IHC2018-Symposium 3

International Symposium on Tropical and Subtropical Vegetable Production: Tackling Present and Future Global Biotic and Abiotic Stressors

ORAL PRESENTATIONS

KEYNOTE 1

Deciphering the roles of dormancy-associated and flowering-time related MADS-box transcription factors during bud dormancy in apple

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Global warming models predict a rise in global mean temperatures with milder winters, which could result in difficulties for the production of temperate fruits such as apples (Malus x domestica Borkh.). This can be explained by the direct relationship between cold exposure and bud dormancy, an adaptive plant mechanism to survive unfavorable climatic conditions, given that dormancy induction and release are assumed to be triggered by low temperatures in apple. Recently, it has been suggested that genes encoding Dormancy-Associated (DAM) and flowering-time related MADS-box transcription factors regulate dormancy, even though their precise mode of action and integration to the process are still unknown. Moreover, the

dormancy process is highly heritable, suggesting a strong genetic control of the trait. The present work aims to characterize apple DAM and flowering-time related MADS-box transcription factors through complementary genetic and molecular approaches. At the genetic level, a target capture sequencing assay is being employed on a French apple core collection in order to identify allelic variations present on genes involved in dormancy and flowering control. A preliminary GWAS analysis refined a previously identified QTL on apple chromosome 9 linked to date of budbreak. These data will also be used to identify allelic variations in MADS-box genes previously identified by the group as candidates to regulate the process. These candidate MADS-box genes have had their transcriptional levels quantified and some of them are co-expressed during different stages of the dormancy process. This is an indicative that their protein products may interact in order to regulate bud dormancy. Within this context, we are currently investigating the formation of transcriptional complexes between their protein products by the utilization of yeast two-hybrid experiments. Together, these approaches will better characterize the molecular regulation of bud dormancy, as well as identify possible resources for breeding programs.

Keywords: Apple treek, adaptation, chilling, heating requirements, proteins, transcription

OS 1-1:

Effects of StP5CS gene overexpression on nodulation and nitrogen fixation of vegetable soybean under salt stress conditions

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Using a quartz sand culture, comparisons were made between the T5 homozygous transgenic lines (HTLs) that overexpress StP5CS (GenBank accession number: JN606861) and their wild-type (WT) host cultivar to examine the differences in the growth and development traits, the concentrations of proline and leghemoglobin (Lb), total nitrogen, total ureide, and the activities of glutamine synthetase (GS) in vegetable soybean [Glycine max (L.) Merrill] under salt stress conditions. Moreover, the relative expression levels of two glutamine synthetase-related genes (GmGS1β1, GmGS1β2), two nodulation-related genes (GmENOD40-1, GmENOD40-2), and one leghemoglobin gene (GmLba) were also measured. The purpose of this research was to provide a theoretical basis for elucidating the mechanisms of nodulation and nitrogen fixation in the roots of transgenic plants under salt stress conditions. Compared with WT plants, the plant height, stem diameter, numbers of flowers, pods and nodules of T5 HTLs significantly increased, and the contents of proline in various tissues of T5 HTLs were also significantly elevated. In addition, the concentrations of Lb in the nodules and the activities of GS in leaves, roots and nodules of the T5HTLs were significantly elevated. Quantitative real-time PCR (qRT-PCR) analysis indicated that the expression levels of GmGS1β1, GmGS1β2, GmENOD40-1, GmENOD40-2,and GmLba were significantly increased in T5 HTLs under salt stress conditions. These results indicate that the

overexpression of StP5CS in T5 HTLs enhanced growth, nodulation and nitrogen fixation in transgenic vegetable soybean under salt stress conditions.

Keywords: Nitrogen fixation, nodulation, salt stress, StP5CS gene, vegetable soybean

OS 1-2:

Salt stress induces Endoplasmic reticulum stress responsive genes in grapevine rootstock,1616C

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Salt stress is one of the most important limitations to plant growth and crop yield. Grapevine that is adapted to semiarid environments, where drought and salinity are common problems, is relatively sensitive to salinity stress. To understand the molecular mechanisms of salt tolerance and ER stress as well as to identify genes commonly regulated by both stresses, we investigated genome-wide transcriptional profiles of leaves of a salt tolerant grapevine rootstock-1616C under salt and ER stress conditions. Transcriptome profiles of leaves were analyzed at 6 and 24 hrs post-treatments. We identified a total of 1643 transcripts differentially expressed in leaves. Of these, 36 were unique to ER stress, while 827 were unique to salt stress. Interestingly, 29 transcripts were common to both stresses. Gene Ontology analysis identified different categories including genes for oxidative stres, protein folding, transmembrane transport, protein phosphorylation, lipid transport, proteolysis, phosynthesis, regulation of transcription. Many genes involved in hormone biosynthesis or encoding transporters, transcription factors were commonly upregulated in response to both ER and salt stresses. Interestingly, a number of ER stress responsive genes were induced by salt stress indicating that salt stress induces ER stress. The differential expression of the selected genes were confirmed by qRT-PCR analysis and were consistent with microarray results.Finally, in the present study, we identified genes involved in salt tolerance and as well as many differentially expressed genes common to both ER ans salt stresses. Our results

provide new insights to understand the mechanisms of salt tolerance and ER stress and may help for genetic improvement and breeding for salt tolerance.

Keywords: ER stress, Vitis vinifera, Abiotic stress, Salt stress, Gene expression

OS 1-3:

The 13-Lipoxygenase CmLOX10 from Oriental Melon Enhances Drought Resistance and Reduces Salt Tolerance in Arabidopsis thaliana

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The lipoxygenase (LOX) pathway is a master regulator for lipid peroxidation, which plays an important role under abiotic stress in plants. Our study showed that CmLOX10, a type of 13-LOX, was up-regulated under drought and high salinity in oriental melon. Besides, CmLOX10 promoter analysis and its transient expression in tobacco leaves suggest that the promoter responds to drought and high salinity stresses. In addition, the overexpressing (CmLOX10-OX) Arabidopsis thaliana plants reduced sensitivity to mannitol during germination stage, and increased the rate of seed germination and cotyledon greening comparing to the wild type. CmLOX10-OX plants have higher anti-drought ability than wild type plants accompanied by increasing survival rate, water content, with up-regulated expression of the allene oxide synthase (AOS), hydroperoxide lyase (HPL) and stress-related gene including DREB2A, NCED3 and MYB2 genes, as well as significantly decreasing the H2O2 and malonaldehyde (MDA) contents. In contrast, under salt stress, CmLOX10 presented a negative influence comparing with wild type, CmLOX10-OX Arabidopsis showed inhibition of seed germination and cotyledon greening under 150 mM NaCl treatment. Beyond that, H2O2 content increased significantly at seedlings stage when treated with 200 mM NaCl for 7 days, with the down-expression of stress-responsive genes, and the expression of AOS and HPL genes changed little. In conclusion, the results above indicate that CmLOX10 may play different roles in response to drought and high salt stresses by regulating expressions of stress-responsive marker genes, lipid peroxidation and H2O2 production.

Keywords: Drought stress, Salt stress, lipid peroxidation, AOS, CmLOX10

OS 1-4:

FcWRKY40 of Fortunella crassifolia functions in salt tolerance by regulating genes involved in ion homeostasis and proline synthesis

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WRKY proteins represent one of the largest families of transcription factors and play a pivotal role in plant response to environmental stresses. Although several WRKYs have been functionally characterized, regulatory role and physiological function of most WRKYs, particularly those from non-model plants, remain largely unknown. In this study, we report that overexpression of FcWRKY40 from kumquat (Fortunella crassifolia) led to enhanced salt tolerance in both tobacco (Nicotiana nudicaulis) and lemon (Citrus lemon). The transgenic lemon lines displayed substantially lower Na+ contents and higher proline levels in comparison with wild type (WT). Furthermore, treatment of the transgenic lemon plants with 24-epi-brassinolide reduced endogenous proline levels and thus compromised salt tolerance. In parallel, mRNA abundances of SOS2 (Salt Overly Sensitive 2) and P5CS1 (D-1-pyrroline-5-carboxylate synthetase 1), two genes involved in ion homeostasis and proline synthesis, respectively, were dramatically enhanced in transgenic lemon. Protein-DNA interaction assays revealed that FcWRKY40 binds directly to W-box elements in the promoters of FcSOS2 and FcP5CS1 and functions as a transcriptional activator. Moreover, FcWRKY40 was up-regulated by both ABA and salt, while salt-induced up-regulation was ABA-dependent. FcABF2 (ABA-responsive element binding factor 2) was shown toregulate FcWRKY40 by interacting with the ABRE (Abscisic acid response element) element in the promoter. Collectively, these results demonstrate that FcWRKY40 plays a positive role in salt tolerance, which may be ascribed to modulation of ion homeostasis and proline biosynthesis by regulating SOS2 and P5CS1. Our findings reveal a transcriptional pathway composed of ABF2-WRKY40-SOS2/P5CS1 that orchestrates salt stress response in plants.

Keywords: Fortunella crassifolia, ion homeostasis, proline biosynthesis, salt stress, transcriptional regulation, WRKY40.

OS 1-5:

Overexpression of BrIQD5 from Chinese cabbage improves the drought tolerance of tobacco plants by the combination of calmodulin binding proteins

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The genes in the IQD family play an important role in various abiotic stress responses. However, the role of IQD gene in the non-biotic stress of the cabbage is still unclear. In this study, BrIQD5 encodes a calmodulin-binding protein and belongs to the IQD gene family. The interaction between IQD5 and BrCaMa and BrCaMb was confirmed by yeast two-hybrid and BIFC techniques. By fragment truncation、amino acid mutation of IQD5, we determined that motif IQ1 of IQD5is a calmodulin binding site, and isoleucine is essential for the interaction of two proteins. The phenotype, physiological index and molecular level of IQD5 transgenic tobacco plants were analyzed and determined. It was proved that BrIQD5 in tobacco had high tolerance to drought and salt stress. In transgenic BrIQD5 plants, the transcriptional level of BrCaMa and BrCaMb homologous genes increased under drought stress compared with wild type plants, but there was no significant difference with wild type under salt stress treatment. Therefore, BrIQD5 can enhance plant drought and salt stress ability; while BrIQD5 enhanced plant drought resistance may be associated with the combination of calmodulin.

Keywords: Chinese cabbage, drought tolerance, calmodulin-binding protein

OS 1-6:

The role of transcription factors in Citrus response to abiotic stresses

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Abiotic environmental stresses such as cold, drought and salinity cause significant economical losses by limiting the growth and development and reducing yield of agriculturally important plants in many regions of the world. Therefore, economically important plants have been bred for improving abiotic stress tolerance for many years. Since abiotic stress tolerance is a quantitative trait controlled by many genes, only limited success has been achieved in some crop plants. Recent development in plant molecular biology and genomics provided new tools for studying complex traits such as cold, drought and salinity tolerance in many agronomically important crops including Citrus. Citrus species show great variation in response to environmental stress tolerance, but most commercially important varieties of Citrus are sensitive to cold, drought and salinity stresses. Recently, mechanisms of cold, drought and salinity stresses have been explored in Citrus. These studies showed that hundreds of genes with variety of functions were induced in response to these stresses. Among these genes, transcription factors (TFs) constitute an important group and are involved in the regulation of gene expression in response to abiotic stresses. The expressions of

various abiotic stress reponsive TFs including bZIP, AP2/ERF, RAV, WRKY and NAC were studied in citrus. Some of these TFs were differentially expressed in response to one or more abotic stresses in different in Citrus genotypes. Since TFs involved in controling multiple pathways, manipulation of expression of these genes can affect the many metabolic pathways. Therefore, use of abiotic stress induced TFs identified resistant and/or tolerant Citrus genotypes could be used for improving abiotic stress tolerance in stress sensitive genotypes.

This study was supported by TÜBİTAK project number 104O158, 106O549 and 215O444.

Keywords: Abiotic Stres Tolerans, Cold, Drought, Salinity, Gene Expression, Citrus, Poncirus, Rangphur lime

OS 1-7:

Genome-wide analysis of the NAC gene family reveals differential expression patterns and stress responses in Prunus mume

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NAC (NAM, ATAF1/2, CUC2) transcription factors (TF) participate in multiple biological processes, play vital roles in plant growth and development, and respond to environmental stress and interact with signaling transduction. To explore further details, we performed a comprehensive functional investigation of the PmNAC TF family, including the molecular characterizations, phylogenetics and expression profiles in different abiotic stresses. 113 high-confidence PmNAC genes were identified, which were clustered into 14 sub-families and distributed in 8 chromosomes, by phylogenetic analyses and genome-wide annotation. We

also found that 19 of all PmNACs were tandenmly and segmentally duplicated. Furthermore, PmNAC genes performed different tissue-specific expression patterns in root, stem, leaf, bud and fruit. The expression profile by RNA-seq showed that 9 PmNAC genes displayed more than two-fold differential expression levels between root and other 4 tissues. Moreover, PmNACs also exhibited different time-specific expression patterns in dormancy of flower buds; 13 PmNAC genes displayed period-specific expression in the process of dormancy breaking of flower buds, which were up-regulated significantly in March compared with November, December and January, suggesting that they might function in flowering and respond to temperature changes. Overall, our findings reveal the genomic landscape and expression profiling of the PmNAC genes in response to temperature and root-specific expression and provide novel insights into the functional analyses and molecular breeding of the Prunus mume NAC gene family.

Keywords: Prunus mume, NAC, expression analysis, environmental stress,signaling transduction

OS 1-8:

Metabolomic analysis of contrasting peaches (Prunus persica L. Batch) in soluble solids content and susceptibility to chilling injury during postharvest in a segregating population

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The chilling injury (CI) is a physiological disorder induced by cold storage in peach. The main symptom of mealiness is the lack of juiciness of the fruit. The soluble solids content (SSC) and susceptibility to develop mealiness are among the most important parameters in the quality of peach fruit. In the present study we evaluated a peach segregating population OxN (O´Henry x NR-053) and its main fruit quality parameters. We selected two groups of peaches, that contrast in the SSC at harvest and another group that contrasts in the mealiness susceptibility after cold storage. To better understand the metabolic changes associated with SSC and juiciness phenotype, untargeted metabolomic profiling was evaluated by GC-TOF-MS. Principal component analysis and partial least squares analysis has been used to visualize changes in metabolomic profile during postharvest. Peaches with high SSC were different in their metabolomic profile in relation to peaches with low SSC. Glucose and citric acid were the metabolites related to low SSC peach. On the other hand, sorbitol and galacturonic acid was the metabolites related to high SSC. After cold storage, mealy peaches were different in they metabolomic profile from the peaches that not presented mealiness symptoms. Aminoacids like isoleucine and valine were metabolites highly correlated with fruit that developed mealiness after cold storage. All the metabolites mentioned are potential candidates for biomarkers of peaches for SSC and mealiness susceptibility phenotype respectively. (Fondef Genoma G13I0005)

Keywords: Peach, biomarkers, soluble solids, mealiness

OS 2-5:

Overexpression of MhMAPK from Malus hupehensis enhanced the tobacco tolerance to abiotic stresses

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The drought, high salinity, heavy metal are the important abiotic stresses limiting plant growth. Mitogen Activated Protein Kinase (MAPK) cascades is the signal transduction regulating stress responses. The MhMAPK gene was isolated from Malus hupehensis (Pamp) Rehd. Var pingyiensis Jiang and its responses to stresses were detected. The transgenic tobacco seedlings which overexpressing MhMAPK in wild type (WT) were obtained and treated with 20% PEG, 200 mM NaCl and 50 μM CuSO4 solution for 7 d. Results showed that the drought, high salinity and CuSO4 stresses inhibited the tobacco growth because the chlorophyll contents, the fresh weight and the root length were reduced and the oxidative damage were increased. However, the transgenic tobacco showed the better growth than the WT and the chlorophyll contents, the fresh weight and the root length of transgenic tobacco seedlings were higher than the WT. The MAPK activity of transgenic tobacco was also higher than the WT. The antioxidant capacity between the transgenic tobacco and the WT showed significant difference. Compared with the WT, the transgenic tobacco seedlings had lower MDA contents and higher antioxidant enzymes activities. Importantly, the overexpression of MhMAPK reduced the cell apoptosis of tobacco roots. Additionally, the contents of free proline and soluble sugar also enhanced after overexpression of MhMAPK. Taken together, the overexpression of MhMAPK enhanced the tobacco tolerance to drought, high salinity and copper stress by the increase of MAPK activity and the decrease of cell apoptosis.

Keywords: MAPK, Malus huphensis, stress, apoptosis

OS 2-6:

White colour is generated by a down-regulation of genes and metabolic pathway of flavonoids

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The colour of olive drupe is a quality trait and affects technological attributes, healthy properties and commercial value of both table and olive oil. Chlorophylls, carotenoids and flavonoids are the main classes of metabolites determining drupe colour at full ripening. Despite the progresses in the comprehension of genetic and physiological mechanisms regulating colour development in many fruits, the knowledge of such mechanisms in O. europaea is still limited. In this study, three cultivars having divergent phenotype for drupe colour were compared along ripening evolution: the “Leucocarpa” with ivory-white colour of pericarp tissues of ripe, in contrast to the reddish of “Buscionetto” and purple-black colour of “Leccino”. The colourless phenotype makes “Leucocarpa” an excellent tool for elucidating the mechanisms regulating flavonoids biosynthesis, since this phenotype is not dependent to a delay in ripening processes, as demonstrated by the loss of firmness and the decrease of chlorophylls and carotenoids, but arise from the lack of anthocyanins (cyanidin-3-glucoside) accumulation and the only trace amount of the flavonol quercetin-3-rutinoside, suggesting a block in flavonoids pathway downstream chalcones biosynthesis. Instead, the pathway of mevalonic acid, involved in the biosynthesis of secoiridoids is not inhibited in the drupe of “Leucocarpa”. Molecular analyses of structural genes expression demonstrated a lack of DFR and ANS genes up-regulation at veraisòn stage and a reduced expression of flavonoid-3-hydroxylase (F3H) gene. Moreover, olive orthologs of the well-characterized classes of transcription factors forming the MBW complex, regulating flavonoids biosynthesis, although modulated during fruit ripening in a cultivar-dependent manner, seems to not be responsible for the ivory mutant phenotype in the drupe of “Leucocarpa”. Interesting, the olive bHLH orthologs of TT8 clade is up-regulated in “Leccino” and “Buscionetto” at the onset of anthocyanins accumulation and mainly expressed in pericarp tissues, in contrast to “Leucocarpa”, where they remain almost constant throughout ripening.

