Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2022) **20**, pp. 1015–1017 doi: 10.1111/pbi.13817

Brief Communication

Chromosome-scale reference genome provides insights into the genetic origin and grafting-mediated stress tolerance of *Malus prunifolia*

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Received 18 January 2022;
revised 27 February 2022;
accepted 15 March 2022.
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Keywords: apple rootstock, genome, genetic origin, stress response, grafting.

Grafting, an ancient horticultural cultivation technique, is essential for commercial apple (*Malus* × *domestica* Borkh.) production. *M. prunifolia* 'Fupingqiuzi' is used as a common apple rootstock due to its known tolerances to abiotic and biotic stresses. However, the genetic origin and evolutionary history of *M. prunifolia* are largely unknown, as are the molecular bases underlying its innate stress tolerance characteristics and ability to graft-transmit stress tolerance to the scion.

Here, we present a high-quality chromosome-scale genome sequence of *M. prunifolia* 'Fupingqiuzi' ($2n = 2x = 34$) assembled using a combined strategy of Illumina short reads, PacBio long reads, and Hi-C data (Figure S1, Tables S1-S3). The total length of the assembly was 712 Mb, with a scaffold N50 of 41.6 Mb (Figure 1a). Approximately 98.6% of the contigs were anchored into 17 pseudochromosomes (Figure 1b). Over 91.36% of non-redundant and single-mapped mate-pair reads were concordant pairs, indicating that Hi-C anchoring has high accuracy. BUSCO revealed a completeness rate of 95.3% (Table S4). A total of 44 997 protein-coding genes were predicted, with an average gene length of 5516 bp. In addition, 1785 non-coding RNA genes and 484 Mb of repetitive sequences were detected in the 'Fupingqiuzi' genome (Figure 1a, Tables S5-S6).

Gene family analysis identified 3880 expanded gene families in *M. prunifolia* (Figure 1c), which were enriched mainly in response to stress, bacterium, abscisic acid (ABA), immune response, and plant–pathogen interaction (Table S7, Data S1), with 56 homologs of *HSP* and 69 homologs of *LRR* were expanded significantly (Table S8).

To explore the genetic origin of the *M. prunifolia*, we sequenced 79 *Malus* accessions (Chen *et al.*, 2021) and downloaded 34 *Malus* resequencing data sets (Duan *et al.*, 2017), including 12 *M. prunifolia*, 58 *M. sieversii*, 18 Chinese landrace, and 25 Chinese wild *Malus* accessions (Table S9). In total, 931.35 Gb of whole-genome sequencing data were obtained, and 14 073 781 SNPs were identified (Figure 1b). Phylogenetic and principal component analysis (PCA) showed that *M. prunifolia* and Chinese landrace accessions clustered together (Figure 1d-e), demonstrating that their genetic structures are very similar and *M. prunifolia* is paraphyly. Genetic structure of *M. prunifolia* accessions exhibited an admixture pattern, compared with *M. sieversii* and *M.* Sect. *Baccatus* (Figure 1f, Figures S2-S4). *D*-statistics analysis of *M. prunifolia* and the other groups showed that *M.* Sect. *Baccatus* shared more alleles with *M. prunifolia* (Figure 1g, Table S10). Furthermore, f_3 statistics illustrated that the *M. prunifolia* had a closer genetic affinity with *M. sieversii* (Figure 1h). These results indicate that *M. prunifolia* has a hybrid origin between *M. sieversii* and *M.* Sect. *Baccatus*.

To further confirm the hybrid origin of *M. prunifolia*, we performed inference of demographic history. The divergence times of *M. prunifolia* versus *M. sieversii* and *M. prunifolia* versus *M.* Sect. *Baccatus* were ~49 614 (95% confidence interval: 37 589–57 888) and ~49 746 (95% CI: 43 526–56 967) years before present, respectively, as estimated by the Multiple Sequentially Markovian Coalescent 2 (MSMC2) (Figure 1i). The two divergence times were almost identical, implying that *M. prunifolia* accessions have hybrid origin. Moreover, the simulation of diffusion approximations for demographic inference (∂a∂i) showed that the hybrid origin model was the best-fitting model (Figure S5, Table S11). The estimated hybrid parameter (f) indicated that ~78% of the nuclear genome of the initial *M. prunifolia* came from *M. sieversii*, and ~22% from *M.* Sect. *Baccatus*, consistent with genetic admixture. Speciation time of

