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Abstracts

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Tomato WRKY81 acts as a negative regulator for drought tolerance by modulating guard cell H₂O₂–mediated stomatal closure

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WRKY transcription factors (TFs) are key regulators in numerous biological processes including stress responses in plants. Here, we explored a critical role of a group III SIWRKY81 TF in regulation of drought stress response. Drought stress gradually increases the transcript levels of SIWRKY81 and impairs leaf water potential and membrane stability in tomato. Analyses of plant phenotypes reveal that silencing of SIWRKY81 in tomato enhances tolerance to drought, while its over-expression in *Arabidopsis* results in an opposite phenotype. Notably, the enhanced tolerance in SIWRKY81-silenced tomato plants is closely associated with rapid and increased stomatal closure. Moreover, such stomatal response in the SIWRKY81-silenced plants is sensitive to ABA alongside drought-induced enhanced accumulation of reactive oxygen species (H₂O₂) in the guard cells. Taken together, these results suggest that SIWRKY81 acts as a negative regulator of stomatal closure by suppressing RBOH-derived H₂O₂ accumulation, which attenuates plant tolerance to drought stress.

Session: Biotic and abiotic stresses

Combined effects of provenance and slow-release fertilizer on nursery and field performance of yellowhorn (*Xanthoceras sorbifolium Bunge*) seedlings

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Yellowhorn (*Xanthoceras sorbifolium Bunge*) has been widely planted for biodiesel production in China, but has frequently shown poor field performance. Container-grown yellowhorn seedlings originating from three Chinese provenances, Wengniute Qi (WQ), Alukeerqin Qi (AQ), and Shanxian (SX), were fertilized with slow-release fertilizer (SRF) at 40, 80, 120, 160 or 200 mg N seedling⁻¹. Tree growth, survival and nutrient content were measured after one year's growth in a greenhouse followed by two years in a field site. Plants from AQ and SX tended to have higher stem and root P contents in the nursery. Higher rates of SRF increased root N, and stem and root P contents. After one year in the nursery, there were a number of interactions between provenance and SRF for plant growth responses and nutrient content in the nursery, however after two years of additional growth in the field, plants from the different provenances generally responded similarly to applied SRF, with few interactions. Final plant height was approximately 10% lower in trees from provenance SX but was not affected by application of SRF. Conversely, final trunk diameter and stem and root biomass were unaffected by provenance but increased with higher rates of applied SRF. Our results indicate that application of SRF may be a useful tool to nutrient load yellowhorn in the nursery and facilitate transplanting performance in the field. Overall, optimal nursery and field performance of yellowhorn were observed in provenance AQ at 120-200 mg N seedling⁻¹ SRF.

Session: Plant signaling and behaviour

Decoding molecular regulators of floral transition in soybean

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The world's population is expected to increase more than 9 billion by 2050, requiring a doubling of food production from the same amount of arable land. Further, climate change threatens our food security since plant reproductive development like flowering and seed production is sensitive to the changes in the external environment. Floral transition in plants is a critical developmental switch as floral induction can impact essential agronomic traits of crops such as yield. Understanding the floral transition is the key to ensuring future food security under changing climate conditions. Soybean is a major legume crop used for human and animal feed and provides 55% of the worldwide oilseed production. Soybean is a photoperiod sensitive crop whose floral transition is triggered by exposure to short-day conditions. Expanding the cultivation areas of crops such as soybean requires optimization of environmental and development responses suitable for changing climate regimes. Our knowledge of key molecular regulators of flower initiation in legumes, including soybean, is limited. We used an integrated bioinformatics and experimental approach to address this gap in our knowledge. Our comparative genomics and RNA-Seq analysis of shoot apical meristem and leaf undergoing floral transition revealed major reprogramming events in leaves and the shoot apical meristem. Our data also revealed an extensive reprogramming of genes associated with the epigenetic chromatin modifications and RNAi gene silencing in the shoot apical meristem during floral transition. Further, our study on lincRNAs suggested the involvement of lincRNAs in signal transduction, developmental, and stress response. Soybean is a paleopolyploid legume with multiple copies of its genes. Our study also revealed the functional divergence of flowering genes.

Session: Dissection of complex quality traits

Gene editing-based modulation of stomatal activity and drought resistance in grapevine

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Grapevine (*Vitis vinifera*) is one of the most important fruit crops in the world, but the cultivars typically planted are not well adapted to abiotic and biotic stresses. As a consequence of climate change, drought is now one of the biggest concerns for agriculture. Indeed, global climate models predict a significant increase in aridity in the near future, and water deficit may become a limiting factor in wine production and quality. As an example, the grape growing region of South Africa recently experienced a severe drought, which had significant negative impacts on agricultural productivity. It is therefore important to investigate how grapevine tolerance to water stress can be improved. In this regard we have focussed our attention on the MYB60 transcription factor. In Arabidopsis, AtMYB60 has been linked to stomatal regulation in response to ABA and, interestingly Arabidopsis myb60 knockouts proved to be more tolerant to drought. The expression of the gene encoding the grapevine ortholog, VvMYB60, appears very specific to the guard cells and is able to revert the phenotype of the Arabidopsis myb60 mutant. The development of genome editing via CRISPR/Cas9 in the last few years has paved the way to new opportunities in the field of crop genetic improvement. The technology is precise, efficient and flexible, and can be used for biotechnological projects, as well as for studies on gene function. Here, we have implemented a genome editing approach to better understand the role of VvMYB60 in grapevine in relation to water stress. CRISPR-P and CRISPR RGEN online software tools were used to select four gRNAs targeting VvMYB60. gRNAs were then cloned into the pDIRECT_22C binary vector that generate multigene knockouts (Voytas Lab Plant Genome Engineering Toolkit). The vector was electroporated in Agrobacterium EHA105 and grapevine (cv 'Chardonnay' and 'Sugraone') transformation was performed. Embryogenic calli are currently in germination medium and molecular analysis of regenerated plants will be performed to detect the presence of Cas9 and mutations in VvMYB60. Expression analysis, phenotypical and physiological characterisation, including field trials, will then follow.

Session: Biotechnology and genome editing

Transcriptome analysis of Chinese chestnut during calcareization

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There were few studies about molecular level in chestnut calcification. In this study, the degree of calcification of chestnut was significantly negatively correlated with moisture content ($r=-0.85$), and the critical value of the seed moisture content of the calcification phenomenon is 35%. Plant cell walls play an important role in determining the flavor of fruit, and pectin, cellulose, and lignin content in cell wall of chestnut were investigated to explore the physiology and biochemistry mechanism of calcification. The transcriptomes from different calcification were analyses to better understand the gene change in chestnut calcification.

Session: Biotic and abiotic stresses

The phenological growth stages of *Sapindus mukorossi* according to BBCH scale

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Sapindus mukorossi is a native tree in southern China that has multiple useful functions, including high landscaping, ecological, esthetic, and economic values. More importantly, its fruits are being rapidly developed as raw materials for bioenergy and saponin production in southern China. To provide a standardized phenological description of the species, this study firstly used the BBCH (Biologische Bundesanstalt, Bundessortenamt und CHEmische Industrie) scale. In total, eight principal stages—i.e., bud, leaf and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity, senescence and beginning of dormancy—and 58 secondary stages were described. It ranges from vegetative bud dormancy to the onset of the next beginning of the dormancy, using a two-digit numerical coding system. In addition to the descriptions, we provide photographic images of some major developmental stages to standardize morphological characteristics and the phenological observation of *S. mukorossi*. This study will be an asset for biological researches and cultivation management in *Sapindus*, and will provide valuable information for other fruit trees.

Session: Plant signaling and behaviour

Five -year assessment of fire blight resistant apple rootstocks in Alabama

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Newly released fire blight resistant apple rootstocks can aid in disease management and improve production sustainability. As part of the multistate NC-140 cooperative research project, an apple rootstock study was established at the Chilton Research and Extension Center near Clanton, Alabama in 2014 aiming to assess 14 newly developed fire blight resistant and size-controlling rootstocks. Low-chill 'Aztec Fuji' apple was the scion cultivar used. The following rootstocks were tested: B.10, G.11, G.202, G.214, G.30, G.41, G.935, G.969, M.26 EMLA, M.9-T337, V.1, V.5, V.6, and V.7. Trees are arranged in a RCBD with ten single tree replications and trained to the highly efficient Tall Spindle training system which has not been tested in Alabama before. Tall Spindle is designed to control the vegetative vigor and optimize the crop production especially in the early stage of tree establishment, while providing early returns to the grower. Our fifth-year results suggest trees grafted on 'V.7', 'G.969', and 'V.5' produced the highest cumulative yield/tree. In general, the Vineland series of rootstocks 'V.5', 'V.6', and 'V.7' had high productivity, and were the most vigorously growing rootstocks in the present study based on data for trunk cross sectional area. Trees on 'G.11' consistently produced the largest mean fruit size ranging between 180 and 206 g through the seasons. 'Aztec Fuji' trees grafted on the weak 'G.214' rootstock had the highest yield efficiency of 1.18 kg/cm² at the end of the fifth season, while trees grafted on 'V.6' and 'V.7' rootstocks had the lowest efficiency of 0.54 and 0.60 kg/cm² respectively. Research is going to continue to assess the production efficiency and fruit quality as a way to achieve more efficient land use and sustainable apple production. This project was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Session: Biotic and abiotic stresses

Draft whole-genome sequence of a “*Candidatus Liberibacter asiaticus*” strain from Yunnan, China

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The unculturable phloem-limited alphaproteobacterium “*Candidatus Liberibacter asiaticus*” is associated with Citrus Huanglongbing (HLB; also called yellow shoot disease), one of the most devastating diseases in citrus production worldwide. HLB was first reported in the Pearl River Delta area of Guangdong Province in China nearly a century ago. As of 2018, HLB has spread and been found in 11 of 19 citrus-producing provinces in southern China. In China, HLB can occur in both low-altitude provinces, such as Guangdong, and high-altitude provinces, such as Yunnan Province. Previous studies have revealed the population variation of “*Ca. Liberibacter asiaticus*” from different geographical locations. Due to the inability to culture this bacterium, the characterization of “*Ca. Liberibacter asiaticus*” has relied heavily on genome analyses. The genome sequence of a strain of “*Ca. Liberibacter asiaticus*” isolated from a low-altitude region (Guangdong Province, China) was reported previously. To further compare the “*Ca. Liberibacter asiaticus*” strains from different geographic locations, here, we report a draft whole-genome sequence of “*Ca. Liberibacter asiaticus*” strain YNJS7C from Yunnan Province, a high-altitude province. “*Ca. Liberibacter asiaticus*” strain YNJS7C was first identified in a navel orange (*Citrus sinensis* L. Osbeck) tree showing typical HLB symptoms in Jianshui City (23°38′N, 102°49′E) of Yunnan Province, China. Total plant DNA was extracted from infected citrus leaf midribs using the plant DNA extraction kit (Omega Bio-tek, Norcross, GA, USA). Library preparation was performed using the Illumina TruSeq version 2 paired-end library preparation kit (300-bp insert size), and sequencing was carried out on the Illumina HiSeq platform (San Diego, CA, USA). Quality control filtering and trimming were conducted using CLC Genomics Workbench 9.5 (Qiagen, Hilden, Germany). The HiSeq sequencing generated a total of 7.87×10^7 reads with an average size of 150 bp. Using the whole-genome sequence of “*Ca. Liberibacter asiaticus*” strain A4 (GenBank accession number CP010804) and those of three “*Ca. Liberibacter asiaticus*” prophages (SC1, SC2, and P-JXGC-3) as references, we identified a total of 110,266 reads using the Bowtie 2 software (version 2.3.4.2) with default settings (end-to-end read alignment mode). De novo assembly was performed using CLC Genomics Workbench 9.5 with default settings. The assembly generated a total of three contigs ranging from 31,541 bp to 1,187,865 bp, with $\sim 15\times$ coverage. The draft genome sequence of “*Ca. Liberibacter asiaticus*” strain YNJS7C comprises 1,258,986 bp, with a G+C content of 36.6%. The strain YNJS7C genome was predicted to have 1,174 open reading frames (ORFs) and 53 RNA genes. Prophage identification by comparing sequences with those of three prophages (SC1, SC2, and P-JXGC-3) revealed YNJS7C to have SC2-like (type 2) and P-JXGC-3-like (type 3) prophages.

Session: Comparative genomics

Improving of stone plant resistance to Plum pox virus by bioengineering approach: from co-suppression to gene editing.

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In modern horticulture Plum pox virus (PPV) imposes serious threats to commercial plantations of a wide range of fruit species belonging to genera *Prunus*. Given the lack of natural genetic resources, which display reliable resistance to PPV infection, there has been considerable interest in using genetic engineering and editing methods for targeted genome modification of stone fruit trees to control Sharka disease caused by PPV. Based on developed protocols of plant regeneration from somatic (leaf) tissue and agrobacterial transformation for some commercial cultivars and rootstock we tested number bioengineering approach for generate PPV resistance. First experiments based on expression of fragments of virus coat protein gene in sense-orientation and self-complementary fragments of gene were used for realization post-transcriptional gene silencing. Apart from the fact that the accumulation of coat protein was evaluated by Western blot assay in most of transgenic lines of cultivar “Startovaya”, we observed absence of PPV only in lines of this cultivar and rootstock” Elita” (*Prunus pumila* L. × *P. salicina* Lindl.) × (*P. cerasifera* Ehrh.) expressed intron hairpin RNAi construct, containing the self-complementary sequences of a fragments of PPV-CP driven by 35SCaMV promoter. Strong induced resistance of these clones confirmed by ten years testing under high infection pressure. The plants of rootstocks 146 (*Prunus pumila* x *Prunus tomentosa*), “Elita” and cultivar Startovaya were transformed by intron hairpin RNAi constructs containing the self-complementary sequences of fragment of factor initiation translation eIF(iso)4G and E genes driven by the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) genes promoter. To clarify the PPV resistance, transgenic plants were inoculated with infected buds by grafting onto one-year-old plants growing in the greenhouse. By the second year of the challenge experiment, transgenic lines demonstrate different range of resistant to PPV as the transgenic events showed no or low level of virus accumulation detected by DAS-ELISA and displayed normal phenotype in greenhouse conditions. Based on these results we start experiments for gene editing methods for knocking out eIF(iso)4G and eIF(iso)4E genes. From about 20 synthesized guide RNA for each genes only 2-3 demonstrated 35-75% efficiency to cut target DNA in in vitro test. Based on these RNAs we create vectors and start transformation of rootstocks and commercial cultivars of stone fruit plants.

Session: Biotic and abiotic stresses

Assessment of the mechanisms of gibberellins in regulating cold tolerance of post-harvest tomato fruit by transcriptome and proteome analyses

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Gibberellins (GAs) are a large group of naturally occurring plant hormones that participate in biological and abiotic stresses in plants. To investigate the role of GA in tomato fruit responses to chilling stress, wild type and GA-deficient mutant (gib-3) tomato fruits were used as experimental material to determine the CI index, malondialdehyde (MDA) levels, ion permeability and activities of phospholipase C (PLC), phospholipase D (PLD), lipoxidase (LOX), polygalacturonase (PG), pectinesterase (PME), superoxidase (SOD) and catalase (CAT). Moreover, eukaryotic mRNA sequencing, isobaric tags for relative and absolute quantitation (iTRAQ) quantitative proteomics analysis and correlation analysis of the transcriptome and proteome were used to determine the mechanisms of the postharvest tomato fruit to chilling stress at the gene and protein levels, thus clarifying the pathway of protein processing in the endoplasmic reticulum and of phenylalanine metabolism and glutathione metabolism that are involved in the process of cold resistance regulation.

Session: Plant signalling and behaviour

Genome-wide identification and expression changes of miRNAs and phased siRNAs in a male-sterile cybrid pummelo

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MiRNAs and phased small interfering RNAs (phasiRNAs) play important regulatory roles in various biological processes of plant development. In our research, genome-wide identification of miRNAs and phasiRNAs was performed in Hirado Buntan pummelo (HBP) and its male sterile somatic cybrid pummelo (G1+HBP) applying sRNA deep sequencing and degradome sequencing data to reveal potential roles of miRNA-PHAS-phasiRNAs-targets network in citrus male sterility. A total of 184 known miRNAs, 22 novel miRNAs and 2235 unique phasiRNAs generated from 280 PHAS loci were identified. A total of 86 and 95 targets of miRNAs and phasiRNA were identified respectively, which was confirmed by degradome analysis. 9 miRNA families were identified as potential triggers of 20 PHAS loci. In addition, 8 phasiRNAs were discovered to be triggers for phasiRNAs production on their targets. Integrating the miRNAs and phasiRNAs analysis data, eleven miRNA-PHAS-phasiRNA-targets and one phasiRNA-PHAS-phasiRNA-targets regulatory cascades were demonstrated. Finally, the expression changes of miRNAs and phasiRNAs in a male sterile line of pummelo were revealed. Compared with the male fertile type HBP, 42 miRNAs and 88 phasiRNAs showed significant different expression in male sterile line G1+HBP. This analysis provided new information about miRNA-PHAS-phasiRNA-targets networks and suggested potential role of miRNAs and phasiRNAs in male sterility of pummelo and contributed to further study of miRNAs and phasiRNA function in citrus and other higher plants.

Session : Comparative genomics

Micropropagation of Iranian jujube (*Ziziphus jujuba* Mill.)

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According to Ayurveda there are large numbers of traditional medicinal uses of *Ziziphus* species and the reasons behind the medicinal potential of this plant are because of some allied substances such as ascorbic acid, thiamine, riboflavin and bioflavonoids and pectin-A. Therefore, this plant is a valuable source of phytochemicals, bioactives, vitamins etc. which makes it to be effective in curing or prevention from various diseases, Thus The present research studied to develop an efficient in vitro propagation system for rapid propagation of these valuable Jujube cultivar Shahzileh, The Shahzileh cultivar is valuable because pulp fruit to seed ration is higher than other cultivars and such as, is the highest fruit production and the highest number of cuttings per year in South Khorasan province, Iran. The results of this study showed, the regenerated plantlets have suitable length and proliferation. According to the results, a combination of BA with low concentration of IBA has the best branching, So, this method can be used for micropropagation of Jujube in commercial scale in our country. Specially, the in vitro cultured plants form variants in stressful culture conditions. The jujube tree can be an appropriate organism to assess the effect of a long-term vegetative propagation in morphological traits by attending to the study's results, and as a consequence, it is recommended to attain stabilized and suitable somaclonal variations in jujube explants subcultures.

Session: Biotechnology and genome editing

Drought-induced molecular responses cause stress-induced flowering in sweet oranges as revealed by RNA-Seq

Zhimeng Gan

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Plants tend to floral induction and flowering if placed under drought and re-watering treatment conditions, but reports on the molecular mechanisms of stress-induced flowering by drought stress are scarce. Here, the transcriptome profiling analysis of sweet oranges (*Citrus sinensis*) plants subjected to mild drought, severe drought and re-watering treatment conditions, showed a total of 5789 differentially expressed genes (DEGs) to all treatments compared with the control. The expression of some DEGs was associated with floral induction by real-time PCR analysis. Gene Ontology (GO) enrichment analysis was used to predict the functions of the drought-responsive genes, these DEGs were enriched in ‘response to wounding’, ‘response to water’, ‘response to water deprivation’, ‘water transport’. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that DEGs mainly belonged to ‘the MAPK signaling pathway-plant’ and ‘Arginine and proline metabolism’ category. as well as genes coding for transcription factors (TFs), protein kinases (PKs) and proteins involved in the abscisic acid (ABA) and ethylene signaling pathways, were differentially regulated by drought stress. Based on these findings, we propose stress-induced plant flowering might be helpful for species preservation. Thus, stress-induced flowering might have biological significance and should be considered as important as other plant flowering control strategy, which will enable us to further understand the molecular mechanism of drought -regulated flowering in citrus.

