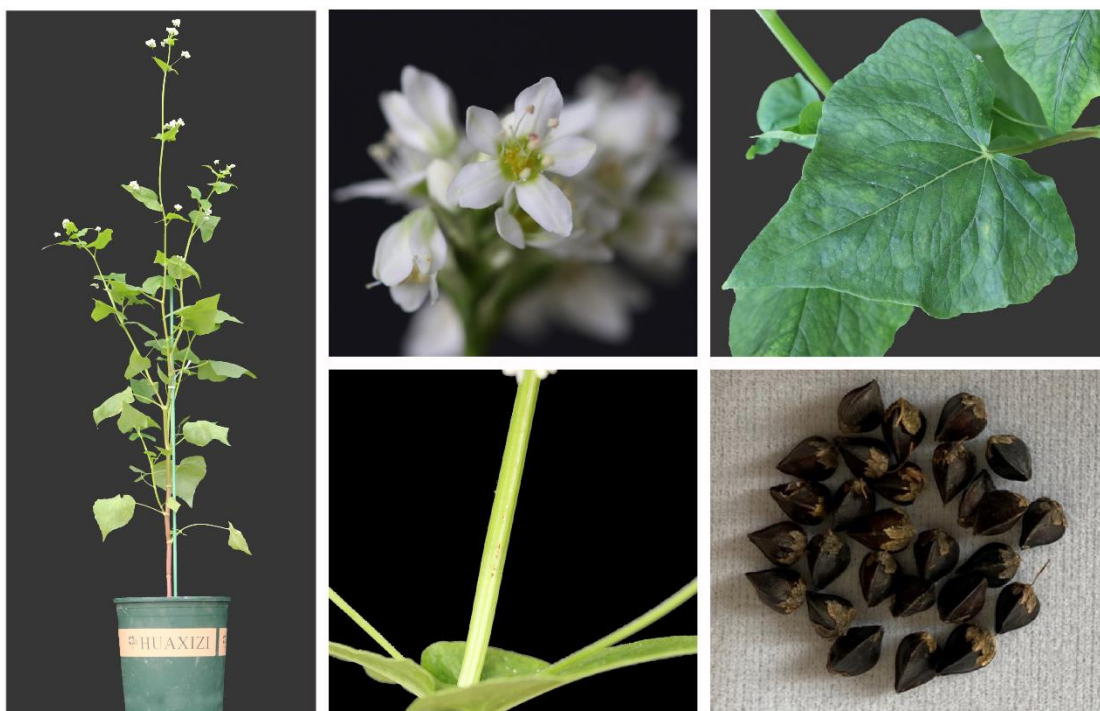
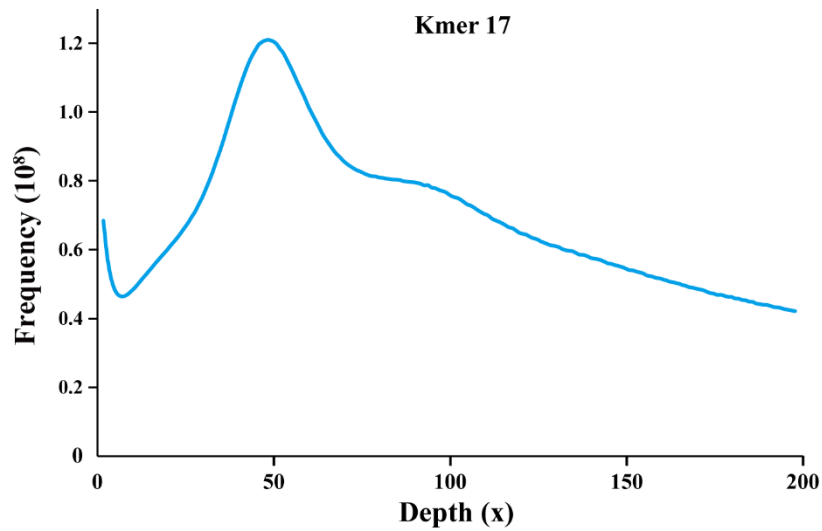


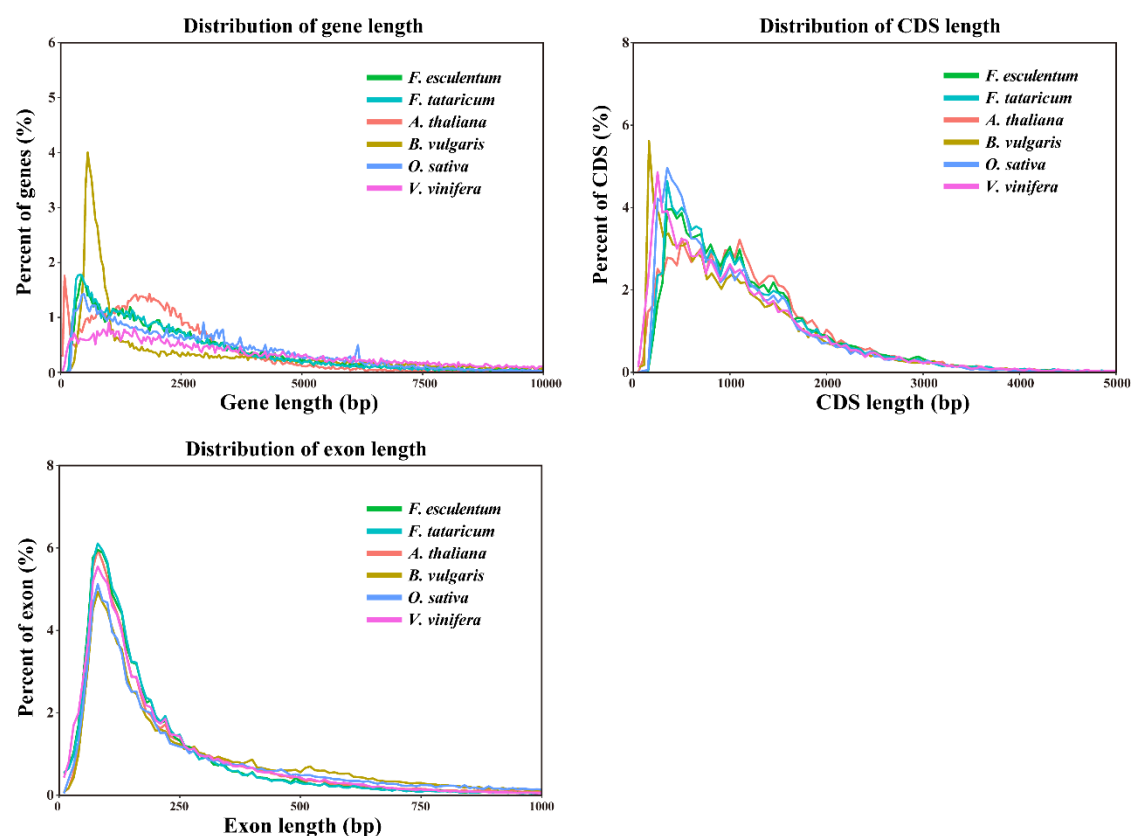


Supplemental Figure 1. Morphology of the plant, flower, stem, leaf, and seed of *F. esculentum*.

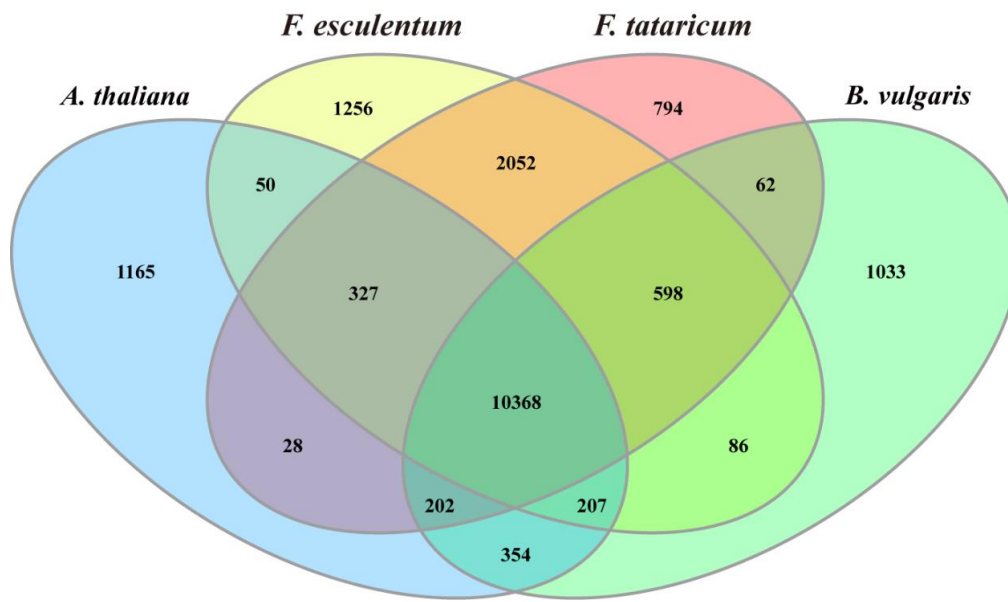


Supplemental Figure 2. Genome size and heterozygosity estimation using 17 K-mer distribution. The X-axis is depth; the Y-axis represents the frequency.

