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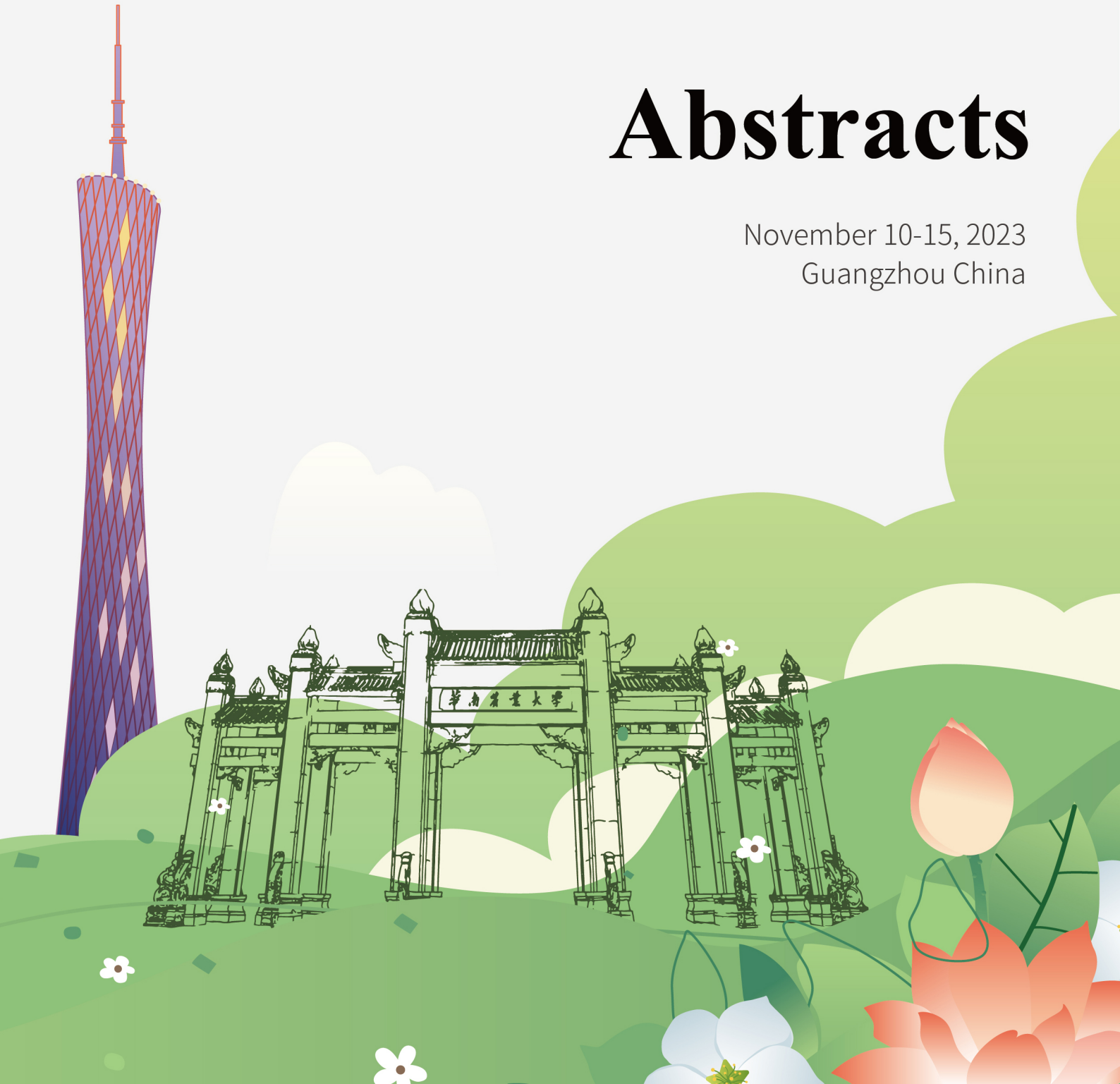

Multi-omics study of wax apple (*Syzygium samarangense*)

Fei Chen[1], Wenquan Wang[1]

[1] *College of Breeding and Multiplication (Sanya Institute of Breeding and Multiplication), Hainan University, Sanya 572025, China*

feichen@hainanu.edu.cn

Abstract

Wax apple (*Syzygium samarangense*), as an emerging tropical fruit, boasts rich nutritional value and significant market potential. This study aimed to explore the wax apple genome to gain a better understanding of the genetic characteristics of this fruit and to provide robust support for future breeding and conservation efforts. We selected the diploid (2n=22) main cultivar, Black Sugar Barbilian, for genome assembly using a combination of high-throughput sequencing technologies. We successfully assembled the genome of wax apple into 11 high-quality, chromosome-level sequences, revealing a gene count of over 40,000 protein-coding genes, including diverse gene families that are likely associated with essential wax apple features. In addition, relying on multiple transcriptomes, we identified key genes involved in wax apple development and fruit flavor. Our research provides substantial support for a deeper understanding of the biological attributes of wax apples. Study of the wax apple genome holds promise for opening up new opportunities for quality improvement, increased yield, and enhanced disease resistance, thereby contributing to sustainable development of the wax apple industry.

Comparative omics analysis reveals that a calcium signaling component and three hormones positively regulate freezing tolerance in potato via CBF- and non-CBF-regulon genes

Lin Chen[1], Tiantian Liu[2], Ye Chen[2], Botao Song[2]

[1] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou 510642, People's Republic of China*

[2] *Key Laboratory of Horticultural Plant Biology, MOE; Key Laboratory of Potato Biology and Biotechnology, MARA; College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, People's Republic of China*

chenlin_304@scau.edu.cn; songbotao@mail.hzau.edu.cn

Abstract

Freezing stress is a major limiting factor in crop production. To increase frost hardiness of crops via breeding, deciphering the genes and metabolites that confer freezing tolerance is vital. Potato cultivars (*Solanum tuberosum*) are generally freezing sensitive, but some wild potato species are freezing tolerant, including *S. commersonii*, *S. malmeanum*, and *S. acaule*. However, the underlying molecular mechanisms that confer freezing tolerance in wild species remain to be deciphered. In the present study, five representative genotypes of the above-mentioned species with distinct levels of freezing tolerance were investigated. Comparative transcriptomics analysis showed that *SaCBL1–like* (*calcineurin B-like protein*) was substantially upregulated in all the freezing-tolerant genotypes. *SaCBL1–like* was shown to confer freezing tolerance without significantly impacting main agricultural traits by increasing the expression of genes in the cold-responsive C-repeat binding factor (CBF) regulon. Further comparative transcriptomics and metabolomics analysis showed that putrescine, SA, and ABA were related to freezing tolerance. Exogenous application of all three hormones could enhance the freezing tolerance of potato. Mechanistic analysis showed that the arginine decarboxylase gene *ADC1*, associated with the putrescine pathway, conferred freezing tolerance in potato by improving the expression of cold-responsive CBF-regulon genes. SA enhanced freezing tolerance of potato by improving expression of *HSFC1*, and ABA promoted freezing tolerance of potato by improving expression of *AREB4* (*ABA-responsive element binding protein*), which induced potato freezing tolerance via trehalose-6-phosphate synthase and glutathione transferase genes. Taken together, our research unravels the calcium signaling component and hormone signaling pathways that confer freezing tolerance in potato.

The contributions of asexual and sexual selections in pineapple domestication

Li-Yu Chen*

Center for Genomics and Biotechnology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

lychen@fafu.edu.cn

Abstract

Most grain crops, vegetables and ornamentals are produced sexually through seed propagation, whereas most fruit trees, tubers and some ornamentals are clonally propagated through grafting, tissue culture, divisions or cuttings. Sexually reproducing species undergo hundreds to thousands of generations of recombination during domestication; this recurrent selection leaves highly tractable signatures in the genome. In contrast, domestication of clonally propagated crops was hypothesized as a single step operation, only one generation away from its wild progenitors. Once a given clone was picked up, the selection was completed. Hence, clonal crops may have undergone zero to a few recombination and selection cycles post domestication, in sharp contrast to sexually reproducing annual crops. Pineapple (*Ananas comosus* (L.) Merr.) is the second most important tropical fruit crop behind banana, and it is clonally propagated using the leafy fruit crown, slips or suckers due to its self-incompatibility. In my talk, I will discuss the domestication of clonally propagated crops using whole-genome resequencing data of 89 *Ananas* accessions. Our findings support co-existence of sexual recombination and one-step operation in domestication of clonally propagated crops. In addition, I will discuss the identification of candidate genes for self-incompatibility in pineapple.

CmABF1-CmHSFA4/CmMYBS3-*CmMYB121* molecular module mediated response of chrysanthemum to salt stress

Xinhui Wang, Yue Wang, Fei Li, Kaili Wang, Jiafu Jiang, Fadi Chen, Sumei Chen*

State Key Laboratory of Crop Genetics and Germplasm Enhancement / Key Laboratory of Flower Biology and Germplasm Innovation, Ministry of Agriculture and Rural Affairs / Key Laboratory of Biology of Ornamental Plants in East China, National Forestry and Grassland Administration / College of Horticulture, Nanjing Agricultural University, China / Zhongshan Biological Breeding Laboratory, China

chensm@njau.edu.cn

Abstract

Excess soil salinity hampers plant growth and development and can even cause death. Chrysanthemum (*Chrysanthemum morifolium*) is easily subjected to salinity stress. We previously found that the heat shock transcription factor *CmHSFA4* was induced by salt, and CmHSFA4 enhanced the salt tolerance of chrysanthemum. We further screened the upstream regulator of *CmHSFA4*, CmABF1, by a Y1H assay. CmABF1 directly bound to the ABRE *cis*-element of the *CmHSFA4* promoter and conferred salt tolerance to chrysanthemum. To explore the genetic relationship between CmABF1 and *CmHSFA4*, transient interference of *CmHSFA4* (CaLCuV-amiR*HSFA4*) was created in *CmABF1*-overexpression transgenic lines (*CmABF1*-OE). The salt tolerance of *CmABF1*-OE/CaLCuV-amiR*HSFA4* transgenic lines decreased, indicating that CmABF1-regulated salt tolerance is partly dependent on *CmHSFA4*. Meanwhile, we found that *CmMYB121* was downregulated in the *CmHSFA4*-overexpressing line compared with the WT by transcriptome sequencing analysis. *CmMYB121* mediated the sensitivity of chrysanthemum to salt stress. CmHSFA4 directly bound to the HSE *cis*-elements in the *CmMYB121* promoter. We further found that the transcriptional repressor CmMYBS3 interacted with CmHSFA4, and the CmHSFA4–CmMYBS3 complex could recruit CmTPL to impair the acetylation level of *CmMYB121* and inhibit its transcription. There was a temporal difference in expression between CmHSFA4 and CmMYBS3 in response to salt stress, and the extent to which CmHSFA4 inhibited *CmMYB121* depended on the amount of CmMYBS3, implying that a precise temporal and dosage-dependent CmHSFA4–CmMYBS3 module may regulate *CmMYB121* in chrysanthemum. In summary, the present study sheds new light on the molecular mechanism of salt-stress response in chrysanthemum.

Functional divergence of CYP76AKs shapes the chemodiversity of abietane-type diterpenoids in genus *Salvia*

Jiadong Hu[1,2], Shi Qiu[1], Junfeng Chen[1], Wansheng Chen[1,2]

[1] *Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

[2] *Department of Pharmacy, Second Affiliated Hospital of Navy Medical University, Shanghai 200003, China*

Chenws126@126.com

Abstract

The genus *Salvia* L. (Lamiaceae) comprises myriad distinct medicinal herbs, with terpenoids as one of their major active chemical groups. Abietane-type diterpenoids (ATDs), such as tanshinones and carnosic acids, are specific to *Salvia* and exhibit taxonomic chemical diversity among lineages. To elucidate how ATD chemical diversity evolved, we carried out large-scale metabolic and phylogenetic analyses of 71 *Salvia* species, combined with enzyme function, ancestral sequence and chemical trait reconstruction, and comparative genomics experiments. This integrated approach showed that the lineage-wide ATD diversity in *Salvia* was induced by differences in oxidation of the terpenoid skeleton at C-20, which was caused by functional divergence of the cytochrome P450 subfamily CYP76AK. These findings reveal a unique pattern of chemical diversity in plants that has been shaped by the loss of enzyme activity and associated catalytic pathways.

The molecular mechanism of *CsHMGR1* mediated mevalonate synthesis to regulate cucumber *Fusarium* wilt resistance

Jun Xu, Zhuoyao Zhao, Jingping Dong, Xiaodong Yang, Xuehao Chen*

School of Horticulture and Landscape Architecture, Yangzhou University, Yangzhou, China

xhchen@yzu.edu.cn

Abstract

Grafting has long been used as the most direct and effective strategy and agronomic measure for prevention of soil-borne and destructive *Fusarium* wilt (*FW*) during production of cucurbitaceous vegetables. Pumpkin (*Cucurbita moschata*), a popular cucurbit crop, has been used as a rootstock to enhance the *FW* resistance of cucumber. However, the potential mechanisms underlying this phenomenon remain largely unknown. Here, we report that grafted cucumber with pumpkin as the rootstock exhibited high resistance against cucumber *FW*. Interestingly, root exudates and rhizosphere microbial communities differed significantly between pumpkin and cucumber inbreeding lines (including highly susceptible and tolerant lines) after cucumber *FW* infection, as revealed by comparative analysis of untargeted metabolomics and meta-genomic sequencing. We found that the root secretions of pumpkin significantly inhibited the growth of cucumber *FW*, and among them, mevalonate was identified as a key anti-fungal metabolite. Mevalonate particularly stimulated the enrichment of rhizosphere *Actinomadura* sp., which effectively and broadly suppress *F. oxysporum* pathogens, especially cucumber *FW*. Through a combination of metabolome, transcriptome, and qRT–PCR analyses, we found that the rate-limiting enzyme of mevalonate synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (CmHMGR1), played potential and key roles in *FW* resistance, consistent with the mevalonate content and gene expression levels between pumpkin and cucumber. Overexpression of *CsHMGR1* in cucumber resulted in a significant increase in mevalonate in root secretions and led to enrichment of *Actinomadura* sp. in soils, conferring high resistance against cucumber *FW* disease. We successfully harvested transgenic cucumber fruits and seeds of non-grafted cucumber after *FW* infection. Our study indicates that *CsHMGR1*-mediated mevalonate synthesis increases cucumber *FW* resistance and could be used as an extremely valuable gene resource for cucumber breeding against *FW* infection.

SlMAPK4-SlMYB75 module-mediated salicylic acid enhances terpenoid synthesis and spider mite tolerance in tomato

Wei Deng[1], Xin Xu, Xu Fang

[1] *School of Life Sciences, Chongqing University*

Dengwei1977@cqu.edu.cn

Abstract

Pests significantly threaten crop yields, destroying nearly 13% of global crop production. Spider mites are pests that can cause extensive damage to more than 1100 plant species and over 140 plant families, including tomato plants. Tomato type VI trichomes can produce and release significant amounts of volatile monoterpenes and sesquiterpenes, some of which can either poison insects or attract natural enemies, thereby preventing infestation. Our experimental results reveal a self-defense mechanism in tomatoes that protects against spider mite infestation. The SlMAPK4–SlMYB75 module mediates SA-promoted β-caryophyllene and α-humulene terpenoid synthesis to inhibit spider mite oviposition, interfere with host selection, and attract predatory mites to improve tomato tolerance to spider mites. This study provides an essential theoretical basis for improving spider mite tolerance and the green control of spider mites.

Progress on molecular mechanisms under peach tree architecture

Bin Tan[1,2], Jun Cheng[1,2], Xiaobei Wang[1,2], Xiaodong Lian[1,2], Haipeng Zhang[1,2], Xianbo Zheng[1,2], Wei Wang[1,2], Langlang Zhang[1,2], Nan Hou[1,2], Xia Ye[1,2], Jidong Li[3], Jiancan Feng[1,2*]

[1] *College of Horticulture, Henan Agricultural University, Zhengzhou, 450046, China*

[2] *Henan Engineering and Technology Center for Peach Germplasm Innovation and Utilization, Zhengzhou, 450046, China*

[3] *College of Forestry, Henan Agricultural University, Zhengzhou, 450046, China*

jcfeng@henau.edu.cn

Abstract

Peach (*Prunus persica* L.) is one of the most important fruit trees in the world. The unique management and horticultural practices of fruit trees, such as pruning, spraying, and harvesting strategies, maximize production and orchard efficiency. A well-shaped fruit tree is critical for optimizing productivity and limiting the labor and inputs needed for orchard management. Plant height, branch number, and branch angle are the major constituents of tree architecture. For plant height, a new single nonsynonymous nucleotide mutation in *PpGID1c* (gid1c^{S191F}) was observed in the GA-insensitive dwarf mutant 'Fenhuashouxingtao'. Furthermore, three genotypes, including gid1c^{W162*}/gid1c^{W162*}, gid1c^{S191F}/gid1c^{S191F}, and gid1c^{W162*}/gid1c^{S191F}, were observed in 13 dwarf cultivars. A high-quality genome of CN14 (temperature-sensitive semi-dwarf, TSSD) was assembled and manually annotated, with 228.82 Mb mapped to eight chromosomes. An aquaporin tonoplast intrinsic protein (*PpTIP2*) was a strong candidate gene for control of TSSD. Sequence variations in the upstream regulatory region of *PpTIP2* were correlated with differences in transcriptional activity at different temperatures. Branch number is another important agronomic trait in peach trees. Transcriptome analysis and transgenic phenotypes indicated that PpTCP18 plays an important role in peach branching by increasing expression of SL biosynthesis genes, including *PpLBO1*, *PpMAX1*, and *PpMAX4*. Moreover, lncRNA5 can target and increase *PpTCP18* expression, resulting in reduced branch number in peach. Pillar trees have vertical branches, and this trait was controlled by *PpTAC1*. Two variations, a 4422-bp insertion in *PpTAC1* and an 11-bp deletion in the *PpTAC1* promoter, were identified in the pillar peach 'Zhaoshouhong' using comparative genomics analysis. Meanwhile, three genotypes were identified in 14 pillar peach germplasms. Furthermore, SL, BR, and IAA significantly affected branch angle in peach trees. These results provide a reference for the molecular mechanism of tree architecture and the improvement of germplasm by molecular design and gene editing in peach.

International standards for sampling and measurement methods in horticultural research in the context of the "big data" era

Ringo (Jinquan) Feng[1], Mike Currie[2], Alistair Mowat[3], Mindy Wang[1], Xiaohui Jia[4], Yunliu Zeng[5], Lindy Guo[1], Stella Si[6]

[1] *The New Zealand Institute for Plant and Food Research Limited, Auckland 1142, New Zealand*
[2] *The Kiwifruit Breeding Centre, 120 Mt Albert Road, Auckland 1025, New Zealand*
[3] *Eurofins Food & Water Testing New Zealand. 137 Tetley Road. Katikati 3166, New Zealand*
[4] *Institute of Pomology, Chinese Academy of Agricultural Sciences, Xingcheng, Liaoning, China*
[5] *College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan, China*
[6] *Anchor Centre for Certification, Pudong, Shanghai, China*

Ringo.Feng@plantandfood.co.nz

Digital technology facilitates collection and storage of large datasets by the international horticulture research communities. Data scientists are developing data management tools for sharing datasets from different geological locations using different genetic materials under various preharvest and postharvest management regimes over many years. This provides an excellent opportunity for variety characterization, breeding, and smart crop management. However, differences in sampling and measurement methods used in different years and by different researchers may lead to confusion and misinterpretation of the analysis of results. Medical science has benefited hugely from international standards in sampling and measurement methods: e.g., blood test results from clinical labs across the world can be interpreted the same way. Horticultural science, on the other hand, lacks international standards for sampling and measurement. This leads to difficulties in comparing and interpreting results from different labs and creates barriers to international collaborations. Here, we use a few fruit quality attributes as examples to demonstrate how measurement results vary because of differences in sampling and measurement methods. Cases of successful standardization of methods are presented, and options to standardize the methods are proposed. We suggest that international horticultural journal editors and industry certification agents could play important roles in promoting standard methods.

Key words: Firmness, Soluble solids content, Dry matter content, Mineral content, Spatial variation, Instrument setting, Certification

Two adjacent NAC transcription factors regulate fruit maturity date and flavor in peach

Ruo-Xi Zhang[1,2†], Yudi Liu[1,3†], Xian Zhang[1,3], Xiaomei Chen[1,3], Juanli Sun[1,3], Yun Zhao[1,2], Jinyun Zhang[4], Jia-Long Yao[5], Liao Liao[1,2*], Hui Zhou[4*], Yuepeng Han[1,2*]

[1] *CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, The Innovative Academy of Seed Design of Chinese Academy of Sciences, Wuhan 430074, China*

[2] *Hubei Hongshan Laboratory, Wuhan, 430070, China*

[3] *University of Chinese Academy of Sciences, 19A Yuquanlu, Beijing 100049, China*

[4] *Key Laboratory of Horticultural Crop Germplasm Innovation and Utilization (Co-construction by Ministry and Province), Key Laboratory of Horticultural Crop Genetic Improvement and Eco-Physiology of Anhui Province, Institute of Horticulture, Anhui Academy of Agricultural Sciences, Hefei 230031, China*

[5] *The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand*

† These authors contributed equally

yphan@wbgcas.cn

Abstract

Although maturity date is an essential factor affecting fresh fruit marketing and has a pleiotropic effect on fruit taste qualities, the underlying mechanisms remain largely unclear. In this study, we functionally characterized two adjacent NAC transcription factor (TF) genes, *PpNAC1* and *PpNAC5*, both of which were associated with fruit maturity date in peach. *PpNAC1* and *PpNAC5* were able to activate transcription of genes associated with cell elongation, cell-wall degradation, and ethylene biosynthesis to promote fruit enlargement and ripening. Furthermore, *PpNAC1* and *PpNAC5* had pleiotropic effects on fruit taste owing to their ability to activate the transcription of genes for sugar accumulation and organic acid degradation. Interestingly, both *PpNAC1* and *PpNAC5* orthologs were found in fruit-producing angiosperms and were arranged adjacently in all 91 tested dicots, but they were absent in fruitless gymnosperms, suggesting their important roles in fruit development. Our results provide insight into the regulatory roles of NAC TFs in maturity date and fruit taste.

SlHB8 negatively modulates cold tolerance in tomato anthers via temperature-dependent regulation of tapetal cell death

Canye Yu[1], Caiyu Wu[1], Yang Yang[1], Hongling Guan[1], Deding Su[2], Zhiqiang Xian[2], Zanlin Pan[1], Da Chen[1], Riyuan Chen[1], Zhengguo Li[2], Yanwei Hao[1]

[1] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*
[2] *Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, Chongqing, China*

yanweihao@scau.edu.cn

Abstract

Cold stress impairs the normal growth and development of plants, especially the reproductive developmental phases. Cold-induced tapetal dysfunction is the main cause of pollen sterility, as it alters timely tapetal programmed cell death (PCD). However, the molecular mechanisms underlying the response of PCD to cold stress remain poorly described. Here, we report that SlHB8, an HD-Zip III family transcription factor, plays a vital role in cold tolerance of the anther. SlHB8 was expressed in tapetal cells and upregulated under low temperature treatment. SlHB8 knockout plants produced by CRISPR/Cas9 gene editing showed higher pollen activity and higher fruit-set rate under cold conditions due to reduced temperature sensitivity of the tapetum. Overexpression of SlHB8 under the control of the 35S promoter reduced pollen activity by affecting tapetum degradation. RNA-seq analysis of tomato anthers at the tetrad stage showed that SlHB8 positively regulates the conserved PCD genetic pathway DYT1–TDF1–AMS–MYB80, as well as other genes related to tapetum and pollen wall development. DNA affinity purification sequencing (DAP-seq), electrophoretic mobility shift assays, yeast one-hybrid assays, and dual-luciferase assays revealed that SlHB8 bound directly to the promoter of *SlMYB80* and activated its expression. Protein–protein interaction results showed that SlHB8 interacted with TDF1, AMS, and MYB80. The interaction between TDF1 and SlHB8 could antagonize their activation function on the *MYB80* promoter. Under cold treatment, SlHB8 was induced in both the wild type and the *slhb8* mutant, whereas DYT1, TDF1, AMS, and MYB80 were induced only in wild-type anthers and failed to increase in *slhb8* mutant anthers, indicating the important role of SlHB8 in the response of PCD to cold stress. Collectively, these findings suggest that SlHB8 negatively regulates cold tolerance in anthers by affecting the tapetal regulatory module in a temperature-dependent manner.

WRKY31-ERF72 network MdWRKY31-MdNAC7 regulatory network: orchestrating fruit softening by modulating cell wall-modifying enzyme MdXTH2 in response to ethylene signaling

Jia-Hui Wang[1,2†], Quan Sun[1†], Chu-Kun Wang[1], Chang-Ning Ma[1], Yu-Wen Zhao[1], Wen-Yan Wang[1], Da-Gang Hu[1*]

[1] *National Research Center for Apple Engineering and Technology, Shandong Collaborative Innovation Center of Fruit & Vegetable Quality and Efficient Production, College of Horticultural Science and Engineering, Shandong Agricultural University, Tai'an, Shandong 271018, China*

[2] *College of Horticulture, Agricultural University of Hebei, Baoding, Hebei, 071000, China*

† These authors contributed equally

fap_296566@163.com

Abstract

Softening in fruits adversely impacts their edible quality and commercial value, leading to substantial economic losses during fruit ripening, long-term storage, long-distance transportation, and marketing. As the apple fruit demonstrates climacteric respiration, its firmness decreases with increasing ethylene release rate during fruit ripening and postharvest storage. However, the molecular mechanisms underlying ethylene-mediated regulation of fruit softening in apple remain poorly understood. In this study, we identified a WRKY transcription factor (TF), MdWRKY31, that is repressed by ethylene treatment. Using transgenic approaches, we found that overexpression of MdWRKY31 delays softening by negatively regulating expression of xyloglucan endotransglucosylase/hydrolases 2 (MdXTH2). Yeast one-hybrid, electrophoretic mobility shift, and dual-luciferase assays further suggested that MdWRKY31 directly binds to the *MdXTH2* promoter via a W-box element and represses its transcription. Transient overexpression of ethylene-induced MdNAC7, a NAC TF, in apple fruit promoted softening by decreasing cellulose content and increasing water-soluble pectin content in fruit. MdNAC7 interacted with MdWRKY31 to form a protein complex, and their interaction decreased the transcriptional repression of *MdXTH2* by MdWRKY31. Furthermore, MdNAC7 does not directly regulate *MdXTH2* expression, but the protein complex formed with MdWRKY31 hinders MdWRKY31 from binding to the *MdXTH2* promoter. Our findings underscore the significance of the NAC7–WRKY31 regulatory complex in ethylene-responsive signaling, connecting the ethylene signal to *XTH2* expression to promote fruit softening. This sheds light on the intricate mechanisms that govern apple fruit firmness and opens avenues for enhancing fruit quality and reducing economic losses associated with softening.

An integrated chemical and genetic approach to improve tomato flavor quality

Harry Klee, Denise Tieman, Li Xiang

University of Florida, Horticultural Sciences, PO Box 110690, Gainesville, FL 32611, USA

hjklee@ufl.edu

Abstract

Although the tomato is ubiquitous in cuisines and one of the highest-value vegetable crops produced worldwide, consumers generally hold negative views on the flavor quality of commercial varieties. Breeders have made tremendous progress in developing varieties with high yield, disease resistance, and postharvest shelf life but have largely neglected flavor because of its ill-defined genetics, expensive phenotyping, and major environmental influences. We took a systematic approach to simplifying flavor, defining the underlying chemistry of consumer preferences and the genetic control of relevant metabolic pathways. We have elaborated the genetic variations that influence the synthesis of flavor-associated chemicals in over 600 accessions of the domesticated tomato and its closest wild relatives. We have assembled a set of genetic markers that can be used to improve the flavor of commercial cultivars, satisfying the desires of both producers and consumers. Superior alleles of flavor-associated genes are being introgressed into a modern commercial variety, significantly increasing favorable volatile content and consumer liking. This analytical/genetic approach is generally applicable to other fruit crops.

Redesigning the tomato fruit shape for mechanized production

Qiang Zhu[1,2,11], Lei Deng[1,2,3,11], Jie Chen[4,11], Gustavo R. Rodríguez[5], Chuanlong Sun[1,2,6], Zeqian Chang[1,2], Tianxia Yang[1,2], Huawei Zhai[6], Hongling Jiang[1], Yasin Topcu[7], David Francis[8], Samuel Hutton[9], Liang Sun[4], Chang-Bao Li[10], Esther van der Knaap[7], Chuanyou Li[1,2,3*]

[1] *State Key Laboratory of Plant Genomics, National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[2] *CAS Center for Excellence in Biotic Interactions, University of Chinese Academy of Sciences, Beijing 100049, China*

[3] *College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China*

[4] *College of Horticulture, China Agricultural University, Beijing 100193, China*

[5] *Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR-CONICET-UNR), Rosario, Argentina*

[6] *State Key Laboratory of Crop Biology, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an 271018, China*

[7] *Institute of Plant Breeding, Department of Horticulture, University of Georgia, GA 30602, USA*

[8] *Department of Horticulture and Crop Science, The Ohio State University, OH 44691, USA*

[9] *Gulf Coast Research and Education Center, University of Florida, FL 33598, USA*

[10] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Ministry of Agriculture, Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*

[11] *These authors contributed equally: Qiang Zhu, Lei Deng, and Jie Chen*

cyli@genetics.ac.cn (C.L.), chuanyouli@sdau.edu.cn

Abstract

Crop breeding for mechanized harvesting has driven modern agriculture. In tomato, machine harvesting for industrial-processing varieties became the norm in the 1970s. However, fresh-market varieties whose fruits are suitable for mechanical harvesting are difficult to breed because of associated reductions in flavor and nutritional qualities. Here, we report the cloning and functional characterization of *fs8.1*, a previously difficult-to-identify quantitative trait locus that controls the elongated fruit shape and crush resistance of machine-harvestable processing tomatoes. *FS8.1* encodes a truncated GT-2 factor that activates the expression of cell-cycle inhibitor genes through the formation of a transcriptional module with the full-length GT-2 factor SlGT-16. The *fs8.1* mutation results in a lower inhibitory effect on the cell proliferation of the ovary wall, leading to elongated fruits with enhanced compression resistance. Our study provides a potential route for introducing a beneficial allele into fresh-market tomatoes without reducing quality, thereby facilitating mechanical harvesting.

Genomics-guided molecular design breeding in apple rootstock

Wei Li

College of Horticulture, China Agricultural University, Beijing 100193, China

liwei0522898@163.com

Abstract

Dwarfing rootstocks have transformed the production of cultivated apples. However, current dwarfing rootstocks have poor adaptability to local conditions in China. Identifying dwarfing genes is an essential prerequisite for molecular design breeding of dwarfing rootstocks. After analyzing the genomic basis of dwarfing traits, we successfully identified a 9,723-bp allele-specific LTR-RT/gypsy transposable element that is present in dwarfing accessions and co-segregates with the dwarfing trait in two segregating populations, implying that it could potentially be used in future dwarfing rootstock breeding. Moreover, the molecular regulatory mechanism of rootstock-induced dwarfing has been systematically elucidated from the perspective of mobile mRNAs, providing important gene sources for molecular design breeding of dwarfing apple rootstocks. Establishing an efficient genetic transformation and genome editing system is another important foundation for achieving molecular design breeding in apple. Using CRISPR/Cas9 technology, we showed that mutating *MdSPL6* could significantly improve shoot regeneration efficiency. Because shoot regeneration is a key step in genetic transformation, targeted mutations of *MdSPL6* may provide a useful approach to facilitate the use of transgenic and gene editing technologies in apple and other rosaceous fruit trees. The dwarfing marker and regeneration-related genes identified in this study have great potential to promote molecular design breeding of apple rootstocks.

MdBT2 regulates nitrogen-mediated cuticular wax biosynthesis via a MdMYB106-MdCER2L1 signaling pathway in apple

Han Jiang[1], Chen-Hui Qi[1,2], Huai-Na Gao[1], Xin-Xiang Xu[1,4], Jian-Ying Cui[1], Xiao-Fei Wang[1], Yan-Hui Lv[1], Wen-Sheng Gao[3], Yuan-Mao Jiang[1], Chun-Xiang You[1], Yuan-Yuan Li[1*]

[1] *National Research Center for Apple Engineering and Technology, Shandong Collaborative Innovation Center of Fruit & Vegetable Quality and Efficient Production, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an, Shandong 271018, China*

[2] *Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*

[3] *Shandong Agricultural Technology Extension Center, Jinan, Shandong 251000, China*

[4] *Yantai Academy of Agricultural Sciences, Yan'tai, Shandong 265500, China*

liyy0912@163.com

Abstract

Cuticular waxes play important roles in plant development and plant–environment interactions. Research on wax biosynthetic pathways has been reported in several plant species, and wax formation is closely related to environmental conditions. However, the regulatory mechanisms that link wax and environmental factors, especially essential mineral elements, are less studied. Here, we found that nitrogen (N) played a negative role in the regulation of wax synthesis in apple. We therefore analyzed wax content, composition, and crystals in BTB-TAZ domain protein 2 (MdBT2)-overexpressing and antisense transgenic apple seedlings and found that MdBT2 could downregulate wax biosynthesis. Furthermore, an R2R3-MYB Transcription Factor 16-like protein (MdMYB106) interacted with MdBT2, and MdBT2 mediated its ubiquitination and degradation through the 26S proteasome pathway. Finally, the HXXXD-type acyl-transferase ECERIFERUM 2-like1 (MdCER2L1) was confirmed as a downstream target gene of MdMYB106. Our findings reveal an N-mediated apple wax biosynthesis pathway and lay a foundation for further study of the environmental factors associated with wax regulatory networks in apple.

GRAS transcription factors regulate fruit development and ripening in tomato

Zhengguo Li, Yudong Liu, Baowen Huang

Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, Chongqing 401331, China

zhengguoli@cqu.edu.cn

Abstract

GRAS family proteins are plant-specific transcription factors that play critical roles in plant development, phytohormone signal transduction, and stress responses. There are 53 *GRAS* genes in tomato, some of which have been functionally identified. Overexpression of *SlGRAS7*, *SlGRAS24*, or *SlGRAS40* disrupted the homeostasis between gibberellin and auxin; a variety of agronomic traits were affected, leading to dwarf plants, lower fruit set, and smaller fruit. Downregulation of *SlGRAS2* inhibited gibberellin biosynthesis and signal transduction, thus inhibiting cell expansion and ovary growth and ultimately causing smaller fruit. Repression of *SlGRAS15* or *SlGRAS26* resulted in a decrease in gibberellin biosynthesis and affected plant architecture. In *SlGRAS38* RNAi fruit, the accumulation of lycopene was reduced, and the activities of cell wall degradation enzymes were also reduced, which prolonged fruit shelf life. Our study revealed that SlGRAS4 acts as a new regulator of fruit ripening by regulating ethylene biosynthesis. SlGRAS4 also mediated a new cold response pathway that confers chilling tolerance in tomato fruit independently of the CBF pathway. We revealed the role of the SlGRAS9 and SlZHD17 transcription factors in controlling chlorophyll and carbohydrate accumulation in tomato fruit. Knock-out or knock-down of *SlGRAS9* or *SlZHD17* resulted in markedly increased chlorophyll content, reprogrammed chloroplast biogenesis, and enhanced accumulation of starch and soluble sugars. Here, we summarize tomato GRAS transcription factors involved in the regulation of fruit development, ripening, and senescence, providing new possibilities for breeding strategies to improve the quality of tomato fruit.

Using *Petunia hybrida* to investigate the mechanism of volatile release

Pan Liao[1,2,3,4], Itay Maoz[5], Meng-Ling Shih[6], Ji Hee Lee[2], Xing-Qi Huang[2], Shaunak Ray[6], Benoît Boachon[7], John A. Morgan[6], Natalia Dudareva[2]

[1] *Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China*

[2] *Department of Biochemistry, Purdue University, West Lafayette, IN 47907-2063, USA*

[3] *State Key Laboratory of Agrobiotechnology, CUHK, Shatin, Hong Kong, China*

[4] *State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong SAR, China*

[5] *Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Volcani Center, PO Box 15159, HaMaccabim Road 68, Rishon LeZion, 7505101, Israel*

[6] *Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN 47907-2100, USA*

[7] *University of Lyon, UJM-Saint-Etienne, CNRS, BVpam Formation de Recherche en Evolution 3727, 42000 Saint-Etienne, France*

panliao@hkbu.edu.hk

Abstract

Plants release volatile organic compounds (VOCs) that serve vital functions in pollination, seed dispersal, and defense against parasites, pathogens, and herbivores. These compounds also contribute to the fragrance of fruits, vegetables, flowers, and leaves. While significant progress has been made in understanding the biosynthesis of VOCs, there is still limited knowledge regarding their release from cells into the environment. Until recently, the release of volatiles was thought to occur through passive diffusion. However, a recent study reported that an ABC transporter plays a role in facilitating volatile emission in petunia flowers, thus presenting evidence of a biologically mediated mechanism. To gain a deeper understanding of this process, we performed a study on *Petunia hybrida* flowers, known for their high levels of phenylalanine-derived volatiles. Our findings revealed that a non-specific lipid transfer protein (nsLTP), specifically PhnsLTP3, plays a role in facilitating the release of VOCs across the cell wall to the cuticle. When we downregulated *PhnsLTP3*, we observed a decrease in VOC emission, as well as a redistribution within the plant, without any impact on the overall VOC pool. In addition, our research demonstrated that the cuticle functions as a sink or concentrator for VOCs, thereby reducing intracellular accumulation and preventing feedback downregulation of the precursor supply of phenylalanine. These findings present new opportunities for the metabolic engineering of VOCs and the mitigation of unwanted plant odors.

SV-GAPS: Structural Variant discovery and Genotyping for *de novo* genome Assemblies on Population Scales

Yi Liao[1] & J.J. Emerson[2]

[1] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*

[2] *Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA*

yiliao@scau.edu.cn

Abstract

Population-scale, gap-free genome assemblies are increasingly being generated for various species, including model organisms, humans, and crop plants. These assemblies serve as valuable resources for comprehensive mapping of genomic variants, particularly structural variants, within a species at the population level. Understanding the phenotypic implications of these variants is crucial for disease diagnosis and crop enhancement. However, current tools for detecting structural variants based on genome assemblies suffer from limitations, as they have been developed and trained using a limited range of aligners and organisms without undergoing comprehensive feasibility evaluations. In this study, we introduce SV-GAPS, a dedicated pipeline designed specifically for the detection and genotyping of structural variants in population-level genome assemblies. We extensively evaluate SV-GAPS across different aligners and species, with a specific focus on plant genomes characterized by varying levels of genomic repetition. Our findings demonstrate that the choice of whole-genome aligner significantly influences the accuracy and consistency of structural variant detection, and its suitability varies depending on the species. SV-GAPS surpasses existing tools in terms of performance on both simulated and real datasets. We demonstrate that SV-GAPS effectively detects genomic variants in plant genomes that exhibit diverse levels of genomic repetition, ranging from *Arabidopsis* to maize. In addition, we emphasize that the identification of genomic variants by SV-GAPS in population-scale assemblies contributes to the evolutionary inference of repetitive genomic regions, such as centromeres.

The delicate regulation of fruit ripening and quality formation by SlBZR1s in tomato

Lihong Liu[1], Fanliang Meng[1], Yuening Li, Haoran Liu[1], Songshen Hu[1], Qiaomei Wang[1*]

[1] *State Agricultural Ministry Laboratory of Horticultural Crop Growth and Development, Department of Horticulture, Zhejiang University, Hangzhou 310058, China*

qmwang@zju.edu.cn

Abstract

Fruit ripening is of both scientific significance in plant development and economic importance in agriculture. One of the main challenges for researchers in both plant biology and agriculture is to offer a ripening product with good visual, flavor, and nutritional quality, as well as a long shelf life, indicated by fruit softening or firmness, to maintain its quality before being consumed. Ethylene, as a ripening hormone, plays a central role in tomato fruit development. However, the involvement of brassinosteroids (BRs) in the regulation of fruit ripening and their relationship with the ethylene pathway are poorly understood. We found that BRs regulate fruit ripening via an ethylene-dependent pathway in tomato. (1) BRs were actively synthesized during tomato fruit ripening, and alterations in the ripening process and fruit quality were observed in *SlCYP90B3* transgenic lines. (2) We determined that the key BR signaling component Brassinazole-resistant1 (SlBZR1) controls a complex but delicate transcriptional cascade to regulate tomato fruit ripening. SlBZR1 directly activates the ethylene biosynthetic genes *SlACO1* and *SlACO3* to contribute to the ethylene burst and ensure normal fruit ripening, and SlBZR1 directly activates the carotenoid biosynthetic gene *SlPSY1* to promote phytoene formation and carotenoid accumulation. (3) Genetic analysis indicated that SlBZR1 and BRI1-EMS-suppressor1 (SlBES1) act redundantly in fruit softening. SlBES1 promotes tomato fruit softening through transcriptional inhibition of *PMEU1*. (4) The target gene of SlBZR1, *TomLOXB*, encodes a chromoplast-localized 9-LOX, producing 9-oxylipin and regulating plastid transformation, mitochondrial dysfunction, and carotenoid accumulation during tomato fruit ripening. These results demonstrate a new role for BR in fruit ripening and establish the molecular mechanism that underlies this important regulation, revealing SlBZR1 as a master regulator of tomato fruit ripening with potential for use in tomato quality improvement and carotenoid biofortification.

A molecular module TGA2-*P5CS1* regulates proline accumulation to enhance cold tolerance in *Poncirus trifoliata*

Wei Xiao, Min Wang, Chunlong Li, Ji-Hong Liu*

National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, China

liujihong@mailh.hzau.edu.cn

Abstract

Cold stress induces the accumulation of proline, but the regulatory mechanisms remain to be elucidated. In this study, we demonstrated that the transcription factor PtrTGA2 enhanced cold tolerance in an SA-dependent manner by regulating *PtrP5CS1* to promote proline synthesis. Transcriptome data revealed that the proline synthetase gene *PtrP5CS1* responds positively to cold stress, and knockdown of *PtrP5CS1* attenuated cold resistance in trifoliate orange. PtrTGA2 was identified in a yeast one-hybrid library screen and confirmed to bind directly to TGACG *cis*-acting elements in the *PtrP5CS1* promoter. PtrTGA2 functions positively in cold tolerance through modulation of proline synthesis by regulating *PtrP5CS1* expression. Moreover, knockdown of PtrTGA2 decreased *PtrICS1* expression and SA content, whereas PtrTGA2-overexpressing lemon showed higher *PtrICS1* transcript levels and SA content. Furthermore, the SA biosynthesis gene *PtrICS1* functions as a direct PtrTGA2 target, forming a positive-feedback regulatory loop to intensify PtrTGA2 transcriptional activation activity. Here, we reveal the mechanistic framework by which an SA–PtrTGA2–*PtrP5CS1* module confers cold-stress tolerance, highlighting the crosstalk between cold and SA signaling pathways in trifoliate orange.

The fruit glossiness locus, *dull fruit* (*D*) encodes a C_2H_2 type zinc finger transcription factor CsDULL in cucumber (*Cucumis sativus* L.)

Xuling Zhai[1], Huazhong Ren[1,2†], Xingwang Liu[1,2†]

[1] *Engineering Research Center of the Ministry of Education for Horticultural Crops Breeding and Propagation, College of Horticulture, China Agricultural University, Beijing 100193, China*

[2] *Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, College of Horticulture, China Agricultural University, Beijing 100193, China*

† These authors contributed equally

xulingzhai@cau.edu.cn; renhuazhong@cau.edu.cn; liuxw01@cau.edu.cn

Abstract

Fruit glossiness is an important external fruit quality trait for freshly consumed cucumber fruit that affects its marketability. Dull fruit appearance is mainly controlled by a single gene, *D* (for *dull fruit*), which is dominant to glossy fruit (*dd*), but the molecular mechanism that controls fruit glossiness is unknown. In the present study, we conducted map-based cloning of the *D* locus in cucumber and identified a candidate gene (*Csa5G577350*) that encodes a C_2H_2-type zinc finger transcription factor, CsDULL. A 4895-bp deletion, including the complete loss of *CsDULL*, resulted in glossy fruit. *CsDULL* is highly expressed in the peel of cucumber fruit, and its expression level is positively correlated with the accumulation of cutin and wax in the peel. Through transcriptome analysis and yeast one-hybrid and dual-luciferase assays, we identified two genes potentially targeted by CsDULL for regulation of cutin and wax biosynthesis/transportation, *CsGPAT4* and *CsLTPG1*. The possibility that CsDULL controls both fruit glossiness and wart development in cucumber is discussed. The present work advances our understanding of the regulatory mechanisms of fruit epidermal traits and provides useful tools for molecular breeding to improve external fruit quality in cucumber.

Disease resistance gene *BcWRKY33A* can improve salt tolerance in Bak choy

Tongkun Liu, Huiyu Wang, Ying Li, Xilin Hou

State Key Laboratory of Crop Genetics & Germplasm Enhancement, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (East China), Ministry of Agriculture and Rural Affairs of China, Engineering Research Center of Germplasm Enhancement and Utilization of Horticultural Crops, Ministry of Education of China, Nanjing Agricultural University, Nanjing 210095, China

Tongkun Liu (liutk@njau.edu.cn)

Abstract

Salinity is a universal environmental stress that causes yield reduction in plants. *WRKY33*, which has been extensively studied in plant defense against necrotrophic pathogens, has recently been found to be important in salt-responsive pathways. However, the underlying molecular mechanisms controlling the involvement of *WRKY33* in salt tolerance have not been fully characterized. Here, we explored the function of *BcWRKY33A* in non-heading Chinese Cabbage (NHCC). Under salt stress, *BcWRKY33A* expression is significantly induced in roots. *BcWRKY33A* exhibits conservative resistance to *Botrytis cinerea* with Arabidopsis *WRKY33*, and promotes root elongation and root hair development under NaCl stress in Arabidopsis. Overexpression of *BcWRKY33A* in Bok choy promotes root elongation and root hair formation, whereas silence of *BcWRKY33A* display inhibited root elongation and abnormal root hair morphology. We further proved that *BcWRKY33A* directly binds to the root development-related genes *BcLRP1* and *BcCOW1*, and then positively regulates them expression. Elevated expression of *BcLRP1* and *BcCOW1* promotes primary root elongation and root hair formation, respectively. Therefore, our data demonstrated that *BcWRKY33A* can promote root development through directly regulating the expression of *BcLRP1* and *BcCOW1* in Bok choy. Furthermore, BcHSFA4A, a protein that interacts with BcWRKY33A, could directly bind to the HSE motif within the promoters of *BcZAT12* and *BcHSP17.6A*, which are involved in the plant response to salt stress. Finally, we found that BcWRKY33A could enhance the transcriptional activity of BcHSFA4A and affect its downstream genes (e.g., *BcZAT12* and *BcHSP17.6A*), and co-overexpression of BcWRKY33A and BcHSFA4A could promote the expression of salt-related genes, suggesting that the regulatory interaction between BcWRKY33A and BcHSFA4A improves salt tolerance in plants. Together, these findings reveal a novel pathway modulating root development, thereby improving the NaCl tolerance of Bok choy.

Mechanism of fruit initiation and diversity in Rosaceae

Lei Guo[1], Junhui Zhou[1,2], Muzi Li[1], Xi Luo[1], Stephen Mount[1], and Zhongchi Liu[1]

[1] *Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20817, USA*

[2] *Peking University Institute of Advanced Agricultural Sciences, Shandong Laboratory of Advanced Agricultural Sciences, Weifang, Shandong 261325, China*

zliu@umd.edu

Abstract

Rosaceae (the rose family) is an economically important family with species prized for high-value fruits and ornamentals. The family also exhibits diverse fruit types, including drupe (peach), pome (apple), drupetum (raspberry), achenetum (strawberry), and others. The ability of homologous floral organs to form distinct fruit types in these four species allows us to investigate the mechanisms by which different fruit types are derived. Through developmental, morphological, physiological, and transcriptomic analysis of each of the four fruit types and subsequent comparative analyses, we identified B- and E-class MADS-box genes and lignification as some of the determining or contributing factors for the evolution of different fruit types. At the same time, this work provides extensive genomic resources from which we built a comparative database called ROFT (ROsaceae Fruit Transcriptome database). ROFT (www.rosaceaefruits.com) enables researchers to query orthologous genes in apple, peach, strawberry, and raspberry, compare their gene expression patterns during different fruit developmental stages, identify tissue- and stage-specific genes, and explore and download consensus co-expression networks.

These genomic resources and foundational understanding also provide us with a framework to investigate the molecular mechanisms of fruit initiation in wild diploid strawberry (*Fragaria vesca*). On the basis of RNA-seq data, we selected *AGL62*, a type I MADS-box transcription factor gene, for further functional studies. Through CRISPR gene editing, we found that *AGL62* plays a critical role in initiating seed and fruit development by inducing auxin biosynthesis in the seed. Our work lays the foundation for future engineering of different fruit types, including parthenocarpic fruits, and promotes both basic understanding and application toward increasing fruit quality and yield.

