these regulatory networks are discussed. Overall, these molecular characterizations can be a useful tool for the evaluation of genetic diversity in different Capsicum species, which may provide an important insight into how Capsicum species evolved.

This work is supported by a grant from the Next-Generation BioGreen 21 Program (No.PJ01115601), Rural Development Administration, Republic of Korea.
The genic male-sterile ms\textsubscript{1} gene is used for commercial F\textsubscript{1} hybrid seed production in paprika (\textit{Capsicum annuum} L.), a colored bell-type sweet pepper. The previously developed CAPS marker, PmsM1-CAPS, was located at about 2 to 3 cM distance from the ms\textsubscript{1} locus. To develop the cosegregating markers for ms\textsubscript{1} gene, we constructed a fine map using 12 high-resolution melting (HRM) markers in an F\textsubscript{2} population consisting of 1,118 individual plants, which were segregated into 867 male-fertile (Ms\textsubscript{1}Ms\textsubscript{1} or Ms\textsubscript{1}ms\textsubscript{1}) and 251 male-sterile (ms\textsubscript{1}ms\textsubscript{1}) plants. The cosegregating region including the ms\textsubscript{1} gene was delimited to be within 869.9 kb genomic region on chromosome 5. Gene prediction analysis revealed that there were 11 putative open reading frames in the region. Interestingly, a strong candidate gene CA05g06780 was identified to be a homolog of \textit{Arabidopsis} MALE STERILITY 1 (MS1) gene, which encodes a PHD-type transcription factor and regulates pollen and tapetum development in \textit{Arabidopsis}. Sequence comparison analysis between male-fertile and sterile plants revealed that there was one nucleotide (T) deletion on the second exon region of CA05g06780 gene in male-sterile plants, which generated a premature stop codon (TGA) on the third exon region of the gene. In conclusion, we developed a cosegregated marker and identified a strong candidate for ms\textsubscript{1} gene.
This study was conducted to select lines tolerant to excessive water injury among pepper germplasm and to investigate the genetic characteristics of those lines in order to contribute to the breeding of pepper (*Capsicum annuum* L.) cultivars with stable productivity in abnormal weather. Among 274 pepper lines and germplasm, 12 pepper lines were selected with 6 resistant and 6 susceptible lines after immersion treatment at seedling stage. Genetic relationship among 6 tolerant and 6 susceptible lines was investigated with RAPD. Each of the tolerant and susceptible lines went through immersion treatment and differentially expressed genes between them were analyzed. In a tolerant line, the expression of A CA02g26670 gene, which is involved in CONSTANS protein pathway and regulation of flowering by day length, increased but that of CA01g21450, CA01g22480, CA01g34470, CA02g00370 and CA02g00380 decreased. In a susceptible line, gene expression increased in CA02g09720, CA02g21290, CA03g16520, CA07g02110 and CA12g17910, which are involved in inhibition of proteolytic enzyme activity, DNA binding, inhibition of cell wall degrading enzyme and nodulin, respectively. The expression of CA02g02820, CA03g21390, CA06g17700 and CA07g18230 in a susceptible line decrease, which are related to calcium-ion binding, high temperature, synthesis of phosphocholine and cold stress, respectively. The expression of genes related to Apoptosis and peroxidase increased and that of CA02g16990 that has a function of nucleoside transporter decreased in both tolerant and susceptible lines. Based on the gene expression that differs between the tolerant and susceptible lines, further studies are needed on breeding abiotic stress tolerance lines.
Chili pepper (Capsicum annuum L.) is one of the most consumed crops in the world. Annual fluctuation of production amount of pepper might be due to various disease outbreak. Bacterial wilt is one of serious diseases in pepper, which is caused by a soil-borne pathogen, Ralstonia solanacearum. The aim of this study was to identify the biovar of 14 isolates of R. solanacearum collected from major pepper cultivated areas in Korea and to compare pathogenicity of the isolates. Biovar analysis of the isolates revealed that they were divided into two groups, biovar 3 and 4. The biovar 3 group included AD-R, YYC-R, and YYP-R isolates, while other 11 isolates belonged to the biovar 4 group. To analyze the pathogenicity of the isolates, 12 Korean commercial pepper cultivars were used including a susceptible ('Geonchowang') and a resistant ('Konesian Hot') controls. Pathogenicity tests suggested that 14 isolates and 12 varieties were divided into six groups based on the pathogenicity and four cultivar groups according to the resistance, respectively. The isolate group I was the strongest virulent strain, while the group VI was the weakest virulent strain. The pepper group B ('Daekwonseoneon'), C ('Meotjinsanai'), and D ('Muhanjilju') were moderate resistant or resistant to some isolates, and the 'Konesian Hot' was resistant to most of isolates. The results of this study provide useful information on the biovar of R. solanacearum in Korea and on the genetic resources available for the bacterial wilt resistance breeding.
P0046 QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO COLLETOTRICHUM ACUTATUM IN CAPSICUM BACCATUM ‘PI 594137’