Keywords: Flavonoids biosynthesis, phenols, anthocyanin, MBW complex gene, Leucocarpa, secoiridoids, DFR gene, ANS gene, F3H gene.

OS 2-7:

Overexpression of MhMAPK from Malus hupehensis enhanced the tobacco tolerance to abiotic stresses

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The drought, high salinity, heavy metal are the important abiotic stresses limiting plant growth. Mitogen Activated Protein Kinase (MAPK) cascades is the signal transduction regulating stress responses. The MhMAPK gene was isolated from Malus hupehensis (Pamp) Rehd. Var pingyiensis Jiang and its responses to stresses were detected. The transgenic tobacco seedlings which overexpressing MhMAPK in wild type (WT) were obtained and treated with 20% PEG, 200 mM NaCl and 50 μM CuSO4 solution for 7 d. Results showed that the drought, high salinity and CuSO4 stresses inhibited the tobacco growth because the chlorophyll contents, the fresh weight and the root length were reduced and the oxidative damage were increased. However, the transgenic tobacco showed the better growth than the WT and the chlorophyll contents, the fresh weight and the root length of transgenic tobacco seedlings were higher than the WT. The MAPK activity of transgenic tobacco was also higher than the WT. The antioxidant capacity between the transgenic tobacco and the WT showed significant difference. Compared with the WT, the transgenic tobacco seedlings had lower MDA contents and higher antioxidant enzymes activities. Importantly, the overexpression of MhMAPK reduced the cell apoptosis of tobacco roots. Additionally, the contents of free proline and soluble sugar also enhanced after overexpression of MhMAPK. Taken together, the overexpression of MhMAPK enhanced the tobacco tolerance to drought, high salinity and copper stress by the increase of MAPK activity and the decrease of cell apoptosis.

Keywords: MAPK, Malus huphensis, stress, apoptosis

KEYNOTE 2

New functions for old fellows: the ASR proteins in plant development and stress responses

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The ASR (Abscisic acid, Stress, Ripening) proteins have been discovered twenty-five years ago as induced by water deficit and fruit ripening in tomato. Since then they have been demonstrated as effectively involved in several developmental stages (seed and pollen grain desiccation, seed germination, senescence, fruit ripening) and in many stress responses (water deficit, cold, salt and osmotic stresses, aluminum tolerance, pathogen infection). The characteristics of ASR as small highly hydrophilic proteins, intrinsically disordered, able to

adopt an ordered structure when they interact with DNA or proteins, displaying a double cytoplasmic and nuclear localization, argue in favor of their dual role, as chaperones in macromolecule protection, as well as transcription factors in gene expression regulation. Here we report a phylogenetic overview of the ASR-family evolution story. The application of global approaches (metabolomics and proteomics) and other targeted biochemical, pharmacological, genetic and epigenetic analyses allows to suggest new molecular roles and biological functions of ASR proteins at the cross talk of metabolic and hormonal signaling, and in the integration of environmental cues.

Keywords: ASR proteins, VvHT1 transcriptional regulation, sugar signaling, nuclear proteome, histones epigenetic marks, water stress

OS 2-1:

Molecular characterization of rd29A: RdreB1BI transgenic strawberry

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Molecular characterization of transgenic plants is crucial for further unintended prediction and phenotype research. In this study, a comprehensive introduction of the RdreB1BI gene to the strawberry genome was identified at nucleic acid level in five selected transgenic lines. Molecular characterization like insertion sites of the exogenousgene in the strawberry genome were determined with high efficient thermal asymmetric interactions PCR (hiTAIL-PCR) and long chain PCR amplification method. One complete and one incomplete fragment of scaffold attachment region (SAR) and multiple backbone fragments of pYH4215-rd29A-RdreB1BI were inserted into the host genome. The single insertion site was found: between 18488014 and 18488027bp on chromosome 4 of Fragaria genome, and 13bp sequence on the host genome was lost during the gene splicing. The detection by the Southern blot and quantitative real time PCR (qRT-PCR) showed that the exogenous gene in five transgenic lines is single copy. The expressionof RdreB1BI gene and other endogenous genes was estimated at transcription levels with qRT-PCR. Expression of the RdreB1BI gene in ‘Benihoppe’ strawberry was significantly lower than the transgenic lines. Moreover, it was also found that the contents of anthocyanin, flavonoids and Phenolic compounds of the transgenic lines are higher than that of control. This is the first time to report the development of molecular characterization technologies were applied in transgenic strawberry plants. Meanwhile, it also reported the insertion at at-rich intergenic region and stable expression of exogenous gene would improve the quality of transgenic fruits. As a consequence, hiTAIL-PCR combined bioinformatic analysis and other PCR methods are reliable, efficient and precise approaches for the safety assessment of strawberry and other genetic modified organisms (GMOs).

Keywords: Transgenic strawberry, RdreB1BI, T-DNA, Safety assessment, Molecular characterization, hiTAIL-PCR

OS 2-2:

MicroRNA143 Involved in Fruit Natural De-astringency of Chinese PCNA Persimmon

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Persimmon(Diospyros kaki Thunb.) fruits accumulate a large amount of proanthocyanidins (PAs) during development, that was the principal component to cause a dry or puckering sensation. Although several miRNAs mediated regulation of PAs polymerization processes were predicted, the involvement of miRNAs and its targets in regulating persimmon astringency removal is poorly understood. DkALDH10 is the potential target of miR143 which was obtained from small RNA libraries derived of ‘Eshi 1’ (Chinese Pollination Constant Non-astringent Persimmon type, C-PCNA). The DkALDH10 gene was also isolated from the ‘Eshi 1’ persimmon by RACE method. Phylogenetic analysis suggested that DkALDH10 belongs to ALDH 10-family subgroup, subcellular localization assays revealed that DkALDH10 was localized in the cytoplasmic membrane. qRT-PCR analysis showed that the expression pattern of DkALDH10 was consistent with that of DkADH and DkPDC which participate in natural astringency loss in C-PCNA. Transcript level expression analysis of DkALDH10 and miR143 showed contrasting divergent expression patterns in ‘Eshi 1’ full fruit development stages. The interaction between DkALDH10 and miR143 was confirmed experimentally through co-transformation of both genes in tobacco (Nicotiana benthamiana) leaves. Furthermore, more soluble PAs conversion into insoluble PAs were observed after transient over-expression of miR143 in ‘Eshi 1’ leaves, while less PAs polymerization after STTM (Short Tanderm Target Mimic)143 transformation. Meanwhile, transient over-expression and gene silence of DkALDH10 and PAs content determination characterized its important role in PAs insolubilization. Our results indicated that miR143 could promote PAs coagulation occurrence through targeting DkALDH10 that repress its expression during C-

PCNA fruit de-astringency process. miR143 may play a critical post-transcriptional regulatory role in PAs metabolism. [This research was supported by National Natural Science Foundation of China (31471846)]

Keywords: Persimmon, miRNA143, DkALDH10, Tannin coagulation, De-astringency, C-PCNA

OS 2-3:

Genetic and chemical diversity in Stevia rebaudiana using microsatellite markers and HPLC analysis

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Stevia rebaudiana Bertoni (2n=22) belonging to genus Stevia and family Asteraceae, is indigenous to Central and South America but its social and economic potential has generated a worldwide interest in its breeding. It is a medicinal and perennial plant whose important bioactive compounds present antibacterial, antifungal, anti-inflammatory, antiviral, antiyeast, cardiotonic, and diuretic properties. Furthermore, their leaf tissues contain sweet steviol glycosides (mainly stevioside and rebaudioside A) that have proven to be a viable and more efficient alternative to other sweeteners. Genetic and/or environmental factors, such as season, altitude, radiation and soil nutrition, affect the metabolic production. Thus, efficient characterization of germplasm and chemistry of these plants is extremely relevant. Currently, few genetic studies on this species are reported and, in most cases, use markers with various limitations such as RAPD, AFLP and ISSRs. In this study, a rapid and efficient protocol was tested to evaluate the genetic diversity of six S. rebaudiana greenhouse grown cultivars collected in July using five microsatellite markers, including two functionally involved in steviol biosynthesis. Optimization of DNA extraction yielded an average DNA yield of 60 µg.g-1 of fresh leaf whose purity was 1.82 and allowed an easy DNA amplification. Primers set was fluorescently labelled with 6-FAM and the amplification fragments generated reproducible bands and electropherograms profiles. In parallel the quantitative profile in stevioside and rebaudioside A was determined for each sample, using water extracts purified by SPE, by normal phase HPLC. Stevioside and rebaudioside A contents varied between 3.85-7.92% and 0.23-1.99%, respectively. The present study concluded that the genetic and

chemical characterization of S. rebaudiana plantsis feasible by microsatellite and HPLC and is important for the selection and conservation of S. rebaudiana Bertoni cultivars.

Keywords: Fingerprint, Natural sweetener, Polymorphism, Rebaudioside A, SSR, Stevioside

OS 2-4:

Agrobacterium-mediated Transformation and Molecular Characterization of Polyacetylene Biosynthesis Genes in the Medicinal Herb Bidens pilosa

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Bidens pilosa is an erect annual plant and is commonly used as herbal tea component or as traditional medicine for treating various disorders including diabetes. To date, around 200 secondary metabolites have been identified from B. pilosa including polyacetylenes, flavonoids, phenylpropanoids and terpenes. Polyacetylenes are the type of compounds with carbon-carbon triple bonds or alkynyl functional groups mainly derived from fatty acid and polyketide precursors. Here we reported the cloning of full-length cDNAs encoding Delta12-fatty acid acetylenase (designated as BPRFAA) and Delta12-oleated desaturase (designated as BPROD), which are key genes in the polyacetylene biosynthetic pathway, from Bidens pilosa var. radiata. Subsequently, 4 vectors including pBPRFAA, pBPROD, pOD-iRNA and pControl (empty vector) were constructed and transformed into B. pilosa via Agrobacterium-mediated method. Over 10 putative transgenic lines were obtained from each construct. Genomic PCR analysis confirmed the presence of transgene and selection marker gene in obtained transgenic lines. Southern hybridization indicated the T-DNA insertion in some transgenic lines was single copy. Furthermore, 4 to 5 FAA genes and 2 to 3 OD genes were detected in wild-type (WT) plant. Quantitative real time-PCR revealed that FAA1, FAA4, FAA14, FAA15, OD1 and OD5 were considered higher expression levels as compared to WT plant. Western blot analysis revealed the OD protein expression in selected transformants. HPLC profiling analyzed the 6 index polyacetylenic compounds and found fluctuation patterns were in those transformants. Taken together, we have established an efficient transformation protocol and then extensive characterized two polyacetylene biosynthesis genes in the medicinal herb B. pilosa.

Keywords: Agrobacterium-mediated transformation; Bidens pilosa; Genetic engineering; Medicinal plant; Polyacetylene

OS 2-5:

Map-based cloning of the pear gene MYB114 identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis

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Abstract body text:

Red fruits are popular and widely accepted by consumers because of an enhanced appearance and enriched anthocyanins. The molecular mechanism of anthocyanin regulation in red-skinned pear (Pyrus) has been studied, and the genes encoding the biosynthetic steps and several transcription factors (TFs) have been characterized. In this study, a candidate R2R3 MYB TF, PyMYB114, was identified by linkage to the quantitative trait loci (QTL) for red skin color on linkage group 5 in a population of Chinese pear (Pyrus bretschneideri). The function of PyMYB114 was verified by transient transformation in tobacco (Nicotinana tabacum) leaves and strawberry (Fragaria) and pear fruits, resulting in the biosynthesis of anthocyanin. Suppression of PyMYB114 could inhibit anthocyanin biosynthesis in red-skinned pears. The ERF/AP2 TF PyERF3 was found to interact with PyMYB114 and its partner PybHLH3 to co-regulate anthocyanin biosynthesis, as shown by a dual luciferase reporter system and a yeast two-hybrid assay. In addition, the transcript abundance of PyMYB114 and PyMYB10 were correlated, and co-transformation of these two genes into tobacco and strawberry led to enhanced anthocyanin biosynthesis. This interaction network provides insight into the coloration of fruits and the interaction of different TFs to regulate anthocyanin biosynthesis.

Keywords: Pear (Pyrus);anthocyanin; QTL ; PyMYB114 ; PyERF3 ;transcription regulatory complex

OS 2-6:

Study on molecular mechanism of ethylene-suppressed anthocyanin accumulation in `Red Zaosu´ pear

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Plant hormones have a crucial role in the regulation of fruit color formation. In this study, light-induced anthocyanin synthesis in postharvest fruit of ‘Red Zaosu’ pear (a hybrid of Pyrus pyrifolia Nakai and Pyrus communis L.) was inhibited by ethylene and promoted by the ethylene receptor inhibitor 1-methylcyclopropene (1-MCP), which is opposite to that in apple, strawberry and some other fruits. To further confirm the results, the same treatments were applied to different pear cultiars ‘Mantianhong’ (Pyrus pyrifolia Nakai) and ‘Hongnanguo’ (Pyrus ussuriensis); and the same results were obtained. qPCR analysis showed that expressions of most structural genes in anthocyanin biosynthesis pathway of ‘Red zaosu’, such as PpCHS, PpCHI, PpF3H, PpDFR and PpUFGT, as well as regulatory genes PpMYB10, PpMYB114, PpbHLH33, PpbHLH3 and PpWD40 were significantly upregulated by 1-MCP treatment, while downregulated by ethephon treatment. Furthermore, the transcription pattern of all the ERFs in pear, important transcription factors in ethylene signaling pathway, were analyzed. A total of 21 ERFs were identified to be correlated with

anthocyanin content, of which one ERF interacted with MYB114. Ethylene significantly induced the expression of this ERF, while 1-MCP inhibited its expression. The expression level of this ERF is negatively correlated with anthocyanin content, inferring that this ERF might play a negative role in anthocyanin biosynthesis.

Keywords: Pear;anthocyanin;ethylene;1-MCP;ERFs

OS 2-7:

Generation of open source genomics resources for African orphan crops by African Orphan Crops Consortium (AOCC), a public-private partnership for promoting food and nutritional security in Africa

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The growing African population needs substantial increase in food production along with nutritious food options to address hunger, malnutrition and stunting. The indigenous or naturalized African fruit tree species are inherently nutritious, fortified with minerals, vitamins and antioxidants with high potential to improve resilience of African food systems. The African orphan crops consortium (AOCC, http://africanorphancrops.org) has prioritized 50 undomesticated or semi-domesticated perennial fruit species and 51 annual species through stakeholder consultations to improve their productivity as well as markets and value chains. The next generation breeding technologies based on genomics assisted selection, holds immense promise to improve their domestication, yield and nutrition traits. It involves generation of reference genome sequence, re-sequencing of 100 diverse accessions and using transcriptome sequencing for genome annotation. At present the AOCC has completed sequencing of six tree genomes; Faidherbia albida (Fa), Artocarpus heterophyllus (Ah), Artocarpus altilis (Aa), Moringa oleifera (Mo), Sclerocarya birrea (Sb), and Annona senegalensis (As), three vegetable species genomes; Lablab purpureus (Lp), Vigna subterranea (Vs) and Solanum aethiopicum (Sa), while other 18 are under various stages of

sequencing. The AOCC is using Illumina's Hiseq2500, Hiseq4000, BGISEQ500 and customized assembly and annotation pipelines for reference genome sequencing. Other technologies such as 10x and PacBio sequencing are also applied if deemed necessary. Among the assembled genomes, the highest scaffold N50 was found to be around 1.54 Mb for Aa, whereas majority of them ranged from 05. To 1 Mb. The initial gene prediction was done usng electronic annotation pipeline, which is now being confirmed using high quality transcriptome sequence from at least 10-12 different tissues per species. After this, re-sequencing will provide information about the single nucleotide polymorphism (SNP), and will aid in trait tagging and genomics assisted breeding.

Keywords: Orphan crops, genomics, next generation sequencing, genomics assisted breeding

OS 2-8:

An integrated `omics´ approach to improve and maintain quality of apples

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Consumption of fresh fruit is increasing as consumers become more aware of the importance of fruit and fruit quality to human health and their roles in disease prevention. In addition to their nutritional benefits, fruits are recognized for their unique flavour, which is important for their enjoyment. Apple fruit quality is dependent on multiple aspects including texture, appearance, color, flavour and nutrition. Among these quality indices, flavour and texture have been recognized as the most significant characteristics determining eating quality of apples. However, the fundamental metabolism responsible for these desirable attributes in apples is not fully understood. In order to reveal mechanisms responsible for fruit firmness and flavor, an integrated multi-disciplinary “omics” approach has been applied to identify genetic markers linked to these traits. These markers can then be exploited through marker-assisted breeding and genome editing to identify quantitative trait loci (QTLs) linked to important traits, such as firmness, flavor and soft scald development. Genome wide association mapping was applied to a collection of 173 unique apple accessions and linked over 55,000 single-nucleotide polymorphisms (SNPs) with 10 phenotypes collected over two years. GWAS revealed several known loci for skin colour, harvest date and firmness at harvest. In addition, several new GWAS associations were detected. A signal for firmness retention after storage on chromosome 10 was detected. Previous reports have suggested that firmness retention is leaked to variation in PG1, a gene repeatedly identified in bi-parental mapping studies and widely believed to underlie a major QTL for firmness on chromosome 10. However, we provide evidence that this major QTL is due to variation in a neighbouring ethylene response factor (ERF) gene. In parallel, gene expression, proteomics and metabolomics based on LC/MS analysis have also been applied to further investigate and evaluate potential markers for fruit ripening and response to postharvest treatments. The present study showcases a multi-disciplinary research platform that utilizes genetics with high resolution of GWAS analysis as well as the use of genomics, proteomics and metabolomics to provide insights that lay the foundation for the accelerated improvement and maintenance of apple quality.