Project “Magic Ploidy”, links cell cycle to tomato quality traits

Fangfang Ma, Zhilong Bao

State Key Laboratory of Crop Biology, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai’An 271018, Shandong, China

fma@sdau.edu.cn; zbo@sdau.edu.cn

Abstract

Plant morphogenesis is spatially and temporally controlled by cell proliferation and differentiation, which affect plant growth and development as well as quality traits of horticultural crops. The cell cycle is the process from the first cell division that generates new cells to the end of the second division, and it plays key roles in maintaining cell size and cell number. Accurate cell-cycle progression, including mitotic cycling and endocycling, results in proper cell division and expansion during plant organ growth and development. The canonical cell cycle consists of four major phases, Gap 1 (G1), synthesis (S), Gap 2 (G2), and mitosis (M). Endocycling is an alternative cell cycle in which genomic replication occurs in the nucleus without cell division, leading to an increase in genomic ploidy, and it is closely related to cell size and organ growth in many plant species. Endocycling occurs mainly in tissues enriched with metabolically active cells, such as the tomato pericarp, implying that the increased ploidy may promote global gene expression and macromolecule production to meet high energy demands and, more importantly, to establish desirable fruit-quality traits. Project “Magic Ploidy”, launched by the joint Lab of Bao and Ma at Shandong Agricultural University, aims to pinpoint core cell-cycle regulators that can bi-directionally change the extent of endocycling in plant cells, which in turn can MAGICALLY regulate tomato quality traits, such as fruit size, flavor, aroma, and color. Via multi-level analyses of tomato mutants with various levels of endocycling, we have established the biochemical roles of the cell cycle, a fundamental cellular event, in the formation of tomato quality traits.

Effects of heat stress on Chinese cabbage at cellular and 3D genome levels

Xiaoxue Sun[1], Wei Ma[1], Jianjun Zhao[1], Qihang Yang[1], Xiaomeng Zhang[1], Hao Liang[1]

[1] *State Key Laboratory of North China Crop Improvement and Regulation, Key Laboratory of Vegetable Germplasm Innovation and Utilization of Hebei, Collaborative Innovation Center of Vegetable Industry in Hebei, College of Horticulture, Hebei Agricultural University, Baoding 071000, China*

xiaoxue_sun@163.com; mawei0720@163.com

Abstract

Chinese cabbage is a semi-cold-tolerant vegetable, and its growth is seriously affected by heat stress. Here, we constructed a single-cell transcriptional map of Chinese cabbage (*B. rapa* L. ssp. *pekinensis* cv. A03) by single-cell sequencing technology and explored the potential heterogeneity of cell types in response to heat stress. In addition, the complex and dynamic 3D structure of chromatin within the cell nucleus poses a challenge to understanding the regulation of gene expression, while also providing potential avenues for analyzing the epigenetic regulation of gene expression. At present, it is believed that plants adapt to environmental fluctuations by modifying chromatin conformation. However, how stress-induced chromatin remodeling dynamically changes remains unclear. Here, we conducted Hi-C, WGBS, and RNA-seq analyses on Chinese cabbage A03 to report the dynamic changes in chromatin during heat stress (HS) processes. Hi-C results revealed rapid chromatin compaction induced by HS, with trends of aggregation in centromeric and telomeric regions. We simulated the distribution of chromatin within the nucleus and inferred a chromatin distribution model for Chinese cabbage seedling leaves. The results revealed a “Rabl” distribution pattern of chromatin in Chinese cabbage. Heat stress led to various levels of chromosome structural changes, including transitions between A/B compartments, alterations in the number and length of TAD-like domains, changes in the quantity of loops, and modifications in methylation levels. These changes in chromatin structure may be associated with the maintenance of chromatin stability under stress conditions. Furthermore, we observed that CHH methylation changes played a crucial role in regulating chromatin morphology. We propose that CHH methylation, by modulating interactions within centromeric regions, plays a pivotal role in controlling chromatin dynamics under heat stress.

Identification of tissue-specific hormonal regulation of root hair formation and response to wounding stress

Xuemin Ma[1], Wang Tao[2], Wenna Zhang[2], Peter Marhavy[1]

[1] *Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden*

[2] *Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, China Agricultural University, Beijing 100193, China*

xuemin.ma@slu.se

Abstract

As sessile organisms, plants are constantly exposed to cellular damage caused by mechanical stresses, sensing soil compaction or invading microbes. Wounding facilitates pathogen infection, but plants have developed a defense mechanism. Our previous studies revealed that the interplay of jasmonate and ethylene plays a vital role in the early response of Arabidopsis roots to local wounding in a tissue-specific manner. Root hairs can be induced shortly after wounding or pathogen infection under the regulation of jasmonate and ethylene. Our preliminary data show that chemical inhibition of jasmonate and ethylene in tomato can compromise wounding- or pathogen-induced root hair formation. Extensive reprogramming of global gene expression, regulated by transcription factors (TFs), occurs in both plant developmental and defense mechanisms. To identify the important TFs that regulate wounding stress and pathogen response in a cell-type-specific manner and regulate root hair induction upon stress, we cross-referenced published datasets of differentially expressed genes upon pathogen infection and cell-specific translatome data from tomato roots. As a result, we identified several genes, including well-known pathogen-response-regulated genes such as *ERF1* in the cortex and *WRKY72* in the exodermis. We also identified *ACO1* and *ACO4,* two ethylene biosynthesis genes expressed in the exodermis, that may function in the ethylene response towards wounding and pathogen-induced root hair formation. Our data from the abiotic stress study showed that the NAC transcription factor SlNAC3 positively regulates *ACO1, ACO4,* and *ERF1*, and may therefore play an essential role in regulating hormone response upon local wounding and root hair induction by the pathogen. We aim to unravel the tissue-specific regulation of root hair formation and response to wounding stress.

TIFF-seq: a universal *Tnt1*-retrotransposon Insertion Flanking Fragment enrichment sequencing strategy for facilitating innovation and utilization of Cucumis germplasm resources

Jian Pan[1,2*], Haifan Wen[2], Yiqun Weng[3], Hongyan Qi[1], Huanle He[2], Run Cai[2], Junsong Pan[2*]

[1] *College of Horticulture, Shenyang Agricultural University, Shenyang, China*

[2] *School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China*

[3] *Horticulture Department, University of Wisconsin, Madison, WI 53706, USA*

panjian@syau.edu.cn

Abstract

Innovating germplasm resources is vital for advancing crop genetic breeding. With the growing demand for genetic variation, induced mutagenesis has been widely employed in plants. *Tnt1* is one of the few well-characterized long terminal repeat retrotransposons in plants that is widely used in the construction of mutant libraries. In earlier studies, we successfully demonstrated that *Tnt1* is very active in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) via transgenic *Tnt1* plants. Specifically, we produced 667 mutant lines in cucumber through tissue culture regeneration. To identify *Tnt1* insertion sites in a high-throughput and cost-effective manner, we devised the TIFF-seq identification strategy—a universal *Tnt1*-retrotransposon Insertion Flanking Fragment enrichment sequencing strategy for deciphering the genome mutations of *Tnt1* mutants. This strategy initially employs GenoBaits technology, which is much more cost-effective than whole-genome sequencing (WGS), to design *Tnt1* sequence-specific capture probes, facilitating the specific capture of *Tnt1* insertion-flanking genomic fragments and obtaining flanking sequences through NGS sequencing. Furthermore, we developed an analysis tool, TNTdetector, to process the NGS data, enabling identification and annotation of insertion-site genomic information and molecular marker design for each site. Through the TIFF-seq workflow, we identified 22,384 insertion sites in cucumber mutant lines, with 37% inserted in gene exons, 10% in introns, 8% in promoter regions, and 45% in intergenic regions, resulting in 8,873 potential insertionally mutated genes. We developed the *Tnt1* insertions genotyping function of TNTdetector to identify homozygous insertions from WGS data. Utilizing this function, we rapidly mapped a glabrous candidate gene from NGS data of a mutant segregation population (n=24) pool. In conclusion, the TIFF-seq workflow consolidates various analysis processes, enhancing the application potential of *Tnt1* transposons for germplasm resource innovation.

Deciphering the mycorrhizal symbiosis code and identifying beneficial microbes in *Citrus*

Zhiyong Pan

Huazhong Agricultural University, National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops

zypan@mail.hzau.edu.cn

Abstract

Citrus plants largely depend on the arbuscular mycorrhizal symbiosis (AMS) with arbuscular mycorrhizal fungi (AMF) to obtain essential nutrients from the soil, but the molecular mechanisms of AMS remain unclear. We revealed that host plants can secrete LysM extracellular (LysMe) proteins into the periarbuscular space between the periarbuscular membrane and the fungal cell wall of the branched arbuscule, thus facilitating symbiosis establishment, possibly through suppression of the chitin-induced plant immune response. The host plants then provide glucose (by the SWEET1b transporter) across the periarbuscular membrane to maintain AMS for a healthy, mutually beneficial symbiosis. In addition to AMF, there are many other beneficial microbes in citrus. We collected rhizosphere soils from five citrus-producing areas of China and created a “citrus rhizosphere microbial biobank” by isolating bacteria from the soils using a limited dilution culture method. The biobank contains 3142 isolates, which were taxonomically categorized into 435 bacterial taxa, with 117 genera and 5 phyla. Based on “rhizosphere microbiome–fruit metabolome” association analysis, we found significant correlations between the abundance of rhizosphere microbial OTUs (operational taxonomic units) and the concentrations of sugars (TSS, Suc, Fru, Glu) and/or organic acids (MA, CA) in citrus. A total of 304 marker OTUs in the citrus rhizosphere were obtained using an established predictive model. To validate the roles of marker OTUs in fruit quality formation, 16 representative strains were selected for a pot-culture experiment, 9 of which played positive roles in sugar (Suc, SS and Fru) accumulation in citrus fruits. Our results suggest that citrus roots may recruit not only AMF for nutrient uptake but also rhizosphere microbes with beneficial effects on fruit quality formation.

The roles of volatiles glucosylation in cold and drought stress in tea plants

Mingyue Zhao[1], Jieyang Jin [1], Wilfried Schwab[2], Chuankui Song[1*]

[1] *State Key Laboratory of Tea Plant Biology and Utilization, International Joint Laboratory on Tea Chemistry and Health Effects, Anhui Agricultural University, Hefei 230036, Anhui, P. R. China*
[2] *Biotechnology of Natural Products, Technische Universität München, Liesel-Beckmann-Str. 1, 85354 Freising, Germany*

sckfriend@163.com

Abstract

Plants have evolved sophisticated defense mechanisms to overcome their sessile nature. However, whether and how volatiles from cold-stressed plants can trigger interplant communication is still unknown. Here, we provide the first evidence for interplant communication via inducible volatiles during cold stress. The VOCs, including nerolidol, emitted from cold-stressed tea plants play key role(s) in priming the cold tolerance of their neighbors via a CBF-dependent pathway. We discovered the first plant UGT (UGT91Q2) in the tea plant whose expression is strongly induced by cold stress and that specifically catalyzes the glucosylation of nerolidol. The accumulation of nerolidol glucoside was consistent with the expression level of *UGT91Q2* in response to cold stress, as well as in different tea cultivars. The reactive oxygen species (ROS) scavenging capacity of nerolidol glucoside was significantly higher than that of free nerolidol. Downregulation of *UGT91Q2* resulted in reduced nerolidol glucoside accumulation, ROS scavenging capacity, and tea plant cold tolerance. Exposure to airborne eugenol triggered a marked increase in *UGT71A59* expression, eugenol glucoside accumulation, and cold tolerance by modulating ROS accumulation and *CBF1* expression. It also promoted drought tolerance by altering ABA homeostasis and stomatal closure. CBF1 and CBF3 play positive role(s) in eugenol-induced cold tolerance, and CBF2 may be a negative regulator of eugenol-induced cold tolerance in tea plants. Results of (Z)-3-hexenol exposure and gene silencing support the hypothesis that (Z)-3-hexenol plays a role in the integration of cold and drought tolerance by stimulating the dual-function glucosyltransferase UGT85A53, thereby altering ABA homeostasis in tea plants. Overall, we present a model for studying the roles of metabolites in plants under multiple stresses and reveal the roles of volatiles in integrating cold and drought stresses in plants.

High-quality ice plant genome analysis provides insights into genome evolution and exploration of important functional genes

Xiaoming Song[1], Shaoqin Shen[1], Nan Li[1], Yujie Wang[1]

[1] *College of Life Sciences, North China University of Science and Technology, Tangshan, Hebei 063210, China*

songxm@ncst.edu.cn

Abstract

Ice plant (*Mesembryanthemum crystallinum*), a member of the Aizoaceae family, is a typical halophyte crop and a model plant for studying the mechanism of transition from C3 photosynthesis to crassulacean acid metabolism (CAM). Here, we report a high-quality chromosome-level ice plant genome sequence. This 98.05% genome sequence is anchored to nine chromosomes, with a total length of 377.97 Mb and an N50 scaffold length of 40.45 Mb. Almost half of the genome (48.04%) is composed of repetitive sequences, and 24,234 genes have been annotated. Subsequent to the ancient whole-genome triplication (WGT) that occurred in eudicots, there has been no recent whole-genome duplication or WGT in ice plants. However, we detected a novel WGT event that occurred in the same order in *Simmondsia chinensis*, which was previously overlooked. Our findings reveal that ice plants have undergone chromosome rearrangements and gene removal during evolution. Through a combination of transcriptomic and comparative genomic data and expression verification, we identified several key genes involved in the CAM pathway and constructed a comprehensive network. As the first genome of the Aizoaceae family to be released, this report will provide a rich data resource for comparative and functional genomic studies of Aizoaceae, especially for studies on salt tolerance and C3-to-CAM transitions to improve crop yield and resistance.

Molecular regulatory network of lily response to heat stress

Ze Wu[1,2], Ting Li[1,2], Liping Ding[1,2], Yinyi Zhang[1,2], Nianjun Teng[1,2]

[1] *Key Laboratory of Landscaping, Ministry of Agriculture and Rural Affairs, Key Laboratory of Biology of Ornamental Plants in East China, National Forestry and Grassland Administration, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China*

[2] *Jiangsu Graduate Workstation of Nanjing Agricultural University and Nanjing Oriole Island Modern Agricultural Development Co., Ltd., Nanjing 210043, China*

wuze@njau.edu.cn; njteng@njau.edu.cn

Abstract

Lily is an important flower with poor thermotolerance, and high summer temperatures seriously affect its growth. Research on the mechanism of heat stress response (HSR) in lily will provide a theoretical basis for developing new cultivars with high thermotolerance. In the past several years, we have identified two lily HSFA3s (LlHSFA3A and LlHSFA3B) that are highly expressed in thermotolerant cultivars and participate in thermotolerance by regulating proline catabolism. Among them, *LlHSFA3B* produces the truncated splice variant LlHSFA3B-II through alternative splicing, which interacts with LlHSFA3A to inhibit its function and fine-tune thermotolerance. Further research has shown that there is a conserved DREB2–HSFA3 regulatory module in lily. By screening the upstream factors of LlHSFA3A, we identified a membrane-associated NAC factor, LlNAC014, which increased thermotolerance by sensing high temperature and translocating to nucleus to activate the DREB2–HSFA3 module. In addition, we also identified a direct regulator of *LlDREB2B*, LlWRKY22, which activates the expression of *LlDREB2B* to participate in HSR. By analyzing differentially expressed genes in response to high temperature, we identified a heat-inducible HD-Zip I transcription factor, LlHB16, from lily, which directly activates expression of *LlHSFA2* and *LlMBF1c* to positively regulate thermotolerance. Another HD-Zip I protein, LlHOX6, can interact with LlHB16 to antagonistically repress *LlHSFA2* and *LlMBF1c*, thereby negatively influencing thermotolerance. At the same time, we found that LlWRKY39 interacts with calmodulin LlCaM3 in a Ca^{2+}-dependent manner, fine-tuning the expression of *LlMBF1c* and participating in the regulation of HSR in lily. We also identified the LlMYB305–LlC3H18–LlWRKY33 high-temperature response regulatory module in lily. Simultaneously, we discovered that the ERF factor LlERF110 negatively affects thermotolerance by interfering with ROS homeostasis and suppressing expression of *HSFA2*. In summary, we have established an HSR network centered on HSFs and identified multiple transcription factors involved in thermotolerance of lily.

Research progress on biological characteristics of *Ginkgo biloba*: pollination and longevity mechanisms

Zhaogeng Lu[1], Jinkai Lu[1], Qingjie Wang[1], Li Wang[1*]

[1] *College of Horticulture and Landscape, Yangzhou University, Yangzhou 225009, China*

Li Wang: liwang@yzu.edu.cn

Abstract

Ginkgo biloba, the only living ginkgophyte among extant gymnosperm lineages, is known as a living fossil plant, and its pollination process retains many primitive characteristics. In addition, ginkgo leaves are rich in flavonoids, and *G. biloba* extracts (GBE) are important raw materials for treating cardiovascular and cerebrovascular diseases. Another significant feature of ginkgo is its long lifespan. There are numerous ancient ginkgo trees distributed worldwide. We therefore conducted research centered on these characteristics of ginkgo and achieved the following results. (1) We identified a total of 101 metabolites in ginkgo pollination drops. These metabolites are indispensable for pollen germination and growth; in particular, organic acids and fatty acids play defensive roles against microbial activity. In addition, we constructed a small-RNA library and found that the interactions of several known miRNAs and their targets in PDs are involved in carbohydrate metabolism, hormone signaling, and defense response pathways. These results provide evidence of extracellular miRNAs that likely function in the PDs of gymnosperms. (2) Only 1-to-5-year-old ginkgo leaves are used for the extraction of medicinal compounds. Nevertheless, the relationship between age and flavonoids in ginkgo leaves is unclear. We found that flavonoid content decreased with age: 82% of the flavonoids exhibited a decreasing trend with increasing age. However, trunk truncation can result in tree rejuvenation; it induced a marked increase in flavonoid accumulation and upregulated expression of genes involved in flavonoid biosynthesis. We used 1-to-1200-year-old ginkgo trees to identify the gibberellin-responsive gene *GbDAL1* as a critical age regulator in controlling flavonoid synthesis. GbDAL1 can inhibit flavonoid synthesis by directly suppressing the expression of *GbFLS* and *GbMYB28*, resulting in reduced stress resistance, suggesting that *GbDAL1* functions as a crucial negative regulator of both flavonoid synthesis and stress resistance.

Mining of heat tolerance genes in grapes and their regulatory mechanisms

Xinna Liu[1,2,3], Haiyang Chen[1,2,3], Lijun Wang[1,2*]

[1] *Beijing Key Laboratory of Grape Science and Enology and State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*

[2] *China National Botanical Garden, Beijing 100093, China*

[3] *University of Chinese Academy of Sciences, Beijing 100049, China*

ljwang@ibcas.ac.cn

Abstract

Heat stress limits the growth and development of crops, including grapevine, which is a popular fruit worldwide. Genetic variability in crop heat tolerance is not well understood. We identified and characterized the heat-stress transcription factor HSFA2 in heat-sensitive *Vitis vinifera* 'Jingxiu' (VvHSFA2) and heat-tolerant *Vitis davidii* 'Tangwei' (VdHSFA2). The transcriptional activation activities of VdHSFA2 were higher than those of VvHSFA2, and a single amino-acid change (Thr315Ile) in the AHA1 motif led to the differences in transcriptional activity between VdHSFA2 and VvHSFA2. Using 41 *Vitis* germplasms, we found that the coding region of HSFA2 differed among heat-sensitive *Vitis vinifera* and heat-tolerant *Vitis davidii* and *Vitis quinquangularis*. Genetic evidence demonstrated that VdHSFA2 and VvHSFA2 are positive regulators of grape heat tolerance, and the former can confer higher heat tolerance than the latter. Moreover, VdHSFA2 can regulate more target genes than VvHSFA2. MBF1c is a target gene of both VdHSFA2 and VvHSFA2; its overexpression enhanced grape heat tolerance, whereas its dysfunction resulted in a heat-sensitive phenotype. Together, our results revealed that VdHSFA2 confers higher heat tolerance than VvHSFA2 and that MBF1c acts as their target gene to induce heat tolerance. VdHSFA2 may be adopted for molecular breeding to improve grape heat tolerance.

SlNAC3 suppresses cold tolerance in tomatoes by enhancing ethylene biosynthesis

Tao Wang[1], Xuemin Ma[2], Cuicui Wang[1], Ying Chen[1], Zhenxiao Xia[1], Zixi Liu[1], Lihong Gao[1], Wenna Zhang[1]

[1] *Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, China Agricultural University, Beijing 100193, China*

[2] *Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden*

zhangwenna@cau.edu.cn

Abstract

Tomato (*Solanum lycopersicum* L.) is one of the main vegetable crops under global facility cultivation and is sensitive to low temperatures (4–10℃). Low-temperature stress can seriously harm the quality and yield of horticultural crops such as tomato. Therefore, improving the low-temperature tolerance of tomato has always been a research hotspot. After plants are subjected to low-temperature stress, they activate the expression of cold-response transcription factors and functional genes, causing a series of physiological and biochemical reactions in the plant. NAC transcription factors are a family of transcription factors unique to plants and have been widely studied in processes such as plant growth and development. However, the molecular mechanisms by which they regulate plant response to low temperature stress have rarely been reported. Through transcriptome and RT–qPCR analysis, we found that *SlNAC3* is the main transcription factor involved in the early low-temperature response of tomato. Through VIGS silencing, CRISPR/Cas9 knockout, and overexpression of *SlNAC3* in transgenic tomato lines, we clarified the function of *SlNAC3* in response to low-temperature stress in tomato. However, our data indicate a negative correlation between the expression level of *CBFs* in *SlNAC3* transgenic lines and cold resistance, indicating that SlNAC3 regulates tomato cold resistance through a CBF-independent pathway. Transcriptome and RT–qPCR analysis, combined with experimental validation, revealed that *SlNAC3* enhances the synthesis of endogenous ethylene in tomato plants by controlling the transcription of ethylene biosynthesis genes, thereby regulating plant cold resistance. Finally, exogenous application of ethylene synthesis and inhibitors, as well as experimental verification of VIGS silencing, clarified that *SlNAC3* participates in the molecular physiological mechanism of ethylene signal response to early low-temperature stress by activating the expression of ethylene biosynthesis genes, providing a new theoretical basis for the study of low-temperature molecular resistance mechanisms in tomato.

An automatic fruit labeling platform and its applications

Wenli Zhang

Beijing University of Technology

The current deep learning-based fruit detection technology has practical application problems such as high cost of manual labeling, poor detection accuracy for dense small targets and obscured targets, and slow detection speed that cannot be deployed in edge devices. In response to the above problems, our research team proposes a highly integrated intelligent orchard service platform. The platform mainly includes the following four modules: (1) the automatic labeling module for multi-category and multi-scene fruit datasets; (2) the fruit detection module for cloud servers and data terminals; (3) the fruit detection module for edge devices and (4) the application task module. Among them, the automatic labeling module automatically generates labeling information for different target domain fruit datasets of multiple categories and scenarios based on a set of labeled source domain datasets. It gets rid of the time-consuming and labor-intensive problem of manual labeling. The fruit detection module for cloud servers and data terminals improves the ability to detect dense small target fruits by improving feature representation and prediction branching. The fruit detection module for edge devices reduces the computational effort in the model by designing a new backbone network. Real-time fruit detection in edge devices is possible. The intelligent orchard service platform proposed by our research team has good performance in different scenarios. The application task module allows for practical needs such as fruit positioning, fruit picking, and yield estimation using the above technologies. It is significant for building an intelligent orchard production system and promoting a higher level of intelligent orchard construction.

Trichoderma asperellum M7 perceives cedrene produced by banana Fusarium wilt Tropical Race 4 for hyperparasitism

Yufeng Chen, Miaomiao Cao, Chen Yufeng, Junting Feng, Wei Yongzan, Jianghui Xie, Wang Wei

Abstract

Banana is an important fruit and grain crop. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) seriously threatens the global banana industry. Chemical methods have difficulty in controlling disease spread, and resistant commercial cultivars are also lacking. Biological control using antagonistic microbes is considered to be a promising strategy. Here, *Trichoderma asperellum* M7 was isolated and identified from the rhizosphere soil of a banana plantation where no disease symptoms of Foc TR4 had been detected for 15 years. The strain exhibited strong antifungal activity, including against Foc TR4. However, the antifungal mechanism of *T. asperellum* M7 is still unknown. We found that *T. asperellum* M7 could directly perceive cedrene produced by Foc TR4, and cedrene treatment induced the production of antifungal 6-pentyl-α-pyrone by *T. asperellum* M7. Genetics analysis revealed that the *Ta363* gene located on the cellular membrane is key for the formation of hyperparasitism. Our results provide a better understanding of the antifungal activity of *T. asperellum* M7 against Foc TR4.

BRAD V3.0: an upgraded Brassicaceae database

Haixu Chen[1], Tianpeng Wang[1], Xiaoning He[1], Xu Cai[1], Runmao Lin[1], Jianli Liang[1], Jian Wu[1], Graham King[2], Xiaowu Wang[1*]

[1] *Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, No. 12, Haidian District, Beijing 100081, China*

[2] *Southern Cross Plant Science, Southern Cross University, Lismore, New South Wales, Australia*

wangxiaowu@caas.cn

Abstract

The Brassicaceae Database (BRAD version 3.0, BRAD V3.0; http://brassicadb.cn) has evolved from the former *Brassica* Database (BRAD V2.0) and represents an important community portal hosting genome information for multiple *Brassica* and related Brassicaceae species. Since the last update in 2015, the complex genomes of numerous Brassicaceae species have been decoded, accompanied by many omics datasets. To provide an up-to-date service, we have performed a major upgrade of the portal. The Model-View-ViewModel (MVVM) framework of BRAD has been re-engineered to enable easy and sustainable maintenance of the database. The collection of genomes has been increased to 25 species, and the user interface has been optimized. Features of the previous version have been retained, and new tools have been added for exploring syntenic genes, gene expression, and variation data. In the 'Syntenic Gene @ Subgenome' module, we have added features for viewing sequence alignments and phylogenetic relationships of syntenic genes. New modules include 'MicroSynteny' for viewing the synteny of selected fragment pairs and 'Polymorph' for retrieval of variation data. The updated BRAD provides substantially expanded genomic data and a comprehensively improved service to the Brassicaceae research community.

Regulation mechanism of lignin and cellulose in stone cell of pear fruit

Shaozhuo Xu[1], Yongsong Xue[1], Cheng Xue[2], Jun Wu[1*]

[1] *College of Horticulture, State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Nanjing Agricultural University, Nanjing, Jiangsu, China*
[2] *College of Horticultural Science and Engineering, Shandong Agricultural University, Taian, Shandong, China*

wujun@njau.edu.cn

Abstract

Lignified stone cell content is a key factor used to evaluate fruit quality, influencing the economic value of pear fruits. The formation of stone cells is accompanied by deposition of lignin and cellulose in the secondary cell wall. However, our understanding of the regulatory networks of stone cell formation is limited owing to the complex secondary metabolic pathways involved. In this study, we identified a hub transcription factor, PbrMYB24 (*Plant Physiology*, 2023), which acted as a positive regulator of stone cell formation, directly activating the expression of lignin and cellulose synthesis genes by combining with different *cis*-acting elements (AC-I, AC-II, and MBS). PbrMYB24 also formed a feedback hierarchical regulatory network with PbrNSC (*Genome Biol*, 2021) and PbrMYB169 (*J Exp Bot*, 2019) to synergistically regulate lignin and cellulose synthesis in stone cells of pear. In addition, we demonstrated that exogenous application of NAA reduced stone cell content and promoted the expression of the auxin response factor *PbrARF13* (*Plant Biotechnol J*, 2023). PbrARF13 was subsequently shown to inhibit *PbrNSC* expression by directly binding to its promoter, thereby reducing stone cell content. Furthermore, PbrNSC was identified as a positive regulator of PbrMYB132 through analysis of a co-expression network of genes related to stone cell formation. PbrMYB132 activated the expression of genes encoding cellulose synthase (PbrCESA4b/7a/8a) and lignin laccase (PbrLAC5) by binding to their promotors. In conclusion, our study shows that a PbrMYB24–PbrNSC–PbrMYB169 and PbrARF13–PbrNSC–PbrMYB132 regulatory cascade mediates the biosynthesis of lignin and cellulose in stone cells of pear fruit, providing new insights into the mechanism of stone cell and plant secondary cell wall formation.

Acetylation of inorganic pyrophosphatase by S-RNase signaling induces pollen-tube tip swelling by repressing pectin methylesterase

Chao Tang, Peng Wang, Shaoling Zhang, Juyou Wu

State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Sanya Institute of Nanjing Agricultural University, Nanjing Agricultural University, Nanjing 210095, China

juyouwu@njau.edu.cn

Abstract

Self-incompatibility (SI) is a widespread genetically determined system in flowering plants that prevents self-fertilization to promote gene flow and limit inbreeding. S-RNase–based SI is characterized by the arrest of pollen tube growth through the pistil. Arrested pollen tubes show disrupted polarized growth and swollen tips, but the underlying molecular mechanism is largely unknown. Here, we demonstrate that the swelling at the tips of incompatible pollen tubes in pear (*Pyrus bretschneideri* [Pbr]) is mediated by the SI-induced acetylation of the soluble inorganic pyrophosphatase (PPA) PbrPPA5. Acetylation at Lys-42 of PbrPPA5 by the acetyltransferase GCN5-related N-acetyltransferase 1 (GNAT1) drives accumulation of PbrPPA5 in the nucleus, where it binds to the transcription factor PbrbZIP77, forming a transcriptional repression complex that inhibits expression of the pectin methylesterase gene *PbrPME44*. The function of PbrPPA5 as a transcriptional repressor does not require its PPA activity. Downregulation of *PbrPME44* resulted in increased levels of methyl-esterified pectins in growing pollen tubes, leading to swelling at their tips. These observations suggest a mechanism for PbrPPA5-driven swelling at the tips of pollen tubes during the SI response. The targets of PbrPPA5 include genes encoding cell wall–modifying enzymes, which are essential for building a continuous sustainable mechanical structure for pollen tube growth. In future studies, we aim to further investigate how the acetyltransferase PbrGNAT1 responds to SI signaling and how the soluble inorganic pyrophosphatase PbrPPA5 accumulates in the nucleus.

Trichomes: the overlooked anti-insect trait in tomato

Shuang Wu[1,2], Minliang Wu[1], Jiang Chang[1], Chao Sun[2], Bin Hua[1]

[1] *College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

[2] *Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

wus@fafu.edu.cn

Abstract

The formation of surface hairs is a common feature of animals, insects, and plants. The hairs in plants are commonly referred to as trichomes, and they exhibit diverse types of different morphologies to protect plants against various stresses. In particular, many trichomes form a glandular head in which herbivore-repelling compounds and metabolites are produced. Using tomato trichomes as a model system, we studied the regulation of organogenesis of multi-cellular trichomes. We identified the developmental switch for trichome formation and provided mechanistic insights into the progressive fate specification of different types of multicellular trichomes. We also elucidated the mechanism of gland formation, as well as the principle of morphological construction of multicellular trichomes. By manipulating trichome formation and metabolism in glandular cells, we have shown that trichomes are an important biological trait that enhances insect resistance in tomato. Our work is therefore important both for genetic engineering of crops with enhanced stress resistance and also for biosynthetic significance.

CsMYB36 is required for a novel lignin-based extracellular barrier formation in glandular trichomes of cucumber for bloom deposition

Ning Hao[1,2], Hongxin Yao[1], Michio Suzuki[2], Baohai Li[3], Chunhua Wang[1], Toru Fujiwara[2], Tao Wu[1*], Takehiro Kamiya[2*]

[1] *College of Horticulture/Yuelu Mountain Laboratory of Hunan Province, Hunan Agricultural University, Changsha 410128, China*

[2] *Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan*

[3] *MOE Key Laboratory of Environment Remediation and Ecological Health, College of Environmental and Resource Science, Zhejiang University, Hangzhou 310058, China*

wutao@hunau.edu.cn

Abstract

Successful biochemical reactions in organisms require the compartmentalization of essential components. Glandular trichomes (GTs) synthesize, store, and secrete diverse specialized metabolites that protect plants against biotic and abiotic stresses and have economic importance for human use. However, the mechanisms underlying compartmentalization remain unclear. Here, we identified a novel structure that is indispensable for the establishment of compartments in cucumber GTs. Silica, a specialized compound, is deposited on the GTs and is visible on the surface of the fruit as a white powder, known as bloom. This deposition confers pathogen resistance and prevents fruit dehydration. Using a cucumber bloomless mutant, we discovered that a lignin-based cell wall structure in GTs, termed the “neck strip”, enables compartmentalization by acting as an extracellular barrier crucial for silica polymerization. This structure is present in the GTs of diverse plant species. Our findings will contribute to a deeper understanding of the biosynthesis of distinct compounds in trichomes and serve as a foundation for enhancing the production of compounds that are beneficial to human health.

Integrated multi-omic data and analyses reveal the pathways underlying key ornamental traits in carnation flowers

Zhang Xiaoni[1,2], Lan Lan[1,2], Luhong Leng[1], Weichao Liu[1,3], Yang Yingxue[1,2], Jiang Heling[1], Bao Manzhu[3], Fu Xiaopeng[3], Wu Zhiqiang[1,2]

[1] *Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Key Laboratory of Synthetic Biology, Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China*

[2] *Kunpeng Institute of Modern Agriculture at Foshan, Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518124, China*

[3] *Key Laboratory of Horticultural Plant Biology, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China*

wuzhiqiang@caas.cn

Abstract

Carnation (*Dianthus caryophyllus*) is one of the most popular ornamental flowers in the world. Although numerous studies on carnations exist, the mechanisms underlying flower color, fragrance, and the formation of double flowers remain unknown. Here, we employed an integrated multi-omics approach to elucidate the genetic and biochemical pathways underlying the most important ornamental features of carnation flowers. First, we assembled a high-quality chromosome-scale genome (636 Mb with a contig N50 of 14.67 Mb) of *D. caryophyllus* 'Scarlet Queen'. Next, a series of metabolomic datasets was generated with a variety of instrumentation types from different parts of the flower at multiple developmental stages to assess spatial and temporal differences in the accumulation of pigment and volatile compounds. Finally, transcriptomic data were generated to link genomic, biochemical, and morphological patterns and propose a set of pathways that control ornamental traits such as petal color, double flowers, and fragrance production. *bHLHs*, *MYBs*, and a *WRKY44* homolog are among the transcription factors proposed to be important for control of petal color patterning, and genes such as *coniferyl alcohol acetyltransferase* and *eugenol synthase* are involved in the synthesis of eugenol. We next assembled a haplotype-resolved genome of 'Aili' and a telomere-to-telomere genome of 'Baltico'. We identified allele-specific expression and found that the length of the genes and CDS numbers were correlated with gene expression ratios and expression levels. The integrated genomics, transcriptomics, and metabolomics datasets presented here provide an important foundation for understanding the mechanisms underlying key ornamental traits, which can in turn be used for selective breeding and gene editing to develop novel carnation cultivars.

Orange-ins and citric acid accumulation of Citrus fruits

Qiang Xu

National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan 430070, P. R. China

xuqiang@mail.hzau.edu.cn

Abstract

Citrus fruits are an important source of nutrients for human health, such as flavonoids, vitamins, carotenoids and citric acid. The *Citrus* genus contains various species, such as sweet orange, and has been grown in more than one hundred countries. The origin of citrus species is a long-standing controversy. In my presentation, I will first show the genome sequencing progress and recent understanding of the origin of citrus species. Our analysis indicates that South Central China is the primary center of origin for the *Citrus* genus. We found substantial variation in the level of fruit acidity in the orange subfamily. Extraordinary accumulation of citric acid in fruit is one of the hallmarks of citrus species. This phenomenon is also unique in the plant kingdom, although the citric acid cycle is part of basic metabolism. We detected variations in sequence and expression of the *PH4* gene in *Citrus* relative to *Citrus*-related genera. Gene editing and biochemical experiments demonstrated a central role for *PH4* in the accumulation of citric acid in citrus fruits. One hundred and fourteen somatic mutants also showed variation in fruit acidity. By genome analysis, we detected 877 TE insertions and found TE insertions in transporters or their regulatory genes, such as *AN1*, associated with variation in fruit acidity. We also compared the transcriptional activation variations of PH4 and AN1 in activation of downstream proton-pumping genes between *Citrus* and *Citrus*-related genera. I will also share insights into the origin and evolution of the orange subfamily and a regulatory mechanism underpinning the evolution of fruit taste.

The calcium signal is required for drought induced flower drop

Tao Xu[1,2]

[1] College of Horticulture, Shenyang Agricultural University, Shenyang 110866, Liaoning, China

[2] Key Laboratory of Protected Horticulture of Ministry of Education, Shenyang, Liaoning Province, China

syauxutao@syau.edu.cn

Abstract

Drought, as the main abiotic stress, induces abscission of flowers and fruits, leading to yield reduction. Auxin and peptide signals play important roles in regulating drought-induced flower drop. However, the regulatory mechanisms by which auxin and peptides function in drought are still unknown. Here, we reveal that drought triggers changes in cytoplasmic Ca^{2+} level to respond to drought, and CAM and CBL-CIPK participate in drought-initiated flower drop by mediating the homeostasis of peptide signals and auxin. These findings provide insights into a previously unknown regulatory mechanism of Ca^{2+} signaling required for drought-induced abscission.

Unlocking diploid seedless watermelons through disruption of essential reproductive genes

Jiao Jiang, Xiner Chen, Qin Feng, Man Liu, Jiafa Wang, Shujuan Tian, Xian Zhang, Li Yuan

Abstract

There have always been challenges in the breeding and production of triploid seedless watermelon, such as the long breeding cycle of tetraploid female parents and the low seed yield, germination rate, and survival rate of triploid seedless watermelon, which have greatly hindered its rapid development. In theory, creation of seedless watermelon at the diploid level could overcome all these problems. We have successfully created diploid seedless watermelon by blocking spore mother cell differentiation, meiotic division, gametophyte development, and embryo development processes. Eventually, we successfully established an induction system for diploid seedless watermelon. Our research provides a solid preliminary foundation for creation of seedless watermelon at the diploid level.

Research and application progress of apple dwarf rootstock breeding and nursery tree propagation technology in China

Dong Zhang[1]

College of Horticulture, Yangling Sub-Center of National Center for Apple Improvement, Northwest A&F University, Yangling, Shaanxi, China

afant@nwafu.edu.cn

Abstract

The apple industry in China is currently undergoing a transformation from a traditionally vigorous cultivation orchard system to a high-density dwarf cultivation orchard system. Orchards are mostly distributed in mountainous and hilly areas with poor soil. A lack of rootstocks and nursery stocks with excellent ecological adaptability has become a key limiting factor for development of high-density dwarf cultivation. The breeding of apple rootstocks in China began in the 1970s. To date, several types of dwarf and semi-dwarf rootstock, such as the SH series, Qingzhen series, and Zhongzhen series, have been bred by over 10 research units involving China Agricultural University, the Shanxi Academy of Agricultural Sciences Pomology Institute, and others through utilization of local wild resources and hybridization of dwarf rootstock. The dwarf rootstocks used in production are principally M26, T337, SH6, GM256, and 'Qingzhen 1'. They are usually used as intermediate stocks in application practices, accounting for more than 90%. Production of apple nursery stocks is mainly concentrated in Shaanxi, Shandong, and other provinces and regions, with a total capacity of about 200 million plants. Vigorous stocks, dwarf intermediate rootstocks, and dwarf self-rooted stocks account for 14%, 55%, and 31%, respectively. The large-scale commercial application of dwarf self-rooted stocks originated in Qianyang County, Shaanxi Province, in 2014. A technical system for propagating apple nursery trees with virus-free dwarf rootstock has been established after 10 years of research; it primarily involves virus detection and elimination, cutting nurseries, layering beds, bench grafting, branching promotion technology, nursery tree storage techniques, and so on. Layering and tissue culture are currently the major approaches used in dwarf rootstock breeding, although cutting and apomixis are also used occasionally. Overall, apple nursery stock propagation in China is shifting away from traditional fragmented vigorous propagation to modern asexual propagation of dwarf stocks.

Chloroplast Acetyltransferase GNAT2 Acts as a Redox-regulated Switch for State Transitions in Tomato

Xiaoyun Wang[1,2,#], Jianghao Wu[3,#], Hongxin Li[1], Ying Liu[1], Dexian Han[3], Danhui Dong[1], Jialong Zhang[1], Lixin Zhang[3,*], Na Zhang[1,*], Yang-Dong Guo[1,*]

[1] *China Agricultural University*

[2] *Beijing University of Agriculture 3. Henan University*

In nature, due to the variability of environmental factors, plants grow in a dynamic light environment which affects the growth and productivity of field crops. The fluctuating light breaks the balance of electron transport causes ROS overaccumulation in chloroplasts and changes the redox state of the PQ pool. It is considered as a kind of abiotic stress. State transitions are a dynamic process to balance the amount of light energy received by photosystem I (PSI) and photosystem II (PSII) to maintain an optimal photosynthetic yield and to minimize photo-damage under a fluctuating light environment. Reversible phosphorylation of the light-harvesting complex of PSII (LHCII) has been considered critical for regulating state transitions. While acetylation of photosynthetic proteins also plays an important role in state transitions but the molecular mechanisms are poorly understood. In this study, we identified a chloroplast lysine acetyltransferase, GNAT2 in *Solanum lycopersicum* and show that *gnat2* mutants are deficient in state transitions and retarded in growth under fluctuating light, and display a late-ripening fruit phenotype when grown in a greenhouse. Quantitative lysine (Lys) acetylome analysis suggests that ^{6}Lys of mature Lhcb2 protein is the target of GNAT2 and is involved in state transitions. ^{131}Cys-related redox changes of GNAT2 affect its acetylation activity on Lhcb2. Therefore, we propose that the chloroplast redox state may regulate the activity of GNAT2 which in turn acetylates ^{6}Lys of Lhcb2 to switch on state transitions in higher plants when facing fluctuating light stress.

Root-to-shoot mobile mRNAs promote JA-Ile biosynthesis to confer chilling tolerance in grafted cucumbers

Wenqian Liu[1†], Cuicui Wang[1†], Shujin Lin[2†], Xiaojun Li[1], Yongzhen Lu[1], Ying Chen[1], Xiao Han[2], Yihan Dong[3], Lihong Gao[1*] and Wenna Zhang[1*#]

[1] *Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, China Agricultural University, Beijing 100193, China*

[2] *College of Life Science and Technology, Fuzhou University, Fuzhou 350116, China*

[3] *Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique, UPR 2357, Université de Strasbourg, 67000, Strasbourg, France*

† These authors contributed equally

zhangwenna@cau.edu.cn; gaolh@cau.edu.cn

Abstract

Grafting is widely used to improve abiotic stress resistance by providing a root system better suited to environmental extremes. The beneficial effects of different graft combinations on plant growth and resistance are different, suggesting that there are systemic signals that enable communication between distant organs. Among known systemic signals, mRNAs are reported to be involved in stress responses by long-distance migration when plants experience various physiological challenges. Cucumber plants (*Cucumis sativus* L.) often experience chilling stress that limits their growth and productivity. Pumpkin rootstock is commonly used to graft cucurbit crops, improving their ability to withstand stress. Although the significance of systemic signals from rootstocks to scions is recognized, the role of root-to-shoot mRNA transport remains understudied. Thus, to address the potential functions of mobile mRNAs in chilling tolerance of grafted cucumber, we investigated phenotypic and physiological changes associated with chilling-response gene expression in above- and belowground tissues of heterografted and homografted cucumber–pumpkin combinations in response to a time course of 4℃ chilling stress. Integrated analysis of the cluster of chilling-induced pumpkin mobile mRNAs with cucumber differentially expressed genes (DEGs) and differentially intensive metabolites (DIMs) in all grafts in response to chilling stress revealed significant enrichment of amino acid biosynthesis and fatty acid metabolism pathways. We identified pumpkin *Ketol-acid reductoisomerase 1* (*CmoKARI1*) and *Choline Kinase 1 (CmoCK1)* as the key mobile mRNAs that specifically travel from the pumpkin rootstock to the cucumber scion upon early chilling stress. Overexpression of *CmoKARI1* and *CmoCK1* resulted in increased JA-Ile signaling and promoted chilling tolerance of heterografts in both cucumber and Arabidopsis. Our results demonstrate the long-distance transport of mRNAs from chilling-tolerant pumpkin to chilling-sensitive cucumber, the first instance of a unidirectional mobile mRNA shown to be triggered by specific environmental cues.

Regulatory mechanism of axillary branch outgrowth in cucumber

Jiacai Chen, Junjun Shen, Xiaolan Zhang*

Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops, Department of Vegetable Sciences, China Agricultural University, Beijing 100193, China

zhxiaolan@cau.edu.cn

Abstract

Lateral branching is one of the most important components of shoot architecture and directly affects crop yield and production cost. During cucumber production for the fresh market, axillary branches must be manually removed to minimize nutritional competition and disease occurrence, and they are therefore undesirable. Here, the regulatory mechanisms of axillary branch outgrowth will be presented. Our data showed that cucumber BRANCHED1 (CsBRC1) inhibits lateral bud outgrowth by directly suppressing CsPIN3 function, and thus auxin accumulation, in axillary buds of cucumber. AGAMOUS-LIKE 16 (CsAGL16) acts as a positive regulator of axillary bud outgrowth. Functional disruption of *CsAGL16* led to reduced bud outgrowth, whereas overexpression of *CsAGL16* resulted in enhanced branching. CsAGL16 binds directly to the promoter of the ABA 8'-hydroxylase gene *CsCYP707A4* and promotes its expression. Cucumber General Regulatory Factor1 (CsGRF1) interacts with CsAGL16 and antagonizes CsAGL16-mediated *CsCYP707A4* activation. Disruption of *CsGRF1* resulted in elongated branches and decreased ABA in axillary buds. Our data suggest that the CsAGL16–CsGRF1 module regulates axillary bud outgrowth via Cs*CYP707A4*-mediated ABA catabolism in cucumber. Utilization of the above-mentioned genes provides a new strategy for manipulation of shoot branching in cucumber breeding.

Advances of theanine biosynthesis, transport and regulation

Zhaoliang Zhang

[1]*State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, Anhui 230036, China*

zhlzhang@ahau.edu.cn

Abstract

Theanine is the most abundant free amino acid in the tea plant. This unique, non-protein amino acid is the most important quality component of tea, as it gives brewed tea its “umami” taste and has a variety of health effects, including calming. Theanine is synthesized mainly in the roots and is transported to new shoots and buds, where it accumulates. Over the course of evolution, the tea plant obtained the ability to synthesize ethylamine and theanine through theanine synthetase (TS) and alanine decarboxylase (AlaDC). Theanine can be synthesized in large quantities only under the synergistic action of CsAlaDC and CsTSI. Theanine is usually stored in tea roots during the winter and transported to the shoots through the vascular system after the emergence of shoots in the spring. This process is mediated by the theanine transporters CsCAT2 and CsAAPs, especially CsAAP1, which was shown to mediate theanine transport from root to shoot. Theanine accumulation is regulated by developmental, environmental, nutritional, and biological factors and changes dynamically in the tea plant. The dynamic accumulation of theanine is regulated at multiple levels. Transcription factors are involved in the transcriptional regulation of theanine biosynthesis. The transcription factors CsMYB40 and CsHHO3 bind to the promoter of *CsAlaDC* to promote and inhibit *CsAlaDC* expression, respectively, giving rise to the “throttle” and “brake” regulatory mechanism that mediates theanine synthesis in response to nitrogen level. Recent studies have shown that theanine may improve the salt tolerance of tea plants by regulating reactive oxygen homeostasis. On the other hand, theanine can also regulate lateral root development in response to nitrogen levels by influencing the accumulation of apoplastic H_2O_2. These findings provide important insight into the biological functions of theanine.

Effects of phosphorus on strawberry quality and regulation of phosphate homeostasis in strawberry plants

Zhihong Zhang

College of Horticulture, Shenyang Agricultural University, China

zhangz@syau.edu.cn

Abstract

Phosphorus (P) plays a vital role in plant growth and reproduction. We found that the P content of strawberry fruit was positively correlated with sugar content, and increasing the P content of strawberry plants by applying P fertilizer could significantly improve the sugar content of strawberry fruit. We studied the regulation of P homeostasis in strawberry plants in order to develop strawberry cultivars through molecular breeding that can better acquire Pi from the soil. The transcription factor PHR1 is the central regulator of P signaling, and strawberry FvPHR1 controls phosphate homeostasis by transcriptionally regulating miR399a. Overexpression of miR399a in strawberry plants increased the sugar content of strawberry fruits. PHO2, which encodes a ubiquitin-binding E2 enzyme, is the target of miR399. We obtained *Fvpho2* mutants using CRISPR/Cas9 gene-editing technology. The P content of mature *Fvpho2* mutant fruit was increased by 40–64% compared with that of wild-type fruit, and fruit quality was significantly increased in the *Fvpho2* mutants. Our findings provide insights for the breeding of high-quality, P-efficient strawberry cultivars through gene editing technology.

Functional genomics research in chinese cabbage

Jianjun Zhao[1], Wei Ma[1], Xiaomeng Zhang[1], Mengyang Liu[1], Xiaoxue Sun[1], Daling Feng[1], Yin Lu[1]

[1] *State Key Laboratory of North China Crop Improvement and Regulation, Key Laboratory of Vegetable Germplasm Innovation and Utilization of Hebei, Collaborative Innovation Center of Vegetable Industry in Hebei, College of Horticulture, Hebei Agricultural University, Baoding 071000, China*

jjz1971@aliyun.com

Abstract

Chinese cabbage is one of the most important leaf vegetables in China. We created and systematically evaluated a single-base mutant library of Chinese cabbage at the phenotypic and molecular levels and constructed a resource-sharing platform for genome and trait data from this mutant population. This work provides a basis for efficient analysis of Chinese cabbage gene functions and mining of new breeding resources. Two key regulatory genes, *BrOPS* and *BrFC2*, were identified from the mutants. The molecular mechanism by which the BrOPS–BrBIN2–BrBES1–BrAS1 module regulates the leafy head of Chinese cabbage was analyzed, and the molecular network through which the BR signaling pathway regulates the development of Chinese cabbage leaf balls was constructed. These findings provide new ideas for the breeding of head-bearing vegetables. In addition, we characterized the molecular mechanism by which the BrFC2–BrPORB module jointly mediates the synthesis of chlorophyll and heme, providing a new understanding of the chlorophyll synthesis pathway.