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1 Full text

Pepper anthracnose caused by Colletotrichum spp. is a serious disease damaging pepper production in many Asian countries including Korea, India, and Thailand. To analyze QTLs for anthracnose resistance, a segregant F₂ population was constructed by crossing between Capsicum baccatum ‘Golden-aji’ (susceptible parent) and C. baccatum ‘PI 594137’ (resistance parent). Red fruits of F₂ plants were inoculated with the C. acutatum ‘KSCa-1’ isolate, and the disease reactions were evaluated by four methods such as disease incidence (DI), true lesion diameter (TLD), overall lesion diameter (OLD), and field resistance (FR). The genetic linkage map of C. baccatum contained 12 linkage group (LG) and covered a total distance of 1,194.4 cM with 478 high resolution melting (HRM) markers. Composite interval mapping (CIM) analysis revealed total five QTLs for anthracnose resistance, including three QTLs (qCaR3.1, qCaR3.2, and qCaR6.1) for DI, two QTLs (qCaR3.2 and qCaR6.2) for OLD, and one QTL (qCaR4.1) for TLD. However, the QTL for FR was not identified. Interestingly, the two QTLs, qCaR3.1 and qCaR3.2, identified in this study were closely linked to the previously reported QTL CaR9. In conclusion, three new QTLs (qCaR4.1, qCaR6.1, and qCaR6.2) were identified in this study. These results will be helpful for marker-assisted selection (MAS) in the anthracnose-resistant pepper breeding program.
Eggplant (*Solanum melongena*) has a high content in phenolic acids, among which chlorogenic acid is the predominant one. Given the beneficial properties for human health of chlorogenic acid, developing new varieties with increased content is a current breeding objective. Exploring the diversity of wild related species for chlorogenic acid content and evaluating the performance of interspecific hybrids may provide relevant information for breeding eggplants with improved bioactive properties. We evaluated 22 accessions from 12 wild eggplant relatives and 42 interspecific hybrids with six cultivated eggplant varieties for chlorogenic acid content and total reducing capacity. A large variation was found among wild species for chlorogenic acid content, with some accessions having up to 60% higher content in chlorogenic acid content than the best *S. melongena* accession. Although chlorogenic acid was the predominant phenolic acid in cultivated eggplant (average of almost 80%), in wild species on average represented less than 50%, indicating that other phenolic acids are also relevant in the wild species. Accordingly, the wild species had a much higher total reducing capacity, on average almost twice as high as that of cultivated eggplant. The interspecific hybrids were intermediate for the characteristics measured, although some of them, like those with *S. insanum*, were closer in the phenolic profile and reducing capacity to the cultivated parent, while those with other species resembled the wild parent. Overall, the results reveal that wild species can contribute to increase the content in chlorogenic acid, as well as other phenolic acids, and reducing capacity in eggplant.
Nine varieties of cultivated eggplant (Solanum melongena) and one of S. insanum, the wild ancestor of eggplant, were crossed in a partial diallel mating design to obtain forty-five F1 hybrids. The hybrids were evaluated under open field cultivation conditions along with the parents and were characterized for 14 morphological and agronomic traits based on EGGNET descriptors and for 14 fruit shape traits with the fruit shape phenomics tool Tomato Analyzer. Plants were distributed in the field in a randomised complete block design. The data revealed a large range of variation in the parents and the hybrids for all traits studied, with generally high values for broad sense heritability. The general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of crosses were highly significant for most of the traits under this study, pointing out that both additive and non-additive genetic components are relevant in traits of morphological and agronomic importance in this crop. Highly significant correlations were found among many pairs of traits, especially among fruit shape traits. Genetic distances among parents based on 7335 SNP markers SNPs revealed that they are not good predictors of the performances of hybrids for most of the traits, including yield. A number of F1 hybrids were heterotic for traits of agronomic interest, like yield. Certain specific cross combinations exceptionally good for presenting an excellent combination of characteristics were identified. These materials will need to be evaluated under multiple locations for further assessing their potential.
MicroRNAs (miRNAs) play roles in various biological processes including growth, development, and defense in plants. Recent studies revealed that some plant miRNAs could produce secondary small interfering RNAs (siRNAs) such as phased, secondary siRNAs (phasiRNAs) and they might regulate cascade of gene expression. We performed genome-wide comparative analysis of miRNAs in Solanaceae plants and their targets regarding to evolutionary history. MiRNAs are mapped onto 12 chromosomes and microsynteny analysis based on miRNAs and their flanking genes revealed about 86% of conserved miRNAs in pepper maintained synteny with that of tomato or potato. Degradome analysis shows many of genes related to transcription or defense response are regulated by miRNAs in Solanum plants. We found novel miRNAs in pepper targeting a number of genes encoding nucleotide-binding leucine rich repeat (NLR) or receptor-like protein (RLP) known as disease-resistant genes. In addition, these novel miRNAs trigger phasiRNAs production indicating amplification of regulation of the disease-resistant gene families. They seem to be generated by duplication of MiRNAs in different mechanisms. Among them, a novel miRNA, can-miR-n033a, targets many NLRs in an expanded subgroup in pepper. Taken together, miRNAs might be generated and evolved to regulate diverse genes involved in plant immunity. This study provides an insight into the de novo evolution of novel miRNAs targeting plant defense genes and possible coevolution with their target genes.