Session: Biotic and abiotic stresses

Cassava is a climate resilient, future ‘smart’ crop

Byju Gangadharan

ICAR-CTCRI

In all the world's economies, agriculture is amongst the most vulnerable of sectors to climate change. Many authors reported that agricultural production could suffer progressive yield losses in the next hundred years due to climate change. The ICAR-CTCRI has been conducting research studies to model the impact of future climate on change in suitabilities of cassava. Geoinformatics tools and ecological niche models such as EcoCrop were used for the study. The paper presents a detailed account of the results of the studies that were conducted in cassava for different future scenarios such as 2030 and 2050 and for different GCMs and RCPs of assessment report four and five (AR4 and AR5). Cassava (*Manihot esculenta* Crantz) is an important food crop of India cultivated in an area of 228,000 ha for food, feed and fuel. Cassava contributes to food security, equity, poverty alleviation and environmental protection. The current climate and future climate projections of 22 GCMs from the SRES-A1B emission scenario were used for the study. A total of 10053 unique coordinates, as points was obtained as cassava presence points in India. The projected change in annual mean temperature and total annual precipitation in the major growing areas ranged from 0.9 to 1.20C and from 6.8 to 112.4 mm respectively. The predicted change in annual mean temperature in Kerala, Tamil Nadu and Andhra Pradesh ranged from -1.2 to +1.40C, -0.4 to +1.30C and -0.1 to +1.40C respectively. In the states of Kerala, Tamil Nadu and Andhra Pradesh, the change in total annual precipitation ranged from 5 to 53 mm (Kerala), 4 to 60 mm (Tamil Nadu) and 6 to 18 mm (Andhra Pradesh). The calibrated data were used to drive the EcoCrop model to find out the suitability of current and future climatic conditions. The change in suitability for all the 22 GCMs used was calculated on pixel basis and the mean suitability change indicate that cassava is actually positively impacted in the current growing areas of India with -2.2 to 15% changes in climate suitability. Out of the 22 GCMs studied, 50% GCMs predicted negative suitability change in Kerala (-1 to -3 %), about 41% GCMs predicted negative suitability change in Tamil Nadu (-19 to -1%) and about 55% GCMs predicted negative suitability change in Andhra Pradesh (-19 to -1%) and the remaining GCMs predicted a positive suitability change in all these states. This study concluded that cassava is potentially highly resilient to future climatic changes and it can become a future crop in India where other crops would fail due to climate change.

Session: Biotic and abiotic stresses

DNA methylation variation of LDOX gene contributes to the floral colour variegation in peach

Zhihong Gao

Nanjing Agricultural University

Peach is an important fruit and ornamental plant in the world. Variegation in flowers often receives consumer attention and variegated plants are of high value in the market. To determine the relationship between DNA methylation and phenotype, we obtained the first single-nucleotide resolution DNA methylation of variegation cultivars in peach by bisulfite sequencing. In our data, a similar methylation rate of 11.96% in red flower buds (RF) and 12.74% in variegated (VF) were determined. The CG methylation is mainly concentrated in transcription regions. We identified 189 differentially methylated regions (DMRs). Among these DMRs, FLS gene expression pattern has significant difference between RF and VF. Associated with the transcriptional and proteome analysis, 106 differently expressed genes and 52 different proteins have varying degrees of methylation. Methylation level of the coding regions is higher than upstream and downstream, and silent genes have higher methylation levels than expressed genes. The leucoanthocyanidin dioxygenase (LDOX) displayed differential methylation. The further experiments showed LDOX gene expression and enzyme activity in RF was higher than VF. Moreover, the methylation state of the FLS and LDOX promoter was opposite based on BSP. The trend was consisted with the flower colour phenotype. Several cis-acting regulatory elements on BSP sequences involved in phytohormones and light responsiveness were methylation, which could affect genes expression. This study identified the context and level of methylation at each site and analysed DNA methylation with BS-Seq and these results are helpful for discovery of the mechanism of the variegated flower petal formation in peach.

Session: Comparative genomics

Characterization of early floral development in extremely early- and late-blooming Malus species and cultivars

Christopher Gottschalk

Michigan State University

Temperate tree fruit crops including apple, cherry, peach, and apricot are sensitive to freezing temperatures in late spring. Increasing evidence suggests that one aspect of climate change is the increased likelihood for late-spring frosts, at least in the major fruit-producing Great Lakes region of the U.S. The introduction of improved cultivars that bloom later in the spring is an obvious proactive step to prevent or reduce crop loss due to spring freezing, but the genes and alleles that influence spring bloom timing are still mostly unknown. Apples, including the domesticated apple (*Malus x domestica* Borkh.) and wild *Malus* species, show extreme variation in the timing of spring bloom. Previous research has implicated both endo- and eco-dormancy as major factors influencing spring bloom dates. However, the influence of the timing of floral initiation and rate of floral development on bloom time has not been investigated. In this study, we documented variation in floral initiation and development between early-blooming and late-blooming apple genotypes using both molecular and anatomical approaches. Our preliminary results suggest that both the timing of floral initiation and rate of floral development, show variation consistent with the observed variation in spring bloom timing. These results suggest that apple cultivar development for resilience to climate change should consider flowering-related loci in addition to dormancy-related loci.

POST

Session: Plant signalling and behaviour

Metabolic adaptation and fruit quality formation of citrus polyploids

Wenwu Guo

Huazhong Agricultural University

Polyploidy is a common phenomenon in nature and has been considered to play a major force in plant evolutionary course. Novel phenotypes following polyploidy have been widely applied in breeding of major economic crops, especially in biomass and stress tolerance improvement. In our citrus breeding program, numerous double diploids via nucellar seedling exploitation were produced. In order to explore the occurrence and extent of metabolic alterations, as well as fruit quality formation following genome doubling, we performed comparative metabolomic analysis between doubled diploid and their corresponding diploids of five citrus genotypes, namely Ziyang Xiangcheng (*Citrus junos*), red tangerine (*C. reticulata*), trifoliate orange (*Poncirus trifoliata* Raf.), precocious trifoliate orange (*P. trifoliata* Raf.) and Ponkan mandarin (*C. reticulata* Blanco). The results showed that polyploidy indeed conferred distinct metabolic phenotypes in all the doubled diploids. About 11-34% of total detected metabolites differentially accumulated in tetraploids and most of these changes were less than 5-fold, reflecting a relatively limited influence exerted by polyploidy on metabolite accumulation. Most of the differentially accumulated metabolites were stochastic. Interestingly, the concentration of most tricarboxylic acid (TCA) cycle intermediates, such as citric acid, malic acid and fumaric acid exhibited a global increase in all tetraploids, indicating that polyploidy likely promotes energy metabolism. Furthermore, an increase trend was observed in primary metabolism, such as organic acid and amino acid metabolism, while a decrease trend in secondary metabolism, such as phenylpropanoid and terpenoid metabolism. For fruits of Ponkan mandarin, primary metabolites especially organic acids tended to accumulate at higher levels in 4x fruit. The expression level of citric acid biosynthesis-related genes was unaltered in 4x fruit compared to the 2x fruit, whereas citric acid transport-related and utilization-related genes were significantly down-regulated during the late stages of fruit ripening, indicating that the transport and utilization instead of biosynthesis of citric acid may have been affected by genome doubling, thus causing the accumulation of citric acid in the 4x fruit. We conclude that primary metabolism takes priority over secondary metabolism for higher plants in order to relieve the “genomic stress” encountered in early stages of genome doubling and to maintain vitality and growth.

Session: Molecular breeding

Overexpression of MiR482c in tomato induces enhanced susceptibility to late blight

Yuhui Hong

Dalian University of Technology

Tomato is the highest-value fruit/vegetable crop worldwide with considerable significance for human nutrition and health. The quality and yield of tomatoes are severely affected by late blight. MicroRNAs (miRNAs), as a class of small RNAs, play significant roles in the plant immune system. Conserved miRNAs mostly exist as gene families in the plant genome. Previous studies have shown that the differences in miRNA family member sequences and structures may result in differences in their targets and mechanisms. MiR482 is an ancient and extensive family whose members perform their regulatory function by cleaving and inhibiting the expression of their target nucleotide binding site and leucine-rich repeat (NBS-LRR) mRNAs. Our previous study demonstrated that miR482b plays a crucial role in the tomato immune system. In this study, a series of experiments were performed to explore the mechanism of miR482c in the resistance of tomato to late blight in detail. The expression patterns of miR482c and its target genes were determined using quantitative real-time PCR (qRT-PCR) upon late blight infection. The abundance of miR482c increased at 6 hours postinfection (hpi) and 24 hpi and subsequently declined at 48 hpi, whereas the expression trends of miR482c-targeted genes contrasted the trend of miR482c. Afterwards, miR482c was transiently overexpressed in tomato, resulting in a larger lesion area than the control plants upon infection. Furthermore, compared with the WT tomato plants, the transgenic tomato plants stably overexpressing miR482c displayed decreased expression of target genes accompanied by lower POD, SOD, and PAL activity activities and higher MDA content, thereby leading to a decline in the ROS scavenging ability and aggravating the damage of lipid peroxidation product accumulation on the cell membrane, eventually enhancing plant susceptibility. This finding indicates that miR482c may act as a negative regulator in tomato resistance by regulating NBS-LRR expression levels and ROS levels. Thus, despite having individual characteristics, miR482c and miR482b may play analogous roles in the plant immune system.

Session: Biotic and abiotic stresses

The complete chloroplast genome sequence of watercress (*Nasturtium officinale* R. Br.)

Xilin Hou

Nanjing Agricultural University

Watercress (*Nasturtium officinale* R. Br.), an aquatic leafy vegetable of the Brassicaceae family, is known as a nutritional powerhouse. Here, we de novo sequenced and assembled the complete chloroplast (cp) genome of watercress based on combined PacBio and Illumina data. The cp genome is 155,106 bp in length, exhibiting a typical quadripartite structure including a pair of inverted repeats (IRA and IRB) of 26,505 bp separated by a large single copy (LSC) region of 84,265 bp and a small single copy (SSC) region of 17,831 bp. The genome contained 113 unique genes, including 79 protein-coding genes, 30 tRNAs and 4 rRNAs, with 20 duplicate in the IRs. Compared with the prior cp genome of watercress deposited in GenBank, 21 single nucleotide polymorphisms (SNPs) and 27 indels were identified, mainly located in noncoding sequences. A total of 49 repeat structures and 71 simple sequence repeats (SSRs) were detected. Codon usage showed a bias for A/T-ending codons in the cp genome of watercress. Moreover, 45 RNA editing sites were predicted in 16 genes, all for C-to-U transitions. A comparative plastome study with Cardamineae species revealed a conserved gene order and high similarity of protein-coding sequences. Analysis of the Ka/Ks ratios of Cardamineae suggested positive selection exerted on the *ycf2* gene in watercress, which might reflect specific adaptations of watercress to its particular living environment. Phylogenetic analyses based on complete cp genomes and common protein-coding genes from 56 species showed that the genus *Nasturtium* was a sister to *Cardamine* in the Cardamineae tribe. Our study provides valuable resources for future evolution, population genetics and molecular biology studies of watercress.

Session: Comparative genomics

UV radiation affected flavonoid metabolism and antioxidant capacity differently in blueberries

Zhixia Hou

Beijing Forestry University

The effect and mechanism of preharvest and postharvest ultraviolet (UV) irradiation on anthocyanin biosynthesis during blueberry development were investigated. The results showed that preharvest UV-B,C and postharvest UV-A,B,C irradiation significantly promoted anthocyanin biosynthesis and the transcripts of late biosynthetic genes (LBG)VcDFR, VcANS, VcUFGT, and VcMYB transcription factor as well as DFR and UFGT activities in anthocyanin pathway in a UV wavelength- and developmental stage-dependent manner. VcMYB expression was positively correlated with that of VcANS and VcUFGT and coincided with anthocyanin biosynthesis responding to the UV radiation. Sugar decreased during postharvest but increased during preharvest UV radiation in mature fruit. Our results indicate that UV-responsive production of anthocyanins is mainly caused by the activation of anthocyanin downstream pathway genes, which could be upregulated by VcMYB. Furthermore, different potential response mechanisms may exist between preharvest and postharvest UV radiation in blueberries, involving a systemic response in living plants and a nonsystemic response in postharvest fruit.

Section: Plant Signalling and behaviour

Response of pomelo nutrients uptake and fruit quality to ratio of CaO and MgO

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Guanxi pomelo orchard in Pinghe County, Fujian Province and Shatian pomelo orchard in Meizhou City, Guangdong Province are suffered from severe soil acidification and soil available Ca^{2+} and Mg^{2+} deficiency. In order to solve the problem we used 15a white fruit Guanxi pomelo and 20a Shatian pomelo as test materials to carry out field plot test. The experiment was conducted from 2017 to 2018, we analyzed the effects of Ca/Mg ratio fertilizers on the yield, quality and absorb efficiency of Ca^{2+} and Mg^{2+} . The main results are as follows: (1) The application of Ca and Mg fertilizers can increase the yield of Guanxi and Shatian pomelo, with increase rate of 5.69% to 61.42%. The application of Ca/Mg ratio (9:1) to Shatian pomelo reached the highest increase rate of 61.42% in 2018 and 33.67% for two years average among all treatments. There was a significant positive correlation between the yield of pomelo fruit and the amount of CaO applied, meanwhile Ca/Mg ratio. (2) The fruit weight, juice rate and yield of Guanxi pomelo increased significantly with the increase of CaO application rate. The MgO application significantly increased the moisture content of fruit valve and decreased the thickness of peel, but the soluble solid concentration of fruit also decreased significantly. As the ratio of calcium to magnesium fertilizers decreased, the thickness of peel became thinner, and the moisture content of peel and valve was significantly increased. The edible rate, fruit shape index and titratable acid of Shatian pomelo significantly increased with the increase of calcium and magnesium fertilizers application. The thickness of peel was significant negative correlation with the amount of CaO applied. The ratio of high Ca/Mg ratio significantly decreased the fruit juice rate. (3) The leaves calcium concentration increased with the leaf age. The leaf Mg concentration of 2017 to 2018 was highest in May, decreased in July and September, and remained relatively stable in November, however, but still deficient widespread. There has a significant positive correlation between leaf Ca/Mg and the corresponding fertilizer application. The leaves Mg concentration decreased with the increase of Ca/Mg ratio significantly in Shatian pomelo orchard but not significantly in Guanxi pomelo orchard. The correlation between leaf Ca and Mg concentration was significantly positively in May and July under low concentration range, and becoming weaker with leaf age increasing, until showing significantly negatively from September to November. This phenomenon indicated that the relationship between the leaves Ca and Mg absorption varies with the Ca/Mg ratio and season. (4) The Ca and Mg concentrations of the peel and capsular valve were significantly higher than that in pulp. Therefore, the Ca and Mg absorption of the peel and valve dominated under application of Ca and Mg fertilizers, the concentrations of Ca and Mg in all parts of fruit (fruit, peel, valve) were significantly positively correlated with the application rate.

Section: Plant Signalling and behaviour

BcMAF2 activates BcTEM1 and represses flowering in Pak-choi (*Brassica Rapa ssp. chinensis*)

Feiyi Huang

Nanjing Agricultural University

Flowering is a key event in the life cycle of plants. Flowering time shows an extensive variation from different Pak-choi (*Brassica Rapa ssp. chinensis*) cultivars. However, the regulation mechanism of flowering in Pak-choi remains rarely known. In this study, a systematic identification and functional analysis of a Pak-choi MADS Affecting Flowering (MAF) gene, BcMAF2, in was carried out. BcMAF2 encoded a protein containing a conserved MADS-box domain, which was localized in the nucleus. QPCR analysis indicated that the expression of BcMAF2 was higher in the leaves and flowers. Overexpression of BcMAF2 in Arabidopsis showed that BcMAF2 repressed flowering, which was further confirmed by silencing endogenous BcMAF2 in Pak-choi. In addition, Tempranillo 1 (TEM1) expression was up-regulated and MAF2 expression was down-regulated in the BcMAF2-overexpressing Arabidopsis. The expression of BcMAF2 and BcTEM1 was down-regulated in BcMAF2-silencing Pak-choi plants. The yeast one-hybrid, dual luciferase and qPCR results revealed that BcMAF2 protein could directly bind to BcTEM1 promoter and activate its expression, which was not reported in Arabidopsis. Meanwhile, a self-inhibition was found in BcMAF2. Taken together, this work suggested that BcMAF2 could repress flowering by directly activating BcTEM1.

Section: Plant Signalling and behaviour

The constitutive expression of a chrysanthemum ERF transcription factor influences flowering time in *Arabidopsis thaliana*

Yaoyao Huang

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AP2/ERF transcription factors (TFs) represent valuable targets for the genetic manipulation of crop plants, as they participate in the control of metabolism, growth and development, as well as in the plants' response to environmental stimuli. Here, an ERF TF encoded by the chrysanthemum (*Chrysanthemum morifolium*) genome, designated CmERF110, was cloned and functionally characterized. The predicted CmERF110 polypeptide included a conserved DNA-binding AP2/ERF domain. A transient expression experiment revealed that the protein was deposited in the nucleus, and a transactivation experiment in yeast suggested that it had no transcriptional activity. The gene was transcribed in the chrysanthemum root, stem and leaf with its transcript level following a circadian rhythm under both long and short days. The effect of constitutively expressing the gene in *Arabidopsis thaliana* was to accelerate flowering. Transcriptional profiling implied that its effect on floral initiation operated through the photoperiod pathway.

Section: Plant Signalling and behaviour

Impact of aluminum (Al³⁺) on tricarboxylic acids cycle and its relationship with resistance mechanisms in Al-contrast genotypes of highbush blueberry (*Vaccinium corymbosum* L.)

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Universidad Católica de Temuco

The tricarboxylic acid cycle (TCA o Krebs) is related to the generation of organic acids (AOs), photosynthetic performance, and tolerance to phytotoxic aluminum (Al³⁺). Aluminum (Al) in acid soils with a pH less than 5.0 is solubilized as a trivalent ion (Al³⁺), which is toxic for the plant. Al³⁺ causes damage of root growth, disrupting metabolism and photosynthesis. Highbush blueberry is an important crop species cultivated in southern Chile. The agronomic potential is based on the excellent quality of its fruit and its high productivity. In this study, we evaluated three cultivars (Star, Al-sensible), Camellia and Cargo (as Al-resistant) for enzymatic activity, gene expression analysis of MALATE DEHYDROGENASE (MDH) NAD and NADP dependent, and CITRATE SYNTHASE (CS) , and organic acid content in nutritive solution. The concentration used was 200 µM as AlCl₃ to pH 4.5 for 0 to 48 h. The results showed significant differences in MDH-NAD activity in roots of Star and Camellia, and in leaves of Cargo increasing at 48h (2.9, 1.6, and 1.3-fold, respectively). In roots MDH-NADP increased in Star and Camellia at 24 h (3.4 and 3.6-fold), whereas, in leaves of Star decreased 77% under Al³⁺. On the other hand, CS reduced its activity in Star and Camellia at 48 h in roots (89-39%) and leaves (70-19%), while Cargo increased 1.17-fold only in roots. MDH-NAD and NADP dependent gene expression decreased in Cargo roots to 50% of both enzymes at 24h) and Camellia a 65-94% at 24 h (MDH-NADP and CS) and of 80% at 48h in CS expression. In leaves, Cargo cultivar increased 0.65, 1.3 and 1.7-fold in MDH-NAD, -NADP, and CS expressions, respectively at 24h. While in Star the MDH-NADP increased around 51% at 24 h and decreased 36% at 48h The Organic acids have the main significant differences (p<0.05) in Star at 0h in roots and in Camellia leaves at 48 h. Finally, these results suggest that Al³⁺ modifies the TCA cycle in blueberry resistant genotypes and that the response is related to changes in the activity and expression of TCA-enzymes, as well as in the concentration of organic acids in tissues. Acknowledgments: FONDECYT Initiation No. 11160355 and FONDECYT No. 1171286 projects.