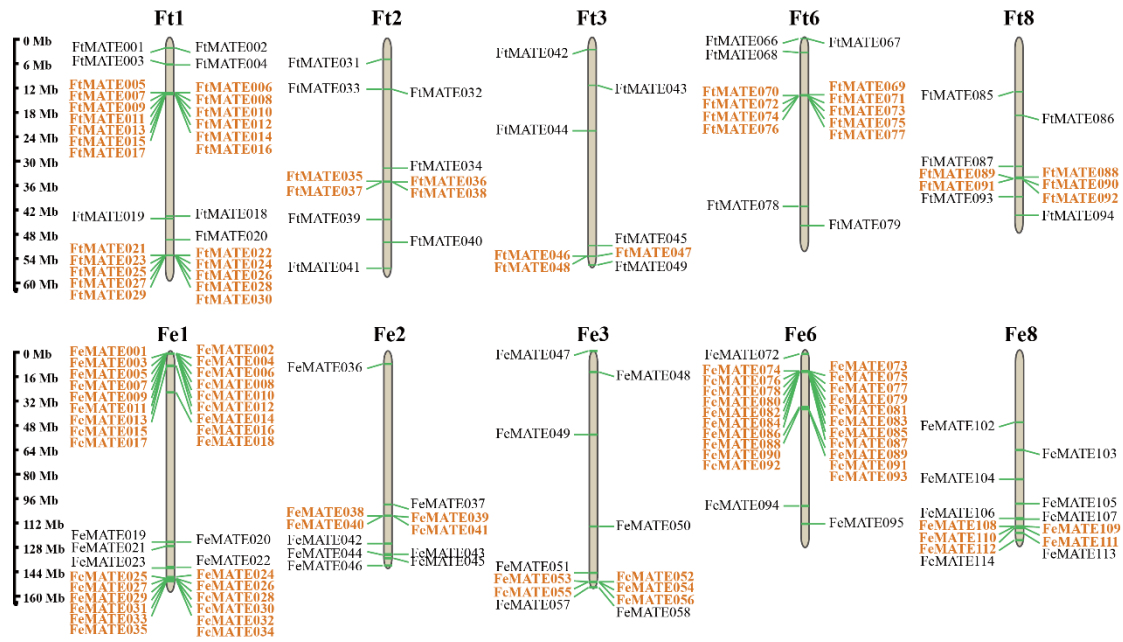
According to the distribution, we estimated that the genome size and heterozygous ratio of *F. esculentum* is approximately 1.28 Gb and 0.9%.



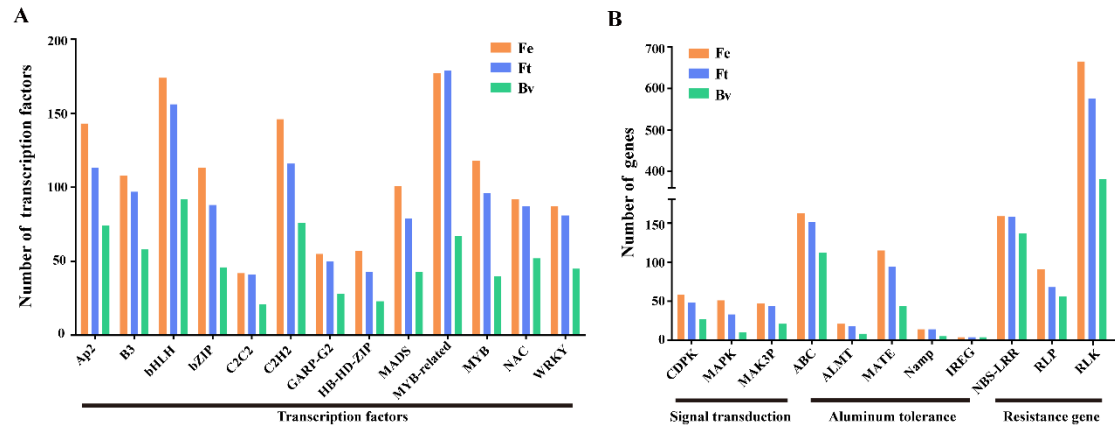
Supplemental Figure 3. Results of gene structure prediction. *F. esculentum* and *F. tataricum* compared with genetic elements of related species. Window refers to the length represented by each point on the horizontal coordinate.



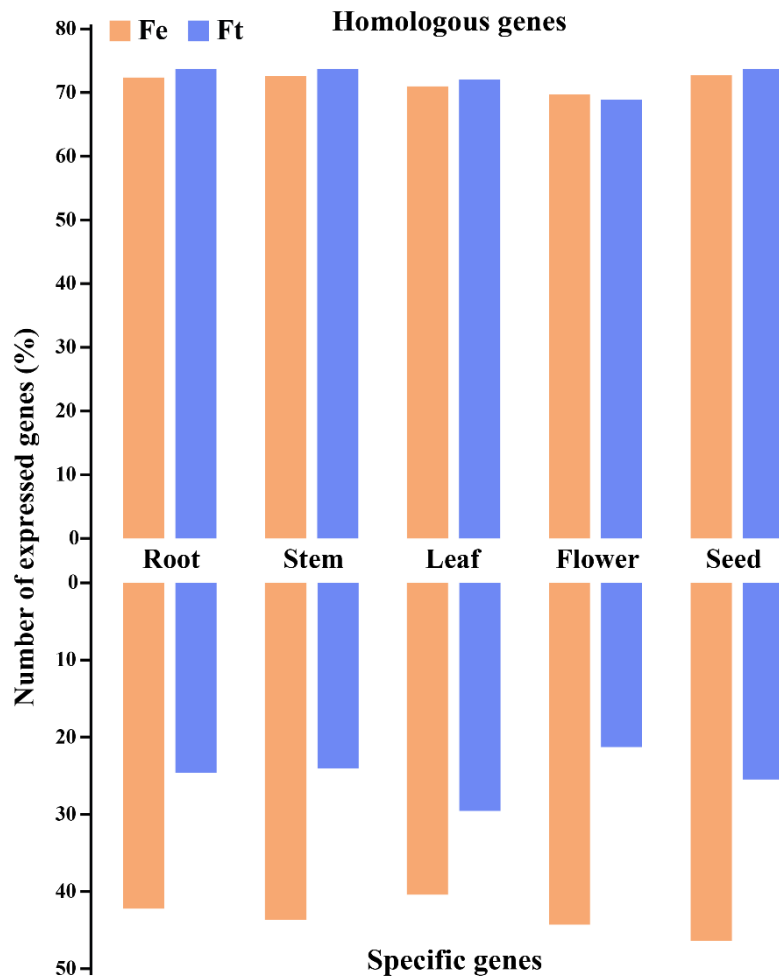
Supplemental Figure 4. Venn diagram shows the number of orthologous gene families in *F. esculentum*, *F. tataricum*, *A. thaliana* and *B. vulgaris*.



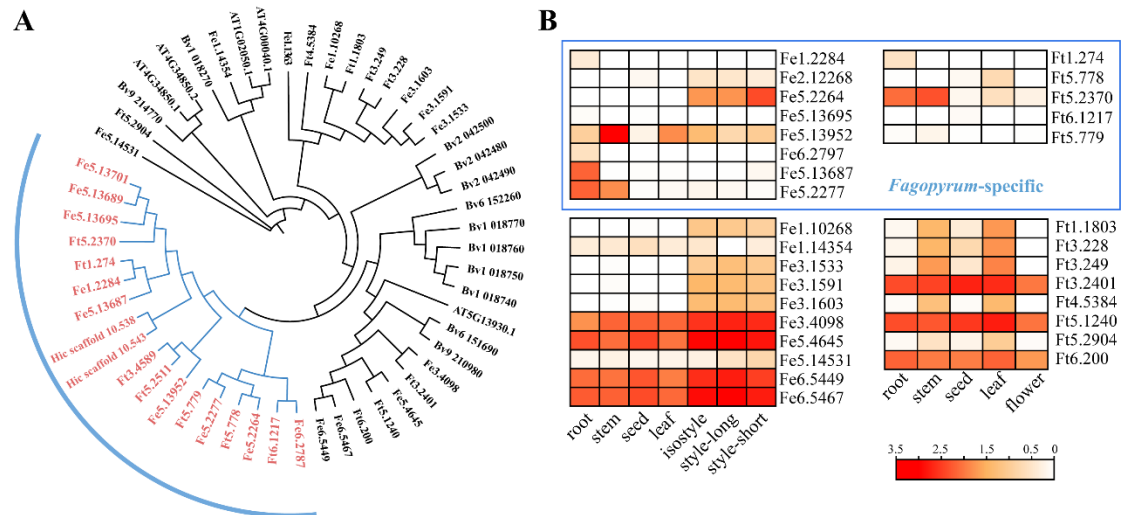
Supplemental Figure 5. Schematic representation of the chromosomal distribution of MATE family members in *F. esculentum* and *F. tataricum*. The genes marked in orange were generated by tandem duplication events.