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Screening the SNPs Related to the Parthenocarpy in Eggplant

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Sequencing based BSA approach was employed to screen the SNP loci related to parthenocarpy of eggplant. F2 population derived from cross between a parthenocarpic line D-7 and a non-parthenocarpic line 896 were obtained. And then we constructed a parthenocarpic pool and a non-parthenocarpic pool from 20 individuals of F2 population respectively. The parental lines and the two pools were all sequenced. The result shows that coverage rate and map reads rate compared to eggplant reference genome were above 90% and 65% respectively. The sequence depths of two parental lines were 15X, and the two F2 pools were 30X. After SNP calling and annotating, quality value of the SNPs below 20 and SNP-indexes below 0.3 and above 0.7 were all removed. Then the non parthenocarpic line 896 was selected as a reference genome, SNP-indexes in positive pool below 0.5 and in negative pool above 0.9 were chose as candidate SNPs loci. Finally, 12 SNPs located in genes were obtained?which related to cyclin-T1-5-like isoform X1, WAT1-related protein At5g07050-like, solute carrier family 35 member F2-like isoform X2, probable sarcosine oxidase-like.

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P0097 SELECTION AND IDENTIFICATION OF EGGPLANT FD STRAINS WITH BOTH FUNCTIONAL MALE STERILITY AND PARTHENOCARPY

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1 Full text

Strains FD21-3, FD28-1, FD07-5 with both functional male sterility and parthenocarpy were obtained in eggplant by using intraspecific crosses?backcross and pedigree selection after years selection and identification. Three male sterility strains F16-5-8, F12-1-1, F13-1-7 and two parthenocarpy strains D-7-1, D-16-3 were selected as parents. The average seedless rate from new combinations of FD strains was 95.4%. These new FD strains will be useful in breeding of seedless eggplant.

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P0121 EFFECTS OF EXOGENOUS 6-BA ON THE PLANT GROWTH, PHOTOSYNTHETIC CHARACTERISTICS AND ENDOGENOUS HORMONE CONTENT IN EGGPLANT (SOLANUM MELONGENA L.) SEEDLINGS UNDER CADMIUM STRESS