Technology-driven auxin research: unexpected findings

Yunde Zhao

Department of Cell and Developmental Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

yundezhao@ucsd.edu

Abstract

Auxin is required for many aspects of plant growth and development. Disruption of auxin biosynthesis, inactivation, transport, or signaling causes severe developmental defects. For example, inactivation of the auxin efflux carrier PIN-FORMED 1 (PIN1) leads to the formation of pin-like inflorescences. Several other Arabidopsis mutants including *pinoid*, *monopteros*, and *npy* also develop pin-like inflorescences and fail to initiate flowers. However, the molecular relationships among the aforementioned genes are not understood. My group has developed several CRISPR/Cas9-based gene-editing technologies that enable precise modifications of plant genes. These technological advances provide unprecedented tools for the study of auxin biology, including the molecular mechanisms of auxin transport and signaling. In this presentation, I will first describe our technologies, including efficient gene-targeting technology and *in situ* tagging of genes with GFP using CRISPR-based homologous recombination. This technology avoids the pitfalls of transgenic approaches such as co-suppression and ectopic overexpression. The technologies also allow us to generate informative new alleles of auxin genes. The new genetic materials we generated recently enabled us to mechanistically connect the aforementioned genes, revealing unexpected mechanisms that govern auxin transport and signaling.

HortGenome Search Engine, a universal genomic search engine for horticultural crops

Sen Wang[1,2#], Shangxiao Wei[1,2#], Yuling Deng[1,2], Shaoyuan Wu[1,2], Haixu Peng[1,2], You Qing[1,2], Xuyang Zhai[1,2], Shijie Zhou[1,2], Jinrong Li[1,2], Hui Zhang[1,2], YunCong Yao[1*], Zhangjun Fei[3,4*], Yi Zheng[1,2*]

[1] *Beijing Key Laboratory for Agricultural Application and New Technique, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China*
[2] *Bioinformatics Center, Beijing University of Agriculture, Beijing 102206, China*
[3] *Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, USA*
[4] *USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA*

yaoyc_20@126.com; zf25@cornell.edu; yz@moilab.net

Abstract

Horticultural crops, comprising fruit, vegetable, ornamental, beverage, medicinal, and aromatic plants, play essential roles in food security and human health, as well as landscaping. With advances in sequencing technologies, genomes for hundreds of horticultural crops have been deciphered in recent years, providing a basis for understanding gene functions and regulatory networks and for improvement of horticultural crops. Despite the availability of valuable genomic data, this information is dispersed across various data warehouses using diverse storage approaches, making it challenging to access and analyze. Consequently, there has been a growing emphasis on using search engines to explore functional genes and gene relationships and enhance our understanding of plant biology. However, standard search engines provide only limited search results when confronted with vast genetic data, leaving a significant amount of genetic information untapped and buried in raw data. To this end, we have developed a lightweight universal search engine, HortGenome Search Engine (HSE; http://hort.moilab.net), that enables users to query genes, functional annotations, protein domains, homologs, and other gene-related functional information for horticultural crops. In addition, four commonly used tools (BLAST, Batch Query, Enrichment analysis, and Synteny Viewer) have been developed for efficient mining and analysis of these genomic data.

Dynamics of the genome cis-regulatory elements revealed by cross-species TF ChIP-seq

Silin Zhong

State Key Laboratory of Agrobiotechnology, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

silin.zhong@cuhk.edu.hk

Abstract

Although the genomes of most important plants have been sequenced, it is still not possible to accurately predict gene expression solely from DNA sequence information. This is because the expression of one gene is often regulated by multiple transcription factors (TFs) that bind to the *cis*-regulatory elements (CREs) located in its promoter open chromatin. To understand how TFs regulate gene expression, we compared the binding of GOLDEN2-LIKE (GLK) TFs across five plant species: tomato, tobacco, Arabidopsis, maize, and rice. Although the function of GLKs is conserved across these species, we found that most of their binding sites are species specific. Interestingly, we found that conserved binding sites are often located near photosynthetic genes dependent on GLK for expression, but sites near non-differentially expressed genes in the *glk* mutant are nevertheless under purifying selection. Our study shows that genome *cis*-variation can cause widespread TF binding divergence and that most of the TF binding sites could be genetically redundant. This poses a major challenge for interpreting the effect of individual sites and highlights the importance of quantitatively measuring TF occupancy. The dynamic nature of TF binding divergence also enabled plant species with similar genes to generate different transcriptional programs, leading to different phenotypes and contributing greatly to species' adaptative and phenotypic plasticity.

Genomic breeding of grapevine

Yongfeng Zhou, Hua Xiao, Zhongjie Liu, Wenwen Liu, Xiaodong Xu, Zhiyao Ma, Hui Xue, Fan Zhang, Bilal Ahmad, Yiwen Wang, Yanling Peng, Zhongxing Jin, Xuejing Cao

National Key Laboratory of Tropical Crop Breeding, Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Key Laboratory of Synthetic Biology, Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

zhouyongfeng@caas.cn

Abstract

The breeding of horticultural crops, especially perennial fruit trees, lags far behind that of annual cereals, largely owing to the delayed application of new life-science technologies, including genomics. The breeding 4.0 strategy aims to design crop genomes by combining beneficial variants of desired agronomic traits while purging deleterious variants. Breeding programs for maize, rice, and potato have been designed under this framework. However, breeding of perennial fruit trees is still at an early stage of marker assisted selection (breeding 2.0). Our investigation of the population genetics of crop domestication revealed that genomic breeding of fruit trees faces unique difficulties. Compared with annual cereals, perennial fruit trees have much more heterozygous genomes, largely due to clonal propagation, which hide deleterious variants, including structural variants, from recessive selection. The breeding of clonal fruit trees requires a more thorough understanding of the genetics of heterozygous genomes because supergenes and regulation disorders may be major causes of agronomic traits. On the basis of our population genetic and quantitative genetic studies of beneficial, deleterious, and structural variants in grapevine, we propose breeding programs with a combination of population genetic simulations.

The Biosynthesis of EGCG, theanine and caffeine in response to temperature is mediated by hormones signal transduction factors in tea plant (*Camellia sinensis* L.)

Qiufang Zhu[1], Lijia Liu[1], Xiaofeng Lu[1], Xinxin Du[1], Ping Xiang[1,2], Bosi Cheng[1], Meng Tan[1], Jiaxin Huang[1], Lijiao Wu[3], Weilong Kong[4], Yutao Shi[1,5], Liangyu Wu[1], Jinke Lin[1]

[1] *College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou, China*

[2] *College of Life and Environmental Science, Hunan University of Arts and Science, Changde, China*

[3] *Institute of Photobiological Industry, Fujian Sanan Sino-Science Photobiotech Co., Ltd, Xiamen, China*

[4] *Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China*

[5] *College of Tea and Food Sciences, Wuyi University, Wuyishan, China*

zqf216@163.com

Abstract

Epigallocatechin-3-gallate (EGCG), theanine, and caffeine are the main flavor components of tea, and their contents are regulated by ambient temperature. However, whether and how the biosynthesis of EGCG, theanine, and caffeine is regulated by endogenous hormones in response to temperature is still unclear. In this study, we investigated the intricate internal relationships among the biosynthesis of these three main taste components, endogenous hormones, and structural genes in tea plants under different temperatures treatments. The results showed that indoleacetic acid (IAA), gibberellin 1 (GA1) and gibberellin 3 (GA3) were significantly correlated with EGCG content; jasmonic acid (JA), jasmonate-isoleucine (JA-Ile) and methyl jasmonate (MeJA) were strongly correlated with theanine content; and IAA, GA1, and gibberellin 4 (GA4) were significantly correlated with caffeine content at different temperatures. According to the results of multi-omics analysis, we propose the following regulatory mechanisms. IAA, GA1, and GA3 upregulated the expression of *chalcone synthase* (*CsCHS*) and *trans-cinnamate 4-monooxygenase* (*CsC4H*) mediated by the signal transduction factors *auxin-responsive protein IAA* (*CsIAA*) and *DELLA protein* (*CsDELLA*), respectively, thus promoting the biosynthesis of EGCG. IAA, GA3, and GA1 upregulated the expression of *CsCHS* and *anthocyanidin synthase* (*CsANS*) mediated by *CsIAA* and *CsDELLA*, respectively, via the transcription factor *WRKY DNA-binding protein* (*CsWRKY*), again promoting the biosynthesis of EGCG. JA, JA-Ile, and MeJA jointly upregulated the expression of *carbonic anhydrase* (*CsCA*) and downregulated the expression of *glutamate decarboxylase* (*CsgadB*) mediated by the signal transduction factor *CsJAZ*, promoting the biosynthesis of theanine. JA, JA-Ile, and MeJA also jointly inhibited the expression of *CsgadB* mediated by *CsJAZ* via the transcription factors *CsWRKY* and *AP2 family protein* (*CsAP2*), promoting the biosynthesis of theanine. IAA inhibited the expression of *adenylosuccinate synthase* (*CspurA*) mediated by *CsIAA* via *CsWRKY*. GA1 and GA4 inhibited the expression of *CspurA* mediated by *CsDELLA* through *CsWRKY*, promoting the biosynthesis of caffeine. These findings reveal that the underlying biosynthetic mechanisms of the major taste components of tea were mediated by hormone signal transduction factors in response to temperature, thereby providing novel insights for improvement of tea quality.

The mechanism of MaABI5-like in the regulation of ripening disorders in banana induced by chilling stress

Zunyang Song[1,2], Xiuhua Lai[1], Hangcong Chen[1], Lihua Wang[1], Xuequn Pang[1], Wangjin Lu[1], Weixin Chen[1], Xueping Li[1*], Xiaoyang Zhu[1*]

[1] *Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Engineering Research Center for Postharvest Technology of Horticultural Crops in South China, Ministry of Education, College of Horticulture, South China Agricultural University, Guangzhou, Guangdong 510642, China*
[2] *Key Laboratory of Food Processing Technology and Quality Control in Shandong Province, College of Food Science and Engineering, Shandong Agricultural University, Tai'an 271018, China*

xiaoyang_zhu@scau.edu.cn

Abstract

Cold stress adversely affects plant production, both qualitatively and quantitatively. Banana is sensitive to cold stress and thus suffers chilling injury when stored below 11°C, causing abnormal fruit softening. However, the mechanism that underlies abnormal fruit softening due to chilling injury remains obscure. The phytohormone abscisic acid (ABA) is essential for plant responses to various environmental stresses, and abscisic acid-insensitive 5 (ABI5) is a key transcription factor in ABA signaling and response. Here, we found that exogenous ABA alleviated chilling injury in 'Fenjiao' banana; induced the accumulation of endogenous ABA, unsaturated fatty acids, and flavonoids; and reduced the content of saturated fatty acids. Moreover, the transcript levels of *MaABI5-like*, fatty acid desaturation genes, and flavonoid synthesis-related genes were upregulated by ABA treatment during cold storage. MaABI5-like protein was able to bind directly to the promoters of genes related to fatty acid desaturation and flavonoid synthesis, activating their expression. Furthermore, transient and ectopic overexpression of *MaABI5-like* in 'Fenjiao' banana fruit and tomato plants enhanced their cold tolerance and upregulated transcript levels of genes related to fatty acid desaturation and flavonoid synthesis. All these results demonstrate that MaABI5-like participates in ABA-induced cold tolerance by increasing the contents of unsaturated fatty acids and flavonoids. Cold stress also severely inhibits the transcript and protein levels of *MaABI5-like*, *MaEBF1*, and fruit softening-related genes. The MaABI5-like protein binds to the promoters of key genes related to starch and cell wall degradation, activating their activities. MaEBF1 physically interacts with MaABI5-like and enhances the transcriptional activity of these starch and cell wall degradation genes but does not ubiquitinate and degrade the ABI5-like protein. These results indicate that the interaction of EBF1 and ABI5-like controls starch and cell wall metabolism in banana, which is strongly inhibited by chilling stress, leading to fruit softening and ripening disorders. These results demonstrate that MaABI5-like is a key player in the chilling stress response, and MaABI5-like coordinates with ethylene F-box protein (EBF1) to regulate chilling-induced softening disorders of 'Fenjiao' banana.

The molecular mechanism underlying tomato adaptation to drought and low temperature stresses

Yingfang Zhu[1], Leelyn Chong[1], Rui Xu, Zhixin Jiao[1]

[1] *State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475001*

zhuyf@henu.edu.cn

Abstract

Abiotic stress has significant effects on crop development, growth, and fruit yield. However, the genetic basis and molecular mechanisms that underlie plant adaptation to abiotic stresses in most crops remain poorly understood. Here, we used phosphoproteomics, genome-wide association study (GWAS), and multi-omics approaches to reveal the mechanisms of tomato adaptation to drought and low-temperature stress. We found that the tomato protein kinase OST1 directly interacts with and phosphorylates VOZ1 to enhance its protein stability and nuclear translocation, thereby promoting *SFT* transcription and early flowering under drought stress. Moreover, we found that a promoter variation in the CONSTANS-like transcription factor *SlBBX31* was significantly associated with cold tolerance in a tomato natural population and was selected during domestication. A 27-bp insertion in the *SlBBX31* promoter prevented its transcriptional activation by SlHY5 and thus the expression of downstream cold-responsive genes. These findings provide valuable insights for the development of hardy tomato varieties.

Cucurbitaceae genome evolution, gene function, and molecular breeding

Lili Ma[1,2†], Qing Wang[1†], Yanyan Zheng[1†], Jing Guo[3], Shuzhi Yuan[1], Anzhen Fu[1], Chunmei Bai[1], Xiaoyan Zhao[1], Shufang Zheng[1], Changlong Wen[1], Shaogui Guo[1], Lipu Gao[1], Donald Grierson[4,*], Jinhua Zuo[1*], Yong Xu[1*]

[1] *Key Laboratory of Vegetable Postharvest Processing, Ministry of Agriculture, Beijing Key Laboratory of Fruits and Vegetable Storage and Processing, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China) of Ministry of Agriculture, Key Laboratory of Urban Agriculture (North) of Ministry of Agriculture, Beijing Vegetable Research Center, Institute of Agro-Products Processing and Food Nutrition, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*

[2] *Department of Food Biotechnology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[3] *Ministry of Education Key Laboratory of Biodiversity Sciences and Ecological Engineering and State Key Laboratory of Genetic Engineering, Institute of Biodiversity Sciences and Institute of Plant Biology, School of Life Sciences, Fudan University, 2005 Songhu Road, Shanghai 200438, China*

[4] *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom*

† These authors contributed equally

zuojinhua@126.com; donald.grierson@nottingham.ac.uk; xuyong@nercv.org

Abstract

Cucurbitaceae is one of the most genetically diverse plant families in the world. Many cucurbits are important vegetables or medicinal plants and are widely distributed worldwide. The rapid development of sequencing technologies and bioinformatic algorithms has enabled the generation of genome sequences for numerous important Cucurbitaceae species. This has greatly facilitated research on gene identification, genome evolution, genetic variation, and molecular breeding of cucurbit crops. To date, genome sequences of 18 different cucurbit species from the tribes Benincaseae, Cucurbiteae, Sicyoeae, Momordiceae, and Siraitieae have been deciphered. This review summarizes their genome sequence information, evolutionary relationships, and functional genes associated with important agronomic traits (e.g., fruit quality). Progress in molecular breeding of cucurbit crops and prospects for future applications of Cucurbitaceae genome information are also discussed.

The miR156/SPL12 module orchestrates fruit color change through directly regulating ethylene production pathway in blueberry

Hongxue Li[1], Shouwen Wang[1], Lulu Zhai[1], Yuhai Cui[2,3], Tang Guiliang[4], Junwei Hou[5], Xuyan Li[1], Shaomin Bian[1]

[1] *College of Plant Science, Jilin University, Changchun 130062, Jilin, China*

[2] *Agriculture and Agri-Food Canada, London Research and Development Centre, London, Ontario, Canada*

[3] *Department of Biology, Western University, London, Ontario, Canada*

[4] *Department of Biological Sciences, Life Science and Technology Institute, Michigan Technological University, Houghton, 49931, MI, USA*

[5] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture and Rural Affairs, Northeast Agricultural University, Harbin, Heilongjiang, China*

shmbian@jlu.edu.cn

Abstract

Color change is an important event during fruit ripening in blueberry. It is well known that miR156/SPLs act as regulatory modules mediating anthocyanin biosynthesis and ethylene plays critical roles in color change, but the intrinsic connections between the two pathways remain poorly understood. Previously, we demonstrated that blueberry VcMIR156a/VcSPL12 affects the accumulation of anthocyanins and chlorophylls in tomato and Arabidopsis. In this study, we first show that VcMIR156a overexpression in blueberry led to enhanced anthocyanin biosynthesis and reduced chlorophyll accumulation, and, intriguingly, concomitant elevation in the expression of ethylene biosynthesis genes and the level of the ethylene precursor ACC. Conversely, VcSPL12 promoted chlorophyll accumulation and suppressed anthocyanin biosynthesis and ACC synthesis in fruits. Moreover, treatment with ethylene substitutes and inhibitors attenuated the effects of VcMIR156a and VcSPL12 on pigment accumulation. Protein-DNA interaction assays indicated that VcSPL12 could specifically bind the promoters and inhibit the activities of the ethylene biosynthetic genes VcACS1 and VcACO6. Collectively, our findings show that VcMIR156a/VcSPL12 alters ethylene production by targeting VcACS1 and VcACO6, thereby governing fruit color change. These findings establish an intrinsic connection between the miR156/SPL regulatory module and the ethylene pathway.

Dual domestication and origin of traits in grapevine evolution

Wei Chen

Abstract

Grapevine cultivation connects strongly with human agricultural history. Here, we explored the domestication and diversification of grapevine using genome-wide variations of 3525 cultivated and wild accessions from major viticultural regions worldwide. We differentiated grape accessions with eight genetic ancestries and found that, in the Pleistocene epoch, harsh climate drove the separation of wild ecotypes due to continuous habitat fragmentation. Domestication occurred concurrently about 11,000 years ago in Western Asia and the Caucasus to yield table and wine grapevines. The Western Asia domesticates had a broad distribution compared to the Caucasus domesticates. The Western Asia domesticates dispersed into Europe with early farmers, received introgression from wild western ecotypes, and subsequently diversified into muscat grape and various western wine grapevine groups. We found that unique grapevine ancestries were established by the end of the Neolithic period and that the process matched the early inception of agriculture across Eurasia. Lastly, analyses of domestication traits revealed novel insights into selection for berry palatability, hermaphroditism, muscat flavor, and berry skin color. Overall, the defined history of grapevine is a testament to how humans interacted with the environment to tame an essential vine.

Jasmonic acid signaling contributes to cold acclimation in jojoba by regulating flavonol synthesis via the JAZ/MYB module

Hongxi Yan, Lamei Zheng, Yijun Zhou, Fei Gao

College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China

gaofei@muc.edu.cn

Abstract

Jojoba (*Simmondsia chinensis*) is a rapidly emerging dryland oil crop. Jojoba seeds contain up to 65% of pale golden, odorless, and highly viscous oil, which possesses significant economic value. China began introducing jojoba in the 1980s, but due to its sensitivity to low temperatures, most introduction trials in the temperate regions in China failed. For most plants, cold acclimation is an effective means to enhance cold tolerance. Previous studies have found that cold acclimation improved the cold tolerance of jojoba, and the improvement of jojoba's low-temperature tolerance after cold acclimation may be associated with the regulation of the flavonoid metabolism pathway. However, the impact of cold acclimation on metabolites and the related molecular regulatory mechanisms are still unclear. Here, metabolomic analysis revealed that various flavonols were accumulated after cold acclimation in jojoba. Time-course transcriptomic and WGCNA analysis demonstrated that flavonol biosynthesis and jasmonic acid (JA) biosynthesis and signaling play crucial roles in the cold acclimation of jojoba. By combining biochemical and genetic analysis, we found an MYB transcription factor that promoted the accumulation of flavonols by activating the expression of functional genes in the flavonol biosynthesis pathway, and the negative regulator JAZ in the JA signaling pathway suppressed this activation effect. Cold acclimation stimulated the production of JA in jojoba leaves. The elevated JA level promoted the degradation of JAZ proteins, subsequently releasing the transcriptional activity of MYB and leading to the accumulation of flavonols with antioxidant activities, thereby enhancing the cold tolerance of jojoba. Our findings unveil the molecular mechanism of JA-regulated flavonol biosynthesis in jojoba and underscore the JA pathway as a promising candidate for improving cold tolerance in breeding and cultivation efforts.

Metabolic profiling and transcriptomic data providing critical flavonoid biosynthesis mechanisms disclose color differences of purple heading Chinese cabbages (*Brassica rapa L.*)

Qiong He, Yihua Xue, Lugang Zhang

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Horticulture, Northwest A&F University, 3 Taicheng Road, Yangling, 712100, Shaanxi, China

cuidy1988@163.com

Abstract

Flavonoids are important secondary metabolites; however, the identities of flavonoids and the related biosynthesis mechanisms of purple heading Chinese cabbage (PHCC) with different degrees of the purple color still remain largely unclear. Here, we conducted combined metabolome and transcriptome analysis and identified 102 flavonoids, including 12 anthocyanins, 6 flavanols, 18 flavanones, 37 flavones, 22 flavonols, 6 isoflavones, and 1 proanthocyanidin. Gallocatechin-gallocatechin, hesperetin C-hexosyl-O-hexosyl-O-hexoside, and tricin O-saccharic acid were the main flavonoids, whereas cyanidin 3,5-O-diglucoside, delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, and pelargonidin 3-O-beta-D-glucoside were the main anthocyanins. Cyanidin 3,5-O-diglucoside, pelargonidin 3-O-beta-D-glucoside, and cyanidin O-syringic acid directly contributed to color differences in both different and identical positions in comparisons of two PHCCs. The main difference occurred in the middle leafhead; meanwhile, 323 flavonoid biosynthesis genes (FBGs) were identified, including 204 differentially expressed genes. Integrative correlation analysis showed that some FBGs were more closely connected with flavonoid content. *BrMYB2* played an important role in anthocyanin production in PHCC, whereas *BrTT8* and *BrMYBL2.1*, and other factors, may synergistically respond to generate color differences, together with a variety of structurally active FBGs (*BrCHS*s, *BrCHI*s, *BrF3H1*, *BrF3'H*, *BrDFR1*, *BrANS1*, *BrUGT*s, *BrAT*s, and *BrGST*s). These results provide valuable clues for understanding flavonoid biosynthesis in *Brassica crops*.

Vacuolar Phosphate Transporter1 (VPT1) may transport sugar in response to soluble sugar status in different types of fruits

Yanjun Zhang[1], Qian Bai[2], Yuanyue Shen[1], Yun Huang[1]

[1] *Beijing Key Laboratory for Agricultural Application and New Technique, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China*

[2] *Ministry of Education Key Laboratory of Silviculture and Conservation, College of Forestry, Beijing Forestry University, 35 East Qinghua Road, Beijing 100083, China*

Abstract

Vacuolar Phosphate Transporter1 (VPT1)-mediated phosphate uptake in vacuoles is essential for plant development and fruit ripening. Here, we find that VPT1 may transport sugar in response to the soluble sugar status of fruits. The VvVPT1 protein isolated from grape (*Vitis vinifera*) berries was tonoplast-localized and contained SPX (Syg1/Pho81/XPR1) and MFS (major facilitator superfamily) domains. Its mRNA expression was significantly increased during fruit ripening and induced by sucrose. Functional analyses based on transient transgenic systems in grape berry showed that VvVPT1 positively regulated berry ripening and significantly affected hexose content, fruit firmness, and ripening-related gene expression. The VPT1 proteins (grape VvVPT1, strawberry FaVPT1, and Arabidopsis AtVPT1) all showed low affinity for phosphate, as verified in a yeast system, while they were different in sugar transport capacity, consistent with fruit sugar status. Recently, we found that the sugar transport capacity of VvVPT1 and FaVPT1 depends on the SPX domain and Glu 522 in the MFS domain. Thus, our findings reveal a role for VPT1 in fruit ripening, which is associated with its SPX and MFS domains in the direct transport of soluble sugar into the vacuole, and our findings open potential avenues for genetic improvements in fleshy fruit.

Polymorphism of BoFLC2 affects flowering time of cabbage in responding to cold

Xiaoyun Liu, Ao Peng, Jiaqin Yang, Jun Si, Xuesong Ren, Hongyuan Song, Qinfei Li

College of Horticulture and Landscape Architecture, Southwest University, 400715, Chongqing, China

feifei1984998@126.com

Abstract

Cabbage is a plant-vernalization-responsive flowering type. In response to cold, *BoFLC2* is an important transcription factor, which allows cabbage plants to remain in the vegetative phase. Here, a 215-bp indel at intron I, three non-synonymous SNPs and a 3-bp indel at exon II were found between $BoFLC2^{E}$ and $BoFLC2^{L}$, cloned from extremely early and extremely late flowering cabbages, respectively. The 215-bp deletion at intron I in $BoFLC2^{L}$ was related to late flowering, as verified in 40 extremely early/late flowering accessions, a diverse set of cabbage inbred lines and two F2 generations by using InDel-FLC2 as a marker. Among the transgenic progenies of seed-vernalization-responsive *Arabidopsis thaliana* (Col) and rapid cycler *B. oleracea* (TO1000, *boflc2*), plants of $BoFLC2^{E}cds$ + $BoFLC2^{L}intron\ I$ showed significantly later flowering time than the others, whereas plants having SNP variance at exon II of $BoFLC2^{E}$ showed earlier flowering time than the others. In addition, the 215-bp sequence enhanced GUS expression in the transiently transfected and stably transformed tobacco, leading to the higher expression of $BoFLC2^{E}$ in the early flowering cabbage than $BoFLC2^{L}$ in the late flowering cabbage. However, in response to cold, $BoFLC2^{E}$ was silenced faster than $BoFLC2^{L}$ via feedback to the core genes of the PHD-PRC2 complex, resulting in their lower transcript levels. These findings indicate that the 215-bp deletion at intron I of $BoFLC2^{L}$ was the main reason for the delay in flowering time, as it slowed its silencing activity via feedback to the core genes of the PHD-PRC2 complex, resulting in late flowering in cabbage.

Ethylene enhances MdMAPK3-mediated phosphorylation of MdNAC72 to promote apple fruit softening

Yun Wei, Aide Wang, Tong Li

College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

litong0327@syau.edu.cn

Abstract

The phytohormone ethylene plays an important role in promoting the softening of climacteric fruits, such as apples (*Malus domestica*); however, important aspects of the underlying regulatory mechanisms are not well understood. In this study, we identified apple MITOGEN-ACTIVATED PROTEIN KINASE 3 (MdMAPK3) as an important positive regulator of ethylene-induced apple fruit softening during storage. Specifically, we show that MdMAPK3 interacts with and phosphorylates the transcription factor NAM-ATAF1/2-CUC2 72 (MdNAC72), which functions as a transcriptional repressor of the cell wall degradation-related gene *POLYGALACTURONASE1* (*MdPG1*). An increase in MdMAPK3 kinase activity was induced by ethylene, which promoted the phosphorylation of MdNAC72 by MdMAPK3. Additionally, MdPUB24 functions as an E3 ubiquitin ligase to ubiquitinate MdNAC72, resulting in its degradation via the 26S proteasome pathway, which was enhanced by the ethylene-induced phosphorylation of MdNAC72 by MdMAPK3. The degradation of MdNAC72 increased the expression of *MdPG1*, which in turn promoted apple fruit softening. Notably, using variants of MdNAC72 that were mutated at specific phosphorylation sites, we observed that the phosphorylation state of MdNAC72 affected apple fruit softening during storage. This study thus reveals that the ethylene–MdMAPK3–MdNAC72–MdPUB24 module is involved in ethylene-induced apple fruit softening, providing insights into climacteric fruit softening.

Methylation of AcGST1 is associated with anthocyanin accumulation in kiwifruit outer pericarp

Xuefeng Zhang[1,†], Kun Zhang[1,†], Yuqi Guo[1], Xiaoyu Lv[1], Meijing Wu[1], Honghong Deng[1], Yue Xie[2], Mingzhang Li[2], Xiulan Lv[1], Hui Xia[1], Dong Liang[1]

[1] *College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China*

[2] *Sichuan Provincial Academy of Natural Resources Sciences, Chengdu 610015, China*

[†] These authors contributed equally.

Abstract

Most red-fleshed kiwifruit cultivars, such as Hongyang, only accumulate anthocyanins in the inner pericarp, and the full-red-flesh trait is a goal that is currently pursued by breeders. In this study, we identified a mutant "H-16" showing red color in both the inner and outer pericarp, and the underlying mechanism was explored. Through transcriptome analysis, a key differentially expressed gene *AcGST1* was identified, which was positively correlated with anthocyanin accumulation in the outer pericarp. The results of McrBC-PCR and bisulfite sequencing showed that the SG3 region (-292 to -597 bp) of the *AcGST1* promoter in "H-16" had a significantly lower CHH cytosine methylation level than that in Hongyang, which was accompanied by low expression of methylase genes (*MET1* and *CMT2*) and high expression of demethylase genes (*ROS1* and *DME*). Transient callus transformation confirmed that the demethylase gene *DML1* can activate the transcription of *AcGST1* to enhance its expression. Overexpression of *AcGST1* enhanced anthocyanin accumulation in the fruit flesh and leaves of transgenic lines. These results suggest that a decrease in the methylation level of the *AcGST1* promoter may contribute to accumulation of anthocyanin in the outer pericarp of "H-16".

VvBBX44 and VvMYBA1 form a regulatory feedback loop to balance anthocyanin biosynthesis in grape

Wenwen Liu[1,2,3,4], Huayuan Mu[1,2,3], Ling Yuan[5], Yang Li[1,2], Yuting Li[1,2,3], Shenchang Li[1,2,3], Chong Ren[1,2], Wei Duan[1,2], Peige Fan[1,2], Zhanwu Dai[1,2], Yongfeng Zhou[4], Zhenchang Liang[1,2], Shaohua Li[1,2], Lijun Wang[1,2]

[1] *Beijing Key Laboratory of Grape Science and Enology, and CAS Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Science, Beijing 100093, China*

[2] *China National Botanical Garden, Beijing 100093, China*

[3] *University of Chinese Academy of Sciences, Beijing 10049, China*

[4] *National Key Laboratory of Tropical Crop Breeding, Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Key Laboratory of Synthetic Biology, Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China*

[5] *Department of Plant and Soil Sciences, Kentucky Tobacco Research and Development Center, University of Kentucky, KY 40546, USA*

wen.wen.liu@hotmail.com

Abstract

Anthocyanins are essential for the quality of perennial horticultural crops such as grapes. In grape, ELONGATED HYPOCOTYL 5 (HY5) and MYBA1 are two critical transcription factors that regulate anthocyanin biosynthesis. Our previous work has shown that VvBBX44, a B-box (BBX) protein, inhibits anthocyanin synthesis and represses *VvHY5* expression in grape calli. However, the regulatory mechanism underlying this regulation was unclear. In this study, we found that loss of *VvBBX44* function resulted in increased anthocyanin accumulation in grapevine callus. VvBBX44 directly repressed *VvMYBA1*, which activated *VvBBX44*. VvMYBA1, but not VvBBX44, directly modulated the expression of grape UDP flavonoid 3-O-glucosyltransferase (*VvUFGT*). We demonstrated that VvBBX44 could repress the transcriptional activation of *VvUFGT* and *VvBBX44* induced by VvMYBA1. However, VvBBX44 and VvMYBA1 did not physically interact in yeast. The application of exogenous anthocyanin stimulated *VvBBX44* expression in grapevine suspension cells and tobacco leaves. These findings suggest that VvBBX44 and VvMYBA1 form a transcriptional feedback loop to prevent overaccumulation of anthocyanin and to reduce metabolic costs. Our work sheds light on the complex regulatory network that controls anthocyanin biosynthesis in grapevine.

Utilization of multi-scale plant phenotyping techniques for fruit trees to enhance smart orchard management

Yue Mu[1], Seishi Ninomiya[1,2], Shutian Tao[3], Wei Guo[2], Hao Yin[3], Shaoling Zhang[3], Gengchen Wu[1], Haitao Li[4], Yibo Zhang[5], Huimin Wang[1], Hao Sun[1]

[1]*Academy for Advanced Interdisciplinary Studies, Collaborative Innovation Center for Modern Crop Production Co-Sponsored by Province and Ministry, Nanjing Agricultural University, Nanjing 210095, China*

[2] *Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Midori-cho, Tokyo 188-0002, Japan*

[3] *Centre of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Nanjing Agricultural University, Nanjing 210095, China*

[4] *College of Artificial Intelligence, Nanjing Agricultural University, Nanjing 210095, China*

[5] *College of Engineering, Nanjing Agricultural University, Nanjing 210031, China*

yuemu@njau.edu.cn

Abstract

Fruit tree phenotyping, which includes dynamically monitoring external geometric characteristics and internal physiological and biochemical information, forms the foundation for decision-making in orchard management and agricultural machine operations. With the development of sensor technology, multi-platform-based fruit tree phenotyping has become increasingly diverse, leading to significant progress in the acquisition of multi-scale information about fruit trees. However, several challenges persist; for instance, the cultivation modes for fruit trees can have substantial impacts on tree architecture, canopy structure, and arrangement of flowering and fruiting; variations in environmental factors, particularly changes in lighting conditions, can significantly affect the image acquisition quality of optical sensors; and dense branch and leaf cover, as well as the clustering of fruits and flowers, can greatly influence object detection accuracy. To address these challenges, it is important to apply multi-source data fusion and artificial intelligence algorithms. This report illustrates applications of multi-source data and artificial intelligence algorithms in the multi-scale phenotyping of fruit trees. It aims to illustrate how phenotyping technology contributes to the development of smart orchards, including the following aspects:

(1) Dynamic monitoring of fruit tree canopy development using UAV platforms.
(2) Generation of colorized three-dimensional orchard maps via dynamic vehicle platforms.
(3) Characterization of tree architecture, branches, and leaves by analyzing the three-dimensional model of fruit trees using static laser scanning.
(4) Extraction of two-dimensional and three-dimensional fruit shape features using an indoor phenotyping platform, thereby enabling automated fruit picking on assembly lines.

Annual hormone and sugar pattern configuration confirms flowering in pitaya (*H. polyrhizus*): A dragon fruit

Kamran Shah, Yonghua Qin

Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou 510642, China

kamranshah801@scau.edu.cn; qinyh@scau.edu.cn

Abstract

Flowering is a complex phase change in a plant's life cycle that is controlled by multiple core stimuli. We found dynamic changes in sugar, hormone, and key sugar metabolism, hormone synthesis, and response-related genes that reveal the natural transmission behavior of phase change in pitaya (*Hylocereus polyrhizus*). Ultra-high performance liquid chromatography (u-HPLC) analysis revealed that sugar (including sucrose, glucose, and fructose) and auxin (AUX) contents were upregulated, while gibberellin-3 and -4 (GA3, GA4) were downregulated during the flower induction stages (early, middle, and late) of pitaya. At each stage, transcriptome analysis was conducted to analyze the transcriptional response of buds. Pathway enrichment was detected in differentially expressed genes (DEGs). Sugar metabolism genes, including *DPE2, BAM1, BAM2, GLU3, CEL1, SUS6, SUS7, SS,* and *SPP2*, were differently expressed at different stages, reflecting that pitaya needs sufficient sugar to flower. Auxin-related genes, including *AUX22D, AUX22, IAA4, IAA8, IAA9, IAA12, IAA13, IAA14, IAA16, IAA26, IAA27, ARF8, ARF9, ARF21, SAUR20, SAUR32, SAUR50, SAUR70*, and GA3-related genes like *CXE6, SCL6, SCL7, SCL13, SCL14, SCL15, SCL18, SCL23, SCL28, CIGR1, GAI1, GID2,* and *GA2OX*, were differently expressed during the flower induction stages. Furthermore, several abscisic acid, brassinosteroid, cytokinin, jasmonic acid, and salicylic acid genes were also detected as differently expressed. These findings showed that AUX promotes pitaya flowering, whereas GA3 inhibits it. In addition, the key floral genes SQUAMOSA Promoter-Binding Protein-Like (*SPL*), Agamous (*AGL*), FT-interacting protein (*FT*), zinc finger protein CONSTANS-LIKE (*CO*), and TCP family transcription factors (*TCP*) were detected as active players in response to hormones during the phase transitions of pitaya. To our best knowledge, there is no report on the dynamic changes that occur in buds annually during the flower induction period of pitaya. This data provides a new theoretical reference for the management of pitaya flowering and an essential foundation for future analysis of the regulation and control of flowering in pitaya.

Analyses of Cullin1 Homologs Reveal Functional Redundancy in S-RNase-Based Self-Incompatibility and Evolutionary Relationships in Eudicots

Linhan Sun[1], Shiyun Cao[2], Ning Zheng[2], Teh-hui Kao[1]

[1] *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA*

[2] *Howard Hughes Medical Institute, Department of Pharmacology, University of Washington, Seattle, WA 98195, USA*

lms6431@psu.edu

Abstract

Self-incompatibility (SI) is an intraspecific reproductive barrier, by which pistils reject self-pollen to prevent inbreeding, but accept nonself pollen to promote outcrossing. In *Petunia* (Solanaceae family), SI is regulated by the polymorphic *S*-locus, which contains the pistil-specific *S-RNase* gene and multiple pollen-specific *S-Locus F-box* (*SLF*) genes. SLFs assemble into E3 ubiquitin ligase complexes known as Skp1–Cullin1–F-box complexes (SCF^{SLF}). In pollen tubes, these complexes collectively mediate ubiquitination and degradation of all nonself S-RNases, but not self S-RNase, resulting in cross-compatible, but self-incompatible, pollination. Previously, using CRISPR/Cas9-mediated genome editing in *Petunia inflata,* we showed that a pollen-specific Skp1 protein in SCF^{SLF}, named PiSSK1, functioned specifically in SI. Here, we report that two pollen-expressed Cullin1 (CUL1) proteins, PiCUL1-P and PiCUL1-B, function redundantly in SI, with PiCUL1-P as the predominant CUL1. This redundancy between CUL1-P and CUL1-B is lost in *Petunia hybrida*, not because of the inability of CUL1-B of *P. hyrbida* (PhCUL1-B) to biochemically interact with SSK1, but due to a reduction in the *PhCUL1-B* transcript level. This is possibly caused by the presence of a DNA transposon sequence in the promoter region of *PhCUL1-B*, which was likely to be inherited from *Petunia axillaris*, one of the parental species of *P. hybrida*. Using phylogenetic and syntenic analyses of *Cullin* genes in various eudicots, we show that three Solanaceae-specific *CUL1* genes share a common origin, with *CUL1-P* dedicated to S-RNase-related reproductive processes in the Solanaceae. However, *CUL1-B* is a dispersed duplicate of *CUL1-P* that has been only found in *Petunia*, and not in the other species of the Solanaceae family we examined. These analyses also suggest that the *CUL1*s involved (or potentially involved) in the SI response in eudicots share a common origin.

Virtual Broccoli Farmland Implementation by Drone-based Phenotyping and Cross-scale Data Fusion

Haozhou Wang[1], Tang Li[1], Erika Nishida[2], Yoichiro Kato[1], Yuya Fukano[1], Wei Guo[1]

[1] *Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
[2] *Graduate School of Horticulture, Chiba University, Chiba, Japan.*

haozhou-wang@outlook.com

Abstract

Broccoli head plays an important role in the vegetable market. Obtaining their growth status in the field in a timely manner is essential for proper crop management decisions and thus has the potential to maximize profits. However, limited by efficiency and human cost, it is not cost-effective to manually investigate the entire farmland. The recent utilization of drone-based aerial phenotyping has provided an efficient means of conducting individual investigations. However, the presence of occlusion prevents access to the invisible portions. In this study, we propose a cross-scale data fusion approach to restore and visualize the invisible parts of each individual broccoli head in the entire farmland. Firstly, we developed a close-range phenotyping pipeline to acquire a comprehensive and high-quality database of 3D broccoli heads. Next, we enhanced the aerial phenotyping pipeline to acquire the morphological characteristics of individual broccoli. Based on the aforementioned pipelines, the closest model was identified from the close-range database for each individual broccoli head from the aerial pipeline. Subsequently, we reintegrated them into aerial field models following geometric transformations. Finally, a 3D virtual visualization of the farmland was presented. Although the proposed template matching approach did not strictly reflect the actual conditions in the field, it offers a potential solution for addressing the invisible parts beneath the canopy. Additionally, it serves as a fundamental technique for the development of digital twins and smart agriculture in the future.

Reciprocal regulation of flower induction by ELF3α and ELF3β generated via alternative promoter usage

Peng Wang, Yu Li, Zhe Liu, Xuhan Li, Shaoling Zhang, Juyou Wu

State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Sanya Institute of Nanjing Agricultural University, Nanjing Agricultural University, Nanjing 210095, China

wangpeng@njau.edu.cn

Abstract

Flowering is critical for sexual reproduction and fruit production. Several pear (Pyrus sp.) varieties produce few flower buds, but the underlying mechanisms are unknown. The circadian clock regulator EARLY FLOWERING3 (ELF3) serves as a scaffold protein in the evening complex that controls flowering. Here, we report that the absence of a 58-bp sequence in the second intron of PbELF3 is genetically associated with the production of fewer flower buds in pear. From rapid amplification of cDNA ends sequencing results, we identified a short, previously unknown transcript from the PbELF3 locus, which we termed PbELF3β, whose transcript level was significantly lower in pear cultivars that lacked the 58-bp region. The heterologous expression of PbELF3β in Arabidopsis (*Arabidopsis thaliana*) accelerated flowering, whereas the heterologous expression of the full-length transcript PbELF3α caused late flowering. Notably, ELF3β was functionally conserved in other plants. Deletion of the second intron reduced AtELF3β expression and caused delayed flowering time in Arabidopsis. AtELF3β physically interacted with AtELF3α, disrupting the formation of the evening complex and consequently releasing the repression of flower induction genes such as GIGANTEA (GI). AtELF3β had no effect in the absence of AtELF3α, supporting the idea that AtELF3β promotes flower induction by blocking AtELF3α function. Our findings show that alternative promoter usage at the ELF3 locus allows plants to fine-tune flower induction.

Study of horticulture from a subcellular perspective: challenges and solutions.

Pengwei Wang

National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, Hubei Province, China.

wangpengwei@mail.hzau.edu.cn

Abstract

To ensure different biochemical reactions take place simultaneously, eukaryotic cells have evolved several membrane-bound organelles, which coordinate their functions through proteins involved in signal transduction, vesicle trafficking, and metabolic pathways. Therefore, studying biological questions from a subcellular perspective will provide useful information for understanding the molecular machinery of various processes. Compared to Arabidopsis, little is known about the subcellular activities of horticultural plants, especially fleshy fruits, which are difficult to study using conventional light microscopy due to their tissue complexity. Here, we introduce some recent advances in the cell biology of fruit-related studies in our lab, such as the application of fluorescent protein labelling, fluorescence lifetime imaging and high-throughput imaging analysis. We also highlight a few examples of inappropriate image acquisition that are likely to produce false conclusions and provide our reasonings and solutions to these issues.

Agrobacterium rhizogenes-Mediated Marker-Free Transformation and Gene Editing System Revealed that AeCBL3 Mediates the Formation of Calcium Oxalate Crystal in Kiwifruit

Pengwei Li[1,†], Yiling Zhang[1,†], Jing Liang[1], Xufan Hu[1], Yan He[1], Tonghao Miao[1], Zhiyin Ouyang[1], Zuchi Yang[1], Abdul Karim Amin[1], Chengcheng Ling[1], Yize Liu[1], Heqiang Huo[2], Yongsheng Liu[1], Wei Tang[1], Songhu Wang[1]

[1] *Anhui Provincial Key Laboratory of Horticultural Crop Quality Biology, School of Horticulture, Anhui Agricultural University, Hefei 230036, China;*

[2] *Mid-Florida Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Apopka, FL 32703, USA.*

[†] These authors contribute equally.

wangsonghu@ahau.edu.cn

Abstract

The transformation and gene editing of the woody species kiwifruit are difficult and time-consuming. To date, a marker-free transformation and gene-editing system for kiwifruit has not yet been developed. Here, we establish a fast and efficient marker-free transformation and gene editing system mediated by *Agrobacterium rhizogenes* for kiwifruit. The transformation of *GUS* and *eGFP* indicated that the hairy root induction efficiency was about 50% and 80% of the induced transgenic hairy roots. Moreover, a removing-root-tip method was developed to significantly increase the regeneration efficiency of transgenic hairy roots. Through *A. rhizogenes*-mediated CRISPR/Cas9 gene editing, the editing efficiencies of *CEN4* and *AeCBL3* achieved 55% and 50%, respectively. Several homozygous knockout lines for both genes were obtained. Our method has been successfully applied in the transformation and gene editing of two different species of kiwifruit. Meanwhile, calcium oxalate (CaOx) crystals are widely present in most photosynthetic organisms, including kiwifruit, and play multiple roles in excess calcium excretion, heavy metal detoxification, and protection against herbivory. However, little is known about how CaOx crystals are formed in plants. Our results indicated that *AeCBL3* overexpression enhanced CaOx crystal formation, but its knockout via CRISPR/Cas9 significantly impaired crystal formation in kiwifruit. In summary, we developed a fast maker-free transformation and highly-efficient CRISPR-Cas9 gene editing system for the genetic modification of kiwifruit. Our work also revealed a novel gene mediating CaOx crystal formation as well as clues to the underlying mechanism.

Loss-of-susceptibility Mutations of Wall-Associated Kinase6 (CsWAK6) Confer Resistance to Necrotrophic Fungal Pathogen *Corynespora cassiicola* in Cucumber, *Cucumis sativus*

Yuhui Wang[1,2], Feifan Chen[1,3], Tusar K. Behera[1,4], Hang Wang[5], Aijun Mao[5], Xiaofei Zhang[5], Jingjing Yang[5], Changlong Wen[5], Jinyi Zhan[2], Ron Dymerski[1], Yugang Wang[1,6], Jinping Zhao[7], Junqi Song[7,8], Yiqun Weng[1,9]

[1] *Department of Plant and Agroecosystem Sciences, University of Wisconsin, Madison WI 53706, USA*
[2] *State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China*
[3] *Horticulture College, Northwest A&F University, Yangling, Shaanxi, China*
[4] *ICAR-Indian Institute of Vegetable Research, Varanasi, 221 305, Uttar Pradesh, India*
[5] *State Key Laboratory of Vegetable Bio-breeding, National Engineering Research Center for Vegetables, Key Laboratory of Biology and Genetics Improvement of Horticultural Crops (North China), Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Science, Beijing 100097, China.*
[6] *College of Horticulture, Shenyang Agricultural University, Shenyang, Liaoning, China*
[7] *Texas A&M AgriLife Research Center at Dallas, Texas A&M University System, Dallas, TX 75252, USA*
[8] *Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843, USA.*
[9] *USDA-ARS Vegetable Crops Research Unit, Madison, WI 53705, USA*

Abstract

Most of the ~300 plant *R* genes cloned so far encode cell-surface or intracellular receptors conferring resistance to biotrophic or hemibiotrophic pathogens. Here, we report the identification and characterization of a single dominant *R* locus, *Cca-4*, in cucumber (*Cucumis sativus* L.), which confers high resistance to the necrotrophic pathogen *Corynespora cassiicola* (CCA), the causal agent of target leaf spot (TLS) disease of many crops. *Cca-4* encodes an RD-type, plasma membrane bound, and wall-associated kinase (WAK) protein CsWAK6. A 1-bp deletion in the susceptible allele (*CsWAK6*^{S}) resulted in the loss of susceptibility, dominant-negative resistance allele (*CsWAK6*^{R}) encoding a truncated protein with a partial kinase domain. The expression of *CsWAK6*^{S} was upregulated in response to CCA infection, and its transcription level was positively correlated with pathogen growth and disease symptom development. We further show that the extracellular domain of CsWAK6 interacts with itself, suggesting that CsWAK6^R may quench the function of CsWAK6^S in TLS susceptibility through the formation of a homo- or heterodimer. Both overexpression of the allele *CsWAK6*^{R} and editing of the allele *CsWAK6*^{S} in "9930" (harboring *CsWAK6*^{S}) reduced the expression level of *CsWAK6* in response to CCA infection and conferred disease resistance. In natural populations, *CsWAK6*^{R} has several haplotypes with India/Pakistan origin that is under breeding selection from landraces to improved varieties. The WAK cluster harboring the *CsWAK6* gene is well conserved, which may be driven by *copia*-like retrotransposon across Cucurbitaceae crops. *CsWAK6*^{R} may represent a novel WAK-type *R* gene for broad-host-range necrotrophic fungal pathogens in plants. The dominant-negative nature of the *Cca-4* resistance allele, and the roles of CsWAK6^R in basal defense with water soaking during early pathogen infection are discussed.