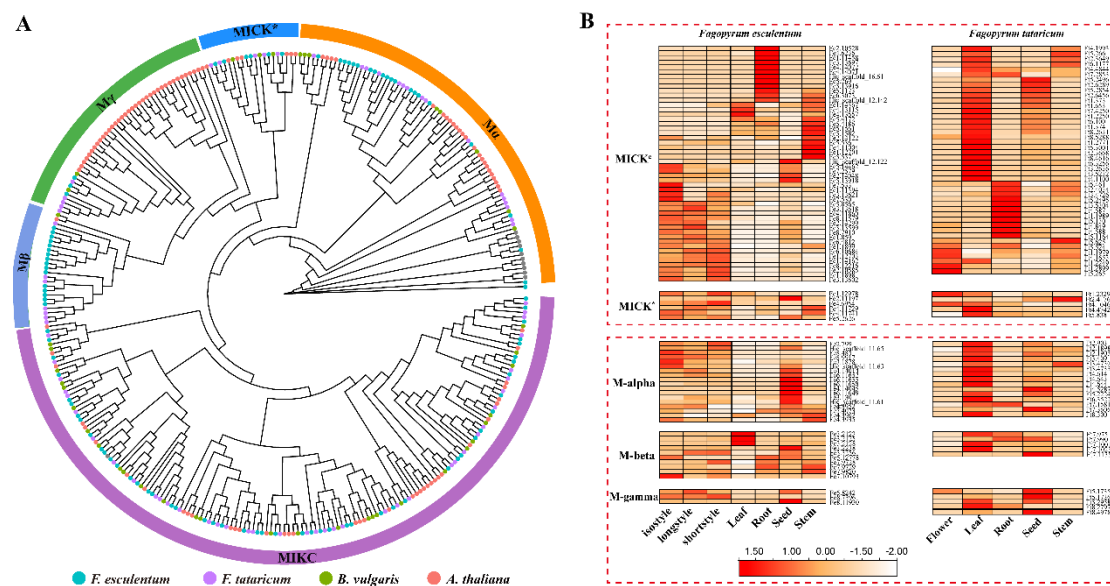
Supplemental Figure 6. The copy numbers of family genes derived from WGD events were analyzed among *F. esculentum*, *F. tataricum*, and *B. vulgaris*. A: Transcription factor family genes were analyzed. B: Stress tolerance-related genes were analyzed.



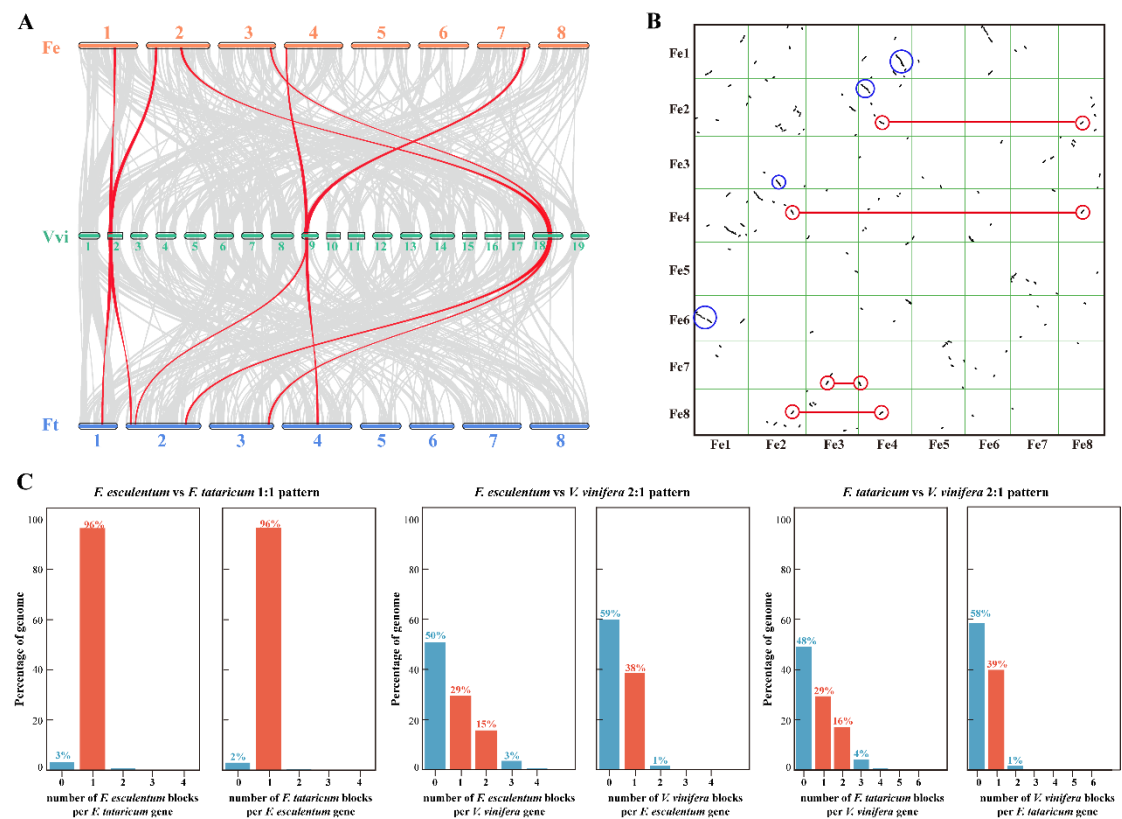
Supplemental Figure 7. Expression levels of homologous genes and specific genes in variant tissues. *F. esculentum* (Fe) and *F. tataricum* (Ft).



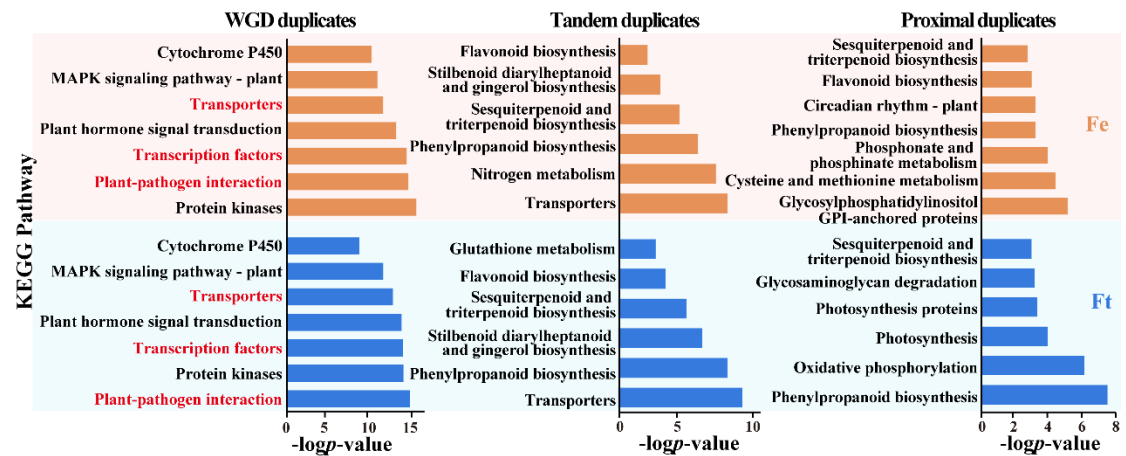
Supplemental Figure 8. The clustering and expression of CHS gene family. A: Neighbor-joining tree of Chalcone synthase (CHS) in four species. *Fagopyrum*-specific genes were shown in red. **B:** The expression level of CHS genes in different tissues of common and Tartary buckwheat. The blue rectangles represent the *Fagopyrum*-specific genes.



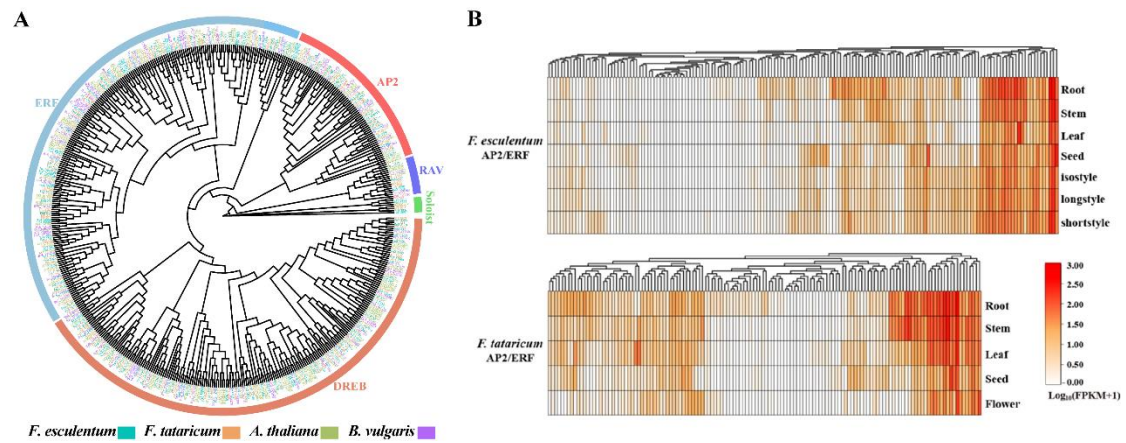
Supplemental Figure 9. Phylogenetic and expressive analyses of MADS-box gene family. A: Neighbor-joining tree of MADS-box genes in four species. B: The expression level of MADS-box genes in different tissues of common and Tartary buckwheat.



