# Genome-wide association study of twenty-three flowering phenology traits and four floral agronomic traits in tree peony (Paeonia section Moutan DC.) reveals five genes known to regulate flowering time

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Abstract

Tree peony is a unique traditional flower in China, with large, fragrant, and colorful flowers. However, a relatively short and concentrated flowering period limits the applications and production of tree peony. A genome-wide association study (GWAS) was conducted to accelerate molecular breeding for the improvement of flowering phenology traits and ornamental phenotypes in tree peony. A diverse panel of 451 tree peony accessions was phenotyped for 23 flowering phenology traits and 4 floral agronomic traits over three years. Genotyping by sequencing (GBS) was used to obtain a large number of genome-wide SNPs (107 050) for the panel genotypes, and 1 047 candidate genes were identified by association mapping. Eighty-two related genes were observed during at least two years for flowering, and 7 SNPs repeatedly identified for multiple flowering phenology traits over multiple years were highly significantly associated with 5 genes known to regulate flowering time. We validated the temporal expression profiles of these candidate genes and highlighted their possible roles in the regulation of flower bud differentiation and flowering time in tree peony. This study shows that GWAS based on GBS can be used to identify the genetic determinants of complex traits in tree peony. The results expand our understanding of flowering time control in perennial woody plants. Identification of markers closely related to these flowering phenology traits can be used in tree peony breeding programs for important agronomic traits.
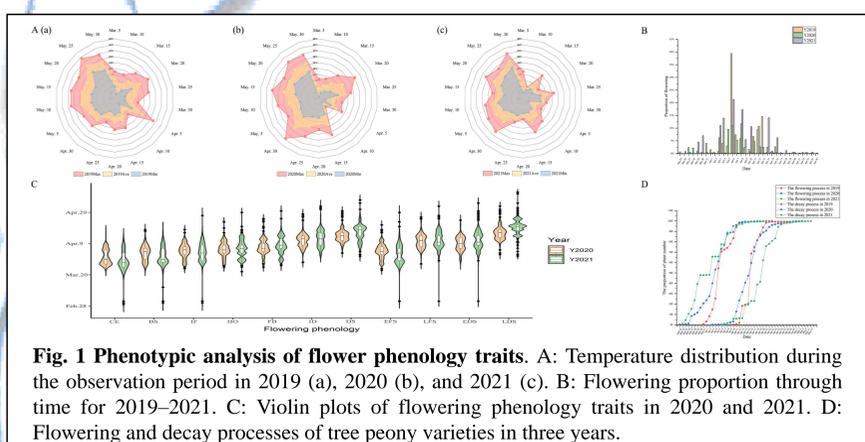

Fig. 1 Phenotypic analysis of flower phenology traits. A: Temperature distribution during the observation period in 2019 (a), 2020 (b), and 2021 (c). B: Flowering proportion through time for 2019–2021. C: Violin plots of flowering phenology traits in 2020 and 2021. D: Flowering and decay processes of tree peony varieties in three years.
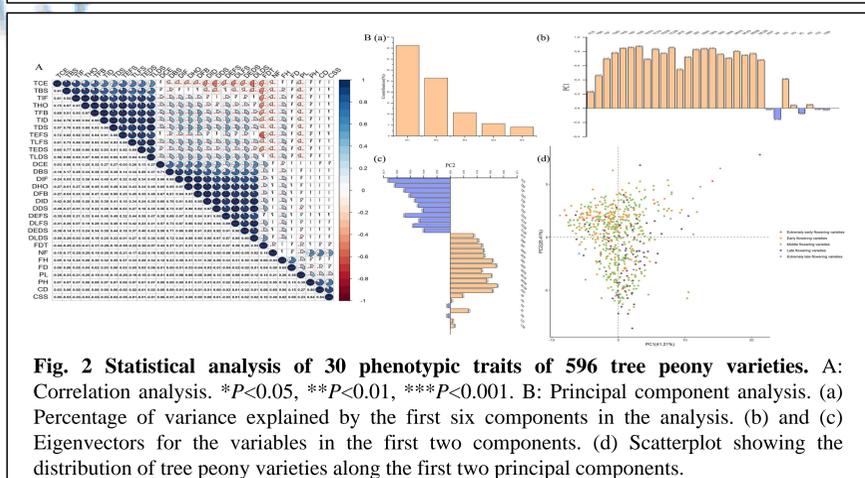