Phylogenomic discovery of deleterious mutations facilitates hybrid potato breeding

Yaoyao Wu[1,2], Dawei Li[1,3], Yong Hu[1,4], Hongbo Li[1†], Guillaume P. Ramstein[5†], Shaoqun Zhou[1], Xinyan Zhang[1], Zhigui Bao[1,6], Yu Zhang[1,7], Baoxing Song[8], Yao Zhou[1,9,10], Yongfeng Zhou[1], Edeline Gagnon[11], Tiina Särkinen[12], Sandra Knapp[13], Chunzhi Zhang[1], Thomas Städler[14], Edward S. Buckler[2,15], Sanwen Huang[1,3†]

[1] *Chinese Academy of Agricultural Sciences*
[2] *Institute for Genomic Diversity, Cornell University*
[3] *Chinese Academy of Tropical Agricultural Sciences*
[4] *The AGISCAAS-YNNU Joint Academy of Potato Sciences, Yunnan Normal University*
[5] *Center for Quantitative Genetics and Genomics, Aarhus University*
[6] *Department of Molecular Biology, Max Planck Institute for Biology Tübingen,*
[7] *School of Agriculture, Sun Yat-sen University*
[8] *Peking University Institute of Advanced Agricultural Sciences*
[9] *Institute of Botany, Chinese Academy of Sciences*
[10] *College of Life Sciences, University of Chinese Academy of Sciences,*
[11] *Technische Universität München, Chair of Phytopathology, TUM School of Life Sciences*
[12] *Royal Botanic Garden Edinburgh*
[13] *Natural History Museum*
[14] *Institute of Integrative Biology & Zurich–Basel Plant Science Center, ETH Zurich*
[15] *USDA-ARS*
[†] These authors contributed equally.

Abstract

Hybrid potato breeding transforms the crop from a clonally propagated tetraploid to a seed-reproducing diploid. Historical accumulation of deleterious mutations in potato genomes has hindered the development of elite inbred lines and hybrids. Here, we assembled the genomes of 32 species (38 genomes) and constructed a deep phylogeny of the botanical family Solanaceae using 92 Solanaceae and its sister clade species (100 genome sequences). Utilizing this whole-genome phylogeny, we employed an evolutionary strategy to identify deleterious mutations. The deep phylogeny revealed the genome-wide landscape of highly constrained sites, which comprised 2.4% of the genome. Based on a diploid potato diversity panel, we inferred 367,499 deleterious variants, of which 50% occurred at non-coding and 15% occurred at synonymous sites. Counterintuitively, diploid lines with relatively-high homozygous deleterious burden can be better starting material for inbred-line development, despite showing less vigorous growth. This will guide the development of good-performing potato inbred lines, which is a key process of hybrid potato breeding. Inclusion of inferred deleterious mutations increased the genomic-prediction accuracy for yield by 24.7%, which can be applied to genomic selection (GS), thereby accelerating hybrid potato breeding. Our study provides insights into the genome-wide incidence and properties of deleterious mutations and their far-reaching consequences for breeding.

Comparative Study of Large-scale Plant Identification Using Deep Learning

Mingle Xu[1,2], Sook Yoon[3], Yongchae Jeong[1], Dong Sun Park[1,2]

[1] *Department of Electronics Engineering, Jeonbuk National University, Jeonbuk, South Korea*
[2] *Core Research Institute of Intelligent Robots, Jeonbuk National University, Jeonbuk, South Korea*
[3] *Department of Computer Engineering, Mokpo National University, Jeonnam, South Korea*

xml@jbnu.ac.kr

Abstract

Identifying plant species is one of the essential steps to maintaining biodiversity, which has been threatened by human activities in recent years. Here, we performed large-scale plant identification using deep learning. A large-scale dataset collected by worldwide experts in a crowdsensing manner, PlantCLEF2022, with 80,000 plant species, 3,995,568 training images, and 55,306 test images, was adopted. The dataset was challenging because of its scale and enormous image variations in plant stages and organs, image scales, and illuminations. We emphasize that the task is worthwhile as it is a kind of real-world application, compared to those tasks with either small datasets or small image variations. With this challenge, several techniques and factors in deep learning were analyzed. The initial ones were architecture, convolution neural networks (CNNs) and vision transformers (ViTs). The transfer learning paradigm was second, while the characteristics of the training datasets were third. Based on these experiments, we contend that plant identification can be considered from the perspective of a computer vision task, yet it has its own particularities, which suggests that some techniques and methods in computer vision are beneficial and that new opportunities and methods are necessary for better performance.

Predicting Desired Fertigation for Greenhouse Crops Using Internet of Things Sensors and Time-Series Model

Mingle Xu[1,2], Sook Yoon[3], Jongbin Park[1,2], Jeonghyun Baek[4], Dong Sun Park[1,2]

[1] *Department of Electronics Engineering, Jeonbuk National University, Jeonbuk, South Korea*
[2] *Core Research Institute of Intelligent Robots, Jeonbuk National University, Jeonbuk, South Korea*
[3] *Department of Computer Engineering, Mokpo National University, Jeonnam, South Korea*
[4] *Rural Development Administration, Jeonbuk 54875, South Korea*

xml@jbnu.ac.kr

Abstract

Greenhouses provide opportunities for large yield with effective and efficient practices. However, many resources are required, such as fertigation, a kind of nutrient in solution. Resources are essential for crop cultivation, and resource shortage hinders plant growth, whereas resource surplus results in waste. In this paper, we investigated the fertigation supply. We found that excess fertigation leads to drainage, which is difficult to purify and threatens the environment. To address this challenge, we predicted the desired amount of fertigation. To achieve this objective, we first established a prototype to record the climate conditions inside a rose greenhouse using Internet of Things sensors. Simultaneously, the desired fertigation amount was obtained with the help of weight scale and historical data of fertigation supply and drainage. Second, a method was proposed to predict the desired fertigation by taking the sensors' data as input, with a time-series model. The experimental results suggest the potential of our method.

Current and Future Plant Disease Datasets in the Era of Deep Learning

Mingle Xu[1,2], Ji Eun Park[1,2], Jaehwan Lee[1,2], Taehyun Kim[3], Sook Yoon[4]

[1] *Department of Electronics Engineering, Jeonbuk National University, Jeonbuk, South Korea*

[2] *Core Research Institute of Intelligent Robots, Jeonbuk National University, Jeonbuk, South Korea*

[3] *Rural Development Administration, Jeonbuk 54875, South Korea*

[4] *Department of Computer Engineering, Mokpo National University, Jeonnam, South Korea*

xml@jbnu.ac.kr

Abstract

Plant disease recognition has witnessed a significant improvement with deep learning in recent years. Although plant disease datasets are essential and many relevant datasets are publicly available, two fundamental questions exist. First, how to differentiate datasets and further choose suitable public datasets for specific applications? Second, what kinds of characteristics of datasets are desired to achieve promising performance in real-world applications? To address these questions, this study explicitly proposes an informative taxonomy to describe potential plant disease datasets. We further provide several directions for the future, creating challenge-oriented datasets, with the ultimate objective of deploying deep learning in real-world applications with satisfactory performance. In addition, the existing related public datasets are non-trivially summarized. We believe that this study will facilitate the community.

Y/DCAR_032551 encodes a RPGE protein that repress carotenoid biosynthesis via interaction with DcAPRR2

Ying-Gang Wang[1], Yu-Min Zhang[1], Kai Zhang[1], Ya-Hui Wang[1], Jing Ma[1,2], Hui Liu[1], Ai-Sheng Xiong[1], Zhi-Sheng Xu[1]

[1] *State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Ministry of Agriculture and Rural Affairs Key Laboratory of Biology and Germplasm Enhancement of Horticultural Crops in East China, College of Horticulture, Nanjing Agricultural University, 1 Weigang, Nanjing, 210095, China*
[2] *College of Horticulture, Qingdao Agricultural University, 700 Changcheng Road, Qingdao, 266109, China*

xuzhisheng@njau.edu.cn

Abstract

The first domesticated carrot was yellow in color and originated from white wild carrot, while orange carrot was selected through this yellow carrot intermediate and predominates the world today. The carrot taproot color change from white to yellow is attributed to carotenoid accumulation and is believed to be triggered by genetic mutation in the quantitative trait locus *Y*. *DCAR_032551* was identified as a candidate for the *Y* gene but has not been functionally validated. Here, we show that *DCAR_032551* encodes a *REPRESSOR OF PHOTOSYNTHETIC GENES* (RPGE) protein, namely DcRPGE1. Expression of DcRPGE1 from wild carrot (DcRPGE1^{W}) in yellow and orange carrots strongly reduced carotenoid accumulation, while knockout of DcRPGE1^{W} from wild carrot promoted carotenoid accumulation and changed the taproot color from white to yellow. DcRPGE1^{W} physically interacts with DcAPRR2 in the nucleus. Through this interaction, DcRPGE1^{W} represses DcAPRR2-mediated transcriptional regulation of *DcPSY1*, *DcPSY2*, and *DcLCYE*, and thus strongly reduces carotenoid biosynthesis. DcAPRR2 directly binds to the newly found RGATTY elements in the promoter regions of *DcPSY1*, *DcPSY2*, and *DcLCYE*, which can be blocked by DcRPGE1^{W}. In yellow and orange carrots, a natural insertion mutation in the second exon region of DcRPGE1 gives rise to alternatively spliced transcripts with premature stop codons. These transcripts encode truncated proteins that could not interact with DcAPRR2 and repress carotenoid accumulation. Collectively, our results provide new insights into the regulation of carotenoid accumulation and effective strategies to breed carotenoid-rich crops.

Unravelling the Multifaceted Control of Apple Fruit Development by microRNA172

Jia-Long Yao[1], Ringo Feng[1], Bin Xia[1], Rongmei Wu[1], Andrew Gleave[1], Tiyi Ding[2], Zhenli Yan[2], Hengtao Zhang[2]

[1] *The New Zealand Institute for Plant and Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand*
[2] *Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, 28 Gangwan Road, Zhengzhou, 450009 China*

Abstract

MicroRNA172 (miR172) is a well-conserved miRNA found across the plant kingdom. Its expression may stem from various gene homologs, exhibiting distinct temporal and spatial expression patterns in each species. It acts by targeting the mRNA of AP2 genes, leading to translational inhibition and mRNA degradation. The AP2 genes themselves have multiple homologs that are expressed in diverse tissues and at different developmental stages. Consequently, the miR172-AP2 regulatory module potentially governs several facets of plant development. To elucidate the role of miR172 in apple fruit development, we generated stable transgenic plants of the cultivar 'Royal Gala' to modulate miR172 expression levels. Plants with overexpression of miR172 produced remarkably small fruit resembling crab apples. This aligns with observations of higher miR172 expression and small fruit size in wild crab apples, and the apple cultivars with much larger fruit that are homozygous for a transposable element insertion in a miRNA172 gene (Yao et al., 2015). Overexpression of miR172 also led to a reduction in anthocyanin accumulation in the fruit skin through the AP2-MYB10 regulatory module (Ting et al., 2022). More recently, we examined transgenic apple plants with suppressed miR172 expression. The data indicated that miR172 silencing delays fruit ripening through the AP2-NAC-ERF regulatory module. Furthermore, miR172 silencing enhances parthenocarpic fruit growth in the absence of pollination or fertilisation. This is consistent with the observation that there is a reduction in fruit growth when miR172 is overexpressed. In conclusion, the miR172-AP2 module governs multiple facets of apple fruit development.

Identification and Characterization of Geranylgeranyl Diphosphate Synthase (GGPPS) Genes Responsible for Carotenoid Biosynthesis in Zinnia elegans Flowers

Chao Zhang, Guo Yu, Jian-Xin Fu

Zhejiang Provincial Key Laboratory of Germplasm Innovation and Utilization for Garden Plants, School of Landscape Architecture, Zhejiang Agriculture and Forestry University, Hangzhou 311300, Zhejiang, China

zhangc@zafu.edu.cn

Abstract

Geranylgeranyl diphosphate (GGPP) plays a pivotal role in the intricate terpene biosynthesis pathway, serving as the precursor for a number of vital phytochemicals, including carotenoids. Carotenoids are important in the petal coloration of *Zinnia elegans* flowers. However, it is unclear how GGPPSs orchestrate the synthesis of carotenoids within *Z. elegans* flowers. In this study, we isolated and characterized four *ZeGGPPS* genes. Conservative domain analysis and homology analysis showed that ZeGGPPS1 and ZeGGPPS4 belonged to the large subunit (GGPPS-LSU) subclade, ZeGGPPS2 belonged to the small subunit I (GGPPS-SSUI) subclade, and ZeGGPPS3 belonged to the small subunit II (GGPPS-SSUII) subclade. All four members were found within chloroplasts, and ZeGGPPS2 also exhibited extra-chloroplastic localization. By employing pigment complementation assays, we successfully demonstrated the catalytic activity of ZeGGPPS1 and ZeGGPPS4, and ZeGGPPS4 was more active than ZeGGPPS1. Yeast two-hybrid assays showed that, similar to ZeGGPPS1, ZeGGPPS4 could interact with ZeGGPPS2 and ZeGGPPS3. Heterodimers' pigment complementation assays illustrated that ZeGGPPS2 reduced the enzymatic activity of ZeGGPPS1 and ZeGGPPS4, while ZeGGPPS3 enhanced the enzymatic activity of ZeGGPPS1 and ZeGGPPS4. Furthermore, the spatiotemporal expression pattern of *ZeGGPPSs* showed that *ZeGGPPS1* and *ZeGGPPS3* were predominantly expressed in leaves, while *ZeGGPPS4* was predominantly expressed in both leaves and petals, and the expression levels of *ZeGGPPS1*, *ZeGGPPS3*, and *ZeGGPPS4* in red flower cultivars were significantly lower than those in yellow flower cultivars. Taken collectively, ZeGGPPS1, ZeGGPPS3, and ZeGGPPS4 assume principal responsibility for carotenoid biosynthesis in the petal coloration of *Z. elegans* flowers. This work lays a solid foundation for future investigations into carotenoid biosynthesis and terpene metabolism in *Z. elegans*.

Carotenoid cleavage dioxygenase ZeCCD4 catalyzes β-carotene degradation to regulate carotenoid accumulation and petal coloration in Zinnia elegans

Chao Zhang, Hong-Sheng Qing, Xin-Yue Liu, Jian-Xin Fu

Zhejiang Provincial Key Laboratory of Germplasm Innovation and Utilization for Garden Plants, School of Landscape Architecture, Zhejiang Agriculture and Forestry University, Hangzhou 311300, Zhejiang, China

zhangc@zafu.edu.cn

Abstract

The content of carotenoids, as a widely distributed natural pigment, influence petal coloration in many plants. Carotenoid cleavage dioxygenase (CCD) directly affects carotenoid content by cleaving the double bond of carotenoids. Common zinnia (*Zinnia elegans*) is an annual ornamental flower belonging to the Asteraceae family, with richly-colored petals from ray floret and excellent ornamental value. Carotenoids are one of the important coloring substances in the ligulate flower color of *Zinnia elegans*. Currently, little is known about the molecular mechanism of carotenoid coloring in the petals of this flower. In this study, the total carotenoid content of petals in 'Dreamland Red' (DRE), 'Dreamland Coral' (DC) and 'Dreamland Yellow' (DY) at different developmental stages was analyzed. With the development of capitulum, the total carotenoid content was highest in DY and lowest in DRE. Three *ZeCCDs* (*ZeCCD1*, *ZeCCD4-1* and *ZeCCD4-2*) were screened from *Zinnia elegans*. The bacterial pigment complementation system revealed that the three ZeCCDs had the ability to cleave β-carotene and other carotenoids (ε-carotene, zeaxanthin and lycopene). Expression analysis showed that the expression patterns of *ZeCCD1* and *ZeCCD4-2* in the petals of different cultivars were significantly negatively correlated with the carotenoid content. Subcellular localization showed that both ZeCCD4-1 and ZeCCD4-2 were localized in the plastid, whereas ZeCCD1 was localized in the cytoplasm. These results indicate that *ZeCCD4-2* is the key gene responsible for the differential accumulation of carotenoids in the petals of different *Zinnia elegans* cultivars.

Serotonin N-acetyltransferase 1 Acts as a Redox-regulated Switch for State Transitions During Photosynthesis Photoreaction in Tomato

Xiaoyun Wang[1], Jianghao Wu[2], Yang-Dong Guo[1], Na Zhang[1]

[1] *China Agricultural University*
[2] *Henan University*

Abstract

In nature, due to the variability of environmental factors, plants grow in a dynamic light environment which affects the growth and productivity of field crops. The fluctuating light breaks the balance of electron transport, which causes ROS overaccumulation in chloroplasts and changes the redox state of the PQ pool. It is considered as a kind of abiotic stress. State transitions are a dynamic process to balance the amount of light energy received by photosystem I (PSI) and photosystem II (PSII) to maintain an optimal photosynthetic yield and to minimize photo-damage under a fluctuating light environment. Reversible phosphorylation of the light-harvesting complex of PSII (LHCII) has been considered critical for regulating state transitions. While acetylation of photosynthetic proteins also plays an important role in state transitions, the molecular mechanisms are poorly understood. In this study, we identify a lysine acetyltransferase, serotonin N-acetyltransferase 1 (SNAT1), in *Solanum lycopersicum* and show that *snat1* mutants are deficient in state transitions and retarded in growth under fluctuating light and display a late-ripening fruit phenotype when grown in a greenhouse. Quantitative lysine (Lys) acetylome analysis suggests that ^{6}Lys of mature Lhcb2 protein is the target of SNAT1 and is involved in state transitions. ^{131}Cys-related redox changes of SNAT1 affect its acetylation activity on Lhcb2. Therefore, we propose that the chloroplast redox state may regulate the activity of SNAT1, which in turn acetylates ^{6}Lys of Lhcb2 to switch on state transitions in higher plants when facing fluctuating light stress.

Harnessing simulation models for achieving smart horticultural production systems

Ningyi Zhang, Shaoling Zhang, Juyou Wu

College of Horticulture, Nanjing Agricultural University, 210095, Nanjing, China

ningyi.zhang@njau.edu.cn

Abstract

Current horticultural crop systems face the challenge of achieving sustainable crop production with reduced labor cost and carbon emission while maintaining high yield and good product quality. This requires a transitioning of the current system into a smart horticultural production system, which is featured by high levels of autonomous control and automatic processing. Simulation models are useful tools for predicting plant growth responses to different environmental conditions, crop yield, and product quality. Thus, simulation models are a core part of smart horticultural production systems in terms of optimizing crop management, developing autonomous decision support algorithms, and designing robotics. This presentation first gives an introduction on simulation models, including two types of models, i.e., process-based models (PBMs) and machine learning (ML). PBMs simulate plant growth and development based on underlying mechanisms and have long been used in crop management optimizations in agricultural systems. ML has become a popular tool in agricultural applications thanks to the development of sensor and communication technologies for data acquisition. We provide an overview of the characteristics of both PBMs and ML, and their applications in different domains of smart horticulture. Next, we present a case study using a 3D modeling tool to facilitate the design of a smart cut-rose production system. Finally, we provide future perspectives in three aspects: (i) the potential of combining PBMs and ML, (ii) modeling of product quality, and (iii) modeling the growth of perennial plants (e.g., fruit trees).

Comparative Phylogenomics and Phylotranscriptomics Provide Insights into the Genetic Complexity of Nitrogen Fixing Root Nodule Symbiosis

Yu Zhang[1,17], Yuan Fu[1,15,16,†], Wenfei Xian[1,†], Xiuli Li[1], Yong Feng[1], Fengjiao Bu[1], Yan Shi[1], Shiyu Chen[1], Robin van Velzen[2], Kai Battenberg[3], Alison M. Berry[3], Marco G. Salgado[4], Hui Liu[5], Tingshuang Yi[5], Pascale Fournier[6], Nicole Alloisio[6], Petar Pujic[6], Hasna Boubakri[6], M. Eric Schranz[2], Pierre-Marc Delaux[7], Gane Ka-shu Wong[8], Valerie Hocher[9], Sergio Svistoonoff[9], Hassen Gherbi[9], Ertao Wang[10], Wouter Kohlen[11], Luis G. Wall[12], Martin Parniske[13], Katharina Pawlowski[4], Normand Philippe[6], Jeffrey J. Doyle[14], Shifeng Cheng[1]

[1] *Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518124, China*
[2] *Biosystematics Group, Department of Plant Sciences, Wageningen University, 6708 PB, Wageningen, The Netherlands*
[3] *Department of Plant Sciences, University of California, Davis, Davis, CA 95616, USA*
[4] *Department of Ecology, Environment and Plant Sciences, Stockholm University, 106 91 Stockholm, Sweden*
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† These authors contributed equally.

chengshifeng@caas.cn; jjd5@cornell.edu

Abstract

Plant root-nodule symbiosis (RNS) with mutualistic nitrogen-fixing bacteria is restricted to a single clade of angiosperms, the Nitrogen-Fixing Nodulation Clade (NFNC), and is best understood in the legume family. Nodulating species share many commonalities, explained either by divergence from a common ancestor over 100 million years ago or by convergence following independent origins over that same time period. Regardless, comparative analyses of diverse nodulation syndromes can provide insights into constraints on nodulation—what must be acquired or cannot be lost for functional symbiosis—and the latitude for variation in symbiosis. However, much remains to be learned about nodulation, especially outside of legumes. Here, we employed a large-scale phylogenomic analysis across 88 species, complemented by 151 RNA-seq libraries, to elucidate the evolution of RNS. Our phylogenomic analyses further emphasize the uniqueness of the transcription factor NIN as a master regulator of nodulation and identify key mutations that affect its function across the NFNC. Comparative transcriptomic assessment revealed nodule-specific upregulated genes across diverse nodulating plants, while also identifying nodule-specific and nitrogen-response genes. Approximately 70% of symbiosis-related genes are highly conserved in the four representative species, whereas defense-related and host-range restriction genes tend to be lineage specific. Our study also identified more than 900 000 conserved non-coding elements (CNEs), of which more than 300 000 are unique to sampled NFNC species. NFNC-specific CNEs are enriched with the active H3K9ac mark and are correlated with accessible chromatin regions, thus representing a pool of candidate regulatory elements for genes involved in RNS. Collectively, our results provide novel insights into the evolution of nodulation and lay a foundation for engineering of RNS traits in agriculturally important crops.

Regulatory mechanism of tomato plants responding to combined abiotic stress

Rong Zhou[1,2], Fangling Jiang[1], Eva Rosenqvist[3], Carl Otto Ottosen[2], Zhen Wu[1]

[1] *Nanjing Agricultural University, Nanjing, China*

[2] *Aarhus University, Aarhus, Denmark*

[3] *University of Copenhagen, Copenhagen, Denmark*

zhour@njau.edu.cn

Abstract

It is a challenge to produce sufficient food to feed the growing population using limited green land under stochastic climate conditions. Disasters from extreme weather can cause remarkable damage to crops and even whole agricultural systems. Crops, including vegetables, usually suffer changes in multifactorial environmental factors instead of individual factors, especially in natural systems. We aimed to clarify the regulatory mechanism of plants to combined abiotic stress, with tomato as an example. After triple screening of approximately 100 wild and cultivated tomato genotypes under different levels of heat stress, we identified two heat-tolerant and two heat-sensitive genotypes. Improved ability to remove reactive oxygen species, regulate stomatal density and area, and stay green of leaves contributed to the heat tolerance. However, the tolerant and sensitive genotypes did not exhibit differences in susceptibility to combined heat and drought stress, showing that the combined stress is a new state that can induce unique responses in tomato compared with single stress. Together with our findings in tomato exposed to combined heat and salinity, combined drought and cold, combined waterlogging and salinity, and combined waterlogging and cadmium stress, the effects of combined stress were not simply the sum of individual effects on plants. These effects depended on species and genotypes as well as stress intensity and duration, where increased CO_2 concentration can disrupt the regulatory mechanisms of plants. Our study will help to improve the climate resilience of plants to combined abiotic stress, to better respond to extreme weather events and to decrease the potential loss in plant production under climate change.

Parallel evolution of transcription factor and cis-element determine the evolution of Solanaceae diversified metabolites

Zhangsheng Zhu[1], Jiali Song[1], Xing Wang Deng[2], Jianjun Lei[1], Changming Chen[1], Bihao Cao[1], Guoju Chen[1], Ruyi Yang[1], Siqi Chen[1]

[1] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou, Guangdong 510642, China*

[2] *Institute of Plant and Food Science, Department of Biology, School of Life Sciences, Southern University of Science and Technology, Shenzhen 518055, China*

zhuzs@scau.edu.cn

Abstract

Plants biosynthesize many species-specific metabolites to adapt to their living environment, many of which have important biological activities. Transcription factors and cis-elements coevolve, leading to diversified specialized metabolites which remain unknown. This study found that the *Catharanthus roseus* IX subfamily ERF transcription factor ORCA3 homologous gene has evolved function to regulate capsaicin in *Capsicum*. Functional analysis found that the ORCA3 homologous gene in the Solanaceae family produces genius-specific metabolites, suggesting they are functionally equivalent and likely interchangeable. We discovered that coevolved ORCA gene expression patterns and biosynthetic promoter elements shape Solanaceae diversified metabolites. This study demonstrates that the coevolution of transcription factors and cis-elements might produce diverse plant metabolites.

Hydrogen peroxide signaling mediates dopamine-induced chromium stress tolerance in tomato

Golam Jalal Ahammed[1,2,3], Shuangsheng Sun[1], Shuangchen Chen[1,2,3]

[1] *College of Horticulture and Plant Protection, Henan University of Science and Technology, Luoyang 471023, China*

[2] *Henan International Joint Laboratory of Stress Resistance Regulation and Safe Production of Protected Vegetables, Luoyang 471023, China*

[3] *Henan Engineering Technology Research Center for Horticultural Crop Safety and Disease Control, Luoyang 471023, China*

ahammed@haust.edu.cn

Abstract

The toxic heavy metal chromium (Cr) reduces crop yield and threatens human health via contamination of the food chain. Dopamine, a bioactive naturally-occurring amine, enhances plant tolerance to a wide range of abiotic stresses; however, the role of dopamine in Cr stress tolerance and the underlying molecular mechanism remain largely unknown. Here, we reveal that root application of dopamine alleviated Cr stress in tomato plants. Cr stress decreased chlorophyll content, Fv/Fm, shoot growth, and biomass accumulation by increasing reactive oxygen species (ROS) accumulation, lipid peroxidation and electrolyte leakage, while exogenous dopamine minimized excessive ROS accumulation, malondialdehyde content and oxidative stress by increasing the activity of antioxidant enzymes, glutathione (GSH) and phytochelatin (PC), and the expression of their encoding genes such as *Cu-Zn SOD, POD, CAT1, APX, GR1, GSH2*, and *PCS* in leaves. Moreover, dopamine increased the expression of *RESPIRATORY BURST OXIDASE HOMOLOG1* (*RBOH1*) and decreased Cr content in leaves. Interestingly, similar to dopamine, exogenous H_2O_2 application also enhanced Cr tolerance; however, the application of an NADPH oxidase inhibitor, diphenyleneiodonium (DPI), aggravated Cr phytotoxicity and attenuated the beneficial effects of dopamine on plant tolerance to Cr stress, suggesting that root-applied dopamine-induced expression of *RBOH1* and associated H_2O_2 signaling mediates the dopamine-induced enhanced Cr tolerance. This work elucidates the fundamental mechanism of dopamine-mediated Cr tolerance and contributes to our existing knowledge of the stress resistance properties of dopamine in plants.

Glutathione mediates melatonin-promoted cadmium tolerance and sequestration in tomato (*Solanum lycopersicum* L.)

Golam Jalal Ahammed[1,2,3], Shuangchen Chen[1,2,3]

[1] *College of Horticulture and Plant Protection, Henan University of Science and Technology, Luoyang 471023, China*

[2] *Henan International Joint Laboratory of Stress Resistance Regulation and Safe Production of Protected Vegetables, Luoyang 471023, China*

[3] *Henan Engineering Technology Research Center for Horticultural Crop Safety and Disease Control, Luoyang 471023, China*

ahammed@haust.edu.cn

Abstract

Glutathione (GSH) plays a critical role in heavy metal tolerance, but the role of GSH in melatonin-induced cadmium (Cd) tolerance remains elusive. Here, we show that GSH is essential for melatonin-promoted Cd tolerance in tomato plants. Cd stress significantly inhibited the CO_2 assimilation rate, Fv/Fm, and plant dry weight, while exogenous melatonin alleviated the negative effects of Cd on photosynthesis and biomass. Importantly, suppression of GSH accumulation by its biosynthetic inhibitor buthionine sulfoximine (BSO) aggravated the Cd-induced phytotoxicity by increasing H_2O_2 accumulation, lipid peroxidation, and electrolyte leakage. The stimulatory effects of melatonin on the activities and transcript levels of antioxidant enzymes, as well as the concentrations of GSH and phytochelatins, were abolished by BSO treatment under Cd stress, which resulted in severe oxidative stress. Moreover, exogenous melatonin failed to reduce Cd accumulation in leaves and roots when GSH biosynthesis was blocked. Further analysis showed that endogenous melatonin deficiency, due to the suppressed expression of *CAFFEIC ACID O-METHYLTRANSFERASE 1* (*COMT1*), also aggravated Cd phytotoxicity; however, exogenous GSH mitigated the deleterious effects of melatonin deficiency under Cd stress. Melatonin deficiency increased Cd accumulation in organelles, but exogenous GSH decreased Cd accumulation in organelles and increased Cd accumulation in soluble and cell-wall fractions in tomato leaves. These results suggest that GSH acts downstream of melatonin in the Cd detoxification pathway and is essential for melatonin-promoted Cd sequestration in tomato plants.

Revealing the HubZIP family: Significance of HubHLH12 and HubHLH13 in orchestrating betalain biosynthesis by regulating the key structure genes in Pitaya

Irfan Ali Sabir, Xinglong Hu, Faiz Ur Rahman, Waqar Ahmed, Jiaxuan Chen, Jiayi Chen, Yonghua Qin

Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China

qinyh@scau.edu.cn

Abstract

The bZIP family, acknowledged as one of the most extensive and diverse groups of transcription factors (TFs), exerts a significant influence on various biological processes, including pigment biosynthesis. However, the molecular properties and functions of bZIP TFs in pitaya betalain biosynthesis have remained largely unexplored. In this study, we present the identification of 74 HubZIP genes within the *Hylocereus undatus* genome. We conducted comprehensive analyses of their chromosomal distributions, physiochemical characteristics, conserved motifs, gene structures, phylogenetic relationships, and synteny. Through phylogenetic analysis, these 74 HubZIP genes were categorized into 28 subfamilies and disseminated across the 11 chromosomes of pitaya. Utilizing pitaya transcriptome data, we identified two candidate genes, HubHLH12 and HubHLH13, which were further validated through real-time quantitative PCR analysis. Both genes exhibited increased expression levels during the coloring period of 'Guanhuahong' and 'Guanhuabai' pitaya, indicating their involvement in betalain biosynthesis. HubHLH12 and HubHLH13 are categorized as group II proteins, each containing a basic leucine zipper domain, and these nuclear proteins demonstrate transcriptional activation activity. Dual luciferase reporter assays and virus-induced gene silencing (VIGS) experiments confirmed that both genes play a pivotal role in promoting betalain biosynthesis by activating the expression of structural genes. The findings of this study not only contribute to our understanding of the regulatory mechanisms governing pitaya betalain biosynthesis but also serve as a fundamental resource for future investigations into the functions of the HubZIP gene family.

The study of the Regulation of the cAMP Signal Pathway on Photomorphogenesis of *Botrytis cinerea* and the Function of Adenylate Cyclase (BAC) PP2C Domain

Yunfei Cai[1,2], Xue Chen[2], Peixuan Li[2], Weiheng Ren[2], Qiang Zhang[2], Yiwen Wang[2], Yina Jiang[2], Pingkuan Zhu[2], Ling Xu[2]

[1] *Zhejiang Institute of Subtropical Crops*

[2] *East China Normal University*

cai41777@126.com

Abstract

Botrytis cinerea is a typical representative of necrotrophic plant pathogenic fungi. It has a broad host range, including many high-value fruits and vegetables such as tomatoes, cucumbers, grapes, and strawberries. The conidia produced under light conditions are key for the pathogenicity, asexual reproduction, stress resistance, and fast spreading of *B. cinerea*. Adenylate cyclase (AC) has been reported in many fungi to regulate growth, reproduction, and pathogenicity by synthesizing cAMP and activating downstream PKA kinase. However, the regulatory mechanism of the cAMP signaling pathway in the production of conidia and the function of the PP2C domain of AC has not been clarified. In a preliminary study, we isolated a *Botrytis cinerea* adenylate (BAC) S1407 site mutation in wild type (B05.10), which altered growth, conidial and sclerotium development, and pathogenicity. In this study, the S1407 site was proved to be an important conserved residue in the PP2C domain, which markedly impacts the phosphorylation status and enzyme activity of BAC, and the cAMP signaling pathway could stabilize the circadian rhythm which is associated with pathogenicity, conidiation, and sclerotium production. Furthermore, a study of the PP2C domain of BAC indicates that it can regulate the phosphorylation status of the *B. cinerea* MAPK signaling pathway component BcSak1 and may regulate *B. cinerea* mycelial growth and conidial germination through this pathway. In summary, a study of the cAMP signaling pathway and the PP2C domain of BAC largely contributes to our understanding of fungal AC and its cAMP signaling pathway. In addition, it provides an experimental basis for developing an efficient control measure for *B. cinerea* in the future.

Na^+-preferential ion transporter HKT1;1 mediates salt tolerance in blueberry

Hifang Song†, Yibo Cao†, Xinyan Zhao, and Lingyun Zhang

State Key Laboratory of Efficient Production of Forest Resources, Key Laboratory of Forest Silviculture and Conservation of the Ministry of Education, Research & Development Center of Blueberry, College of Forestry, Beijing Forestry University, Beijing 100083, China

† These authors contributed equally.

caoyibo@bjfu.edu.cn

Abstract

Soil salinity is a major environmental factor constraining the growth and productivity of highbush blueberry (*Vaccinium corymbosum*). Leaf Na^+ content is associated with variation in salt tolerance among blueberry cultivars; however, the determinants and mechanisms conferring leaf Na^+ exclusion are unknown. Here, we observed that the blueberry cultivar 'Duke' was more tolerant than 'Sweetheart' and accumulated less Na^+ in leaves under salt stress conditions. Through transcript profiling, we identified a member of the high-affinity K^+ transporter (HKT) family in blueberry, *VcHKT1;1*, as a candidate gene involved in leaf Na^+ exclusion and salt tolerance. *VcHKT1;1* encodes a Na^+-preferential transporter localized to the plasma membrane, which is preferentially expressed in root stele. Heterologous expression of *VcHKT1;1* in Arabidopsis (*Arabidopsis thaliana*) rescued the salt hypersensitivity phenotype of the *athkt1* mutant. Decreased *VcHKT1;1* transcript levels in blueberry plants expressing antisense-*VcHKT1;1* led to increased Na^+ concentrations in xylem sap and higher Na^+ contents in leaf compared with wild-type plants, indicating that VcHKT1;1 promotes leaf Na^+ exclusion by retrieving Na^+ from xylem sap. A naturally occurring 8-bp insertion in the promoter increased the transcription level of *VcHKT1;1*, thus promoting leaf Na^+ exclusion and blueberry salt tolerance. Collectively, we provide evidence that VcHKT1;1 promotes leaf Na^+ exclusion and propose natural variation in *VcHKT1;1* will be valuable for breeding Na^+-tolerant blueberry cultivars in the future.

Innovations and applications of carbon stock refurbishment in improving soil health and enhancing efficient vegetable production

YE Cao, WH Li, CX Liu

School of Wine & Horticulture, Ningxia University, Yinchuan, 750021, China

caohua3221@163.com

Abstract

With the increase of chemical agriculture practices, pollution, secondary salinization deterioration of structure, and deactivation in soil are worse than ever before. To effectively alleviate soil degradation, we applied different carbon stocks, including in-situ vermicomposting with biochar into soil. The reconstruction of carbon stock increased organic matter content, total nitrogen, available nitrogen, total phosphorus, available phosphorus, and available potassium content in the root-soil complex, while urease, phosphatase and sucrase activities were also improved. Based on these findings, the amount of autotoxic compounds, such as benzoic acid, showed significant decreases in the treatment of in-situ vermicomposting with biochar, suggestive of a positive alleviating effect on soil continuous cropping obstacles. In terms of management of root-knot nematodes, we found 10% leachate of earthworm to exhibit the best inhibitory effect on second-stage juveniles, with a mortality rate of 56.67% within 24 hours and 82.22% within 48 hours. When 7.5% leachate of earthworm was applied, the nematode egg hatching rate was the lowest, at only 17.48%. To further investigate the compounds of leachate of earthworm, four nematocidal compounds, including benzaldehyde, palmitic acid, benzene compounds, naphthalene, and its derivatives, were found. In summary, we have developed new biological products and formulations, including microbial compounds against disease, earthworm peptides, conditioners of wormcast with probiotics, and composite nematode-resistant agents such as benzaldehyde, palmitic acid, and naphthoic acid. This study provides a foundation for the further study of carbon stock refurbishment in improving soil health.

DELLA regulates reflowering of tree peony through interaction with UBQ1

Yanting Chang, Wenbo Zhang, Zehui Jiang, Tao Hu

Key Laboratory of National Forestry and Grassland Administration/Beijing for Bamboo & Rattan Science and Technology, Ornamental Plants and Landscape Institute, International Center for Bamboo and Rattan, Beijing 100102, China

cytcyt123456@126.com

Abstract

Tree peony (Paeonia suffruticosa Andr.) is an ornamental plant native to China, which is known as the "king of flowers" due to its ornamental, medicinal, and oil value. However, due to the short flowering period and once-a-year flowering characteristics of conventional varieties, the short effective ornamental period of tree peony restricts its commercial application. In this study, the once-a-year flowering variety 'Fengdan' and the twice-a-year flowering variety 'Haihuang' were used to analyze floral bud development using transcriptome and metabolome data. The regulatory network of plant hormones involved in the re-flowering of tree peony was constructed, and gibberellin and abolic acid, which regulate the key stages of floral bud development in the secondary flowering process, were identified. A key gene, *DELLA*, related to the secondary development of floral buds, was identified through co-expression network analysis. The ubiquitin enzyme gene *UBQ1* was found to interact with DELLA by yeast two-hybrid screening. Allogeneic expression of DELLA in *Arabidopsis thaliana* proved that the gibberellin pathway was involved in regulating the re-flowering process of peony. This study provides evidence for the regulatory mechanism of flowering in tree peony and also provides a new candidate gene for subsequent flowering-modified breeding in tree peony.

Morphological and Physiological Indicators and Transcriptome Data Analyses Reveal the Mechanism of Selenium Multilevel Mitigation of Cadmium Damage in *Brassica juncea*

Linling Li[1,2], Shiyan Wang[1,2], Shuai Wu[1,2], Shen Rao[1,2], Li Li[1,2], Shuiyuan Cheng[1,2], Hua Cheng[1,2]

[1] *School of Modern Industry for Selenium Science and Engineering, Wuhan Polytechnic University, Wuhan 430048, China*
[2] *National R&D Center for Se-rich Agricultural Products Processing, Wuhan Polytechnic University, Wuhan 430023, China*

12621@whpu.edu.cn

Abstract

Cadmium (Cd) is a common agricultural soil pollutant, which seriously harms the environment and the human body. In this study, *Brassica juncea* was treated with different concentrations of $CdCl_2$ and Na_2SeO_3. Physiological indexes were measured and transcriptome data were studied to reveal the mechanisms by which selenium (Se) reduces the inhibition and toxicity of Cd in *B. juncea*. The results showed that Se alleviated the inhibitive Cd effects on seedling biomass, root length, and chlorophyll content, and promoted the adsorption of Cd by pectin and lignin in the root cell wall (CW). In addition, Se alleviated the oxidative stress induced by Cd and reduced the MDA content in cells. As a result, SeCys and SeMet alleviated the transport of Cd to the shoots. Transcriptome data showed that the bivalent cation transporter MPP and the ABCC subfamily participated in the separation of Cd in vacuoles, CAL1 mediated the chelation of Cd in the cytoplasm, and ZIP transporter 4 reduced the transport of Cd to the shoots. These results indicate that Se alleviates the damage of Cd in plants and decreases its transport to the shoots by improving the antioxidant system, enhancing the ability of the CW to adsorb Cd, reducing the activity of Cd transporters, and chelating Cd.

Phytoremediation of petroleum contaminated soils by sea-buckthorn in the loess plateau of China

Qiuxiao Duan[1], Gang Han[1], Bixiao Cui[2,3], Heinz Rennenberg[4]

[1] *College of Forestry, Northwest A&F University, Yangling 712100, Shaanxi, China*

[2] *College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, Shaanxi, China*

[3] *Luoyang Open University, Luoyang 471000, Henan, China*

[4] *Center of Molecular Ecophysiology (CMEP), College of Resources and Environment, Southwest University, Chongqing 400715, China*

qiuxiao.duan@nwafu.edu.cn

Abstract

Sea-buckthorn (*Hippophae rhamnoides*) is widely domesticated and cultivated in orchards worldwide. With the advantages of large root systems and symbiosis of fibrous roots with Frankia, a group of N_2 fixing bacteria which form nodules with roots, sea-buckthorn can grow in harsh environments under extreme weather conditions. However, its tolerance to petroleum contamination in the soil has so far not been studied. To elucidate whether sea-buckthorn can be employed for the phytoremediation of petroleum contamination, its remediation efficiency as well as its growth and metabolic traits in petroleum-contaminated soil were examined in an experiment with potted plants. Sea-buckthorn seedlings were planted in soils with four different petroleum concentrations from April to September in a pot experiment. The responses of growth, gas exchange and the antioxidative system were examined on three sampling dates. After 5.5-months of exposure, soil petroleum remediation was evaluated in planted and unplanted soils. The petroleum decontamination rate was significantly enhanced by *H. rhamnoides* in soils with petroleum contamination up to 15 g kg^{-1} after one growing season. The root-to-shoot ratio increased continuously with the increase of the petroleum concentration as a consequence of reduced biomass allocated to the shoots, probably as a mechanism to counteract stress by the petroleum contamination of the soil. Photosynthesis was enhanced by lower petroleum concentrations in the soil ($\leqq$15 g kg^{-1}) in September. Short-term exposure (in July) to high petroleum concentrations (20 g kg^{-1}) in the soil enhanced ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) activities and increased ascorbic acid (AsA) and glutathione (GSH) contents, indicating elevated activity of the Foyer-Haliwell-Asada cycle. However, similar effects were not observed after long-term exposure. Our results prove that sea-buckthorn is a promising tool for phytoremediation of petroleum-contaminated soils up to 15 g kg^{-1} in loess plateau soil in China.

Effect of UV-C and LED Treatments on Postharvest Quality and Flavor of Pepino Fruit During Storage

Zixin Lin[1,2], Pang xi[1,2], Yaqi Zhao[1,2], Bihong Feng[1*], Qing Wang[2*],

[1]*College of Agricultural, Guangxi University, Nanning 530004, China;*
[2]*Key Laboratory of Vegetable Postharvest Processing, Ministry of Agriculture and Rural Affairs, Beijing Key Laboratory of Fruits and Vegetable Storage and Processing, Institute of Agri-food Processing and Nutrition, Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*

cissylin99@163.com

Abstract

Pepino (*Solanum muricatum* Aiton) fruit, a South American Solanaceae perennial herb, is rich in vitamins and phenols, offering notable antioxidant and nutritional value. However, storage issues affect the industry's progress by impacting post-harvest quality. Ultraviolet-C (UV-C) and Light-emitting diode (LED) treatments have gained recognition as secure and cost-effective postharvest preservation for fruits and vegetables. Our study utilized 'Changli' pepino fruit as the experimental material, with a control group left untreated. Seven other groups of fruits were subjected to treatments involving 1.0, 1.5, 2.0, and 3.0 $kJ \cdot m^{-2}$ UV-C, as well as 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ red, blue, and white LED irradiation. Results revealed that a 1.5 $kJ \cdot m^{-2}$ UV-C treatment effectively delayed fruit senescence, preserved its firmness, reduced respiration rates and ethylene production, and maintained levels of soluble solids, chlorophyll, vitamin C, flavonoids, and total phenols while enhancing antioxidant enzyme activity reducing MDA levels. Using electronic nose and headspace-gas chromatography-mass spectrometry, volatile compound analysis showed that UV-C treatment potentially preserves fruit flavor by promoting the synthesis of alcohols and esters while stabilizing acid, aldehyde, and ester levels. In addition, LED treatments, particularly red and white light, promoted post-harvest ripening of pepino fruit. Red, white, and blue LED irradiation stimulated peel and pulp coloration, softening, respiration, and ethylene release. It also facilitated the accumulation of various compounds, such as TSS, glucose, fructose, sucrose, anthocyanin, flavonoids, and carotenoids. Transcriptomics and metabolomics analysis revealed the role of LED red light and white light in up-regulating carotenoid biosynthesis genes, enhancing carotenoid accumulation. Meanwhile, LED blue light significantly boosted genes associated with flavonoid and anthocyanin biosynthesis, resulted in higher flavonoid and anthocyanin levels. To conclude, UV-C treatment effectively preserves the quality of pepino fruit, while LED treatment enhances the postharvest storage quality of immature pepino fruit. This research paves the way for new techniques in storage and preservation.

Effects of different storage periods and high-temperature healing treatments on browning of fresh-cut yams

Xi Pang[1,2], Zixin Lin[1,2], Yaqi Zhao[1,2], Tian Tian[1,2], Huafeng Liang[1,2], Bihong Feng[1], Qing Wang[2]

[1] *College of Agriculture, Guangxi University, Nanning 530004, China*

[2] *Key Laboratory of the Vegetable Postharvest Treatment of Ministry of Agriculture, Beijing Key Laboratory of Fruits and Vegetable Storage and Processing, Institute of Agri-Food Processing and Nutrition (IAPN), Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*

fbh@gxu.edu.cn

Abstract

The fresh-cut yam industry is expanding rapidly, but the issue of browning during manufacturing is negatively impacting its commercial viability. To address the issue of browning in fresh-cut yams, it is essential to conduct research on yam cultivars that are suitable for fresh-cutting and processing, as well as to develop effective strategies for preventing browning. In the initial phase of the study, vegetable yam and iron stick yam were stored at 4 °C. Fresh-cutting was carried out on days 0, 30, and 60 of storage. Subsequently, the yam was cut into small uniform pieces and placed in an incubator at 35 °C for 7 days for healing (treatment group), while the control yam group was stored at 4 °C and fresh-cutting was carried out at the end of high-temperature healing. After chopping, the yam was stored at 4 °C for 8 days. Throughout this period, sensory evaluation and sampling were carried out every two days. As the storage time increased, vegetable yam showed a decreased browning index, while iron stick yam showed an increased browning index. The respiration rate, ethylene production, lignin content, phenolic content, chlorogenic acid content, and browning enzyme activity increased in iron stick yam; however, the respiration rate and lignin content of vegetable yam decreased with prolonged storage, and browning enzyme activity increased and then decreased. As a result, vegetable yam is a better choice for fresh-cutting and processing. The low browning index, respiration rate, ethylene production, total phenol and lignin content, reduced enzyme activity associated with browning, and superior shelf-life quality were all preserved in fresh-cut yam treated with high-temperature healing.

Two new allelic variants of the ClLMI1 gene are responsible for the non-lobed leaf phenotype in watermelon (*Citrullus lanatus*)

Ying Wang[1], Yang Shao[1], Ming Xu[1], Ziying Wang[1], Yu Guo[1], Xiujie Liu[3], Jixiu Liu[3], Yue Gao[3], Meiling Gao[1, 2]

[1] *College of Life Sciences, Agriculture and Forestry, Qiqihar University, Qiqihar, 161006, China*

[2] *Heilongjiang Provincial Key Laboratory of Resistance Gene Engineering and Protection of Biodiversity in Cold Areas, Qiqihar,161006, China*

[3] *Qiqihar Horticultural Research Institute, Qiqihar, 161006, China*

gaomeiling0539@163.com

Abstract

Plants are comprised of various leaf shapes, and watermelon leaves are normally lobed, which is controlled primarily by the *ClLL1* gene (*Cla97C04G076510*). The presence of a 24-bp deletion in one coding region has been shown to be responsible for non-lobed leaves. In this study, to explore the mechanism behind the formation of lobed leaves, two contrasted watermelon lines, the natural mutant line (*DW-2*) with non-lobed leaves and the wild-type line (DG-2) with lobed leaves, were crossed to generate the bi-parental F_2 mapping populations. Genetic segregation analysis suggested that the non-lobed leaf (*nll*) phenotype was controlled by a single recessive gene. The *nll* gene was mapped to a 0.7-Mb interval on chromosome 4 by BSA-seq. Fine genetic mapping with a large F_2 population (n=1069) and screened recombinants exposed the delimited candidate region of 98.23 kb with eight functionally annotated genes. Sequence analysis in this region suggested that *Cla97C04G076510* is the most likely candidate gene for regulating *nll*, which encodes a homeobox leucine zipper (HD-Zip) transcription factor, a homolog of *LATE MERISTEM IDENTITY1* (*LMI1*) in Arabidopsis. A single nucleotide deletion (Cla97Chr04: 24,040,586, T/-) in the second exon of *nll* was mainly responsible for the non-lobed leaf phenotype, which encoded a truncated protein of 149 aa. However, sequence alignment showed that there was no deletion in another non-lobed leaf line GWAS-158. Therefore, the allelic test indicated that the non-lobed leaf trait of GWAS-158 and *DW-2* was controlled by the same gene *Cla97C04G076510*. The nucleotide polymorphisms of this gene among lobed and non-lobed lines showed that a C-to-T mutation in the second intron in GWAS-158 resulted in alternative splicing, which encoded a larger protein of 233 aa.