Section: Biotic and abiotic stresses

The competing endogenous RNAs network influences tomato cracking

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Fruit cracking, occurs easily under unsuitable environmental conditions, is one of the main disorders in fruit production. It is widely accepted that plant have developed a defense mechanisms or regulatory networks in response to abiotic stress. This involves perceiving, integrating and responding to stress signals by modulating the expression of related genes. Fruit cracking is also a kind of physiological disease caused by abiotic stress. Previously reported a single or two genes may influence fruit cracking. However, almost none of these efforts have involved cracking regulatory network. Here, irrigation treatments resulted in differential expression of mRNAs, microRNAs, lncRNAs and circRNAs in cracking-resistant and cracking-susceptible tomatoes. The GO pathway of the differentially expressed mRNAs are mainly enriched in ‘hormone metabolic process’, ‘cell wall organization’, ‘oxidoreductase activity’ and ‘catalytic activity’. Based on these mRNAs and related microRNAs, lncRNAs and circRNAs, a ceRNA network that influences tomato cracking were constructed.

Section: Biotic and abiotic stresses

New class of genome editing technologies in horticultural crops to evolve new crop varieties rely on Engineered Endonucleases (EENs)

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Class of Genome Editing These technologies in Horticultural crops to evolve new crop varieties rely on Engineered Endonucleases (EENs) that cleave DNA in a sequence-specific manner because of the presence of a sequence-specific DNA-binding domain or RNA sequence. Through recognition of the specific DNA sequence, these nucleases can efficiently and precisely cleave the targeted genes. The double-strand breaks (DSBs) of DNA consequently result in cellular DNA repair mechanisms, including homology-directed repair (HDR) and error-prone non-homologous end joining breaks (NHEJ), leading to gene modification at the target sites. Zinc Finger Nucleases (ZFNs) in gene modification ZFNs are the first generation EENs that were developed following the discovery of the functional principles of the Cys2-His2 zinc finger (ZF) domains. Each Cys2-His2 ZF domain consists of 30 amino-acid residues that fold into a bba configuration TALENs in gene modification. Recently, a new EEN, i.e., Transcription Activator - Like Effector Nucleases (TALENs), has rapidly emerged as an alternative to ZFNs for genome editing. The broad applications of TALENs were based on the recognition of the functional principles of the type III transcription activator-like (TAL) effectors that are secreted by the plant pathogenic bacteria *Xanthomonas*. CRISPR/Cas in gene modification more recently, a new class of genome-editing technology, i.e., the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/ CAS (CRISPR-Associated) system has been developed. CRISPRs were firstly identified in the *Escherichia coli* genome in 1987 as an unusual sequence element consisting of a series of 29-nucleotide repeats separated by unique 32-nucleotide “spacer” sequences. Central to this process is the activity of MYB-bHLH-WD repeat (MBW) complexes that regulate the transcription of anthocyanin genes. Therefore, new crop varieties with high concentrations of anthocyanin could be generated by modifying some of these genes through genome-editing technologies.

Section: Biotechnologies and genome editing

Improving of stone plant resistance to plum pox virus by bioengineering approach: from co-suppression to gene editing.

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Plum pox virus (PPV) imposes serious threats to commercial plantations of a wide range of fruit species belonging to genera *Prunus*. Given the lack of natural genetic resources, which display reliable resistance to PPV infection, there has been considerable interest in using genetic engineering and editing methods for targeted genome modification of stone fruit trees to control Sharka disease caused by PPV. Based on developed protocols of plant regeneration from somatic (leaf) tissue and agrobacterial transformation for some commercial cultivars and rootstock we tested number bioengineering approach for generate PPV resistance. First experiments based on expression of fragments of virus coat protein gene in sense-orientation and self-complementary fragments of gene were used for realization post-transcriptional gene silencing. Apart from the fact that the accumulation of coat protein was evaluated by Western blot assay in most of transgenic lines of cultivar “Startovaya”, we observed absence of PPV only in lines of this cultivar and rootstock” Elita” ((*Prunus pumila* L. × *P. salicina* Lindl.) × (*P. cerasifera* Ehrh.) expressed intron hairpin RNAi construct, containing the self-complementary sequences of a fragments of PPV-CP driven by 35SCaMVpromoter. Strong induced resistance of these clones confirmed by ten years testing under high infection pressure. The plants of rootstocks 146 (*Prunus pumila* x *Prunus tomentosa*), “Elita” and cultivar Startovaya were transformed by intron hairpin RNAi constructs containing the self-complementary sequences of fragment of factor initiation translation eIF(iso)4G and E genes driven by the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) genes promoter. To clarify the PPV resistance, transgenic plants were inoculated with infected buds by grafting onto one-year-old plants growing in the greenhouse. By the second year of the challenge experiment, transgenic lines demonstrate different range of resistant to PPV as the transgenic events showed no or low level of virus accumulation detected by DAS-ELISA and displayed normal phenotype in greenhouse conditions. Based on these results we start experiments for gene editing methods for knocking out eIF(iso)4G and eIF(iso)4E genes. From about 20 synthesized guide RNA for each genes only 2-3 demonstrated 35-75% efficiency to cut target DNA in in vitro test. Based on these RNAs we create vectors and start transformation of rootstocks and commercial cultivars of stone fruit plants.

Section: Biotic and abiotic stresses

Downregulation of RdDM during strawberry fruit ripening

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Background: Recently, DNA methylation was proposed to regulate fleshy fruit ripening. Fleshy fruits can be distinguished by their ripening process as climacteric fruits, such as tomatoes, or non-climacteric fruits, such as strawberries. Tomatoes undergo a global decrease in DNA methylation during ripening, due to increased expression of a DNA demethylase gene. The dynamics and biological relevance of DNA methylation during the ripening of non-climacteric fruits are unknown. Results: Here, we generate single-base resolution maps of the DNA methylome in immature and ripe strawberry. We observe an overall loss of DNA methylation during strawberry fruit ripening. Thus, ripening-induced DNA hypomethylation occurs not only in climacteric fruit, but also in non-climacteric fruit. Application of a DNA methylation inhibitor causes an early ripening phenotype, suggesting that DNA hypomethylation is important for strawberry fruit ripening. The mechanisms underlying DNA hypomethylation during the ripening of tomato and strawberry are distinct. Unlike in tomatoes, DNA demethylase genes are not upregulated during the ripening of strawberries. Instead, genes involved in RNA-directed DNA methylation are downregulated during strawberry ripening. Further, ripening-induced DNA hypomethylation is associated with decreased siRNA levels, consistent with reduced RdDM activity. Therefore, we propose that a downregulation of RdDM contributes to DNA hypomethylation during strawberry ripening. Conclusions: Our findings provide new insight into the DNA methylation dynamics during the ripening of nonclimacteric fruit and suggest a novel function of RdDM in regulating an important process in plant development. Keywords: DNA methylation, Fruit ripening, Strawberry, RdDM, siRNA

Section: Plant Signalling and behaviour

Elucidating the molecular mechanisms underpinning a novel acyanic trait in apple

Justin Lashbrooke

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Anthocyanin pigments are the major contributing molecules for colour development in apple fruit. Red, bicolour and blushed apples all accumulate anthocyanins to varying degrees, while green fruited varieties show no anthocyanin accumulation in the fruit at harvest. While important for consumer-linked traits, these pigments are also bioactive and have numerous important physiological and ecological functions in plants, such as attraction of pollinators and seed dispersers, protection from high light irradiation, and scavenging free radicals produced in cells during stress conditions. In this study we identify a novel absence of anthocyanin (acyanic) trait in apple, which shows no anthocyanin formation in all tissue types and developmental stages. The underlying gene for this trait ('a') was mapped in multiple cross populations and shown to be recessive in its inheritance for loss of function for anthocyanin accumulation. Making use of microsatellite markers we were able to successfully identify a 3.4 MB region of the genome as the location of a and identify an important international apple variety as a carrier of a. Genome analysis and annotation of the identified genomic region followed by gene expression analysis and pigment profiling via HPLC identified a candidate gene for a. Finally, DNA sequencing of the genomic context of this gene identified an hAT transposable element present in the coding sequence of a in acyanic plants. This transposon could be directly attributed to the disruption of the normal functioning of this gene and the resultant acyanic phenotype.

Section: Plant Signalling and behaviour

Elevated CO₂ delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit

Dong Li

Zhejiang University

Color is an important quality attribute for the consumer's acceptability of fruit. Elevated CO₂ was applied to strawberry fruit to explore its influence on chlorophyll catabolism and anthocyanin synthesis. The results showed that 20% CO₂ delayed the changes of a* and b* value in strawberry fruit. The degradation of chlorophyll was delayed in CO₂ treated fruit by inhibiting the activities of chlorophyllase and down-regulating the expression of FaChl b Reductase, FaPAO and FaRCCR. In addition, lower concentration of anthocyanins and lower activity of PAL, C4H, 4CL and CHS were recorded under the effect of 20% CO₂. Meanwhile, qRT-PCR analysis showed that 13 genes involved in phenylpropanoid pathway and flavonoid biosynthesis pathway were also down-regulated under CO₂ stress. However, no residual effect on pigment metabolism was observed when elevated CO₂ was removed. Our study provided new insights into the regulation of elevated CO₂ on the role of pigment metabolism in postharvest.

Section: Plant Signalling and behaviour

The effect of substrate composition on solanaceous fruits growth in the indoor vertical farm

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The totally closed plant factories offer controllable environment for plants, so the plant growth are highest compared with other protected and open field cultivation. In the highly effective growth system, the internal air circulation usually causes the high humidity, and the evaporation of substrate is reduced correspondingly. Thus, the irrigation frequency and irrigation amount will decreased, which leads to the amount of the nutrient elements absorbed by plant is limited. The substrate composition directly affected the evaporation of substrate. The different ratios of peat and perlite combining with different irrigation frequencies were tested. It is found that the higher irrigation frequency (once per day) improve the growth of cucumber, pepper and tomato seedlings. Compared with normal ratio of peat and perlite, decreasing peat and increasing perlite will improve the seedling growth, cooperated with higher frequency of irrigation. The leaf number and the fresh weight of cucumber, pepper and tomato seedlings are significantly increased. The time of first flower is earlier, too. Moreover, the holding water capacity of substrate was decreased, and the air permeability was increased. Thus, it is presumed that the higher ratio of perlite and lower ratio of peat have benefits for water evaporation in substrate in the plant factory with high humidity, so higher irrigation frequency can be applied to offer more nutrient elements for cucumber, pepper and tomato seedlings, and the growth of these seedlings was improved.

Section: Biotic and abiotic stresses

Transcriptome analysis of embryos at two developmental stages reveals important insight into oil body development and oil accumulation in radish

Shisheng Li

Huanggang normal university

Radish is widely used as root vegetable and also suitable for producing edible oil. While the mechanism on development of the oil body and the accumulations of oil in seeds of radish are rarely studied. Here the ultrastructural investigation was used to trace the formation of oil body during embryo development in radish. The results show that peripheral cell around globular embryo began to differentiate and oil body start to appear at bent-embryo stage. The robust increases of oil body were found in the mature cotyledon stage. Thus embryos at bent and the mature cotyledon stage were chose for transcriptome analysis to uncover the regulation of oil accumulation. A total of 88864 unigenes with an average length of 805 bp and an N50 of 1390 bp were obtained from two different embryos in radish: bent embryo and mature embryos after de novo sequencing. The KOG classification of unigenes assigned 18,418 unigenes to 25 functional categories. In total, of these, 5,902 sequences containing 7,055 potential EST-SSRs were identified from 80,521 consensus sequences. There were 6694 up-regulated DEGs and 7826 down-regulated DEGs via comprehensive analysis. Differential genes potentially related to lipid metabolism were validated by qPCR. These findings identify candidate genes involved in oil biosynthesis which may be useful for underlying the accumulation of oil in radish.

Section: Plant Signalling and behaviour

Growth and metabolic adaptation strategies of riparian plant *Distylium chinense* to submergence: a study of survival and recovery dynamics

Xiaoling Li

CHINA THREE GORGES UNIVERSITY

Distylium chinense is a dominant native evergreen shrub in the riparian zone of the Three Gorges Reservoir region of China. To evaluate growth and metabolic adaptation mechanisms of *D. chinense* to anti-season flooding, one field study and one controlled experiment were conducted during autumn, winter and spring. In the field study performed in the artificial water-level-fluctuation zone (WLFZ) of Xiangxi River, China, all the seedlings at altitudes <170 m above m.s.l. eventually died after the floodwaters receded. Seedling survival was 91.67% at altitude 173 m above m.s.l. with 86 d of complete submergence and a 2-m water depth. In the controlled experiments, the survival of all the seedlings under 30-d and 60-d submergence treatments almost reached 100% but fell to 67.5% and 47.5% under the 90-d and 120-d submergence treatment, respectively. In terms of the relative dry mass accumulation, *D. chinense* was investing more in roots and stems than in leaves under the control conditions. Dry mass accumulation of roots, stems and leaves of *D. chinense* seedlings decreased significantly and increased gradually during the subsequent 60-day recovery period. And *D. chinense* accumulated substantial amounts of carbohydrate (starch and soluble sugar) before submergence and invested more in roots and stems than in leaves under the control conditions, which showed that *D. chinense* allocated more carbohydrate to the organs (roots and stems) for acquisition of most limiting resources, both during submergence and after de-submergence. Different submergence duration treatments also led to a significant reduction of starch and soluble sugar content in the submerged roots, stems and leaves for the 90- and 120-d submerged seedlings, but didn't decrease and had a significant increase for the 30- and 60-d submerged seedlings compared with the controls after submergence treatments. The activities of PDC, ADH and LDH enzymes showed significant increase after 30, 60, 90 and 120 d of submergence but decreased with the prolonged submergence time, and activities of ADH were higher than those of LDH in the roots and leaves in the corresponding submergence treatments, respectively. The results showed that the respiration of roots and leaves of *D. chinense* gradually changed from the mitochondrial aerobic respiration to the anaerobic respiration mainly based on ethanol fermentation. After returning to the control conditions, the recovery patterns of carbohydrate (starch and soluble sugars) were coincided with those of dry mass accumulation of the roots, stems and leaves of *D. chinense* and the 30- and 60-d submerged seedlings showed faster and stronger recovery than the 90- and 120-d submerged seedlings for dry mass and carbohydrate accumulation after de-submergence. A significant regression equation derived through multivariate stepwise regression analysis, root starch content and root dry mass accumulation were the major factors affecting the seedling survival. The significantly positive correlations between seedling survival and functional traits and their significant relationships with each other probably reflect covariation among this suite of functional traits. Thus, *D. chinense* may have adopted this metabolic adaptation strategy together with growth adaptations to survive long-term submergence, where allocation to carbohydrate reserves in roots and root dry mass accumulation as a basis of

survivorship and re-growth may be an effective strategy of submergence tolerance . Therefore, *D. chinense* could be considered to be as native pioneer tree species in the re-vegetation in the WLFZ and the other similardegenerative riparian area and stream ecosystem.

Section: Biotic and abiotic stresses

Key factors identification in fruit ripening and rubbery texture caused by 1-MCP in papaya

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South China Agricultural University

Ethylene promotes fruit ripening whereas 1-methylcyclopropene (1-MCP), a non-toxic antagonist of ethylene, delays fruit ripening via the inhibition of ethylene receptor. However, unsuitable 1-MCP treatment can cause fruit ripening disorders. In this study, we show that short-term 1-MCP treatment (400 NL•L⁻¹, 2 h) significantly delays papaya fruit ripening with normal ripening characteristics. However, long-term 1-MCP treatment (400 NL•L⁻¹, 16 h) causes a “rubbery” texture of fruit. The comparative transcriptome analysis showed that a total of 5,529 genes were differently expressed during fruit ripening compared to freshly harvested fruits. Comprehensive functional enrichment analysis showed that the metabolic pathways of carbon metabolism, plant hormone signal transduction, biosynthesis of amino acids, and starch and sucrose metabolism are involved in fruit ripening. 1-MCP treatment significantly affected fruit transcript levels. A total of 3,595 and 5,998 differently expressed genes (DEGs) were identified between short-term 1-MCP, long-term 1-MCP treatment and the control, respectively. DEGs are mostly enriched in the similar pathway involved in fruit ripening. A large number of DEGs were also identified between long-term and short-term 1-MCP treatment, with most of the DEGs being enriched in carbon metabolism, starch and sucrose metabolism, plant hormone signal transduction, and biosynthesis of amino acids. Furthermore, we found that long-term treatment with 1-MCP significantly inhibited the expression of CpEBF1, an EIN3 binding F-box-1 gene. Protein interaction analysis with yeast two-hybrid, BiFC and GST pull-down showed that CpEBF1 interacts with CpMADS1/3 and CpEIL1 proteins. The interaction of CpEBF1 with CpMADS1/3 further activated the activities CW-degradation genes promoters. Subcellular localization showed that these proteins were localized in the nucleus. Additionally, the expression levels of CpMADS1/3, CpEIL1, and several CW degradation-related genes were significantly downregulated by long-term 1-MCP treatment. Therefore, we propose that the inhibited expression of CpEBF1 and CpMADS1/3 resulted in repressing the activation of CW- degradation related genes by their interactions, thereby resulting in fruit softening disorders.

Section: Plant Signalling and behaviour

Agrobacterium-mediated vacuum infiltration and floral dip transformation of Brassica Rapa

Ying Li

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Rapid-cycling *Brassica Rapa* (RCBr), also known as Wisconsin Fast Plants, are small robust plants with a short lifecycle that are widely used in biology teaching. RCBr have been used for decades but there are no published reports of RCBr genetic transformation. *Agrobacterium*-mediated vacuum infiltration has been used to transform pakchoi (*Brassica Rapa ssp. chinensis*) and may be suitable for RCBr transformation. The floral dip method, an improved version of vacuum infiltration, could make the procedure easier. Based on previous findings from *Arabidopsis* and pakchoi, plants of three different ages were inoculated with *Agrobacterium*. Kanamycin selection was suboptimal with RCBr; a GFP screen was used to identify candidate transformants. RCBr floral bud dissection showed that only buds with a diameter less than 1 mm carried unsealed carpels, a key point of successful floral dip transformation. Plants across a wide range of inflorescence maturities but containing these immature buds were successfully transformed, at an overall rate of 0.1% (one per 1000 T1 seeds). Transformation was successful using either vacuum infiltration or the floral dip method, as confirmed by PCR and Southern blot. A transformation system for RCBr was established in this study. This will promote development of new biology teaching tools as well as basic biology research on *Brassica Rapa*.

Section: Biotechnology and genome editing

Performance of substrate heating in Chinese solar greenhouse for overwintering tomatoes cultivation: experiment and root-zone temperature field simulation

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Temperature distribution of root zone and its effect on the growth and quality of soilless tomato under different temperatures were studied by localized heating, heating temperatures at 22°C, 28°C and 34°C, respectively. The results showed that the average temperature of perlite-embedded rock wool and coconut bran could be increased to 22.74°C, 23.09°C, 29.92°C, 29.63°C, 35.17°C and 35.38°C at night by setting different heating temperatures respectively, when perlite-embedded rock wool and coconut bran were used as substrates. Temperature treatments at 28°C and 22°C had significant effects on the growth and physiological quality of tomato plants. The plant height, stem diameter, leaf number, root activity, ascorbic acid and soluble sugar increased significantly, but the energy consumption at 22°C was significantly lower than 28°C. At the same temperature, the stem diameter of tomato plants using coconut bran as substrate was significantly higher than that using perlite-embedded rock wool as substrate. The simulation results of root temperature were further analyzed by CFD software.