Keywords: Malus domestica, genetcis, genomics, quantitative proteomics, metabolomics, quality, firmness, soft scald, disorders

OS 2-9:

Discovering Genes Involved in the Synthesis of Secondary Metabolites from the Seeds of Moringa oleifera Lam. through Transcriptome Analysis

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Moringa oleifera Lam. is an outstanding crop having seeds with a myriad of benefits including nutrition, health and phytoremediation. These benefits are attributed to the compounds present in the seeds including secondary metabolites considered as antioxidants. Exploring the transcriptome of M. oleifera seeds paved the way for the discovery of the expressed secondary metabolites. Although various phytochemical researchers reported the presence of secondary metabolites in the seeds of M. oleifera, such information is lacking at the transcriptomic level. In the present study, RNA sequencing was used to analyze the transcriptome of the mature embryo of M. oleifera. More than 41 million sequencing reads were generated and de novo assembled using Trinity and SOAP assemblers. Annotation was performed through the NCBI non-redundant database. Furthermore, analysis of the levels of gene expression in M. oleifera seeds using FPKM was performed. Trinity produced 177,417 contigs and SOAP-de novo assembly produced 49,170 contigs. Based on gene ontology functions, the highly expressed genes for secondary metabolites are mostly involved in catalytic activities, metabolic processes, and single-organism processes. Biological pathway analysis using KEGG-KAAS revealed genes encoding 18 enzymes of the phenylpropanoid pathway, 11 enzymes involved in the flavonoid pathway, and 19 enzymes in the alkaloid pathway. FPKM analysis revealed upregulated genes encoding for enzymes peroxidase and betaglucosidase suggesting that M. oleifera seeds have nutritional and pharmacological benefits. Hence, M. oleifera seeds should be incorporated in the regular diet because seeds are not often eaten as much as the leaves. This study provides the first transcriptome profile of the candidate genes involved in the biosynthesis of secondary metabolites in the M. oleifera seeds. This is a baseline information that may open the potentials for improving the yield of secondary metabolites in the M. oleifera seeds. Validation of the expression of the aforementioned genes using qRT-PCR is underway.

Keywords: Transcriptome, contigs, secondary metabolites, qRT-PCR

OS 2-10:

New sweet cherry genomic tools and their use in marker-assisted breeding

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Thanks to the new sequencing technologies, a great number of genomic data such as genome sequences, high density genetic maps and transcriptomic data, have become available for genetic studies in sweet cherries. Using all these resources, it is possible to detect QTLs covering a very small chromosomic region and to find molecular markers tightly linked to traits of interest. Moreover, based on fine mapping and RNASeq analyses, candidate genes can be easily identified with a higher accuracy. Hence, marker-assisted breeding (MAB) has now become a reality for this species. Given that sweet cherry has a long period of juvenility and that large areas are needed to evaluate thousands of new hybrids, MAB will allow breeders to rationalize their programs and plant only those hybrids with favorable allelic combinations for the most critical agronomic traits. The main goal of our team is to understand sweet cherry adaptive responses to climate change in order to create sweet cherry varieties well adapted to the global warming, with a good yield and good fruit quality. We focus on complex traits such as chilling and heat requirements for flowering as well as the fruit weight, firmness and additional fruit quality traits in order to meet farmer's needs. In this study, we will present the new Regina genome sequence using a combination of sequencing strategies (PacBio RSII sequencing and BioNano optical mapping). The efficiency of the two genotyping technologies, 15K SNP arrays and Genotyping By Sequencing (GBS) will be compared for the construction of high density linkage maps. Moreover, new perspective offered by the genomic selection methodology to select hybrids for traits difficult to phenotype on many hybrids and in a short period of time will be presented. These genomics tools will considerably decrease the cost and the duration of our sweet cherry breeding program.

Keywords: Prunus avium, genomic, genetic, QTL, marker assisted breeding, adaptation to climate change

OS 2-11:

Genetic Relationship Between Some Strawberry Varieties and F1 Population Obtained from

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In this study; three local ('Karaçilek', 'Tüylü', 'Deli') and three standard ('Kabarla', 'Sweet Ann' and 'Sweet Charlie') strawberry varieties were used as pollinators for ‘Osmanlı’ local cultivar.Molecular analyzes with fourteen UBC-ISSR primers were carried out to determine the polymorphism levels between the parents used in the hybridization, fifty-two selected F1 genotypes and one Fragaria chiloensis for comparison. A total of 76 bands were obtained from primers. Out of 76 bands, 60 bands were polymorphic. The data used for statistical analysis were obtained by the evaluation of ISSR bands. The data obtained from the evaluation of ISSR-PCR bands were analyzed in Popgene32 and MEGA5.0 computer package program and dendrogram was obtained according to UPGMA method. Similarity index dendograms were divided into two main groups at the level of 20% difference, one large and the other small. While Fragaria chiloensis, Tüylü-2 and two F1s (CC48 and CA97 code numbered) were in the small main group, other F1 genotypes and parent parents were in the large group.

Keywords: Hybridization breeding, ISSR, molecular, Osmanlı cultivar

OS 2-12:
Temporary silencing targeted plant genes with exogenous application of dsRNA

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Silencing of plant genes through double stranded RNA (dsRNA) techniques is well entrenched in the molecular biologists toolkit. Standard techniques to study knockouts include transformation, resulting in genetically modified plant lines, and Viral Induced Gene Silencing, which gives a transient silencing effect, but is reliant on a compatible viral vector system. Recent advances in crop protection studies have shown the capacity for dsRNA to enable targeted silencing even when applied exogenously to the plant, going so far as to provide viral resistance weeks after application of dsRNA (Mitter et al., 2017, Tenllado et al., 2003). To date very few studies have focused on harnessing exogenous dsRNA application to target endogenous genes, and manipulate the gene expression of the plant itself.

In my research I have targeted Arabidopsis endogenous genes with in vitro transcribed dsRNA to knock-down specific endogenous gene expression and to alter the plant phenotype.

Targeted genes include the reporter gene Phytoene desaturase (Qin et al., 2007) and genes in the Jasmonic acid signalling pathway, which is a negative regulator of adventitious root formation (Gutierrez et al., 2012). Development of this technique will lead to the ability to temporarily knock out genes of interest for any plant species, without the need to create a genetically modified plant, or to develop a compatible viral system.

Keywords: Double-stranded RNA, Silencing, Arabidopsis, Exogenous application

OS 2-12:
From the Outside-In: topical application of microRNAs to avocado

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The study of gene function in horticulturally relevant crops is a major scientific bottleneck. There is a district lack of molecular tools optimised for what are often physiologically complex organisms. Therefore, many studies in horticultural crops are limited to examining gene expression under a variety of experimental conditions to infer regulation over a trait. Some horticultural crops are amendable to genetic modification technologies, such a transformation, and in these crops CRISPR will contribute to revolutionising our understanding of genetic regulation underpinning important horticultural traits. However, many crops, such as avocado that we work on in our group, to date will not regenerate from callus placing GM technologies out of reach, requiring for new and innovative tools to be optimised for studying gene function. Therefore, we have been investigating the topical application of artificial microRNA to specifically knock-down gene expression in tissue cultured avocado shoots. We have shown that topically applied microRNAs can be uptake into the shoot and the 21nt mature microRNA excised and methylated, determined with qPCR and validated with Northern Blots. This can be achieved for both endogenous miRNAs and miRNAs for reporter genes such as GFP and GUS. The broad applicability of this technique across horticulturally relevant species suggests strong potential as a useful tool in studying gene function in complex organisms.

Keywords: Avocado, topical, microRNA

KEYNOTE 3

New insights into the distinct S-RNase-based self-incompatibility mechanisms in Prunus

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Most fruit tree species in *Prunus* (Rosaceae) show the S-RNase-based self-incompatibility (SI) system, which utilizes S-RNase and F-box proteins as the pistil *S* and the pollen *S*, respectively. Although similar molecules are involved in specificity determination of the SI recognition reaction across the three plant families, Rosaceae, Solanaceae, and Plantaginaceae, accumulating data suggest the presence of distinct SI recognition mechanisms in *Prunus*. The pistil *S*determinant S-RNase is considered to have a cytotoxic effect commonly in the three plant families. However, the pollen *S*determinant F-box proteins are suggested to have different functions in the genus *Prunus* and in the other taxa that show the S-RNase based SI. Pollen *S* in *Prunus* is assumed to release cytotoxicity of self S-RNase, while in the other taxa, pollen *S* is considered to be involved in S-RNase degradation and detoxification. Since whole genome sequence data of various plant taxa have been available, it is now possible to utilize new approaches such as evolutionary analysis and genome re-sequencing to uncover molecular mechanism of SI. This report presents new insights to SI mechanism in *Prunus* obtained based the evolutionary and genome-wide DNA sequence analyses. The novel gene for self-compatibility found recently in *Prunus* is also discussed

Keywords: F-box protein, MGST, SFB, SLFL, S-RNase

OS 3-1:

MicroRNA0137 Targeting to a AP2/ERF Family Transcription Factor ERF14 Mediates the Resistance against Botryosphaeriadothidea Infection in Malus

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MicroRNA (miRNA)-mediated post-transcriptional regulation plays a fundamental role in plant defense responses against pathogen infection. The accumulation of Malus-specific miR0137 was decreased in leaves infected by the fungi pathogen Botryosphaeriadothidea. miR0137 silenced a AP2/ERF family transcription factor ERF14 by direct cleavage. ERF14 could bind specifically to the GCC-box and the DRE/CRT sequences, which commonly exist in the promoter of pathogenesis-related genes. Over-expression of ERF14 or inhibiting miR0137 induced the expression of several PR genes under normal conditions in tobacco and apple seedlings, while silencing ERF14 or over-expression miR0137 had the opposite effects. Yeast one hybrid demonstrated that ERF14 could bind to the promoter of those PR genes. The

mature miR0137 in resistant variety Malus hupehensis was accumulated less than that of susceptible variety Malus domestica 'Fuji' with or without B.dothidea infection, while the expression of pre-miR0137 had no significant difference in the two varieties. We also detected three SNPs in the precursor of the miRNA affected the secondary structure of the MIR0137 foldbacks of the two varieties. The different secondary structure of the MIRNA foldbacks contributes to the different efficiency and accuracy of the processing of mature miRNA. The G89 SNP near the terminal loop is revealed to play a key role.

Keywords: ERF14; Botryosphaeriadothidea; Defense responses; Malus; miR0137

OS 3-2:
miRNAs participation in prickly pear (Opuntia sp) fruit development

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microRNAs (miRNAs) are small and non-coding RNA that regulate genetic expression at posttranscriptional level. Fruit development and ripening is one of the process regulated by miRNAs. The miRNAs play a vital regulatory function in eukaryotic gene expression by binding specific sequences in target genes and suppressing their expression. Little is known about miRNA on non-climacteric development against climacteric fruits and only one previous work has been published about miRNA in Opuntia for prickly pear production. Prickly pear development and ripening has particular features about ripening time across all different morphospecies, depending on the time required for full fruit development from anthesis to physiological maturity. Due to nutritional value, economic potential and the characteristics to consider prickly pear a model to study, it is necessary to get appropriate integration of information about physicochemical, biochemical and molecular characteristics

in this fruit, in order to understand prickly pear fruit ripening. The aim of this work was to identified the miRNAS that participate in prickly pear fruit development. Bioinformatics and molecular biology methods were combined to isolate, identify and select candidate miRNAs in order to analyze their expression profiles during prickly pear development.miRNA expression analysis through microarray and sRNA-seq technology for Opuntia morphospecie Robusta was performed and miRNAs expression was analyzed by RT-PCR. Here, we report 43 miRNAs y 26 targets with function in fruit development, including processes such as sexual organs development, flower induction, enhanced seed yield, biosynthesis of antioxidant and modulation of ripening through sulfate assimilation which is a critical factor for ethylene biosynthesis.

Keywords: Cactus fruit, fruit ripening, Opuntia robusta, small RNAs, mir159

OS 3-3:

Deciphering the roles of dormancy-associated and flowering-time related MADS-box transcription factors during bud dormancy in apple

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Global warming models predict a rise in global mean temperatures with milder winters, which could result in difficulties for the production of temperate fruits such as apples (Malus x domestica Borkh.). This can be explained by the direct relationship between cold exposure and bud dormancy, an adaptive plant mechanism to survive unfavorable climatic conditions, given that dormancy induction and release are assumed to be triggered by low temperatures in apple. Recently, it has been suggested that genes encoding Dormancy-Associated (DAM) and flowering-time related MADS-box transcription factors regulate dormancy, even though their precise mode of action and integration to the process are still unknown. Moreover, the dormancy process is highly heritable, suggesting a strong genetic control of the trait. The present work aims to characterize apple DAM and flowering-time related MADS-box transcription factors through complementary genetic and molecular approaches. At the genetic level, a target capture sequencing assay is being employed on a French apple core collection in order to identify allelic variations present on genes involved in dormancy and flowering control. A preliminary GWAS analysis refined a previously identified QTL on apple chromosome 9 linked to date of budbreak. These data will also be used to identify allelic variations in MADS-box genes previously identified by the group as candidates to regulate the process. These candidate MADS-box genes have had their transcriptional levels quantified and some of them are co-expressed during different stages of the dormancy process. This is an indicative that their protein products may interact in order to regulate bud dormancy. Within this context, we are currently investigating the formation of transcriptional complexes between their protein products by the utilization of yeast two-hybrid experiments. Together, these approaches will better characterize the molecular regulation of bud dormancy, as well as identify possible resources for breeding programs.

Keywords: Apple treek, adaptation, chilling, heating requirements, proteins, transcription

OS 3-4:
Transcriptional analysis of tomato-interactions with RNA viruses

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Tomato is one of the most important agricultural crops in the world and Turkey. There are a number viruses causing disease in tomato. RNA viruses cause many major diseases of tomato and adversely affect tomato production in various tomato grown regions of the world. The most effective way of controlling viral diseases is the genetic resistance. Since only limited resistant genes were identified for certain viruses and some of the resistance genes identified were broken in the field, alternative resistant strategies need to be developed. Development of alternative resistance requires understanding plant-virus interactions. Therefore, different genomics methods including SSH, microarray and RNA-seq were used for identification of virus responsive genes in RNA virus infected tomato plants. Tomato plants were infected with tomato chlorosis and tomato spotted virus by different methods such as mechanical or graft inoculation, or vector transmission based on the virus. Changes in gene expression were also

effected by the time of virus infection. Therefore, gene expression was analyzed at different stages such as early, mid and late periods of RNA virus infection. Analysis showed that expression of a number of genes involved in different cellular functions and/or metabolic activities were changed by virus inoculation at different time. Expression profile of many differentially expressed genes were dependent on virus used for infection, methods of inoculation or method of gene expression. However, some differentially expressed genes were the same regardless of the virus, methods of inoculation and expression analysis. This suggested that they were involved in disease development process of different viruses. These genes could be used or targeted for developing alternative broad-spectrum resistance strategies against RNA viruses in tomato or other plants.

This study was supported by TÜBİTAK project number 111O546, 115O561 and 215O257.

Keywords: Tomato, RNA virus, transcriptome, resistance, microarray, RNA-Seq

OS 3-5:

A prolyl 4 hydroxylase plays a role in the regulation of the tomato (Solanum lycopersicum) fruit growth program

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Prolyl 4 hydroxylases (P4Hs) catalyze a post-translational modification in glycoproteins such as Arabinogalactan proteins (AGPs) and extensins which are involved in a plethora of developmental programs. RNAi silencing as well as over-expression of tomato P4H3 resulted in important phenotypes such as fruits of smaller diameter, lower number of seeds and alterations in the progression of flower and fruit abscission. Specifically, the smaller fruits could be attributed to cell expansion inhibition of the parenchymatic, epidermal and sub-epidermal cells. Moreover, endoreduplication analysis of the tomato fruits showed no differences in the endoreduplication index compared to the control. Immunolocalization as well as Western blot analysis showed mostly a decrease in the content of AGPs and extensins in the three RNAi lines during fruit growth indicating that silencing of P4H3 leads to either lower levels of the substrate-proteins or alterations in their structure. Over-expression of P4H3 resulted mostly in the increase of the content of AGPs and extensins according to immunolocalization and western blot analysis during fruit growth. These results indicate that regulation of expression of P4Hs may regulate

glycoprotein levels such as AGPs and extensins resulting in pleiotropic effect in growth and development programs.

Keywords: Tomato, P4Hs, AGPs, fruit growth, abscission, RNAi

OS 3-6:

Characterization of the pollen-part modifier gene involved in self-incompatibility reaction in sweet cherry

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Self-incompatibility (SI) prevents self-fertilization and promotes outcrossing to generate genetic diversity within a plant species. However, SI can be an obstacle to production and breeding of cultivated plants. Amongst various SI systems in higher plants, three plant families, the Solanaceae, Plantaginaceae and Rosaceae, share theS-RNase-based gametophytic SI system. The Rosaceae includes Malus, Pyrus and Prunus genera, which contain commercially important fruit tree species. Although they commonly use S-RNase and F-box protein for self and non-self recognition, the underlying mechanism of SI recognition in Prunus is different from that of other species. Many self-compatible (SC) mutants were found and have been utilized extensively for commercial production. Analyses of these SC mutants have given us important clues to understand SI recognition mechanism. This study focused on a SC sweet cherry (Prunus avium) cv. Cristobalina, which is supposed to have a mutated pollen-part modifier gene located outside of the S locus. Several SI cultivars and ‘Cristobalina’ F1 populations segregating for SC and SI individuals were subjected to Illumina genome sequencing. Obtained reads were subdivided into 35-bp subsequences called k-mers. K-mers thus obtained were cataloged into SC and SI pools, and SC-specific k-mers were extracted. Then, the original reads containing the SC-specific k-mers were assembled into candidate contigs containing SC locus of ‘Cristobalina’. Analyzing the obtained SC-specific contig sequences, we found a transposon-like insertion in the promoter region of a gene that showed downregulated transcription in SC individuals. Although this gene is orthologous to ParMDO, which is a pollen-part modifier candidate in apricot (P. armeniaca), the mode of mutation in ‘Cristobalina’ appears to be different from that of SC apricot.

Functional characterization of the modifier gene will expand our understanding of the Prunus-specific SI recognition system.

Keywords: S-RNase, Modifier gene, Prunus, k-mer analysis

OS 3-7:

Abscisic acid-mediated transcriptional regulation of dormancy- associated MADS-box genes during peach bud dormancy

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Crop damages due to spring frosts may be devastating to peach producers in both cold and warm climates. Most frost events are usually preceded by unexpected warm weather in early spring that induces bud break in most deciduous trees. The co-occurrence of frost with the transition period from dormancy to growth has disastrous consequences on the emerging flowers. One of the mechanisms that has been suggested to avoid frost injury is to delay bloom date until the frost risk period passes. Flower phenology in peach and other deciduous trees is largely governed by bud dormancy. Dormancy- associated MADS-box genes (DAM), particularly DAM5 and DAM6, are strong candidate genes for the control of bud dormancy and bloom date in peach and other Prunus species. Transcript profiles of DAM genes during dormancy cycle show distinct patterns in early- versus late-bloom peach varieties. However, the transcriptional regulation of these genes by plant signaling molecules, e.g. plant hormones, have yet to be fully elucidated. Abscisic acid (ABA) is one of plant hormones that is known to control dormancy initiation, maintenance, and release in deciduous woody species. In the present research, we applied ABA (ProTone, from Valent BioSciences at 18 g/gal) and a surfactant (Regulaid, from KALO at 1.9 ml/gal) to an early-bloom peach cultivar (‘Century’ on Lovell rootstock) grown in Winchester, Virginia, the USA. Trees were sprayed twice a month from August to February (three trees per month; three branches per tree, and at least 50 buds on each branch). Each month, two sets of samples were collected from treated and untreated trees: One set for gene expression analyses and hormone profiling; and the other set to calculate chilling requirements and the percentage of bud break. Our results indicate that the timing of ABA application has differential effects on the induction kinetics of DAM genes and the bloom date in peach.