Phenotypic Variation of Flowers for the Cultivars of Camellia reticulata

Hongxing Xiao, Jiachen Min, Xueqin Wu, Di Huang, Shengtong Chen, Longqing Chen, Fang Geng

School of Landscape and Horticulture of Southwest Forestry University, Southwest Landscape Engineering Technology Research Center of State Forestry and Grassland Administration, Yunnan Functional Flower Resources and Industrialization Technology Engineering Research Center, Kunming, 650224 China

Abstract

Phenotypic variation, including directions and degrees, of 12 floral traits from 38 cultivars of *C. reticulata* were explored by comparing their distributions and deviations in three different dimensions: floral organ number, size, and shape. The results indicated that there were rich variations in the 12 floral traits of different cultivars, with the coefficient of variation (*CV*) ranging from 13.00% to 67.00%, an average *CV* of 27.24%, the smallest *CV* for petal length/petal width of 13.00%, and the largest *CV* for petal number of 67.00%. The weight calculation using the *CV* showed that the variability of pistil number (20.485%) had the greatest impact, while the variability of petal length/petal width (3.99%) had the smallest impact. Correlation analysis showed that there was a highly significant correlation between all 12 traits, with the greatest correlation between flower diameter and petal width (r = 0.822). Principal component analysis showed that the feature values extracted from the first four principal components were all greater than 1.00, with a cumulative contribution rate of 76.484%. Cluster analysis divided 38 cultivars into three groups. Group I had larger mean values for all traits, indicating a relatively lower degree of petalization. Group II had smaller mean values for all traits, but the highest average *CV*, demonstrating a relatively high degree of petalization and variability. The average value of each trait in group III fell within the moderate range, and the average number of variations was small, indicating the degree of petalization was relatively low and the variability was small. The three groups of 38 cultivars were also analyzed by using box plots and frequency distribution functions. Only nine traits showed significant differences. The floral traits of group I all showed upward distributed box bodies in box plot analysis, whereas those of group III showed downward distributed box bodies. However, the 12 traits in the three groups exhibited significant differences in frequency distribution functions. The variation degrees were quantitatively characterized by numbering traits > sizing traits > shaping traits, as well as by horizontal dimensions > radial dimensions.

Evolution of antimicrobial cysteine-rich peptides in plants

Huizhen Ma[2,3,4], Yong Feng[2], Qianqian Cao[2,3,4], Jing Jia[2,3,4], Muhammad Ali[1], Dilip Shah[5], Blake C. Meyers[5,6], Hai He[1], Yu Zhang[1]

[1] *School of Agriculture, Sun Yat-Sen University, Shenzhen 518107, China*

[2] *Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518124, China*

[3] *State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475004, China*

[4] *Shenzhen Research Institute of Henan University, Shenzhen 518000, China*

[5] *Donald Danforth Plant Science Center, Saint Louis, MI 63132, USA*

[6] *Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211, USA*

hehai@mail.sysu.edu.cn

Abstract

Plants produce cysteine-rich peptides (CRPs) that have long-lasting broad-spectrum antimicrobial activity to protect themselves from a variety of pathogens. We analyzed 240 plant genomes, ranging from algae to eudicots, and discovered that CRPs are widely distributed in plants. Our comparative genomics results revealed that CRP genes have been amplified through both whole genome and local tandem duplication. The copy number of these genes varied significantly across lineages and associated with the plant ecotype, which may be due to their resistance to changing pathogenic environments. The conserved and lineage-specific CRP families contributed to diverse antimicrobial activities. Furthermore, we investigated the unique bi-domain CRPs that resulted from unequal crossover events. Our findings provide unique evolutionary perspectives on CRPs as well as insights into their antimicrobial and symbiotic characteristics.

Effects of Exogenous Betaine and Melatonin on Postharvest Storage Performance of Longan Fruit

Zhang Ruimin, Hu Kaixue, Qiu Qiuping, Liu Shunzhi, Hu Weirong

College of Life Sciences, Guangzhou University, Guangzhou 510006, China

weironghu@163.com

Abstract

As one of the most favorite subtropical fruits in China, longan (*Dimocarpus longan*) matures in the summer during high temperature and humidity. However, it is susceptible to deterioration by pericarp browning, pulp breakdown and pathogenic bacteria inoculation after harvest due to its rapid metabolism. To study the effects of combined treatment, namely betaine and melatonin, on the storability of postharvest longan, 'Chuliang' longan fresh fruits were treated with 5, 10, 15 mmol/L betaine with 300 mmol/L melatonin and stored at 4℃ for 42 days. The changes of the weight loss rate, total soluble solids (TSS), titratable acid (TA), ascorbic acid (VC), free proline, malondialdehyde (MDA), catalase (CAT) activity, peroxidase (POD) activity, and superoxide dismutase (SOD) content were investigated. The results showed that, compared with the melatonin control group, the composite treatment group exhibited higher commercial preservation and higher contents of TSS, TA, VC and free proline but lower weight loss rates in longan fruit. Meanwhile, treatment with betaine and melatonin showed lower MDA content and POD activity, but higher SOD and CAT activities ($P < 0.05$). Among these regimens, the preservation effect of 10 mmol/L betaine with 300 mmol/L melatonin was best for restraining the spoilage of postharvest longan fruit.

Orphan gene BR1 regulates bolting resistance in Chinese cabbage

Mingliang Jiang[1], Xiaonan Li[2], Xiangshu Dong[3], Wenxing Pang[2], Zhongyun Piao[2], Hong Lang[1]

[1] *School of Agriculture, Jilin Agricultural Science and Technology College, Jilin, 132101, China*

[2] *College of Horticulture, Shenyang Agricultural University, Shenyang, 110866, China*

[3] *School of Agriculture, Yunnan University, Kunming, 650504, China*

jiangmingliang@jlnku.edu.cn

Abstract

Orphan genes (*OGs*) are unique to a specific taxon and important for species-specific traits and stress responses. An *OG* designated *BOLTING RESISTANCE 1* (*BR1*) was identified and found through gene structural variation analyses to be more highly conserved in Chinese cabbage than in other available accessions. The expression of *BR1* was increased in bolting-resistant Chinese cabbage and decreased in bolting non-resistant Chinese cabbage, and the expression of some genes was consistent with the bolting resistance phenotype. *BR1* is primarily expressed in leaves at the vegetative growth stage, and the highest *BR1* expression levels during the flowering stage were observed in the flower buds and siliques compared to other tissue types. The overexpression of *BR1* in Chinese cabbage was associated with enhanced bolting resistance under long day (LD) conditions, and the gene knockout plants '*br1*' showed early flowering compared to the control plants. The identification of downstream interacting proteins and upstream transcription factors suggests that BR1 may regulate bolting resistance through the photoperiodic pathway. Taken together, we propose that the orphan gene *BR1* functions as a novel regulator of flowering time, and these results suggest that *BR1* may represent a promising candidate gene to support the selective breeding of Chinese cabbage cultivars with enhanced bolting resistance.

Characterization and expression analysis of key abscisic acid signal transduction genes during kiwifruit development

Wenbin Kai

Postharvest Key Technology and Quality Safety of Fruits and Vegetables in Jiangxi Province, Jiangxi Key Laboratory for Postharvest Technology and Non-destructive Testing of Fruits & Vegetables, Jiangxi Agricultural University, China

kaiwb@jxau.edu.cn

Abstract

Actinidia chinensis cv 'Hongyang' kiwifruit, one of the most favorite fruits, is a promising commercial cultivar due to its unique flesh color and nutrition value. To characterise the regulation of the abscisic acid (ABA) signalling pathway during fruit development and ripening, we investigated the key genes involved in this pathway in kiwifruit (*Actinidia chinensis*), which included 18 *AcPYLs* genes encoding ABA receptors, 7 *AcPP2Cs* genes encoding type 2C protein phosphatases, and 7 *AcSnRK2s* genes encoding members of the SNF1-related protein kinases 2 family, as identified in the kiwifruit reference genome. *AcPYLs*, *AcPP2Cs*, and *AcSnRK2s* from kiwifruit are putative homologues of the corresponding Arabidopsis and tomato proteins. Phylogenetic analysis revealed that *AcPYLs* and *AcSnRK2s* clustered into three subfamilies. Transcript levels indicated that the expression pattern of *AcNCED4* was consistent with the ABA content of the pulp during kiwifruit development. Furthermore, the ABA content of the axile placenta was higher than that of the sarcocarp in kiwi pulp, and the transcript levels of most ABA signal transduction genes, including *AcPYLs*, *AcPP2Cs*, and *AcSnRK2s*, were higher in the axile placenta. Moreover, AcPP2C3 was localised in the nucleus and could interact with all *AcPYLs* and *AcSnRK2s* in an ABA-independent manner, as revealed by subcellular localisation and yeast two-hybrid assays. These findings enhance our understanding of ABA signal transduction genes in kiwis and provide the necessary background for further exploration of the molecular mechanisms at play during kiwifruit development.

AP2/ERF transcription factor PtrERF110 orchestrates cold tolerance by directly regulating sugar and sterol accumulation in Poncirus trifoliata (L.) Raf.

Madiha Khan, Chunlong Li, Ji-Hong Liu

Abstract

Ethylene responsive factors (ERFs) play a key role in the orchestration of the cold stress response. However, the transcriptional regulatory mechanisms and downstream target genes of most ERFs remain to be elucidated. In the present study, we identified a cold-induced ERF, designated as PtrERF110, from *Poncirus trifoliata* (L.) Raf., an elite cold-hardy plant closely related to citrus. PtrERF110 is a nuclear protein with transcriptional activation activity. Overexpression of *PtrERF110* remarkably enhanced cold tolerance in transgenic lemon (*Citrus limon*), a cold-sensitive species, whereas VIGS (virus-induced gene silencing)-mediated knockdown of *PtrERF110* drastically impaired cold tolerance of *Poncirus trifoliata*. RNA-sequence analysis revealed that overexpression of *PtrERF110* resulted in global transcriptional reprogramming of a range of stress-responsive genes. Three genes, including *PtrERD6L16* (early responsive dehydration 6-like transporters), *PtrSPS4* (sucrose phosphate synthase4), and *PtrUGT80B1* (UDP-glucose: sterol glycosyltransferases80B1), were confirmed as direct targets of PtrERF110. Consistently, *PtrERF110*-overexpressing transgenic plants exhibited higher expression levels of *SPS4*, *ERD6L16*, and *UGT80B1*, and accumulated more sugars and sterols compared to the wild type. In addition, silencing of *PtrSPS4*, *PtrERD6L16*, and *PtrUGT80B1* compromised the cold tolerance of *Poncirus trifoliata*. Taken together, our findings indicate that PtrERF110 positively regulates cold tolerance by modulating the accumulation of sugars and sterols via transcriptional targeting of *PtrERD6L16*, *PtrSPS4,* and *PtrUGT80B1*. The cold-responsive regulatory module (ERF110-*ERD6L16*/*SPS4*/*UGT80B1*) unraveled in this study advances our understanding of the molecular mechanisms underlying the accumulation of sugars and sterols under cold conditions in plants.

Selection of Citrus Rootstocks Resistant to Huanglongbing Disease

Faisal Hayat, Nazir Ahmed, Yang Peng, Juan Li

College of Horticulture, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, China

13751774213@139.com

Abstract

Citrus fruits have a prominent position among tree fruits and are widely grown in tropical and subtropical regions. Biotic and abiotic stresses hamper its production throughout the world, and citrus greening (huanglongbing; HLB), one of the most devastating citrus diseases, is caused by phloem-limited unculturable fastidious bacteria, namely *Candidatus* Liberibacter species borne by psyllids, small plant-feeding insects. The disease reduces citrus tree yield and fruit quality, making it unproductive and contributing to death. Currently, there is no effective strategy to completely eradicate HLB disease, and short-term management is only achievable via a "three-pronged" approach. Moreover, conventional breeding faces difficulties because of cross-species incompatibility and prolonged juvenile periods. To reduce the damage caused by HLB, many investigators have employed disease-resistant rootstocks to boost the tolerance of citrus plants to HLB. This research utilized three citrus rootstock cultivars and four grafted varieties (scion/rootstock graft combinations) as the experimental subjects. The side grafting method was employed to inoculate citrus plants with *Las bacterium*. It was found that the levels of chlorophyll a, chlorophyll b, and total chlorophyll, as well as photosynthesis and chlorophyll fluorescence, were all lowered in different citrus varieties following infection with CLas. After the inoculation of CLas, there was a marked elevation in the production of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) in various citrus varieties. Moreover, the activities of peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO) were significantly higher with lower CLas titers in 'Jiu Bing Le' and 'Hong Jiang'/'Jiu Bing Le' seedlings compared with the other tested treatments. In summary, through the analysis of variations in physiological indicators and CLas content in different citrus varieties before and after infection, it was determined that 'Jiu Bing Le' and 'Hong Jiang'/'Jiu Bing Le' were the least impacted by HLB, suggesting that 'Jiu Bing Le' plants exhibit strong resilience to HLB and have the potential to boost the disease resistance of the scion to a certain degree.

Functional Characterization of VvTPS54.1 Gene Related to Muscat Aroma in Grapes (*Vitis vinifera L.*)

Yongzhou Li, Caili Yang, Liangliang He, Yinhua Song, Peng Zhang, Sanjun Liu

Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, 450009, China

liyongzhou@caas.cn

Abstract

Fruit aroma is an important quality with respect to consumer preference, but the most important aroma compounds and their genetic regulatory mechanisms remain elusive. The *VvTPS54.1* gene has been identified in grape; however, the regulation of muscat aroma in grape by *VvTPS54.1* is still unclear. In this study, we identified 215 aroma compounds, including 88 esters, 64 terpenes, and 29 alcohols by SPME-GC-MS in muscat-scented grapes from the progeny of the 'Muscat Hambug' × 'red globe' population. Skin transcriptome data for muscat-scented grape in hybrid populations were generated and subjected to aroma compound-gene correlation analysis. The combined transcriptomic analysis and terpene profiling data revealed 20 candidate genes, which were assessed in terms of their involvement in aroma biosynthesis regulation. Among the differentially expressed genes for terpene synthesis, *VvTPS54.1* (VIT_00s0271g00010) was the most significantly differentially expressed gene. Subcellular localization analysis detected *VvTPS54.1* in the cell membrane. The coding sequence of *VvTPS54.1* caused premature termination of transcription due to mutations in SNPs in grapes with different aromas, and different coding sequences were transgenic in M-Tom, with great differences among terpenoids. A marker specific to the *VvTPS54.1* coding sequence was developed to distinguish grape cultivars and F1 progeny. We identified 2 transcription factors, VvMYB24 and VvGATA2, that could interact with *VvTPS54.1*, whose encoding gene was highly expressed in muscat grape. The results of this study shed light on the volatile compounds and “anchor points” of synthetic pathways in the skin of muscat grape and provide new insights into the regulation of muscat aromas in grapes.

Appropriate Soil Nitrogen Content Promotes Growth of Paphiopedilum micranthum by Interfering with the Bacterial Community

Yao Feng[1], Cui-Ying Wang[2], Zong-Yan Li[1], Yin Lia, Jia-Xue Hua, Yue-Hong Ruana

[1] *Landscape Engineering Institute of National Forestry Bureau, Southwest Forestry University, 650224, China*
[2] *School of Biotechnology and Engineering, West Yunnan University, Lincang 677000, China*

Abstract

Paphiopedilum micranthum, a perennial herb of the Orchidaceae family, has high ornamental value and is used in gardens; however, it is listed as one of the most endangered species in the Orchidaceae family due to its unsustainable collection and discontinuous distribution in the tropical and subtropical regions of Asia. *P. micranthum* is only found in the karst region of southeast Yunnan, southwest Guizhou, northern and western Guanxi, and northern Vietnam. A study of its field habitat is helpful to better protect and utilize the germplasm resources of Genus *Paphiopedilum* in southeast Yunnan. Soil leaching in the karst region results in nutrient impoverishment, which is inherently low in available nitrogen and phosphorus. The rhizosphere microbial community plays an important role in promoting the decomposition of soil organic matter as well as the conversion of nutrients and maintenance of nutrient cycling in soils to ensure the healthy growth of orchids. However, the composition and dynamics of bacterial communities in the rhizosphere of *Paphiopedilum* in different habitats have not been fully revealed. In this study, the Illumina MiSeq high-throughput sequencing platform was used to monitor the dynamic changes of rhizosphere bacterial communities in different habitats from two locations (Xiaobazi and Shedu of Wenshan county), which independently represents its distribution under half-shady shrubs, shady trees and on stone walls. Meanwhile, the soil physicochemical properties were also determined to further analyze their differences. Among the six physical and chemical properties of soil in four habitats, there was no significant difference in pH, organic matter, hydrolyzed nitrogen, total nitrogen, nitrate nitrogen and ammonium nitrogen. *P. micranthum* was characterized by its small population and narrow regional distribution in the field. Analysis of variance suggested that *Paphiopedilum* rigorously chooses to grow in some specific types of soil, which has high similarities in soil physical and chemical properties, although under different habitats. 16S rRNA gene sequencing indicated that the soil had no significant difference in community richness (Sobs, Chao1 and ACE index) and community diversity (Shannon and Simpson index). A total of 728 genera were detected in the four habitats, of which 263 genera were common. Linear discriminant analysis effect size (LEfSe) showed that the significantly enriched phyla (LDA>2, $p<0.05$) were Actinobacteriota (42%) Proteobacteria (26%) and Acidobacteriota (12%). At the genus level, the abundances of *Crossiella* (4%), *Pseudonocardia* (3%) and *Mycobacterium* (3%) decreased significantly. Based on the correlation analysis between physical and chemical properties and soil bacteria, it was found that there was a significant correlation between bacteria in four habitats and nitrogen-related indexes. This research provides insights into the nitrogen demands of *P. micranthum* distributed in karst heterogenic habitats and the characteristics of its soil bacterial community. Thus, it will be helpful to use sensible and economical fertilization for an appropriate nitrogen supply in its cultivation.

CiWRI2: A potential key regulator of pecan fruit initiation via very long-chain fatty acids-related cuticle metabolism

Qiaoyan Wang[1,†], Miao Shi[1,†], Junhao Chen[1,2,†], Andrey Vaulin[3], Wenlei Guo[1], Jie Luo[1], Yifei Xie[1], Xin Zhang[1], Yongrong Li[4], Zhengjia Wang[1], Jarkko Salojärvi[3,5], Kean-Jin Lim[1]

[1] *State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou, 311300, China*

[2] *Department of Biology, Saint Louis University, St. Louis, MO 63104, USA*

[3] *School of Biological Sciences, Nanyang Technological University, 63755, Singapore*

[4] *Nanjing Green Universe Pecan Science and Technology Co. Ltd., Nanjing 210007, China*

[5] *Organismal and Evolutionary Biology Research Program, Faculty of Biological and Environmental Sciences, Viikki Plant Science Centre, University of Helsinki, Helsinki, 00014, Finland*

[†] These authors contributed equally.

keanjin.lim@zafu.edu.cn

Abstract

Pecan (*Carya illinoensis*), an important crop plant, produces nuts rich in unsaturated fatty acids. While studies have elucidated the mechanism of fatty acid synthesis in pecan fruit development, specific facets, such as differences in fruit size and fatty acid content, continue to invite further exploration. We analysed three varieties—Stuart, Mahan, and Pawnee—each with distinct fruit morphologies and unsaturated fatty acid profiles. Our comparative transcriptomics analysis of these three accessions during the last four stages of fruit development identified a member of the AP2/ERF WRINKL*ED (WRI) family, CiWRI2, which showed an expression peak at stage 5 and a progressive decrease towards the subsequent stages. Subsequent network analysis further linked this gene to lipid biosynthesis. However, the role of WRI2 is poorly characterised in woody plants, although it has been linked with grain size in monocot crop plants such as rice. Motivated by this observation, we further explored the function of *CiWRI2* in pecan fruit development, particularly in lipid metabolism. Quantitative PCR across all 8 pecan fruit developmental stages revealed a significant increase in *CiWRI2* expression during stages 1 to 4, suggesting involvement in early fruit development. To gain additional insights, we overexpressed *CiWRI2* in transgenic Arabidopsis and performed transcriptomic sequencing on the early-, middle- and late-stage seeds. Transcriptomic analysis indicated a significant differential expression of genes associated with cuticle metabolism, such as very long-chain fatty acids (VLCFA) and seed maturation during the early and middle seed development stages. Interestingly, recent studies have shown that VLCFA, a key member of cuticle metabolism, is related to plant organ development. Based on these findings, we hypothesise that *CiWRI2* has a role in early fruit development in pecan through the regulation of cuticle metabolism, highlighting an area for further exploration of pecan fruit biology and lipid metabolism.

Evolutionary analysis of the SMXL genes in Rosaceae: Further insights for their origin, expansion, diversification and role in regulating pear branching

Liu Chunxiao, Li Xiaogang

Institute of Pomology, Jiangsu Academy of Agricultural Sciences/Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, Nanjing, Jiangsu, China

chuntianxiaozi@163.com; xiaogangli@aliyun.com

Abstract

SMXL genes act as both suppressors and transcription factors. They constitute a conserved gene family, which is ubiquitous in eukaryotic systems from liverworts and mosses to green plants, and they are involved in the regulation of plant branching, leaf elongation, and anthocyanin biosynthesis. In the absence of SLs, SMXLs perceive SL signals and form a complex with TPL, which binds directly to the promoters of *SMXLs*, repressing their expression. SMXLs also function as transcription factors. In the presence of SLs, D14 binds to SLs to promote the formation of the SMXLs-D14-SCF-MAX2 complex, triggering the ubiquitin-mediated degradation of SMXLs and relieving their transcriptional repression. In this research, a total of 121 *SMXL* genes were obtained from 16 genomes, including 7 species from *Rosaceae* (pear, apple, loquat, *Gillenia trifoliate*, peach, strawberry and Chinese rose), and some representative species of *Asterids*, monocots and basal angiosperms, including *Ficus microcarpa*, *Arabidopsis thaliana*, soybean, tomato, grape, greater yam, rice, water lily and *Amborella trichopoda*. Among the 121 genes, the number of nonredundant *SMXL* genes varied from 3 (*A. trichopoda*) to 18 (soybean). Phylogenetic analysis demonstrated that the *SMXL* genes were classified into 4 groups (groups I–IV). Gene structure analysis showed that the *SMXL* genes had very conserved gene structures, with no more than two introns for each. *SMXL* genes in *Rosids*, *Asterids*, monocots and basal angiosperms showed gene expansion and evolution clues. Phylogenetic trees and syntenic network analyses were consistent with previous classification and evolution analyses of the *SMXL* genes. Three-dimensional structure prediction of the SMXL proteins showed specific structures among the groups but also similarities within group members, suggesting that the functions of *SMXLs* in the same group may be redundant or similar, consistent with the results of secondary structure analysis. Expression analysis of 10 *PpySMXL* genes at different times of pear sprouting and branching showed differential expression after artificial fruit thinning. RT-qPCR analysis revealed that the expression of the 10 *SMXL* genes was significantly induced by fruit thinning treatment. Collectively, our study is a comprehensive investigation of *SMXL* genes in *Rosaceae*, especially in pear. These findings enrich our knowledge of pear tree sprouting and branching and offer suggestions on pear tree planting in order to promote tree shaping, pruning, and fruit production.

AP2/ERF family gene PtrRAP2.13 from trifoliate orange encodes a transcription activator that promotes root growth and enhances drought stress tolerance in plant

Dechun Liu, Shiguo Wan, Yong Liu

Department of Pomology, College of Agronomy, Jiangxi Agricultural University, Nanchang, 330045, China

ldc873380800@163.com

Abstract

Citrus quality and yield are severely limited by drought stress. Studies on the roles of APETALA2/ethylene responsive factor (AP2/ERF) family transcription factors (TFs) on plant drought resistance have made some progress. However, only a few AP2/ERF family TFs have been shown to be involved in the drought response in citrus. The current paper identified the AP2/ERF family gene *PtrRAP2.13* from trifoliate orange. PtrRAP2.13 exhibited high identity with RAP2.13 proteins found in other plant species, and PtrRAP2.13 possessed the conserved domain AP2. PtrRAP2.13 is a nuclear protein with transactivation activity. PEG6000 and ABA treatments significantly induced *PtrRAP2.13* expression in leaves and roots of trifoliate orange. Under drought stress, Arabidopsis ectopic-overexpressing *PtrRAP2.13* exhibited elevated ABA content, better developed roots, and enhanced antioxidant enzyme activities, as well as reduced accumulation of malondialdehyde (MDA) and reactive oxygen species (ROS) compared with WT plants. However, opposite trends in these physiological indices were observed in the *PtrRAP2.13* homolog silencing lemon. Furthermore, transgenic Arabidopsis showed significantly increased expression levels of genes associated with ABA biosynthesis, ROS scavenging and drought response. However, these genes exhibited decreased expression in the *PtrRAP2.13* homolog silencing lemon. The findings of this study indicate that PtrRAP2.13 plays a positive role in regulating plant drought tolerance by promoting root development and ROS sequestration.

Evaluation of apricot fruit aroma by electronic nose

Jiacheng Liu, Weisheng Liu, Ning Liu, Qiuping Zhang, Yuping Zhang, Yujun Zhang, Ming Xu, Xiaoxue Ma, Haijuan Zhao, Bijun Wang, Shuo Liu

Liaoning Institute of Pomology, Xiongyue, Yingkou City, China

Abstract

To determine the differences in fruit aroma between different apricot germplasm resources, and to provide a reference for aroma evaluation and quality improvement of apricot fruits, we evaluated aroma components in 47 apricot varieties using an electronic nose system (PEN 3). The response values of different sensors were obtained, followed by principal component analysis (PCA), linear discriminant analysis (LDA) and loading analysis (LO). LO analysis showed that five sensors, namely W5S (sensor of NOx compounds), W1S (sensor of methane compounds), W1W (sensor of hydrogen sulfide), W2S (sensor of broad alcohols), and W2W (sensor of organic sulfide), played major roles in the evaluation of apricot fruit aroma. PCA and discrimination values indicated that the aromas of three varieties, Jipin, Yinxiangbai and Naimanwanshu, showed obvious differences compared to the other tested varieties, and the first and second main axes were 94.61% and 3.58%. LDA indicated that the apricot with white flesh color showed a significant difference ($P < 0.05$) from the other two flesh color types (yellow and orange). However, LDA was not applied to discriminate the province of origin. These results show that the soft solute type had a significant effect on apricot fruit aroma, the soft solute type of apricot aroma was unique, and different flesh colors have different apricot aromas.

Magnesium promotes tea plant growth via enhanced glutamine synthetase-mediated nitrogen assimilation

Qunfeng Zhang[1], Yutao Shi[1,2], Hao Hu[3,4], Jianyun Ruan[1], Alisdair R. Fernie[5], Mei-Ya Liu[1]

[1] *Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China*
[2] *Wuyi University, Wuyishan 354300, China*
[3] *Department of Botany and Plant Sciences, Institute of Integrative Genome Biology, University of California, Riverside, CA, USA*
[4] *Key Laboratory for Biology of Horticultural Plants, Ministry of Education, College of Horticulture & Forestry Sciences, Huazhong Agricultural University, Wuhan, China*
[5] *Max-Planck-Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany*

yummilyliu@163.com; liumeiya@tricaas.com

Abstract

The plantation soil of acidic tea (*Camellia sinensis*) usually suffers from magnesium (Mg) deficiency, and the application of fertilizer containing Mg can substantially increase tea quality by enhancing the accumulation of nitrogen (N)-containing chemicals such as amino acids in young tea shoots. However, the molecular mechanisms underlying the promoting effects of Mg on N assimilation in tea plants remains unclear. Here, both hydroponic and field experiments were conducted to analyze N, Mg, metabolite contents, and gene expression patterns in tea plants. We found that N and amino acids accumulated in tea plant roots under Mg deficiency, while metabolism of N was enhanced by Mg supplementation, especially under a low N fertilizer regimen. ^{15}N tracing experiments demonstrated that assimilation of N was induced in tea plant roots following Mg application. Furthermore, WGCNA analysis of RNA-seq data suggested that genes encoding glutamine synthetase isozymes (*CsGSs*), key enzymes regulating N assimilation, were regulated by Mg treatment. Overexpression of *CsGS1.1* in Arabidopsis (*Arabidopsis thaliana*) resulted in a more tolerant phenotype under Mg deficiency and increased N assimilation. These results validate our suggestion that Mg transcriptionally regulates *CsGS1.1* during the enhanced assimilation of N in tea plants. Moreover, results of a field experiment demonstrated that high Mg and low N had positive effects on tea quality. This study enhances our understanding of the molecular mechanisms underlying the interactive effects of Mg and N in tea plants while also providing both genetic and agronomic tools for the future improvement of tea production.

HuNAC20 and HuNAC25, Two Novel NAC Genes from Pitaya, Confer Cold Tolerance in Transgenic Arabidopsis

Xinglong Hu, Fangfang Xie, Wenwei Liang, Yinhao Liang, Zhike Zhang, Jietang Zhao, Guibing Hu, Yonghua Qin

Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou 510642, China

qinyh@scau.edu.cn

Abstract

NAC transcription factors are one of the largest families of transcriptional regulators in plants, and members of the gene family play vital roles in regulating plant growth and development, including biotic/abiotic stress responses. However, little information is available about the NAC family in pitaya. In this study, we conducted genome-wide analysis, and a total of 64 NACs (named *HuNAC1–HuNAC64*) were identified in pitaya (Hylocereus). These genes were grouped into fifteen subgroups with diversity in gene proportions, exon–intron structures, and conserved motifs. Genome mapping analysis revealed that *HuNAC* genes were unevenly scattered across all eleven chromosomes. Synteny analysis indicated that the segmental duplication events played key roles in the expansion of the pitaya NAC gene family. The expression levels of these *HuNAC* genes were analyzed under cold treatments using qRT-PCR. Four *HuNAC* genes, i.e., *HuNAC7*, *HuNAC20*, *HuNAC25*, and *HuNAC30*, were highly induced by cold stress. *HuNAC7*, *HuNAC20*, *HuNAC25*, and *HuNAC30* were localized exclusively in the nucleus. *HuNAC20*, *HuNAC25*, and *HuNAC30* were transcriptional activators, while *HuNAC7* was a transcriptional repressor. Overexpression of *HuNAC20* and *HuNAC25* in *Arabidopsis thaliana* significantly enhanced tolerance to cold stress by decreasing ion leakage, malondialdehyde (MDA) content, and H_2O_2 and O_2^- accumulation, accompanied by the upregulation of the expression of cold-responsive genes (*AtRD29A*, *AtCOR15A*, *AtCOR47*, and *AtKIN1*). This study presents comprehensive information on the NAC gene family and candidate genes that can be used to breed new pitaya cultivars with tolerance to cold conditions through genetic transformation.

SnRK2.4-mediated phosphorylation of ABF2 regulates ARGININE DECARBOXYLASE expression and putrescine accumulation under drought stress

Jie Song[1], Peipei Sun[2], Weina Kong[1], Zongzhou Xie[1], Chunlong Li[1], Ji-Hong Liu[1]

[1] *Key Laboratory of Horticultural Plant Biology (MOE), College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China*

[2] *School of Horticulture, Anhui Agricultural University, Hefei 230036, China*

Abstract

Arginine decarboxylase (ADC)-mediated putrescine (Put) biosynthesis plays an important role in plant abiotic stress response. SnRK2s (SNF1-related protein kinases 2s) and ABFs, ABA-response element (ABRE) binding factors, are core components of the ABA signaling pathway involved in drought stress response. We previously reported that *ADC* of *Poncirus trifoliata* (*PtrADC*) functions in drought tolerance. However, whether and how SnRK2 and ABF regulate *PtrADC* to modulate putrescine accumulation under drought stress remains largely unclear. Here, we employed a set of physiological, biochemical, and molecular approaches to reveal that a protein complex composed of PtrSnRK2.4 and PtrABF2 modulates putrescine biosynthesis and drought tolerance by directly regulating *PtrADC*. *PtrABF2* was upregulated by dehydration in an ABA-dependent manner. PtrABF2 activated *PtrADC* expression by directly and specifically binding to the ABRE core sequence within its promoter and positively regulated drought tolerance by modulating putrescine accumulation. PtrSnRK2.4 interacted with and phosphorylated PtrABF2 at Ser93. PtrSnRK2.4-mediated PtrABF2 phosphorylation was essential for the transcriptional regulation of *PtrADC*. Furthermore, PtrSnRK2.4 was shown to play a positive role in drought tolerance by facilitating putrescine synthesis. Taken together, this study sheds new light on the regulatory module SnRK2.4-ABF2-*ADC* responsible for fine-tuning putrescine accumulation under drought stress, which advances our understanding of the transcriptional regulation of putrescine synthesis.

Combined Analysis of Transcriptome and Metabolome in Blackberry Under Heat Stress

Lingfeng Jiang[1], Xing Guo[2], Siqi Huang[1], Wei Guo[2], Qingzhu Hua[2], Wenpei Song[2]

[1] *College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

[2] *College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

2267922163@qq.com

Abstract

Blackberry (*Rubus fruticosus* L.), a characteristic fruit tree of the Rubus genus in the Rosaceae family, is currently a popular "third generation fruit" worldwide. It has a unique taste, high processing value, high market demand, and high content of nutrients, with high medicinal value. High temperature stress is one of the most severe forms of abiotic stress in blackberries. High temperatures have caused a sharp decline in production and quality. To reveal the potential mechanism of blackberry under high temperature stress, this study conducted a joint analysis of the transcriptome and the metabolome of the heat-resistant variety Shuangji (SJ) and the heat-sensitive variety Three Triple Crown (SGW). The results showed that 6822 and 3191 differentially expressed genes (DEGs) were identified in SJ and SGW, respectively. A total of 3191 upregulated genes and 3631 downregulated genes were identified by SJ. A total of 2981 upregulated genes and 3262 downregulated genes were identified by SGW. We also identified 562 and 613 differential metabolites (DAMs) in SJ and SGW, respectively. SJ had 294 upregulated and 268 downregulated metabolites. SGW had 330 upregulated and 283 downregulated metabolites. There were many common heat stress (HS) response genes in both blackberry varieties, accounting for approximately 43%, and the expression patterns of these HS responsive genes were different in the two varieties. However, the expression changes of the most common HS-response genes in SJ were more significant, which may explain why SJ is more heat-resistant. Similar results were obtained for metabolomic data, especially for the expression of flavonoids, phenolic acids, lipids, and amino acids. The changes in multiple genes and metabolites highlight the complex mechanisms in blackberry during HS. The glutathione metabolism pathway and plant hormone signaling pathway play key roles in the response of blackberries to HS, and the high expression of related genes and the high accumulation of metabolites may be the main reasons for blackberry heat tolerance.

Tracking organelle activities through efficient and stable root genetic transformation system in woody plants

Jinli Gong[1,2], Yishan Chen[1,2], Yanna Xu[1,2], Miaofeng Gu[1,2], Haijie Ma[1,2], Xiaoli Hu[1,2], Xiaolong Li[1,2], Chen Jiao[3], Xuepeng Sun[1,2]*

[1]*Collaborative Innovation Center for Efficient and Green Production of Agriculture in Mountainous Areas of Zhejiang Province, College of Horticulture Science, Zhejiang A&F University, Hangzhou 311300, Zhejiang, China*

[2]*Key Laboratory of Quality and Safety Control for Subtropical Fruit and Vegetable, Ministry of Agriculture and Rural Affairs, Zhejiang A&F University, Hangzhou 311300, Zhejiang, China*

[3]*Institute of Biotechnology, Zhejiang University, Hangzhou 310058, Zhejiang, China*

xs57@zafu.edu.cn

Abstract

Due to the protracted transgenic timeline and low efficiency in stable genetic transformation of woody plants, there has been limited exploration of real-time organelle imaging within stable transgenic woody plant cells. Here, we established an efficient *in vivo* genetic transformation system for woody plants using *Agrobacterium rhizogenes*-mediated approach. This system was successfully validated in multiple perennial woody species. Using citrus as a model, we introduced organelle-targeted fluorescent reporters via genetic transformation and investigated their subcellular localization and dynamics using advanced imaging techniques, such as confocal microscopy and live-cell imaging. Moreover, we subjected transgenic MT-GFP-labeled mitochondria in root cells to stress conditions simulating agricultural adversities faced by fruit crops. The stress-induced experiments revealed notable alterations in mitochondrial morphology. Our study contributes novel insights into membrane trafficking processes, protein localization dynamics, and cellular physiology in woody plants, while also providing stable and efficient genetic transformation methods for perennial woody species.

Pbr3RAV2 transcription factor regulates pear resistance to Botryosphaeria dothidea through the autophagy pathway

Fei Wang, Yun Wang, Shaoling Zhang, Xun Sun

Center of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

Abstract

Pear ring rot, caused by *Botryosphaeria dothidea* (*B. dothidea*), is the most serious disease affecting pears. In recent years, the rapid development of genomics and transcriptomics has significantly improved our understanding of the genetic basis of plant disease resistance. However, the molecular mechanisms underlying the resistance of pears to *B. dothidea* remain elusive. Here, we demonstrate that the pear autophagy-related gene, *PbrATG1a*, plays a key role in autophagic activity and resistance to *B. dothidea*. Based on the promoter of *PbrATG1a*, we screened the transcription factor Pbr3RAV2 by yeast one-hybrid assay. The results of yeast one-hybrid assay, dual-luciferase reporter assay, and EMSA assay showed that Pbr3RAV2 binds the CAACA element of *PbrATG1a* promoter directly. Specifically, overexpression of *Pbr3RAV2* enhanced autophagic activity and resistance to *B. dothidea* in pear callus. Transient silencing of *Pbr3RAV2* led to compromised autophagic activity and resistance to *B. dothidea* in *Pyrus betulaefolia*. Additionally, we identified PbrTTG1, which interacts with Pbr3RAV2, using a yeast two-hybrid assay. Luciferase complementation imaging assay and Co-IP assay demonstrated that PbrTTG1 interacts with Pbr3RAV2. Simulated pathogen infection enhanced the interaction between Pbr3RAV2 and PbrTTG1. The Pbr3RAV2-PbrTTG1 complex significantly increased the binding capacity of Pbr3RAV2 to the promoter of *PbrATG1a* and its transcription. These findings offer new insights into the molecular mechanisms underlying the resistance of pears to disease and suggest potential targets for genetic manipulation to bolster resistance in pears.

Large-scale proteogenomic atlas of pear

Peng Wang[1], Xiao Wu[1], Zebin Shi[2], Juyou Wu[1], Shaoling Zhang[1]

[1] *State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Sanya Institute of Nanjing Agricultural University, Nanjing Agricultural University, Nanjing 210095, China*
[2] *Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*

slzhang@njau.edu.cn

Abstract

Pear is an important fruit tree that is widely distributed around the world. The first pear genome map was reported from our laboratory approximately 10 years ago. To further study global protein expression patterns in pear, we generated pear proteome data based on 24 major tissues. The tissue-resolved profiles provided evidence of the expression of 17,953 proteins. We identified 4,294 new coding events and improved the pear genome annotation via a proteogenomic strategy based on 18,090 peptide spectra with peptide spectrum matches >1. Among the 8 randomly selected new short-coding open reading frames that were expressed in the style, 4 promoted and 1 inhibited the growth of pear pollen tubes. Based on gene coexpression module analysis, we explored the key genes associated with important agronomic traits such as stone cell formation in fruits. The network regulating the synthesis of lignin, a major component of stone cells, was reconstructed, and receptor-like kinases were implicated as core factors in this regulatory network. Moreover, we constructed the online database PearEXP (http://www.peardb.org.cn) to enable access to the pear proteogenomic resources. This study provides a paradigm for in-depth proteogenomic studies of woody plants.

Allopolyploidization from two dioecious ancestors leads to recurrent evolution of sex chromosomes and reversion to autosomes

Li He[1,†], Yuàn Wang[1,†], Yi Wang[1,2], Ren-Gang Zhang[3], Yuán Wang[1], Elvira Hörandl[4], Judith E. Mank[5], Ray Ming[6]

[1] *Eastern China Conservation Centre for Wild Endangered Plant Resources, Shanghai Chenshan Botanical Garden, Shanghai, 201602, China*
[2] *Laboratory of Systematic Evolution and Biogeography of Woody Plants, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, China*
[3] *Yunnan Key Laboratory for Integrative Conservation of Plant Species with Extremely Small Populations, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
[4] *Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), University of Goettingen, Göttingen, Germany*
[5] *Department of Zoology and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, Canada*
[6] *Centre for Genomics and Biotechnology, Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Key Laboratory of Genetics, Breeding and Multiple Utilization of Crops, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[†] These authors contributed equally

lhe@cemps.ac.cn

Abstract

Polyploidization presents an unusual challenge for species with sex chromosomes, as it can lead to complex combinations of sex chromosomes that disrupt reproductive development. This is particularly true for allopolyploidization between species with different sex chromosome systems. Here, we assembled haplotype-resolved chromosome-level genomes of a female allotetraploid weeping willow (*Salix babylonica*) and a male diploid *Salix dunnii* using Hi-C and PacBio HiFi reads. We used phylogenomics of nuclear and plastid genomes to show that weeping willow arose from crosses between a female ancestor from the *Salix* clade, having XY sex chromosomes on chromosome 7, and a male ancestor from the *Vetrix* clade, having ancestral XY sex chromosomes on chromosome 15. Our analysis revealed that weeping willow has one pair sex chromosomes, ZW on chromosome 15, that derive from the ancestral XY sex chromosomes in the *Vetrix*-clade male ancestor and the X chromosomes on chromosome 7 from the *Salix*-clade female ancestor that has reverted to an autosome. Taken together, our results point to rapid evolution and reversion of sex chromosomes following allopolyploidization in weeping willow.

Machine learning-based population genetics analyses reveal the important impact of introgressed beneficial and deleterious variants on grape breeding

Hua Xiao[1], Zongjie Liu[1], Nan Wang[1], Qiming Long[1], Shuo Cao[1], Guizhou Huang[1], Wenwen Liu[1], Yanling Peng[1], Summaira Riaz[2], Andrew M. Walker[2], Brandon S. Gaut[3], and Yongfeng Zhou[1,4]

[1]*State Key Laboratory of Tropical Crop Breeding, Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Key Laboratory of Synthetic Biology, Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China*

[2]*Department of Viticulture and Enology, University of California, Davis, Davis, CA, USA*

[3]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, 95616, USA*

[4]*State Key Laboratory of Tropical Crop Breeding, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, China*

Xiaohua01@caas.cn

Abstract

Grapevine is one of the first domesticated crops. During grape breeding, wild grapes are often used as sources for key agronomic traits. Previous studies have revealed genomic signals of introgression from wild to cultivated grapes in Europe. The gene flow (introgression) from wild grapes to cultivars not only brought beneficial variants but also many deleterious variants. However, our understanding of the consequences, genetic architecture, and traits affected by introgression is still quite limited. Thus, we studied resequencing data from samples spanning the distributional range of wild (*Vitis vinifera* ssp. *sylvestris*) and cultivated (*Vitis vinifera* ssp. *vinifera*) grapes. Based on population genetic analyses including machine learning methods, we predicted the time, mode, genomic pattern, and biological impacts of these introgression events. We discovered a consistent gene flow between European wild grapes (EU) and cultivated grapes over the past ~2000 years, especially from EU to wine grapes. Gene pathways associated with the synthesis of aromatic compounds were enriched in regions that were both selected and introgressed, suggesting that EU wild grapes were an important resource for improving the flavor of cultivated grapes. Despite the potential benefits of introgression in grape improvement, the introgressed fragments introduced a higher deleterious burden, with most deleterious SNPs and structural variants (SVs) hidden in a heterozygous state, which increased the genetic load in wine grapes, As a result, this could hampers the breeding of grapes, especially through crossing in the future. We further used forward simulation to simulate the dynamics of introgressed alleles and found that introgression may have played a historically significant process for improving clonal systems like domesticated grapes. In general, our study of the beneficial and harmful effects of introgression is critical for the genomic breeding of grapevine to utilize the advantages of wild resources.

Establishment of a direct somatic embryogenesis regeneration system using immature cotyledon explants in Camellia sinensis cv. Shuchazao

Tianyuan Yang

State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, Auhui 230036, China

yangtianyuan@ahau.edu.cn

Abstract

Tea is one of the most popular non-alcoholic beverages, while tea plant is also an important cash crop in the world. Somatic embryogenesis (SE) is a powerful tool for producing large scale vegetative propagation, but the unestablished SE regeneration system greatly impedes the mass production of tea seedlings, thereby affecting the development of tea industry. In this study, we established a high-efficiency direct SE system in tea that includes somatic embryo initiation, maturation and germination. Somatic embryo initiation was affected by tea cultivars, explant age and plant growth regulators. Cotyledons of tea seeds at 270 days after flowering had the highest globular embryo induction efficiency as explants. Somatic embryos were induced well in embryo initiation medium after adding 1 mg/L NAA and 0.5 mg/L 6-BA. The optimum induction rate of globular embryos to cotyledonary embryos was 12.62 ± 1.57%, when the globular embryos was transferred to the hormone-free medium supplemented with 2 g/L activated carbon. Mature embryos germinated to produce plantlets on MS medium containing 1 mg/L 6-BA and 0.1 mg/L NAA with a generation frequency of 20.2 ± 0.1%. In addition, six SE-related genes were identified, and the expression level of these genes during the SE of tea were analyzed by quantitative real-time RT-PCR (qRT-PCR). These genes showed higher expression levels in the initiation embryo stage than in the maturated embryo stage, which indicated that these genes are crucial in the initiation of SE in tea. Taken together, the establishment of a direct SE regeneration system for tea plant can promote their large-scale propagation and genetic improvement as well as studies about SE-related genes, laying the foundation for further studies on the molecular mechanism of SE in tea plant.

Exploration of the mechanism underlying lipid alterations in the yellowing leaves of 'HAES344' macadamia

Weihai Yang, Xiaopeng Li, Qin Shao, Qiusheng Xiao, Na Chen

College of Life Science and Resources and Environment, Yichun University, Yichun, Jiangxi, 336000, China

seayang2004@126.com

Abstract

Macadamia variety 'HAES344' (*Macadamia integrifolia*) is a high-value, evergreen nut tree that is grown commercially in tropical and subtropical regions around the world for its premium edible kernel. However, the shoots of 'HAES344' are prone to generate etiolated leaves under continuous high temperatures in summer, which eventually results in a decrease of yield. Lipids are the important subcellular components and participate in a variety of physiological processes. However, the profiles and metabolic mechanisms of lipids in leaf yellowing of 'HAES344' remain unclear. In this study, three 8-year-old 'HAES344' macadamia trees with similar canopy size, grown in Zhanjiang, China, were selected for an experimental block, and the newly mature leaves on June 1 (green leaf), 20 (moderately yellow leaf with an etiolation area between 50% and 70%) and 28 (severely yellow leaf with an etiolation area nearly 100%) were collected and used for the analysis of lipid composition and transcript expression of related genes in lipid metabolism. Widely targeted metabolomics analysis identified 20 differential accumulated lipids, including 5 free fatty acids, 8 glycerol esters, 5 lysoPC and 2 lysoPE, and all these lipids were significantly downregulated in the yellowing leaves. Transcriptomic analysis revealed 108 differential expressed genes related to lipid metabolism, including 22 upregulated and 69 downregulated genes in the severely etiolated leaves compared to the green leaves. Of these 108 genes, the largest proportion of genes was involved in cutin, suberine and wax biosynthesis, followed by linolenic acid metabolism, fatty acid degradation, and glycerolipid metabolism, while the smallest proportion of genes was involved in sphingolipid metabolism. Through the qRT-PCR analysis of 20 key functional genes, the relative expression levels of *SDP1*, *ACX1*, *S-ACP-DES6*, *FATB1*, *LACS4*, *accD*, and *ALDH3F1* were increased during leaf yellowing, while those of *LOX1.5*, *LACS1*, *LACS2*, *BCCP2*, *KCS12*, *LDH3H1*, *DGAT2*, *GPAT8*, and *GDPD2* were decreased. Additionally, the expression levels of *LOX2*, *LOX1.6* and *ADH* increased and then decreased, while the expression trend of *OLE1* was opposite. In yellow leaves, the transcription levels of *LOX1.5*, *LACS1*, *KCS12*, *DGAT2* and *GPAT8* were significantly decreased, while those of *ACX1*, *S-ACP-DES6*, *FATB1*, *accD*, and *ALDH3F1* were significantly increased. Compared with green leaves, the expression levels of *SDP1*, *LACS4*, *LOX2*, *LOX1.6* and *ADH* were significantly increased in moderately and/or severely etiolated leaves, while those of *OLE1*, *LACS2*, *BCCP2*, *LDH3H1*, and *GDPD2* were significantly reduced only in moderately or severely yellow leaves. These results of the reduced lipids and the differentially expressed genes show the distinctive characteristics of lipid metabolism during leaf yellowing, which lays a foundation for the further exploration of the molecular mechanisms of macadamia leaf yellowing under heat stress.