Section: Biotic and abiotic stresses

Anthocyanins and acidity, two interconnected qualitative traits of Citrus genera under the control of shared transcription factors

Concetta Licciardello

CREA – OFA

The presences of anthocyanins and fruit acidity represent important quality traits of Citrus: the former has recognized health-promoting value, whereas the latter is a major determinant of fruit taste where acidless (sweet and with low or no acidity in the flesh) genotypes represent a good opportunity to investigate the genes responsible for the traits. Recently, it has been proposed that both characters are controlled at the transcriptional level by MBW (Myb, bHLH, WD40) complexes sharing the same bHLH transcription factor. Anthocyanin pigmentation characterizes young leaves and flower buds of many citrus genotypes having citron (*Citrus medica*) as a parent. In addition, some blood oranges (*C. sinensis*), namely Moro, Tarocco and Sanguinello, accumulate anthocyanins in fruit flesh and rind, even though they are derived phylogenetically from complex and interspecific crosses between ancient mandarin (*C. reticulata*) and pummelo (*C. maxima*). Pigmentation is not only limited to the genus Citrus, but it is also diffused among citrus relatives. Even though several studies have described the involvement and the characterization of structural genes responsible for anthocyanin biosynthesis in blood and common oranges, no information has been reported for tissues different from flesh/juice and rind nor for species different from sweet orange, where the purple colour specifically is visible in young leaves and flowers. The present work aims to investigate the transcriptional regulation of structural genes (PAL, CHS, CHI, F3H, DFR, ANS, UFGT, GST) by regulatory (Ruby-Myb type and Noemi-bHLH type) genes responsible of anthocyanin pigmentation in eight different pigmented tissues (flesh/juice, rind, young leaves, flower bud, petals, stamen, style, stigma) of twenty-eight genotypes belonging to fourteen species (*C. medica*, *C. sinensis*, *C. limon*, *C. limonia*, *C. aurantifolia*, *C. latifolia*, *C. meyeri*, *C. latipes*, *C. celebica*, *C. hystrix*, *Microcitrus australasica*, *Murraya paniculata*, *Severina distica*, *S. buxifolia*), through a RT-qPCR analysis approach. On the other hand, the linked interaction between Ruby (Myb responsible of the anthocyanin pigmentation) and Noemi (bHLH controlling the acidity trait) has been reported and the role of structural (CsPH1 and CsPH5) and regulatory (Noemi, CsPH4, CsPH3) genes determining acidity trait were investigated in a subset of sweet orange varieties, traditionally acidic and acidless ones.

Section: Dissection of complex quality traits

High efficiency genome editing in Phalaenopsis orchid MADS genes by using CRISPR/Cas

Choun-Sea Lin

ABRC/Academia Sinaca

These authors contributed equally. In this report, we established a high efficient CRISPR gene editing in Phalaenopsis orchid. Triple, double, and single mutants can be obtained by different sgRNA construction and transformation strategies which could reduce the labor of transformation. The null MADS mutants of Phalaenopsis orchid, a heterozygous genome and long juvenility crop, were obtained. Not only this protocol can be useful to orchid industry, these strategies can be applied to the other perennial plants for gene family studies.

Section: Biotechnology and genome editing

A deletion mutation in *CsLH* confers long hypocotyl in cucumber

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A common problem with greenhouse lighting is not enough light. At weak light condition, cucumber seedlings exhibit over-elongated hypocotyl. These long hypocotyl seedlings are low tolerant to stress and their stems are easily broken, which are problems for industrialized plant nursery process. Here, we report a cucumber mutant that has three times longer hypocotyl than wild type in white light. To fine map the *lh* gene, one BC1 population and one F2 segregating populations were constructed using three inbred parental lines (*lh* × LH and *lh* × 9930). With the two populations, we investigated the inheritance patterns and chromosomal locations of *lh* in cucumbers. Consequently, a single recessive gene was identified that controls long hypocotyl. Molecular markers which were derived from resequencing data of two parents (*lh* and LH) and the reference cucumber genomes “9930”, were used to map the target gene *lh* to a 140-kb region that contained 16 genes flanked by two SNP-based markers, Snp13 and Snp14, respectively. By the Indel analysis with resequencing data, we found that a 7-bp deletion at *CsLH* codon region, which leads to protein translation stopping, and this result was confirmed by Sanger sequencing. Phylogenetic analysis showed that *CsLH* is a homologous gene to phytochrome. Fine mapping of *CsLH* will enrich the theoretical knowledge of hypocotyl elongation, and provide theoretical and technology basis for the improvement of cucumber breeding in industrialized nursery process.

Section: Dissection of complex quality traits

Overexpression of MiR482c in tomato induces enhanced susceptibility to late blight

Yushi Luan

Dalian University of Technology

Tomato is the highest-value fruit/vegetable crop worldwide with considerable significance for human nutrition and health. The quality and yield of tomatoes are severely affected by late blight. MicroRNAs (miRNAs), as a class of small RNAs, play significant roles in the plant immune system. Conserved miRNAs mostly exist as gene families in the plant genome. Previous studies have shown that the differences in miRNA family member sequences and structures may result in differences in their targets and mechanisms. MiR482 is an ancient and extensive family whose members perform their regulatory function by cleaving and inhibiting the expression of their target nucleotide binding site and leucine-rich repeat (NBS-LRR) mRNAs. Our previous study demonstrated that miR482b plays a crucial role in the tomato immune system. In this study, a series of experiments were performed to explore the mechanism of miR482c in the resistance of tomato to late blight in detail. The expression patterns of miR482c and its target genes were determined using quantitative real-time PCR (qRT-PCR) upon late blight infection. The abundance of miR482c increased at 6 hours postinfection (hpi) and 24 hpi and subsequently declined at 48 hpi, whereas the expression trends of miR482c-targeted genes contrasted the trend of miR482c. Afterwards, miR482c was transiently overexpressed in tomato, resulting in a larger lesion area than the control plants upon infection. Furthermore, compared with the WT tomato plants, the transgenic tomato plants stably overexpressing miR482c displayed decreased expression of target genes accompanied by lower POD, SOD, and PAL activity activities and higher MDA content, thereby leading to a decline in the ROS scavenging ability and aggravating the damage of lipid peroxidation product accumulation on the cell membrane, eventually enhancing plant susceptibility. This finding indicates that miR482c may act as a negative regulator in tomato resistance by regulating NBS-LRR expression levels and ROS levels. Thus, despite having individual characteristics, miR482c and miR482b may play analogous roles in the plant immune system.

Section: biotic and abiotic stresses

Application of genome editing for improving disease resistance in apple: Cas of DIPM for resistance to fire blight

Mickael Malnoy

FEM

Fire blight, caused by the bacterium *Erwinia amylovora* (E.a.), is one of the most economically important and invasive diseases affecting apple (*Malus x domestica*). The molecular mechanisms of E.a.-apple interaction are widely elucidated but the management of the disease remains still arduous. Apple protein MdDIPM4 interacts with the DspA/E effector, mandatory for the pathogenesis of *Erwinia amylovora*, but its biological function is still unknown. In this work, the knock-out of MdDIPM4 has been produced in two *Malus x domestica* susceptible varieties, by using the CRISPR/Cas9 system delivered via *Agrobacterium tumefaciens*. Fifty-seven transgenic lines were analyzed using a Next Generation Sequencing in order to identify the CRISPR/Cas9-induced mutations. Some edited plants with a loss of function mutation were selected and inoculated with the pathogen. An increased resistance was observed, demonstrating that MdDIPM4 is involved in plant susceptibility to fire blight. Moreover, with the aim of producing transgene-free plants, we used a heat shock-inducible FLP-FRT recombination system designed specifically to remove the entire T-DNA in those plants with increased pathogen resistance. For the first time, our data demonstrated the possibility to produce apple varieties more resistant to fire blight and free from exogenous DNA by using the CRISPR/Cas9 technology. These plants may be used in further analysis to better elucidate how MdDIPM4 is involved in the onset of the disease

Section: Biotechnology and genome editing

Identification and function analysis of tomato circular RNAs responsive to late blight

Jun Meng

Dalian University of Technology

Circular RNAs (CircRNAs) are a class of endogenous noncoding RNAs that have been proved to play essential roles in many cellular and disease resistance processes by acting as microRNAs (miRNAs) sponge. MiRNAs have been shown to trigger target mRNA degradation or translational inhibition. Mounting studies had reported CircRNA-miRNA-mRNA cascades played significant roles in plant growth and resistance to abiotic/biotic stresses. With the development of highthroughput sequencing and bioinformatics, circRNAs have been widely analysed in animals, but the understanding of characteristics and function of circRNAs is limited in plants, especially in tomato. Here, a total of 68 circRNAs were identified in transcriptome of tomato (*Solanum pimpinellifolium*, Sp). Sequencing of tomato infected by *Phytophthora infestans* (*P. infestans*) (SpPi) were analyzed and the differential expressed genes analysis showed that 36 circRNAs differential expressed in SpPi compared with in Sp. Among them, nine were up regulated and 27 were down regulated. Gene ontology of circRNA parental genes revealed that they are closely related to genes such as ethylene synthesis, defense response, and post-transcriptional gene silencing. Two up-regulated circRNAs, circRNA45 and circRNA47, relating to defense response were selected for further study. Two disease-related miRNA, miR477-3p and miR9478-5p, which were targeted by circRNA45 and circRNA47 were selected as follow-up targets. Real-time quantitative PCR was performed to study the expression patterns of circRNA45, circRNA47, miR477-3p and miR9478-5p. The results showed that upon *P. infestans* infection, the expression trends of circRNA45 and circRNA7 were negatively correlated with the expression trends of miR477-3p and miR9478-5p. In addition, circRNA45, circRNA7, miR477-3p and miR9478-5p were transiently overexpressed in tomato separately. In tomato plants overexpressing circRNA45 and circRNA47, the expression levels of miR477-3p and miR9478-5p were significantly decreased and the target genes of two miRNAs were significantly up-regulated. In tomato plants overexpressing miR477-3p and miR9478-5p, the target genes were down-regulated. The above results demonstrated that circRNA45 and circRNA47 can indirectly regulate mRNA expression by silencing miRNA, and thus play a significant role in plants-pathogens interaction. In summary, our study identified circRNAs in tomato and preliminarily explored the role of tomato circRNA-miRNA-mRNA cascade in tomato-pathogen interactions. Our work laid a solid foundation for future research.

Section: Biotic and abiotic stresses

Molecular Cytogenetic Mapping in Sweet Orange (*Citrus sinensis*)

Luke Miao

Huazhong Agricultural University

Citrus sinensis is one of the most important citrus cultivation in China and the world. The orange chromosomes are small and similar in morphology, lacking effective markers, which makes its cytological research relatively lagging, therefore little is known about cytogenetic information. This study intends to screen the molecular markers that can recognize sweet orange chromosomes by bioinformatic analysis and FISH (Fluorescence in situ hybridization) experiments on cultivated diploid and double haploid line of sweet orange. On the one hand, the mitosis metaphase chromosomes can be distinguished by combining the molecular markers with DAPI/CMA staining; the other hand, a high-resolution molecular cytogenetic map is constructed on the pachytene chromosomes, which can improve genomic information and integrate linkage groups with cytological map. We used the sweet orange specific antibody CsCenH3 to immunoprecipitate centromere binding protein and have obtained the centromere sequence 5-1 and another repeat sequences CL-6 after sequencing. We have screened another high-copy sequence CL-2 in genome. It was confirmed by FISH and CMA/DAPI staining that CL-2 was located on three chromosomes of D, F and C, respectively, while CL-6 only had a bright signal at the near centromere of D-type chromosome. Besides, we also selectively designed oligonucleotide probes on chromosome different regions to recognize specific chromosome. We will do double/multi-FISH to integrate probes, such as high copy gene 45SrDNA, tandem repetitive sequence, and oligo probes, to create and enrich the chromosome karyotype and finally construct high-resolution molecular cytogenetic map in sweet orange. The discovery of molecular markers and the construction of molecular cytogenetic maps are expected to lay the foundation for studying chromosome structural variation, species evolution and genetic improvement of sweet orange.

Section: molecular breeding

Bioinformatic approaches to identify target-specific guide RNAs for CRISPR-Cas genome editing of *Vitis vinifera* L.

Loredana Moffa

Uniuud

Grapevine (*Vitis vinifera* L. ssp. *Sativa*) is an economically important cultivated species for the production of wines, raisins, and juices. It is characterized by thousands of different varieties, the majority of which are highly susceptible to several fungal pathogens. Among them, *Erysiphe necator*, the causal agent of powdery mildew, can cause severe crop and quality losses if no control measures are carried out. In most conventional-managed vineyards, powdery mildew and other diseases are mainly treated by chemical fungicides, making viticulture one of the agricultural activities with the greatest chemical input. To reduce the environmental impact of grapevine cultivation many efforts are ongoing through biological or biotechnological approaches. In this context, the grapevine genetic improvement is gaining great benefit from the New Plant Breeding Technologies, which resemble traditional breeding but with shorter time and less impact on the varieties's genomes. In order to reduce the fungal susceptibility, we plan to produce two different knock-out mutants for two members of the MLO gene family (VvMLO6 and VvMLO7), well known for their involvement in powdery mildew susceptibility. Moreover, to further evaluate their functional effect(s), three different editing events will be performed: each gene will be switched-off independently and we will produce a third mutant carrying both the mutations. The last editing event is designed in order to evaluate the possibility of any synergistic outcome. The above mentioned purposes will be achieved by using CRISPR-Cas technology on Glera, Pinot Nero and Sangiovese varieties. Despite the ability of this technique to introduce Insertion and/or Deletion (InDel) in very specific target sequences, off target activities can occur. To minimize the latter possibility, we took advantages from different bioinformatics tools in order to: i) design guides, ii) predict on-target editing efficiency and iii) minimize unspecific editing events. First, SNPs calling analysis of the above mentioned varieties was performed using the near-homozygous Pinot PN40024 genome as reference. Then, guide RNAs were designed using CRISPRdirect tool. Guides specificity was analysed using CRISPR_P tool and, as final criteria for guide selection, absence of unspecific target on genomic DNA was evaluated with CRISPOR and RGEN. The two selected gRNAs will be implemented into expression plasmids, together with the Cas9 and used to perform Agrobacterium-mediated transformation of embryogenic calli.

Section: Biotechnology and genome editing

New insights into grapevine Esca syndrome: environmental microbiology studies and (near-) future eco-friendly control strategies

Luca Nerva

CREA - Research Centre for Viticulture and Enology

Europe is the world largest grape producer, but in recent years, the report of diseases due to infection by grapevine trunk pathogens (GTPs) is becoming one of the main constraints for viticulture. Among grapevine trunk diseases (GTDs), esca syndrome is one of the most complex characterized by simultaneous infection of several fungi, which lead to important reduction in yield and quality. To date, it is worth noting that no curing approach is available for esca syndrome. Previous characterization of fungal isolates associated to esca syndrome lead to the recognition of at least three important players: *Phaeoacremonium minimum*, *Phaemoniella chlamydospora* and *Fomitiporia mediterranea*. We created, for the first time, a curated fungal endophytes collection of which we known the virome state. We identified several fungal viruses (i.e. mycoviruses) of which we are now studying the biological impact. In specific, some of these mycoviruses were recovered from several *P. minimum* and *F. mediterranea* isolates. In addition, we analyzed the bacterial and fungal communities of the bulk soils associated with esca-symptomatic and asymptomatic vines. Results showed no significant differences in richness between the two types of samples. Conversely, we observed that esca-related pathogens and grapevine trunk disease (GTD) pathogens were more abundant in the bulk soils of symptomatic plants, suggesting that the soil could represent an important source of inoculum. New approaches to control esca syndrome, such as the use of biological control agents or RNA interference (RNAi) technique are now under evaluation. In light of the ongoing climate changing scenario, these approaches could represent promising exploitable tools for a more sustainable disease management in viticulture.

Section: abiotic and biotic stresses

Kissing cousins: Comparative genomics and population genetics of pathogenic *Pseudoperonospora cubensis* and *Ps. humuli*, to unbraid the species

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Pseudoperonospora cubensis and *Ps. humuli*, two closely related Oomycetes species, cause major losses among the horticultural crops with the ensuing downy mildews in cucurbits and hops, respectively. Several discordant hypothesis were formulated as to those species identity and taxonomic relationships based on the phytopathological evidence. With the availability of draft genome sequences of both pathogens, we aimed at determining the species delimitation. Current and historical (herbarium specimens) symptomatic host plant leaf samples were subjected to DNA extraction and genotyping with *Ps. cubensis*-based gSSRs. Subsequent capillary electrophoresis, analytical sequencing of extracted PCR products, and a host of population genetics tools were employed. Out of the 24 gSSRs tested for cross-amplification into *Ps. humuli*, 21 successfully amplified with their PCR products range within the *Ps. cubensis* range, but barely any cross-amplification was achieved for other tested Oomycetes gDNA samples. Sequencing results of PCR products for selected loci showed strong evidence for differences among the two pathogens. Population genetics provided evidence for primarily sexual reproduction in the *Ps. humuli* populations and the historical *Ps. cubensis*, but current *Ps. cubensis* was mostly clonal in character both in Europe and in North America. We also observed a population divergence in the current North American populations, confirming the 2005/2006 epidemics and the re-emergence of this pathogen. As expected from the closely related species, significant synteny was observed in the comparative genomics analysis both at the gDNA and translated sequence level, with *Ps. humuli* genome showing evidence of tandem expansion. Results of this study will have impacts on the plant protection strategies and pathogen management in these economically important crops.

Section: Comparative genomics

***MYB103* as candidate gene for male sterility in leaf chicory, radicchio**

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We report the first high-density linkage map construction through genotyping-by-sequencing (GBS) in leaf chicory (*Cichorium intybus* subsp. *intybus* var. *foliosum*, $2n = 2x = 18$) and the SNP-based fine mapping of the linkage group region carrying a recessive gene responsible for male-sterility (*ms1*). An experimental BC1 population, segregating for the male sterility trait, was specifically generated and 198 progeny plants were preliminary screened through a multiplexed SSR genotyping analysis for the identification of microsatellite markers linked to the *ms1* locus. Two backbone SSR markers belonging to linkage group 4 of the available *Cichorium* consensus map were found genetically associated to the *ms1* gene at 5.8 and 12.1 cM apart. A GBS strategy was then used to produce a high-density SNP-based linkage map, containing 727 genomic loci organized into 9 linkage groups and spanning a total length of 1,413 cM. 13 SNPs proved to be tightly linked to the *ms1* locus based on a subset of 44 progeny plants analyzed. The map position of these markers was further validated by sequence-specific PCR experiments using an additional set of 64 progeny plants, enabling to verify that four of them fully co-segregated with male-sterility. A mesosynteny analysis revealed that 10 genomic DNA sequences encompassing the 13 selected SNPs of chicory mapped in a peripheral region of chromosome 5 of lettuce (*Lactuca sativa* L.) spanning about 18 Mbp. Since a MYB103-like gene, encoding for a transcription factor involved in callose dissolution of tetrads and exine development of microspores, was found located in the same chromosomal region, this orthologous was chosen as candidate for male-sterility. The amplification and sequencing of its CDS using accessions with contrasting phenotypes/genotypes (i.e., 4 male sterile mutants, *ms1ms1*, and 4 male fertile inbreds, *Ms1Ms1*) enabled to detect an INDEL of 4 nucleotides in its second exon, responsible for an anticipated stop codon in the male sterile mutants. This polymorphism was subsequently validated through allele-specific PCR assays and found to fully co-segregate with male-sterility, using 64 progeny plants of the same mapping BC1 population. Overall, our molecular data could be practically exploited for genotyping plant materials and for marker-assisted breeding schemes in leaf chicory.