Keywords: DAM genes, peach, bloom date, Abscisic acid

OS 3-8:

Mechanism of male organ differentiation mechanism in the sex determination system of kiwifruit

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The sexuality of kiwifruit (Actinidia spp) is determined by a heterogametic male (XY) system. The Y chromosome of kiwifruit is hypothesized to carry two genetic factors conferring maleness, which would be defined as the suppressor of feminization (SuF) and the male promoting factor (M). Microscopic observations of male organ differentiation have indicated that delay in degeneration of tapetal cells is likely to make the pollen sterile in female individuals, which is supposed to be due to the lack or disruption of the M gene. However, the M gene and its molecular function have yet to be identified. We focused on the M gene candidate, temporarily named KYP (Kiwifruit Y-encoded polysaccharide-related protein), and investigated its functions. First, we assessed the KYP expression pattern during the anther development using a male A. deliciosa cultivar, ‘Tomuri’. The KYP was significantly expressed immediately before the maturation of tapatal cells and on or immediately after the degradation of them. RNA in situ hybridization has been conducted to monitor the localization of the KYP. Furthermore, using the CRISPR-Cas9 system, we have developed and analyzed gene-edited Arabidopsis lines lacking Arabidopsis KYP orthologs to unveil the physiological functions of the KYP. The KYP-lacked lines showed male sterility due to the fails in tapetum degradation similar to in kiwifruit female flower. These results collectively suggested that the KYP can act for proper degradation of the tapetal cells, making it an indispensable gene for pollen fertility.

Keywords: Actinidia, dioecy, sex chromosome, gene editing

OS 3-9:

Flower colour patterns in Cymbidium orchid linked with expression of a Myb transcription factor during flower development

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Orchid flowers have complex flower morphology with fascinating pigmentation patterns. Sepals and petals tend to be similar in shape and colour, but the central petal at the base of the flower, also called the lip, is highly modified and often has a distinctive pigmentation pattern. Not only does pigment concentration vary but there may be quite distinct zones of coloured spots and/or stripes. Differences in colour, pigment profile and concentration have been described but the factors regulating pigment patterning have only been considered recently. The expression of different Myb transcription factors has been linked to specific colour patterns in Dendrobium and Phalaenopsis. In Cymbidium orchids, anthocyanins are the major pigment in cultivars with red coloured flowers. They may be present as a blush colour in the petals, or co-localised with other pigments to give a wide range of flower colours. Lip tissues of cymbidiums in all the major flower colour groups often accumulate anthocyanins, even when anthocyanins are not present in other petals. Here we report changes in expression of a key flavonoid biosynthetic gene (ChDFR) and a Myb transcription factor (ChMYB1) from Cymbidium hybrida. A comparison is made between sepal/petal and lip tissues across flower development and betweena ‘white’ cultivar (Jungfrau dos Pueblos, JDP) and a ‘red’ cultivar (Clarisse Austin South Pacific, CASP). Higher levels of expression in both genes were detected in CASP compared to JDP, which was consistent with the different levels of anthocyanin accumulation between the two cultivars.

Keywords: Cymbidium. colour patterns. Myb transcription factor. dihydroflavanol reductase.

OS 3-10:

Transcriptome comparison among three types of annual roots of Malus hupehensis Rehd

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The roots of fruit tree contain perennial roots and annual roots grouped by growth years. Annual roots were divided into absorbing roots, extensive roots and conductive root by

function. Transcriptome analysis of the annual roots of Malus hupehensis Rehd. was performed using Illumina HisSeq 2500 RNA-seq. 52217996 clean reads were obtained after filtering, which contained 10.54G Base in total. 54 396 unigenes were generated after assembling original reads. The unigenes were further blatsed using the BLAST and BLAST2GO software in Nr、Swiss-Prot、GO、COG and KEGG database for homology comparison. 31530 unigenes were found to be homologous with the genes in these databases and accounted for 57.96% of the total unigenes. There are significant differences among the expression level of a large amount of genes of the absorbing roots, extensive roots and conductive roots. It shows that the expression level of genes producing Ammonium transporters protein, Subtilisin-like protease, aquaporin protein and casparian strip membrane protein is higher in absorbing roots than that in the extensive roots or conducting roots. The expression level of Xyloglucan endotransglucosylase/hydrolase gene is higher in extensive roots than that in conducting roots. In addition, the expression levels of many extension genes are higher in extensive roots than those in absorbing roots or conducting roots. The expression level of genes producing heat shock protein, sugar transport protein and pectate lyase is higher in conducting roots than that in the extensive roots or absorbing roots. The difference of these genes expressions coincides with the functions and growth characteristics of these three types of roots.

Keywords: Malus hupehensis Rehd.; RNA-Seq; function annotation; transcriptome; root

OS 3-11:

Molecular Markers Development and Application in Garlic

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Garlic (Allium sativum L.) as a clonally propagated crop has considerable amount of genetic diversity and very large genome which limits genetic and molecular studies in this crop. Molecular markers have been successfully applied for revealing genetic diversity, constructing linkage map, and developing treat associated markers in garlic. The genetic diversity among 48 garlic clones has been determined with eight isozyme, 183 amplified fragment length polymorphism (AFLP), 80 random amplified polymorphic DNA (RAPD) markers, and simple sequence repeat (SSR) markers generated from expressed regions of the garlic genome. The tree topologies of all markers systems were concordant but RAPD and isozyme dendrograms reflected less polymorphism. Molecular markers demonstrated that genetic diversity among the garlic clones is high and garlic genotypes were clustered into 10 groups at %60 similarity level. A DNA marker (Bltm) significantly associated with bolting phenotype of garlic was developed from mitochondrial genome. The Bltm marker discriminated bolting garlic clones from non-bolting genotypes efficiently. The first genetic

maps of garlic were constructed in two mapping populations with AFLP markers and gene specific markers. The first genetic maps of garlic demonstrated that garlic genome consists of high amount of duplications and generated linkage groups can be utilized to map of important traits.

Keywords: Garlic, genetic diversity, genetic maps, molecular markers

OS 3-12:

Overexpression of blue light receptor AaCRY1 improves artemisinin content in Artemisia annua L

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Artemisinin, an effective antimalarial compound, is isolated from the medicinal plant Artemisia annua L. However, the supply of artemisinin is limited because of the low content of artemisinin in A. annua. Previous studies show that the artemisinin biosynthesis is promoted by light in A. annua. Cryptochrome1 (CRY1) is involved in many processes in the light response. In this study, AaCRY1 was cloned from A. annua. Overexpressing AaCRY1 in Arabidopsis thaliana cry1 mutant resulted in blue-light dependent short hypocotyl phenotype and short coleoptile under blue light. Yeast two-hybrid and subcellular co-localization showed that AaCRY1 interacted with AtCOP1. Overexpression of AaCRY1 in transgenic A. annua increased the artemisinin content. When AaCRY1 was overexpressed in A. annua driven by the CYP71AV1 promoter, the artemisinin content was 1.6 times higher than that of the control. And the expressions of the artemisinin biosynthesis pathway genes including ADS, CYP71AV1 and DBR2 were significantly increased in transgenic lines. Furthermore, we expressed the C terminal of AaCRY1(CCT) involved a GUS-CCT fusion protein in A. annua. The results showed that the artemisinin content was increased by 0.7- to 1.4-fold in GUS-CCT transgenic A. annua plants. These results demonstrate that overexpression of AaCRY1 is an effective strategy to increase artemisinin production in A. annua.

Keywords: Artemisinin, Cryptochrome 1, Artemisia annua, sesquiterpene

IHC2018-Symposium 3

International Symposium on Tropical and Subtropical Vegetable Production: Tackling Present and Future Global Biotic and Abiotic Stressors

POSTER PRESENTATIONS

P-3:Genome-wide identification of microRNAs related to blossom-end rot (BER) in tomato fruit

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Diverse microRNAs (miRNAs) found in plants play as negative regulators in gene expression at the transcriptional and post-transcriptional levels to control growth and development, or response to biotic and abiotic stresses. Blossom-end rot (BER) is a calcium-related physiological disorder commonly occurring in the areas for commercial production of tomato (Solanum lycopersicum) and often causes economic losses. As compared to the known physiological mechanisms of BER, the knowledge about the molecular mechanism of BER is quite limited, especially in the field of miRNAs. In order to figure out the miRNAs-guided BER-related regulatory networks, we sprayed tomato fruits with and without calcium chloride (CaCl2) solution. In comparison to the control fruit, which was not sprayed with CaCl2 solution, BER incidence was significantly reduced in the fruit sprayed with CaCl2 solution. Those fruits were subjected to RNA isolation and high-throughput sequencing analysis. With high-throughput sequencing analysis, we identified several BER-related miRNAs which were differentially expressed between tomato fruits with and without CaCl2 treatment. The expression levels of those miRNAs were further confirmed by quantitation RT-PCR (qRT-PCR) analysis. This research identified several BER-related miRNAs, which may lead us to reveal miRNAs-guided BER-related regulatory networks in the future.

Keywords: Blossom-end rot, microRNA, tomato

P-7:Genome-wide identification and expression analysis of mitogen-activated protein kinase gene family in Vitis vinifera

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Mitogen-activated protein kinase (MAPK) genes are evolutionary highly conserved ancient cascades in all eukaryotes.MAPKs are universal signaling molecules in eukaryotes that mediate the intracellular transmission of extracellular signals resulting in the induction of appropriate cellular responses. In plants, MAPKs control cell's fate by playing important roles in prominent functions including cytokinesis, differentiation, proliferation, cell development, abiotic and biotic responses, pathogen defense, hormonal responses. MAPK cascades are composed of four protein kinase modules: MAPKKK kinases (MAPKKKKs), MAPKK kinases (MAPKKKs), MAPK kinases (MAPKKs), and MAPKs. In this report, we performed a complete inventory of MAPK cascades genes in Vitis vinifera, by comparison with MAPK, MAPK kinases, MAPK kinase kinases and MAPK kinase kinase kinase kinase members of Arabidopsis thaliana. We identified 14 MAPKs, 5 MAPKKs, 62 MAPKKKs, and 7 MAPKKKKs in Vitis vinifera genome. We identified orthologs of V. vinifera putative MAPKs in different species, and ESTs corresponding to members of MAPK cascades in various tissues. In addition, we constructed a phylogenetic tree including individuals of V. vinifera MAPK gene family with MAPK genes from other plants. Finally, we examined their expression profile in different organs at different developmental stages by Real-time PCR. Interestingly, the expression of all V. vinifera MAPKK and MAPKKKK genes in the grape berry have the same expression profile during veraison and ripening indicating their regulation by growth and development. The present study could help elucidate the biological and physiological functions of these proteins in V. vinifera.

Keywords:MAP kinase, Vitis vinifera, signal transduction, protein phosphorylation

P-9:Agrobacterium-mediated transient transformation in apple fruits

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Apple (Malus domestica) is one of the most widely cultivated temperate fruits around the world. Improvement of the quality characteristics of the flesh of the apple fruits is a major objective in apple breeding. Now, rapid identification of gene function has been becoming the most important research topic in improvement fruit quality of apple. Gene function studies in apple fruits (Malus domestica) have been hindered by the long juvenile period of fruit trees. Transient gene expression is a simple and universal method and has been broadly applied in gene functional analyses in several plant species. However, few studies have reported the systemic method of transient expression in apple fruits. We developed a highly efficient and robust Agrobacterium-mediated transient expression system in apple fruit, which achieves rapid analysis of diverse gene functions in apple fruits. Using β-glucuronidase (GUS) as a reporter for Agrobacterium-mediated transformation assay, the results showed that the GUS gene was successfully expressed in tested apple fruits. And we show that the most suitable infiltration pressure for gene function assay in 'Red Fuji', 'Granny Smith' and 'Royal Gala' fruits is -90 kPa, and in 'Golden delicious' fruits is -70 kPa. Meanwhile, the infiltrated efficiency of 'Golden delicious' was higher than that in other apple cultivars. Moreover, transient silencing of MdMYB10 alters the anthocyanin content in 'Red Fuji' which demonstrate that the vacuum infiltration of Agrobacterium can be used to gene function assay. In conclusion, this method may have the potential as a general tool for the efficient regulation of gene expression in fruits.

Keywords:apple, fruits, gene function, transient expression, infiltration

P-11:Genome-wide identification of graft-transmissible small RNAs in Solanum lycopersicum and Nicotiana benthamiana

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Grafting, an ancient horticulture technique, brings various advantages for improvement of crop production. Small RNAs (sRNA) play important roles in the growth, development and stress responses of plant by regulating gene expression at the transcriptional and post-transcriptional levels. Recent studies showed that some sRNAs are phloem-mobile. RNA silencing mediated by mobile sRNAs may shorten breeding process with the mobile sRNAs being transmitted through the phloem and across the grafting junction and performing the regulation in the distal site. To identify graft-transmissible sRNAs, heterografting between tomato (Solanum lycopersicum) and Nicotiana bethamiana was performed, and homografted plants were used as the controls. With high-throughput sequencing, a lot of graft-transmitted sRNAs (gtsRNAs) were identified, and their mobility was further confirmed by quantitative RT-PCR (qRT-PCR) analysis. Interestingly, the sRNA movement occurred more frequently from shoots to roots than from roots to shoots. As microRNAs (miRNAs) have been widely studied and well annotated among sRNA species, we then focused on graft-transmissible miRNAs (gtmiRNAs). The potential targets of gtmiRNAs were predicted via bioinformatics approaches. Then, the expression levels of some putative targets in the grafting materials were analyzed by qRT-PCR analysis. The results proved that the predicted targets of gtmiRNAs were down-regulatively expressed in the presence of the gtmiRNAs coming from the hetero-counterpart of the heterografted plant. The detailed results would be displayed and discussed further in this study. Transgrafting, a method to join wild-type and transgenic plants through grafting, will brings transgenic advantages to the wild-type counterpart. Combination of transgrafting with gtsRNAs may become a novel, workable and efficient approach for crop production improvement in the future.

Keywords:RNA silencing, Grafting, high-throughput sequencing, RT-PCR, microRNAs, Transgrafting

P-14:Plastochron is a key component in the regulation of flowering time in tomato

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The flowering time of the tomato is usually evaluated by the number of leaves preceding the first inflorescence (LN). However, leaf initiation rate could regulate flowering time. Therefore, the flowering time would vary even if LN were the same. In the previous study, we detected quantitative trait loci (QTLs) controlling days to flowering (DTF) on chromosomes 1, 3, 4, 6 and 7, and those controlling LN on chromosomes 3, 6 and 7. To investigate the relationship between DTF and leaf initiation rate, we conducted QTL analysis on plastochron (the time between the initiation of successive leaf primordia at the apical meristem) by using one hundred and eleven lines of a BC1F8 population developed from Solanum lycopersicum 'M570018' (SL) and Solanum pimpinellifolium (PI124039, SP). In another experiment, we constructed two near isogenic lines each harboring a single SP chromosome segment on chromosomes 1 (87-132) or 4 (1-123), both of which are associated with DTF QTLs. The two near isogenic lines, SP and SL were grown in a greenhouse and inspected microscopically for the number of initiated leaves to evaluate the effect of the QTLs on plastochron. As a result, two additive QTLs and a pair of epistatic QTLs of plastochron were detected by QTL analysis. Furthermore, one of two additive QTLs was located in the same region as DTF QTL on chromosome 1. Plastochrons of 87-132 and 1-123 were shorter than that of SL, whereas no significant difference in LN were found among these lines. These results indicate that DTF QTLs on chromosomes 1 and 4 affect the flowering time via controlling plastochron. In the present study, QTLs of plastochron were detected on the same region as DTF QTLs where LN QTLs were not detected in the previous study.

Keywords:quantitative trait loci, leaf initiation rate, flowering time, tomato, near isogenic line

P-15:ROS, as a critical signaling, is the upstream of NO signaling to respond iron-deficiency in apple rootstock

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Iron (Fe), an essential micronutrient, is absolutely necessary for plant growth and development. To cope with Iron deficiency, plants come up with a wide range of adaptive responses. It has been reported that reactive oxygen species (ROS) and nitric oxide (NO) are both iron deficiency signals in Malus xiaojinensis (an iron-efficiency apple genotype)respectively. Here, we used M.xiaojinensis and M.baccata (an Fe-deficiency genotype) to study the interaction between these two signaling pathways in the iron-deficiency mechanism. Under iron-deficiency condition, ROS burst mildly at 6h and decreased, afterwards NO fortified significantly from 1d to 6d, while there is no significant difference on the ROS content at 6h in M.baccata and at 6d NO increased slightly. The expression of ROS donor RESPIRATORY BURST OXIDASE HOMOLOGS (RBOH) family members and NO synthetic genes were consistent with the content changes in both materials. Moreover, compared with M.baccata, the NADPH oxidase (NOX) inhibitor, diphenyleneiodonium (DPI) treatment reduced the relative content of NO dramatically and blocked the expression of the NO-related genes, like MdNOA, MdNIR and MdGSNOR under iron-stravation at 1d in M.xiaojinensis. The Fe-related gene expression was also repressed markedly by exposure to DPI in Malus xiaojinensis. These results strongly suggest that in Malus xiaojinensis RBOH-meidited ROS as a critical signaling is the upstream of NO signaling to respond iron-deficiency.