Telomere-to-telomere gap-free reference genome of wild blueberry (*Vaccinium duclouxii*) provides high soluble sugar and anthocyanin accumulation

Tuo Zeng[1], Zhijiao He[2], Jiefang He[1], Wei Lv[1], Shixiang Huang[1], Jiawen Li[3], Liyong Zhu[4], Shuang Wan[5], Wanfei Zhou[4], Zhengsong Yang[2], Yatao Zhang[1], Cong Luo[1], Jiawei He[2], Caiyun Wang[1], Liangsheng Wang[6,7,8]

[1] *School of Life Sciences, Guizhou Normal University, Guiyang 550000, China*
[2] *Institute of Alpine Economic Plant, Yunnan Academy of Agricultural Sciences, Lijiang 674199, Yunnan, China*
[3] *School of Advanced Agricultural Sciences, Peking University, 100871 Beijing, China*
[4] *National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, College of Horticulture & Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China*
[5] *Wuhan Benagen Technology Co., Ltd, Wuhan 430070, China*
[6] *Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*
[7] *China National Botanical Garden, Beijing 100093, China*
[8] *University of Chinese Academy of Sciences, Beijing 100049, China*

zengtuo@gznu.edu.cn

Abstract

Vaccinium duclouxii, endemic to southwestern China, is a berry-producing shrub or small tree belonging to the Ericaceae family, with high nutritive, medicinal, and ornamental value, abundant germplasm resources and good edible properties. In addition, *V. duclouxii* exhibits strong tolerance to adverse environmental conditions, making it a promising candidate for research and offering wide-ranging possibilities for utilization. However, the lack of the *V. duclouxii* genome sequence has hampered its development and utilization. Here, a high-quality telomere-to-telomere genome sequence of *V. duclouxii* was de novo assembled and annotated. All of the 12 chromosomes were assembled into gap-free single contigs, providing the highest-integrity and -quality blueberry assembly reported so far. The *V. duclouxii* genome is 573.67 Mb in length and encodes 41,953 protein-coding genes. Combining transcriptomics and metabolomics analyses, we have uncovered the molecular mechanisms involved in sugar and acid accumulation and anthocyanin biosynthesis in *V. duclouxii*. This study provides essential molecular information for further research on the quality of *V. duclouxii*. Moreover, the high-quality telomere-to-telomere assembly of the *V. duclouxii* genome will provide insights into the genomic evolution of *Vaccinium* and support advancements in blueberry genetics and molecular breeding.

Phospholipid remodeling of apple dormant buds during cryopreservation and regrowth

Zhang Jinmei, Wang Yingmin, Xin Xia

National Crop GeneBank of China, Institute of Crop Science, Chinese Academy of Agricultural Sciences

zhangjinmei@caas.cn

Abstract

Cryopreservation protocols of apple dormant buds were developed and optimized in the National Crop GeneBank of China (Institute of Crop Science, Chinese Academy of Agricultural Sciences) in a previous study. However, the mechanisms that maintain cell integrity and regrowth potential have not been well explained. Using the UPLC-TQ-S platform with MassLynx software data analysis and quantification, 12 comparative lipidomics datasets of apple dormant buds during cryopreservation treatments were obtained. Results showed that dehydration and programmed cooling treatments stimulated the protective mechanisms against extreme low temperature by promoting the expression of membrane lipid metabolism genes and the accumulation of phosphatidic acid (PA), phosphatidylcholine (PC), and phosphatidylethanolamine (PE), indicating the induction of membrane lipid remodeling in dormant buds of apple during cryopreservation. Furthermore, PA may be involved in the regulation of low-temperature stress as a signaling molecule. This study provides new information on the regrowth response mechanism of apple dormant buds during cryopreservation, and also provides a technical framework for the preservation of other germplasm resources.

Effects of Extreme Root Restriction on the Nutritional and Flavor Quality, and Sucrose Metabolism of Tomato (*Solanum lycopersicum L.*)

Danxia Liu[1,†], Jinxiang Chen[1,†], Yanwei Hao[1], Xiaolong Yang[1], Riyuan Chen[1,2], Yiting Zhang[1]

[1] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*

[2] *Huangpu Innovation Research Institute, South China Agricultural University, Guangzhou 510642, China*

[†] These authors contributed equally.

yitingzhang@scau.edu.cn

Abstract

Root restriction is suitable for horticultural soilless cultivation characterized by high efficiency and quality in the case of high density and low node order pinching. However, little research is available on the mechanism of root restriction in improving the flavor and nutritional quality of tomatoes. We investigated the effects of Extreme Root Restriction (ERR, 750 mL/plant) on the content of metabolites, activity of enzymes, and expression level of genes involved in sucrose metabolism in different clusters of two tomato types. The fruit diameter and single fruit weight of common tomato at CIII were reduced by 5.6% and 14% under ERR, respectively, and as a result, the fruit uniformity throughout the whole plant was improved. ERR enhanced the accumulation of metabolites in tomato fruits, such as soluble sugars, amino acids, vitamin C, lycopene, and polyphenol, which was caused by the 'concentration effect' that occurred with the reduction of fruit size. The activities of enzymes (SS, SPS, NI, AI) at CIII and CIV of cherry tomatoes increased by 3–4 fold under ERR. ERR enhanced the accumulation of sucrose, glucose, and fructose in tomato fruits not only by modulating activities of metabolizing enzymes but also by inducing the expression of sucrose metabolism genes, including sucrose synthase genes (*SS1, SS3–6*) in common tomato, fructokinase genes (*FKs*), hexokinase genes (*HKs*), and sucrose phosphate synthase genes (*SPSs*) in cherry tomato. The above results are expected to provide a theoretical basis for root restriction cultivation techniques and practical guidance for high-quality tomato production in industrialized cultivation.

Extraction and functional analysis of key regulatory genes for adventitious bud regeneration from watermelon cotyledons induced by 6-BA

Na Zhang[1], Xing Huang[3], Hongxia Zeng[1], Yuhong Sun[1], Jian Ren[1], Mi Tang[1*]

[1] *Wuhan Academy of Agricultural Sciences, Wuhan 430345, China*

[2] *Huazhong Agricultural University, Wuhan 430070, China*

[3] *Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China*

zhangna@wuhanagri.com(N.Z.); huangxing@catas.cn(X.H.); zenghongxia@wuhanagri.com(H.Z); sunyh68@163.com;renjian@wuhanagri.com(J.R.); tangmi@wuhanagri.com(M.T.)

Abstract

6-BA is one of the most effective plant growth regulators for watermelon adventitious bud induction and plays a key role in watermelon regeneration. However, no in-depth molecular biological studies have been conducted to reveal its induction mechanism. In previous studies, we verified the functions of the WOX and SAUR gene families in the process of adventitious bud regeneration by real-time quantitative PCR, but the throughput was much lower than that of transcriptomics. To solve this problem, we analyzed the morphological changes, physiological responses, and transcriptome changes in adventitious bud regeneration from watermelon cotyledons induced by 6-BA. The phenotypic results indicated that 10 to 21 days after 6-BA treatment was the key period for adventitious bud regeneration from watermelon cotyledons. After 6-BA induction, the contents of five cytokinins reached maximum values on the 17th day after treatment, and this may be a key time point for exploring the response of watermelon cotyledon adventitious bud regeneration to 6-BA treatment. Transcriptome analysis identified 33,868 differentially expressed genes (DEGs) in 13 comparison groups, 15,108 of which were upregulated and 18,760 downregulated. GO functional enrichment analysis showed that the DEGs were mainly involved in metabolic processes, cell processing, biological regulation, catalytic activity, and binding activity. KEGG metabolic pathway enrichment analysis showed that the DEGs were mainly associated with plant hormone signal transduction, ribosomes, am.

Non *S*-locus *F-box* gene breaks self-incompatibility in *Citrus*

Zonghong Cao[1], Yu Hu[1], Mengmeng Xu[1], Zongzhou Xie[1], Junli Ye[1], Xiuxin Deng[1,2], Lijun Chai[1,2]

[1]*National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan, Hubei 430070, China*
[2]*Hubei Hongshan Laboratory, Wuhan 430070, China*

caozonghong@webmail.hzau.edu.cn; chailijun@mail.hzau.edu.cn

Abstract

Self-incompatibility (SI) is a crucial mechanism that prevents self-fertilization and inbreeding in flowering plants. *Citrus*, one of the most important fruit crops, employs *S*-RNase-based gametophytic SI (GSI). In *Citrus*, some studies have been performed on the transition from SI to SC, but the mechanism remains unclear. Here, we identified a non-*S* locus F-box protein (Sli) by using stamen transcriptome datasets of self-compatibility (SC) 'Shatangju' mandarin (*Citrus reticulata* Blanco). The *in vitro* interaction assay results showed that Sli could interact with S_2-RNase and S_{30}-RNase of 'Shatangju' as well as multiple S-RNases from *Citrus* and other species. Expression pattern analysis showed that *Sli* was highly expressed in the pollen of SC varieties but not in the pollen of SI varieties, indicating that the expression level of *Sli* is highly correlated with the SC phenotype. Furthermore, the overexpression of the *Sli* gene in SI 'Mini-Citrus' resulted in the SC phenotype. Analysis of *Sli* promoter haplotypes and activities indicated that the allelic variation of the *Sli* promoter influenced the expression level of *Sli* in different citrus and thus, regulate the SI to SC transition in some citrus. Identification of *Sli* significantly advances the understanding of the genetic basis of the SI system in citrus.

Actinobacteria–Important Friends of a Centuries-old Apple Tree (*Malus prunifolia*)

Jieqiang He, Qingmei Guan

State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China

jieqiang.he.dawn@gmail.com

Abstract

Malus prunifolia, a unique germplasm with a strong tolerance to stress, is widely used as an apple rootstock in China. In Fuping Country, Shaanxi Province, China, a centuries-old *M. prunifolia* apple tree was found by chance to be still productive every year. The genome of this longevity apple tree and the molecular mechanisms of its resistance were explored in our previous studies. It is unknown whether the microbiome, especially the rhizobacteria, an important factor influencing plant growth and health, plays an important role in the long-lived *M. prunifolia* tree. In this study, to understand whether unique rhizobacteria are at work in the root system of the long-lived tree, two young *M. prunifolia* trees aged 15 years, descendants of the old tree in the same orchard, were used for comparison to exclude the influence of environment and descent. Firstly, the 16S rRNA data showed that the composition of the root bacteria community was influenced by age. Furthermore, the abundance of 56 ASVs was significantly increased in the rhizosphere soil of the old tree relative to the two young trees, and 29 of these ASVs were exclusive to the old tree. Functional annotations showed that these differential ASVs were mainly and significantly related to carbohydrate metabolism, lipid metabolism, environmental adaptation and nitrification. Additionally, two strains similar to the differential ASVs belonging to Actinobacteria were isolated and found to promote the growth of primary roots and the number of lateral roots by inoculating the Arabidopsis root, and this root-promoting phenotype is still present in tomato and monocotyledonous wheat. Our results reveal the potential role of Actinobacteria in apple tree longevity, and the isolation of strains from the long-lived tree provides a new application source for crop production.

A calcium-dependent protein kinase33, phosphorylate a bHLH transcription factor89, enhances drought tolerance in tomato

Jiahui Hu, Juan Wang, Baike Wang, Tao Yang, Ning Li, Haitao Yang, Qinghui Yu

Institute of Horticultural Crops, Xinjiang Academy of Agricultural Science (Key Laboratory of Horticulture Crop Genomics Research and Genetic Improvement in Xinjiang), Urumqi, China

hujiahui@163.com

Abstract

Plants have evolved multiple strategies in response to various environmental stimuli to enhance vitality. Calcium-dependent protein kinase (CPK) appears to play a pivotal role in abiotic stress signaling. To date, studies on the specific substrates recognized by CPK in response to drought stress have been limited. In this study, we reveal a potential interaction between CPK and a bHLH transcription factor under drought stress and confirm that both have positive roles in plant response to drought stress. First, we identified a CPK gene (named *SpCPK33*) from wild tomato *Solanum pennellii* and transformed it into tomato (*S. lycopersicum*) to enhance drought stress tolerance. Through yeast two-hybrid screening, we discovered that SpCPK33 interacts with a bHLH transcription factor (named *SpbHLH89*), which also exhibits positive response to drought stress. To further investigate this interaction, we conducted experiments, including BiFC (Bimolecular Fluorescence Complementation), pull-down assays, and Co-IP (Co-immunoprecipitation), all of which revealed a possible interaction between SpCPK33 and SpbHLH89 at the cytoplasmic membrane. RNA-seq analysis of transgenic lines overexpressing both *SpCPK33* and *SpbHLH89* under drought stress demonstrated their involvement in the MAPK pathway, which is associated with drought stress. Additionally, these transgenic lines showed improved tolerance through activation of antioxidant enzymes and regulation of osmoregulatory substances, leading to more efficient scavenging of reactive oxygen species (ROS). Furthermore, the hybrid tomato line co-expressing *SpCPK33-SpbHLH89* exhibited greater tolerance against drought conditions and reduced water loss by regulating leaves stomatal aperture in response to abscisic acid (ABA). Conversely, anti-expression lines (*anti-cpk33* and *anti-bhlh89*) demonstrated increased sensitivity to drought stress. Overall, our findings uncover the essential role of the SpCPK33-SpbHLH89 module in response to drought stress in tomato. This study provides new evidence on the substrate specificity of the CPK signal transduction pathway and offers insights into new strategies for breeding drought-tolerant tomato varieties.

JAs-inducible *PpZAT5* and *PpBBX32* modulate temperature-dependent and tissue-specific anthocyanin accumulation in peach fruit

Dan Huang[1], Lei Xue[1], Yueqin Lu[1], Kui Lin-Wang[2], Andrew C. Allan[2,3], Bo Zhang[1], Kunsong Chen[1], Changjie Xu[1]

[1] *Zhejiang Provincial Key Laboratory of Horticultural Plant Integrative Biology, Zhejiang University, Zijingang Campus, Hangzhou 310058, China*

[2] *New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand*

[3] *School of Biological Sciences, University of Auckland, Auckland, New Zealand*

hdan@zju.edu.cn; chjxu@zju.edu.cn

Abstract

Anthocyanins have multiple biological functions and contribute significantly to the appearance and nutritional value of fruits. Anthocyanin accumulation was observed in the mesocarp around the peel (MP) of peach (*Prunus persica* cv. Zhonghuashoutao) fruit stored at 16°C. We found that there was differential anthocyanin accumulation in the peel (P), MP and mesocarp around the stone (MS). A key transcription factor, PpMYB10.1, is known to be involved in peach anthocyanin regulation. However, the regulatory factors upstream of *PpMYB10.1* and the mechanisms underpinning temperature-dependent and tissue-specific anthocyanin accumulation remain elusive. Here, by cross-analysis of different sets of RNA-seq data, the transcription factor genes *PpBBX32* and *PpZAT5* were identified to be related to anthocyanin accumulation. Both PpBBX32 and PpZAT5 positively regulate anthocyanin biosynthesis, as confirmed by homologous or heterologous transient injection and stable tobacco transformation experiments. Dual-luciferase, yeast one-hybrid and EMSA analyses showed that both PpBBX32 and PpZAT5 could directly bind to specific sequences in the *PpMYB10.1* promoter and activate its expression. PpBBX32 and PpZAT5 were also observed to interact at the protein level. Through molecular docking, firefly luciferase complementation imaging (LCI) and dual-luciferase combination assays, we found that PpBBX32, PpZAT5, and PpMYB10.1 are predicted to form a protein complex, which induces anthocyanin biosynthesis. *In silico* analyses of the PpBBX32 and PpZAT5 promoters revealed the presence of *cis*-acting elements related to the plant hormones ABA, auxin, SA and MeJA. When these four hormones were injected into peach flesh, anthocyanin accumulation was induced by MeJA, accompanied by the enhanced expression of anthocyanin biosynthesis-related genes, *PpMYB10.1*, *PpBBX32* and *PpZAT5*. Endogenous JA and JA-Ile contents were measured and found to be higher in anthocyanin-accumulating tissues, including MP from fruit stored at 16°C, MS, and flesh around the infiltration site of MeJA. In summary, PpBBX32 and PpZAT5 are upstream regulators of *PpMYB10.1* that allow JAs to take part in temperature-dependent and tissue-specific anthocyanin accumulation in peach by modulating their expression.

High-quality *Bougainvillea* genome provides new insights into the evolutionary history and pigment biosynthesis pathways in Caryophyllales

Lan Lan[1,2,3,†], Huiqi Zhao[4,5,†], Suxia Xu[6], Shenglong Kan[3], Xiaoni Zhang[1,3], Weichao Liu[3,7], Xuezhu Liao[1,3], Luke R. Tembrock[8], Yonglin Ren[2], Wayne Reeve[2], Jun Yang[4,5], Zhiqiang Wu[1,3]

[1] *Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, 518120, China*

[2] *College of Science, Health, Engineering and Education, Murdoch University, 6150, Western Australia, Murdoch, 90 South Street, Australia*

[3] *Kunpeng Institute of Modern Agriculture at Foshan, Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518124, China*

[4] *Sanya Institute, Hainan Academy of Agricultural Sciences, Sanya, 572025, China*

[5] *Institute of Tropical Horticulture Research, Hainan Academy of Agricultural Sciences, Haikou, 571100, China*

[†] These authors contributed equally.

lanlan@caas.cn; zhaohuiqi@hnaas.org.cn; juny83@126.com; wuzhiqiang@caas.cn

Abstract

Bougainvillea is a perennial ornamental shrub that is highly regarded in ornamental horticulture around the world. However, the absence of genome data limits our understanding of the pathways involved in bract colouration and breeding. Here, we report a chromosome-level assembly of the giga-genome of *Bougainvillea* × *buttiana* 'Mrs. Butt', a cultivar thought to be the origin of many other *Bougainvillea* cultivars. The assembled genome is ~5 Gb with a scaffold N50 of 151,756,278 bp and it contains 86,572 genes which have undergone recent WGD. We confirmed that multiple rounds of whole genome multiplication have occurred in the evolutionary history of Caryophyllales, reconstructed the relationship in Caryophyllales at whole genome level, and found discordance between species and gene trees as the result of complex introgression events. We investigated betalain and anthocyanin biosynthesis pathways and found instances of independent evolutionary innovations in nine different Caryophyllales species. To explore the potential mechanism of diverse bract colours in *Bougainvillea*, we analyzed the genes involved in betalain and anthocyanin biosynthesis and found extremely low expression of *ANS* and *DFR* genes in all cultivars, which may limit anthocyanin biosynthesis. Our findings indicate that the expression patterns of betalain biosynthesis-related genes did not directly correlate with the bract colour, and the higher expression of betalain biosynthesis-related genes is required for the coloured bract. The improved understanding of the correlation between gene expression and bract colour allows plant breeding outcomes to be predicted with greater certainty.

StMADS11 subfamily plays essential roles in flower organogenesis and flowering time in hickory

Caiyun Li, Zhichao Sun, Di Wu, Bo Zhang, Hongmiao Jin, Kean-Jin Lim, Zhengfu Yang, Zhengjia Wang

State Key Laboratory of Subtropical Silviculture, College of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou, 311300, China

licaiyun@stu.zafu.edu.cn

Abstract

StMADS11 subfamily genes play crucial roles in regulating plant flowering, maintaining normal flower development, and controlling bud dormancy. These genes also exhibit functional differences between annual plants and perennial woody plants. Despite their importance, their roles are understudied in trees of the walnut family, particularly in hickory (*Carya cathayensis* Sarg.). Motivated by this knowledge gap, we comprehensively investigated the hickory genome, delving into gene clustering, sequence characteristics, and expression pattern analysis. Our analysis revealed five *StMADS11* subfamily genes in the hickory genome: *CcSVP*-like, *CcAGL24*-like1, *CcAGL24*-like2, *CcJOINTLESS*-like1 and *CcJOINTLESS*-like2. Expression analysis indicated that the *StMADS11* subfamily genes exhibit the highest expression level in female flower buds, with a distinct seasonal pattern. When these genes were overexpressed in *Arabidopsis*, they caused different abnormalities in floral organs and pods. Overexpression of *CcSVP*-like lines caused a delay in flowering, while overexpression of the remaining *StMADS11* subfamily lines promoted flowering. Subsequent protein interaction studies showed that *StMADS11* interacts with CcFUL-like proteins. However, only CcFUL-like, CcSVP-like, CcJOINTLESS-like1 and CcJOINTLESS-like2 proteins could bind to the *CcSOC1*-like promoter, consequently suppressing the expression of *CcSOC1*-like. Our results suggest that *CcSVP*-like can inhibit the flowering of hickory by suppressing the expression of *CcSOC1*-like and participating in the regulation of the hickory flower formation gene network. Conversely, other members of the *StMADS11* subfamily appear to promote flowering, indicating a complex interplay of these genes in floral regulation. These insights highlight the *StMADS11* subfamily's complex role in floral development and timing in hickory. They invite further studies to elucidate the underlying molecular pathways and to enhance our understanding of flowering processes in hickory and other perennial woody plants.

Fluorescence characteristics of tomato (*Lycopersicon esculentum* Mill.) leaves under different high-temperature stress and nitrogen application at flowering and fruiting stages

Chunying Li[1,2], Zaiqiang Yang[1], Chunlong Zhang[3], Jing Luo[1], Nan Jiang[1], Fengyin Zhang[1]

[1] *School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing, 210044, China*

[2] *School of Environment and Tourism, West Anhui University, Lu'an, 237000, China*

[3] *School of Electrical and Optoelectronic Engineering, West Anhui University, Lu'an, 237000, China*

20211108008@nuist.edu.cn; yzq@nuist.edu.cn

Abstract

To study the effects of nitrogen application on the photosynthetic structure and light system of tomato (*Lycopersicon esculentum* Mill.) leaves in the recovery stage after heat stress, OJIP curves and JIP parameters were determined through a control experiment in an artificial climate chamber. Tomato variety "Jin fen No. 1" was planted and exposed to 4 day/night temperature levels (25 °C/15 °C as control CKT; 30 °C/20 °C, as LHT; 35 °C/25 °C, as MHT; 40 °C/30 °C, as SHT) for 7 days under five nitrogen supply levels (N1–N5: 0, 1.3, 1.95, 2.6 and 3.75 g/plant, respectively; N4 as control). Different high temperature/nitrogen combination treatments resulted in obvious differences at points O, K, J, I, and P. Compared with CKTN4, nitrogen application increased the fluorescence intensity of SHTN2-SHTN5 in P, I and J phases, and decreased that of MHTN1-MHTN4. At point P, SHTN5 increased by 13.27% and SHTN3 by 10.10%. At point I, SHTN5 increased by 13.52% and SHTN3 by 12.21%. At point J, SHTN5 increased by 20.16% and SHTN3 by 26.18%. There were significant differences ($p < 0.01$) among the responses of high temperatures to nitrogen supply levels. On the first day of the recovery period, N had no significant effect on F_v/F_M, F_v, $F_o$and F_M; however, their interaction was significant ($p < 0.05$). HT and N for F_o, F_M, F_V, F_O/F_M, F_V/F_M, F_V/F_O, ABS/RC and DI_O/RC had no significant interaction effects on the eighth day of the recovery period. F_v/F_o was sensitive to high temperature and nitrogen application. Under all 5 nitrogen applications, temperature played a significant role in increasing DI_O/RC, especially for N2 and N3. According to the leaf pipeline model, while decreasing the nitrogen application under SHT, there was more active RC, and the higher value of the specific energy flux (ABS/RC, TR_O/RC and DI_O/RC) showed the increased ability to RC the reduction of plastoquinone.

Comparative Analysis of Chloroplast Genome Structure and Phylogenetic Relationships Among Six Taxa Within the Genus *Catalpa* (Bignoniaceae)

Feng Li[1,2], Ying Liu[1], Junhui Wang[1], Peiyao Xin[2], Jiangtao Zhang[3], Minggang Zhang[4], Wenjun Ma[1,*]

[1] *State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, National Innovation Alliance of Catalpa Bungei, Beijing, China,*

[2] *Key Laboratory of National Forestry and Grassland Administration on Biodiversity Conservation in Southwest China, Southwest Forestry University, Kunming, China, and College of forestry, Guizhou University, Guiyang, Guizhou, China/Institute for Forest Resources and Environment of Guizhou, Guizhou University, Guiyang, Guizhou, China*

[3] *Henan Academy of Forestry, Zhengzhou, China*

[4] *Guizhou Academy of Forestry, Guiyang, China*

lifeng53@126.com

Abstract

Species within the genus *Catalpa* are mostly semievergreen or deciduous trees with opposite or whorled leaves. *C. bungei*, *C. fargesii* f. *duclouxii* and *C. fargesii* are sources of traditional precious wood in China. Due to a lack of phenotypic and molecular studies and insufficient sequence information, intraspecific morphological differences, common DNA barcodes and partial sequence fragments cannot clearly reveal the phylogenetic or intraspecific relationships within *Catalpa.* Therefore, we sequenced the complete chloroplast genomes of six taxa of the genus *Catalpa* and analyzed their basic structure and evolutionary relationships. The chloroplast genome of Catalpa showed a typical tetrad structure, with a total length ranging from 157,765 bp (*C. fargesii*) to 158,355 bp (*C. ovata*). The length of the large single-copy (LSC) region ranged from 84,599 bp (*C. fargesii*) to 85,004 bp (*C. ovata*), that of the small single-copy (SSC) region ranged from 12,662 bp (*C. fargesii*) to 12,675 bp (*C. ovata*), and that of the inverted repeat (IR) region ranged from 30,252 bp (*C. fargesii*) to 30,338 bp (*C. ovata*). The GC content of the six chloroplast genomes was 38.1%. The 113 genes included 79 protein-coding genes, 30 tRNA genes and 4 rRNA genes. Five hypervariable regions (*trnH-psbA, rps2-rpoC2, rpl22, ycf15-trnl-CAA and rps15*) were identified by analyzing chloroplast nucleotide polymorphisms, which might serve as potential DNA barcodes for the species. Codon usage patterns were highly similar among the taxa included in the present study. In addition to the stop codons, all codons showed a preference for ending in A or T. Phylogenetic analysis of the entire chloroplast genome showed that all taxa within the genus *Catalpa* formed a monophyletic group, clearly reflecting the relationships within the genus. This study provides information on the chloroplast genome sequence, structural variation, codon bias and phylogeny of *Catalpa*, which will facilitate future research efforts.

Insights into the impact of cluster bagging on flavonoid compounds in Kolor grape berries: A comprehensive comparative analysis of metabolomics, gene regulation, and chromatic attributes

Huiqing Li[1,2], Xiaotong Gao[1,2], Jun Wang[1,2]

[1] *Center for Viticulture & Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

lhq510604578@cau.edu.cn

Abstract

Light conditions greatly influence the ripening of grape berry and the accumulation of flavonoid compounds. In recent years, due to the increasing incidence of damage by birds and bees, early-ripening grape varieties such as Kolor (*Vitis vinifera* L. cv.) have exhibited heightened vulnerability to such harm. Bagging, when compared to bird netting, has proven to be a more effective method for averting such damage. To investigate the impact of bagging treatment, flavonoids were isolated by HPLC-QqQ equipped with an EIS. The bagging treatment could modify the microclimate within grape clusters, not only by blocking light but also by elevating temperature. The bagging treatment had a notable impact on anthocyanins and flavanols in Kolor berries, but it did not significantly affect flavonols. The total concentration of flavanols in the berries decreased significantly, whereas the concentration of malvidins in the skin declined, and the proportion of peonidins in the pulp exhibited a significant increase. Following E-L 36, the expression levels of *HYH* and *HY5* in the bagged skin were significantly lower than those in the control group. The expression levels of *F3H-2*, *LDOX*, *UFGT*, and *CHI-1* in the skin exhibited a consistent pattern with the accumulation of anthocyanins. The expression of light-regulated factors in the pulp was diminished and significantly downregulated subsequent to bagging treatment. The expression levels of *UFGT*, *F35H-2*, *MYB4a*, and six other genes demonstrated correlations with the concentrations of anthocyanins and flavanols, with correlations exceeding 80%. The skin of bagged grapes exhibits an elevated b* during veraison, contributing to an intensified yellow color, whereas the a* of mature berries declines and the b* increases, resulting in a more pronounced orange color in the bagged pulp. This study lays the groundwork for a thorough evaluation of the suitability of bagging technology.

Transcriptome analysis and hormone reveal the key pathways and regulatory network involved in early fruit development of *Gleditsia sinensis* Lam. (Fabaceae)

Qiao Liu[1,2,3,4], Yang Zhao[1,2,3,4], Ju Yang[1,2,3,4], Feng Xiao[1,2,3,4], Xiurong Wang[1,2]

[1] *College of Forestry, Guizhou University, Guiyang550025, Guizhou, China*

[2] *Institute for Forest Resources & Environment of Guizhou, Guizhou University, Guiyang, Guizhou, China*

3 Key Laboratory of Forest Cultivation in Plateau Mountain of Guizhou Province, Guiyang, Guizhou, China

[4] *Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Guizhou University, Guiyang, Guizhou, China*

gs.liuqiao20@gzu.edu.cn

Abstract

Gleditsia sinensis Lam. (Fabaceae) is a medicinal legume with scolus and pods rich in saponin, polysaccharose and multiple specialized metabolites for potential medicinal and industrial value. Low fruit setting rate of artificial economic forest seriously hinders its development and utilization. Understanding the cellular events, physiology and biochemistry and molecular regulatory processes of fruit initiation and early fruit development is essential for improving the yield. However, such information of *G. sinensis* remains largely unknown. In this research, we divided the critical time period from eflorescence to early fruit development and then generated high-resolution spatiotemporal gene expression profiles in the ovary. Comparative transcriptomics and weighted gene co-expression network analyses revealed specific genes and gene modules at specific developmental stages, indicative of distinct genetic programming. Furthermore, the dynamic changes of non-structural carbohydrates and endogenous plant hormones in the ovary across fruit set and early fruit development were investigated. In summary, we identified the potential regulatory network of fruit initiation and followed the development as well as the sets of candidate genes involved. These results provide a valuable reference for the application of exogenous substances during key fruit development periods, such as hormones and sugars, and insights on the development of molecular tools for improving yield.

Haplotype-resolved *Camellia gigantocarpa* genome assembly provides insights into the chromosome structure and evolution of the *Camellia* genus

Cui Lu[1], Wei Li[2], Yu-wei Cui[1], Zheng-tao Wang[1], Xiang-yu Li[1], Ge-ran Hu-tang[3], Hai-Yang Wang[1], Qun-Jie Zhang[1]

[1] *Institution of Genomics and Bioinformatics, South China Agricultural University, Guangzhou 510642, China*

[2] *College of Life Sciences, Institute of Life Sciences and Green Development, Hebei University, Baoding 071000, China*

[3] *Plant Germplasm and Genomics Center, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*

lucui77@163.com

Abstract

Camellia gigantocarpa is an oil-tea tree whose seeds are used to extract high-quality vegetable oil. It is part of the Sect. *Furfuracea*, which is sister to the commonly cultivated oil tea belonging to Sect. *Oleifera* and Sect. *Camellia*. We used HiFi and Hi-C date to generate a haplotype-resolved *Camellia* genome with a contig N50 exceeding 15 Mb that represents the highest quality genome sequence available to date. Our results revealed a significant degree of complementarity between the two sets of chromosomal haplotypes. Recent and extensive amplification of segmental duplications (SDs) in the genome included genes involved in fundamental processes such as protein translation and photosynthetic efficiency. A comparative analysis with other species within the *Camellia* genus revealed that the genomic structure of *C. gigantocarpa* has remained stable compared to the *C. sinensis* genome. Evidence for this comes from the observation that the *C. gigantocarpa* genome lacks two inversions shared by Sect. *Oleifera* and Sect. *Camellia* genomes. Oil metabolic pathways in *C. gigantocarpa* share similar expression patterns and evolutionary history with these other high-oil species, suggesting *C. gigantocarpa* could also serve as a woody oil crop. Our results highlight evolutionary features of the *Camellia* genome, such as a high heterozygosity, many repetitive sequences, and a stable chromosomal structure, with SDs serving as a driving force in genome evolution. Our work also demonstrates the importance of collecting and preserving diverse germplasm resources for oil tea breeding.

Leaf-derived caffeine as a new weapon targeting rhizosphere microbiota to improve plant performance under nitrogen stress

Yuanyuan Luo[1], Xinyuan Liu[1], Huanyun Peng[1], Tianyuan Yang[1], Shupei Zhang[1], Huimin Guo[1], Xiangchun Zhang[3], Jianyun Ruan[3], Xiangde Yang[3], Xuehang Yan[3], Shilai Bao[4], Xiaochun Wan[1], Ruibo Sun[2], Zhaoliang Zhang[1]

[1] *State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Science & Technology, Anhui Agricultural University, Hefei, Anhui 230036, China*

[2] *Anhui Province Key Laboratory of Farmland Ecological Conservation and Pollution Prevention, College of Resources and Environment, Anhui Agricultural University, Hefei, 230036, China*

[3] *Tea Research Institute, Chinese Academy of Agriculture Sciences, Key Laboratory of Tea Biology and Resource Utilization of Tea, The Ministry of Agriculture, Hangzhou 310008, China*

[4] *State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

767791934@qq.com

Abstract

Mobile signals play important roles in mediating the cooperation between shoots, roots, and the rhizosphere microbiome in response to biotic or abiotic stress. However, the potential signals that coordinate the shoot-root-microbiome response to nitrogen (N) stress remain unknown. Caffeine, the dominant alkaloid in beverage plants (such as tea and coffee), fluctuates in root exudates and phloem exudates under aluminum stress and additional nitrogen (N) fertilization. In this study, we used ^{15}N-labeled caffeine to track N stress-induced long-distance transport in tea plants. We found that root exudated more caffeine under N stress. We showed that feeding leaves with caffeine could improve root N uptake and selectively enrich N-cycling bacteria in the rhizosphere under N stress. Similarly, in different tea cultivars, the bacteria correlated with the caffeine content in the root and rhizosphere soil were also associated with soil N-cycling. We collected soil treated with different concentrations of N fertilizer, and the alteration of the microbiota in N-poor soils was further exacerbated by the addition of caffeine compared to N-applied soils. These findings unravel the long-distance transport of leaf-derived caffeine induced by the root perception of N stress, thus manipulating the rhizosphere microbial community and playing an important role in plant N uptake. Our work indicates that the call for help from below-ground promotes the formation of root-shoot-microbiota circuits, thus helping the host to adapt to changing environmental conditions.

Comparison of fruit important traits and genetic analysis of hybrid offspring of different male parents of Jujube

Yujia Luo[1], Yilin Pan[1], Wannian Chen[1], lixin Ge[1], Jiurui Wang[2], Mengjun Liu[3] and Fenfen Fen[1]

[1] *The National and Local Joint Engineering Laboratory of High Efficiency and Superior-Quality Cultivation and Fruit Deep Processing Technology of Characteristic Fruit Trees in Southern Xinjiang, College of Horti-culture and Forestry, Tarim University, Alar 843300, China*

[2] *College of Forestry, Hebei Agricultural University, Baoding 071001, China*

[3] *College of Horticulture, Hebei Agricultural University, Baoding 071001, China*

lluoyujia@163.com; yanfening@163.com

Abstract

Two hybrid combinations ('Yuhong' × 'Jiaocheng 5', 'Yuhong' × 'Xingtai 16') with male sterile jujube germplasm resource ' Yuhong ' as the female parent were used as materials to observe and determine 31 traits such as fruit descriptive traits, fruit kernel size traits and fruit intrinsic nutritional traits of parents and offspring. The results of the quality traits were combined to reveal the genetic differences of offspring caused by different male parents, which provided a reference for screening excellent hybrid offspring and germplasm resource innovation. Among them, the coefficient of variation, genetic transmission and heterosis of fruit size traits of 'Yuhong' × 'Jiao 5' were higher than those of 'Yuhong' × 'Xing 16', and the offspring of 'Yuhong' × 'Jiao 5' had a wide range of transgressive segregation, suggesting that the male parent was one of the important factors affecting the fruit size of the offspring. There was rich genetic diversity in the intrinsic nutritional traits of hybrid fruits, and the variation range was between 6.94% and 37.61%. The heritability of the traits of hybrid offspring was different, and the heritability of fruit transverse diameter and total phenol content was the highest, which was greater than 100%. The dominance rate was -22.39% to 12.90%, and the dominance rate was variable in different hybrid offspring. The single nucleus weight and fruit core diameter were genetically stable in the two combinations, and the heritability of single nucleus weight was 206.21% and 125.24%, respectively. Some traits of hybrid progenies showed a wide range of separation, with fruit size, fruit shape and fruit titratable acidity greatly affected by the male parent. Our findings provide a reference for parent selection and jujube hybrid breeding.

Revealing novel perspectives on the regulation of norisoprenoid biosynthesis of winegrape

Xiao Meng[1,2], Xuechen Yao[1,2], Yi Wei[1,2], Yachen Wang[1,2], Xinjie Lyu[1,2], Changqing Duan[1,2], Qiuhong Pan[1,2]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*
[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

mengxiao2020@cau.edu.cn

Abstract

Norisoprenoids, such as *β*-damascenone and *β*-ionone, are volatile apocarotenoids that play a crucial role in the aromatic profile of wines, contributing to their complex floral and fruity aroma. Carotenoids act as direct precursors for norisoprenoid synthesis. However, the transcriptional regulation controlling the expression of structural genes involved in carotenoid metabolism remains poorly understood, particularly in grapes.

This study analyzed the volatile metabolome and transcriptome of grapes covering 17 stages of berry development to identify the key factors that regulate the production of norisoprenoids. Using K-means clustering, we identified carotenoid metabolism-related genes that were co-expressed with norisoprenoids. The plant carotenoid cleavage oxygenase (CCO) family is an enzyme group that facilitates the cleavage of carotenoids and participates in many important physiological functions. This family includes two subgroups: carotenoid cleavage dioxygenases (CCDs) and 9-cis-epoxide carotenoid dioxygenases (NCEDs). While CCD directly cleaves carotenoids to produce apocarotenoids, NCED is reported to be involved in abscisic acid (ABA) synthesis. We found that *VvNCEDs* were strongly correlated with the accumulation of norisoprenoids. Overexpression of *VvNCEDs* significantly increased *β*-damascenone content, in addition to promoting ABA synthesis. Further experiments showed that exogenous ABA treatment promoted the accumulation of norisoprenoids, whereas the opposite result was observed with NDGA (ABA inhibitor) treatment. These findings suggest that the regulation of norisoprenoids by *VvNCEDs* could be mediated by ABA. In addition, we identified a MADS-box family transcription factor named VvTM6, which was highly correlated with the contents of several norisoprenoids and the expression levels of key structural genes in the carotenoid metabolic pathway. Further investigation showed that overexpression of *VvTM6* activated the expression of genes upstream of carotenoid metabolism, enhancing the accumulation of norisoprenoids in grape berries. This is the first report of a transcription factor positively regulating norisoprenoid metabolism in grapes.

Regulatory endogenous peptides in Juglandaceae fruit development: A peptidomics approach

Zhaohui Niu[1], Caiyun Li[1], Zhengfu Yang[1], Kean-Jin Lim[1], Jianqin Huang[1], Dong Pei[2], Zhengjia Wang[1]

[1] *State Key Laboratory of Subtropical Silviculture, College of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou, 311300, China*

[2] *Chinese Academy of Forestry, Beijing, 100091, China*

2020202011011@stu.zafu.edu.cn

Abstract

Since the discovery of plant peptide hormones in the 1990s, these compounds have received much attention due to their crucial roles in plant development. Most studies, however, have focused on crops and model species, leaving the economically important woody oil crops of the Juglandaceae family (*Carya cathayensis*, *C. illinoensis* and *Juglans regia*) underexplored. To address this knowledge gap, we first developed endogenous peptide extraction methods to counter challenges posed by plant tissue secondary metabolites, protein degradation and low peptide concentration. Next, we employed high-throughput mass spectrometry-based peptidomics to investigate Juglandaceae endogenous peptides during fruit development. Endogenous peptides were identified by comparison with UniProt and Portal of Juglandaceae protein databases. Our extraction methods required just over 2 g of plant tissue samples. However, only 18.71% of the protein in the fruit could produce endogenous peptides. Our analysis identified 2,249 endogenous peptides in *C. cathayensis*, 2,474 in *C. illinoensis* and 5,973 in *J. regia*. While the endogenous peptides in hickory and pecan displayed similarities, those in walnuts exhibited differences. Throughout fruit development, the early stage demonstrated a greater quantity and variety of peptides than the middle and mature stages, indicating an important role for endogenous peptides in the regulation of early development. Among these endogenous peptides, approximately 21% had special modifications, 18% underwent N-terminal acetylation, and 3% underwent hydroxylation. Gene ontology (GO) enrichment analyses linked the precursor proteins of these endogenous peptides to signal transduction function, aligning with the characteristics of the endogenous regulatory peptides. The GO analysis suggests further study on the roles of peptide-producing precursor proteins. Our work identified Juglandaceae endogenous peptides and laid a foundation for future research into plant peptide hormones. The challenge ahead lies in verifying the biological functions of these peptides and utilising them in production.

Unraveling the Sulfur-Infused Aroma of Durian Fruit: Insights into DzMGL Function and Transcriptional Regulation

Pinnapat Pinsorn[1], Kamonpan Sanachai[2], Thanyada Rungrotmongkol[3,4], Rainer Hoefgen[5], Mutsumi Watanabe[5,6], Franziska Brueckner[5], Ryo Nakabayashi[7], Tetsuya Mori[7], Akira Oikawa[7,8], Ryosuke Sasaki[7], Jeerus Sucharitakul[9], Kazuki Saito[7], and Supaart Sirikantaramas[1,10]

[1] *Center of Excellence in Molecular Crop, Department of Biochemistry, Faculty of Science, Chulalongkorn University; 10330 Bangkok, Thailand*
[2] *Department of Biochemistry, Faculty of Science, Khon Kaen University; 40002 Khon Kaen, Thailand*
[3] *Program in Bioinformatics and Computational Biology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand*
[4] *Center of Excellence in Structural and Computational Biology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[5] *Max Planck Institute of Molecular Plant Physiology; 14476 Potsdam-Golm, Germany*
[6] *Present address: Nara Institute of Science and Technology Graduate School of Biological Sciences; 8916-5 Takayama-cho, Ikoma, 630-0192 Nara, Japan*
[7] *RIKEN Center for Sustainable Resource Science; 1-7-22 Suehiro-cho, Tsurumi-ku, 230-0045 Yokohama, Japan*
[8] *Graduate School of Agriculture, Kyoto University, Uji, 611-0011 Kyoto, Japan*
[9] *Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University; 10330 Bangkok, Thailand*
[10] *Omics Sciences and Bioinformatics Center, Chulalongkorn University; 10330 Bangkok, Thailand*

6172894323@student.chula.ac.th

Abstract

Durian (*Durio zibethinus* L.) is an economically important fruit crop in Southeast Asia. In Thailand, more than 200 durian varieties are found. Among them, two commercial cultivars, Chanee and Monthong, are mainly cultivated. Durian fruit releases a strong sulfury odor containing ethanethiol, methanethiol, and hydrogen sulfide during ripening. Methionine γ-lyase (MGL) is an enzyme responsible for the α, γ-elimination of L-methionine into methanethiol, ammonia, and α-ketobutyrate. The gene encoding MGL is highly expressed in durian pulps during ripening, coinciding with methanethiol production during ripening. However, the function of durian MGL (DzMGL) has not been characterized. Thus, enzymatic activity assays of L-methionine, L-cysteine, and ethionine were performed. Besides α, γ-elimination of L-methionine and ethionine, durian MGL also had α, β-elimination to degrade cysteine into hydrogen sulfide, ammonia, and pyruvate. Cysteine content in Chanee was higher than in Monthong, which corresponds to the higher production of hydrogen sulfide. This evidence suggests that one of the factors is related to the difference in odor strength between the two cultivars. The computational study also provided amino acid residues related to the substrate binding ability in the active site of DzMGL and AtMGL. Moreover, transcriptional regulation of DzMGL was elucidated using yeast one-hybrid screening and dual-luciferase activity. Here, DzHD-ZIP1.8 acts as an activator of the MGL promoter and controls gene expression. To the best of our knowledge, HD-ZIP transcription factors were first identified to regulate MGL expression in durian fruit during ripening. These results contribute to the understanding of durian MGL function and its transcriptional regulation of gene expression in durian fruit, which contributes to the production of sulfur-containing volatile compounds.

Assessment of locally available seaweeds as sustainable organic compost fertilizer resources

Durlave Roy

School of Agriculture, MS student, Bangladesh Open University

kdr2021@gmail.com

Abstract

Marine red algae from the Bangladesh Bay of Bengal Hypnea Sp have been used as organic materials due to the presence of multiple plant growth-stimulating compounds. The effect of various seaweed species on plant growth and development, with an emphasis on the use of this renewable bio-resource in the sustainable agriculture of fertilizer raw materials, was investigated. Organically made fertilizers play an important role in increasing crop yield, and crop quality promises improvements considering climate adaptation. Seaweed-waste compost was used in evaluation trials at Sreemangal, Bangladesh, to evaluate its efficacy and find out the optimum dose for profitable Betel leaf production. This part of the study was directed toward the analysis of future trends and performances of composting seaweed wastes. A field study was conducted using three treatments given by khasia farmers of the Sreemangal khasia betel leaf cultivation community of Bangladesh. Seaweed wastes mixed with compost organic fertilizer at 50 g per support tree were used. The highest betel leaf yield was obtained from seaweed wastes mixed with compost organic fertilizer and applied to plants. This study suggests that seaweed wastes mixed with organic fertilizer are suitable for betel leaf cultivation. In summary, area-based conservation is a key tool for achieving the sustainable development goals of responsible production and consumption and climate action.

Application of Improved Unet Model in Cucumber Downy Mildew Spot Segmentation

Shi Dongyuan[1,2], Chen Xiaohui[1,3], Yang Xinting[2], Li Ming[1,2], Diao Ming[1]

[1] *Department of Horticulture, Agricultural College of Shihezi University/Key Laboratory of Special Fruits and Vegetables Cultivation Physiology and Germplasm Resources Utilization of Xinjiang Production and Construction Corps, Shihezi 832003, China*
[2] *Research Center of Information Technology, Beijing Academy of Agriculture and Forestry Sciences/National Engineering Research Center for Information Technology in Agriculture/National Engineering Laboratory for Agri-product Quality Traceability/Meteorological Service Center for Urban Agriculture, China Meteorological Administration-Ministry of Agriculture and Rural Affairs, Beijing 100097, China*
[3] *Chemical Engineering Department, University of Almería, Almería 04120, Spain*

Abstract

Cucumber disease control is a pressing concern in the realm of smart agriculture, with one of its pivotal aspects being the precise identification and quantitative segmentation of cucumber leaf diseases. To address the issue of indistinct areas afflicted by cucumber downy mildew disease, we employed deep learning to analyze pixel-level phenotypic features (spot distribution). We introduced an effective image-based segmentation technique for cucumber leaf diseases to achieve both efficient and precise segmentation and localization of these obscured spots. To facilitate this, we assembled datasets of diseased leaves and contributed corresponding pixel-level annotations. These datasets served as the basis for training and optimization purposes. Next, we incorporated the transformer encoder into the U-Net network structure and used the ResNet50 network as the backbone feature extraction network, and superimposed Multiple Residual Dilated Convolution (MRDC) in the hopping connection modules to increase the receptive field and enhance the model's ability to extract underlying features. To prevent network neuron loss, we judiciously applied the SeLU function. Following the expansion of the diseased leaf dataset through data augmentation techniques, we trained the improved U-Net model by selectively freezing specific network layers using a transfer learning approach. Our results indicate that, compared to other classical segmentation networks on a cucumber leaf disease segmentation dataset we constructed ourselves, the improved U-Net model achieved an accuracy rate exceeding 95% in the task of segmenting disease spots and yielded favorable outcomes on various open-source datasets as well. This method outperforms in the segmentation of cucumber downy mildew spots and offers valuable insights for plant disease phenotyping.

Vineyard microclimate alterations induced by black inter-row mulching through transcriptome reshaped the flavoromics of Cabernet Sauvignon grapes

Meng-Bo Tian[1,3,†] , Yu Wang[1,2,†] , Jun Wang[1,3]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*
[2] *Anhui Engineering Laboratory for Agro-products Processing, College of Tea & Food Science and Technology, Anhui Agricultural University, Hefei 230036, China*
[3] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*
[†] These authors contributed equally.

jun_wang@cau.edu.cn

Abstract

To alter the vineyard microclimate and produce quality wine under a semi-arid climate, black geotextile inter-row mulch (M) was applied for two vintages (2016–2017). The integration of metabolome and transcriptome analysis identified the key genes responsible for the increased concentrations of organic acids, phenylalanine, glutamine, ornithine, arginine, and C6 alcohols, and for the decreased concentrations of glucose, fructose, ε-viniferin, anthocyanins, flavonols, terpenes and norisoprenoids in M grapes. The upregulated genes related to antenna proteins in photosynthesis and heat shock proteins confirmed that M weakened the total light exposure, and grapes suffered severe heat stress. The effects of metabolites and the transcriptome were more evident in vintage grapes with weaker light. In addition, the potential key transcription factors regulating the biosynthesis of the metabolites, including *VviGATA11*, *VviHSFA6B*, and *VviWRKY03*, were identified through weighted correlation network analysis (WGCNA). Taken together, this study provides a valuable overview of metabolic and transcriptomic responses of grapes exposed to inter-row mulch treatment in semi-arid climate, which could facilitate the understanding of the complex regulatory network of metabolites in response to microclimate changes.