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1 Full text

The effects of exogenous 6-BA on the growth, contents of chlorophyll, photosynthetic characteristics and endogenous hormone contents of eggplant (Solanum melongena L.) seedlings of ‘Huqie 08-1’ by substrates culture were studied under cadmium stress. Results showed that the plant growth, chlorophyll a (Chl a) content, chlorophyll b (Chl b) content, total chlorophyll (Chl a+b) content, the photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), cytokinins (CTK) and indoleacetic acid (IAA) contents, the ratio of CTK/ABA and IAA/ABA of eggplant seedling were significantly decreased, while intercellular CO2 concentration (Ci), abscisic acid (ABA) content were significantly increased under cadmium stress compared with the control. The longer the treatment time, the higher the Cd concentration, the greater the change. Under cadmium stress, exogenous 6-BA significantly decreased Ci, ABA content compared with cadmium stress, while Chl a content, Chl b content, Chl a+b content, Pn, Gs, Tr, contents of CTK and IAA, the ratio of CTK/ABA and IAA/ABA were significantly increased. The results suggested that exogenous 6-BA played an important role in enhancing the resistance to cadmium stress of eggplant seedlings and elevated the tolerance of plants through keeping high chlorophyll content, photosynthesis and hormone content.
The scarlet (Solanum aethiopicum) and gboma (S. macrocarpon) eggplants are neglected crops related to the common eggplant (S. melongena). In order to get insight into their diversity we performed a morphological characterization of 63 accessions of scarlet eggplant and 12 accessions of gboma eggplant, including the wild ancestors of both crops, using 45 morphological descriptors. Also, a subset of 39 accessions of scarlet eggplant and seven of gboma eggplant were characterized with 11 SSR and 39 SNP transcriptome-derived markers. Both species are clearly distinct at the morphological level, with significant differences for many traits. In addition, a large morphological diversity was observed in each species and also among and within the different cultivar groups of S. aethiopicum (Aculeatum, Gilo, Kumba, and Shum), and also between each of the crops and their respective wild ancestors. All SSR and SNP markers were polymorphic in the collection, although SSRs were generally more informative than SNPs, with average values of the PIC for SSRs being more than two-fold greater than those of SNPs. Observed heterozygosity with both markers was low, resulting in high values for the fixation index, suggesting a mostly autogamous reproduction. Although scarlet and gboma eggplants were clearly distinct with both SSR and SNP markers, contrarily to morphological traits, no clear pattern of molecular differentiation was observed among cultivar groups or between the wild ancestor and cultivated accessions. The results obtained reveal that a large morphological and molecular diversity exists in both African eggplants, which can be exploited for selection and breeding.
P0148 INTERSPECIFIC HYBRIDIZATION BETWEEN SOLANUM ELAEAGNIFOLIUM AND S. MELONGENA AND POTENTIAL FOR EGGPLANT BREEDING

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Silver-leaf nightshade (Solanum elaeagnifolium) is an invasive weed highly tolerant to drought considered as part of the tertiary gene pool of eggplant (S. melongena). Although it is native to the New World, it has spread to many dry areas of the world. In an attempt to introgress S. elaeagnifolium drought tolerance into the cultivated eggplant we made multiple crosses of two accessions of S. elaeagnifolium with six different accessions of S. melongena. When using S. melongena as a female, F1 plantlets obtained by embryo rescue, as well as morphologically normal F1 seed, was obtained from a cross between one of S. elaeagnifolium and one S. melongena accession. The F1 hybrids had a pollen viability estimated with FDA of 21.6%, compared to values over 60% for both parents. The F1 hybrids were backcrossed to the S. melongena parent using the F1 as female parent. A high degree of success was obtained in the backcrosses, with a fruit set percentage of over 40% and a number of seeds per fruit between 4 and 40. Germination of the BC1 seed was around 50%. The morphological characterization revealed multiple differences between the parents for both vegetative, flower and fruit traits. The hybrids were intermediate, although they are more similar to the wild S. elaeagnifolium in fruit size traits. Also, S. elaeagnifolium had a total reducing capacity eight-fold higher than S. melongena. Overall the results indicate that introgression breeding using S. elaeagnifolium can be of interest for the genetic improvement of multiple traits in eggplant.
P0227 GOLDEN2-LIKE TRANSCRIPTION FACTOR REGULATES DIVERSE METABOLIC CHANGES IN PEPPER

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1 Full text

Pepper and tomato provide suitable models for comparisons of fruit ripening processes. We present distinct molecular patterns of ripening between pepper and tomato by transcriptomic analysis. Our analyses unveil potential mechanisms of non-climacteric ripening and pepper-specific pigmentation due to defect of regulators and ethylene synthesis. The Golden2-like (GLK2) transcription factor is shown to distinct expression pattern rationalizing differential ripening pattern. The silencing effect of the GLK2 in pepper fruit is analogous to the uniform mutation. GLK2 also regulates unique metabolic changes in pepper fruits. The integrated analysis allows us to better understand differential genetic factors of fruit development and ripening in pepper and tomato.
P0231 SMYB1 INTERACT WITH SMBHLH1 TO REGULATE ANTHOCYANIN ACCUMULATION IN EGGPLANT

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1 Full text

The purple eggplant varieties possess an high content of health-promoting anthocyanins in the fruit skin, mainly delphinidin 3-(p-coumaroylrutinoside)-5-glucoside (nasunin) and delphinidin 3-rutinoside (tulipanin). At transcriptional level, the biosynthesis of anthocyanin is controlled by the common regulatory ternary complex ‘MBW’, composed of Myb and basic helix-loop-helix (bHLH) transcription factors as well as WD repeat proteins. Previous studies demonstrated the involvement of SmMyb1 in controlling anthocyanin synthesis in eggplant tissues, however the functional role of the formerly isolated basic-helix-loop-helix protein (SmbHLH1) and the Wd40 partner (SmAN11) still needs to be elucidated.