Keywords:reactive oxygen species (ROS), nitric oxide (NO), iron deficiency, Malus xiaojinensis

P-17: Comparative expression profiles of Mh-ACO1 and Mh-ACO2 RNAi banana fruits by RNA-seq

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Banana (Musa acuminata) is an economically fruit crop in subtropical and tropical regions. As banana is a typical climacteric fruit, the quality as well as storage life of banana is largely affected by

ethylene. Ethylene production in banana fruits is regulated by the activity of ACC synthase and ACC oxidase. Two ACC oxidase genes, Mh-ACO1 and Mh-ACO2, were found to contribute to the increase of ethylene production and be differentially expressed in banana fruits. In this research, RNA interference (RNAi) targeting one of the two ACC oxidase genes was used to further investigate the differential functions of Mh-ACO1 and Mh-ACO2 during fruit ripening. Gene expression profiles have been analyzed during banana fruit ripening by taking advantage of the next-generation high-throughput sequencing to sequence the transcripts of Mh-ACO1 and Mh-ACO2 RNAi transgenic banana fruits. Comparative analysis of gene expression in Mh-ACO1 and Mh-ACO2 RNAi transgenic banana plants showed that Mh-ACO1, Mh-ACO2 and Mh-ACS1 related to ethylene biosynthesis were down-regulated in ripening fruits. Furthermore, the expression levels of genes related to ethylene signaling in ripening banana fruits were strongly influenced by the expression of genes associated with ethylene biosynthesis. Furthermore, we found that the expression patterns and levels of genes involved in carbohydrate metabolism and ethylene production were closed linked in Mh-ACO1 and Mh-ACO2 RNAi transgenic banana, although both pathways were distinct in terms of biochemical function. In this study, we also report that expression levels of genes related to starch and sucrose metabolism in ripening banana were strongly influenced by the expression of genes associated with ethylene biosynthesis.

Keywords:fruit ripening; ethylene production; genetic transformation; RNA interference

P-20:Production of hemagglutinin of Avian Influenza Virus as oral vaccine in transgenic carrot

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Avian influenza, or bird flu, is an infectious and severe respiratory disease of birds caused by Avian Influenza Viruses (AIV). The current virus-inactivated vaccines for avian influenza are produced in chicken embryos and time-consuming. Transgenic plants have been employed successfully as a low-cost, low risk of contamination and large-scale production system of safe and biological therapeutically proteins, including antibodies, antigens and hormones. In view of the advantages of

plant-based oral vaccine production, this study focuses on using transgenic carrots (Daucus Carota L.) as an antigen-delivery system for subunit vaccines against AIV. The gene encoding the AIV surface glycoprotein (hemagglutinin, HA), a major antigen of AIV agent, was introduced into carrots by Agrobacterium tumefaciens-mediated transformation methods. In order to optimize the expression of this gene in transgenic plants, HA gene was under control of the duplicated Cauliflower Mosaic Virus (CaMV) 35S promoter and Tobacco Etch Virus (TEV) enhancer. Escherichia coli heat-labile enterotoxin B subunit (LTB) gene was linked to hemagglutinin (HA) gene to produce peptide as adjuvant. Coding sequence of endoplasmic reticulum (ER) retention signal, HDEL, was added to the 3' end of ha gene. This transgene was codon-optimized by referring with codon usage bias of carrot nuclear genome. After transformation, calli derived from transformed carrot hypocotyls were selected by antibiotic kanamycin and regenerated into plantlets. The putative transformants were confirmed by GUS histochemical staining, polymerase chain reaction (PCR), and Southern blot analysis. The detection of LTB-HA protein was performed by Western blot analysis.

Keywords:genetic transformation, plant-based vaccine, oral delivery, codon optimization

P-21:Genetic analysis of Light-Independent Anthocyanin Biosynthesis Mutants Induced by Ethyl Methane Sulfonate in Turnip 'Tsuda' (Brassica rapa)

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Solar light is a critical environment factor for anthocyanin biosynthesis in plant flowers and fruits. Intriguingly, short-wavelength light (UV-A and blue +UV-B light) contribute to anthocyanin accumulation significantly in both hypocotyls and swollen root peels of 'Tsuda' Turnip (Brassica rapa ssp.rapa). To investigate this mechanism, an 15,000 EMS-generated mutant population was constructed. After the screening of the M2 plants, seven mutants with anthocyanin-related phenotypic variation were classified into two groups: the white mutants and red mutants. Consistent with phenotypic variations, the expression levels of anthocyanin-related genes were decreased in white mutants and increased in red mutants compared with wild-type plants at both upperground and underground parts of swollen root peels. A stop-gained mutation on BrMYB4 in r30 were identified by using TILLING platform, which generated a truncated protein and lost its function on

regulating BrC4H expression. Genetic analysis showed that single recessive genes controlled the phenotypes of three white mutants w9, w68, w204 and one red mutant r15, respectively. Based on mapping-by-sequencing analysis, the mutant traits of w9 and w68 were primarily mapped to chromosome 7. By using the HRM (High Resolution Melting) and CAPS (Cleaved Amplified Polymorphism Sequences) we compressed target regions of w9 locus into ~140kb that contained ~50 candidate genes in which 12 genes (BraA07003264、BraA07003268、BraA07003273、BraA07003280、BraA07003283、BraA07003286、BraA07003288) were predicted to have SNPs in their CDS regions. The w68 locus was primarily mapped to a region from 20.71 to 23.37Mb. RNA-seq analysis suggested that the candidate gene in w68 probably involved in regulation of ion channel to promote light-induced anthocyanin biosynthesis in turnips.

Keywords: Short Wavelength Light, Anthocyanin Biosynthesis, Mapping-by-sequencing, Map-based cloning

P-23:Preliminary study on molecular mechanism of ethylene-suppressed anthocyanin accumulation in 'Red Zaosu' pear

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Plant hormones have a crucial role in the regulation of fruit color formation. In this study, light-induced anthocyanin synthesis in postharvest fruit of 'Red Zaosu' pear (a hybrid of Pyrus pyrifolia Nakai and Pyrus communis L.) was inhibited by ethylene and promoted by the ethylene receptor inhibitor 1-methylcyclopropene (1-MCP), which is opposite to that in apple, strawberry and some other fruits. To further confirm the results, the same treatments were applied to different pear cultiars 'Mantianhong' (Pyrus pyrifolia Nakai) and 'Hongnanguo' (Pyrus ussuriensis); and the same results were obtained. qPCR analysis showed that expressions of most structural genes in anthocyanin biosynthesis pathway of 'Red zaosu', such as PpCHS, PpCHI, PpF3H, PpDFR and PpUFGT, as well as regulatory genes PpMYB10, PpMYB114, PpbHLH33, PpbHLH3 and PpWD40 were

significantly upregulated by 1-MCP treatment, while downregulated by ethephon treatment. Furthermore, the transcription pattern of all the ERFs in pear, important transcription factors in ethylene signaling pathway, were analyzed. A total of 21 ERFs were identified to be correlated with anthocyanin content, of which one ERF interacted with MYB114. Ethylene significantly induced the expression of this ERF, while 1-MCP inhibited its expression. The expression level of this ERF is negatively correlated with anthocyanin content, inferring that this ERF might play a negative role in anthocyanin biosynthesis.

Keywords:pear;anthocyanin;ethylene;1-MCP;ERFs

P-27:Comparative transcriptome analysis in the leaf and seed of two buckwheat species

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Buckwheat (Fagopyrum spp.) is a short-season filed crop with high contents of rutin and other medical-valued phenolic compounds in the genus Fagopyrum, Polygonaceae. To gain insights into the gene expressional diversity between common buckwheat (F. esculentum) and tartary buckwheat (F. tataricum) the Illumina next-generation sequencing (NGS) technology on transcriptome of these two species was performed. The reads were de novo assembled and annotated after blasting to the sequences in the NCBI and buckwheat genome databases. In the common buckwheat, 97,200 and 74,544 contigs were obtained from the seed and leaf, respectively. In the tartary buckwheat, the seed and leaf transcriptomes consisted of 117,545 and 80,945 contigs, respectively. Within 31,473 differentially expressed genes (DEG) between the seed and leaf transcriptomes in the common buckwheat, 25,282 were up-regulated and 6,191 were down-regulated. Similar result was obtained in tartary buckwheat with 25,692 up-regulated and 4,506 down-regulated genes coming to 30,198 DEGs. To understand the molecular mechanisms associated with the biosynthesis of rutin in two buckwheat species 10 homologous genes encoding enzymes involved in the flavonoid biosynthesis were archived from the transcriptome libraries. The results indicated that there were 110 and 70

candidate genes in the common and tartary buckwheat, respectively. The expression level of these genes in the tartary buckwheat was higher than those in the common buckwheat. Therefore, our sequence collection is an exceptional resource for physiological research and breeding work for the buckwheat.

Keywords:RNA-seq, flavonoid biosynthesis, rutin

P-29:Repression of TERMINAL FLOWER1 primarily mediates floral induction in pear (Pyrus pyrifolia Nakai) concomitant with the changes in gene expression of plant hormone-related genes and transcription factors

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Floral induction is an important event in the annual growth cycle of perennial fruit trees. For pear, this event directly affects fruit production in the following year. The flower buds in many species are induced by FLOWERING LOCUS T (FT), whose effect is repressed by the meristem-expressed gene TERMINAL FLOWER1 (TFL1). In this study, we investigated the functions of pear FT and TFL1 genes during floral development. Pear FTs (PpFT1a and PpFT2a) expression in reproductive meristems was not obviously induced prior to floral initiation, while TFL1s (PpTFL1-1a and PpTFL1-2a) expression rapidly decreased. The induction of the productive meristem identity MADS-box gene AP1 after PpTFL1s repression suggested a primary role for PpTFL1 in floral induction. RNA-seq analysis suggested that plant hormone-related genes and several transcription factors that were co-expressed with PpTFL1,were potentially involved in the PpTFL1-mediated floral induction. Our data indicated the essential function of TFL1 in pear floral induction and added another species within the Rosaceae family in addition to strawberry and rose that shows a role for TFL1 in floral induction.

Keywords: Pear; floral induction; TERMINAL FLOWER1; signal transduction; transcription factor; plant hormone-related genes

P-30:Molecular analysis of the lower actinidin phenotype in yellow-fleshed kiwifruit (Actinidia chinensis)

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Actinidin is a predominant protein contained in kiwifruit (Actinidia spp.). While uptake of actinidin is beneficial to help gastric protein digestion with the cysteine protease activity, the protein is also recognized as a major elicitor of allergy which induces tingling in oral cavity and occasionally severe anaphylactic reactions. Given that fresh consumption of kiwifruit has been increased globally, breeding Actinidia cultivars with lower level of actinidin is required to reduce the risk of allergenicity. Here, we searched variations in the actinidin level in Actinidia varieties. Among several varieties having trace amounts of actinidin, A. chinensis 'Kohi' was targeted to be analyzed for the molecular basis for the phenotype. 'Kohi' had an extremely low transcript level of Act1a, a critical gene for actinidin level. The upstream region of Act1a in 'Kohi' constituted different sequences from that of A. deliciosa 'Hayward' which has an active promoter for the high expression of Act1a. The 'Kohi' sequence in the diverged region was found to be rich in cytosine residues. Bisulfite sequencing revealed that cytosines in the diverged region in 'Kohi' are methylated at higher levels than in 'Hayward'. Our data provides a possibility of epigenetic regulation to reduce the actinidin level. This cultivar could be a new choice as a genetic resource in breeding to develop cultivars with controlled actinidin levels.

Keywords:allergenicity, Act1a, epigenetic regulation

P-31:The cuticular wax biosynthesis is regulated by MIXTA1 protein in Eustoma grandiflorum

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Eustoma grandiflorum is a very important horticulture plant, in which the fresh durations of cut flower is a key market trait. Plant cuticular wax plays an important role in plant self-protective barrier, avoidance of nonstomatal water loss, protecting tissue from abiotic and biotic stress tolerances and UV irradiation.Many key enzymes and transcription factors involved in the cuticular waxes biosynthesis pathway have been well characterized, but the detailed molecular mechanism remains unclear. In previous work we identified MIXTA1, an MYB-type transcription factor, from Eustoma grandiflorum and found that over-expression of MIXTA1 resulted in an increase of total wax loads in leaves, and decrease of leaves water loss significantly. In this work, we investigated the involvements of MIXTA1 in the regulation of waxy synthesis and response to drought stress using the over-expression lines of MIXTA1 through phenotypic, physiological, biochemical and molecular approaches. We figured out the downstream target genes by RNA-seq and Chromatin immunoprecipitation analysis. We found that KCS3 and CER3 are the key target genes related to MIXTA1 mediated wax biosynthesis. These results not only reveal novel molecular mechanism of MIXTA1 in cuticular waxes biosynthesis and drought tolerance, but also provide a new idea for extending the durations of Eustoma grandiflorum cut flowers.

Keywords: Eustoma grandiflorum, MIXTA1, Cuticular wax, Drought tolerance

P-32:Characterization and Analysis of a Senescence-associated Gene Metallothionein in Transgenic Tobacco

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Leaf senescence is the final stage of leaf development. During this stage, the most visible symptom is leaf yellowing that is due to the chloroplast breakdown and chlorophyll loss. Hydrolysis of macromolecules, such as proteins, nucleic acids and lipids, occurs concomitantly. The hydrolyzed components are recovered by transporting these materials from senescent parts to the growing parts of plants. Senescence occurs in response to aging, however, certain stresses and hormones can hasten senescence. Dark, drought, detachment, and the hormones abscisic acid (ABA) and ethylene are all able to induce leaf yellowing. The genes of which expression level is upregulated during this stage are referred to as senescence-associated genes (SAGs). A cDNA isolated from senescent leaves of sweet potato (Ipomoea batatas cv. Tainong 57) contained an open reading frame with 201

nucleotides and encoded 66 amino acids. Amino acid sequence comparison indicated the cDNA encodes a type I metallothionein (MT)-like protein. The gene was named as SPMT1. Using SPMT1 as a probe in Northern hybridization, the mRNA expression was found exclusively in senescent leaves including yellowing and completely yellow leaves but not in mature green leaves. Thus, the MT-like gene is a senescence-associated gene. In order to reveal the function or the role of this MT-like gene playing in the process of leaf senescence, the antisense of the SPMT1 gene was inserted in a pBI121 based pAMT plasmid and the pAMT plasmid was introduced into the tobacco genome via Agrobacterium-mediated transformation. Southern blot suggested all the transformants, except MT1R1, might contain 1 to 2 copies of transgene. Northern hybridization showed the transgene expressed in five out of six transformants and expressed more actively in MT1R13. While growing in pots, most of the transformants presented greener leaves and the progress of leaf senescence seemed to be delayed comparing to the wild-type plants. The detached mature leaves of wild-type tobacco plant floated on ABA and ethephon (precursor of ethylene) (50 and 100 mM) solutions were yellowing after 5 days; by contrast, leaves of MT1R13 were still green under the same treatment. Besides possessing greener and smaller leaves, the MT1R13 was sterile. The loss of fertility resulted from the abnormal development of its stamen, especially the shortened filaments. The flower lifetime of MT1R13 was longer than that of wild type. In general, the transformants expressing antisense MT gene had greener leaves, and their leaf yellowing process was delayed no matter under hormones treatment or untreatment conditions. The unexpected function of chimeric antisense MT construction in prolonging plant senescence may open a new direction in senescent-related researches.

Keywords: Agrobacterium-mediated transformation; Leaf senescence; Metallothionein; Tobacco; Transgenic plant

P-34:Identification of S-Alleles in Selected Almond Genotypes by PCR Based Marker

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Almond is a fruit species that shows self-incompatibility controlled by S-Allele. Recently, molecular techniques for almonds has been developed to determine self-incompatibility. In this paper, we presented the results of a sub-project belong to main project titled “The breeding of almond rootstocks resistant to biotic and abiotic stress conditions”, supported by TUBITAK (The Scientific and Technological Research Council of Turkey). In this sub-project, it was determined the self-incompatibility by molecular markers based on PCR primers in 42 almond genotypes selected from Isparta region (Turkey) as a results of previously breeding study. The identification of S-Alleles will be useful in selecting appropriate parents in breeding studies as well as orchard managements.

Keywords: Prunus amygdalus, self-incompatibility, molecular, genotype, breeding

P-35:Molecular cloning and expression analysis of a novel tea terpene synthase

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Semi-fermented oolong tea is an important feature of teas in Taiwan with characteristic aroma that affects tea quality, and volatile terpenes account for more than 50% of the aroma compounds. During semi-fermented tea manufacturing process, relative content of floral and fruity aroma compounds, including some volatile terpenes, increase in a fluctuating profile; however, the associated molecular mechanism and enzymatic reactions remain unclear. In order to identify key enzyme involved in the biosynthesis of tea volatile terpenes, one novel unigene annotated to Arabidopsis thaliana terpene synthase 4 (AT1G61120), a geranyllinalool synthase, was singled out for further investigation based on the transcriptome database established in former study. This unigene

was designated as Camellia sinensis terpene synthase 4 (CsTPS4). CsTPS4 contained a 2,517 bp open reading frame, which can be translated to protein sequence with 839 amino acids, and the predicted molecular weight was 97 kDa. With the use of primer sets designed according to CsTPS4 unigene sequence, the cloning of this gene had been completed. In the process of semi-fermented tea manufacturing, the expression level of CsTPS4 was up-regulated during solar withering and fluctuated during indoor withering and shaking procedure. The maximum expression level of CsTPS4 appeared in late stages of fermentation, corresponding to high volatile terpene contents and suggesting the potential role of this gene to participate in volatile terpene biosynthesis. Further research on CsTPS4 characterization will provide clearer evidence on the function of this gene in the formation of tea terpenes, especially during manufacturing process.

Keywords: Camellia sinensis, semi-fermented tea, volatile organic compounds, terpene synthase

P-37:SSR markers correlated with fatty acids traits in olive

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Olive oil is one of the essential dietary supplements in the Mediterranean region. Olive oil yield and quality depend on both genetic and environmental factors and enhancement of the yield and quality of olive oil in olive cultivars are important breeding goals. Molecular markers linked or significantly associated with agronomic traits could be useful in olive breeding programs to reduce time and cost. In this study, fatty acid contents of olive cultivars were measured with high-performance liquid chromatography (HPLC) method and polymorphisms among the olive cultivars were detected with simple sequence repeat (SSR) markers. Significant correlations between fatty acid contents in olive oil were determined. Structure analysis grouped olive genotypes into two gene pools. General linear model was applied to determine correlation between SSR markers and fatty acids traits. Five SSR markers were found to be significantly correlated with stearic, oleic, linoleic and linolenic acids of olive oil.