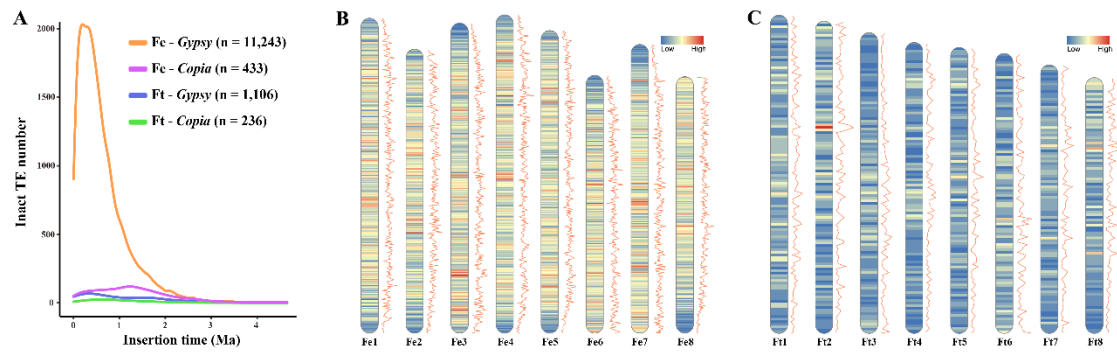
Supplemental Figure 10. The analysis of whole genome duplications. A: Synteny between the genomic regions of *Fagopyrum* species and *V. vinifera*. A collinear relationship is highlighted by presenting one syntenic set in red. B: Intra-genomic collinear plot of *F. esculentum*. C: The syntenic depth ratios between three species gene pairs.



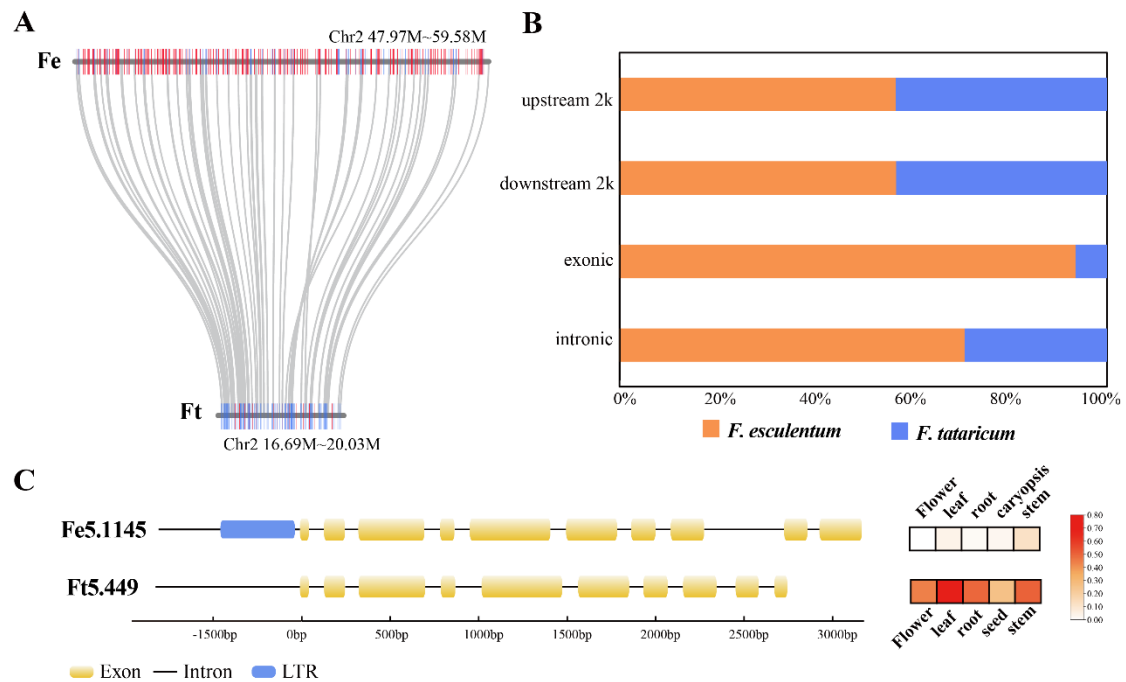
Supplemental Figure 11. KEGG enrichment analysis of genes derived from WGD duplicates, tandem duplicates, and proximal duplicates in *F. esculentum* and *F. tataricum*.



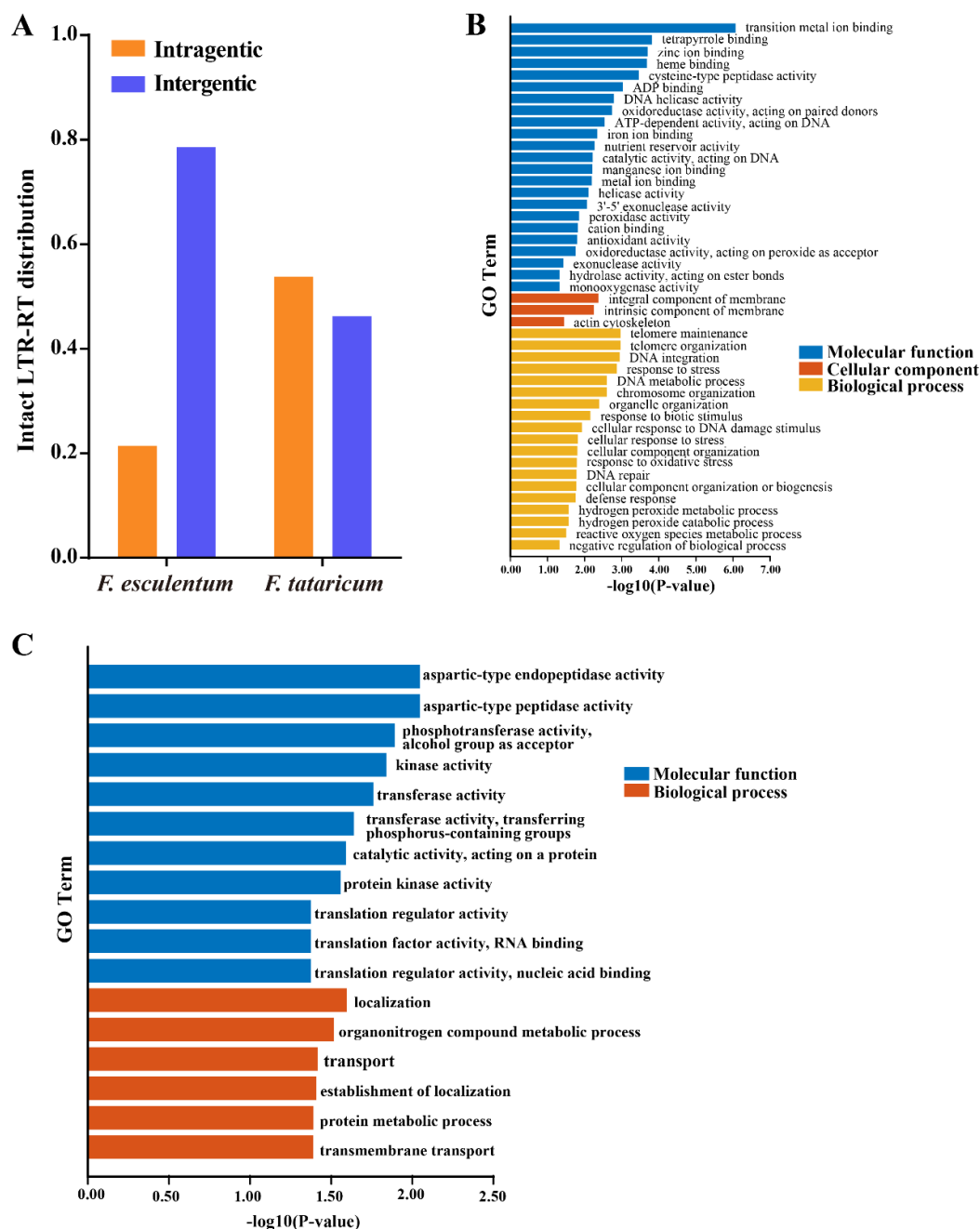
Supplemental Figure 12. Phylogenetic and expressive analyses of AP2 genes in *F. esculentum*, *F. tataricum*, and *B. vulgaris*. A: Neighbor-joining tree of AP2 genes in four species. B: The expression level of MADS-box genes in different tissues of common and Tartary buckwheat.



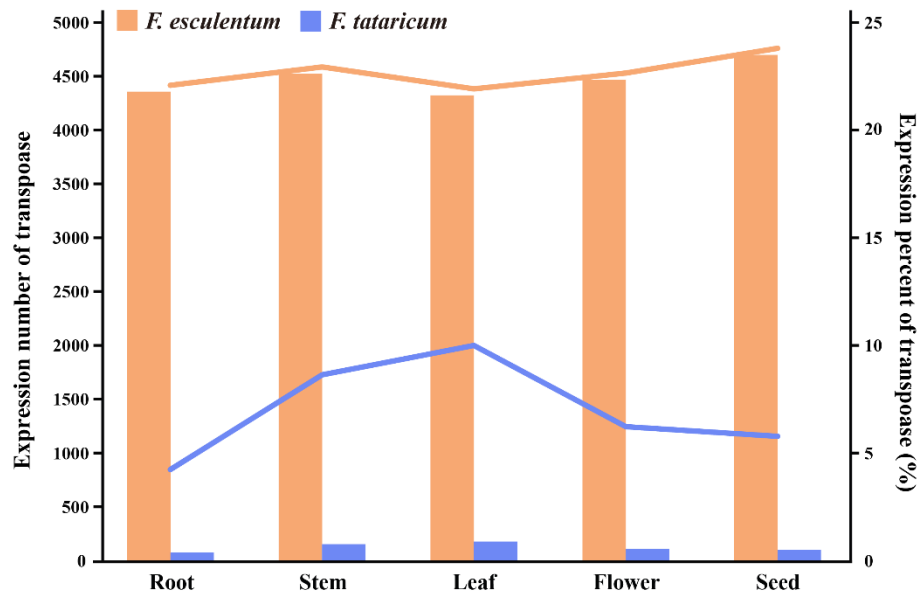
Supplemental Figure 13. The number and distribution of LTR in *F. esculentum* and *F. tataricum* genome. A: Insertion time of *Gypsy* and *Copia* elements in *F. esculentum* and *F. tataricum*. The numbers of LTR used for this analysis are provided in parentheses. B-C: The density of LTR in *F. esculentum* and *F. tataricum* genome (100 Kb window).