Section: Molecular breeding

Annotating the epigenome of strawberry based on comprehensive analysis of histone modifications

Qinwei Pan

Nanjing Agriculture University

Chromatin is a complex of DNA and various modified histones and non-histones in eukaryotic cells, which affect gene expression and various processes. However, the information on chromatin structure of fleshy fruits is limited. Here we present a chromatin landscape for woodland strawberry *Fragaria vesca* based on eight histone modifications and DNA methylation, summarized by twelve chromatin states. Through the comprehensive analysis combined with other data (short/long RNA long products etc.), it reveals the relationship of chromosome, gene expression and other functional domains. Our systematic profiling and integrative analysis of chromatin structure will serve as a resource for future experimental investigations of genome structure and function for Rosaceae species.

Section: Comparative genomics

Fire blight resistance in apple/pear hybrids may be related to their genomic structure.

Giulia Pasqualetto

Fondazione Edmund Mach

Although *Malus* and *Pyrus* are closely related, with highly co-linear genomes, the two genera are characterised by many specific differences, including disease resistances, secondary metabolites, fruit texture, flavour and shape. Hence, intergeneric hybrids between apple and pear provide a unique germplasm resource for genetic analysis, as well as new cultivar development, using advanced genomic breeding strategies. Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora* (Enterobacterales; Erwiniaceae), affects apple and pear production worldwide. A number of resistance loci against this disease have been located on genetic maps of both apple and pear. We investigated fire blight resistance in apple-pear hybrids, by studying 31 putative hybrids raised from a ‘Cox’s Orange Pippin’ x ‘Old Home’ cross at The New Zealand Institute for Plant and Food Research Limited. We inoculated up to eight replicates of each hybrid grafted on ‘M9’ rootstock and compared these with ‘Cox’s Orange Pippin’ and ‘Imperial Gala’ grafted on ‘M9’ rootstock and ‘Old Home’ and ‘Williams’ grafted with *Pyrus calleryana*, using the cut-leaf method (Maas Geesteranus and Heyting, 1981) for inoculation with *E. amylovora* (Ea 236 at 1×10^6 cfu/ml), as it is widely applied in both apple and pear. Disease progress was observed in the period from 2 to 6 weeks after inoculation. Level of disease was quantified by expressed necrosis length as a percentage of the total shoot length, both measured downwards from the point of inoculation. The result clearly showed that all of the 31 putative hybrids were resistant to fire blight, while the parents and controls exhibited the expected range of resistance and susceptibility according to previous work. Preliminary results using high-resolution melting marker analysis of the seedling genomes indicate there is a hybrid apple/pear genomic region on LG2, while LG7 is represented by apple DNA. Interestingly, fire blight resistance has been reported on LG2 of pear ‘Old Home’ (Montanari et al., 2016), while Khan et al. (2007) have located fire blight resistance on LG7 of the apple ‘Fiesta’, which is related by descent from ‘Cox’s Orange Pippin’. Our next step is to analyse recombination events during the crossing of apple and pear, using the IRSC 9K apple/pear SNP array: this will enable us to further investigate the relationship of these reported QTL resistances to fire blight infection within the genomic structure of our 31 apple/pear hybrids.

Section: Molecular breeding

MITE insertion of IRT1 regulates iron uptake in citrus

Shu'ang Peng

Huazhong Agricultural University

Citrus plants take up iron from the soil by IRON-REGULATED TRANSPORTER 1 (IRT1) high-affinity iron transporter at the root surface. We previously reported that the citrus rootstock 'Zhique' (*Citrus wilsonii* Tanaka, ZQ) showed tolerance to iron deficiency under calcareous soil condition. Here, we found that the citrus IRT1-GFP fusion protein was located on plasma membrane and the IRT1 gene showed higher transcript expression level in ZQ than another common rootstock 'Zhike' (*Poncirus trifoliata* (L.) Raf. ZK). Compared with that in ZK, the promoter regions of IRT1 in ZQ were inserted by two miniature inverted-repeat transposable element (MITE) sequences with different lengths. Furthermore, the citrus IRT1 comprising two alleles with or without MITE insertion could be structurally divided into six types. Genotyping PCR analysis of 24 citrus original and breeding varieties revealed that the IRT1 allele with the MITE insertion was iron deficiency resistance, suggesting that the MITE insertion in IRT1 promoter may play an important role in iron uptake by regulating transcript expression of IRT1.

Keywords: Citrus, iron deficiency, IRT1, MITE

Section: Biotic and abiotic stresses

Construction of Brassica-specific genes over-expression mutant library in *Arabidopsis thaliana*

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Orphan genes, also called lineage-specific genes (LSGs), are important for responses to biotic and abiotic stresses, and are associated with lineage-specific structures and biological functions. We first identified and characterized Brassica-specific genes (BSGs), Cruciferae-specific genes (CSGs), and evolutionary-conserved genes (ECGs) using the genome of *Brassica Rapa*. To explore the BSGs functions, the expression patterns of BSGs in various tissues and their responsive patterns to *Plasmodiophora brassicae* were analyzed using previously published RNA sequencing data and semi-qPCR in *B. Rapa*. To study the functions of BSGs, a BSGs over-expression (BSGsOE) mutant library consisting of 127 genes in *Arabidopsis thaliana* were constructed. A total of 78 BSGs displayed visible phenotype variations. Observation of BSGsOE mutants revealed that phenotype variations of BSGsOE *Arabidopsis* lines showed Chinese cabbage specific traits, such as wavy, hairy, upward or downward curving leaves, serrated margins and numerous leaves. Flowering time variations are rich in mutant library, especially delayed flowering, indicating that BSGs caused delayed flowering time might be related to bolting tolerance of Chinese cabbage. Furthermore, BSG37, caused a strong delayed flowering time phenotype, which might played vital roles in bolting tolerance. And different BSGs play general and specific roles in the response to pathogen and environmental stresses. This work extensively elucidates the functions of BSGs for the first time, and reinforces the idea that BSGs are a valuable resource for identifying useful genes related to species-specific traits and stress response.

Section: Biotechnology and genome editing

The heterologous expression of a chrysanthemum TCP-P transcription factor CmTCP14 suppresses organ size and delays senescence in Arabidopsis thaliana

Yixin Qu

Nanjing Agricultural University

TCP transcription factors are important for plant growth and development, but their activity in chrysanthemum (*Chrysanthemum morifolium*) has not been thoroughly explored. Here, a chrysanthemum TCP-P sequence, which encodes a protein harboring the conserved basic helix-loop-helix (bHLH) motif, was shown to be related phylogenetically to the *Arabidopsis thaliana* gene AtTCP14. A yeast-one hybrid assay showed that the encoding protein had no transcriptional activation ability, and a localization experiment indicated that it was localized in the nucleus. Transcription profiling established that the gene was most active in the stem and leaf. Its heterologous expression in *A. thaliana* down-regulated certain cell cycle-related genes, reduced the size of various organs and increased the chlorophyll and carotenoid contents of the leaf which led to delayed senescence and a prolonged flowering period. Moreover, by screening the cDNA library of chrysanthemum, we found that the CmTCP14 can interact with CmFTL2 and some CmDELLAs.

Section: Biotechnology and genome editing

Impact of exogenously applied MeJA on stomata density and photosynthetic performance in two *Vaccinium corymbosum* L. cultivars under aluminum and manganese toxicity

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Universidad de La Frontera

Aluminum and manganese toxicity have been described as the most important growth-limiting factor for plants in many acid soils around the world. Under acidic pH conditions, the Al^{3+} and Mn^{2+} concentrations increase, becoming potentially phytotoxic for plants. As an essential micronutrient, Mn takes place in several metabolic processes, such as photosynthesis by forming part of the structure of proteins in the oxygen-evolving complex, participating in the H_2O photolysis, electron transport and also as an enzyme antioxidant-cofactor. However, Mn excess can lead to damages of the photosynthetic machinery, specifically PSII subunits. On the other hand, Al^{3+} toxicity results in root growth reduction, inducing chloroplast malformations, decreases in total chlorophyll content and photosynthetic rates accompanied by partial inhibition of photosynthetic electron transport in photosystem II (PSII). There is a need to evaluate new strategies to counteract the negative effects of these elements in crop plants growing under acid soils. The exogenous application of Methyl jasmonate (MeJA) has shown to be a promising tool to improve the harmful effects of different stress factors such as Al, Cd, among others. Therefore, we aimed to evaluate the contribution of an exogenously applied MeJA on the performance of two *Vaccinium corymbosum* cultivars under Al and Mn toxicity. Two contrasting blueberry cultivars (Camellia, resistant and Star, sensitive to Al) were conditioned in nutrient solution at pH 4.8 with and without MeJA (0.01 mM) application under toxic Mn (1000 μM) and Al (200 μM) for 21 days as following: Control, MeJA, Al, Mn, Al+Mn, Al+MeJA, Mn+MeJA and Al+Mn+MeJA. Photosynthetic parameters were evaluated *in vivo*, and leaves were collected to evaluate photosynthetic pigments (PP) by HPLC-DAD and total hormones (TH) as IAA equivalents. Besides, leaves were fixed with Glutaraldehyde 2.5% to evaluate stomata density (SD) by VP-SEM microscopy. The results showed a differential response between cultivars with MeJA application under Al and Mn stress and their combinations. Similar SD is shown in the non-stressed condition in both genotypes. However, MeJA alone regulates the number of stomata negatively in both cultivars, decreasing significantly in Star (48%). Despite SD decline, A (net photosynthesis), g_s (stomatal conductance) and E (transpiration) did not change with MeJA treatment in Star and Camellia. However, Star photosynthetic performance, total chlorophylls, chlorophyll ratio, and β -carotene showed a high decrease (until 70%) under Al and Mn treatments (showing sensitivity to both treatments), recovering with the exogenous MeJA application. Regarding TH, Camellia and Star showed similar behavior under control and MeJA; however, when exposed to Al alone, both cultivars increased its values. Star cultivar appears to be sensitive to Al and Mn toxicity, improving its performance with MeJA treatment. Interestingly, MeJA alone negatively regulates the SD without changing the photosynthetic parameters in both cultivars. However, in the presence of stress conditions, the MeJA application improves the performance of the sensitive cultivar Star. Acknowledgments: FONDECYT-1171286

Section: Biotic and abiotic stress

The potential of ectomycorrhizal fungi for enhancing successful vegetation restoration on heavy-metal tailings

Liang Shi

Nanjing Agricultural University

Whether the huge external hyphal system of ectomycorrhizae that promotes host plants' acquisition of water and nutrients can selectively inhibit their transport of heavy metals at the same time remains unclear. In the root-bag test, external hyphae took the initiative into the Cu/Cd-contaminated bulk soil, absorb and transport Cu and Cd to the rhizosphere soils and further transport it to the shoots of the host plants. Inoculation with ectomycorrhizal fungi (EMF) also promoted the uptake of nutrients by host plants, thereby increasing their biomass and improving Cu/Cd tolerance compared with non-inoculated plants. Inoculation with EMF species with higher Cu or Cd tolerance generated more phytostabilization and phytoextraction of Cu or Cd by host plants. Therefore, we concluded that EMF do not act as a barrier inhibiting the absorption of heavy metals by host plants, but rather promote this absorption. On the other hand, vegetation reconstruction on heavy-metal tailings dumps is a very difficult process. The survivorship of non-mycorrhizal (NM) seedlings after 6 months was only 56%. The survivorships of *Laccaria laccata* and *Hebeloma vinosophyllum* seedlings were 89% and 100%, respectively. Inoculation with EMF significantly promoted the absorption of nutrients, such as Pi, by host pine seedlings. Pine seedlings increased the numbers of soil bacterial communities and the activities of soil urease and acid phosphatase. *H. vinosophyllum* seedlings had the greatest effect among all of these soil indicator parameters. Mycorrhizal pine seedlings significantly reduced the availability of heavy metals in the soil and increased the amount of heavy metals transferred to host plant tissues. Cd-tolerant EMF were especially efficient promoters of these activities. Thus, the cultivation of ectomycorrhizal pine trees can improve soil quality in mining wastelands and increase the chances of successful vegetation restoration.

Section: Biotic and abiotic stress

PtrABF2 is a substrate of PtrSnRK2.4/2.6 kinase and positively regulates putrescine accumulation in Poncirus trifoliata responding to drought tolerance

Jie Song

Huazhong Agricultural University

PtrABF2 is a substrate of PtrSnRK2.4/2.6 kinase and positively regulates putrescine accumulation in Poncirus trifoliata responding to drought tolerance Jie Song, Tonglu Wei, Ruhong Ming, Ji-Hong Liu*Key Laboratory of Horticultural Plant Biology, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, ChinaArginine decarboxylase (ADC)-mediated putrescine biosynthesis play important roles in plant growth and development, crop yield and quality, as well as responses to abiotic stresses. The Abscisic acid (ABA) is a multifunctional hormone in Poncirus trifoliata in response to drought stress. However, the exact mechanism by which ABA regulates putrescine accumulation is largely unknown in citrus. Here we identified a member of Abscisic acid-responsive element (ABRE)-binding factors (ABFs)-ABF2 which can interact with and may be phosphorylated by PtrSnRK2.4/2.6. Virus-induced gene silencing (VIGS) was used to obtain PtrABF2-silencing Poncirus. The VIGS lines was more seriously injured under drought stress, in consistent with lower free putrescine levels and accumulation of more ROS, suggesting that PtrABF2 functions as a positive regulator in ABA signaling-mediated drought response. Furthermore, expression level of putrescine biosynthesis gene PtrADC were repressed by PtrABF2 directly binding to its promoter. Taken together, these results demonstrate that PtrABF2 is a positive regulator of putrescine biosynthesis and positively regulate the drought stress response, at least in part, via the modulation of putrescine-associated reactive oxygen species homeostasis.Key Words: PtrABF2 , PtrSnRK2.4/2.6 kinase, putrescine, Poncirus, drought

Section: Biotic and abiotic stress

Targeted and untargeted metabolomics research coupled with genome-wide association analyses reveal genetic and biochemical control mechanism of phenolic compounds in apple fruit

Jun Song

Agriculture and Agri-Food Canada

Apples are a nutritious food source with significant amounts of polyphenols that contribute to human health and wellbeing, primarily as dietary antioxidants. Although numerous pre- and post-harvest factors can affect the composition of polyphenols in apples, genetics is presumed to play a major role since polyphenol concentration varies dramatically among apple cultivars. Here we investigated the genetic architecture of apple polyphenols by combining high performance liquid chromatography (HPLC) as well as untargeted metabolomic analysis employing mass spectrometry obtaining data with approximately 250,000 single nucleotide polymorphisms (SNPs) from two diverse apple populations. We found that polyphenols can vary in concentration by up to two orders of magnitude across cultivars, and that this dramatic variation is often predictable using genetic markers and frequently controlled by a small number of large effect genetic loci. Using GWAS, we identify candidate genes for the production of quercitrin, epicatechin, catechin, chlorogenic acid, 4-o-caffeoylquinic acid and procyanidins B1, B2 and C1 with HPLC analysis. With the aid of untargeted LC/MS from 1400 chemical features, we additionally identified the genetic control regions for quercetin, 2-hexylglucoside and quercetin 3-(6"-malonyl-glucoside). This study reveals new insights into the genetic and biochemical control mechanism of phenolic compounds in apples. Our observation that a relatively simple genetic architecture underlies the dramatic variation of key polyphenols in apples suggests that breeders may be able to improve the nutritional value of apples through marker-assisted breeding or gene editing.

Section: Dissection of complex quality traits

Calcium alleviates temperature stress by regulating nitrogen and respiratory metabolism in *Malus baccata* roots

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The effects of calcium on respiratory and nitrogen metabolism of apple roots (*Malus baccata* Borkh.) exposed to temperature stress (5 °C ~ 20 °C ~ 0 °C) were investigated. Seedlings were treated with distilled water (control), calcium chloride (CaCl₂) or calmodulin antagonist trifluoperazine (TFP) before temperature stress. Temperature was increased from 5 °C to 20 °C (1 °C h⁻¹) and then decreased to 0 °C (1 °C h⁻¹). Temperature stress decreased root vitality and increased root malondialdehyde (MDA) concentration, the effect of which was exacerbated by TFP treatment. Treatment with CaCl₂ improved root vitality and decreased root MDA concentration. At 20 °C, exogenous CaCl₂ alleviated the negative effects of temperature stress on the total respiration rate by enhancing the activity of tricarboxylic acid cycle (TCA). Activities of key enzyme in nitrogen metabolism were strongly inhibited by temperature stress. Exogenous CaCl₂ significantly increased key enzyme activities of nitrogen metabolism compared to the control. However, the TFP treatment markedly reduced the activity of glutamate synthase (GOGAT) at 20 °C and noticeably inhibited glutamate dehydrogenase (GDH) activity during the entire temperature stress period. The data showed that the Ca²⁺-calmodulin (Ca²⁺-CaM) signal system was involved in increase of GOGAT and GDH activity that occurred with an increase in temperature, and played a role in the increase in the total respiration rate and GDH activity which occurred with a decrease in temperature. Cultural practices that improve plant calcium (Ca) status in the early spring may mitigate damage induced by temperature stress.

Section: Biotic and abiotic stress

Protective effect of Se-enriched red radish sprouts on carbon tetrachloride-induced liver injury in mice

Nana Su

Nanjing Agricultural University

This study aimed to investigate the effect of Se (Selenium) treatment on nutritional quality in radish sprouts. The results showed that 15 μ M sodium selenite significantly increased phenolics compounds, flavonoids compounds, anthocyanins and some essential amino acid content, while improving the total antioxidant capacity of radish sprouts. Besides, the Se-enriched radish sprouts significantly alleviated the liver damage caused by carbon tetrachloride (CCl₄) in mice and improved the antioxidant capacity of the liver in mice, while the Se-enriched radish sprouts alleviated the inflammatory reaction and apoptosis caused by CCl₄. These results imply that Se-enriched radish sprouts have a positive impact on mice with CCl₄-induced liver injury, and that in future Se-enriched radish sprouts could be developed into an effective food and health care product for the liver injury prevention.

Section: Plant signalling and behaviour

An exploration on sex determination mechanism of monoecious pistacia chinensis bunge

Shuchai Su

Beijing Forestry University

Pistacia chinensis Bunge is a pioneer tree for afforestation, and of high value as an ornament and for timber and medicine. It has also become the preferred biofuel tree in northern China in current years, because its oil content of fruit is more than 40%. However, it is dioecious and lacks good varieties, leading to waste of manpower and field, thus resulting in low and unstable yield. Rare monoecious individuals were found, to explore its biological characteristics, origin and floral development mechanism, dioecious and monoecious materials were used to explore the phenotypic characteristics of different sex types of *P. chinensis* in this study. Moreover, DNA molecular markers, sex stability, and inflorescence bud differentiation were studied. Additionally, the inflorescence buds of different sex types during the critical stage of sex differentiation were selected for RNA transcriptome and small RNA sequencing, and the key genes affecting sex determination were screened. Two candidate genes were selected for the sequences analyses and functional identification.

Section: Plant signalling and behaviour

Cotton fiber elongation requires the transcription factor GhMYB212 to regulate sucrose transportation into expanding fibers

Wenjie Sun

Shanghai Jiao Tong University

Cotton is white gold across the globe and composed of fiber cells derived from the outer integument of cotton ovules. Fiber elongation uses sucrose as a direct carbon source. The molecular mechanism transcriptionally controlling sucrose transport from ovules into the elongating fibers remains elusive. In this study the involvement of GhMYB212 in the regulation of sucrose transportation into expanding fibers was investigated. GhMYB212 RNAi plants (GhMYB212i) accumulated less sucrose and glucose in developing fibers, and had shorter fibers and a lower lint index. RNAseq and proteinDNA binding assays revealed that GhMYB212 was closely linked to the pathways of sucrose and starch transportation and metabolism, directly controlling the expression of a sucrose transporter gene GhSWEET12. GhSWEET12 RNAi plants (GhSWEET12i) possessed similar fiber phenotypes to those of GhMYB212i. Exogenous sucrose supplementation in ovule cultures did not rescue the shorter fiber phenotype of GhMYB212i and GhSWEET12i. This finding supported the idea that the attenuated rate of sucrose transport from the outer seed coat into the fibers is responsible for the retardation of fiber elongation. Current investigations support the idea that GhMYB212 functions as the main regulator of fiber elongation by controlling the expression of GhSWEET12, and therefore it is important to study cell expansion and sugar transportation during seed development.