Keywords: Olive, SSR markers, Structure analysis, fatty acids

P-41:Molecular cloning of Heptahelical protein(HHP) gene family, characterization and expression analysis of BcHHP1 involved in responses to multiple abiotic stresses in Pak-choi （Brassica rapa ssp. chinensis）

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Heptahelical protein (HHP) signaling pathway is involved in cold acclimation responsive to low temperature and some other stresses. HHP transcription factor family is the key component of this signaling pathway. In this study, five HHP-like genes, BcHHP1, BcHHP2, BcHHP3, BcHHP4 and BcHHP5, were isolated from non-heading Chinese cabbage (Brassica rapa ssp. chinensis cv. suzhouqing). Multiple sequence alignment and phylogenetic analysis showed that BcHHP proteins were highly homologous to HHP proteins from Arabidopsis thaliana, Brassica oleracea and Brassica rapa. Some of these HHP proteins might share similar parts of the function. Furthermore, real-time quantitative PCR (qPCR) analysis showed that BcHHP1 was induced and co-expressed under cold and salt treatments. Besides, BcHHP1 was also accumulated in response to abscisic acid (ABA) and salicylic acid (SA) treatments, indicating that BcHHP1 gene might participate in response to hormone treatments. In addition to that, a BcHHP1-YFP fusion protein was localized to the nucleus and cytoplasm. These results indicated that five BcHHP genes were isolated from Pak-choi, which might constitute a functional HHP signaling pathway, in which BcHHP1 plays an important role in response to cold treatment in Pak-choi. This work might be useful for future functional analysis of other HHP-like genes in Pak-choi.

Keywords: Heptahelical protein. Expression analysis. Abiotic stress. Subcellular localization. Pak-choi

P-49:miRNAS ANALYSIS DURING (Tagetes erecta) FLOWER DEVELOPMENT

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miRNAs regulate gene expression in eukaryotes, they are involved in the control of many plant developmental processes, including fruit development. The information available on miRNAs, related to expression, abundance, and conservation in several species, provides a new opportunity to study the role of miRNAs in non-model species, such as Tagetes erecta (marigold). In this work, were combined bioinformatics and molecular biology methods (microarray hybridization and RNA sequencing) to isolate, identify and select candidate miRNAs in order to analyze their expression profiles during prickly pear development. The profiling comparison detected the expression of miRNAs clustered in different expression patterns, during flower development. A Gradual increase in the expression of several miRNAs, including miR172, was observed during flower development. Stable expression analyses in Arabidopsis thaliana suggest that miR172 plays diverse roles during flower development. The results described in this work, represent the first report of miRNA expression profiles during marigold development.

Keywords: Arabidopsis thaliana, gene expression, microarray, miR172, transcriptome,

P-51:ELISA and RT-PCR detection of Apple chlorotic leafspot virus (ACLSV) and Apple mosaic virus (ApMV) and determination of viral variation in South Africa

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Objective: Apple chlorotic leafspot virus (ACLSV) and Apple mosaic virus (ApMV) result in reduced yield and unmarketable fruit symptoms. The first objective of this study was to compare the double antibody sandwich enzyme-linked immune-sorbent assay (DAS-ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) techniques for the detection of ApMV and ACLSV. The second objective was to determine genetic variation in South Africa of ApMV and ACLSV in pome- and stone fruit.

Materials and Methods: Samples from various areas were tested using DAS-ELISA and RT-PCR and the number of positive samples as well as dilution ranges of samples detected by each technique, were compared. The factor of one technique being more sensitive than the other was also determined. In the second part of this study, the genetic variation of ApMV and ACLSV isolates from South Africa was investigated. RNA of ACLSV and ApMV was extracted and used for RT-PCR of the coat protein genes and then sequenced. Phylogenetic trees were constructed using these and other sequences deposited on GenBank.

Results and Discussion: RT-PCR was more sensitive than DAS-ELISA by a factor of about 5 to 100 times in the case of ACLSV and 100 to 1 000 times for ApMV.

Phylogenetic analysis revealed that five clades of ACLSV exist worldwide and that South African isolates group into 3 of these clades. It also indicates that isolates of ACLSV clades are not restricted either pome- or stone fruit and that that cross-infection between pome- and stone fruit is a strong possibility. Phylogenetic analysis of ApMV isolates, indicated two major groups occurred in South Africa. The first group consisted of only 4 isolates, grouped together with isolates from India, whilst the second large group, consisting of 33 isolates, grouped together with isolates from Czech Republic and India.

Keywords: ApMV, ACLSV, ELISA, RT-PCR

P-52:Comparative transcriptome analysis of gibberellin-induced sex determination in bitter gourd

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Bitter gourd (Momordica charantia L., 2n = 2x = 22) is an important tropical and sub-tropical Cucurbitaceae crop. Bitter gourd is a monoecious plant whose unripe fruits contain many medicinal

ingredients. As the first step to understand the sex determination mechanism of bitter gourd the transcriptomes of GA3-spread seedlings at 6-8 leaf stage and seeds soaked with GA3 were compared in aid of next-generation sequencing technology. Pooled reads from four samples were de novo assembled by Trinity. 13,2707 contigs were obtained and N50 reached 2921 bp. 10,298 putative genes were obtained after functional annotation by Trinotate pipeline. According to TPM (transcripts per million) calculated by kallisto, GA3 foliar application caused 1,104 genes expressed twice more and 1,139 genes expressed twice less than the control; seeds soaked with GA3 caused 547 genes expressed twice more and 374 genes expressed twice less than the control. To gain insights into the mechanism of sex determination, all genes involved in biosynthesis and signal transduction pathways of phytohormones were chosen for comparison of gene expression level. Transcription factors related with multiple phytohormones might play important roles in the induction of sex determination in bitter gourd.

Keywords:GA3, next-generation sequencing, monoecious crop

P-54:Genome-wide identification, characterisation and expression analysis of D-type cyclin gene family in Prunus mume

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Cyclins, an important class of cell division regulators, plays an extremely important role in plant growth and development. D-type cyclins (CYCDs) are the rate-limiting components through the G1 phase. In plants, studies of CYCDs are mainly concentrated in herbaceous plants, yet little information is available about genes in perennial woody plants, especially in ornamental plants. Here, 12 Prunus mume CYCD genes (PmCYCDs) were identified and characterized, which were named according to identity percentages of the corresponding orthologs in Arabidopsis thaliana and Populus. The genomic organization of each subgroup CYCD was similar to their orthologs in A. thaliana and Populus. The expression levels of PmCYCDs genes were analyzed in roots and leaves under different treatments including NAA, 6-BA, ABA, and sugar. Differential expression characteristics of the PmCYCDs in response to sugar and phytohormones were measured. Though PmCYCDs were induced by sucrose, their extents of induction among PmCYCDs subgroups varied. The induction of PmCYCD1;1 and PmCYCD1;2 by hormones depended on the presence of sucrose. PmCYCD3;1 was greatly stimulated by NAA and the strengthening effect on induction was observed

when sugar and hormones were added together. Furthermore, PmCYCDs performed different tissue-specific expression patterns in root, stem, leaf, bud, and fruit, respectively. PmCYCD1;1 is primarily highly expressed in buds and stems, however the expression level of PmCYCD7;1 is lowest in buds and stems. Taken together, our studied demonstrates that PmCYCDs are functional in plant development and provides a basis for further study of cyclin gene family.

Keywords: Prunus mume, Cyclin, CYCD, Expression analysis, Cell division

P-58:Transcriptome analysis and SNP/SSR marker information of the garlic (Allium sativum L.)

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Garlic as a second biggest crop after onion among allium species is grown all in the world. However, the genomic information available for garlic are still very limited. Recently, next-generation sequencing technology has provided a powerful approach for analyzing the transcriptome, and for shedding light on the molecular biology of no-model crops, as garlic.In the present study, transcriptome of the bulbs of two garlic (Allium sativum L.) accessions, T17 and T36, was sequenced with short reads on Illumina Genome Analyzer platform. We generated approximately 48 million sequencing reads and assembled de novo, yielding 91748 high quality unigenes with an average length of 567 bp. Among these unigenes, 27,191 were identified as putative homologs of annotated sequences in the public protein databases, 66943 and 78431 unigenes were found to be highly abundant in T17 and T36, respectively. A total 912 differentially expressed genes were revealed between the two accessions. A total of 2017 simple sequence repeat (SSR) motifs were revealed based on unigenes and a total of 1735 candidate single nucleotide polymorphisms (SNPs) with high confidence were identified from T17 vs T36. Among the SSR motifs, 132 primers were designed and 16 of them were polymorhpic and generated 44 alleles among 104 accessions of garlic. The garlic

transcripts set generated here provides a resource for gene discovery and development of functional molecular markers.

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Keywords: De novo assembly ; garlic ; transcriptome ; illumina sequencing ; SSR; SNP

P-61:Transgenic expression of ATPG4 gene in chrysanthemum enhances plant growth and delays plant senescence

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AT-hook proteins of plant is involved in growth and development through the modification of chromatin architecture and the alteration of gene expression. Recently many genes encoding AT-hook protein identified and their involvement in yield increase and senescence delay was reported. Overexpression of chromatin architecture controlling ATPG4 (AT-hook protein of Genomine 4) gene in arabidopsis conferred seed yield increase, senescence delay, and drought and oxidative stress tolerance. In this study, we have generated chrysanthemum transgenic plants overexpressing ATPG4 gene via Agrobacterium-mediated transformation and investigated the characteristics of transgenics related with plant growth and senescence. A total of 24 transgenic chrysanthemum plants were produced under the selection of phosphinothricin. Based on PCR and RT-PCR analysis of bar and ATPG4 gene, 19 independent transgenic lines were selected. In the phenotypic analysis of transgenics after flowering in a greenhouse, compared to wild-type plants, the transgenics exhibited

the increase in plant height, ray flower number and fresh weight of cut flower. In the analysis of postharvest life of cut flowers, the transgenic lines showed the extended postharvest life and delayed chlorophyll reduction compared to wild-type plants. The results of this study indicate that the introduction of ATPG4 gene in chrysanthemum may have the positive effect on plant growth and senescence delay. * This work was supported by a grant (116080-3) from IPET through Agri-Bio Industry Technology Development Program, funded by MAFRA, South Korea

Keywords: Chrysanthemum, ATPG4, Plant growth, Senescence delay

P-65:miRNA and target interaction in ethylene biosynthesis pathway for prickly pear ripening

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MicroRNAs (miRNAs) are small and non-coding RNA molecules that regulate genetic expression included fruit development and ripening. Prickly pear developmental time has features across all different Opuntia morphospecies and some of them ripe early in 80 days after flowering (daf) but others ripe late until 140 daf. In this work, we performed miRNA expression analysis through microarray and bioinformatics for Opuntia Robusta which has an early fruit ripening profile. Here, we report the target interactions with role related to ripening by the identity of sexual organs (miR156/160/398), flower induction (miR172), enhanced seed yield (miR397), biosynthesis of antioxidant (miR157/529/535/837) and modulation of ripening through the control of sulfate flux and assimilation but also repressing ETHYLENE INSESITIVE 3 (miR395/171) and such interactions miRNA-target together may play a critical role for ethylene biosynthesis.

Keywords:ripening, miR395, EIN3, ethylene, sulfur flux

P-90:Transcriptomic and metabolomic analysis reveal novel cellular responses of harvested strawberry fruit exposed to short-term high CO2

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In order to improve storability of strawberry fruit, postharvest technology of short-term 30% CO2 treatment was applied to fruit. By using transcriptomic and metabolomic analysis, we first reported new cellular responses induced by short-term 30% CO2 in harvested strawberry fruit. Fruits were stored at 10oC for 10 days after 30% CO2 exposurefor 3 h as a treatment or air for 3 h as a control, respectively. Thirty-percent CO2 showed an effect on inhibiting fruit decay and fruit softening. Transcriptomic analysis revealed that expression levels of expansin, pectinesterase, and b-xylosidase which are cell degradation enzymes were significantly reduced and heat shock proteins were significantly increased by 30% CO2. Metabolite profilings revealed that glucose, quinic acid, succinic acid, and arabinose were significantly increased by 30% CO2, suggesting that fruit ripening was delayed by 30% CO2. Transmission electron microscopy showed that disintegration of middle lamella in cell wall was inhibited by 30% CO2. Pectin content in the cell wall of 30% CO2 treated strawberry fruit was 46% higher than that in the control at 3 days after storage. We therefore could confirm that short-term 30% CO2 reduces pectin decomposition of cell wall by deactivating cell wall degradation enzyme activities and induces abiotic stress-defensive genes in harvested strawberry fruit.

Keywords:carbon dioxide, Fragaria x ananassa, metabolites, plant cell wall, postharvest

P-94:Establishment of an efficient breeding system for Ceratocystis canker resistance fig (Ficus carica)

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Fig (Ficus carica) is one of the important fruit crops in Mediterranean countries, especially in Turkey, the top fig producer over the world. Fig is a gynodioecious plant including caprifig type (male flowers and short-style female flowers) and fig type (long-style female flowers), and the female fig fruits are mainly consumed. Therefore, it is necessary to select female plants in the breeding programs. Under high temperature and humidity conditions, on the other hand, Ceratocystis canker caused by Ceratocystis ficicora is one of the most disruptive diseases for fig. Highly resistance cultivars are therefore required for stable productions of fig in the regions such as Japan. The aim of this study is to establish an efficient breeding strategy for selection of female and Ceratocystis canker resistance lines, for which genomics-based breeding technology would be helpful. To identify genetic loci for the disease resistance, breeding populations were generated from crosses between common figs and the fig wild relative, F. erecta, which is a donor of the disease resistance. The populations were analyzed by a double-digest restriction-site associated DNA sequencing (ddRAD-Seq) method to obtain genome-wide SNPs, in which the genome sequence of fig published from our group was used as a reference. Subsequent genome-wide association study (GWAS) and whole-genome resequencing (WGRS) analysis were able to identify the causative gene candidates. In parallel, another population derived from a cross between a fig and a caprifig was also analyzed with ddRAD-Seq, GWAS, and WGRS to reveal the sex determinant locus on the high-density genetic map for fig. SNPs linked to the disease resistance and sex determinant were converted into conventional DNA markers. Accordingly, an efficient breeding system for fig based on SNP analysis was established to select female and disease resistance lines from breeding materials.

Keywords: Ceratocystis canker, Fig, Genomics-based breeding

P-98:Physiological control of dormancy in sweet cherry flower buds

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In the context of global changes, temperate fruit trees are affected by contradictory effects: increased productivity in relation with longer growing season and insufficient chilling during winter. It is therefore essential to better understand the mechanisms controlling phenology and its response to environmental conditions. Optimal timing and quality of flowering directly depend on adequate dormancy progression during winter and spring, regulated by a combination of chilling and warm temperatures.

Physiological, genetic and functional genomic studies have shed light onto the mechanisms underlying dormancy control in deciduous trees. Notably, internal signals such as hormones and sugars were shown to play a key role in dormancy establishment, maintenance and release. In order to further study how these signaling pathway control dormancy progression, we combined physiological and transcriptional analyses of sweet cherry flower buds during the dormancy period. In particular, quantification of abscisic acid (ABA) and gibberellins (GAs) was compared to the expression patterns for genes involved in the hormonal pathways. Results suggest that ABA is critical for dormancy maintenance and we propose that the complex balance between ABA and GA pathways regulate the timing for dormancy release.

Keywords: Prunus avium, bud dormancy, hormonal pathways, metabolomic analyses, adaptation to climate change

P-103:Analysis of PRR genes expression and its relationship with the resistance to citrus canker disease in citron C-05

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Citrus canker disease, caused by Xanthomonas citri subsp. citri (Xcc), is a worldwide quarantine disease, and the resistance to the pathogen is one of the main citrus breeding goals. During the last decade, a citrus resistant genotype - citron C-05 (JY) has been identified. RNA-seq was then performed and resistance related genes were found induced by the pathogen. Some of the genes are PRRs. The expression of FLS2, CERK1 and Lyp2 were analyzed to confirm the RNA-seq results. After being inoculated with Xcc, the expression all the analyzed PRR genes obviously upregulated in the resistant citron C-05 in comparison with that of the susceptible 'Bingtang' sweet orange (BTC), which might imply that the expression of FLS2 in JY is normal while it's inhibited by the bacterial pathogen in BTC. The inoculation of different concentrations of Xac indicated that PRRs expression was positively related to Xac concentrations and reached the highest level at 1010 cfu·ml−1 of inoculunm while negatively to severity of symptom. The PRR gene sequences and promoters of the resistant and susceptible genotypes were further analyzed, and possible mechanism was suggested.

Keywords:citrus canker disease; PRRs; gene expression; resistance

P-113:Application of Molecular Marker Technology on Identification of Cabbage Varieties and Study of Cabbage Immature-bolting Characteristics

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In order to establish DNA fingerprinting to identify the purity of cabbage hybrid,the genomic DNAs of 4 hybrids (Xiaguang, Zaoxia 16, Zhengchun, Hanguang 2) and its parents were used as group of template DNA respectively. In 4 groups, 3 efficacious primers were gained in each group, they were S15, S42, S147 and S42, S78, S88 and S42, S103, S193 and S42, S89, S151 respectively. The specific RAPD fingerprints in the 4 groups could be established with S42 primer and then transformed into digital fingerprinting.BSA method was applied to study the immature-bolting characteristic and find its linkage RAPD markers. Through selecting of 260 RAPD primers, we gained 4 primers, which can amplicate markers linked to the immature-bolting gene of cabbage. There were 5 linked markers showed from the relationship analysis result of F2 population with the 4 primers, and the linkage distances of the markers are different, the closest one is 8.58 cM. Three markers that were closest to immature-bolting gene correspondingly were examined of sequence and named S97-392, U16-446, and U16-562 according to fragment size and primer source. The result of sequence comparison between the 3 markers showed that there was little comparability, and no same DNA fragment or gene in GeneBank.

In order to establish DNA fingerprinting to identify the purity of cabbage hybrid,the genomic DNAs of 4 hybrids (Xiaguang, Zaoxia 16, Zhengchun, Hanguang 2) and its parents were used as group of template DNA respectively. For each of the 4 groups, 3 efficacious RAPD primers were obtained: S15, S42, S147for group ??, S42, S78, S88 for group ???, S42, S103, S193 for group ???, and S42, S89, S151 for group ???, respectively. The specific RAPD fingerprints in the 4 groups could be established with S42 primer?????? (what means?) and then transformed into digital fingerprinting. BSA method was applied to study the immature-bolting characteristic and find its linkage RAPD markers. Through selecting of 260 RAPD primers, we identified 4 primers, which can amplicate markers linked to the immature-bolting gene of cabbage. There were 5 linked markers showed from the relationship analysis result of F2 population with the 4 primers, and the linkage distances of the markers are different, the closest one is 8.58 cM. Three markers that were closest to immature-bolting gene correspondingly were examined of sequence and named S97-392, U16-446, and U16-562 according to fragment size and primer source. The result of sequence comparison between the 3 markers showed that there was little comparability, and no same DNA fragment or gene in GeneBank.