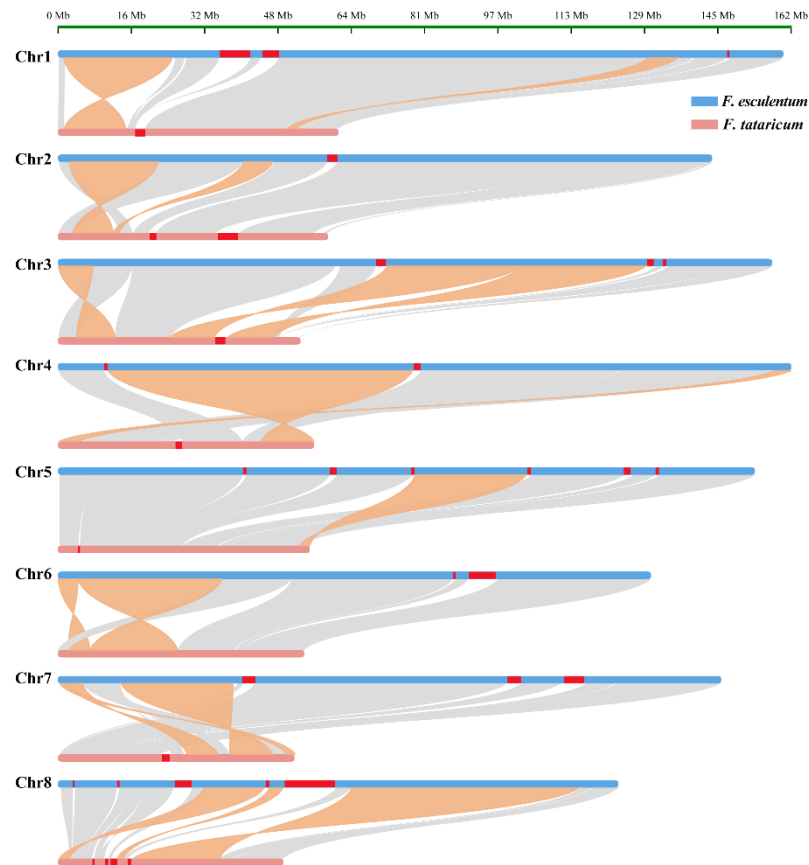
Supplemental Figure 14. The impacts of LTR on genome evolution. A: Microsynteny comparisons between *F. esculentum* and *F. tataricum* in Chr2. The red and blue lines represent intact LTR-RTs and genes, respectively. B: Number of genes with LTR in different genic regions of the *F. esculentum* and *F. tataricum* genomes. C: The homologous genes with different gene structures and expression levels possess different LTR insertion at the gene 2 kb regions upstream in common and Tartary buckwheat.



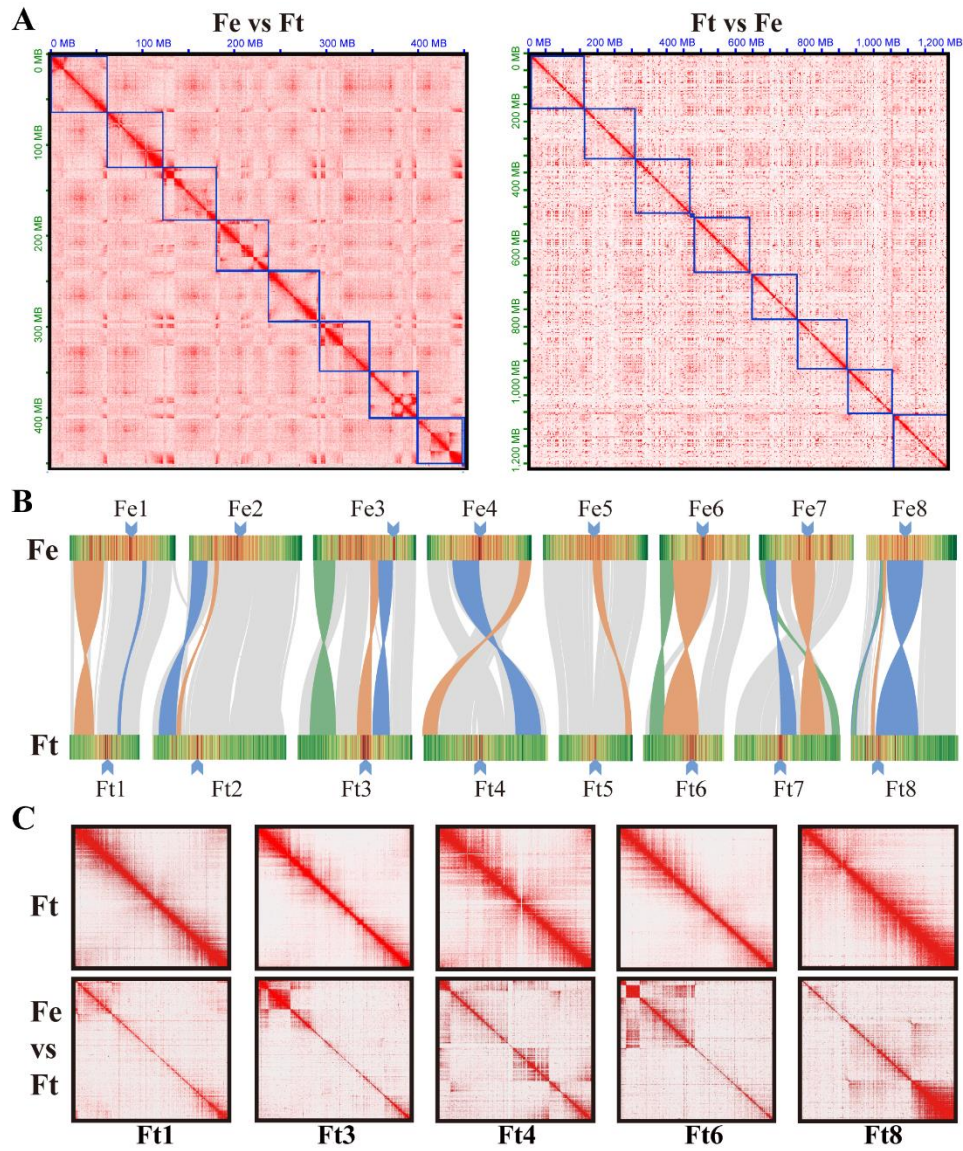
Supplemental Figure 15. GO enrichment of genes associated with LTR. A: The distribution of intact LTR-RT in *F. esculentum* and *F. tataricum* genome. B: GO enrichment analysis of the genes with LTR insertion in *F. esculentum*. C: GO enrichment analysis of the genes with LTR insertion in *F. tataricum*.



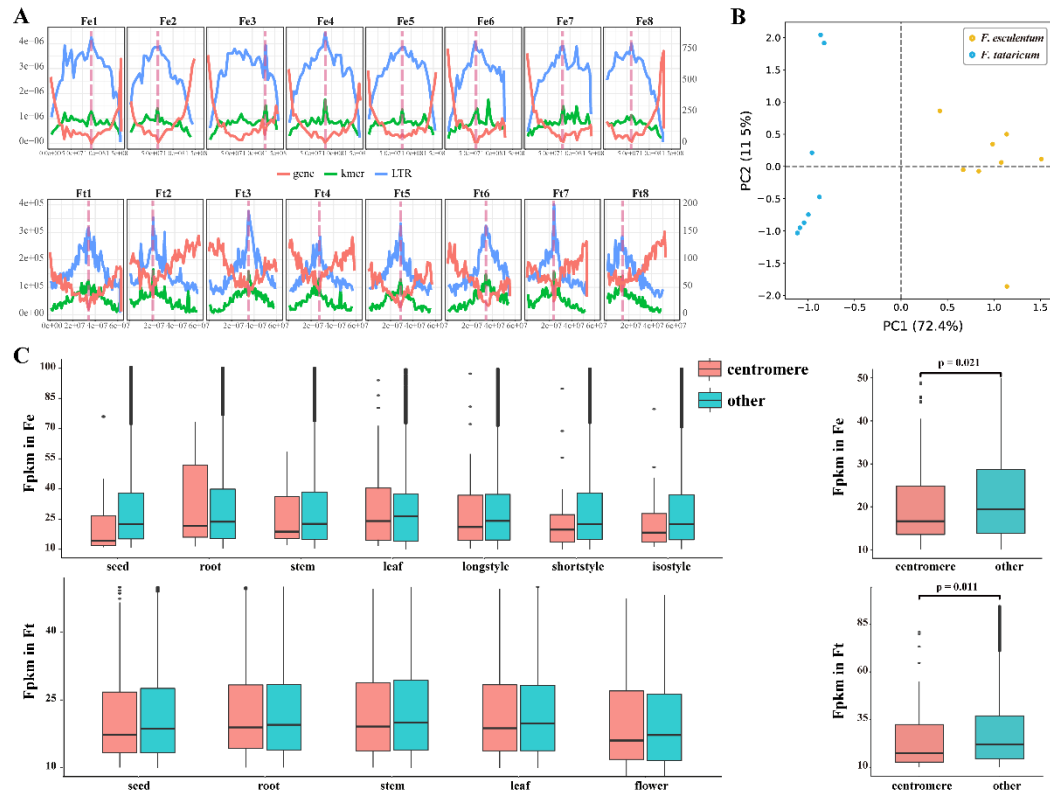
Supplemental Figure 16. The number and the expression proportion of transposase in different tissues of *F. esculentum* and *F. tataricum*.