BRCA1, a DNA-damage repair-related gene, inhibits secondary xylem development in *Populus*

Xiaqin Wang, Jin Zhang, Mengzhu Lu

State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou 311300, China

wxq@stu.zafu.edu.cn

Abstract

Wood formation, as a feature of woody plants, is an important process of secondary xylem development, which includes vascular cambium cell division and differentiation, cell expansion, secondary cell wall deposition, and programmed cell death (PCD). The secondary xylem mainly consists of tracheary elements (TEs) and fibers. TEs function in water transport and undergo DNA degradation, content loss, and cell death, which is a typical PCD event. PCD is essential for wood formation. However, the current understanding of PCD in the last step of wood formation is insufficient and requires further investigation. The breast cancer susceptibility gene 1 (BRCA1) was found to have a function in cell proliferation regulation and genome integrity safeguard in *Arabidopsis*. In this study, we found that *PagBRCA1*, an orthologous gene of *Arabidopsis* BRCA1 from the hybrid poplar (*Populus alba* × *P. glandulosa*) clone 84K, negatively regulates secondary xylem development. Phylogenetic analysis showed that only one *BRCA1* gene was found in each analyzed species, indicating the indispensable role of *BRCA1*. The GUS staining assay showed that *PagBRCA1* was specifically expressed in the vascular cambium of the stem. The transgenic 84K poplar overexpressing *PagBRCA1* exhibited decreases in stem diameter and leaf size but increases in plant height and internode length compared to the nontransgenic 84K poplar. Protoplast transfection indicated that *PagBRCA1* acts as a transcriptional activator that is different in Arabidopsis. To investigate the mechanism of *PagBRCA1* in regulating xylem differentiation, the bimolecular fluorescence complementation assay (BiFC) showed that PagBRCA1 interacts with PagRBR and PagXND1, respectively, which both function as negative regulators of PCD in secondary xylem development. Overall, our study suggests that PagBRCA1 might regulate secondary xylem development by interacting with PagRBR and PagXND1 to inhibit PCD. Further studies are needed to understand this regulatory network.

Ethylene-responsive VviERF003 modulates glycosylated monoterpenoid synthesis by upregulating *VviGT14* in grapes

Ya-Chen Wang[1,2], Yi Wei[1,2], Xiang-Yi Li[1,3], Hui-Min Zhang[1,2], Xiao Meng[1,2], Chang-Qing Duan[1,2], Qiu-Hong Pan[1,2]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

[3] *Department of Plant Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China*

bs20213060548@cau.edu.cn

Abstract

Terpenoids are important components contributing to aromas of grapes and wines. Grapes contain terpenoids in both volatile free and non-volatile glycosylated forms, with the latter being more abundant. Glycosylated terpenoids possess latent aromatic potential for their essential role in adding to the flowery and fruity bouquet of wines. Nevertheless, the transcriptional regulatory mechanism responsible for glycosylated terpenoid biosynthesis remains poorly understood. Our previous study showed a strong correlation between the expression of the glycosyltransferase gene *VviGT14* and the accumulation of glycosylated monoterpenoids in developing grapes. Additionally, we discovered the ABA-inhibited WRKY transcription factor, VviWRKY40, which had a negative regulatory effect on the expression of *VviGT14* and the production of glycosylated monoterpenoids. This study identified an AP2/ERF transcription factor, VviERF003, through DNA pull-down screening using the *VviGT14* promoter. The investigation demonstrated that both genes were co-expressed and synchronized with the accumulation of glycosylated monoterpenoids during grape maturation. Furthermore, VviERF003 was found to bind to the *VviGT14* promoter and promote its activity according to the results of yeast one-hybrid and dual-luciferase assays. VviERF003 upregulated *VviGT14* expression in vivo, leading to increased production of glycosylated monoterpenoids, as demonstrated by transient overexpression in grape leaves and stable overexpression and transient RNAi interference in grape calli. Moreover, ethylene induced the expression of both *VviERF003* and *VviGT14*, elevating glycosylated monoterpenoid levels and ABA biosynthesis in grapes. However, *VviERF003* was not ABA-responsive, in contrast to *VviWRKY40*. The findings suggest that VviERF003 is ethylene-responsive and stimulates glycosylated monoterpenoid biosynthesis by upregulating *VviGT14* expression. In summary, we propose an underlying mechanism wherein VviERF003 and VviWRKY40 regulate the production of glycosylated monoterpenoids by targeting *VviGT14* in grapes.

MdMYB3 represses anthocyanin biosynthesis in apple

Yarong Wang[1], Zhenli Yan[1], Hengtao Zhang[1], Jialong Yao[1,2]

[1] *Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, 32 Gangwan Road, Zhengzhou 450009, China*

[2] *The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand*

wang_ya_rong@163.com

Abstract

It is well known that MdMYB10 positively regulates anthocyanin biosynthesis in apple. However, we found that some wild apples could not accumulate anthocyanin in fruit skin, although *MdMYB10* was highly expressed in the fruit skin. In these cases, *MdMYB3* was also highly expressed. We postulate that MdMYB3 may inhibit anthocyanin biosynthesis as it is grouped into the repressor MYB clade. We confirmed this repressive role of MdMYB3 using both tobacco and apple transgenic plants. Transgenic tobacco plants with overexpression of *MdMYB3* showed reduced anthocyanin accumulation in flower petals. Transgenic apple plants with overexpression of *MdMYB3* showed reduced anthocyanin accumulation in leaves when the plants were grown in tissue culture medium containing a high level (10%) of sucrose. Transcriptome analysis of these transgenic apple plants showed that overexpression of *MdMYB3* decreased the expression of anthocyanin biosynthesis genes such as *MdC4H*, *MdCHI*, *MdDFR* and *MdUGT*. It was further showed that MdMYB3 could bind to the promoters of *MdCHI*, *MdC4H*, *MdUGT* and *Md4CL* by yeast one hybrid assays and could reduce promotor activity by dual-luciferase assays. These results indicate that MdMYB3 inhibits anthocyanin synthesis by binding to the promoters of anthocyanin biosynthesis genes and inhibiting the transcription of these genes.

Integrated Analysis of Transcriptome and Metabolome to Unveil Mechanisms for Enhancing Grape Aroma Quality with Synthetic Auxin: Spotlight the Mediation of ABA in Crosstalk with Auxin

Nong-Yu Xia[1,2], Xue-Chen Yao[1,2], Wan-Hui Ma[1,2], Ya-Chen Wang[1,2], Yi Wei[1,2], Lei He[1,2], Hao-Tian Cheng[1,2], Wei-Ming Yang[3], Chang-Qing Duan[1,2], Qiu-Hong Pan[1,2]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

[3] *Yuanshi Vineyard, Yinchuan 750026, China*

panqh@cau.edu.cn

Abstract

Global warming brings challenges to the fields of viticulture and enology and results in higher sugar concentration and lower acidity in harvest grapes, accompanied by wines with elevated alcohol content and decreased flavor quality. This phenomenon is visible in the continental arid/semi-arid regions such as Xinjiang and Ningxia in western China. To maintain optimal wine quality, specific cultivation techniques have been implemented to postpone the grape ripening process. It is widely accepted that pre-véraison application of naphthaleneacetic acid (NAA), which is a synthetic auxin, can delay the ripening of grapes and improve their quality. However, the mechanism behind the effects of NAA on grape aromas remains unclear. This study incorporated the analyses of the aroma metabolome, phytohormones, and the transcriptome of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes in the commercial vineyards at the east foot of Helan Mountain in Ningxia. The analyses demonstrated that pre-véraison NAA application (10–40 mg/L) increased *β*-damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), two important varietal aroma components, by upregulating *VvPSY1* and *VvCCD4b*. Additionally, NAA treatment decreased 2-isobutyl-3-methoxypyrazine (IBMP), which contributes to a green unripe scent, by downregulating *VvOMT2*. Notably, abscisic acid (ABA) levels increased in NAA-treated grapes during véraison, which triggered further changes in these aroma metabolisms. The ABA-responsive factor VvABF2 was potentially involved in the positive modulation of *VvPSY1,* while the auxin response factor VvARF10 may play a role in *VvCCD4b* upregulation and *VvOMT2* downregulation during NAA induction. Based on these observations, VvARF10 possibly mediates crosstalk between ABA and auxin signaling pathways following NAA treatment, exerting an essential role in enhancing the aroma quality of grapes. These findings also provide a reference for the implementation of retarding berry maturation in this region.

ClFSC Encodes Protein Kinase and Regulates Formation of Fruit Peel Stone Cells in Watermelon (*Citrullus lanatus* L.)

Tiantian Yang

Northeast Agricultural University

Abstract

The peel stone cell layer in watermelon fruit is a product of high lignin accumulation, which plays an important tissue-hardening function in the peel. However, an in-depth genetic basis and inheritance analysis has not been reported yet. In the present study, we found that more stone cell formation is a dominant trait, and it is regulated by a major QTL. Bulk segregant analysis (BSA) primarily identified a major genetic effect of the *ClFSC* locus within range of ~1.39 Mb on chromosome 2, and linkage mapping narrowed down the *ClFSC* locus within a 166.93-kb region. An expanded F_2 mapping population consisting of 504 individuals, which delimited the *ClFSC* locus to a 41.59-kb region, harboring four candidate genes. Among these genes, only one gene (*Cla97C02G044130*) had a non-synonymous mutation that caused the protein alteration. Subcellular localization shows that the protein is located in the cell membrane, sequence variation analysis of coding regions and gene expression levels validated *Cla97C02G044130* as the most possible candidate for *ClFSC*, which encodes a protein kinase. Hence, we believe that our results provide a valuable genetic resource for investigating the stone cell of watermelon, with *ClFSC* function promoting lignin synthesis and increasing lignin accumulation, thereby forming peel stone cell tissue.

Terroir Shapes Aroma Style in Wine Grapes: Insights into Spatiotemporal Regulatory Network of 'Marselan' Aroma in the East Foot of Helan Mountain

Xuechen Yao[1,2], Xiao Meng[1,2], Nongyu Xia[1,2], Changqing Duan[1,2], Qiuhong Pan[1,2]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

yaoxch@cau.edu.cn

Abstract

Terroir encompasses a variety of factors within a vineyard, such as climate, soil, topography, and environmental microorganisms. These factors collectively influence grape berry metabolism, thereby impacting wine quality and contributing to its unique regional characteristics. Despite the widely acknowledged significance of terroir in wine production, this field still lacks scientific research in certain areas. The complex and diverse nature of terroir makes it challenging for researchers to accurately identify the most influential factors contributing to grape and wine characteristics. Furthermore, the metabolic pathways and regulatory networks involved in grape flavor are not yet completely understood, which restricts researchers from investigating how terroir influences grape flavor. Consequently, this impedes the advancement in agronomic practices. In this study, we establish a terroir dataset by selecting five plots at the East Foot of Helan Mountain. This dataset includes meteorological and soil data from the five plots, as well as profiles of aroma and their precursor metabolites, phytohormone levels, and transcriptomes throughout the entire development stage of 'Marselan' berries. Through integrated analysis, we constructed a spatiotemporal map of the aroma metabolism of the 'Marselan' grape and established a regulatory network linking environmental factors, gene expression, and aroma compound accumulation. Furthermore, we conducted in vitro experiments to determine the period of heat stress affecting the accumulation of β-damascenone, and we identified *VvADH* as a key structural gene in the β-damascenone synthesis pathway.

participates in the biosynthesis of bGenome-wide association study uncovers a new candidate gene–*VvGGPPS* oth monoterpenoid and norisoprenoid

Hui-Min Zhang[1,2], Zheng-Yang Sun[1,2], Qi Sun[1,2], Xin-Jie Lyu[1,2], Lei Sun[3], Hai-Ying Xu[3], Chang-Qing Duan[1,2], Qiu-Hong Pan[1,2]

[1] *Center for Viticulture & Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

[3] *Beijing Academy of Forestry and Pomology Sciences, Beijing 100093, China*

zhanghui_min@cau.edu.cn

Abstract

Grapes (*Vitis vinifera*) are among the most widely produced and consumed fruits globally due to their unique taste and flavor. Aroma, an important attribute of grapes, directly influences consumer preference. As a result, breeders have long been committed to developing grape varieties with rich and diverse aroma compounds. Monoterpenoids and norisopreoids, which contribute to grape's floral, sweet and citrus-like scent, have garnered significant attention from both researchers and planters. As is typical for quantitative traits, the biosynthesis and regulatory mechanism of the two compound groups in grapes are incompletely understood. In this study, we investigated a hybrid population of *Vitis vinifera* L. Mascat of Alexandria, a Muscat cultivar, and *V. vinifera* L. Christmas Rose, a neutral aroma cultivar. We resequenced the genome of this population and acquired SNP markers, while also detecting the concentrations of 13 monoterpenoids and 11 norisopreoids over two consecutive years. Genome-wide association study (GWAS) identified that *VvGGPPS*, a geranylgeranyl diphosphate synthase located at the intersection of monoterpenoid and norisoprenoid biosynthesis pathways, was associated with both monoterpenoids and norisoprenoids. Transient overexpression of *VvGGPPS* in grape berries and leaves resulted in increased concentrations of these aroma compounds, indicating its involvement in their synthesis. We confirmed two SNP loci in the coding sequence region and 5′-UTR of the *VvGGPPS* gene using a grapevine germplasm population containing 97 varieties. These loci were verified through the kompetitive allele specific PCR (KASP) assay, and it was evident that their varied genotypes indeed correspond to different content levels of monoterpenoids and norisoprenoids. Our findings suggest that these two SNP loci have the potential to be used as molecular markers for grapevine breeders focused on selecting varieties with pleasant aromas.

Identification of WOX family in blueberry and its potential involvement of adventitious root development and pH stress response

Botian Zheng, Tianran Shi, Shaomin Bian

College of Plant Science, Jilin University, Changchun, China

shmbian@jlu.edu.cn

Abstract

WUSCHEL (WUS)-related homeobox (WOX) protein family members play indispensable roles in plant growth, development, and stem cell differentiation. While the roles of some WOX proteins in the regulation of meristematic cells have been extensively studied in model plants such as Arabidopsis and rice, the expression and function of WOX members in woody plant blueberries remain largely unexplored. Blueberry (Vaccinium spp.) is recognized as one of the five major healthy foods for humans and is often referred to as the "king of the world fruit" due to its remarkable nutritional and health potential. In this study, we conducted a comparative analysis of the blueberry genome data, identifying a total of 39 *WOX* genes. Furthermore, we performed a systematic phylogenetic analysis of blueberry *VcWOX* genes, encompassing their phylogenetic relationships, conserved motifs, identification of differentially expressed *WOX* genes during adventitious root development, and their expression profiles. Notably, we found that among *VcWOX* genes, there were 3 pairs of duplicated genes and 8 pairs of quadruplicated genes, exhibiting homologies of greater than 95%. Moreover, we examined the transcriptional accumulation of *VcWOX* genes during adventitious root development in blueberries, revealing distinct expression patterns, indicative of their diverse functions in this process. Additionally, our analysis demonstrated three distinct types of transcriptional profiles for *VcWOX* genes, suggesting their varied functional roles. Remarkably, we also investigated the expression of *VcWOX* genes under different pH conditions (pH 4.5 and pH 6.5) and observed differential transcriptional accumulation for certain *VcWOX* genes. Notably, some *VcWOX11s* exhibited different transcriptional responses to pH stress (pH 6.5) in pH-sensitive and pH-tolerant species, suggesting their potential role in adapting to pH stress.

DNA methylation-dependent epigenetic regulation between *Brassica rapa* host and *Plasmodiophora brassicae* interaction

Jingyi Zheng, Xiaonan Li, Zhongyun Piao

College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

amberz0301@163.com

Abstract

Clubroot, known as the "cancer" of crucifers, is a serious soil-borne disease caused by *Plasmodiophora brassicae* Woron. It has become one of the main diseases limiting the production of Brassica crops, as it can impede the physiological functioning of the root system and result in slow growth, wilting or death of the plant. DNA methylation, an important and common epigenetic modification, is involved in a variety of biological processes such as plant growth and development, disease resistance and stress tolerance. However, there are few reports on the pathological mechanism of *P. brassicae* at the epigenetic level. In this study, we conducted whole-genome bisulfite sequencing (WGBS) and generated single-base high-resolution DNA methylation maps for a pair of NIL exhibiting clubroot susceptibility ('BJN3-2', referred to S) and resistance ('CR BJN3-2', referred to R) at different *P. brassicae* infection stages, combined with transcriptome data. We found that the overall methylation level of disease-resistant materials was lower than that of susceptible materials, and both of them showed a trend of down-regulation of methylation during the course of the infection, which was more pronounced especially at the early stage. Next, we used the methylation inhibitor 5-AzaC for exogenous treatment and found that the S material exhibited reduced disease extent and delayed disease duration. Therefore, it could be inferred that reducing DNA methylation levels could potentially benefit the disease resistance response. In addition, transcriptome data revealed a consistent expression trend of key genes involved in DNA methylation and demethylation pathways between R and S materials. Specifically, the important demethylation enzyme BraDME responded to *P. brassicae* infection earlier than the others. Some of the differentially expressed genes (DEGs) were found to be accompanied by differentially methylated regions (DMR) and more present in the CHH context, most of which were enriched in plant hormone pathways. The hormone contents after inhibitor treatment with 100 μM 5-AzaC showed a similar trend to those of the R materials and showed more consistent trends in ABA, JA, GA and SA. Subsequently, WGCNA analysis of hormone content as trait data identified a module with very high correlation with ABA, JA, and GA. One of the hub genes, ERF109, was negatively correlated with ABA content and having significant differential methylation level of the promoter region in the context of the CHH. Therefore, we consider that ERF109 may positively regulate host resistance to *P. brassicae* through the ABA pathway and its expression level may be influenced by DNA methylation in the promoter region.

SnRK2.3-AREB1-TST1/2 cascade activated by cytosolic glucose regulates sugar accumulation across tonoplasts in apple and tomato

Lingcheng Zhu[1], Fengwang Ma[1], Yong-Ling Ruan[1,2], Mingjun Li[1]

[1] *State Key Laboratory of Crop Stress Biology in Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Xianyang, China*

[2] *Division of Plant Sciences, Research School of Biology, The Australian National University, Canberra, ACT, Australia*

zhulingcheng316@nwsuaf.edu.cn

Abstract

Soluble sugars are the core components of fruit quality, and the degree of sugar accumulation is largely determined by tonoplast-localized sugar transporters. We previously showed that two classes of tonoplast sugar transporters, MdERDL6 and MdTST1/2, coordinately regulate sugar accumulation in vacuoles. However, the mechanism underlying this coordination remains unknown. Here, we discovered that two transcription factors, MdAREB1.1/1.2, regulate *MdTST1/2* expression by binding their promoters in apple. The enhanced *MdAREB1.1/1.2* expression in *MdERDL6-1*-overexpressing plants resulted in an increase in *MdTST1/2* expression and sugar concentration. Further studies established that MdSnRK2.3, whose expression could be regulated by expressing *MdERDL6-1*, interacted with and phosphorylated MdAREB1.1/1.2, thereby promoting the MdAREB1.1/1.2-mediated transcriptional activation of *MdTST1/2*. Finally, the orthologous *SlAREB1.2* and *SlSnRK2.3* exhibited similar functions in tomato fruit as in their apple counterparts. Together, our findings provide insights into the regulatory mechanism of tonoplast sugar transport exerted by SnRK2.3-AREB1-TST1/2 for fruit sugar accumulation.

Biosynthesis of EGCG, Theanine and Caffeine in Response to Temperature is Mediated by Hormones Signal Transduction Factors in Tea Plant (Camellia sinensis L.)

Qiufang Zhu[1], Lijia Liu[1], Xiaofeng Lu[1], Xinxin Du[1], Ping Xiang[1,2], Bosi Cheng[1], Meng Tan[1], Jiaxin Huang[1], Lijiao Wu[3], Weilong Kong[4], Yutao Shi[1,5], Liangyu Wu[1], Jinke Lin[1]

[1] *College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou, China*
[2] *College of Life and Environmental Science, Hunan University of Arts and Science, Changde, China*
[3] *Institute of Photobiological Industry, Fujian Sanan Sino-Science Photobiotech Co., Ltd., Xiamen, China*
[4] *Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China*
[5] *College of Tea and Food Sciences, Wuyi University, Wuyishan, China*

zqf216@163.com

Abstract

As the main flavor components of tea, the contents of epigallocatechin-3-gallate (EGCG), theanine and caffeine are regulated by ambient temperature. However, whether the biosynthesis of EGCG, theanine and caffeine in response to temperature is regulated by endogenous hormones and their mechanisms are still unclear. In this study, we investigated the internal intricate relationships between the biosynthesis of these three main taste components, endogenous hormones, and structural genes in tea plants at different temperatures. The results showed that IAA, GA1 and GA3 were significantly correlated with the content of EGCG; JA, JA-Ile and MeJA were strongly correlated with theanine content; IAA, GA1 and GA4 were significantly correlated with caffeine content at different temperatures. According to the results of multi-omics analysis, we speculate the following regulatory mechanisms: IAA, GA1 and GA3 upregulated the expressions of *chalcone synthase* (*CsCHS*) and *trans-cinnamate 4-monooxygenase* (*CsC4H*) mediated by the signal transduction factors *CsIAA* and *CsDELLA*, respectively, which promoted the biosynthesis of EGCG; IAA, GA3 and GA1 upregulated the expression of *CsCHS* and *anthocyanidin synthase* (*CsANS*) mediated by *CsIAA* and *CsDELLA*, respectively, via the transcription factor *CsWRKY*, and promoted the biosynthesis of EGCG; JA, JA-Ile and MeJA jointly upregulated the expression of *carbonic anhydrase* (*CsCA*) and downregulated the expression of *glutamate decarboxylase* (*CsgadB*) mediated by the signal transduction factor *CsJAZ*, and promoted the biosynthesis of theanine; JA, JA-Ile and MeJA also jointly inhibited the expression of *CsgadB* mediated by *CsJAZ* via transcription factors *CsWRKY* and *CsAP2*, which promoted the biosynthesis of theanine; IAA inhibited the expression of *adenylosuccinate synthase* (*CspurA*) mediated by *CsIAA* via the transcription factor *CsWRKY*; GA1 and GA4 inhibited the expression of *CspurA* mediated by *CsDELLA* via the transcription factor *CsWRKY*, which promoted the biosynthesis of caffeine. These findings reveal that the underlying mechanism of biosynthesis of the main taste components in tea plant in response to temperature is mediated by hormone signal transduction factors, thereby providing novel insights into improving the quality of tea.

Carotenoid and transcriptome profiles of a novel citrus cultivar ‘Jinlegan’ reveal mechanisms of yellowish fruit formation

Hongyan Chen[1], Huiyu Ji[1], Shiping Zhu[2], Kaijie Zhu[1], Junli Ye[1], Xiuxin Deng[1]

[1] *National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan 430070, China*
[2] *National Citrus Engineering Research Center, Southwest University, Chongqing 400715, China*

chenhy@webmail.hzau.edu.cn

Abstract

Citrus fruit coloration is a vital quality trait that is determined primarily by the composition and content of carotenoids. Natural citrus mutants with different fruit pigments are available to study carotenoid metabolism. Here, ‘Jinlegan’ (MT) tangor, a spontaneous bud mutant derived from ‘Shiranuhi’ (WT) with distinctive bright yellowing fruit, was studied. High performance liquid chromatography (HPLC) analysis revealed that the yellowish MT flavedo and pulp were primarily caused by the decrease in total carotenoid content. The total carotenoid content in MT flavedo was reduced by 75% (79.98 μg g^{-1} DW) compared with that in WT (318.40 μg g^{-1} DW), including approximately 84%, 80%, and 60% reductions in the contents of *β*-cryptoxanthin, violaxanthin and zeaxanthin, respectively. The total carotenoid content in MT pulp was 60% lower (10.09 μg g^{-1} DW) than that in WT pulp (26.61 μg g^{-1} DW), which was mainly due to decreases of 70% and 30% in the contents of *β*-cryptoxanthin and zeaxanthin. To explore the molecular mechanism underlying carotenoid variation in MT, RNA-seq analyses were performed on the flavedo and pulp of WT and MT at five developmental stages. The reduced expression of phytoene synthase (*PSY*) and *β*-carotene hydroxylase 1 (*BCH1*) in the flavedo and pulp of MT at the breaker stage might explain the reduction in carotenoids. Weighted gene co-expression network analysis (WGCNA) further identified 23 key transcription factors that are closely associated with carotenoid accumulation. This study offers a comprehensive picture of the metabolic and transcriptional alterations in a unique citrus mutant with yellowish fruit, which provides new insights into the molecular regulation of carotenoid accumulation in citrus fruit.

Impact of plant fertility on population adaptive evolution

Jialei Chen[1,2], Jialu Wang[1], Yifei Pei[1], Xue Feng[1], Xiwen Li[1]

[1] *Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*

[2] *College of Medicine, Henan University of Chinese Medicine, Zhengzhou 450046, China*

chenjialeicjl@163.com

Abstract

Population adaptability in plants is closely linked to gene polymorphisms, which can be enhanced through gene exchange and facilitated by hybridization. At different evolutionary stages, plant populations require varying levels of fertility to ensure effective gene exchange. In this review, we summarize male sterility models, which affect the development of tapetum cells by affecting reactive oxygen species levels and energy replenishment. Additionally, we highlight plant fertility-related genes and investigate how they impact fertility by regulating factors, such as flower morphology, pollen characteristics, physicochemical properties and allelopathy, ultimately affecting the hybridization and invasion efficiency of species. Furthermore, we propose an adaptive evolution round (AER) model wherein fertility-related genes regulate fertility levels to affect adaptive evolution in plant populations. We discuss the evolutionary significance of fertility-related genes and a novel perspective is provided for future research on plant fertility.

From challenges to opportunities: unveiling the secrets of pitaya through omics studies

Jiaxuan Chen, Irfan Ali Sabir, Yonghua Qin

Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou, Guangdong, 510642, China.

jxchen0127@163.com

Abstract

Pitaya (pitahaya or dragon fruit), which belongs to the genus *Hylocereus* or *Selenicereus* in the Cactaceae family, is well-known all over the world, especially in tropical and subtropical regions, for its health-benefiting properties. Although the pitaya traits have been extensively investigated, only a few studies have addressed the molecular mechanisms underlying the formation of fruit quality traits in pitaya. The breeding improvement of pitaya is a complex task due to various factors, including its generation cycle, polyploidy, high heterozygosity, and complex physiological structure. Furthermore, the absence of effective methods and resources exacerbates the challenges in improving the breeding of this fruit crop. In recent years, advancements in biotechnology and sequencing have played a vital role in supporting and expediting conventional breeding techniques, as well as providing insights into the molecular mechanisms and evolutionary processes of pitaya. This review highlights the latest developments in pitaya research, including genome, transcriptome, metabolome, and proteome sequencing; functional gene identification; and regulatory network analysis; as well as the novel tools, platforms, and programs that have emerged in this field. It paves the way for studying gene functions and molecular breeding methods with desirable fruit quality traits, which will help to accelerate pitaya breeding programs.

LlWRKY33-LlHSFA4-LlCAT2 module confers resistance to *Botrytis cinerea* in lily

Liping Ding[1,2,†], Ze Wu[1,2,†], Jun Xiang[1,2], Xing Cao[3], Sujuan Xu[1,2], Yinyi Zhang[1,2], Dehua Zhang[1,2], Nianjun Teng[1,2]

[1] *Key Laboratory of Landscaping, Ministry of Agriculture and Rural Affairs, Key Laboratory of Biology of Ornamental Plants in East China, National Forestry and Grassland Administration, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China*

[2] *Jiangsu Graduate Workstation of Nanjing Agricultural University and Nanjing Oriole Island Modern Agricultural Development Co., Ltd., Nanjing 210043, China*

[3] *College of Architecture, Yantai University, Yantai, 264005, China*

[†] These authors contributed equally.

2280653382@qq.com; njteng@njau.edu.cn

Abstract

Gray mold caused by *Botrytis cinerea* is one of the major threats to lily production. However, limited information is available about the underlying defense mechanism against *B. cinerea* in lily. Here, we characterized a nuclear-localized class A heat stress transcription factor (HSF)-LlHSFA4 from lily (*Lilium longiflorum*), which positively regulated the response to *B*. *cinerea* infection. The *LlHSFA4* transcript and its promoter activity were increased by *B*. *cinerea* infection in lily, indicating its involvement in the response to *B*. *cinerea*. Virus-induced gene silencing (VIGS) of *LlHSFA4* impaired the resistance of lily to *B. cinerea*. Consistent with its role in lily, overexpression of *LlHSFA4* in Arabidopsis enhanced the resistance of transgenic Arabidopsis to *B*. *cinerea* infection. Further analysis showed that LlWRKY33 directly activated *LlHSFA4* expression. We also found that both LlHSFA4 and LlWRKY33 positively regulated the plant response to *B*. *cinerea* by reducing cell death and H_2O_2 accumulation and activating the expression of the reactive oxygen species (ROS) scavenging enzyme gene *LlCAT2* via binding to its promoter, which may contribute to reducing H_2O_2 accumulation in the infected area. Taken together, our data suggest that there may be a LlWRKY33-LlHSFA4-LlCAT2 regulatory module, which confers *B*. *cinerea* resistance by reducing cell death and ROS accumulation.

Molecular Mechanism of RhLHY in Inhibiting Rose Flower Formation under Weak Light by Activating the RhTPPF Gene Related to Trehalose Synthesis

Yingdong Fan[1,2], Wuhua Zhang[1,2], Jie Dong[1,2], Jinzhu Zhang[1,2], Tao Yang[1,2], Daidi Che[1,2]

[1] *College of Horticulture and Landscape Architecture, Northeast Agricultural University, Harbin 150030, China*

[2] *Key Laboratory of Cold Region Landscape Plants and Applications, Harbin 150030, China*

fanying_dong@163.com

Abstract

Flowering is the process by which plants transition from vegetative growth to reproductive growth. Plants integrate environmental factors and endogenous signals, resulting in complex but precise networks that regulate flowering through long-term evolution. However, this network is not yet clear in *Rosa hybrida*. Short light period and weak light intensity have both caused the flowering time of *Rosa hybrida* cv 'Carola' to be delayed and the endogenous sugar content to decrease. Through transcriptome sequencing data analysis, it was identified that the expression of the trehalose phosphatase gene *RhTPPF* may be related to the delayed flowering of roses under low light conditions. Overexpression of *RhTPPF* in tobacco inhibited the flowering of tobacco, while knockdown of *RhTPPF* in rose promoted the flowering of rose. The key gene *RhLHY* in the biological clock can bind to the *RhTPPF* promoter, thereby promoting *RhTPPF* expression. Gene silencing experiments have confirmed that *RhLHY* is a negative regulatory factor for rose flowering. These results indicate that the *RhLHY-RhTPPF* pathway plays a crucial role in the response of rose to weak light signals in flower formation. Our results not only reveal that there is another layer of *RhTPPF* in the flowering of perennial woody plants, but that it can also enrich plant light response mechanisms.

GAMYB transcription factor LoMYB65 from lily plays a vital role in pollen development

Xinyue Liu[1,2,3], Ling He[1,2,3], Ze Wu[1,2,3], Qianqian Fang[1,2,3], Nianjun Teng[1,2,3]

[1] *Key Laboratory of Landscaping Agriculture, Ministry of Agriculture and Rural Affairs, College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China*
[2] *Key Laboratory of Biology of Ornamental Plants in East China, National Forestry and Grassland Administration, College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China*
[3] *Jiangsu Graduate Workstation of Nanjing Agricultural University and Nanjing Oriole Island Modern Agricultural Development Co., Ltd., Nanjing, Jiangsu 210043, China*

njteng@njau.edu.cn

Abstract

Lily (*Lilium spp.*) is an important horticultural crop, but its use is limited due to severe pollen contamination problems. There are many studies on pollen development in model plants, but few on flower crops such as lilies. Gibberellin (GA) is a member of a large class of hormones that play important roles in plant vegetative growth and reproductive development. GAMYB is a member of the R2R3-MYB family that is upregulated by gibberellin, and it plays an important role in anther development. Here, we isolated a novel *GAMYB*, named *LoMYB65*, from lily, which was closely related to *AtMYB65* and *AtMYB33* in Arabidopsis. Fluorescence quantitative PCR results showed that *LoMYB65* was mainly expressed in lily anthers. *LoMYB65* could be activated by 288 μmol L^{-1} GA_3 treatment, and the LoMYB65 protein was located in the nucleus and cytoplasm, and it had transactivation activity in yeast and tobacco leaf cells. A conserved motif within 226 amino acids of the C-terminus of LoMYB65 contributed to its transactivation. Overexpression of *LoMYB65* caused dwarf phenotype, abnormal tapetum development, and fewer siliques in transgenic Arabidopsis plants, whereas the transgenic plants showed male sterility. Simultaneous knockdown of *LoMYB65* with VIGS (Virus Induced Gene Silencing) in lily anthers caused abnormal pollen development and reduced pollen amount. Given that overexpression of *LoMYB65* in Arabidopsis and knockdown of *LoMYB65* in lily resulted in decreased pollen counts, we speculate that the effects of *LoMYB65* may be dose dependent. Overall, these findings suggest that *LoMYB65* plays an important role in anther development and pollen formation in lily. *LoMYB65* may be a candidate gene for the pollenless breeding of lily.

Synergistic effects of earthworms and cow manure under reduced chemical fertilization modified the microbial community structure to mitigate the continuous cropping effects on Chinese flowering cabbage

Fucheng Gao, Lin Ye, Xiaoguo Mu, Lei Xu, Zhenfang Shi, Yuan Luo

College of Wine and Horticulture, Ningxia University, Helanshan Xilu No. 489, Yinchuan 750021, China

gfc3207@163.com; yelin221112@126.com

Abstract

The substitution of chemical fertilizers with organic fertilizers is a viable strategy to enhance crop yield and soil quality. In this study, we investigated the changes in soil microorganisms, soil chemical properties, and growth of Chinese flowering cabbage under different fertilization treatments involving earthworms and cow manure. Compared with the control (100% chemical fertilizer), CE (30% reduction in chemical fertilizer + earthworms) and CFE (30% reduction in chemical fertilizer + cow dung + earthworms) treatments at soil pH 8.14 and 8.07, respectively, and CFC (30% reduction in chemical fertilizer + cow manure) and CFE treatments increased soil organic matter (SOM), total nitrogen (TN), available nitrogen (AN), and available potassium (AK) contents. Earthworms and cow manure promoted the abundance of *Bacillus* and reduced that of the pathogens *Plectosphaerella* and *Gibberella*. The mantle test revealed that pH was not correlated with the microbial community. Random forest analysis verified that AN, SOM, and TN were important factors that jointly influenced bacterial and fungal diversity. Overall, the synergistic effect of earthworms and cow manure increased soil fertility and microbial diversity, thereby promoting the growth and development of Chinese flowering cabbage. This study enhances our understanding of how bioregulation affects the growth and soil quality of Chinese flowering cabbage, and thus provides guidance for the optimization of fertilization strategies to maximize the yield and quality of Chinese flowering cabbage while reducing environmental risks.

Deciphering the Regulatory Network of Auxin-mediated Inhibition of Fruitlet Abscission in Litchi

Zidi He[1,4], Xingshuai Ma[1,4], Jianguo Li[1,2,3,4], Minglei Zhao[1,2,3,4]

[1] *Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, 510642, China*
[2] *State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou, 510642, China*
[3] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China*
[4] *Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China*

hzd@stu.scau.edu.cn; jianli@scau.edu.cn; zml503@scau.edu.cn

Abstract

Excessive precocious fruit abscission poses a significant challenge in the litchi industry. The hormone auxin plays a crucial role in inhibiting organ abscission; however, the regulatory networks involved in the auxin-mediated regulation of organ abscission in litchi remain poorly understood. To address this, we conducted a comprehensive study integrating transcriptome sequencing analysis, determination of relevant physiological indicators, bioinformatics analysis, and molecular biology experiments. By doing so, we constructed a regulatory network that elucidates the auxin-mediated regulation of litchi fruitlet abscission. Through this network, we identified 31 key transcription factors that regulate abscission by modulating various abscission-related pathways. Additionally, we confirmed the role of a key R2R3-MYB transcription factor, LcMYB62, as a positive regulator of litchi fruitlet abscission using Virus-Induced Gene Silencing (VIGS) and heterologous overexpression approaches. The auxin signal was observed to inhibit the expression of genes involved in cell wall degradation and the separation of abscission zone cells by partially suppressing the transcription of *LcMYB62*, thereby maintaining fruit retention. Collectively, our findings provide novel insights into the molecular mechanisms underlying the auxin-mediated regulation of abscission.

Quebrachitol: Synthesis, Metabolism, and Physiological Functions in *Litchi chinensis*

Liangliang Huang[1], Renfang Zeng[1], Huicong Wang[1,2]

[1] *Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China*

[2] *Department of Life Sciences and Technology, Yangtze Normal University, Fuling 408100, China*

wanghc1972@263.net

Abstract

Quebrachitol (2-*O*-methyl-chiro-inositol) is a kind of inositol methyl ether, and its isomers include bornesitol (1-*O*-methyl-myo-inositol), pinitol (3-*O*-methyl-chiro-inositol), ononitol (4-*O*-methyl-myo-inositol), and sequoyitol (5-*O*-methyl-myo-inositol). The synthetic pathway, metabolic fate, and biological function of inositol methyl ethers in plants have recently attracted much attention. Our previous study revealed that quebrachitol is an important photosynthetic product, which is synthesized in litchi leaf. It is transported through the phloem and is widely distributed in various tissues. LcIMT1 is the key enzyme catalyzing the precursor of quebrachitol, bornesitol. In this study, we confirm that *LcIMT1* is a crucial gene involved in the reaction of bornesitol through prokaryotic expression and in vitro enzymatic activity analysis. The optimal pH for LcIMT1 recombinant protease activity was 8, with an optimal reaction temperature of 25 °C. *LcBEPa* and *LcBEPb* were targeted as the enzyme genes in catalyzing the two-step reaction to convert bornesitol into quebrachitol in litchi. The contents of quebrachitol and bornesitol in litchi root significantly increased in parallel with the elevated expression levels of *LcIMT1* and *LcMIPS* in response to drought. When *LcIMT1* was overexpressed in *Arabidopsis*, the transformed lines demonstrated increased survival rates under drought stress. These results suggest the important role of maintaining osmotic balance and drought resistance.

Screening and comprehensive evaluation of 25 excellent individuals of *Camellia oleifera* Abel.

Siqi Huang[1], Fang Li[2], Caiqin Li[2], Bipei Zhang[2], Panfeng Tu[2], Yongquan Li[2], Wenpei Song[2]

[1] *College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

[2] *College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

14118126@njau.edu.cn

Abstract

Camellia seed oil possess high nutrition and health value, but there is a widespread phenomenon of extensive management and 'depend on Heaven for food' in the production of *Camellia oleifera* growers, resulting in low yield and serious problems in on-and-off years of *C. oleifera*. In this study, 25 common *C. oleifera* monocultures with high fruit set and different fruit traits were selected and sampled at the late stage of fruit development, and their biological traits (e.g., crown width, fruit weight, fruit shape index, number of seeds, seed weight, pericarp thickness) and economic traits (e.g., yield, oil content of seeds) were investigated and analyzed by principal component analysis, with the aim of screening out monocultures with excellent overall traits and providing a theoretical basis for low-yield forest transformation. The results showed that H2 monocultures had better overall performance among all traits and could be selected as a priority; H5 and H16 were second only to H2 and might need to be cultivated by horticulture to improve yield; H6, H9, H7 and H18 monocultures could be considered as alternatives; H15, H20 and H22 monocultures were considered for elimination. This study analyzed and evaluated the comprehensive traits of 25 *C. oleifera* monocultures to provide theoretical support for the selection and breeding of good oil tea varieties and production practice in the future, but the yield data was only collected for one year. Given that there are long and short periods, it is possible that recording data such as plant yield for 3–5 years can provide more accurate and reliable results.

Comparative Test of Different Varieties of Pumpkin in Plateau Open Field Cultivation

Yongqiang Jiang[1,2], Xuemei Sun[1,2], Jiang Li[1,2]

[1] *Academy of Agriculture and Forestry Sciences, Qinghai University*

[2] *Key Laboratory of Qinghai Province Vegetable Genetics and Physiology Xining 810016, Qinghai, China*）

jiangyongqiang0951@163.com

Abstract

To enrich pumpkin planting types in plateau areas and provide more consumption and planting options for local areas, we comparatively examined 28 pumpkin varieties. The results showed that the 28 pumpkin varieties had their own characteristics and could be planted together to provide consumers with more choices. The results showed that the comprehensive quality of Lizi, Weihuang 4 and Xiyangyang varieties was better, the single fruit size was suitable, the fruit shape was beautiful, and the yield was high, which was suitable for planting in the Xining region. Although the yield of Bantian Beibei No.1 and Bantian Beibei No.3 was low, the comprehensive nutritional quality was high, and they could be planted according to specific needs. Other tested varieties performed generally well and could be planted according to market and production needs.

Evaluation of Tolerance of Six Introduced Blackberries Under Heat Stress

Lingfeng Jiang[1], Xing Guo[2], Wenda Su[1], Wei Guo[2], Haocheng Ruan[2], Wenpei Song[2]

[1] *College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

[2] *College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, 510230, China*

2267922163@qq.com

Abstract

Blackberry (*Rubus fruticosus* L.) is currently a popular "third generation fruit" in the world, with a unique taste, high processing value, large market demand, and rich nutrition. Blackberries are native to the northern temperate zone and most varieties have weak heat resistance. This study comprehensively evaluated the heat resistance of six blackberries planted in Deqing Forest Farm, Guangdong Province, which were introduced from the Institute of Botany, Chinese Academy of Sciences, Jiangsu Province. Robust and equally-growing plants were selected as the test materials. The temperature gradients of 25 ℃, 30 ℃, 35 ℃, 38 ℃, and 42 ℃ were applied to plants in an artificial climate box for 2 days at 12 hours day/12 hours night. By measuring eight physiological and biochemical indicators, including conductivity, chlorophyll, malondialdehyde, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), soluble protein, and proline, it was found that the semi lethal temperature of 'Shuangji' was the highest (49.08 ℃), with the lowest being 'Triple Crown' (44.56 ℃). Using factor analysis and membership function analysis, combined with principal component analysis, the heat resistance of six introduced blackberries was comprehensively ranked from strong to weak as follows: 'Shuangji' > 'Ningzhi No. 3' > 'Ningzhi No.4' > 'Kiowa' > 'Hull' > 'Triple Crown'. This study provides a theoretical basis for the introduction of blackberries in southern China, and the findings are of great significance for the screening and promotion of heat-resistant blackberries, as well as the development of the blackberry industry.

Research on the mechanism of fruit coloration promoted by abiotic stress in orange

Zhenghua Jiang[1], Ranran Wei[1], Zhengchen He[1], Zongzhou Xie[1], Junli Ye[1], Lijun Chai[1], Pengtao Yue[1], Xiuxin Deng[1,2]

[1] *National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan, Hubei 430070, China*
[2] *Hubei Hongshan Laboratory, Wuhan 430070, China*

zionhua_jiang@163.com; louisaimeyue@126.com; xxdeng@mail.hzau.edu.cn

Abstract

'Newhall' Navel orange is one of the main cultivated varieties of citrus in China. Its bright-red color and unique taste are preferred by customers. However, the peels of matured 'Newhall' orange fruits can be plagued by pale and uneven coloration, decreasing the fruit value. Carotenoids are natural pigments that affect the color of fruits and vegetables. Different carotenoids accumulate in peels and determine the color diversity of citrus fruits. We report that reddish citrus varieties, such as 'Newhall' navel orange, accumulate high levels of β-citraurin, a bright-red colored carotenoid, and the *CCD4b* gene is responsible for the accumulation of this pigment. Environmental factors can affect pigment biosynthesis and accumulation, and thus, fruit coloration. In this study, we harvested 'Newhall' orange fruit at 210 DFAB (days after full blossom), treated fruit with different concentrations of NaCl and H_2O_2, and stored the fruit at room temperature for 15 d with sampling every 5 d. Untreated fruit served as the control. The color change and carotenoid content in the fruit peels were analyzed. NaCl and H_2O_2 treatments both induced fruit coloration and β-citraurin accumulation compared with the control. At 15 DAT (days after treatment), the level of β-citraurin was 84.81 μg/g and 54.01 μg/g in NaCl and H_2O_2 treated fruits, respectively, while its level was 26.08 μg/g in the control. In addition, the levels of phytoene, violaxanthin, 9-cis-violaxanthin and zeaxanthin were significantly higher in the treated fruit than in the untreated fruit. Taken together, NaCl and H_2O_2 treatments induce the coloration in 'Newhall' navel orange and therefore improve its economic value. To explore the mechanism used by NaCl and H_2O_2 to induce β-citraurin biosynthesis, we will examine the expression of β-citraurin biosynthetic genes and identify the transcription factors whose expression is closely related to the expression of β-citraurin biosynthetic genes in the future.

(Z) -3-hexenol integrates drought and cold stress signaling by activating abscisic acid glucosylation in tea plants

Jieyang Jin, Yan Hu, Xiaoyu Liang, Chenru Wei, Zhengwei Luo, Kai Wang, Lanxin Luo, Cuilin Xie, Chuankui Song

State Key Laboratory of Tea Plant Biology and Utilization, International Joint Laboratory on Tea Chemistry and Health Effects, Anhui Agricultural University, 230036, Hefei, Anhui, China

jjyjieyang@163.com

Abstract

Cold and drought stress severely limits crop production, and both forms can occur simultaneously. Although some transcription factors and hormones have been identified in plants subjected cold and drought stress, the role of metabolites, especially volatiles, in response to cold and drought stress exposure is rarely studied due to lack of suitable models. Here, we established a model for studying the role of volatiles in tea (*Camellia sinensis*) plants experiencing simultaneous cold and drought stress. Using this model, we showed that volatiles induced by cold stress promote drought tolerance in tea plants by mediating reactive oxygen species and stomatal conductance. Needle trap micro-extraction combined with GC-MS identified the volatiles involved in the crosstalk and showed that cold-induced (Z)-3-hexenol improved the drought tolerance of tea plants. In addition, silencing *CsADH2* (*Camellia sinensis alcohol dehydrogenase 2)* led to reduced (Z)-3-hexenol production and significantly reduced drought tolerance in response to simultaneous cold and drought stress. Transcriptome and metabolite analyses, together with plant hormone comparison and abscisic acid (ABA) biosynthesis pathway inhibition experiments, further confirmed the roles of ABA in (Z)-3-hexenol-induced drought tolerance of tea plants. (Z)-3-hexenol application and gene silencing results supported the hypothesis that (Z)-3-hexenol plays a role in the integration of cold and drought tolerance by stimulating the dual function glucosyltransferase UGT85A53, thereby altering ABA homeostasis in tea plants. Overall, we present a model for studying the roles of metabolites in plants under multiple stresses and reveal the roles of volatiles in integrating cold and drought stress in plants.

Lily membrane-associated NAC transcription factor LlNAC014 is involved in thermotolerance via activation of the DREB2-HSFA3 module

Wu Ze[1,2,3,†], Li Ting[1,2,†], Xiang Jun[1,2], Teng Renda[1,2], Zhang Dehua[1,2], Teng Nianjun[1,2]

[1] *Key Laboratory of Landscaping Agriculture, Ministry of Agriculture and Rural Affairs, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China*

[2] *Jiangsu Graduate Workstation of Nanjing Agricultural University and Nanjing Oriole Island Modern Agricultural Development Co., Ltd., Nanjing 210043, China*

[3] *College of Agriculture, Nanjing Agricultural University, Nanjing 210095, China*

[†] These authors contributed equally.

wuze@njau.edu.cn; njteng@njau.edu.cn

Abstract

NTL (NAC with transmembrane motif 1-like) transcription factors with a conserved transmembrane motif are members of the NAC family and important in plant development and stress response. However, knowledge of their regulatory pathways is scarce, especially under heat stress. Here, we cloned and identified a novel lily (*Lilium longiflorum*) NTL gene, *LlNAC014*, that increases thermotolerance. High temperature repressed *LlNAC014* expression but activated its protein. LlNAC014 contained a typical transmembrane motif at its far C-terminus and was normally located on membranes, but under heat stress it entered the nucleus as a transcription factor. LlNAC014 also has a transactivation domain at its C-terminus, and its active form, LlNAC014ΔC, could function as a trans-activator in both yeast and plant cells. *LlNAC014ΔC* overexpression in lily and Arabidopsis increased thermotolerance and also caused growth defects; silencing *LlNAC014* in lily decreased thermotolerance. LlNAC014ΔC could constitutively activate the heat stress response by inducing the expression of heat-responsive genes, some of which were dependent on the HSF (heat stress transcription factor) pathway. Further analysis showed that LlNAC014 was a direct regulator of the DREB2-HSFA3 module and bound to the CTT(N7)AAG element in the promoters of LlHSFA3A, LlHSFA3B, and *LlDREB2B* to activate their expression. Thus, LlNAC014 increases thermotolerance by sensing high temperature and translocating to the nucleus to activate the DREB2-HSFA3 module.