Keywords: Cabbage; Hybrid; Identify; Fingerprinting; Immature-bolting; BSA; Linkage marker;

P-114:Developing apple genetically resistant to fire blight disease via CRISPR-Cas genome editing

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Apple production is often plagued by a number of diseases, including fire blight caused by bacterium Erwinia amylovora. Development of apple genetically resistant to fire blight is highly desired and is a fundamental and long term solution to the disease problems. Genome editing using CRISPR/Cas9 has become a popular approach to induce targeted mutations for crop trait improvement. We initiated a research to develop CRISPR-Cas genome editing technology for apple aiming at fire blight resistance. Establishment of an efficient and reliable in vitro regeneration system is a prerequisite for genetic transformation and subsequently for genome editing. We tested different types of tissue culture media and different concentrations of plant growth regulator and developed a highly efficient regeneration system with greater than 80% leaf explants produced shoots. To establish CRISPR/Cas9 genome editing in apple, constructs containing guide RNAs targeting the apple phytoene desaturase (PDS) gene were made. The PDS gene encodes an enzyme in the carotenoid biosynthesis pathway and disruption of this gene results in dwarf and albino phenotypes in Arabidopsis. This feature could be used to detect genome editing events to facilitate technology development. We also made constructs targeting an apple endogenous gene that may be involved in fire blight pathogen infection. Mutating this gene could potentially confer resistance to the disease. Transient expression of the genome editing constructs in Nicotiana benthamiana leaves via agro-infiltration confirmed nuclear localization of Cas9 protein. The genome editing constructs have been introduced into tissue culture system via Agrobacterium-mediated transformation. Plant transformation and genome editing will be analyzed once plants are developed. Our long term goal is to mutate apple endogenous genes interacting with pathogens via CRISPR-Cas and generate apple for fire blight and other disease resistance.

Keywords: Apple, fire blight disease, CRISPR-Cas genome editing, tissue culture, transformation

P-115:Development of a molecular marker tightly linked to the C locus conferring a white bulb color in onion (Allium cepa L.)

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To develop molecular markers linked to the C locus determining a white bulb color in onion, a combination of bulked segregant analysis and RNA-Seq was performed using bulked RNAs extracted from 12 plants each of yellow and white F2 bulbs. Ninety-seven genes showed at least five-fold increased expression in the yellow bulk. Eleven previously isolated structural genes were identified from these genes. Two homologous genes coding for phenylalanine ammonia lyase and chalcone isomerase were also identified. In addition, three glycosyltransferases genes and two acyltransferases genes showed increased transcription in the yellow bulk. Increased expression of 18 structural genes in the yellow F2 individuals were further confirmed by RT-PCR. Regarding regulatory genes, a gene encoding a WRKY transcription factor was most significantly down-regulated in the white bulk. In the MBW complex, known to control a flavonoid biosynthetic pathway, 47, 4, and 8 genes encoding putative MYB, bHLH, and WD40 proteins, respectively, were identified from transcriptome. Among them, 2 MYBs, 1 bHLH, and 1 WD40-coding genes showed upregulation in the yellow bulk and high similarity with other known regulators of flavonoid biosynthesis. Among 1,829 contigs containing SNPs or InDels, five contigs were shown to be linked to the C locus. In addition, SNPs in the gene encoding glutathione S-transferase also showed linkage relationship. Six molecular markers were developed and a linkage map was constructed using 586 F2:3 individuals. The GST1 marker based on the GST-coding gene showed perfect linkage to the C locus.

Keywords:onion(Allium cepa L.),white bulb, molecular marker, C locus

P-117:Mining and comparison of the genes involved in strigolactones biosynthesis /signaling and expression analysis in columnar and standard apple

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Columnar apple is a valuable resource for genetic improvement of cultivated apples due to its special tree architecture. Strigolactones (SLs) is a novel class of plant hormone controlling shoot branching. SLs concentration exhibited higher levels in columnar apple. In this study the members of major genes implicated in SLs biosynthesis and signaling were identified from apple genome sequences, and their expression profiles were characterized in columnar and standard apple using quantitative reverse transcription polymerase chain reactions.

Materials. The plant materials comprised four columnar apples, four standard apples. Buds or shoots were collected from each sampling tree in the field from February to August, were kept in ice box during transportation, and then were put into liquid nitrogen.

Methods. The main methods include gene predication and EST analysis, transcription profiling by quantitative reverse transcription PCR (qRT-PCR), strigolactone quantification, gene transformation etc.

Results. The higher expression level of and MdMAX4-3 in both buds and shoots of columnar apple could suggest that transcript abundance of MAX3 and MAX4 could result in high SLs hormone content. MdD53-4, an inhibitor of the SLs, showed lower expression levels in columnar apple and had weak inhibitory effect on SLs signal transduction. MdCo31 could be a strong candidate gene for the control of columnar habit. Overexpressions of MdCo31 in tobacco increased SLs contents and weakened the inhibition of SLs signal transduction by increasing expression of MAX3 and down regulating the transcription of D53, and resulted in regulating the columnar character through SLs mediated manner.

Keywords: Key Words: Strigolactones; MdCo31; Columnar apple; Standard apple; Expression

P-118:Gene expression analysis of pecan (Carya illinoinensis) flower structures using RNA-Seq

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Pecan (Carya illinoinensis)is a profitable nut belonging to Juglandaceae family with production over 125 tons in the United States in 2017. Pecan originated in North America and has been introduced to Europe, Asia, South America, South Africa and Australia. Transition from vegetative phase to reproductive phase in pecan trees can take up to 10 to 30 years. The mechanism(s) responsible for flower initiation and the shift to produce flowers are unknown. Furthermore, alternate bearing, defined as high production of small sized pecans in 'on' years and lower production of large sized pecans in 'off' years, is a significant constraint in pecan production. Based on transcriptome data of buds from pecan fruiting and non fruiting shoots during the summer of 2016 from mature 'Western' pecan trees, there is evidence that flower initiation may occur in a two-step pattern. The first step occurs the summer prior to pistillate flower bloom and the second step occurs in the beginning of Spring when the flowers bloom. Our previous RNA-Seq experiments have investigated the putative flowering genes that may have role in flowering. Due to the fact that pecan trees are monoecious, it is unknown whether the putative flowering genes are involved in pistillate or catkins flowering. Comparison of bud transcriptomes from protandrous ('Western') versus protogynous ('Wichita') may help differentiate the staminate and pistillate flower genes. RNA-Seq analysis of fruiting buds, non fruiting buds, catkins and pistillate flowers were also performed. Gene expression analysis of these transcriptomes allow us compare the genes involved in their development and increase our understanding of alternate bearing.

Keywords: Pecan, flower initiation, transcriptome, pistillate flower, catkin flower, flower buds

P-123:Identification of genes related to mesocarp development in cucumber by transcriptome profiling

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Cucumber (Cucumis sativus L.), an agriculturally and economically important vegetable crop worldwide, which are consumed with its immature fruits.The cucumber mesocarp is important

for fruit estabilishment, which determines fruit thickness, and edible ratios of cucumber. In present study, the thick mesocarp line “D8” was selected to analyze the gene expression profiling of mesocarp at 0, 3, 6, 9 days after pollination (DAP) by RNA-sequencing. Analysis of the differentially expressed genes revealed that the genes mainly related to carbon metabolism, biosynthesis of amino acids, citrate cycle, oxidative phosphorylation were up-regulated during the first 3 DAP. From the 3 DAP to 6 DAP, the genes related to photosynthesis, oxidative phosphorylation and plant hormone signal transduction were highly induced. From 6 DAP to 9 DAP, the genes related to plant hormone signal transduction, ubiquitin mediated proteolysis, regulation of autophagy, carotenoid biosynthesis were highly induced. The differentially expressed genes linked to cyclin, hormone biosynthesis/signaling, carbon metabolism, transcription factors MYB and WRKY were up-regulated during the 9 DAP. Quantitative real-time polymerase chain reaction was applied to verify the transcriptome results. Finally, the gene expression profile were compared between “D8” and line “XUE1” which show thin mesocarp. The results of this study should lead to a better understanding of the mechanism of mesocarp formation in cucumber.

Keywords: Cucumber; fruit thickness; RNA-seq

P-135:Identification and Expression Analysis of multidrug resistance-related ABC transporter genes (MDR) in Oyster Mushroom (Pleurotus Osteradus)

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ATP Binding Casette proteins, which are one of the structures that control many metabolic pathways at cell membrane and constitutes one of the greatest protein families, take role at substance transportation by using ATP energy. ABC proteins provide the required energy to carry out its biological function by hydrolyzing ATP. Moreover, a major part of ABC proteins which are also known as ABC transporters, take role in transportation of a variety of substrates through biological membranes. Soluble ABC proteins are not involved in transmembrane transportation however; they take an important role in cellular processes such as ribosomal biogenesis and translation of mRNA. Multidrug Resistance (MDR) subfamily which is one of the subfamily of ABC proteins, take role in antimicrobial peptides and lipid transport, mating factors (pheromones) transport, mitochondrial porphyrin uptake, multidrug resistance, eukaryotic peptide export, processing of antigens, protection from the oxidative stress, heavy metals tolerance, etc. In this study, eight genes encoding MDR proteins were identified by the bioinformatics analysis of oyster mushroom (Pleurotus osteradus) genome, and the phylogenetic tree of these genes were constituted for the first time. In addition PoMDRs expression level at different organs were analyzed by qPCR.

Keywords: ABC transporters, MDR Subfamily, gene expression

P-136:Identification and expression analysis of PDR/ABCG subfamily of ABC transporters in Agaricus Bisporus

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ATP Binding Casette (ABC) transporters are one of the greatest and various membrane protein families. ABC transporter proteins are very important as they function in transportation of specific substances that take role in the development and resistance against pathogens of plants. Development of resistance against biotic and abiotic stress, excretion of endogenous and exogenous toxins, and the variety of the metabolic products of inert plants are thought to be regulated by ABC proteins. Pleiotropic Drug Resistance (PDR) subfamily of ABC proteins take role in drug resistance, sterol intake, stress response, toleration to weak organic acids and terponoids, translocation of membrane phospholipids and quorum sensing. In this study, we identified seven genes encoding PDR proteins by the bioinformatics analysis of Agaricus bisporus genome. The phylogenetic tree of these genes was constructed by MEGA software. In this search, AbPDRs expression profile at different parts of the body were analyzed by qPCR.

Keywords: Agaricus Bisporus, gene expression, ABC transporters, PDR subfamily

NO PAYMENT

P-1:Transcriptome Analysis of monoterpene biosynthesis pathway in petals of Lilium `Siberia´ at different flowering stages

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Lilium ‘Siberia’, a typical Oriental hybrid lily with strong fragrance, emits a large amount of monoterpnes, which showed a significantly developmental emission, but the mechanisms are largely unknown. In this study, we used RNA-seq technique to determine the petal transcriptome at four stages, including bud stage (BS), half-bloom stage (HS), full-bloom stage (FS), and late-bloom stage (LS), and analyzed differentially expressed genes (DEGs) to investigate the molecular mechanism of monoterpene biosynthesis. The transcriptome sequencing analysis indicated that totally 56.28 Gb clean base and 223.40 Mb clean read were obtained, and were assembled into 124233 unigenes, of which 35749 unigenes were annotated. The genes in the terpenoid backbone biosynthesis pathway showed significantly different expression levels at different flowering stages. The gene expression levels of 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), and geranyl diphosphate synthase (GPS), firstly

increased and then decreased along with the flowering stages, and the gene expression of ocimene synthase (OCS) exhibited the similar pattern, whose maximum appeared at FS, which was consistent with the monoterpene emission in our previous study. The gene expression of HMG-CoA reductase (HMGR) in MVA pathway presented the same pattern. The gene expression pattern of solanesyl-diphosphate synthase (SDS) and geranylgeranyl diphosphate reductase (GGDR) was opposite and displayed the minimum at FS in branched pathway downstream of monoterpene biosynthesis. We demonstrated that the gene expression of the key enzymes in the MEP pathway regulated the biosynthesis of monoterpenes regularly along with the flower development, resulting in the high release amount at FS. Moreover, the high activation level of MVA pathway, and the depressed branched metabolic pathway of ubiquinone and other terpenoid-quinone at FS may partly contribute to the monoterpene biosynthesis.

Keywords: Lilium 'Siberia'; Floral scent; Monoterpene; Transcriptome sequencing; DEGs analysis

P-37:Genome-wide analysis of the family of light-harvesting chlorophyll a/b-binding proteins in pomegranate (Punica granatum L)

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The light-harvesting chlorophyll a/b-binding (LHC) proteins, higher plant light-harvesting antenna, play a key role in photosynthesis and development of plant. Here, genome-wide analysis of the LHCs in pomegranate is conducted. Total 16 pomegranate LHCs (PgLHCs) were identified by HMMER program. Amino acid sequences of these proteins were ranged from 128 to 342 in length. Syntenic test indicated that most PgLHC genes were from gene duplication. An Neighbor-Joining tree was constructed and the result showed that the sixteen LHC genes could be divided into 5 different groups. Further analysis demonstrated that LHC genes in pomegranate are slightly different in terms of gene structure and conserved motif. This is the first report of identification of LHCs in pomegranate at the genome level, and the conclusion presented here would be useful for molecular breeding of pomegranate varieties with high yield or high resistence to stresses.

Keywords: Pomegranate; LHC gens family; phylogeny; genome

P-53:Antioxidant and Neuronal Cell Protective Effects of Extract from Rootbark Morus alba L

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Mulberry (Morus alba L.) is widely known to have medicinal properties such as treating fever, liver protection, improvement eyesight, joints strength and lowering blood pressure. This study investigated total phenolic contents (TPC), total flavonoid contents (TFC) of the branches, rootbark and leaves. Results demonstrated that rootbark of Mulberry (Morus alba L.) has highest amount of TPC (35.0±1.5 mg GAE/g) and TFC (7.9±0.2 mg QE/g) among three different parts. The antioxidant properties of 70% ethanol extract of root bark from Mulberry were determined through 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Rootbark of Mulberry has radical scavenging activity dose dependently. Neuroprotective effect against H2O2-induced cell viablity on differentiated PC12 cells was analyzed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The result showed that rootbark of mulberry has significant cell protective effect on differentiated PC12 cells against H2O2. On the basis of these results we obtained, rootbark of mulberry showed high antioxidant and neuro-protective effects.

Keywords: Mulberry rootbark, antioxidant, cell protection, nerutonal PC12 cells

P-95:A possible MYB-bHLH proteins complex formed to activate anthocyanin productions in blood orange fruit

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Anthocyanin production is transcriptionally regulated by the MYB-bHLH-WD40 (MBW) complex, which is composed of R2R3-MYB protein, bHLH type protein as well as WD40 repeat protein to activate several of the late flavonoid biosynthetic genes in many fruit trees. But it is still unknown about a MBW modulation in Citrus. In this study, the transcriptome or proteome analyses on fruit pulp from the blood orange and the navel orange were performed to study anthocyanin-related molecular changes in Citrus sinensis fruit during consecutive developmental periods, including young fruit, fruit-coloring onset and fruit delayed-harvest for two months, during which fruit remained on the trees. Three possible regulators Cs6g17570 (encoding R2R3-MYB protein), Cs5g31400 (encoding bHLH type protein) and Cs9g04810 (WD40 repeat protein) showed correlations with anthocyanin production during blood orange fruit development and ripening. In particular, R2R3-MYB, bHLH-type and WD40 proteins from blood orange were interacted in vitro and in vivo, respectively. Additionally, the relative expression levels of Cs6g17570 and Cs5g31400 were higher in a partial of mutated fleshes with greater anthocyanin accumulated than the remaining fleshes with less anthocyanin contents in a 'Mosaic' blood orange fruit. Moreover, the bHLH gene and dihydroflavonol 4-reductase, a key enzyme involved into anthocyanin biosynthesis pathway, had greater transcription levels in different blood orange cultivars and also in the transgenic Arabidopsis thaliana by Cs6g17570 ectopic expression. Furthermore, Cs6g17570 and Cs5g31400 in blood orange were up-regulated by cold induction and then had similar expression profiling during cold storage. In conclusion, Cs6g17570 and Cs5g31400 encoding proteins might be involved into anthocyanin regulation in blood orange by MYB-bHLH complex.

Keywords: Citrus sinensis, blood orange, navel orange, anthocyanin, transcriptome, proteome

P-100:Genome-wide identification of strigolactones biosynthetic genes and expression analysis in columnar and standard apple

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Columnar apple is a valuable resource for genetic improvement of cultivated apples due to its special tree architecture. This columnar habit is characterized by short internodes, reduced long branching and increased lateral spurs on branches. MdCo31 is a strong candidate gene for control of columnar habit. Strigolactones (SLs) is a novel class of plant hormone controlling shoot branching. In order to further study the relationship between SLs and columnar traits, and whether MdCo31 can regulate the SLs biosynthesis gene expression. Based on previous reports, twenty-three SLs biosynthesis and signaling related genes were identified in apple genome data following TBLASTN with amin acid sequences from Arabidopsis, pea, rice and petunia. These genes included five MdMAX1-related genes, one MdMAX2, two MdMAX3, three MdMAX4, five MdD14, three MdD27 and four MdD53. The hormone content of columnar was higher than that of standard apple. The transcripts patterns of twenty-three SLs biosynthesis and signaling relate geneswere detected using real-time PCR from different development buds and shoots. The results showed that the expression profile of MdD27-2, MdMAX1-3, MdMAX3-1, MdMAX4-3, MdD54-1 and MdD54-2had difference between with columnar and standard apple. The expression level of MdD27-2, MdMAX1-3, MdMAX3-1, MdMAX4-3 in columnar were higher than that in standard apple. However, thetranscriptof MdD54-1 and MdD54-2 was lower than that of standard apple. The result of overexpression of MdCo31indicated that expression of MdCo31gene was associated with the short plant height and short internode length in tobacco (Nicotiana benthamiana). The expression levels of NbMAX3 was up regulated and NbD54 down regulated in transgenic tobacco. Our results suggested the SLs was related to columnar traits. The MdCo31 gene increases the SLs content by up regulating MdMAX3 gene expression, on the other hand by inhibiting the expression of the MdD54 gene, thereby inhibiting the degradation of hormone signaling, and then increasing the SLs effect.