Fig. 2 Statistical analysis of 30 phenotypic traits of 596 tree peony varieties. A: Correlation analysis. *$P<0.05$, **$P<0.01$, ***$P<0.001$. B: Principal component analysis. (a) Percentage of variance explained by the first six components in the analysis. (b) and (c) Eigenvectors for the variables in the first two components. (d) Scatterplot showing the distribution of tree peony varieties along the first two principal components.
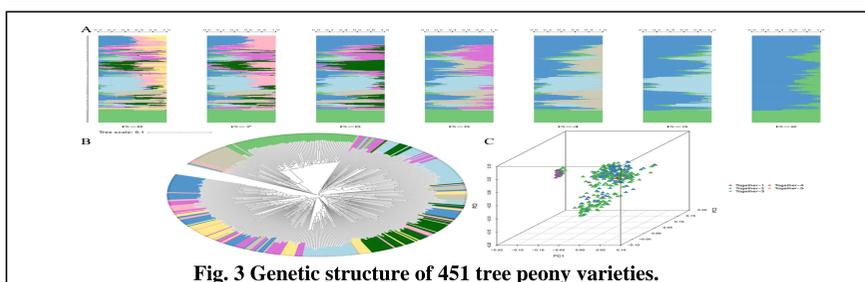

Fig. 3 Genetic structure of 451 tree peony varieties.
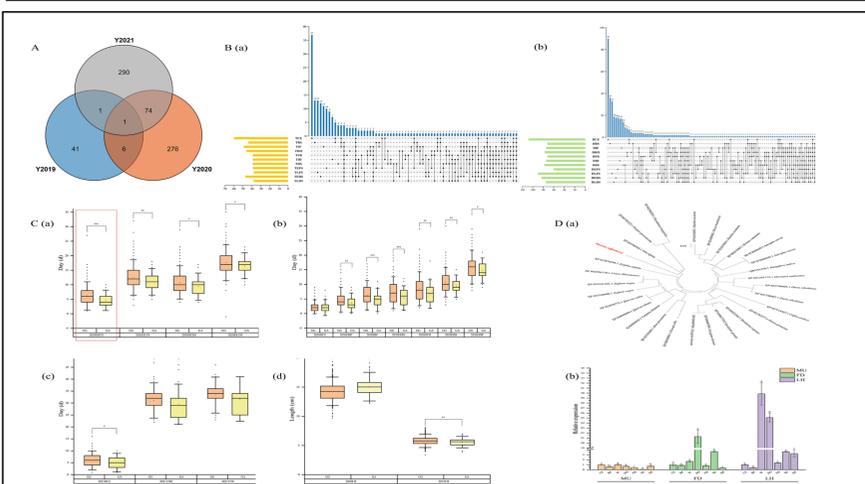
Fig. 4 Profile of loci associated with flowering-related traits of the tree peony population over three years. A: Venn diagram of loci associated with 23 traits over three years (2019–2021). B: (a) Bar chart of the associations of 11 flowering phenology timing-related traits and their interactive upset plot. (b) Bar chart of the associations of 11 