Dissecting the regulatory mechanism of shoot branching by strigolactones and abscisic acid in *Nervilia fordii* based on metabolite profiling and transcriptome analyses

Shuxuan Liu, Jiayi Chi, Ruoting Zhan, Rui He

Research Center of Chinese Herbal Resource Science and Engineering, Guangzhou University of Chinese Medicine, Key Laboratory of Chinese Medicinal Resource from Lingnan (Guangzhou University of Chinese Medicine), Ministry of Education, Joint Laboratory of National Engineering Research Center for the Pharmaceutics of Traditional Chinese Medicines, Guangzhou, China

a1048516947@qq.com

Abstract

Strigolactones (SLs) and abscisic acid (ABA) are two important phytohormones involved in shoot branching, morphology and seed dormancy. *Nervilia fordii* (Hance) Schltr, one of the most well-known herbs in the *Lingnan* region of China, has long been used as *Qingtiankui* for curing pulmonary diseases in traditional Chinese medicine. However, because of the particularity of its growth, that is, it develops only one leaf per year combined with the excessive harvesting by farmers, the yield of *N. fordii* is getting lower and lower. Here, we investigated the effects of SLs and ABA on the branching of *N. fordii* to address the problem of its low yield. Transcriptome analyses of corms (C), petioles (P) and leaves (L) of *N. fordii* were performed. A total of 9916, 17878 and 10736 differentially expressed genes (DEGs) were identified among three comparisons C *vs* P, C *vs* L and P *vs* L, respectively. Network analysis pointed out an enriched interaction cluster, "carotenoid biosynthesis", and several SLs and ABA biosynthesis and signal transduction related genes were upregulated or downregulated. Furthermore, analysis of phytohormone levels showed that single-leaf *N. fordii* had higher levels of SLs and ABA than multiple-leaf *N. fordii*. The present study indicates that the increase of endogenous SLs and ABA resulted in the *N. fordii* branching phenotype. The transcriptome and metabolome also provide valuable resources for settling the shortage of *N. fordii*.

A single nucleotide deletion in the coding sequence of *ClphyB* leads to short lateral branch mutation in watermelon

Sitong Liu[1], Yaru Duan[1], Yu Guo[1,2], Xiuping Bao[1,2], Ziying Wang[3], Xiujie Liu[3], Jixiu Liu[3], Wen Zhao[3], Meiling Gao[1,2]

[1] *College of Life Science, Agriculture and Forestry, Qiqihar University, Qiqihar, Heilongjiang, 161006, China*

[2] *Heilongjiang Provincial Key Laboratory of Resistance Gene Engineering and Protection of Biodiversity in Cold Areas, Qiqihar, Heilongjiang, 161006, China*

[3] *Qiqihar Agricultural Technology Extension Center, Qiqihar, Heilongjiang, 161006, China*

1327389334@qq.com; gaomeiling0539@163.com

Abstract

Plant branching habit is a unique morphological trait of watermelon that regulates plant architecture as well as crop yield, but an in-depth understanding of the molecular regulation of the branching habit in watermelon is unavailable. In this experiment, two contrasted watermelon lines with different phenotypes, a normal branching line (GWAS-38) and a mutant short lateral branched (*slb*) line, were crossed to derive the bi-parental F_2 mapping populations. Genetic segregation analysis of collected phenotypic data depicted a good-fit 3:1 segregating ratio, which indicated that the *slb* locus in the mapping population was regulated by one recessive gene. Bulk segregant sequencing analysis (BSA-seq) and genetic linkage mapping with F_2 mapping populations (336 plants) identified the 1.63-Mbp region on chromosome 5. Fine genetic mapping with an expanded F_2 mapping population (1020 plants) narrowed down the candidate *slb* locus to a 25,355bp interval with two predicted genes. The sequence variations indicated a 1 bp deletion (A→-), causing a truncated protein lacking the conserved domains for the phytochrome PAS2 structural domain and the HATPase structural domain in the mutant *slb* line, which was also co-segregated with the short lateral branched phenotype. Haplotype analysis and gene expression analysis suggested that the *Cla97C05G088180.1* gene encoding phytochrome B is the major gene regulating the short lateral branched trait in the mutant *slb* line. Furthermore, CRISPR/Cas9-mediated mutation of *CsphyB* led to hypocotyl elongation, reduced leaf angle, and inhibited branch elongation, suggesting that *CsphyB* is mainly responsible for the regulation of branch elongation in cucumber plants. To the best of our knowledge, this is the first report of the cloning and molecular characterization of the *ClphyB* gene in the regulation of branch elongation in watermelon. We believe that our results provide important genetic insights for understanding the possible role of *ClphyB* in the architecture of watermelon plants.

LcDOF5.6-LcRbohD regulatory module controls the ROS-mediated fruitlet abscission in litchi

Xingshuai Ma[1,2,3,4†], Xianlin Xie[1,4†], Zidi He[1,4], Fei Wang[1,4], Ruixin Fan[1,4], Qingxin Chen[1,4], Hang Zhang[1,4], Zhiqiang Huang[1,4], Hong Wu[1,2*], Minglei Zhao[1,2,3,4*] and Jianguo Li[1,2,3,4*]

[1] *Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, 510642, China*

[2] *State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou, 510642, China*

[3] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China*

[4] *Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou, 510642, China*

† These authors contributed equally.

953609421@qq.com

Abstract

Reactive oxygen species (ROS) have emerged as key regulators of plant organ abscission. However, the mechanism underlying the regulation of ROS in the abscission zone (AZ) is not completely established. Here, we report that the DOF (DNA binding with one finger) transcription factor LcDOF5.6 can suppress litchi fruitlet abscission by repressing ROS accumulation in fruitlet AZ (FAZ). The expression of *LcRbohD*, a homolog of the *Arabidopsis* RBOHs that are critical for ROS production, was significantly increased during litchi fruitlet abscission, in parallel with an increased accumulation of ROS in FAZ. By contrast, silencing of *LcRbohD* reduced ROS accumulation in FAZ as well as fruitlet abscission in litchi. Using *in vitro* and *in vivo* assays, we revealed that LcDOF5.6 could inhibit the expression of *LcRbohD* via direct binding to its promoter. Consistently, silencing of *LcDOF5.6* increased the expression of *LcRbohD*, concurrently with higher ROS accumulation in FAZ and increased fruitlet abscission. Furthermore, the expression of key genes (*LcIDL1*, *LcHSL2*, *LcACO2*, *LcACS1*, and *LcEIL3)* in IDA (INFLORESCENCE DEFICIENT IN ABSCISSION) and ethylene pathways were altered in LcRbohD-silenced and LcDOF5.6-silenced FAZ cells. Taken together, our results demonstrate an important role of the LcDOF5.6-LcRbohD module during litchi fruitlet abscission. Our findings provide new insights into the molecular regulatory network of organ abscission.

Identification and characterization of the CONSTANS-like gene family in the day-neutral plant *Litchi chinensis* Sonn.

Xifen Pan, Biyan Zhou

College of Horticulture, South China Agricultural University

307103187@qq.com

CONSTANS (CO) and *CONSTANS-like*(*COL*) genes play a crucial role in regulating flowering in photoperiod-sensitive plants. However, there is limited information about their function in day-neutral plants. In this study, we identified ten *LcCO/COL* genes in litchi, which were divided into 3 groups, and investigated their diurnal and tissue-specific expression patterns. Our findings demonstrated that *LcCO/COL*s exhibit conserved structural similarity consistent with their counterparts in other plant species. Expression analysis specific to the flower induction stage indicated that *LcCO/COL*s in group 1 and group 2 exhibit significant transcriptional accumulation during the pre-induction and mid-induction stages in leaves. To investigate whether all lychee varieties exhibit day-neutrality, we conducted photoperiod experiments under short-day, long-day, and full-day conditions using the 'Sanyuehong' variety. The results revealed that the flowering time under short-day conditions was significantly earlier than under full-day conditions, but there was no significant difference compared to long-day conditions. Furthermore, the percentage of flowering shoots under short-day conditions was significantly higher than under full-day conditions, with no significant difference compared to long-day conditions. Additionally, we performed RT-qPCR analysis to examine the flower induction process at different stages in leaves and buds of different varieties. The findings demonstrated that LcCO/COLs in group 3 exhibited a significant accumulation of expression levels in the buds of the 'Guiwei' and 'Nuomici' late-maturing varieties during the late induction stage. Conversely, LcCO/COLs in group 2 showed a significant accumulation of expression levels in the buds of the 'Sanyuehong' and 'Feizixiao' early-maturing varieties during the late induction stage. In summary, our study provides new insights into the *CONSTANS-like* gene family in the day-neutral plant *Litchi chinensis* Sonn., highlighting their potential role in flower induction and suggesting the presence of day-neutrality in the 'Sanyuehong' variety. Further research should explore the regulatory mechanisms and potential applications of these genes in day-neutral plants.

Establishment of somatic embryogenesis regeneration system and transcriptome analysis of early somatic embryogenesis in Litchi chinensis

Yaqi Qin†, Bo Zhang†, Shiqian Wang, Wuyan Guo, Zhike Zhang, Yonghua Qin, Jietang Zhao, Guibing Hu

[1] *State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (South China), Ministry of Agriculture and Rural Affairs/ Guangdong Litchi Engineering Research Center, College of Horticulture, South China Agricultural University, Guangzhou 510000, China*

† These authors contributed equally.

jtzhao@scau.edu.cn; guibing@scau.edu.cn

Abstract

Litchi chinensis Sonn. is an important economic fruit tree in tropical and subtropical regions. Regrettably, the efficiency of plant regeneration via somatic embryogenesis in litchi is typically low due to the poor conversion of embryos to plants. The purpose of this study was to establish a regeneration system via somatic embryogenesis from immature embryos explants in 'Heiye' cultivar of litchi. Our results demonstrated that MS medium supplemented with 2.0 mg· L^{-1} 2,4-D was optimal for callus induction. For somatic embryo (SE) induction, MS medium containing 0.5 g· L^{-1} activated charcoal (AC) was the most effective, while the use of zeatin (ZT) and thidiazuron (TDZ) resulted in abnormal somatic embryos. Rooting and regeneration rates of 2.15% and 17.5%, respectively, were achieved using MS medium supplemented with 0.5 mg· L^{-1} AC. Furthermore, transcriptome analysis was performed on embryogenic callus (EC), globular embryo (GE), and heart embryo (HE) to explore the molecular mechanisms of early somatic embryogenesis. A total of 2587 common DEGs between EC_vs_GE and EC_vs_HE were identified, and the expression patterns of these common DEGs were separated into 12 major clusters. GO annotation and KEGG pathway analysis revealed that these common DEGs were implicated in plant hormone signal transduction, auxin-activated signaling pathway, and other biological processes. Furthermore, differentially expressed transcription factors were identified, and the function of *LcBBM2*, which is highly expressed during early somatic embryogenesis, was verified. Overexpression of *LcBBM2* in tomato promoted callus and shoot formation. Therefore, this study provides a theoretical basis and a technical strategy for the improved genetic breeding of litchi.

Citrus ichangensis WRKY27 functions in cold tolerance by modulating lignin synthesis through transcriptional regulation of *CiCAD*

Jing Qu, Ji-Hong Liu

National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China

Abstract

WRKY comprises a large family of transcription factors in plants, but most WRKY members are still poorly understood. In this study, we report the functional characterization of a Group Ⅱe WRKY gene (*CiWRKY27*) from *Citrus ichangensis*. *CiWRKY27* was stongly induced by cold and presented as a transcriptional activator. Overexpression of *CiWRKY27* in tobacco (*Nicotiana nudicaulis*) conferred enhanced cold stresses tolerance, whereas virus-induced gene silencing (VIGS)-mediated knockdown of CiWRKY27 greatly elevated the cold sensitivity of Ichang papeda. Global transcriptome profiling revealed that CiWRKY27 silencing resulted in extensive transcriptional reprogramming of stress-responsive genes associated with metabolic pathways, plant hormone signal transduction and phenylpropanoid biosynthesis. In addition, a total of 3403 promoter-located peaks were detected as high-confidence CiWRKY27 binding regions by DNA affinity purification sequencing (DAP-seq) technology. Conjoint analysis of RNA-seq and Dap-seq excavated 717 potential CiWRKY27 regulated downstream target genes during cold stress. CiWRKY27 was further verified to directly and specifically bind to the promoter of *Cinnamyl-alcohol dehydrogenase* (*CiCAD*). Cold treatment upregulated the *CiCAD* expression level, elevating CAD enzyme activity and lignin accumulation. Moreover, yeast two-hybrid screening found that CiWRKY27 interacted with CiRAP2.7, an AP2 transcription factor, thereby activating the expression of *CiCAD* to resist the cold in *Citrus ichangensis*. Taken together, our results demonstrate that CiWRKY27 functions as a positive regulator in cold tolerance by regulating genes involved in lignin biosynthesis.

Effect of photoperiod on flower formation of passiflora

Dongyu Sun, Biyan Zhou

College of Horticulture, South China Agricultural University, Guangzhou 510642, China

484856356@qq.com

Abstract

Passiflora undergoes competition between reproductive growth and vegetative growth during flower bud differentiation, and in the short-day winter season, most flower buds in lotus japonicus will abort before flowering, severely affecting the development of the Passiflora industry. Based on this phenomenon, we conducted a photoperiod experiment on 'Tainong No. 1' and measured the physiological parameters, flower bud morphology, anatomical observations, and leaf physiological indicators, and performed transcriptome sequencing. The results confirmed that under long-day conditions (16 hours of light/8 hours of darkness), the flower buds of 'Tainong No. 1' could continuously develop and enlarge until flowering, while under short-day conditions (8 hours of light/16 hours of darkness), the flower buds stopped developing. The results showed that under long-day conditions, the number of secondary branches, number of leaves per branch, number of internodes per branch, and length of branches per branch were significantly higher than those under short-day conditions. Under long-day conditions, the lotus flower buds continued to differentiate and enlarge until the bracts burst open, while under short-day conditions, both the flower buds and leaf buds stopped developing. Through transcriptome analysis, key candidate genes for flowering were identified, such as *TEMPRANILLO 1* (*TEM1*), *CONSTANS-LIKE 4* (*COL4*), and *AGAMOUS-LIKE 24* (*AGL24*). To study the function of *PeTEM1*, *PeCOL4*, and *PeAGL24*, overexpression vectors of *35S::PeTEM1*, *35S::PeCOL4*, and *35S::PeAGL24* were constructed and transformed into wild-type *Arabidopsis thaliana*. The results showed that, compared to wild-type plants, *35S::PeTEM1* transgenic plants exhibited a significantly late-flowering phenotype, *35S::PeCOL4* transgenic plants exhibited a significantly early-flowering phenotype, and *35S::PeAGL24* transgenic plants not only exhibited a significantly early-flowering phenotype but also had degenerated petals, elongated styles, continued elongation at the apex, and development of fewer pods. Photoperiod can affect the flowering of some lotus varieties, but there are few reports on related research achievements, and further research is urgently needed to study the flowering mechanism.

Melatonin Boosts Tomato Cold Resistance via ABA Accumulation

Guangzheng Wang[1], Zhongqi Tang[1*], Jihua Yu[1,2]

[1] *College of Horticulture, Gansu Agriculture University, Lanzhou 730070, China;*

[2] *State Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou 730070, China*

wanggz@st.gsau.edu.cn

Abstract

Cold stress is a common abiotic challenge in greenhouse vegetable cultivation, significantly affecting the normal growth of horticultural crops. Melatonin is a well-known multifunctional molecule, and some studies suggest that exogenous melatonin application can improve tomato tolerance to cold stress. However, the molecular mechanisms underlying melatonin's enhancement of cold resistance in tomato remain understudied. The aim of this study is to reveal the physiological and molecular changes associated with exogenous melatonin application in the context of tomato response to cold stress. In the experiment, plants grown under normal conditions served as the control group (CK), and two treatments were applied: cold stress (T1) and cold stress with external melatonin application (T2). By comparing RNA sequencing transcriptome profiles, we found differential expression of genes (DEGs) in the comparisons CK vs T1, CK vs T2, and T1 vs T2, resulting in 2,997, 3,682, and 588 DEGs, respectively. A Venn diagram revealed that there were 135 common DEGs across all treatments. Further analysis uncovered that genes associated with ABA metabolism, such as *CYP707A1*, were significantly upregulated during low-temperature stress, and their expression was significantly suppressed after melatonin application. Additional gene searches identified the differential expression of *CYP707A2* and *NCED1*, which are involved in ABA synthesis. *CYP707A2* exhibited a similar expression trend to *CYP707A1*, while *NCED1* was significantly upregulated under low-temperature stress, and its expression further increased after melatonin application. The endogenous ABA content in tomatoes increased by 24.83% at 48 hours after melatonin application compared to the control without melatonin treatment. qPCR results also indicated that tomato pretreated with melatonin for 3 hours exhibited a sixfold increase in *NCED1* expression compared to the non-melatonin-treated control. By contrast, *CYP707A1* and *CYP707A2* were downregulated by 30-fold and 44-fold, respectively. In summary, exogenous melatonin regulates the expression of genes involved in ABA synthesis and metabolism, leading to ABA accumulation, and ultimately enhancing the plant's ability to resist cold stress.

Mobile HY5 regulates the light-induced accumulation of carotenoids and soluble sugars during tomato fruit ripening

Jiachun Wang[1,2], Xiaomeng Li[1], Jiajia Li[1], Han Dong[1,2], Yanhong Zhou[1,3], Zhangjian Hu[1,3], Jingquan Yu[1,2,3]

[1] *Department of Horticulture, Zijingang Campus, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China*

[2] *College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China*

[3] *Hainan Institute, Zhejiang University, Sanya 572000, China*

Jiachun0529@zju.edu.cn; zjhu90@zju.edu.cn; zjhu90@zju.edu.cn

Abstract

Fruit ripening in tomatoes is a complicated process characterized by significant changes in carotenoids and soluble sugars content. While the significance of light in tomato growth is acknowledged, its specific influence on ripening has remained elusive. In this study, we report that the absence of light led to a reduction in carotenoid and soluble sugar synthesis in immature tomato fruits. The light-responsive transcription factor HY5 was identified as a pivotal player involved in tomato fruit ripening. Notably, at the fruit breaker stage, the transcript and protein levels of HY5 exhibited an increase, significantly enhancing the accumulation of carotenoids and soluble sugars during ripening. Further studies demonstrated that HY5 directly binds to the promoters of key carotenoid biosynthesis genes, such as *PSY1* and *PDS,* as well as the sucrose invertase genes *LIN5* and *LIN6*, thereby activating their expression. In addition, we conducted grafting experiments by attaching shoot sections bearing *HY5*-deficient flowers onto the rootstock that overexpressed *HY5* and observed a remarkable induction of the HY5 protein in the fruit scion, strongly suggesting that HY5 can function as a mobile signal, transferring from the rootstock to the fruit scion. Consequently, the mobile HY5 significantly promoted the accumulation of carotenoids and sugars in fruits originating from the rootstock that overexpressed *HY5*, in stark contrast to the fruits from self-grafting *hy5* plants. In summary, our study not only describes the mechanism of how light-induced HY5 acts as a mobile signal to contribute to fruit ripening, but also provides new insights into improving tomato fruit quality.

Investigation of the optimization of cryopreservation protocols for apple dormant buds and their resposes of antioxidant status

Wang Yingmin, Zhang Jinmei, Xin Xia

National Crop GeneBank of China, Institute of Crop Science, Chinese Academy of Agricultural Sciences

82101215041@caas.cn

Abstract

Cryopreservation of dormant buds is important for long-term storage of woody plant germplasm resources. The National Crop GeneBank of China (Institute of Crop Science, Chinese Academy of Agricultural Sciences) conducted a study on the cryopreservation of important vegetative propagules, such as apple, mulberry, and potato. In this study, apple dormant buds were collected from a germplasm resource nursery in December, January, and February; dehydrated to a moisture content of 20%, 30%, or 40%; cooled at programmed rates; exposed to liquid nitrogen; and preserved in the tank. After thawing and rehydration, the cryopreserved dormant buds were grafted onto rootstocks in the field and the regrowth rates of apple dormant buds were evaluated. Results showed that the regrowth rates of apple dormant buds reached 90% and 86.67% in December and January, respectively, and 53.33% in February, which indicated the successful establishment of cryopreservation protocols for apple dormant buds. The relevant oxidation products and antioxidant status were also assessed during cryopreservation. This study will facilitate the long-term preservation of apple germplasm resources.

Molecular Insights Into DzMYB1: A Novel Regulator of Flavonoid Biosynthesis in Durian (*Durio zibethinus*) Fruit

Kamonwan Weerawanich[1], Heidi Halbwirth[2], Supaart Sirikantaramas[3]

[1] *Center of Excellence in Molecular Crop, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand*

[2] *Institute of Chemical, Environmental & Bioscience Engineering, Technische Universität Wien, Vienna, Austria*

[3] *Omics Sciences and Bioinformatics Center, Chulalongkorn University, Bangkok, Thailand*

Kamonwan.wee@gmail.com

Abstract

Flavonoids, a class of phenolic compounds, are known for their potent antioxidant properties. While various transcription factors (TFs) regulate flavonoid biosynthesis, MYB TFs are pivotal in controlling key genes in this pathway. In this investigation, we identified potential MYB TFs from the transcriptome database of 'Monthong' cultivar durian pulp. MYBs showing upregulation at the ripe stage were classified as transcriptional activators due to their positive correlation with both flavonoid biosynthetic genes and flavonoid accumulation levels in ripe durian pulps. Among these, DzMYB1, the most highly expressed candidate MYB activator at the ripe stage, was chosen for functional characterization. LC-MS/MS analysis revealed an increase in flavonoid content in *Solanum lycopersicum* cv. Micro-Tom fruit transiently expressing DzMYB1 compared to the GFP control. Furthermore, our results demonstrated that DzMYB1 controls flavonoid biosynthesis by regulating the promoters of various biosynthetic genes, including chalcone synthase, chalcone isomerase, and flavanone 3-hydroxylase, thereby promoting their transcriptional activation. Additionally, we observed that DzMYB1 acts as a homodimer and forms a complex with homodimerized DzbHLH1 in the regulation of flavonoid biosynthesis. These discoveries offer a more profound understanding of the functional roles of MYB proteins in governing the flavonoid pathway in durian pulps.

C2H2-type zinc finger protein ZAT12 of *Poncirus trifoliata* acts downstream of CBF1 to regulate cold tolerance

Yang Zhang[1, 2,†], Wei Xiao[1,†], Min Wang[3], Madiha Khan[1], Ji-Hong Liu[1]

[1] *National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, China*

[2] *Hubei Key Laboratory of Germplasm Innovation and Utilization of Fruit Trees, Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences, Wuhan 430064, China*

[3] *College of Life Sciences, Gannan Normal University, Ganzhou 341000, China*

[†] These authors contributed equally.

liujihong@mail.hzau.edu.cn

Abstract

The Cys2/His2 (C2H2)-type zinc finger family has been reported to regulate multiple aspects of plant development and abiotic stress response. However, the role of C2H2-type zinc finger proteins in cold tolerance remains largely unclear. Through RNA-sequence analysis, a cold-responsive zinc finger protein, *PtrZAT12*, was identified and isolated from trifoliate orange (*Poncirus trifoliata* L. Raf.), a cold-hardy plant closely related to citrus. Furthermore, we found that *PtrZAT12* was markedly induced by various abiotic stresses, especially cold stress. PtrZAT12 is a nuclear protein, and physiological analysis suggests that overexpression of *PtrZAT12* conferred the enhanced cold tolerance in transgenic tobacco (*Nicotiana tabacum*) plants, while knockdown of *PtrZAT12* by virus-induced gene silencing (VIGS) increased the cold sensitivity of trifoliate orange and repressed the expression of genes involved in stress tolerance. The promoter of *PtrZAT12* harbors a DRE/CRT *cis*-acting element, which was verified to be specifically bound by PtrCBF1 (*Poncirus trifoliata* C-repeat BINDING FACTOR1). VIGS-mediated silencing of *PtrCBF1* reduced the relative expression levels of *PtrZAT12* and decreased the cold resistance of trifoliate orange. Based on these results, we propose that *PtrZAT12* is a direct target of CBF1 and plays a positive role in modulating cold stress tolerance. The findings provide new insights into the regulatory module composed of CBF1-*ZAT12* in the response to cold stress and advances our understanding of cold stress response in plants.

Metabolome and Transcriptome Analyses Reveal the Correlation between Fructan Changes and Phytohormone Regulation During Tuber Sprouting of *Helianthus tuberosus* L.

Ya Wen[1,2], Zhenjie Zhou[1,2], Juan Li[1,2], Gui Wang[1,2], Xiaozhu Guo[1,2], Xuemei Sun[1,2]

[1] *Academy of Agriculture & Forestry, Qinghai University, Xining 810016, Qinghai, China Qinghai University, Xining 810003, China*

[2] *Laboratory for Research and Utilization of Germplasm Resources in Qinghai Tibet Plateau*

sunxuemei1008@163.com

Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) is an asexually reproducing plant with tubers rich in fructan-based carbohydrates that serve as the primary processing organ. Phytohormones have been suggested to regulate tuber sprouting. However, the dynamic fructan changes and phytohormone regulatory mechanisms during tuber sprouting remain unclear. In this study, two different cultivars of Jerusalem artichoke were used to analyze the changes in the proportion of carbohydrate in total sugar, especially fructans, during sprouting. Furthermore, metabolomic and transcriptomic analyses of phytohormones were conducted at three selected sprouting periods. From sprouting to true leaf formation, the proportion of fructan in total sugar increased, while sucrose decreased. After true leaf formation, the proportion of fructan in tubers decreased, while the free sugars (sucrose and fructose) increased. Metabolome and transcriptome analyses screened 92 differential genes related to the signaling of eight phytohormones, with auxin and brassinolide, two growth promoting hormones, having the most differential genes. Moreover, jasmonic and salicylic acid contents increased significantly before and after sprouting, and significantly correlated with the differential gene expression in the corresponding pathway. The proportion of carbohydrate in tubers during sprouting was highly correlated with the expression of phytohormone-related genes in the buds. In conclusion, phytohormones may have a regulatory effect on carbohydrates during the sprouting of Jerusalem artichoke tubers.

Research on the Pineapple Flowering Process and its Regulatory Diversity

Jing Wu[1], Wei Zhang[1], Ziqin Zhong[1], Junhu He[2], Yan Leng[1], Hui Liu[1], Yehua He[1], Aiping Luan[2]

[1] *South China Agricultural University, Guangzhou 510642*

[2] *Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101*

1185587799@qq.com

Abstract

The pineapple plant (*Ananas* Tourm. ex Linn.), despite being a perennial herbaceous species, displays distinctive features in its flowering, such as difficulty in achieving flowering, pronounced seasonality, and irregular flowering patterns. In pineapple cultivation, ethylene is commonly used to promote flowering, shorten the vegetative growth phase, achieve uniform and consistent flowering, and eliminate seasonal variations in flowering. Although there is considerable research on pineapple ethylene-induced flowering globally, the mechanisms and regulatory networks governing natural and ethylene-induced flowering remain unclear. Hence, exploring the molecular control mechanisms of pineapple flowering holds vital significance for improving production efficiency and advancing pineapple biotechnology for breeding. To study pineapple flowering, we used 10-month-old ‘Jingtong' edible pineapple plants with 20–25 leaves. After treating them with ethrel for 6 hours, *AcEIN3*/*EIL* showed a sharp increase in expression. After 12 hours, genes associated with flowering, such as *AcAP1* and *AcFT*, increased, indicating the start of flower bud development. To elucidate the flowering mechanism of pineapple, we utilized the early-flowering mutant variety *yll* as the material. We conducted reciprocal crosses between *yll* and the normal vegetative phase variety ‘MD-2', resulting in a total of 262 F_1 plants. Further RNA-seq analysis of the apical meristem during the flowering transition of *yll*, combined with differential gene expression, gene function enrichment, and co-expression network analysis, revealed the enrichment of 13 flower bud differentiation-related or flowering-specific genes within a single module. In this module, except for *AcERF24*, all other 12 genes have been confirmed in other plants to be associated with flowering. Therefore, it is considered that *AcERF24* specifically participates in the regulation of pineapple flowering.

Progress of research on organic acid metabolism in fruits

Wu Jiale, Wei Liyao, Zhang Zhike

College of Horticulture, South China Agricultural University

934224230@qq.com

Abstract

Organic acid is an important component of fruit flavor material composition, and acid content and proportion are important factors affecting fruit quality, as well as one of the important sensory indicators determining consumer choice. Therefore, understanding the relationship between organic acid metabolism and accumulation in fruit is key to improving fruit quality. Organic acid metabolism is not regulated by a single gene, but by multiple genes regulated by synergistic effects with multiple structural genes and transcription factors, in which genetic traits affecting organic acid accumulation contain multiple transcription factors in addition to functional genes related to synthesis, catabolism, utilization, and translocation of organic acids. In this paper, we review the characteristics of organic acid accumulation in different fruit trees, the roles of key enzymes and related genes of acid metabolism in the regulation of fruit acid content, and the mechanisms of transcription factors in regulating acids. It aims to provide a theoretical reference for regulating fruit acid content and improving fruit quality.

Integrated Cytological, Physiological, and Transcriptome Analyses Provide Insight into the Albino Phenotype of Chinese Plum (*Prunus salicina*)

Weiwei Xie[1], Dantong Xu[1], Fangce Chen[1], Zhengpeng Wang[1], Jiandong Luo[1], Yehua He[1], Qianming Zheng[2], Chaoyang Liu[1]

[1] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*

[2] *Institute of Pomology Science, Guizhou Academy of Agricultural Science, Guiyang 550006, China*

20213137120@stu.scau.edu.cn

Abstract

Seed propagation is a cost-effective way to produce offspring with high genetic diversity, which plays an important role in the new variety breeding of plums. Albino seedlings that arise during seed reproduction can have a significant impact on plant growth and breeding. In this research, we present the first report of albino occurrences in the seed reproduction process of *Prunus salicina* and describe the cytological, physiological, and transcriptomic changes observed in albino seedlings. The albino seedlings could not grow normally and exhibited lethal characteristics; differences in the proportions of albinos were observed among different plum varieties. The albino seedlings, which were observed in several plum cultivars, exhibited abnormal chloroplast ultrastructure and perturbed stomatal structure. Compared to normal seedlings, the photosynthetic pigment contents in albino seedlings decreased by more than 90%, accompanied by significant reductions in several chlorophyll fluorescence parameters. Furthermore, substantially altered photosynthetic parameters indicated that the photosynthetic capacity and stomatal function were impaired in albino seedlings. Additionally, the activities of the antioxidant enzyme were drastically altered against the background of higher proline and lower ascorbic acid in leaves of albino seedlings. A total of 4048 differentially expressed genes (DEGs) were identified through transcriptomic sequencing, and the downregulated DEGs in albino seedlings were greatly enriched in the pathways for photosynthetic antenna proteins and flavonoid biosynthesis. *GLK1* and *FTSZ* were identified as candidate genes responsible for impaired chloroplast development and division in albino seedlings. Moreover, the significant downregulation of the nuclear genes encoding photosynthetic antenna proteins and the PEP-dependent chloroplast genes involved in photosystem I and II could give rise to the notably reduced photosynthetic capacity of the albino seedlings. Our findings shed light on the intricate physiological and molecular mechanisms driving albino plum seedling manifestation, which will contribute to improving the reproductive and breeding efforts of plums.

Molecular Mechanism of miR160a-ARF Module Regulating Banana Response to Low Potassium Stress

Tang Yi

Huaqiao University, Institute of Horticultural Science and Engineering, Xiamen, 362021

w_mingyuan@163.com

Abstract

Potassium (K) is a necessary element for plant growth and development, accounting for 2–10% of the total dry weight. It has fundamental physiological functions, such as regulating cell osmotic pressure, activating enzyme reactions, maintaining charge balance, promoting photosynthesis, and improving stress resistance. Currently, approximately 22.5% of soils worldwide are affected by mineral deficiencies, with 40% of soils having low potassium levels. In China, more than 35% of cultivated land is affected by low potassium stress. Banana (Musa nana Lour.), the highest traded fruit globally, producing 1.19 billion tons annually, with nearly one-third of the output coming from China and India, possesses significant economic value. Bananas are rich in potassium, with demand for soil potassium levels five to seven times that of crops such as rice and corn. When there is insufficient potassium in the soil, bananas may exhibit varying degrees of potassium deficiency symptoms. In this study, we screened the key gene miR160a, which responds to low potassium stress, from banana low-potassium-stress transcriptome data. Overexpression of miR160a affected plant root growth and potassium accumulation. To further explore its regulatory mechanism, we used bioinformatics analysis to predict the downstream target gene ARF18-like-2 of miR160a and found that after low-potassium-stress treatment, miR160a expression was significantly reduced, whereas ARF18-like-2 showed an opposite trend. Moreover, tissue-specificity analysis revealed that miR160a and ARF18-like-2 exhibited the highest abundance in roots. Through transient tobacco expression and dual-luciferase reporter gene experiments, we verified the interaction between miR160a and ARF18-like-2, with miR160a negatively regulating the expression of ARF18-like-2. To further investigate the biological function of miR160a and ARF18-like-2, we overexpressed miR160a and ARF18-like-2 in Arabidopsis. Under normal potassium treatment, the growth of Arabidopsis overexpressing ARF18-like-2 was inhibited and the primary root was shorter. However, under low potassium treatment, overexpression of ARF18-like-2 in Arabidopsis resulted in significant increases in primary root length and growth recovery. Overexpression of miR160a in Arabidopsis resulted in normal growth, but the primary root length decreased under low potassium treatment. We speculate that the expression of ARF18-like-2 enhances plant tolerance to low potassium stress while inhibiting normal development. Thus, ARF18-like-2 is specifically expressed under low potassium stress while being suppressed by miR160a under normal conditions.

VvMYBD acts as a negative regulator of norisoprenoid biosynthesis in grape*

Wei Yi[1,2], Meng Nan[3], Wang Yachen[1,2], Meng Xiao[1,2], Yao Xuechen[1,2], Pan Qiuhong[1,2]

[1] *Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

[2] *Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*

[3] *Key Laboratory of Brewing Molecular Engineering of China Light Industry, Beijing Technology and Business University, Beijing 100048, China*

weiyi292266@163.com

Abstract

Norisoprenoids, a class of apocarotenoids with ubiquitous flavor, are the main components contributing to floral-fruity aroma and non-aroma type wines. However, the mechanism underlying the regulation of norisoprenoid biosynthesis in grape berry remains unclear. MYB1R1s are R1-type MYB transcription factors with a single MYB domain, some of which are involved in anthocyanin accumulation. In recent years studies have indicated that MYB1R1 may be involved in the regulation of carotenoids. However, little is known about the function of VvMYB1R1 in grape (*Vitis vinifera*). Our team previously reported that VvMYB1R1 directly regulated a gene in the flavonoid pathway. Here, we identified a VvMYB1R1 homolog (VvMYBD) in grape berry, which may participate in the regulation of norisoprenoid metabolism. It encoded a 300-amino acid protein with an EAR motif, which was homologous to AtMYBD. VvMYBD was located in the nucleus, and it acted as a repressor, with no transcriptional activation domain in yeast self-activation assay. The expression profile showed that VvMYBD was mainly expressed in berries before véraison and the expression was inhibited by light and high temperature in the leaves of plantlets. Transient overexpression in *Vitis quinquangularis* leaves and stable overexpression in grape callus decreased the norisoprenoid content. *VvMYBD*-overexpressing tomato also showed less norisoprenoid content in Br 3 fruits. Moreover, in transgenic tomato and grape callus, the accumulation of carotenoids, flavonols, and PAs were also regulated. According to transcriptome and qRT-PCR analyses, we speculate that VvMYBD may regulate isoprenoid and flavonoid metabolism by regulating the expression of *VvPIF4* and *VvMYBC2-L1*.

Protection and innovative utilization of the germplasm resources for *Begonia benariensis* 'BIG'

ZHAO Yi[1], CHEN Caixia[1,2], CAO Chuanqu[2], Wang Jisheng[2], GAO Junshan[1]

[1] *School of Life Sciences, Anhui Agricultural University, Hefei 230036, China*

[2] *Hefei Hualü Seedling Co. Ltd, Hefei 230041, China*

gaojsh@ahau.edu.cn

Abstract

The Begonia benariensis 'BIG' variants are highly valuable both economically and ornamentally. Because the 'BIG' series varieties in China currently rely on imports from abroad, the domestic flower industry is currently experiencing a "bottleneck" issue with germplasm resources. Therefore, it is critical to produce independent intellectual property varieties, conserve outstanding genetic resources, and construct a bank of germplasm resources. The solution to this issue has been found to be plant in vitro preservation. To establish a rapid propagation system for *B. benariensis* 'BIG', this study used MS and 1/2MS as the basic media and various plant hormones to study the effects of combination media on adventitious bud differentiation, regeneration, and rooting. The results showed that the optimal medium for adventitious bud differentiation was MS+1 mg·L^{-1} 6-BA+0.1 mg·L^{-1} 2.4-D+0.2 mg·L^{-1} NAA. The optimal medium for adventitious bud subculture was MS+1 mg·L^{-1} 6-BA+0.1 mg·L^{-1} NAA. The optimal medium for rooting was 1/2 MS+1.0 mg·L^{-1} NAA. To identify the best method for producing mutant plants with chromosome doubling, the chromosome doubling technology of *B. benariensis* 'BIG' produced by colchicine was investigated. Colchicine was applied to the *B. benariensis* 'BIG' callus as a mutagen. The colchicine mutation efficiency at various concentrations and treatment durations was compared. According to the findings, treating the callus for 4 hours with 0.05% colchicine produced the strongest mutagenic effect, with a 37.5% mutagenic rate. The outcomes offer a solid theoretical foundation for breeding and improving *B. benariensis* 'BIG' cultivars.

Genetic Diversity and Population Genetic Structure Analysis of Jerusalem Artichoke Germplasm Resources Based on GBS Sequencing

Wang Ying, Zhong Qiwen, Sun Xuemei

[1] *Academy of Agriculture & Forestry, Qinghai Xining University*
[2] *Laboratory for Research and Utilization of Germplasm Resources in Qinghai Tibet Plateau*

996255388@qq.com

Abstract

GBS is a rapid, easy and low-cost genotyping method to obtain SNP information by analyzing the fragment sequences after enzymatic cutting of genomic DNA for high-throughput sequencing. In this research, 256 samples of Jerusalem artichoke germplasm resources were sequenced and typed to analyze their genetic diversity and population genetic structure. Finally, 166.7 Gb of valid data and 229,499 SNP loci were obtained. The genetic structure, phylogenetic tree and principal component analysis all classified the 256 samples of Jerusalem artichoke germplasm resources into five subgroups. Group 2 had the highest degree of genetic diversity, whereas groups 2 and 5 had the highest degree of genetic differentiation. This research lays the foundation for the breeding of new varieties of Jerusalem artichoke and the screening of excellent traits.

Comparison of Expression Profile of MaXTHs between a Tolerant and a Sensitive Banana Cultivars when Responded to *Fusarium oxysporum* and Low Temperature

Yehuan Tan[1], Huiling Zan[1], Houbin Chen[1,2], Xiaoquan Li[3], Zuxiang Su[3], Chengjie Chen[1], Hui Liu[1], Yilin Chen[1], Ziyue Zhao[1], Yingyan Xiao[1], Jing Liu[1], Yafang Zhao[1], Chunxiang Xu[1]

[1] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*

[2] *Guangdong Laboratory for Lingnan Modern Agriculture, Maoming Branch, Maoming 525000, China*

[3] *Guangxi Academy of Agricultural Sciences, Nanning 530007, China*

chxxu@scau.edu.cn

Abstract

Banana (*Musa* spp) production is seriously threatened by *Fusarium oxysporum* (*Foc*) and low temperature (LT). Xyloglucan endotransglycosylase/hydrolases (XTHs) are chief enzymes in cell wall remodeling and play a central role in stress responses. However, the role of MaXTHs in stress resistance is not clear. Here, the identification and characterization of MaXTHs were carried out, followed by prediction of their cis-acting elements in the promoter region and protein-protein interactions. In addition, candidate *MaXTHs* involved in the resistance/tolerance of banana to *Foc* or LT were screened through comparison of their responses to stresses between a resistant/tolerant and a sensitive cultivars by RNA-seq analysis. Moreover, immunofluorescence labeling was employed to compare changes in the temporal and spatial distribution of different types of xyloglucan components between the two pairs of cultivars upon stress. In total, 53 *MaXTHs* were identified, and all were predicted to be located in the cell wall, with 14 MaXTHs also in the cytoplasm. Sixteen MaXTHs possessed low temperature responsive cis-acting elements. Many MaXTHs contained elements related to defense and stress responsiveness and/or plant hormones. Only 11 MaXTHs were found to interact with other proteins, including those potentially involved in Foc tolerance. MaXTH10 showed higher expression in the resistant cultivar compared to the susceptible one before and after wounding and pathogen infection. Similar results were observed with non-XXXG-type xyloglucan recognized by the CCRC-M87 antibody. Among the MaXTHs with LT responsive elements, MaXTH7/26/32/50 showed higher expression in the CT cultivar compared to the CS cultivar before and after LT stress. These results reveal that MaXTH7/10/26/32/50 are candidate genes involved in banana tolerance/resistance to LT or Foc.

Naturally occurring promoter variation of γ-glutamyl peptidase synthesis gene (*BrGGP1*) contributes to the 3-butenyl glucosinolate contents in *Brassica rapa*

Tianyu Zhang, Siwen Ma, Zhongyun Piao, Xiaonan Li

College of Horticulture, Shenyang Agricultural University

gracesleexn83@syau.edu.cn

Abstract

An increase of 3-butenyl glucosinolate (GNA) content enhances the unique spicy taste of *Brassica rapa* crops but reduces taste and flavor quality. In this study, we used Chinese cabbage inbred line '59-1' with low GNA as the recurrent parent and turnip 'ECD04' with extremely high GNA as the donor parent for multiple generations of backcross and selfing. SSR marker assisted selection was used to construct a set of chromosome segment substitution lines and their 3-butenyl glucosinolate composition were determined. Finally, the line 'CSSL05' with significantly increased GNA content was selected, and it was found that the A01 chromosome of ' CSSL05' line contained a single insertion fragment from the 'ECD04' genome. Thus, the locus controlling GNA content was initially located in the A01 chromosome interval and named as *qGSL1*. The enlarged derived F_2 population constructed with ' CSSL05' and '59-1' were used to fine-locate it to 3.34 Mb-3.36 Mb on chromosome A01. The *qGSL1* region was analyzed and found to contain a homologous structural gene (*Bra011201*) related to Arabidopsis glucosinolate synthesis gene *AtGGP1*, named *BrGGP1*. By gene cloning, sequencing, GNA content detection and gene expression analysis of *BrGGP1* gene between the two parents, it was found that the *BrGGP1* gene sequence was the same between 'ECD04' and '59-1', but their promoter sequence showed multiple base polymorphisms. In a large *B. rapa* natural variation population, the *BrGGP1* promoter carried either the ECD04 allele or the 59-1 allele, which was associated with *BrGGP1* expression and GNA content. Furthermore, plants overexpressing the *BrGGP1* gene were developed, and the GNA content was found to increase significantly. In conclusion, we reveal an important but previously unknown natural variation in the *BrGGP1* gene promoter sequence that contributes to GNA synthesis, thus providing a theoretical basis for the molecular regulation of GNA synthesis in *B. rapa* crops.

Differences in anthocyanin accumulation and regulatory characteristics in *Ananas* tissues and organs

Wei Zhang[1], Ziqin Zhong[1], Jing Wu[1], Junhu He[1], Mingzhe Yang[1], Hui Liu[1], Chaoyang Liu[1], Yehua He[1], Aiping Luan[2]

[1] *South China Agricultural University, Guangzhou 510642, China*

[2] *Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, China*

zw130403@163.com

Abstract

The color of the *Ananas* (*Ananas* Tourm. ex Linn.) organ is one of the important indicators to evaluate its commercial value, and the organ color has obvious specificity in the existing germplasm resources. For example, only inflorescences accumulate anthocyanins, leaves show persistent or seasonal accumulation, and peels show stage accumulation and whole plant accumulation in some primitive varieties. There are also mutant germplasms in which inflorescences or petals do not accumulate and color chimera. These findings indicate that with the evolution of *Ananas*, the key genes that regulate anthocyanin accumulation may have differentiated, resulting in the diversity of anthocyanins accumulated in tissues and organs. However, the mechanism of anthocyanin accumulation in *Ananas* is still unknown. Therefore, exploring the regulatory mechanism of the differential accumulation of anthocyanins in *Ananas* tissues and organs will not only help us understand the domestication of *Ananas* cultivars but also provide a theoretical basis for the creation of new colorful *Ananas* germplasm. Based on RNA-seq analysis of multi-tissue and multi-developmental stages of 'Shenwan' (*A. comosus* cv. SW), *Ananas* R2R3-MYB transcription factor family analysis, and molecular biology identification such as qPCR, we screened four R2R3 MYB genes (*AcMYB262\263\266\267*) that specifically regulate the accumulation of anthocyanins in different tissues such as leaf and peel, and were clustered together. In addition to the above findings, multi-omics analysis of the 'BTH' (*A. comosus* cv. BTH) fruit development in 3 stages found two additional transcription factors, AcMYB12 and AcHOX21, whose expression levels were downregulated as key factors leading to the decrease of anthocyanin accumulation with the increase of fruit ripeness. The above research opens the door to the accumulation of *Ananas* anthocyanins and also lays the foundation for subsequent more in-depth molecular regulation research. The anthocyanin accumulation of *Ananas* is interesting, and it warrants further in-depth study.

Research on carotenoid accumulation in citrus peel based on a new red-peel orange mutant

Zhehui Zhang[1], Yongjun Hu[1], Zhenghua Jiang[1] Junli Ye[1], Xiuxin Deng[1,2]

[1] *National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan, Hubei 430070, China*
[2]*Hubei Hongshan Laboratory, Wuhan 430070, China*

zhangzhehui@webmail.hzau.edu.cn

Abstract

Citrus, one of the most important fruits in the world, has high economic value. The bright red peel influences consumer choice and can increase their desire to purchase the fruit. The orange pigments of the citrus peel color are mainly carotenoids. Different carotenoid species contribute to the rich appearance of citrus fruits. In a previous study, a red-fleshed citrus mutant with deep red peel was found in the production area. At 210DAF, the peel of the mutant was deeper than the common variety. It was demonstrated that the mutant did not differ from the common variety in terms of soluble sugars, organic acids and ripening period. However, the carotenoid content of the peel was higher than that of the common variety, especially the β-citraurin content, which mainly contributes to the red appearance. The level of β-citraurin was 585.23 μg/g in mutant, whereas the level was 330.10 μg/g in the common variety. Furthermore, there was no significant difference in the carotenoid content of the other compounds such as phytoene, violaxanthin, 9-cis-violaxanthin and zeaxanthin, suggesting that the deeper red peel is mainly due to higher levels of β-citraurin. It was further found that the expression of the *CCD4b* gene, a key gene that mainly determines the production of β-citraurin, was also significantly higher in the mutant than in the common variety. Through transcriptome data mining, an ERF family transcription factor was found to promote the expression of the *CCD4b* gene, and its expression was significantly higher in the mutant than in the common variety. This study reveals the molecular mechanism by which the ERF gene regulates the expression of the *CCD4b* gene and thus participates in the carotenoid metabolism of citrus peels.

Pineapple leaf color types and their genetic variations

Ziqin Zhong[1,3], Wei Zhang[1], Jing Wu[1], Junhu He[1], Mingzhe Yang[1], Hui Liu[1], Yehua He[1], Aiping Luan[2]

[1] *South China Agricultural University, Guangzhou 510642*

[2] *Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101*

[3] *Sichuan Agricultural University, Chengdu 611130*

1959784654@qq.com

Abstract

Bromeliads (*Ananas* spp.) are significant tropical crops, and their leaf color holds important ornamental value. Edible bromeliads are predominantly green leaves, while the ornamental plants exhibit a broader spectrum of colors. At present, the creation of vibrant fresh varieties through hybridization is a pivotal breeding strategy. The leaf color analysis of over 1,200 pineapple germplasm samples revealed that pineapple leaves can be categorized into seven types: white (yellow), yellow green, light green, green, light red, purple-red, and chimeric. Their color is determined by the content and ratio of chlorophyll, anthocyanins, and carotenoids. Anthocyanins are prevalent in the epidermal and water-storage cells, while chlorophyll is mostly in mesophyll cells. We created a hybrid population using red-leaf 'ZY' (*A*. lucidus cv 'Ziye') and green-leaf 'BTH' (*A*. comosus cv 'Bingtanghong'). The 2,746 orthogonally crossed and 1,154 reverse-crossed F_1 plants showed a segregation ratio of 9 Green:7 Red. In hybrids from over 100 varieties leaf lacking anthocyanin accumulation, no red leaves were observed. Our previous research indicated that *AcMYB262* is the key transcription factor for anthocyanin accumulation in leaf. Based on the F_1 segregation and qRT-PCR analysis, the absence of anthocyanin in leaf was linked to suppressed *AcMYB262* expression. Crossbreeding green-leaf and light-green-leaf varieties resulted in a 1 Green:1 Light-Green ratio in the F_1. However, breeding two green-leaf varieties consistently produced green leaves. Studies on green-leaf 'LZZ' and its light-green mutant 'HZZ', RNA-seq and qRT-PCR analysis indicate the chlorophyll synthesis gene *AcGLK* might dictate their color differences. While their *AcGLK* ORF sequences were identical, 'HZZ' had deletions in its promoter, possibly causing its light-green color. In summary, anthocyanins and chlorophyll in pineapple leaves are found in separate cells and are governed by different metabolic pathways and genes. Our study suggests *AcMYB262* and *AcGLK* are pivotal genes for the leaves' red and green colors.

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