Keywords: Strigolactones; MdCo31; Columnar apple; Standard apple; Expression

P-104:Characterization and development of EST-SSR markers in Jerusalem artichoke (Helianthus tuberosus L.) using transcriptome sequences

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Jerusalem artichoke is distributed throughout most regions of the world owing to its extensive adaptability. In recent years, Jerusalem artichoke has been developed as a novel source of sugar, biofuel, and animal feed, and these uses have received widespread attention. In order to understand the general characteristics of simple sequence repeat (SSR) markers in Jerusalem artichoke EST sequences and accelerate the use of SSR markers in Jerusalem artichoke research, this study used 40,370 sequenced unigene fragments and MISA software to identify SSR loci. A total of 1,204 SSR loci were identified with 13 different types of repeats, distributed among 1020 EST sequences, of which trinucleotide repeats were the most common, accounting for 38.21% of the total SSR loci. Among the 44 repeat motifs, AG/CT, AAG/CTT, and ATC/ATG motifs had the highest frequencies, accounting for 22.45%, 14.71%, and 7.84% of all motifs, respectively. There were great differences in repeat number among motifs. Dinucleotide repeats exhibited the highest number of repeats. From these sequences, 48 pairs of EST-SSR primers were designed, and 22 primer pairs for loci with high polymorphism were selected to analyze the genetic diversity of 45 Jerusalem artichoke germplasm sources. Cluster analysis was conducted using the genetic similarity coefficient, revealing significant genetic differences between Asian and European genetic material. Cluster analysis revealed a relationship between the genotypes and geographic origins of Jerusalem artichoke. The Jerusalem artichoke EST-SSR marker system established in this study provides an effective molecular marker system for future research focused on Jerusalem artichoke genetic diversity and breeding new varieties.

Keywords: Helianthus tuberosus L.·EST-SSR marker·Genetic diversity·Germplasm Resources

P-109:Galactinol synthase (AnGolS1) of A.nanus can improve the germination ability and rate of tomato seeds, and the resist cold stress ability of tomato plants under low-temperature

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Galactinol synthase (GolS) is important in the physiology of plant stress resistance, photosynthate translocation and seed physiology. A galactinol synthase (AnGolS1) which was isolated from Ammopiptanthus nanus seedlings was transformed into tomato. In previous studies, we found that AnGolS1 could increase galactinol content, but not increase the content of raffinose and stachyose in the AnGolS1 overexpression tomato (AnGolS1-OX) seeds. Interestingly, the germination ability and rate of the AnGolS1-OX seeds were improved compared with wild tomato seeds under low-temperature stress, while it was not different under normal temperature. In addition, AnGolS1 can improve the cold tolerance of the AnGolS1-OX seedlings and maturity plants under low-temperature.

It indicates that AnGolS1 maybe anticipate in the maturity and germination of tomato seeds and helps tomato plant to resist cold damage. Thus, it is important to define its metabolic functions that will help us to further understand the molecular mechanism of cold tolerance of tomato.

Keywords: Galactinol synthase; tomato; cold stress

P-110:RNA-seq reveals the influence of low night temperature to tomato locule number involves in the change of transcription levels of hormones and sugar metabolism

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Background: The size and shape of tomato (Solanum lycopersicum L.) fruit is determined by the locule number, which not only controlled by genetic factors, but also regulated by the environment. However, the molecular mechanisms involved in the response of tomato locule number of suboptimal night temperature is not yet clear. This work analysis the response of transcription level of low night temperature using Illumina RNA-seq and qRT-PCR aim to explore the relationship between temperature and tomato locule number.

Results: This study with multi-locule 'MLK1' as the experimental materials. Under sub-low night temperature (28/10℃), the locule number of first inflorescence and second inflorescence of tomato was significantly increased ($p < 0.05$), and there was no significant difference between suboptimal night temperature (28/15 ℃) and nomal night temperature (28/ 20 ℃) on the locule number of tomato. We identified 65060425, 57752715, 47913998, 47523527, 54114303, 47801791, 59583787 unigenes, and 148, 243, 1327, 2776, 2125, 2128 differentially expressed genes (DEGs) in tomato at 0d, T10-10d, T15-10d, T20-10d, T10-20d, T15-20d, T20-20d, respectively. Gene Ontology (GO) analysis showed that the main enrichment were "catalytic activity", "metabolic process" and "binding". KEGG analysis revealed that "plant hormone signal transduction" and "starch and sucrose metabolism" were most enriched out of the total pathways. Then GA, IAA, CTK pathways and sugar metabolism were further analyzed to observe the expression pattern of differentially expressed transcripts. Our data speculate that KAO (solyc10g007860) and GA2ox5 (solyc07g061730), GA2ox7 (solyc02g080120), YUCCA (solyc09g074430), LOG7 (solyc10g082020), CKX6 (solyc12g008900), SUS (solyc12g009300), lin5 (Solyc09g010080) can be used as candidate genes to control the number of

tomato locule. qRT-PCR assay results for 18 selected transcripts validated the data obtained by RNA-seq, the results were basically consistent with transcriptional data**.**

Conclusions:

Taking into account the results above, suboptimal night temperature induces the change of transcription levels of GA, IAA, CTK and sugar metabolism closely relates to tomato locule number. Our research may provide powerful help to understand the molecular mechanism of tomato locule formation to environment.

Keywords: Tomato ; low night temperature; locule number, fruit malformation; gibberellin; sugar

P-111:Transcriptomics Studies of Banana for Candidate Gene Identification in a Genetic Improvement Program Framework in Ecuador

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Modern breeding techniques including genetic transformation and genome editing needs the identification of genes relevant for the improved character. In Ecuador, routinely banana tissue culture and Agrobacterium-mediated transformation is established. Several methods could be used for gene identification and function elucidation. Gene expression analysis were performed in different banana cultivars including ‘Williams’ (AAA) and the black sigatoka resistant accessions ‘Calcutta-4’ and ‘Tuu Gia’. Two techniques have been applied for gene identification including the Suppression Subtractive Hybridization (SSH) and RNA sequencing (RNA-seq). Tissues used for

transcriptomic analysis were leaves from grown banana plants in pots with at least three fully developed leaves and 15 cm in height, derived from in vitro culture. For identification of candidate gene resistance to black sigatoka disease two different bioassays were performed after inoculation of Pseudocercospora fijiensis conidia including: i) a SSH technique on 'Calcutta-4', ii) and a RNA-seq with Illumina® in 'Williams' and 'Tuu Gia' after inoculation of P. fijiensis conidia. Furthermore, SSH was performed after application of a biofertilizer in banana plants. Several genes were identified and evaluated for differentially expression in banana. In silico functional annotation revealed that different pathways in banana plants are affected including plant growth processes and related to biotic and abiotic stresses. Candidate genes involved in relevant pathways could be used in a genetic improvement program in banana through Agrobacterium-mediated transformation or could be targets for genome editing, for the generation of bananas resistant to black sigatoka, the main constraint in banana production in Ecuador.

Keywords:

Musa, Agrobacterium tumefaciens, Transcriptome, RNA-seq, SSH, banana

P-112:Delineating the gene cascade involved in floral induction and repression in mango

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In our earlier communications (Vyavahare et al. and Krishna et al. 2017) we had demonstrated that there are three florigen encoding (Flowering locus T) genes namely MiFT1, MiFT2 and MiFT3 and two anti florigen Terminal Flower 1 (TFL1) like genes in mango. The expression patterns of MiFT genes and TFL1 like genes under natural field conditions and in plants treated with gibberellic acid (that suppresses flowering) and paclobutrazol (that ensures flowering) was studied. The studies showed

that MiFT1 and MiFT3, that encode an identical protein, function as inducers of flowering while the MiFT2 is never expressed. Of the two TFL1 like genes, MiTFL1a appeared to be the suppressor of flowering. These studies have been extended to show that MiFT1/3 is capable ofrestoring early flowering in Arabidopsis ft-10 (late flowering) mutant strengthening our conclusion that MiFT1/3 encode the florigen equivalent in mango. Interestingly the non-expressing MiFT2with its introns when transformed into Arabidopsis ft-10 mutant was indeed transcribed, spliced but still could not restore early flowering in the ft-10 mutant.

The downstream target genes of the FT-FD complex namely the MADS-box transcription factors SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1), APEATALA1 and LEAFY were characterized and their expression during the flowering in mango studied. These studies hint towards a possible role of these genes in promoting the floral meristem identity. The genes functioning upstream of FT namely GIGANTEA and CONSTANS have also been characterized and their possible function in relation to mango flowering will be discussed.

Keywords: Mango, Flowering Genes, Flowering locus T, Terminal Flower 1

P-116: A novel method for the production and screening of CRISPR/Cas9-mediated non-transgenic mutants of perennial plants

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Developing CRISPR/Cas9-mediated non-transgenic mutants in asexually propagated perennial crop plants is challenging but highly desirable. Here, we report a highly useful method using an Agrobacterium-mediated transient CRISPR/Cas9 gene expression system to create non-transgenic mutant plants without the need for sexual segregation. We have also developed a rapid, cost-effective, and high-throughput mutant screening protocol based on Illumina sequencing followed by high-resolution melting (HRM) analysis. Using tetraploid tobacco as a model species and the phytoene desaturase (PDS) gene as a target, we successfully created and expediently identified mutant plants, which were verified as tetra-allelic mutants. We produced pds mutant shoots at a rate of 47.5% from tobacco leaf explants, without the use of antibiotic selection. Among these pds plants, 17.2% were confirmed to be non-transgenic, for an overall non-transgenic mutation rate of 8.2%. Our method is reliable and effective in creating non-transgenic mutant plants without the need to segregate out transgenes through sexual reproduction. This method should be applicable to many economically important, heterozygous, perennial horticultural crop species that are more difficult to regenerate.

Keywords: CRISPR, Transient-Expression, Non-Transgenic Mutants, Agrobacterium, Perennial Plants

P-121:Identification of novel chemical compounds regulating strawberry fruit development and ripening

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The ripening of non-climacteric fruit is a complex regulation. No single master switch controlling ripening initiation, such as the role played by ethylene in climacteric fruits, has yet been found for non-climacteric fruits. To understand the mechanism of non-climacteric fruit ripening, we have used a chemical genetics approach to isolate the chemical compounds acting on fruit ripening and quality in strawberry, a non-climacteric model fruit. Chemical genetics is a high-throughput approach for determining gene function using small bioactive molecules to activate/inactivate gene products (i.e., proteins). This approach has been used to better elucidate hormonal signaling in Arabidopsis. We are using this approach for studying fruit development and ripening. We have obtained more than 20 chemical compounds affecting strawberry fruit ripening. We observed a variety of effects on fruit color, firmness and ripening in strawberry fruit. Our data suggest that the chemical genetics approach is useful for accelerating the gene discovery and crop improvement of horticultural crops.

Keywords: Fruit ripening, strawberry, chemical genetics

P-124:Sphingolipids during olive fruit ripening

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Plant sphingolipids are involved in the building of the matrix of cell membranes and in signalling pathways of physiological processes and environmental responses. However, information regarding their role in fruit development and ripening, a plant-specific process, is unknown. The present study seeks to determine whether and, if so, how sphingolipids are involved in fleshy-fruit development and ripening in an oil-crop species such as olive (Olea europaea L. cv Picual). Here, in the plasma membranes of live protoplasts, we used flourescence to examine various specific lipophilic stains in sphingolipid-enriched regions and investigated the composition of the sphingolipid long-chain bases (LCB) as well as the expression patterns of sphingolipid-related genes, OeSPT, OeSPHK, OeACER,and OeGlcCerase, during olive-fruit development and ripening. The results demonstrate increased sphingolipid content and vesicle trafficking in olive-fruit protoplasts at the onset of ripening. Moreover, the concentration of LCB [t18:1(8Z), t18:1 (8E), t18:0, d18:2 (4E/8Z), d18:2 (4E/8E),

d18:1(4E), and 1,4-anhydro-t18:1(8E)] increases during fruit development to reach a maximum at the onset of ripening, although these molecular species decreased during fruit ripening. On the other hand, OeSPT, OeSPHK, and OeGlcCerase were expressed differentially during fruit development and ripening, whereas OeACER gene expression was detected only at the fully ripe stage. The results provide novel data about sphingolipid distribution, content, and biosynthesis/turnover gene transcripts during fleshy-fruit ripening, indicating that all are highly regulated in a developmental manner.

Keywords: Alkaline dihydroceramidase, fruit ripening, glucosylceramidase, olive, serine palmitoyltransferase, sphingolipid, sphingosine kinase, vesicle trafficking

P-125:Brassinosteroid-induced modulation of sphingolipid long-chain base composition and gene expression during early olive-fruit development

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Sphingolipids are abundant membrane components and signalling molecules in various aspects of plant development. However, the role of sphingolipids in early fleshy-fruit growth has been rarely investigated. In this study, we first investigated the temporal changes in sphingolipid long-chain base (LCB) content, composition, and gene expression that occurred during flower opening and early fruit development in olive (Olea europaea L. cv Picual). Moreover, the interaction between sphingolipid and the plant hormone, brassinosteroid (BR), during the early fruit development was also explored. For this, BR levels were manipulated through the application of exogenous BRs (24-epibrassinolide,

EBR) or a BR biosynthesis inhibitor (brassinazole, Brz) and their effects on early fruit development, sphingolipid LCB content, and gene expression were examined in olive fruit at 14 days post-anthesis (DPA). We here show that sphingolipid with C-4 hydroxylation and Δ8 desaturation with a preference for (E)-isomer formation are quantitatively the most important sphingolipids in olive reproductive organs.In this work, the total LCB amount significantly decreased at the anthesis stage, but olive sphingosine-1-phosphate lyase (OeSPL) gene was expressed exclusively in flower and upregulated during the anthesis, revealing an association with the d18:1(8E) accumulation. However, the LCB content increased in parallel with the up-regulation of the expression of genes for key sphingolipid biosynthetic and LCB modification enzymes during early fruit development in olive. Likewise, we found that EBR exogenously applied in olive tree significantly accelerated the fruit growth rate associated with reduced levels of sphingolipid LCB content and gene expression in olive fruit after 7 and 14 days of treatment, whereas Brz slowed the fruit growth rate and boosted the sphingolipid LCB content and gene expression during early growth. Thus, our data indicate that endogenous sphingolipid LCB and gene-expression levels are intricately controlled during early fruit development in olive and also suggest a possible link between BR, the sphingolipid content/gene expression, and early fruit development.

Keywords: Brassinosteroid, early fruit growth, olive, serine palmitoyltransferase, sphingolipid, sphingosine-1-phosphate lyase

P-126:Sphingolipid and sterol accumulation during mature-fruit abscission in olive

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Sphingolipids, found in membranes of eukaryotic cells, have been demonstrated to carry out functions in various processes in plant cells. However, the roles of these lipids in fruit abscission remain to be determined in plants. Biochemical and fluorescence microscopy imaging approach has been adopted to investigate the accumulation and distribution of sphingolipids during mature-fruit abscission in olive (Olea europaea L. cv. Picual). Here, a lipid-content analysis in live protoplasts of the olive abscission zone (AZ) was made with fluorescent dyes and lipid analogs, particularly plasma membrane sphingolipid-enriched domains, and their dynamics were investigated in relation to the timing of mature-fruit abscission. In olive AZ cells, the measured proportion of both polar lipids and sphingolipids increased as well as endocytosis was stimulated during mature-fruit abscission. Likewise, mature-fruit abscission resulted in quantitative and qualitative changes in sphingolipid long-chain bases (LCBs) in the olive AZ. The total LCB increase was due essentially to the increase of t18:1(8E) LCBs, suggesting that C-4 hydroxylation and Δ8 desaturation with a preference for (E)-isomer formation were quantitatively the most important sphingolipids in olive AZ during abscission. However, our results also showed a specific association between the dihydroxylated LCB sphinganine (d18:0) and the mature-fruit abscission. These results indicate a clear correlation between the sphingolipid composition and mature-fruit abscission. Moreover, measurements of endogenous sterol levels in the olive AZ revealed that it accumulated sitosterol and campesterol with a concomitant decrease in cycloartenol during abscission. In addition, underlying the distinct sterol composition of AZ during abscission, genes for key biosynthetic enzymes for sterol synthesis, for obtusifoliol 14α-demethylase (CYP51) and C-24 sterol methyltransferase2 (SMT2), were up-regulated during mature-fruit abscission, in parallel to the increase in sitosterol content. The differences found in AZ lipid content and the relationships established between LCB and sterol composition, offer new insights about sphingolipids and sterols in abscission.

Keywords: Abscission, fruit, olive, sphingolipid, sterol, vesicle trafficking

P-127:OLIVE FRUITS ENRICHED IN PHENOLICS BY PRE-HARVEST ABSCISIC ACID TREATMENT

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The aim of this research was to study the effect of abscisic acid pre-harvest treatment on the phenolic composition of olive fruits. To that end we applied abscisic acid (i.e., 50 mg/L and 100 mg/L) on Arbequina and Picual olive trees. Two different days of harvesting (i.e., day 3 and 6 after treatment) were also included in the study. Although the results obtained depended on the cultivar and on the day of harvesting a general trend was established. The treatment with 50 mg/L of abscisic acid resulted in higher total phenol content but significant decrease in the DPPH activity. In contrast, olives treated with 100 mg/L abscisic acid resulted in higher total phenol content, DPPH activity and contents of oleuropein, hydroxytyrosol and phenolic acids as compared with controls. The best values of total phenol content and IC50 were obtained for treated Picual olives (727.75 mg gallic kg-1 and 889.72 µg/ml, respectively) whereas the highest values of oleuropein and hydroxytyrosol were measured for treated Arbequina olives (508.94 and 559.67 mg kg-1, respectively). Phenolic acid content was also higher in Picual olives treated with 100 mg/L of abscisic acid. Particulary, values ranged from 7.26 mg kg-1 for caffeic acid to 92.38 mg kg-1 for chlorogenic acid. Exogenous abscisic acid applied to olive trees is a promising agronomic practice to obtain olives enriched in antioxidants.

Keywords: Elicitors, preharvest treatment, olives, phenolics, antioxidant, functional

P-128:METHYL JASMONATE IMPACT ON THE OLIVE ANTIOXIDANT COMPONENTES AND PROPERTIES

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We here investigate the effects of the application of methyl jasmonate to olive trees on antioxidant composition of olive fruits. Two cultivars (ie, Arbequina and Picual) were evaluated in our study. As a result, the total phenol content increased significantly with the treatment in Arbequina (from 155.89 to 434.22 mg gallic acid kg-1) whereas decreases were observed in Picual (from 338.27 to 127.71 mg gallic acid kg-1). Similarly, decreases in phenolic acid content were measured in Arbequina whilst no effect was observed in Picual olives. However, the contents of oleuropein and hydroxytyrosol did not increase with the pre-harvest methyl jasmonate for both Arbequina and Picual. Also for both cultivars the treatment of the olive trees increased the free radical scavenging activity of the olive fruits (IC50 from 514.36 to 1125.46 µg/mL in Arbequina and from 611.98 to 114.55 µg/mL in Picual)

Keywords: Olive fruit, olive tree, methyl jasmonate, antioxidant, pre-harvest treatment, phenolics, quality