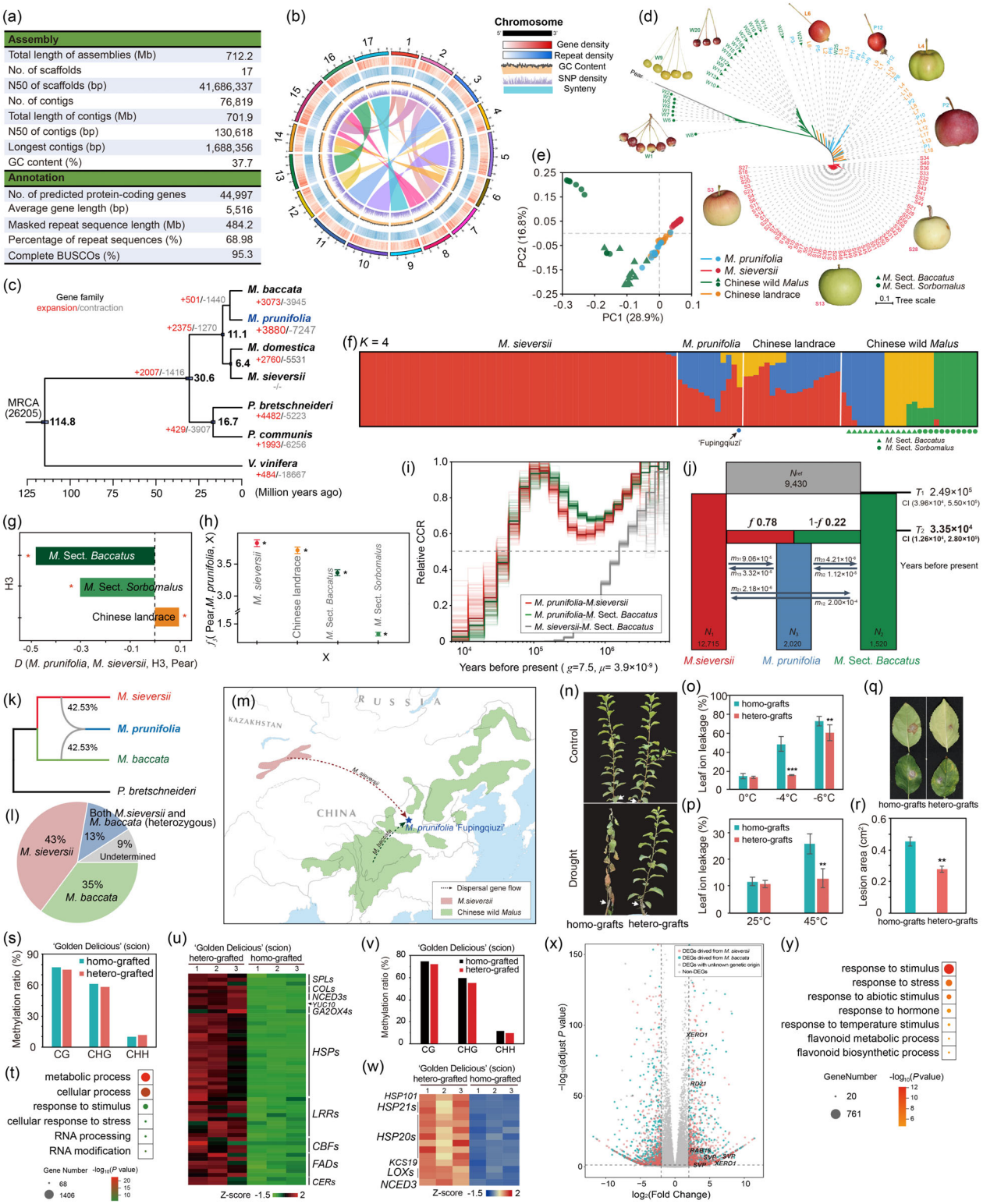

Figure 1 (**a**, **b**) Genome assembly, annotation (a), and features (b) of *M. prunifolia* 'Fupingqiuzi'. (**c**) Gene family analysis. (**d**-**f**) Phylogenetic tree (d), PCA (e), and admixture analysis (f) of all 113 *Malus* accessions. (**g**, **h**) *D* and f_3 statistics analyses. Error bars represent SE. (**i**) Inferred relative cross-coalescence rate among *Malus* populations. (**j**) Simulation results of the best ∂a∂i model. (**k**) ML-bootstrap network. (**l**, **m**) Genetic contribution of the two main ancestors to 'Fupingqiuzi'. (**n**) Drought, (o) Freezing, (**p**) Thermotolerance, and (**q**, **r**) Disease resistance of homo- and hetero-grafts. Data are means ± SD. (**s**, **v**) Relative proportions of mCs (methylated cytosines) in homo- and hetero-grafted 'Golden Delicious' under control (s) and drought conditions (v). (**t**) GO enrichment analysis of DMR-related genes between homo- and hetero-grafted 'Golden Delicious'. (**u**, **w**) Expression of DEGs between homo- and hetero-grafted 'Golden Delicious' under (u) control and drought conditions (w). (**x**, **y**) Genetic origins (x) and functional enrichment (y) of DEGs in hetero-grafted 'Fupingqiuzi' in response to drought stress. Three biological replicates were prepared for each condition in (s-y).

M. prunifolia was dated to ~33 500 years before present, close to the results by MSMC2 (Figure 1j, Figure S6, and Table S12).