The transcripts of anthocyanin biosynthetic (SmCHS, SmDFR, SmANS) and regulatory (SmMyb1, SmbHLH1 and SmAN11) genes were analyzed in the fruit skin of purple, green and white eggplant berries, and their expression levels were higher in pigmented than in non-pigmented tissues. The SmbHLH1, SmAN11 and SmMyb1 genes were cloned in the expression vector pEAQ-HT and transiently transformed into N. benthamiana. Significant anthocyanin accumulation was detected 4 days after leaf infiltration with SmMyb1, individually or in combination with SmbHLH1 or SmAN11 or both. Vice versa, no anthocyanin accumulation was spotted in leaves infiltrated individually with SmbHLH1 or SmAN11.

The ability of SmMyb1 to interact with SmbHLH1 was appraised through a yeast two-hybrid assay. Only yeast cells co-transformed with both SmMyb1 and SmbHLH1 were able to grow on selective medium lacking leucine, tryptophan and uracile, thus highlighting their interaction.

The present results provide a first evidence for the role of ‘MBW’ complex in regulating anthocyanin synthesis in eggplant.
Chilli peppers, *Capsicum annuum*, are globally the most widely grown spice product. Several quality traits are important in *C. annuum*, including colour retention. Carotenoids are responsible for these colour traits, however post-harvest storage results in degradation of fruit carotenoid pigments, affecting crop quality. Therefore, the colour retention trait is essential for breeders to produce an economically valuable crop.

Carotenoid composition is directly linked to the red colour phenotype: capsanthin, and its esters, are primarily responsible for the red colour. An understanding of mechanisms associated with carotenoid degradation in *C. annuum* will determine how colour is lost over time.

It is hypothesised that molecular, biochemical and structural factors influence colour retention. Carotenoid profiling has identified lines within a population with increased carotenoid retention, and GC-MS is being used to assess broader metabolism of these lines. These lines will be further analysed to determine the genetic basis of this trait.

Looking at general quality traits, preliminary microscopy studies have revealed that *C. annuum* fruit cuticle structure may be associated with post-harvest quality, and metabolomic analysis also supports this finding. The underlying mechanism resulting in cuticle structure correlating with post-harvest quality will be studied, though preliminary evidence suggests this may be linked to levels of endogenous lipid peroxidation.

This study will provide detailed analysis of colour retention mechanisms in *C. annuum*, which will be instrumental in the breeding of high colour retention varieties, and result in greater understanding of carotenoid degradation mechanisms in this economically valuable crop.
P0300 BIOUSLUMINESCENT IMAGING AS A SENSITIVE TOOL FOR REAL-TIME EVALUATION OF RALSTONIA SOLANACEARUM INFECTION DYNAMICS IN RESISTANT AND SUSCEPTIBLE PEPPER LINES