Section: Plant signalling and behaviour

Heat-induced regulation of linear and cyclic electron flow protects photosystem I in grape leaves

Yongjiang Sun

Beijing Forestry University

In order to investigate the photosynthetic strategy of grape to adapt heat stress, chlorophyll a fluorescence and the change in absorbance of P700 were analyzed. Heat stress resulted in a significant decrease of the photosystem II (PSII) potential quantum efficiency and linear electron flow through PSII and PSI, but had little effects on photosystem I activity. With the temperature increased, the rates of P700+ dark re-reduction and the initial rate of the post-illumination increase in chlorophyll fluorescence enhanced significantly, indicating that heat stress induced the cyclic electron flow (CEF) around PSI. Besides, both pH-dependent high-energy state quenching (qE) and P700 oxidation increased under heat stress conditions, and showed a well correlation with CEF activity. These results suggested that overclosure of PSII reaction centers at high temperature resulted in the photoinhibition of PSII, while the down-regulation of linear and activation of cyclic electron flow during heat stress played a role in protecting PSI through contributing to the generation of a proton gradient and P700 oxidation, respectively.

Section: Biotic and abiotic stresses

Effects of exogenous abscisic acid on primary metabolite profile of postharvest tomato during ripening

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Phytohormone abscisic acid (ABA) plays an important role in tomato ripening. However, the regulation of primary metabolites by ABA during tomato ripening is still obscure. In the present research, fruit were infiltrated with ABA (1.0 mM) and deionized water (control), and kept in darkness for 15 d, and the effects of exogenous ABA on the relative contents of sugars, organic acids, amino acids and the expressions of key genes related with sugar and organic acid metabolisms were analyzed. The results showed that exogenous ABA accelerated tomato ripening, accompanying with the enhanced changes of fruit colour and firmness, the higher levels of respiration rate and ethylene production. Meanwhile, it also enhanced the relative levels of some primary metabolites, such as glucose (3.05-fold), lyxose (2.64-fold) and fructose (3.25-fold), citric acid (2.99-fold), citramalic acid (1.80-fold), ascorbic acid (1.70-fold) and gluconic acid (1.70-fold), as well as serine (1.91-fold), pyroglutamic acid (1.93-fold), GABA (1.90-fold), glutamic acid (2.60-fold), aspartic acid (2.16-fold), asparagine (1.72-fold) and glutamine (1.69-fold). Additionally, the expressions of most key genes were stimulated (\log_2 fold change = 1.06 to 3.64) by exogenous ABA during tomato ripening. These results indicate ABA is systematically involved in the regulation of primary metabolites and the expression of related genes, which may be a useful regulation target to improve tomato taste and flavor.

Section: Plant signalling and behaviour

Effect of exogenous additives on embryo rescue and development of ovules of seedless grape

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The young ovules from Ruby Seedless× Muscat Hamburg, Himrod× Muscat Hamburg were taken as experimental materials, to investigate the effects of exogenous additives (6-BA, glycine and activated carbon) on ovule and embryo development of seedless grape. The results showed that the concentration of 0.5 mg/L 6-BA treatment gained the highest embryo development rate from Ruby Seedless× Muscat Hamburg, which was 15.89%; 4.00 mmol/L glycine was the optimum concentrations for Ruby Seedless× Muscat Hamburg and 1.00 mmol/L glycine concentration was the best for Himrod × Muscat Hamburg, the embryo development rate were 13.33% and 17.33%, respectively. The optimum concentration of activated carbon was 1.0 g/L for two combinations.

Section: Biotechnology and genome editing

Titanium dioxide nanoparticles increase the photosynthetic performance, but not the antioxidant system in *Solanum lycopersicum* L.

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Light response curve and antioxidant systems were evaluated by titanium dioxide nanoparticles (NPs TiO₂) applied to seeds of *S. lycopersicum*. Doses of 1000 and 2000 mg L⁻¹ of NPs and 2000 mg L⁻¹ of TiO₂ microparticles (μPs) and deionized water as control were used. All treatments of NPs TiO₂ were sonicated and stirred by 30 min each for adequate suspensions, immediately before seeds application on petri dish at 48 h for its imbibition. The seeds of each treatment were sown in individual pots with peat: perlite 3:1, irrigated by capillarity in a grown chamber at 25 °C, 55% RH, 200 μmol photons m⁻²s⁻¹ source and UV-A light from led, and 16/8 h photoperiod. After 30 days of sowing, the light curves were measured by IRGA (LI-COR 6400 xt) and light compensation point (LCP) and light saturation point (LSP) were calculated. Leaves were harvested for biochemical analysis as photosynthetic pigments, lipid peroxidation (LP, through TBARS-method), total phenols (TP, by Folin-Ciocalteu-method) and antioxidant activity (AA, through DPPH-method). The main results showed a higher LCP and LSP by the effect of 2000 NPs and 2000 μPs TiO₂ respect to the control. In addition, the treatment of 1000 NPs reached a higher light curve over 1000 μmol photons m⁻²s⁻¹ compared to the others treatments. In this context, Chl a+b and Chl b were higher at 1000 NPs compared to 2000 μPs, whereas, Chl b and carotenoids decreased at 2000 μPs. No differences were observed in Chl a and Chl a/b. A slight increase of antioxidant system seems to be observed at the highest dose, but without differences between the treatments. Finally, TiO₂ treatments, do not affect the antioxidant system, conversely, the photosynthetic performance measured through light response curve and Chl a+b and Chl b concentration was increased mainly in 1000 NPs on *S. lycopersicum*. Acknowledgment: VIPUCT N°201GI-CI-01 project and CONICYT-PCHA/Doctorado Nacional/2016-21160984

Section: Biotechnology and genome editing

The impacts of light exposure and vine balance on ripening parameters in cool climate grown (*Vitis vinifera* L.) Merlot

Josh Vanderweide

Michigan State University

Viticulture practices are commonly utilized in vineyards to alter fruit quality. In cool climates, this often involves the modulation of the source-to-sink balance or an increase in fruit exposure to sunlight to aid in ripening progression, especially in red cultivars. The influence of both radiation and vine balance on fruit quality have been extensively studied in grapevines, however, little research has been carried out to decipher the involvement of both parameters in improving fruit chemistry. To study this interaction, Merlot vines in Michigan, USA and Udine, IT were subjected to the same leaf removal treatments at fruit set during the 2018 growing season: C (control with no defoliation), AB-5 (after-bloom removal of five leaves), or AB-8 (after-bloom removal of 8 leaves). Vine balance was significantly lowered with increasing levels of defoliation, but this was accomplished through the change in shoot leaf area and not cluster weight. Therefore, vines with fruit zone exposed to increasing levels of radiation also had decreasing values for vine balance. Basic fruit chemistry (°Brix and pH) were not changed, but titratable acidity was decreased by AB-8 only at harvest. HPLC analysis of glucose, fructose, and sucrose in berry skins and juice was performed. Phenylpropanoids were evaluated in skin samples via untargeted UHPLC-MS, and 52 compounds were identified in samples from Udine. In addition, 19 genes involved in flavonoid biosynthesis were analyzed via RT-qPCR at six time points between fruit set and harvest. In general, all phenolic groups detected (simple phenolics, flavonols, flavan-3-ols, and anthocyanins) saw an increase with increasing defoliation during veraison, however, only the flavonol glucosides were significantly different among treatments at harvest. A pattern was observed among metabolites that saw the greatest increase in 4' substituted phenylpropanoids compared to compounds having 3'4' or 3'4'5' substituted B-rings in response to increasing levels of defoliation. This was strongly correlated to the ratios of FLS5/F3'Ha and FLS5/F3'5'H relative expression, respectively, which was significantly separated among the three treatments. While analysis is still underway, our data reaffirms the important role of fruit exposure to radiation in promoting higher fruit quality for red vinifera cultivars grown in cool climates.

Section: Dissection of complex quality fruit traits

Study on miRNA-mediated seed and stone hardening development regulatory networks and their modulation of GA-induced seedless-berries in grapevine (*Vitis vinifera* L.)

Chen Wang

Nanjing Agricultural University

A significant body of evidence supports the potential role of grape (*Vitis vinifera*) microRNAs (VvmiRNAs) during grape berry development. In spite of this, the molecular mechanisms underlying VvmiRNA role during grape stone hardening development remain unknown. Stone hardening stage (SHS) is a crucial developmental stage in grape for the protection and dispersal of seed; meanwhile, this also affects the grape berry size and quality. Here, a total of 161 conserved and 85 species-specific VvmiRNAs/VvmiRNAs* were identified using Solexa sequencing. Among these, 30 VvmiRNAs were identified as SHS-specific, while another 52 VvmiRNAs exhibited a differential expression across various stages of grape berry development. Interestingly, 44.1% of VvmiRNAs displayed high SNP variance in their sequences, especially, VvmiR156, 166, 167, 168 and 479 families, which might be one of the reasons for the generation of new VvmiRNA family members. The obtained VvmiRNA sequences were used to identify their target genes. The miRNAs' targets were verified by using RLM-RACE and qRT-PCR. Expression analysis revealed that GA might repress the stone hardening and embryo development by inducing the expression of VvmiR31-3p and VvmiR8-5p, to negatively regulate the activity of VvCCoAOMT and VvDCAF1 lignin biosynthesis enzymes, as a key molecular mechanism involved in modulation of GA-induced grape seedless berry development. GO and KEGG pathway analysis indicated VvmiRNA-targeted genes were mainly involved in several pathways associated with the modulation of berry and seed development, of these, 13 miRNAs putatively take part in regulation of embryo development, 11 ones were identified to participate in the pathway of lignin and cellulose biosynthesis, and another 28 potentially involve in modulation of hormone signals, sugar and proline metabolism, and thus an assumed schematic mode of miRNA-mediated stone and seed development was proposed in this work. This is the first report about the regulatory role of VvmiRNAs in the regulation of stone hardening stage of grape berries, which provides valuable genetic information for the production of new seedless grape varieties.

Section: Plant signalling and behaviour

Analysis of transcriptomic and metabolomic profiles reveals the key genes and metabolites that contribute to the flavors in sand pear

Hong Wang

Jiangsu Academy of Agricultural Sciences

Sand pear (*Pyrus. pyrifolia* (Burm.) Nakai) is a major economic fruit crop in China. Sucui1 (SC1), a sand cultivar, is one of progenies generated from a cross between Cuiguan (CG) and Huasu (HS). SC1 has been cultivated widely in Yangzi River, and displays excellent Chinese-prefer flavors, including crisp, tender richer juice, sweetness with 12.5~13.0% soluble solids content and yellow greenish skin without rust. To understand the underlying molecular mechanism of distinguished flavors in this cultivar, we performed transcriptomic analysis and metabolic profiling to define gene sequences, expression and metabolite divergences between the progeny and its parents. Our data show that genes annotated as MYB family, RLK protein kinase and cellulose synthase were significantly altered in numbers between progeny and parents. Genes and metabolites, which shift in expression levels and contents, were further highlighted by significant enrichment of sucrose, amino acid and cell wall components. Taken together, our results shed light on molecular insights that govern the fruit flavors and provide a foundation for fruit quality improvement for future breeding in sand pear.

Section: Dissection of complex quality fruit traits

The loss of a single residue from CmFTL3 leads to the failure of florigen to flower

Lijun Wang

Nanjing Agricultural University

The product of CmFTL, a gene represented by multiple transcripts, is an important determinant of floral development in chrysanthemum. Here, a new transcript CmFTL3ps4 which contains three different amino acid residues compared to CmFTL3 was characterized. When driven by the *Arabidopsis thaliana* FT promoter, CmFTL3ps4 expression did not rescue the late flowering phenotype of the *A. thaliana* ft-10 mutant. When the variant sequences CmFTL3Q130K, CmFTL3G136A and CmFTL3D145N were heterologously expressed in *A. thaliana*, both CmFTL3G136A and CmFTL3D145N were shown to accelerate flowering, although to a different extent. There was no significant difference in the number of leaves which had formed before the flowering of either the CmFTL3Q130K or the CmFTL3ps4 transgenic lines. Neither the transgenic expression of CmFTL3ps4 or CmFTL3Q130K was able to rescue the ft-10 mutant phenotype. A bimolecular fluorescence complementation assay confirmed that CmFTL3Q130K did not interact with CmFDL1, a homolog of the bZIP transcription factor FD. The conclusion was that a novel residue change affected FT activity through its disruption of the interaction with CmFDL1.

Section: Biotechnology sand genome editing

Ammonia-oxidizing archaea and bacteria response to different manure application rates during organic vegetable cultivation in Northwest China

Zhan Wang

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Ammonia-oxidization is a critical process in organic vegetable field nitrogen cycling that involves ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the effects of different amounts manure on ammonia-oxidizing microorganism (AOM) communities during production of organic vegetables in northwest China remains unclear. Here, we used the *amoA* gene to quantify AOM abundance and community structure in organic vegetable fields. Quantitative PCR (qPCR) revealed that AOB showed remarkably larger abundance than AOA, with an AOB/AOA ratio of 1.08–20.67 across all soil samples. Moreover, the pairwise analyses revealed that the potential nitrification rate (PNR) was significantly positively correlated with AOB abundance, but not correlated with AOA abundance, suggesting that the AOB community might make a greater contribution to nitrification than the AOA community in organic vegetable fields. Clone library-based analyses showed that AOB sequences were affiliated with *Nitrosomonas* and *Nitrospira*, with *Nitrospira* predominant in treatments that received manure nitrogen at >900 kg ha⁻¹ and *Nitrosomonas* dominant in those that received <600 kg ha⁻¹. Ammonia-oxidizing archaea sequences were classified into *Nitrosopumilus* and *Nitrososphaera*, with *Nitrosopumilus* being dominant when manure was added and *Nitrososphaera* showing the opposite trend. Principal coordinate analysis (PCoA) also showed that a closer application ratio of manure resulted in more similar AOM community structures. Electrical conductivity, total carbon, total nitrogen, nitrate, available phosphorus, available potassium and total organic carbon contents were potential key factors influencing AOM abundance and compositions in organic vegetable fields. Our findings increase the understanding of AOM distribution patterns in organic vegetable fields.

Section: biotic and abiotic stresses

VvWRKY transcription factors modulate norisoprenoids biosynthesis by directly regulating carotenoid cleavage dioxygenase gene in grape berries

Yi Wei

China Agricultural University

The norisoprenoids such as β -damascenone and β -ionone in grape berries (*Vitis vinifera*, L.) are considered to be the main components contributing floral-fruity aroma to non-aroma type wines as the concentration of these substances in wine usually exceeds their perception threshold. However, the mechanism underlying the regulation of norisoprenoid biosynthesis in grape berry remains unexplored. Carotenoid cleavage dioxygenase1 (VvCCD1) is a critical enzyme in norisoprenoid biosynthesis pathway and it can catalyze the symmetrical cleavage of zeaxanthin and lutein to generate 3-hydroxy- β -ionone. VvCCD1 is highly expressed at the early stages of the grape berry development, and then the gene expression remains basically stable throughout the ripening stage. The highest transcript level of VvCCD1 were found in functional leaves, five fold higher than that in young berries, followed by flowers, tendril, root, stem and young leaves. To identify the transcription factor regulating the expression of VvCCD1, we bioinformatically analyzed the promoter sequence of VvCCD1 and found two series of W-box that have been thought as the DNA-binding sites of WRKY transcription factors. Using the W-box sequence as a bait in a yeast one-hybrid system to screen the sequence-binding proteins from grape berry, we identified a WRKY transcription factor, WRKY33-like, which directly bound to the W-box sequence of VvCCD1 promoter. This WRKY gene was isolated from the berries of *Vitis vinifera* cv. Cabernet Sauvignon, encoding 603 amino acids, with two conservative WRKY domains. Nuclear location of this protein was demonstrated in onion epidermal cells. Dual luciferase assay indicated that this transcription factor significantly inhibited the activity of VvCCD1 promoter. According to these results, we initially infer that this WRKY is involved in the negative regulation of the biosynthesis of norisoprenoid in grape berry.

Section: Plant signalling and behaviour

SIMYB72 regulates the accumulation of chlorophyll and carotenoid, and transition of chloroplast to chromoplast in tomato

Mengbo Wu

Chongqing University

MYB transcription factors family is large and functionally diverse in plant kingdom and the R2R3 MYBs constitute the largest subfamily of MYB family and exhibit a wide diversity of functions. Recently, some R2R3 MYBs were reported to be involved in chlorophyll and carotenoid accumulation. Tomato is second largest vegetable crop in the world and has been a classical model system for understanding fruit development and ripening. In tomato, the ripening process includes the conversion from chloroplast to chromoplast, breakdown of chlorophyll and carotenoid accumulation (Egea et al. 2011). The chlorophyll content of fruit is correlated with the nutrient, and carotenoids are contribute to the color and nutritional quality of fruits. Here we demonstrated that an R2R3-MYB transcriptional factor (SLMYB72) regulates chlorophyll and carotenoid metabolism by directly. We found that MYB72 gene directly binding to the promoter of POR to negative regulated the POR gene expression. In RNAi-SIMYB72 lines the POR expression increased twelve fold compared with WT and the chlorophyll accumulation are significantly elevated. This results indicated that MYB72 could affected the chlorophyll accumulation by negative regulative the POR expression. In our study down regulation MYB72 result to a yellow spots at red fruit stage, which is similar with Baoshan's report. It has reported that PSY, Z-SIO, PDS and so on are the key enzymes in the carotenoid biosynthesis pathway. We have demonstrated that MYB72 directly binding to the PSY1 and Z-SIO's promoter then regulate these genes expression. In RNAi-SIMYB72 lines PSY1 expression are reduced a half and Z-SIO expression also reduced dramatically, which may result in a decrease of lycopene content and a yellow spots phenotype in red fruit stage. In this study RNAi-SIMYB72 lines exhibit higher total contents of flavonoids and phenolics than WT, we found that the expression of 4CL and PD(Solyc06g074530) increased 4-fold and 8-fold respectively compared with WT. From the HPLC result analysis we found the content of rutin and quercetin are increased dramatically we have demonstrates that SLMYB72 could regulated the CHS1 expression by directly binding to the genes promoter. acid and gallic acid than WT which may be due to the increased of P-COUMAROYL 3-HYDROXYLASE (C3H Solyc03g097030.3.1) and CCR-like4 (Solyc06g061280.3.1). this results indicated that SLMYB72 involved in regulation of phenolic acid biosynthetic.