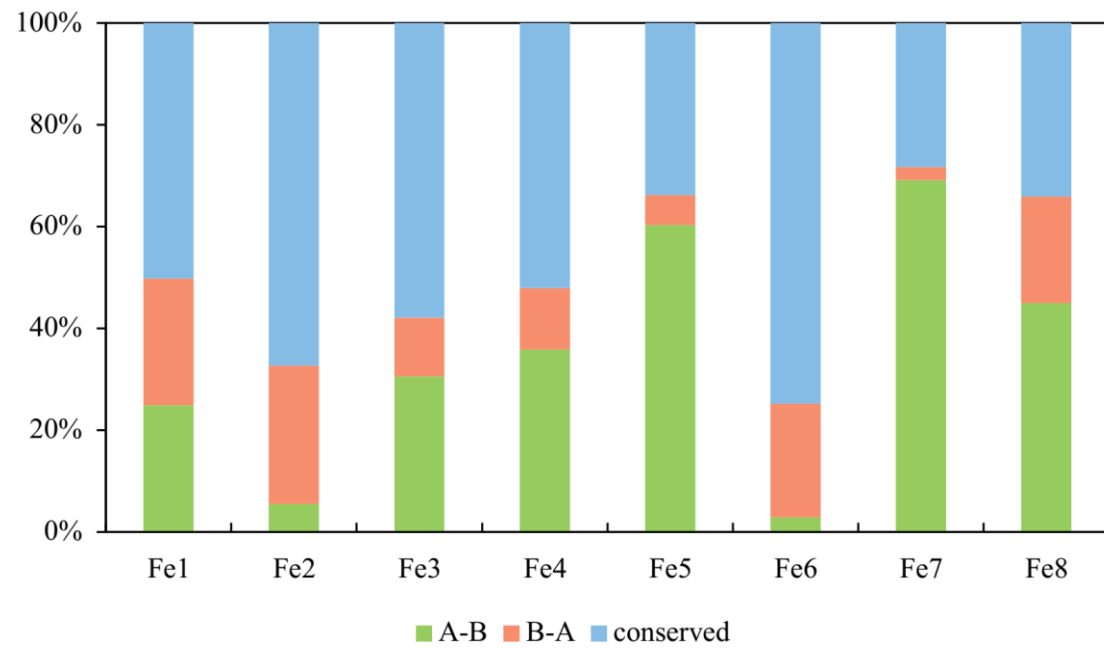
Supplemental Figure 17. Macrosynteny between two buckwheat species karyotypes. The large fragment inversion and insertions were marked in orange and red, respectively.



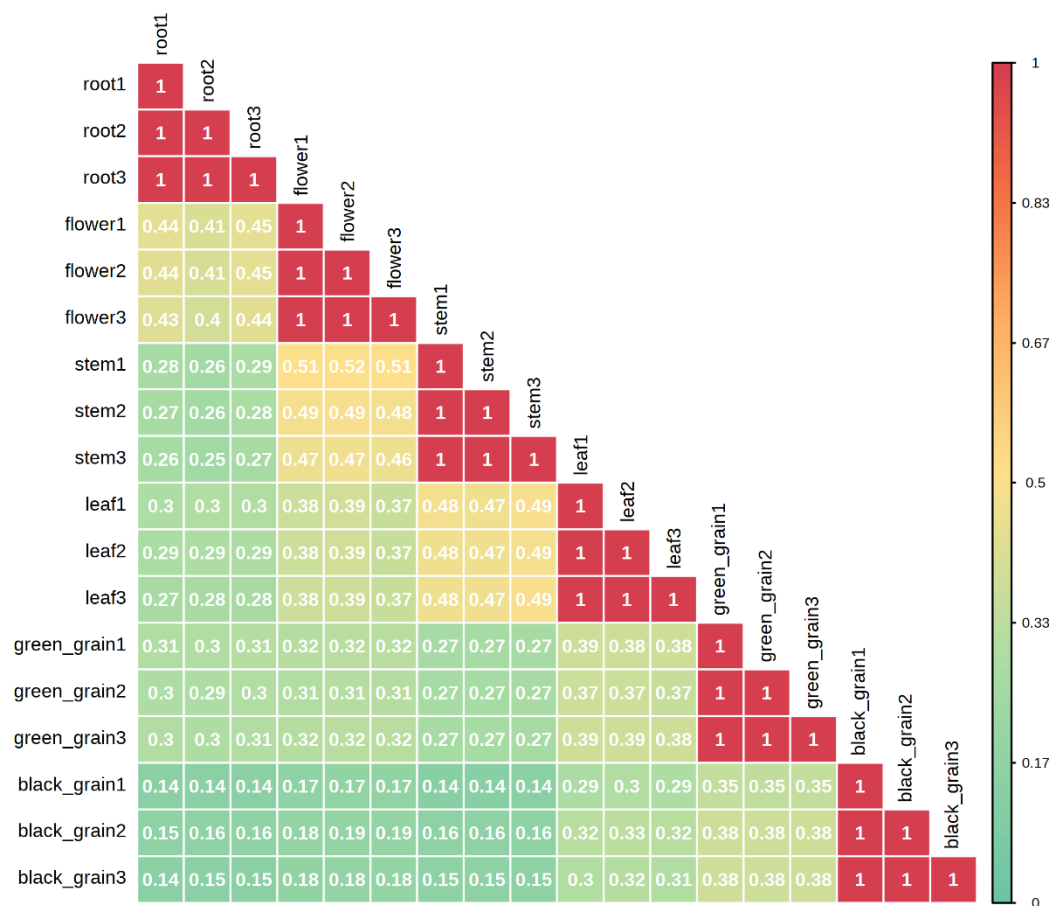
Supplemental Figure 18. Verification of large fragment inversions. A: The inter-genomic Hi-C heatmap between common and Tartary buckwheat. B: Macrosynteny between two buckwheat species karyotypes. The heatmap tracks for each chromosome represent the LTR coverage per 100 kb. The large fragment inversion was marked in different colors. C: Large fragment inversions was verified by using inter-genomic Hi-C heatmap in Chromosome 1, 3, 4, 6 and 8.



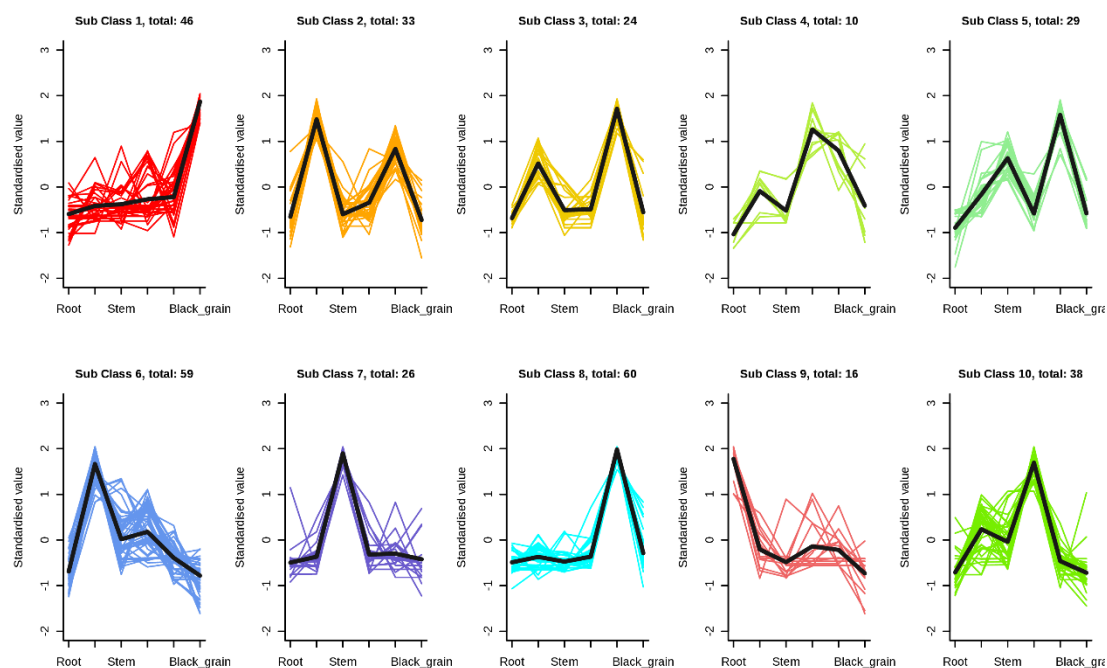
Supplemental Figure 19. Analysis of centromere regions. A: Identification of the candidate centromere region. The red, green, and blue lines represent gene density, 20-mer frequency and LTR density. B: PCA analysis of 20-mer sequence in candidate centromeres in each chromosome between two buckwheat species. C: Comparison of the gene expression level of centromere region and non-centromere region in different tissues.