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1 Full text

Bacterial wilt, caused by Ralstonia solanacearum, is a major disease affecting pepper (Capsicum annuum) production worldwide. In this study, we evaluated the resistance of 100 pepper lines using R. solanacearum strain Rs-SY1 (phytype I, isolated from sweet pepper in South China) and identified one resistant pepper line, BVRC 1. In order to study the bacterial spatial-temporal distribution in planta in real time, we generated a strongly bioluminescent R. solanacearum strain (BL-Rs7) using pXX3 carrying luxCDABE genes. BL-Rs7 was similar to its parent strain Rs-SY1 in morphology and pathogenicity. Furthermore, luminescence intensity of BL-Rs7 strongly correlated with bacterial population in planta ($R^2 = 0.88$). The utility of bioluminescence assay was validated by real-time in vivo monitoring of R. solanacearum infection dynamics in resistant and susceptible pepper lines BVRC 1 and BVRC 25, respectively, following root inoculation. The luminescence signals were detected in susceptible pepper plants from 3 days post inoculation (dpi), while little signal was detected in roots or stem of the resistant pepper line over 10 dpi and only a very low titer of bacteria was detected in the root. The results suggested that pepper line BVRC 1 exhibits resistance by interfering with translocation and multiplication of R. solanacearum in the stem.
Nitrogen (N) is the most important factor limiting plant growth and crop yield in both natural and agricultural environments. Plant's ability to acquire and utilize N from soil solution is a critical step limiting nitrogen use efficiency (NUE). To improve NUE, genotypes identification with high ability to N uptake and utilization, is a pivotal challenge in sustainable agriculture. In the present study, 19 eggplant (Solanum melongena L.) accessions, grown in hydroponic solution for 18 days, have been screened to identify efficient and inefficient genotypes, at low (0.5mM) and high (10mM) nitrate (NO₃⁻) concentrations. Morphological root parameters were measured and primary metabolism profiling were investigated by GC-MS. An efficient (#222) and inefficient (#22) genotypes were identified for several morphological traits potentially involved in NUE and then chosen to investigate their metabolome. In particular, #222 genotype appeared to be well adapted to low NO₃⁻ increasing several root traits and showing high nitrogen utilization efficiency (NUE), aNUE component. Interestingly, metabolomic analysis pointed out, at both concentrations, significant differences between genotypes. In particular, #222 genotype showed, under low NO₃⁻, higher glutamine, glutamate, alanine, aminoacids involved in nitrogen metabolism, glucose, sucrose, fructose as well as citric, α-ketoglutaric and malate acids (TCA cycle), in roots. According to our results, several studies proved that genotypes with a higher alanine content in root were characterized by higher NUE (Good et al., 2007). Moreover, the data confirmed C/N link for improving plant’s ability to N uptake and utilization. Finally, molecular characterization of eggplant genes involved in the nitrate uptake and utilization is underway.

P0349 PRELIMINARY STUDY OF THE EFFECT OF MATURATION AND GROWING CONDITIONS ON THE VOLATILE COMPOSITION OF CAPSICUM PEPPERS

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1 Full text

Quality in fruits and vegetables may be affected by genotype, growing conditions and ripening. Despite many compounds related to taste and nutritional value have been studied, volatile fraction knowledge is very scarce. In this sense, flavour of organic products is considered of better quality compared to conventional fruits, although this preconception must be clarified.

This work shows preliminary results of a study aimed to assess the effect of maturation and organic cultivation on the volatiles of Capsicum peppers. Volatiles of unripe and fully ripe fruits from one Capsicum annuum (Cuneo) and one C. frutescens (Bol-144) accessions cultivated under organic and conventional growing practices were extracted by head space solid phase microextraction (HS/SPME) and analysed by gas chromatography/mass spectrometry (GC/MS).

In general, organic conditions provided higher total volatile content and number of compounds compared to conventional conditions, except for Bol-144 fully ripe fruits. Thus, the number of volatiles in both unripe and fully ripe fruits of Cuneo had a relation organic/conventional above 1.6 and around 1.1 in unripe fruits of Bol-144 while in Bol-144 fully ripe fruits the relation was around 0.7. Additionally, considering volatile profile according to maturation, individual volatiles presented several differences being detected only in one maturity state within the same genotype, as in the case of 2-Methoxy-3-isobutylpyrazine for Bol-144, registered only in fully ripe fruits. The effect of genotype-by-environment interaction is also discussed.

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The term Chiltepin refers to wild ancestors of common peppers (Capsicum annuum var. glabriusculum). They are found in a range of environmental conditions along the center of domestication of C. annuum. From humid forests of Chiapas to the dry Chihuahuan Desert, and also the highlands of Mexico. To study their diversity and phylogenetic relationships is of paramount importance to assess the domestication of C. annuum as well as to exploit their genetic pool. In this work, a collection of Capsicum accessions, including the five cultivated species of this genus and wild chiltepins was genotyped using Genotyping-by-Sequencing (GBS). Phylogenetic analysis was performed relying on SNP-based genetic distances to assess the similarity between cultivated peppers and wild chiltepins. Our findings indicated that there is a considerable amount of genetic variability among samples included in the study. As expected, all C. annuum cultivars clustered together. Meanwhile, C. baccatum, C. chinense, C. frutescens and C. pubescens showed a wide diversity among them and clearly differed from C. annuum (both cultivated and wild forms). Furthermore, C. annuum var. glabriusculum accessions clustered in two separate groups. One subgroup seems to have a closer relationship to C. annuum cultivars, while the other clustered closer to C. frutescens. These results suggest that chiltepins encompass a huge genetic pool, including not only close ancestors to C. annuum, but also materials related to C. frutescens, domesticated in the Amazonian Basin and the Caribbean region.