Section: Plant signalling and behaviour

Gene expression and DNA methylation profiles associated with nucellar embryo initiation of citrus

Xiaomeng Wu

Huazhong Agricultural University

As a unique way of apomixis native in citrus, nucellar embryogenesis (NE) confers development of asexual embryos along with the zygotic embryo in individual seeds. The 6-7 layers of nucellar cells surrounding the embryo sac with thickened cell wall and the condensed cytoplasm, namely nucellar embryo initiation (NEI) cells, spontaneously develop into embryos genetically identical to the maternal parent. Nucellar polyembryony (NPE) produces clonal offspring, which is beneficial for rootstock production, but hinders the efficiency of citrus cross breeding. The mechanisms of NPE has been widely investigated, the NPE locus of the Citrus genus was initially mapped within a 380 kb region on chromosome 4 (Nakano et al. Plant Science 2012). Recently, the candidate NPE-controlling gene CitRWP was identified by our institute, from a more accurate locus of 80 kb, according to BSA-Seq of the F1 segregation population of a cross between the mono-embryonic pummelo and the poly-embryonic mandarin, and the GWAS analysis of 108 citrus germplasms (Wang et al. Nature Genetics 2017). Despite the notable progresses in genetic study of the locus controlling NPE, the genes and biological processes involved in NPE of citrus still need to be explored to elucidate the processes of nucellar embryo initiation and development. In this study, to identify genes and epigenetic modifications involved in initiation of citrus NPE, the RNA-Seq and BS-Seq analyses were applied on the apomictic cells and sporophytic cells that were accurately collected by Laser microdissection (LMD), soon before (Pre-NEI stage) and after (NEI stage) the NEI cells are observable in ovules under microscope. The results are as follows: 1) At Pre-NEI stage, nucellar tissues of polyembryonic (Poly-NC) and monoembryonic (Mono-NC) from the segregating F1 population of HB pummelo × Fairchild mandarin cross were collected separately. A total of 532 DEGs were identified, among which 444 were up-regulated in Poly-NC, the enriched biological processes include translation, ribosome biogenesis, ubiquitin-dependent protein catabolic process, response to oxidative stress. 2) At NEI stage, NEI cells and the surrounding sporophytic ovule (SO) cells of Ponkan mandarin were collected separately. Among the 2,854 DEGs, 1,483 were up-regulated in the NEI cells compared with SO cells; and the enriched biological processes include embryonic development, callose deposition during defense response and methylation. 3) DNA methylation level was lower in Poly-NC compared with Mono-NC, and was also obviously lower in NEI than in SO, especially in CHH context, which indicated that an overall loss of DNA methylation during nucellar embryogenesis. The CHH methylation difference was mainly at TE body and the flanking regions, especially at class I TE. The identification of genes and epigenetic modifications associated with NPE are expected to serve rootstock propagation and breeding of citrus, to deepen the understanding of plant apomixis, and to develop technologies for heterosis fixation of crops in the future. Keywords: citrus; nucellar initiation; laser microdissection; RNA-Seq; BS-Seq

Section: Plant signalling and behaviour

Effects of light intensity and zinc and potassium nutrition on physiology and quality of soilless tomato in greenhouse

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The effects of zinc fertilizer on the growth and development, photosynthetic characteristics, quality and yield of tomato under different light supplementation intensity and different concentration of potassium were studied. The results showed that the growth index, quality and yield of tomato plants increased first and then decreased with the increase of potassium and zinc fertilizer concentration, but the photosynthetic index and pigment content increased gradually under the condition of $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ supplementation. When potassium ion concentration was 12 mmol/L and zinc ion concentration was $1.54 \mu\text{mol / L}$, VC content, soluble solids and sucrose acid ratio of tomato fruit were increased effectively. When the concentration of potassium ion was 16 mmol / L and the concentration of zinc ion was $1.155 \mu\text{mol / L}$, the salt stress caused by excessive concentration of potassium fertilizer could be alleviated significantly. The net photosynthetic rate of tomato was the highest, the organic matter accumulated most, and the titratable acid was decreased effectively, and the ratio of sugar to acid was increased. In general, when the light intensity is $380 \mu\text{mol m}^{-2} \text{s}^{-1}$, the concentration of potassium ion is 16 mmol / L, and the concentration of zinc ion is $1.155 \mu\text{mol / L}$, the comprehensive quality of tomato fruit is the best and the yield is the highest.

Section: Plant signalling and behaviour

Citrus triploid recovery based on 2x × 4x Crosses via an Optimized Embryo Rescue Approach

Kai-dong Xie

Huazhong Agricultural University

Abstract: Seedlessness is a primary breeding objective for citrus fresh fruit market, and triploids have been proven to have great value to produce seedless fruits. In this study, aiming to produce triploid plants for developing some seedless cultivars, four 2x × 4x interploid crosses were conducted using three elite but seedy cultivars as seed parents and one newly flowered doubled diploid and two allotetraploid somatic hybrids as pollen parents. As a result, a total of 1,454 developed and 3,409 undeveloped seeds from 341 fruits were obtained. Using an optimized embryo rescue approach, 669 developed and 1,301 undeveloped seeds germinated in vitro, with an average germination rate as 52.5% for the crosses using ‘Nadorcott’ tangor and ‘Bendizao’ tangerine (polyembryonic) as seed parents and 31.0% for the crosses using ‘Orah’ mandarin (monoembryonic) as seed parent. Then by shoot and root induction, totally 1,354 plantlets were regenerated, among which 401 and 54 plants were proved to be triploids and tetraploids by flow cytometry (FCM) analysis and chromosome counting. The hybrid nature of selected triploid progenies, as well as two different origins (doubled diploid and hybrid origins) for selected tetraploid progenies was further confirmed by single nucleotide polymorphism (SNP) markers. **Key words:** Citrus; seedless breeding; interploid crosses; ploidy analysis; SNP markers

Section: Molecular breeding/biotechnology

Functions of transcription factors CsAGL6 and CsPIF8 in regulating citrus fruit qualities

Juan Xu

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Both primary and secondary metabolites play critical roles in determining fruit quality. However, a single transcription factor (TF) that regulates a network of distinct metabolic pathways has not yet been reported in plants. In this study, we demonstrate that in citrus, the MADS-box TF CsAGL6 directly binds the promoter and regulates the transcription of two genes that contribute to primary metabolism and 11 genes that contribute to secondary metabolism. The overexpression of CsAGL6 in citrus callus significantly altered the levels of primary and secondary metabolites. Furthermore, we found that CsAGL6 directly interacts with important proteins from citrus (CsCLPB1-4, CsHSP70-2/5, CsNDPK3, CsSlrP and CLPX1) that may regulate and degrade AGL6. Taken together, our findings demonstrate that CsAGL6 can affect the accumulation of metabolites that contribute to fruit quality and provide evidence for a complex network that regulates metabolism in plants. Moreover, we propose a model for the posttranslational modification and degradation of AGL6. Moreover, the functions of CsPIF8 in regulating SOD and chlorophyll degradation were also reported.

Section: Dissection of complex quality traits

Mechanism analysis of lotus flower bud abortion induced by low light

Yingchun Xu

Nanjing Agricultural University

Under weak light, flower bud of lotus is easy to abort and cannot bloom. Compared with whole sun, shading treatment forced the growth of the lotus flower to be blocked, the flowering amount decreased significantly, and the flowering period was delayed and shortened. 'Snow Love' cultivar had the lowest light compensation point. The content of soluble sugar decreased under light, and the sucrose content was the most affected by shading; the total amount of chlorophyll increased, and the total amount and growth of 'Snow Love' was the most significant; correlation analysis showed that the flowering amount of lotus and the light compensation point of lotus. There was a significant positive correlation between total sugar content and leaf number. It was confirmed by abortive flower bud section observation and tunnel staining that apoptosis was the main cause of flower bud abortion caused by weak light. Transcriptomic analysis screened for two key genes in response to low light, NnTPS1 and NnSnRK1. Two genes were expressed in mature leaves, young leaves, petioles, flowers, stalks, roots and rhizomes, and expression levels in flower stalks and leaves were relatively high, indicating that two genes are in different organs. There are differences in expression and synergy. NnTPS1 and NnSnRK1 strongly responded to weak light stress, and there was antagonistic effect between them. Expression of NnTPS1 increased with the increase of sucrose concentration in a certain sucrose concentration. NnTPS1 and NnSnRK1 genes were successfully isolated from the lotus flower. The ORFs of the two genes were 2830 bp and 1680 bp, respectively, which were closely related to Arabidopsis and rice. The pFastR05-NnTPS1 over-expression vector was constructed and inoculated with Agrobacterium-mediated tobacco leaf disc method. The NnTPS1 over-expressing transgenic lines were obtained by Kana antibiotic and RT-PCR. The tobacco has increased tillering and flowering period compared to wild type.

Section: Plant signalling and behaviour

Draft genome of red bayberry provides new insights into sex determination

Wang Yan

Zhejiang University

Red bayberry (*Morella rubra*) is an important evergreen fruit tree in southern China. A large number of simple sequence repeat markers have been developed by genome sequencing survey and subsequently were used in genetic diversity evaluation. Recently, we assembled the first high-quality genome for both a female and a male individual of red bayberry. Based on the whole genome sequence, we acquired a linkage map including 3,075 single nucleotide polymorphisms, 118 sequence repeat markers and a female specific marker named MrFT2_BD-SEX, providing a valuable foundation for molecular biology, genetics and modern breeding. By whole-genome comparison between the sequenced female and male and bulk segregant analysis of natural female and male population, we found a 59Kb female specific region that contains seven putative genes, four of which are related to sexual floral development. We propose that sex determination follows the ZW model for female heterogamety. Tests of the female specific marker MrFT2_BD-SEX on hybrids derived from two female cultivars, Biqi (sex genotype W-b/Z) and Dongkui (sex genotype W-d/Z), showed 3:1 ratio of female (W-) to male (ZZ) offspring and 1:1:1:1 ratio of W-b/W-d: W-b/Z: W-d/Z: Z/Z as expected. All the flowering and fruiting seedlings are fit to their genotypes, demonstrating the ZW sex-determining model is credible. Our findings provide the new insight that the sex differentiation mechanism in red bayberry is controlled by a single female specific gene (W-) which is located in chromosome 8 and can provide a valuable resource for plant sex chromosome evolution in Myricaceae family. Most importantly, the offspring with WW genotype could be an excellent resource for commercial breeding as it would have 100% female individuals for fruit production.

Section: Comparative genomics

Water stress analysis of strawberry using hyperspectral imaging and artificial intelligence algorithm

Myongkyoon Yang

Seoul National University

Development of smart agricultural analysis technology using convergence technology is required to increase agricultural competitiveness in response to rapidly changing climate conditions and to ensure stable production of crops. As the securing of core technology becomes important and the analysis of complex quality characteristics has become possible, the importance of phenomics as a research field that covers the phenotype such as physiological, morphological, biochemical characteristics of crops is emerging. The shape and color that appear on the surface like morphology are significant because it contains various information in phenotype analysis of the quality of crops. In this study, analysis models were developed that applies various algorithms based on multiple sensor information for changes in water stress in crops. Strawberry seedlings were used as experimental crops for about 30 days. Experiments were conducted on individual pots containing soil for the independence of each. During the experimental period, the crops were divided into four groups, from no-moisture to sufficient. Except for the amount of water, the external environment of the experiment was kept the same for all crops, and various sensor networks such as temperature, humidity, and soil moisture sensor were constructed to collect environmental data in real-time. Hyperspectral images (400 ~ 1000nm) and high-resolution images obtained for the changes of seedlings were combined with environmental sensor data and applied to algorithm application and model development of water stress. Several preprocessing processes have been performed on hyperspectral images containing a lot of information. Image processing and machine learning algorithms have been used to utilize these various types of data. As a result, a model was developed to discriminate the characteristics of crops according to the amount of water. It is expected that this will be used to perform accurate non - destructive analysis of crop characteristics such as moisture, as well as various stresses and diseases.

Section: Biotic and abiotic stresses

ABRE BINDING FACTOR 3 mediates ABA-induced bud dormancy maintenance through DAM gene

Qinsong Yang

Zhejiang University

Bud dormancy establishment and maintenance are essential for perennial plants to survive cold winters, while dormancy release is important for the resumption of growth and flowering. Dormancy maintenance is a status during the transition from endo-dormancy to eco-dormancy. Studies have shown that abscisic acid plays important role in dormancy establishment and maintenance and inhibit bud dormancy release while DAM genes are also considered the key regulators of bud dormancy. However, the mechanism of ABA-regulated dormancy maintenance remains largely unknown. Here, we performed ABA and fluridone (FLU, an inhibitor of ABA biosynthesis) treatments on pear buds and confirmed the positive role of ABA in pear bud dormancy maintenance. Then, we identified that an ABA-responsive bZIP transcription factor, ABRE BINDING FACTOR 3 (PpyABF3), could bind to the promoter of pear DAM3 gene and up-regulate its expression. Besides, ABF3 also up-regulates PpyCBF4, which was previously proved to regulate DAM expression, to indirectly regulate pear bud dormancy. Overexpression of PpyABF3 in pear callus also induced DAM3 expression and inhibited callus growth as the same phenotype observed in DAM3 overexpression lines. Ectopic expression of PpyABF3 in Arabidopsis enhanced seed dormancy and inhibited seed germination in an ABA-dependent manner. Notably, another ABA-induced ABF protein, PpyABF2, interacts with PpyABF3 and interferes PpyABF3 binding and activating DAM3 promoter, indicating a precise timing and control of the regulation of DAM expression. These findings give new insight into the regulation of DAM genes and detailed mechanism of ABA-induced dormancy maintenance.

Section: Plant signalling and behaviour

Nucleolar codominance in citrus somatic hybrids

Li Yao

Huazhong Agricultural University

Somatic hybridization, also called protoplast fusion, is a type of genetic modification in plants by which two distinct species of plants are fused together to form a new hybrid plant with the characteristics of both, a somatic hybrid. This kind of breeding method has been used in Citrus to overcome barriers in the traditional sexual cross breeding, namely nucellus embryo interference, male or female sex organ abortion, and distant sexual hybridization incompatibility, which has expanded the range of species for hybridization and greatly enriched the diversity of citrus. In addition, the citrus somatic hybrids provide new multi-resistant rootstocks, precious scion breeds and citrus species for cultivating triploid seedless varieties. However, genetic information of citrus somatic hybrids remain rarely known. Citrus sinensis 'Hamlin' (HA), Citrus jambhiri (RL), Citrus sinensis 'Bonanza' (PN), Citrus aurantium (GT), HA + RL somatic hybrid (HR), PN + RL somatic hybrid (PR), PN+GT somatic hybrid (PG) were used as experimental materials in our study. The results of CMA/DAPI double fluorescence staining in somatic hybrids did not display as sum of the parental chromosomes, which indicates that chromosomes probably translocated during somatic hybrid formation. Combined with Chromosomes silver staining and 45SrDNA Fluorescent in situ hybridization(FISH), we found a phenomenon that ribosomal RNA genes with expression activity in parents are all expressed in somatic hybrids. We named it nucleolar co-dominance. The results of identifying rDNA affinities by aligning the non-conserved sequence ITS shows that both parental ITS sequences were identified in somatic hybrids, which also supports the phenomenon of nucleolar co-dominance. We give a hypothesis that epigenetics maybe involved in the mechanism of nucleolar co-dominance. Immunofluorescence staining of anti-DNA-methylation monoclonal antibody 5mC and anti-histone-acetylated-protein antibody H3K9ac showed that in the parental and somatic hybrids the site of active 45S rDNA was lowly methylated, and in the inactive 45S rDNA site the signal was high. Acetylation in all 45S rDNA sites in the nucleus of the parental and somatic hybrids was low during interphase. During mitotic, the transcriptional activity of 45S rDNA was highly acetylated, and in concentrated 45S rDNA locus the signal of acetylation was low. These results demonstrate that DNA methylation, histone H3K9ac modification is associated with nucleolar co-dominant 45S rRNA gene expression. The results of the cold resistance test of the somatic hybrids of Citrus sinensis and Citrus aurantium also showed co-dominance. The capacity of somatic hybrid was between the parents but more like the one with stronger cold resistance. We will further elucidate the mechanism of nucleolar co-dominance by detecting changes in total RNA level, ribosomal RNA level, 45S rDNA promoter methylation, and rDNA transcriptional regulatory genes in somatic hybrids and their parents. Studies have shown that the expression level of ribosomal RNA in rice is positively correlated with its cold resistance and climatic adaptability. Therefore, it is necessary to search the resistance feature of ribosomal RNA expression in citrus, with which useful rootstocks and scion species can be selected.

Section: comparative genomics

Peach-gum: A promising alternative to delay the ripening and senescence of peach fruits

Junli Ye

Huazhong Agricultural University

This study aims to evaluate the potential of peach-gum coating in delaying postharvest fruit ripening and softening. Effects of peach-gum treatment on firmness, ethylene production and water-loss rate of peach fruits were studied during cold storage for two consecutive years. Compared to the control, treatments with different concentrations (1%, 5% and 10%, v/v) of peach-gum all delayed ethylene production and fruit softening, and prevented water losing to some extent. However, peach-gum treatments didn't alter the content of malic acid, citric acid, quinic acid, glucose, fructose and sucrose, except that it repressed the reduction of sorbitol. Transcriptome analysis revealed that expression of plenty genes related to fruit softening and cell wall degradation were repressed by peach-gum treatment, in accordance with the delayed softening. Meanwhile, the expression of senescence associated genes, chitinase genes and pathogenesis-related genes that were up-regulated during cold-storage, were also inhibited by peach-gum treatment. Among those differentially expressed genes between peach-gum treated and control fruits, genes involved in IAA (Indole-3-acetic acid) transport and auxin-response were relatively statistical overrepresented, suggesting that auxin-mediated signaling pathway might involve in the physiological and molecular responses of peach fruits to peach-gum treatment. A total of 90 transcription factors belonging to 26 families, i.e. MYB, ERF, WRKY, bZIP etc., were also included. An interesting finding is that 21 of 23 zinc finger proteins from four TF families of C2H2, C3H, CO and Dof were up-regulated in peach-gum treated fruits, implying their potential functions in regulating the process of peach fruit ripening and senescence. Additionally, content of ABA (abscisic acid) and IAA was detected to be much lower in peach-gum treated fruits than control group. Taken together, our study has proved that the peach-gum could potentially serve as a new edible coating for peach fresh-keeping. These results established the basis for future exploitation of improved peach-gum based edible coatings by incorporating with other effective compounds, and provided valuable information for further investigation on the regulatory mechanism underlying fruit ripening and senescence in peach.

Section: Plant signalling and behaviour ????

A novel MADS-box transcription factor Transparent Testa16 from citrus regulates anthocyanin accumulation

Ye Lixia

Huazhong Agricultural University

Transparent Testa16 (TT16), a transcription factor belonging to the MADS-box family proteins, plays an important role in endothelial cell specific-cation and proanthocyanidin (PA) accumulation in *A. thaliana*. However, our understanding of its roles in xylophyta is limited. In this study, we characterized a novel MADS-box regulatory gene from citrus, CiMADS43, which is homologous to the TT16 gene in Arabidopsis. CiTT16 is a typical transcription factor. The subcellular localization results indicate that the gene is localized in the nucleus, and it has self-activating activity in yeast. Moreover, in different organs of citrus, CiTT16 is mainly expressed in flowers and fruits. In Arabidopsis, *tt16* mutant seed testa cannot accumulate anthocyanins and Testa transparent. Overexpression of CiTT16 in the mutant, the accumulation of anthocyanins was restored, indicating that CiTT16 can participate in the synthesis of anthocyanins. In addition, overexpression of CiTT16 in Arabidopsis, the color of testa is normal, but the leaves become curled. In tomato, there was no obvious change in the color of the fruit, but the anthocyanin content of the seed testa of some strains was significantly increased. Y1H results indicated that UNE10, a bHLH transcription factor, can bind to the promoter of CiTT16 directly, and Y2H results showed that AGL9 protein can interact with TT16 protein. However, the mechanism of these genes in the synthesis of anthocyanins remains to be further studied. Overall, our study elucidates the important roles of CiTT16 and will better our understanding of the precise role of TT16 in xylophyta.