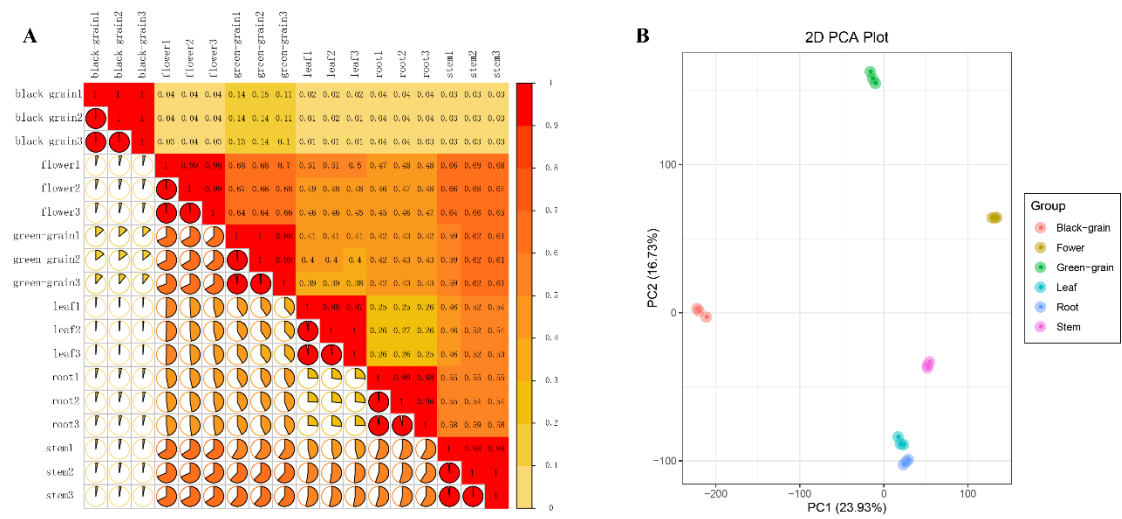
Supplemental Figure 20. The transformation of A/B compartment in the collinear region of *F. esculentum* and *F. tataricum*.



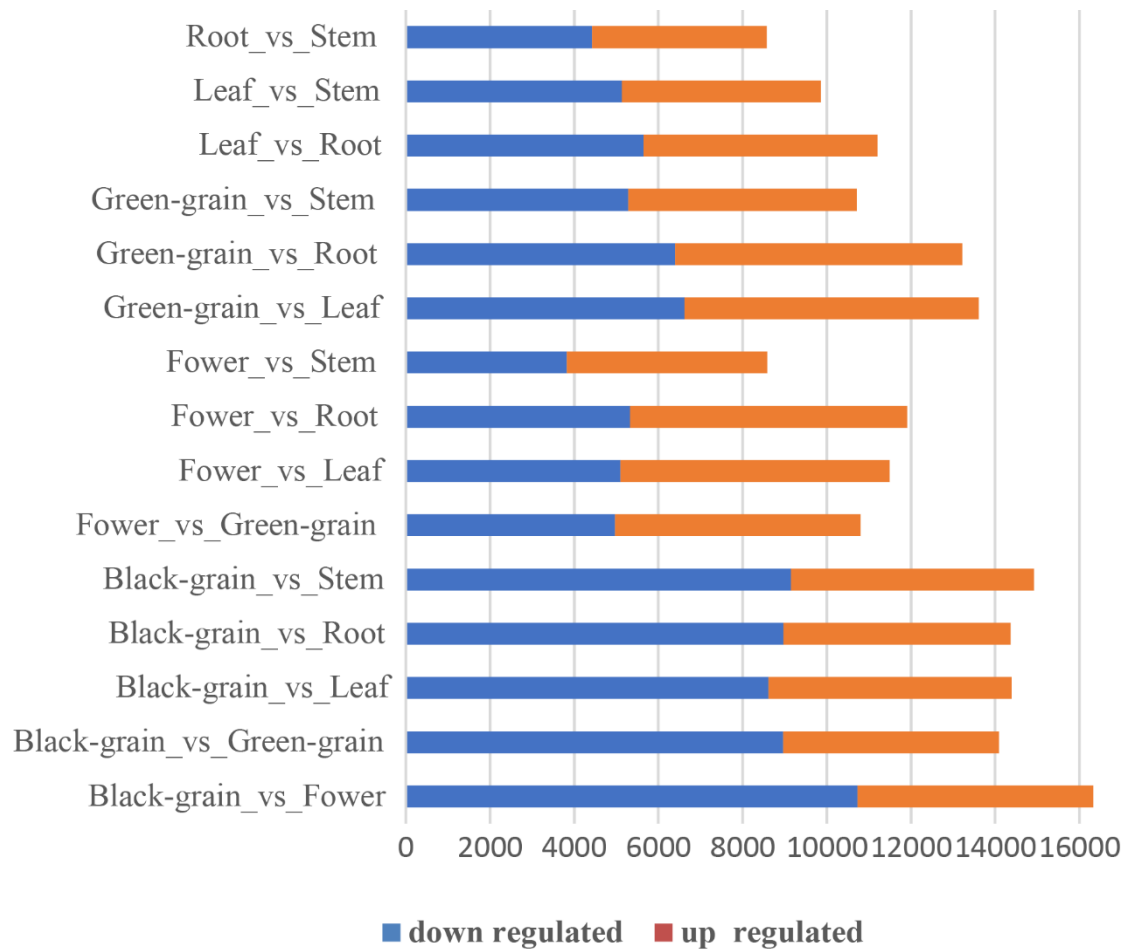
Supplemental Figure 21. Correlation analysis of the relative flavonoid content among six tissues.



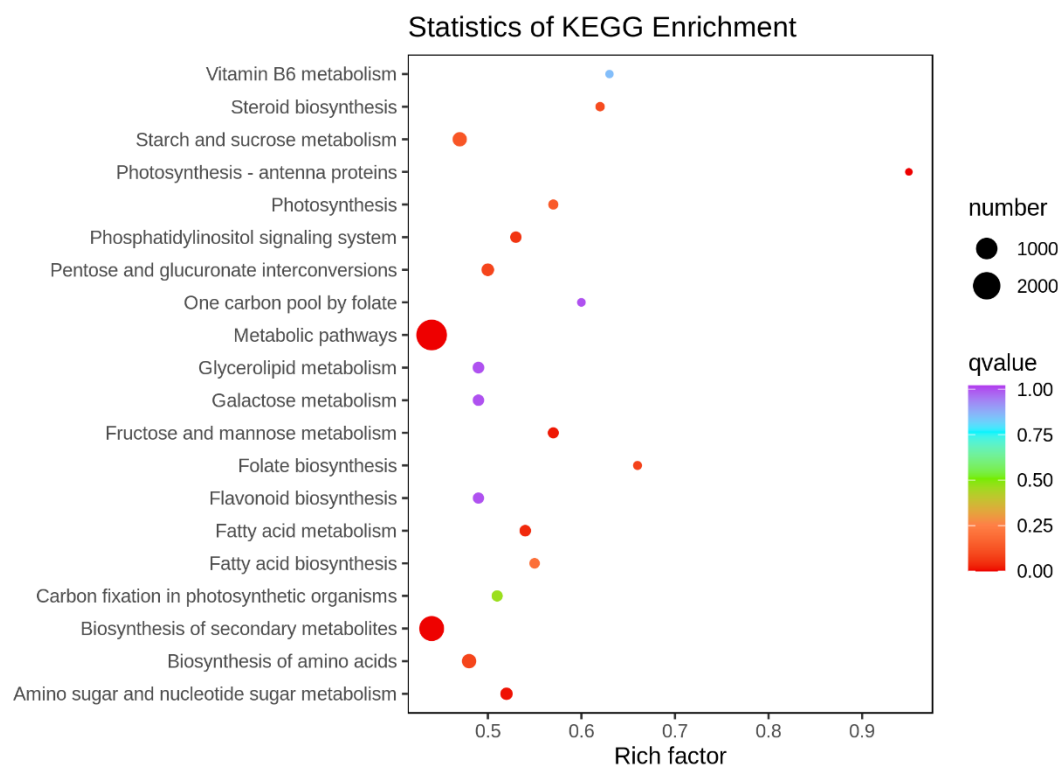
Supplemental Figure 22. K-means clustering groups of the differential metabolites of six tissue.



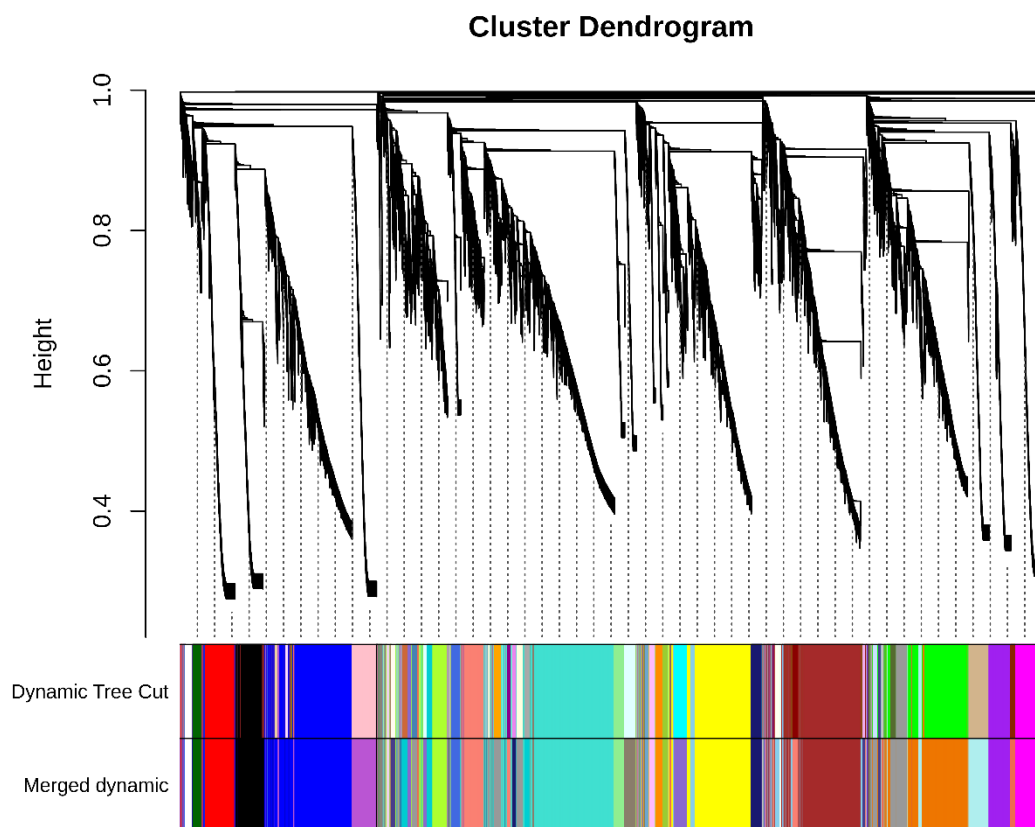
Supplemental Figure 23. The correlation and PCA analysis of transcriptome sample correlation and PCA analysis.