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1 Full text

ABSTRACT

In China the cultivated area of hot pepper has covered more than 20 million hectares. But most of the cultivars are conventional varieties with low yield and poor resistance. In order to solve this problem, we carried out the research study in new variety breeding of hot pepper from 2004. After five years, we developed a new variety of hot pepper- Yanjiao 425 which is the first single birth and upward pepper in China derived from 481-4-1-1and 750-1-1-1 cross. It is a new F1 hybrid of hot pepper which is suitable for processing. Yanjiao 425 has higher yield, stress resistance, strong growth and middle-late Mature. The plant is about 91.8 cm in height, 84.5 cm in width. The characteristics of fruit is small, lanceolate, single birth and upward. The length/width of fruit is 8.9 cm and 1.1 cm with a flesh wall thickness of 0.14 cm in mature period. The colour of immature fruit is green, and the colors of mature fruit is red. Moreover, Yanjiao 425 with high capsaicin (2.79 g/kg) and capsanthin (0.866 g/kg), good quality and excellent flavor. The average yield is about 30 t per hm². This new variety is suitable to be cultivated in Southwest of China, HuBei, HuNan and etc. The planting scale is more than 7000 ha/year in China

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Traditional tomato varieties have been widely cultivated in Southern Europe for centuries, and many of them are credited for their excellent organoleptic quality. Nevertheless, they present a number of agricultural limitations, most remarkably the lack of resistance to diseases. On the other hand, modern tomato hybrids have been developed for high yield, resistance to a wide range of diseases and enhanced fruit shelf-life. As a consequence, nowadays the tomato market is dominated by modern varieties.

To capture the best of both worlds -traditional and modern- a set of 90 F1 hybrids were developed by crossing 25 traditional tomato varieties with up to 7 modern breeding lines harbouring a set of dominant resistance genes against the most relevant diseases in the tomato crop. This set of 90 F1 hybrids and the 32 parental lines were exhaustively characterized for yield, fruit weight and firmness, Brix and titratable acidity. Additionally, a comprehensive metabolomic profiling was performed on fruits from those genotypes, including all the compounds contributing to fruit flavour and visual appearance and most of those with an impact on human’s health. The different metabolomic platforms used allowed the classification of the hybrids based on their levels of volatile compounds, primary metabolites, a wide number of secondary metabolites and antioxidant ability in the red ripe fruit. The combining ability of the hybrids was determined for each of these traits.
OP09: COFFEE

P0363 TARGETED CAPTURE OF DREB SUBFAMILY GENES AS CANDIDATES GENES FOR DROUGHT TOLERANCE POLYMORPHISM IN NATURAL POPULATION OF COFFEA CANEPHORA

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1 Full text

Coffea canephora, (Robusta), provides 33% of worldwide coffee production, 80% and 22% of Ugandan and Brazilian coffee production, respectively. Abiotic stress such as temperature variations or drought periods, aggravated by climate changes, are factors that affect this production. This sensitivity threatens both the steady supply of quality coffees and the livelihood of millions of people producing coffee.

The natural genetic diversity of C. canephora offer a potential for detecting new genetic variants related to drought adaptation. In particular, modifications occurring in genes related to abiotic stress tolerance make these genes candidate for breeding programs in order to enhance the resilience to climate change.

C. canephora transcription factors from the DREB subfamily (Dehydration Responsive Element Binding Protein) have been recently identified as candidate genes. Indeed, in the C. canephora Conilon group, the CcDREB1D gene showed an increased expression in response to drought in the leaves of a drought tolerant clone¹. The objectives of this study are to identify and characterize the allelic diversity (Single Nucleotide Polymorphism, SNPs) within drought-tolerant candidate genes with a special focus on the DREB subfamily genes. These genes will be annotated on the reference genome sequence of C. canephora⁵ and on anew assembly. A targeted capture array will be designed for these entire genes, and their flanking regions. These captured regions will be sequenced in a set of wild Ugandan populations. Subsequent detection of SNPs for the whole set will be used to test correlation of these SNPs with traits related to drought tolerance.