flowering phenology duration-related traits and their interactive upset plot. C: The allelic effect at 059892F:42007 for the significantly associated target traits in different years ($P < 0.05$) in the association panel. D: The candidate gene *PoFY* was analyzed by evolutionary tree (a) and its expression profile during flowering (b).
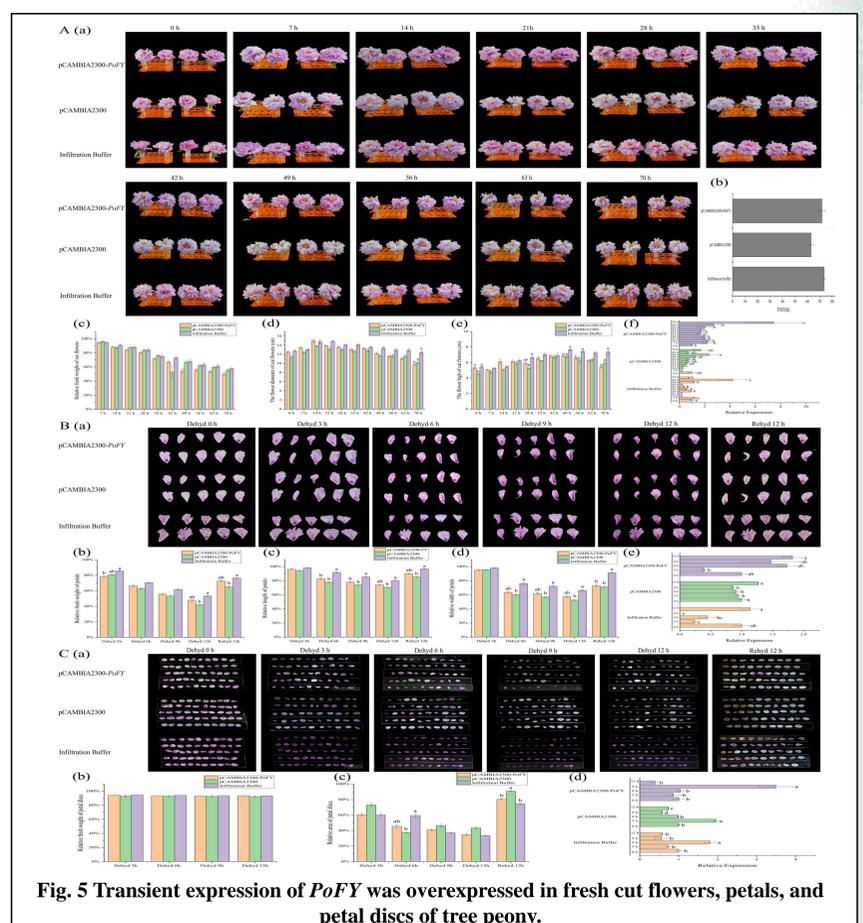
Fig. 5 Transient expression of *PoFY* was overexpressed in fresh cut flowers, petals, and petal discs of tree peony.
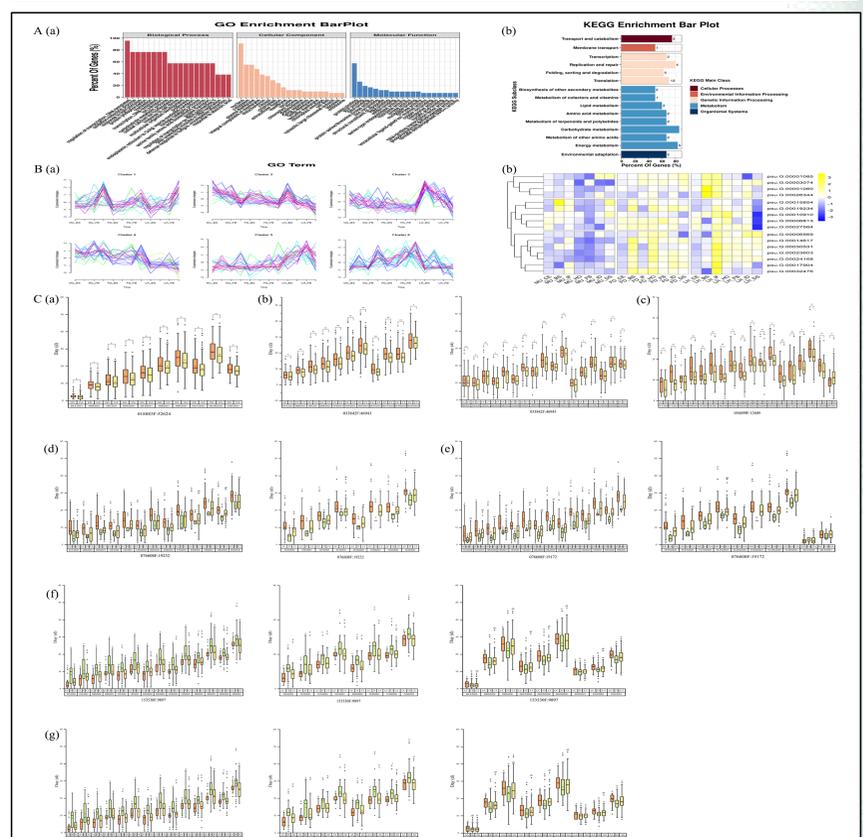
Fig. 6 Integration of GWAS analysis with transcriptome sequencing. A: GO (a) and KEGG (b) enrichment of 200 candidate genes. B: Cluster profiles of differential gene expression (a) and the expression profiles of selected gene candidates during the flowering process (b). C: Allelic effects at seven candidate loci for the significantly associated target trait in different years ($P < 0.05$).