Phylogenetic networks from the 2756 orthologous single-copy gene tree topologies showed reticulately evolutionary relationships among 'Fupingqiuzi', *M. sieversii*, and *M. baccata* genomes (Figure 1k), indicating a hybrid origin for 'Fupingqiuzi'. A window-based genetic distance approach also showed that approximately 43% of the *M. prunifolia* genome was derived from *M. sieversii* and 35% derived from *M. baccata* (Figure 1l), further supporting the hybrid origin of 'Fupingqiuzi' from ancient admixture of *M. sieversii* and *M. baccata* (Figure 1m, Figure S7, Table S13).

To investigate the influence of 'Fupingqiuzi' (as rootstocks) on stress resistance of scion, we generated homo-grafts (*M.* × *domestica* 'Golden Delicious' grafted onto itself) and hetero-grafts ('Golden Delicious' grafted onto 'Fupingqiuzi'). We found that compared with homo-grafts, hetero-grafts were more tolerant to drought, extreme temperatures, and pathogen infection (Figure 1n-r).

To reveal the molecular basis of grafting-mediated stress tolerance, we analysed the genome-wide DNA methylation and gene expression of homo- and hetero-grafted 'Golden Delicious'. Compared with homo-grafted 'Golden Delicious', CG and CHG methylation (H = adenine, thymine, or cytosine) were higher, whereas CHH was lower in the hetero-grafted 'Golden Delicious' but not to a significant level (Figure 1s, Figure S8). Differentially methylated regions (DMRs) analysis between hetero- and homo-grafted 'Golden Delicious' identified 4002 DMRs and 3188 DMR-related genes, with their encoded proteins significantly enriched in terms of 'cellular response to stress' (Figure 1t, Data S2-S3). Among the DMR-related genes, 652 genes were involved in the environmental adaptation, and some genes were related to flowering and ABA biosynthesis (Data S2). In addition, compared with homo-grafts, hetero-grafts exhibited 2750 differentially expressed genes (DEGs) in scions. Among these DEGs, we identified flowering-related genes including homologs of *SPL4*, *SPL13*, *CONSTANS-LIKE 2* (*COL2*), and genes related to the biosynthesis of hormones including homologs of *NCED3*, *YUC10*, *GA2OX6,* and *GA2OX1* (Data S4). In addition, the up-regulated genes contained stress-responsive genes, including homologs of *HSPs*, *LRRs*, *CBFs*, *FADs*, and cuticular wax biosynthesis genes (Figure 1u). DNA methylation modifies chromatin structure and thereby affects the transcriptional regulation of genes (Zhang *et al*., 2018). Among the DMR-associated DEGs, genes related to flowering and stress adaptation in hetero-grafted 'Golden Delicious', including homologs of *FAD4*, *SPL5*, *LRR*, and *DJC76*.

To further investigate the response of grafting combination to stress tolerance, we performed drought stress treatment on homo- and hetero-grafted 'Golden Delicious'. Genome-wide methylation levels were reduced in all CG, CHG, and CHH sequence contexts in hetero-grafted 'Golden Delicious' relative to homo-grafted 'Golden Delicious' under drought conditions (Figure 1v, Figure S9). We identified a total of 2512 DMR-related genes, which were mainly related to stimulus (Figure S9, Data S5-S6). Moreover, there are 497 DEGs in hetero-grafted 'Golden Delicious' compared with homo-grafted 'Golden Delicious', which are strongly associated with abiotic stresses (Figure S9, Data S7-S8). Notably, we found that genes related to molecular chaperones, biosynthesis of cuticular wax and ABA, and lipoxygenase were highly expressed in hetero-grafted 'Golden Delicious', these included homologs of *HSP*, *KCS19*, *LOX*, and *NCED3* (Figure 1w).

We also sequenced the transcriptomes of homo- and hetero-grafted 'Fupingqiuzi' (rootstocks) under drought treatment. A total of 3102 DEGs were significantly enriched to the abiotic stresses (Figure 1x-y, Data S9 and S10). More importantly, 1271 and 1147 DEGs were derived from *M. sieversii* and *M. baccata,* respectively (Figure 1x). Notably, DEGs derived from *M. sieversii* including homologs of *XERO1* and *RAB18* were up-regulated whereas expression of genes derived from *M. baccata* including homologs of *RD21* and *SVP* was increased in 'Fupingqiuzi' under drought stress, suggesting the different gene contributions of *M. sieversii* and *M. baccata* to the 'Fupingqiuzi' (Figure 1x, Data S11).

In summary, these findings provide insights into the genetic origin of Chinese-originated crabapples and lay the foundation for a better understanding grafting-mediated stress tolerance.

Acknowledgement

We thank the National Key Research and Development Program of China (2018YFD1000100) for funding resources.

Conflict of interest

No conflict of interest was declared.

Author contributions

Q.G., Q.X., and F.M. conceived the study. Other authors carried out experiments and analyses. Z.L. and J.H. wrote the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1-S11 Supplementary Figures.
Data S1-S11 Supplementary Datasets.
Methods S1 Supplementary Methods.
Table S1-S13 Supplementary Tables.