We expect to understand the adaptive strategies developed by crops in the wild in order to respond to climate change and use the genetic resources within wild populations, as a basis for transferring drought- and heat-tolerance traits.
Large-scale omics information facilitates studies of molecular interactions and functions and the development of novel strategies in plant breeding. We have maintained databases to efficiently provide comprehensive omics information in tomato and other model plants.

The TOMATOMICS database (http://bioinf.mind.meiji.ac.jp/tomatomics/index.php) provides genome and transcriptome information for tomato. We have predicted 54,783 transcripts and their gene structures in the Heinz 1706 genome sequence (SL 2.5), and assigned locus identifiers (using the prefix TMCS). TOMATOMICS also provides information on cDNA clones obtained from the MicroTOM cultivar.

We have also constructed gene expression networks (GENs) and integrated knowledge-based functional gene annotations from model plants and crops including tomato. The knowledge-based annotations have been accumulated through natural language processing (NLP) and manual curation of the published literature. The GENs and knowledge-based functional annotations are accessible from our database Plant Omics Data Center (PODC; http://bioinf.mind.meiji.ac.jp/podc/).

Information about genes that are highly expressed in a particular spatiotemporal pattern facilitates the identification of key genes in particular biological processes. We have developed an algorithm to statistically detect such spatiotemporally expressed genes from RNA-Seq data. By using RNA-Seq data from SRA, we identified approximately 70,000 spatiotemporally expressed genes in ten plant species including tomato. To provide information on the candidate genes, we have developed a database, CATchUP (http://plantomics.mind.meiji.ac.jp/CATchUP). These databases can help us to understand the biological functions of genes and to identify new genes concerned with agronomic traits.

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**P0146 EGMI DB: THE EGGPLANT MICROSATELLITE DATABASE**

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1 Full text

Microsatellites or simple sequence repeats (SSRs) represent one of the most informative, versatile and practical DNA-based markers used in plant breeding programs, since they are easy to score and have wide genomic distribution, codominant inheritance and a multi-allelic nature. Pseudomolecules as well as unmapped scaffolds, of the recently developed high quality reference eggplant genome, were used for the bulk mining of SSR markers and for the construction of the first microsatellite marker database *EgMiDB* (Eggplant Microsatellite Database - [http://www.eggplantmicrosatellite.org](http://www.eggplantmicrosatellite.org)). From the ~1.1 Gb of the ungapped eggplant genomic sequence, we identified 132,831 perfect SSR motifs (density of about 120 SSR/Mb), which included 20,670 (15.6%) compound SSRs while the imperfect SSR motifs were over 178,400. Dinucleotides were the most common, representing 42.8% of all microsatellites, followed by tri- (37.0%), mono (8.4%) and tetranucleotides (7.1%). Penta- and hexanucleotide repeats were the least frequent, together representing less than 5% of the set of perfect SSRs. *EgMiDB* is an user-friendly and freely accessible tool, which offers chromosome wise as well as location wise search of primers by implementing Primer3, and represents a one-stop resource for the global community of scientists and breeders. The database has been projected to gain benefit of the genome sequence linked to pseudomolecules/scaffold and, having user need-based primer designing facilities with mobile-friendly features, will facilitate rapid selection of suitable custom markers for a wide range of genetic analyses.
P0296 PEPPERHUB, AN INFORMATICS HUB FOR THE CHILI PEPPER RESEARCH
COMMUNITY

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1 Full text

Pepper is an important vegetable with versatile uses and has great diversity in fruit morphology. However the basic research in pepper is significantly lagged behind its agricultural and scientific importance. Recent publication of pepper genome paved the road to explore the functional genomics in this species. To provide a rich resources for investigation of molecular mechanisms of pepper genes in a diverse biological process, we conducted large scale transcriptome sequencing using an elite pepper breeding line 6421. Messenger RNAs from over one hundred ninety samples were sequenced in triplicates, which were sampled from different organ or tissue over a time course of development and from pepper young seedlings over a time course of different stress or hormone treatment. Over three terabytes mRNA-sequencing data were generated. To provide access to these transcriptome data and other omics data for pepper research, we constructed PepperHub, an informatics hub for the chilli pepper researcher community. The PepperHub platform currently hosts pepper genome, transcriptome, small RNA, proteome, and variome data and web-based analytic tools for users analyze those data. The utility of PepperHub was demonstrated by analyses of pepper MYB gene family and the discovery of MYB transcription factor genes potentially involved in pepper fruit color-break. PepperHub is available at http://www.hnivr.org/pepperhub.
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