Section: Plant signalling and behaviour

Transcriptome analysis of cultivated and wild soybean seedlings under flooding stress and the functions of GsGSTU24 and GsGSTU42 genes involved in submergence tolerance

Bingjun Yu

Nanjing Agricultural University

Flooding stress at the early seedling stage is a universal environmental constraint for soybean production. The plant-specific and ubiquitous tau class of glutathione S-transferase (GSTU) is encoded by a large gene family, and often has highly stress-inducing expression characteristics, which plays an important protective role in plant tolerance to different environmental stresses. To explore the mechanisms underlying initial flooding tolerance in soybean, in this study, we constructed leaf tissue transcriptional profile after 2 days of submergence stress using a flooding-tolerant *Glycine soja* accession and flooding-sensitive *Glycine max* cultivar and identified GsGSTU24 and GsGSTU42 as the two potential submergence stress-responsive genes. We analyzed and revealed the flooding-tolerant physiological function by over-expressing GsGSTU24 or GsGSTU42 in overexpressed transgenic soybean and *Arabidopsis thaliana* under submergence stress. The results showed that, over-expression of GsGSTU24 or GsGSTU42 in the hairy roots composite soybean plants conferred enhanced flooding tolerance under submergence stress by increasing levels of GSH and activities of GST, SOD, POD and CAT antioxidant enzymes, along with decreased H₂O₂ and O₂⁻ level in the leaves and roots, an which resulted in recovered plant growth. Similarly, over-expressing of both genes in *Arabidopsis thaliana* could also alleviate the stress injury by reducing ROS levels and increasing chlorophyll content in leaves. The positive function may be related to the decreased expression level of AtRBOHD associated with reactive oxygen species (ROS) production and AtORE1 associated with chlorophyll degradation, which resulted in lower accumulation of ROS and higher photosynthesis efficiency in leaves of transgenic *Arabidopsis thaliana*. In addition, the expression levels of endogenous GSTU genes, such as AtGSTU11 and AtGSTU24 were up-regulated in GsGSTU24 or GsGSTU42-transgenic *Arabidopsis* under submergence stress, which is correlated to the higher GST activity. It can indicate that, GsGSTU24 or GsGSTU42 gene exhibiting flood tolerance of *G. soja*, is mainly related to the involvement in regulating glutathione metabolism, reducing oxidative damage caused by flooding stress, and improving photosynthesis efficiency.

Section: Biotic and abiotic stresses

The involvement of ethylene in calcium-induced adventitious root formation in cucumber under salt stress

Jian Yu

Gansu Agricultural University

Calcium and ethylene are essential in plant growth and development. In this study, we investigated the effects of calcium and ethylene on adventitious root formation in cucumber explants under salt stress. The results revealed that 10 μM calcium chloride (CaCl_2) or 0.1 μM ethrel (ethylene donor) treatments have a maximum biological effect on promoting the adventitious rooting in cucumber under salt stress. Meanwhile, we investigated that removal of ethylene suppressed calcium ion (Ca^{2+})-induced the formation of adventitious root under salt stress indicated that ethylene participates in this process. Moreover, the application of Ca^{2+} promoted the activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and ACC Oxidase (ACO), as well as the production of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene under salt stress. Furthermore, we discovered that Ca^{2+} greatly up-regulated the expression level of CsACS3, CsACO1 and CsACO2 under salt stress. Meanwhile, Ca^{2+} significantly down-regulated CsETR1, CsETR2, CsERS, and CsCTR1, but positively up-regulated the expression of CsEIN2 and CsEIN3 under salt stress; however, the application of Ca^{2+} chelators or channel inhibitors could obviously reverse the effects of Ca^{2+} on the expression of the above genes. These results indicated that Ca^{2+} played a vital role in promoting the adventitious root development in cucumber under salt stress through regulating endogenous ethylene synthesis and activating the ethylene signal transduction pathway.

Section: Biotic and abiotic stresses

Study on tissue culture method of plants

Ping Zha

Institution of Botany, CAS

Aiming at the existing shortcomings of tissue culture technology, this paper studies a plant tissue culture method, the explants is not easy to drown, the growth rate of seedlings is fast. In addition, it solves the problem that the roots are difficult to clean and easy to be hurt before planting. This method saves labor, improve efficiency and reduce costs.

Section: Biotechnology and Genome editing

One C2H2-type zinc finger proteins from trifoliate orange (*Poncirus trifoliata* (L.) Raf.) functions in the cold tolerance by CBF-dependent pathway

Yang Zhang

Huazhong Agricultural University

The Cys2/His2-type zinc finger family was recently shown to regulate multiple aspects of plant development and abiotic stress. However, the roles of the C2H2-type zinc finger proteins in plant cold stress remain largely unclear. Through the RNA-seq analysis, a cold-responsive zinc finger protein gene was identified and cloned from the *Poncirus trifoliata*, named PtrZAT12. Furthermore, we found that PtrZAT12 markedly induced in various abiotic stresses, especially cold treatment. And it was localized in the nucleus, but with no transcriptional activation, which was caused by the EAR motif in the C terminus. The cis-element analysis of its promoter revealed that there were two DRE/C-repeat elements, recognized by the PtrCBF1. In addition, physiological analysis suggested that overexpression of PtrZAT12 conferred enhanced cold tolerance in transgenic tobaccos. Based on these results, we propose that PtrZAT12 functions in the cold stress tolerance by CBF-dependent pathway. Keywords: *Poncirus trifoliata*, cold stress, C2H2 zinc finger, CBF-dependent

Section: Biotic and abiotic stresses

PtrABF4 and PtrNAC29 transcription factors regulate PtrBAMs expression and modulate drought response through soluble sugar accumulation in *Poncirus trifoliata*.

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Abiotic stress, such as drought, high salinity and cold stress, is one of the most important constraints to plant growth in the world. Starch is a key metabolite in mediating plant response to abiotic stress. Moreover, the ABA pathway is central to drought and salt stress responses in plant. Sugar can also act as signaling molecules, which cross-talk with the ABA-dependent pathway to activate downstream genes under drought stress. Starch degradation mediated by beta-amylase (BAM) is the main source of soluble sugar. Our previous studies have shown that the PtrBAM1, which is involved in starch degradation, is induced by drought and ABA. But how ABA signal is regulated by PtrBAM1 under drought stress is not clear. Here, PtrBAM1-overexpressing exhibited higher drought tolerance phenotypes and more soluble sugar accumulation, whereas down-regulation of PtrBAM1 in *Poncirus trifoliata* by VIGs resulted in elevated drought sensitivity. Then we performed Y1H library screening using the promoter of PtrBAM1 as bait, identified a ABF family gene named ABF4 and a NAC family gene named NAC29. Y1H, LUC and EMSA assay indicated that these two TFs can bind to the promoters of PtrBAM1 and activate its expression. Moreover, PtrABF4 and PtrNAC29 overexpressed tobaccos promoted higher soluble sugars and more drought tolerance. While, knockdown them with RNAi or VIGs caused a sensitive phenotype under drought treatment. These results illustrated that PtrABF4 and PtrNAC29 are positive regulators in drought stress by interacting with PtrBAM1 to promote soluble sugar accumulation through ABA-dependent pathway. Key Words: Drought stress, PtrBAM1, PtrABF4, PtrNAC29, soluble sugar

Section: Biotic and abiotic stresses

The Phenological Growth Stages of *Sapindus mukorossi* According to BBCH Scale

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Sapindus mukorossi is a native tree in southern China that has multiple useful functions, including high landscaping, ecological, esthetic, and economic values. More importantly, its fruits are being rapidly developed as raw materials for bioenergy and saponin production in southern China. To provide a standardized phenological description of the species, this study firstly used the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale. In total, eight principal stages—i.e., bud, leaf and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity, senescence and beginning of dormancy—and 58 secondary stages were described. It ranges from vegetative bud dormancy to the onset of the next beginning of the dormancy, using a two-digit numerical coding system. In addition to the descriptions, we provide photographic images of some major developmental stages to standardize morphological characteristics and the phenological observation of *S. mukorossi*. This study will be an asset for biological researches and cultivation management in *Sapindus*, and will provide valuable information for other fruit trees.

Section: Plant Signaling and Behavior

Identification of key genes controlling important traits in Chinese cabbage using EMS mutants

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Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*), a Brassica plant originated from China, is one widely grown vegetable crop in the world. However, little is known about the molecular mechanisms regulating agriculture traits in Chinese cabbage. By using EMS mutagenesis, we have constructed a pool of Chinese cabbage mutants and identified some key genes regulating important agriculture traits through Mutmap approach. The dg mutant shows dark-green leaves and high chlorophylls. The DG gene encodes BrFC2 which locates on the thylakoid membrane. The dg mutation occurs in the conserved C-domain (Chlorophyll -binding motif, CAB), causing an incensement of the catalytic efficiency of BrFC2 and the heme content. To our knowledge, this is the first report for single-base-changed mutant with dark-green leaf color, and the first example for dark green-leaf color caused by deficiency of chlorophyll synthesis pathway. The non-heading mutant fg-1 exhibits flat heading leaves. In fg-1 plants, the epidermal cells on the adaxial surface are significantly smaller, while those on the abaxial surface are much larger than in wild-type plants, causing the heading leaves flat similar to rosette leaves. The segregation of the heading phenotype in the F2 and BC1 population suggests that the mutant trait is controlled by a pair of recessive alleles. Phytohormone analysis at the early heading stage showed significant decreases in IAA, ABA, JA and SA, with increases in methyl IAA and trans-Zeatin levels, indicating that these hormones may coordinate leaf adaxial-abaxial polarity, development and morphology. RNA-sequencing analysis at the early heading stage showed a decrease in expression levels of several genes involved in auxin transport (BrAUX1, BrLAXs, BrPINs) and responsive. Transcript levels of ABA responsive genes, including BrABF3, were up-regulated in mid-leaf sections, suggesting that both auxin and ABA signaling pathways play important roles in regulating leaf heading. In addition, a significant reduction in BrIAMT1 transcripts in fg-1 might contribute to leaf epinastic growth. The expression profiles of 19 genes with known roles in leaf polarity were significantly different in fg-1 leaves compared to the wild type, suggesting that these genes might also regulate leaf heading in Chinese cabbage. In conclusion, leaf heading in Chinese cabbage is controlled through a complex network of hormone signaling and abaxial-adaxial patterning pathways. These findings increase our understanding of the molecular basis of head formation in Chinese cabbage.

Section: Plant Signaling and Behavior

Comprehensive characterization of a floral mutant reveals the mechanism of hooked petal morphogenesis in *chrysanthemum morifolium*

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The diversity of form of the chrysanthemum flower makes this species an ideal model for studying petal morphogenesis, but as yet, the molecular mechanisms underlying petal shape development remain largely unexplored. Here, a floral mutant, which arose as a bud sport in a plant of the variety ‘Anastasia Dark Green’, and formed straight, rather than hooked petals, was subjected to both comparative morphological analysis and transcriptome profiling. The hooked petals only became discernible during a late stage of flower development. At the late stage of ‘Anastasia Dark Green’, genes related to chloroplast, hormone metabolism, cell wall and microtubules were active, as were cell division-promoting factors. Auxin concentration was significantly reduced, and a positive regulator of cell expansion was down-regulated. Two types of critical candidates, boundary genes and adaxial–abaxial regulators, were identified from 7937 differentially expressed genes in pairwise comparisons, which were up-regulated at the late stage in ‘Anastasia Dark Green’ and another two hooked varieties. Ectopic expression of a candidate abaxial gene, CmYAB1, in chrysanthemum led to changes in petal curvature and inflorescence morphology. Our findings provide new insights into the regulatory networks underlying chrysanthemum petal morphogenesis.

Section: Plant signalling and behaviour

The resistant mechanism of *Musa acuminata* ‘Pahang’ against *Fusarium oxysporum* f. sp. cubense tropical race 4

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Bananas (*Musa spp.*), not only are an important cash crop for export in many developing countries, but also are a food security crop in some tropical and subtropical areas. However, the global banana industry is threatened by a soil-borne fungal disease, Fusarium wilt of banana, caused by *Fusarium oxysporum* f. sp. cubense (Foc), especially by tropic race 4 (TR4), is a typical vascular and soil-borne disease. Identifying a reliable Foc TR4 resistance source and understanding the underlying defense mechanism is essential for future banana resistance breeding programs. In the present study, AmCyan gene was stably transferred into wild type Foc TR4 (WT-Foc TR4) and obtained the engineering strain AmCyan-Foc TR4, which is consistent with the biological characteristics and pathogenicity of the WT-Foc TR4. Two genotypes of banana Pahang and Brazilian were inoculated with AmCyan-Foc TR4 and the infection processes were subsequently monitored. We found that, in a wounding inoculation system, Foc TR4 mycelia invaded root vascular bundles via wounds at 3 dpi, and reached the corms at 7 dpi in both accessions. However, the amount of fungal biomass and the degree of necrosis in Pahang corms were significantly less than their levels in Brazilian at 14 dpi. By 7 dpi the infection processes in roots of Pahang and Brazilian were similar, but that later, the colonization in Pahang corms was significantly delayed, compared with infection in Brazilian. The result indicates that suppression of further fungal proliferation in the corm is a possible mechanism of Fusarium wilt resistance in Pahang. Microscopic examination reports that, in a wounding inoculation system, the Foc TR4 infection processes in roots of Pahang (resistant) and a triploid cultivar Brazilian (susceptible) were similar by 7 days post inoculation (dpi), but significant differences were observed in corms of both genotypes at 14 dpi. We compare transcriptomic responses in the corms of Pahang and Brazilian, and show that Pahang exhibited constitutive defense responses before Foc TR4 infection and inducible defense responses prior to Brazilian at the initial Foc TR4 infection stage. Due to ancient whole genome duplications, a relevant number of *Musa* genes are present in multiple copies with expected functional redundancy. Consequently, paralogous genes associated to DEGs were searched to assess their impact when considering gene expression additive effects. Among the 17 differentially expressed genes (DEGs) identified in Pahang under Foc TR4 infection, 4 DEGs have no paralog in the *Musa* genome. Their gene expression was not submitted to further analysis. For the other 13 DEGs, gene expression of the paralog was considered to calculate the global gene expression. Most key enzymatic genes in the phenylalanine metabolism pathway were up-regulated in Brazilian, suggesting that lignin and phytotoxin may be triggered during later stages of Foc TR4 infection. This study unravels a few potential resistance candidate genes whose expression patterns were assessed by RT-qPCR assay and improves our understanding the defense mechanisms of Pahang response to Foc TR4.

Section: Biotic and abiotic stresses

Genome-wide identification and functional analysis of chitinase gene family in grape

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Chitinases, the important resistance-related proteins, are crucial hydrolytic enzymes, which attack fungal pathogens by catalyzing the fungal cell wall degradation. As a large gene family, the VvChis have not been systematically analyzed and effectively investigated in grape. In this study, we identified 42 VvChis in grape by searching the conserved domains, and divided them into A, B, C, D and E groups according to phylogenetic relationships, gene structure and conserved domains analysis. Quantitative real-time PCR (qRT-PCR) and publicly available microarray data analysis revealed distinct temporal and spatial expression patterns of VvChis in various tissues of different developmental stages. The transcriptional level of most genes was high in the root of ‘Koyho’ and ‘Summer Black’. Combining cis-elements in the promoter, GO and KEGG analysis, and prediction of interaction proteins, we revealed the function of Chitinase. After the pathogen infecting the leaves and berries of grape, the expression levels of VvChis in A, B and E groups showed a significant upward trend, of which VvChi5, VvChi25, VvChi11 (leaf) and VvChi16 (fruit) were the most up-regulated. The interaction between Chi-17 and Metallothionein (MTL) was confirmed by yeast two-hybrid system and bimolecular fluorescence complementation (BiFC). This study clarified the member composition and expression pattern of VvChi family in grape, and initially explored the disease resistance function of VvChi, which laid a foundation for constructing the functional regulation network of VvChi in grapes.

Section: Comparative genomics

Comparative transcriptome profiling of the early response to chestnut gall wasp in durable resistant and susceptible Chinese chestnut genotypes

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Chinese chestnut (*Castanea mollissima* BL.) can be infested by *Dryocosmus kuriphilus* Yasumatsu that results in gall formation and great loss in production and economy. Research on investigating formation and control of gall-wasp using genomics approach is rarely reported. In this study, RNA-Seq was used to investigate the dynamic changes of genes in two cultivars of chestnut (*C. mollissima* BL) 'HongLi' which has gall susceptible trait and 'Shuhe Wuyingli' which has gall resistance trait infected with *D. Kuriphilus*. By comparative analysis, there are 25358 genes were co-expressed in all samples, 521 and 424 genes were only expressed in HL_Gall_A and SHW_Gall_A samples. The gene function annotation showed 120 DTFs (differentially expressed TFs) between the 'HL' control and the infected material, while there were 193 DTFs between the 'SHW' control and the infected material. The massive parallel transcriptome profiling in this study will serve as a valuable resource for detection of molecular networks of chestnut gall resistance in Chinese chestnut.

Section: Biotic and abiotic stress

Key factors identification in fruit ripening and rubbery texture caused by 1-MCP in papaya

Xiaoyang Zhu

South China Agricultural University

Ethylene promotes fruit ripening whereas 1-methylcyclopropene (1-MCP), a non-toxic antagonist of ethylene, delays fruit ripening via the inhibition of ethylene receptor. However, unsuitable 1-MCP treatment can cause fruit ripening disorders. In this study, we show that short-term 1-MCP treatment (400 nL•L⁻¹, 2 h) significantly delays papaya fruit ripening with normal ripening characteristics. However, long-term 1-MCP treatment (400 nL•L⁻¹, 16 h) causes a “rubbery” texture of fruit. The comparative transcriptome analysis showed that a total of 5,529 genes were differently expressed during fruit ripening compared to freshly harvested fruits. Comprehensive functional enrichment analysis showed that the metabolic pathways of carbon metabolism, plant hormone signal transduction, biosynthesis of amino acids, and starch and sucrose metabolism are involved in fruit ripening. 1-MCP treatment significantly affected fruit transcript levels. A total of 3,595 and 5,998 differently expressed genes (DEGs) were identified between short-term 1-MCP, long-term 1-MCP treatment and the control, respectively. DEGs are mostly enriched in the similar pathway involved in fruit ripening. A large number of DEGs were also identified between long-term and short-term 1-MCP treatment, with most of the DEGs being enriched in carbon metabolism, starch and sucrose metabolism, plant hormone signal transduction, and biosynthesis of amino acids. Furthermore, we found that long-term treatment with 1-MCP significantly inhibited the expression of CpEBF1, an EIN3 binding F-box-1 gene. Protein interaction analysis with yeast two-hybrid, BiFC and GST pull-down showed that CpEBF1 interacts with CpMADS1/3 and CpEIL1 proteins. The interaction of CpEBF1 with CpMADS1/3 further activated the activities CW-degradation genes promoters. Subcellular localization showed that these proteins were localized in the nucleus. Additionally, the expression levels of CpMADS1/3, CpEIL1, and several CW degradation-related genes were significantly downregulated by long-term 1-MCP treatment. Therefore, we propose that the inhibited expression of CpEBF1 and CpMADS1/3 resulted in repressing the activation of CW- degradation related genes by their interactions, thereby resulting in fruit softening disorders.

Section: Plant signalling and behaviour

Characterization of transcriptomics reveals metabolism-related pathways and EST-SSR markers related to *Colletotrichum fructicola* infection in strawberry (*Fragaria ×ananassa* Duchase)

Xiaohua Zou

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Strawberry anthracnose caused by *Colletotrichum fructicola* is an important disease in Asia that results in significant economic losses in quality and production. However, the molecular mechanism of strawberry to infection with *C. fructicola* is still poorly understood. Based on our previous research, here, we focused on the comparative transcriptomes of differentially expressed genes (DEGs) which were annotated with GO terms and KEGG pathway enrichment analysis. The most enriched categories were identified as energy metabolism, carbohydrate metabolism, and the biosynthesis of other secondary metabolites. We further examined the expression of key DEGs by quantitative PCR in two varieties with different responses to *C. fructicola* inoculation. Additionally, we detected 3019 potential cultivar specific simple sequence repeats (SSRs) from the transcriptomic data of Jiuxiang. 13 EST-SSRs were identified as differentially expressed EST-SSRs (DE-EST-SSRs) after *C. fructicola* infection. Furthermore, DE-EST-SSRs have polymorphisms in the SSRs allele in different varieties and wild species. Our data provided molecular insight into *C. fructicola* infection in strawberry which are important supplement to previous research, and could provide molecular information relating to the defense response in strawberry.

Section: Biotic and abiotic stress