The 8th International *Horticulture Research* Conference

A Chromosome-Scale Reference Genome Provides Insights into the Genetic Origin and Grafting-Mediated Stress Tolerance of *Malus prunifolia*

Zhongxing Li[1,†], Lun Wang[1,†], Jieqiang He[1,†], Xuewei Li[1,†], Nan Hou[1], Junxing Guo[1], Shengjun Liu[2], Jidi Xu[1], Mickael Malnoy[3], Cecilia Deng[4], Xiangqiang Zhan[1], Yang Dong[5,6], Michitaka Notaguchi[7], Fengwang Ma[1,*], Qiang Xu[2,*], Qingmei Guan[1,*]

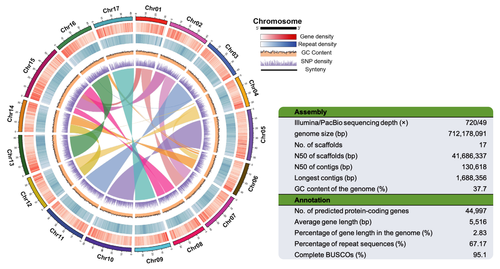
[1] State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, China
[2] Key Laboratory of Horticultural Plant Biology (Ministry of Education), Huazhong Agricultural University, Wuhan 430070, China
[3] Department of Genomics and Biology of Fruit Crops, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige 38010, Italy
[4] The New Zealand Institute for Plant and Food Research Limited, Auckland 1142, New Zealand

Background

Malus prunifolia 'Fupingqiuzi' is a unique germplasm with strong tolerance to stress environments and even affects tolerance of scion after grafted. It is widely used as an apple rootstock in China. Despite its importance for the improvement of cultivated apple, its genetic origin and evolutionary history are largely unknown, as are the mechanisms that underlie its acquisition and transmission of stress tolerance.

Results

I. Genome Sequencing, Assembly, and Annotation



Assembly	
Illumina/PacBio sequencing depth (×)	720/49
genome size (bp)	712,178,091
No. of scaffolds	17
N50 of scaffolds (bp)	41,686,337
N50 of contigs (bp)	130,618
Longest contigs (bp)	1,688,356
GC content of the genome (%)	37.7
Annotation	
No. of predicted protein-coding genes	44,997
Average gene length (bp)	5,516
Percentage of gene length in the genome (%)	2.83
Percentage of repeat sequences (%)	67.17
Complete BUSCOs (%)	95.1

Tab. 1 Summary of genome assembly and annotation for the M. prunifolia 'Fupingqiuzi'.

Fig. 1 Genome features of M. prunifolia 'Fupingqiuzi'. Circular representation of the chromosome karyotype, gene density, repeat density, GC content, SNP density, and synteny blocks in the *M. prunifolia* genome.

II. Genome Evolution

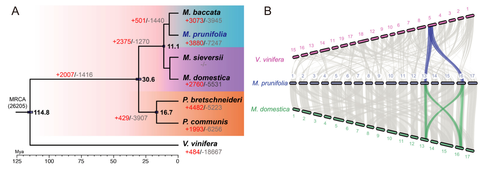


Fig. 2 Genomic evolution of *M. prunifolia*. (**A**) Construction of a phylogenetic tree and estimation of divergence times (Mya, black numbers) of *M. prunifolia* and six other plant species. Expansions (red numbers) and contractions (grey numbers) of gene families among six plant species were shown. The number at the root (26,205) denotes the total number of gene families predicted in the most recent common ancestor (MRCA). (**B**) Synteny blocks of *M. prunifolia*, *M.* × *domestica* , and *Vitis vinifera*. Blue lines represent one-to-two synteny blocks between *V. vinifera* and *M. prunifolia*, and green lines indicate one-to-one synteny blocks between *M. prunifolia* and *M.* × *domestica*.

III. Genetic origin of *M. prunifolia*

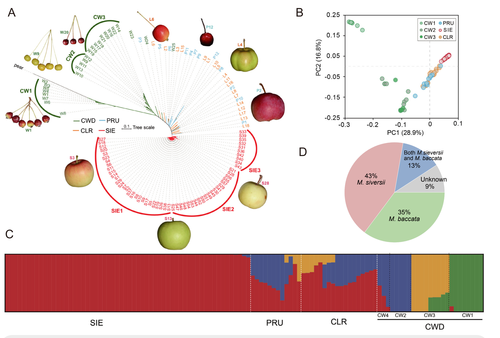


Fig. 3 Genetic structure analysis of *M. prunifolia*. (**A**) Neighbor-joining (NJ) phylogenetic tree constructed using SNPs at four-fold degenerate sites (4D SNPs). Each accession group, including *M. prunifolia* accessions (PRU), Chinese wild accessions (CWD), Chinese landrace accessions (CLR), and *M. sieversii* accessions (SIE) is color coded. (**B**) Principal component analysis (PCA) of all 113 apple accessions. (**C**) Admixture analysis of all apple accessions. The length of each colored segment represents the proportion of the individual genome inferred from ancestral populations (K=4). (**D**) Summary of the genomic contributions of the two major ancestors of 'Fupingqiuzi'.

IV. Graft-mediated resistance phenotypes of *M. prunifolia*

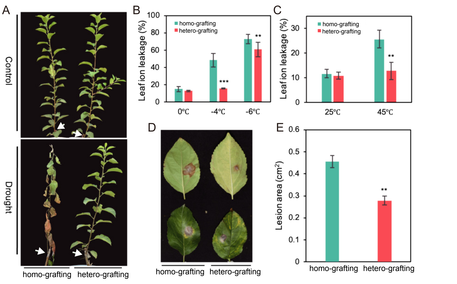


Fig. 4 Stress tolerance of *M. domestica* 'Golden Delicious' grafted onto *M. dometica* 'Golden Delicious' or *M. prunifolia* 'Fupingqiuzi'. (**A**) Drought stress tolerance. White arrows indicate the graft unions. (**B**) Freezing tolerance. (**C**) Thermotolerance. (**D**) Disease resistance (inoculated with *Alternaria alternata f. sp. mali*) (**E**) Quantitative data in (D). Data are means ± SD [*n*=10 in (A), (B) and (C); n = 15 in (D) and (E)]. Student's t-test was performed, and statistically significant differences are indicated by **P* < 0.05, ***P* < 0.01, or ****P* < 0.001.

V. DNA methylation and transcription changes in *M. domestica* after grafting onto *M. prunifolia*

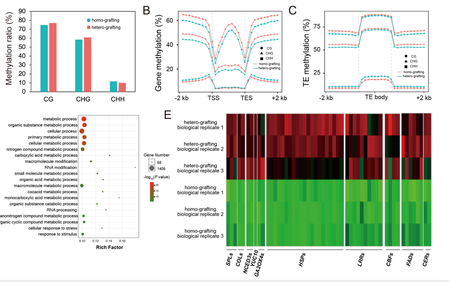


Fig. 5 Grafting-mediated DNA methylation and gene transcription in the *M. domestica* 'Golden Delicious' scion. (**A**) Relative proportions of mCs (methylated cytosines) in three sequence contexts (CG, CHG, and CHH) in homo-grafting and hetero-grafting. (**B**) Global distribution of DNA methylation levels among gene coding regions and their 2-kb upstream and downstream regions. (**C**) Percentage of methylation levels among TE regions and its 2-kb upstream and downstream sequences. (**D**) Gene ontology (GO) enrichment analysis of differentially methylated genes (DMGs) in hetero-grafted 'Golden Delicious' compared to homo-grafted 'Golden Delicious' . (**E**) Heatmap showing the expression levels of selected genes related to flowering, hormone biosynthesis, and stress response in homo-grafting and hetero-grafting.

VI. Grafting-mediated DNA methylome and transcriptome in response to drought stress

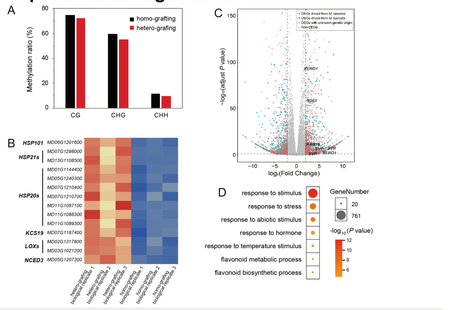


Fig. 6 Landscape of grafting-mediated changes in scion and rootstock under drought conditions. (**A**) Relative proportions of three mCs (methylated cytosines) sequence contexts in homo-grafted and hetero-grafted 'Golden Delicious' under drought conditions. (**B**) Heatmap of selected differentially expressed genes (DEGs) related to molecular chaperones, lipoxygenase, biosynthesis of cuticular wax and ABA in homo-grafted and hetero-grafted 'Golden Delicious' under drought conditions. (**C**) Volcano diagram showing DEGs (adjust *P* value<0.05; $|\log_2(\text{Fold Change})|>2$) in hetero-grafted *M. prunifolia* compared to homo-grafted *M. prunifolia* and their genetic origins from *M. sieversii* and *M. baccata* under drought conditions. (**D**) Gene ontology (GO) terms of enriched DEGs in hetero-grafted *M. prunifolia* in response to drought stress.

Conclusion

Acknowledgement

National Key Research and Development Program of China (2018YFD1000100)
National Natural Science Foundation of China (31872080)

In this study, we assembled a 712-Mb high-quality, chromosome-scale *M. prunifolia* genome and analyze sequences of 113 *Malus* accessions. We clarify the two major gene flows of *M. prunifolia*: *M. sieverii* and *M. baccata*. In addition, scions grafted onto *M. prunifolia* show increased tolerance to drought, cold, heat stress, and pathogen attack. Differentially methylated regions (DMRs) and differentially expressed genes (DEGs) under control and drought stress conditions are enriched in stress response in the scion. Notably, 1,217 and 1,147 drought-responsive DEGs are contributed from *M. sieverii* and *M. baccata* in response to drought stress, respectively. Some of these DEGs are related to biosynthesis of ABA and cuticular wax, root growth, oxidoreductase, and molecular chaperone. Moreover, mobile mRNAs that move from rootstock to scion under control and drought stress encode key regulatory genes associated with stress tolerance. Together, these findings provide insights into genetic origin of Chinese originated crabapples and lay the foundation for better understanding the grafting-mediated stress tolerance.