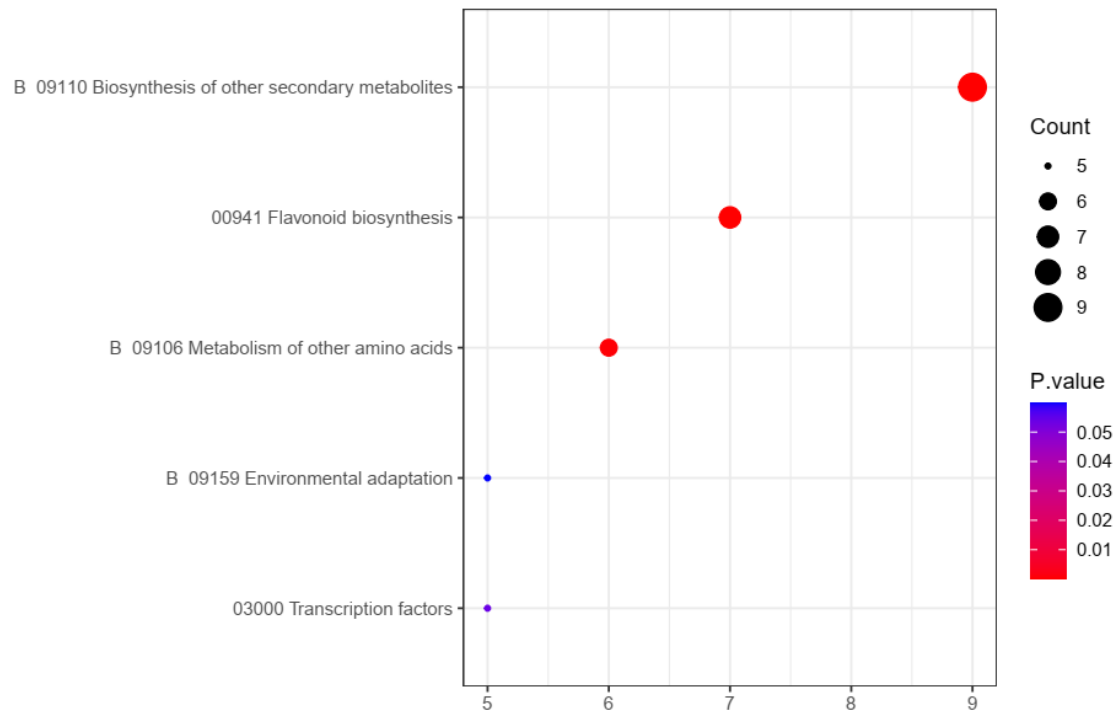
Supplemental Figure 24. Number of DEGs in different groups.



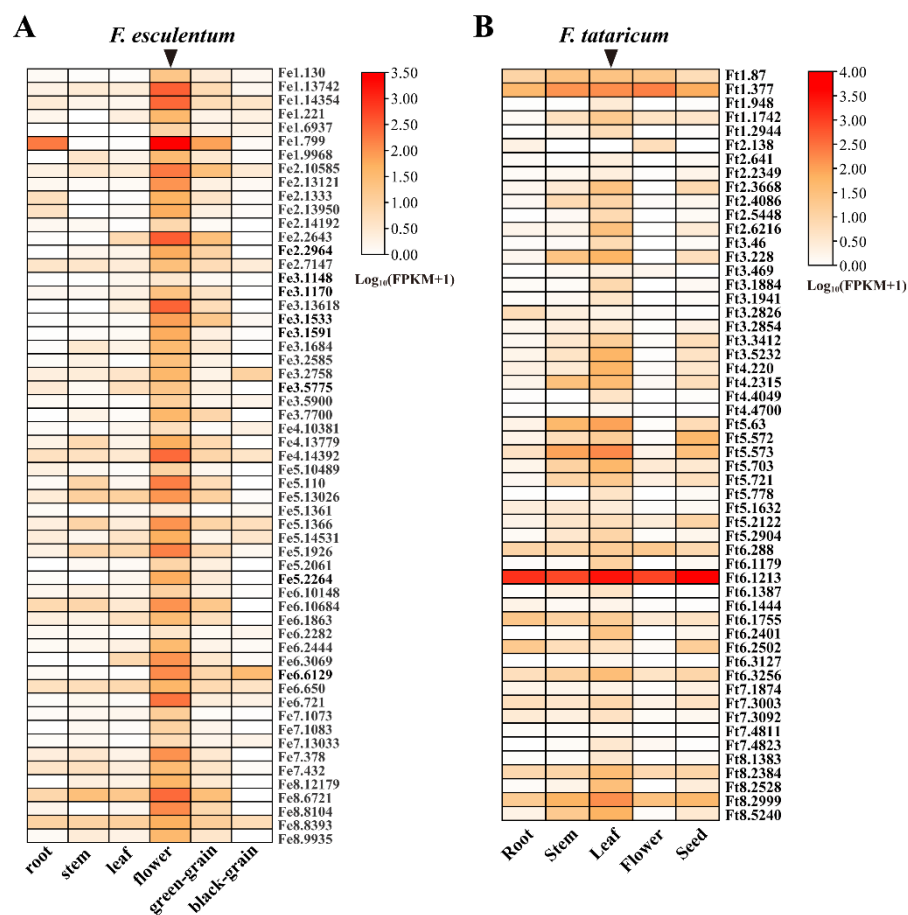
Supplemental Figure 25. KEGG enrichment of DEGs in black grain vs green grain.



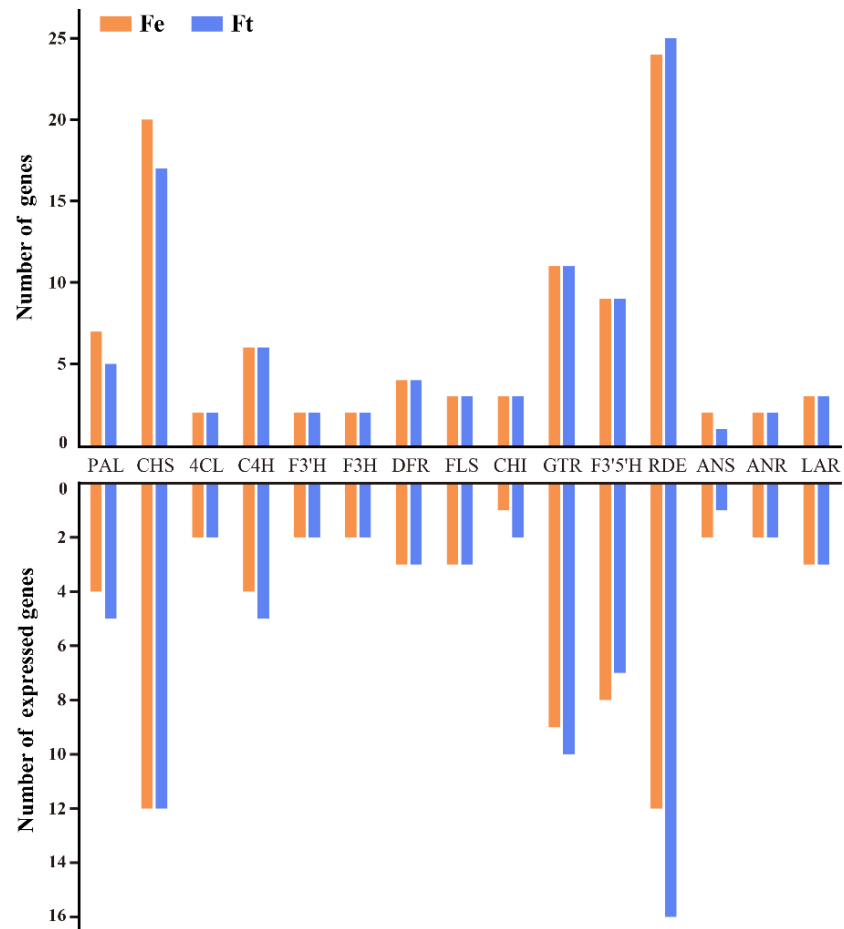
Supplemental Figure 26. Gene cluster dendrograms and module detecting.



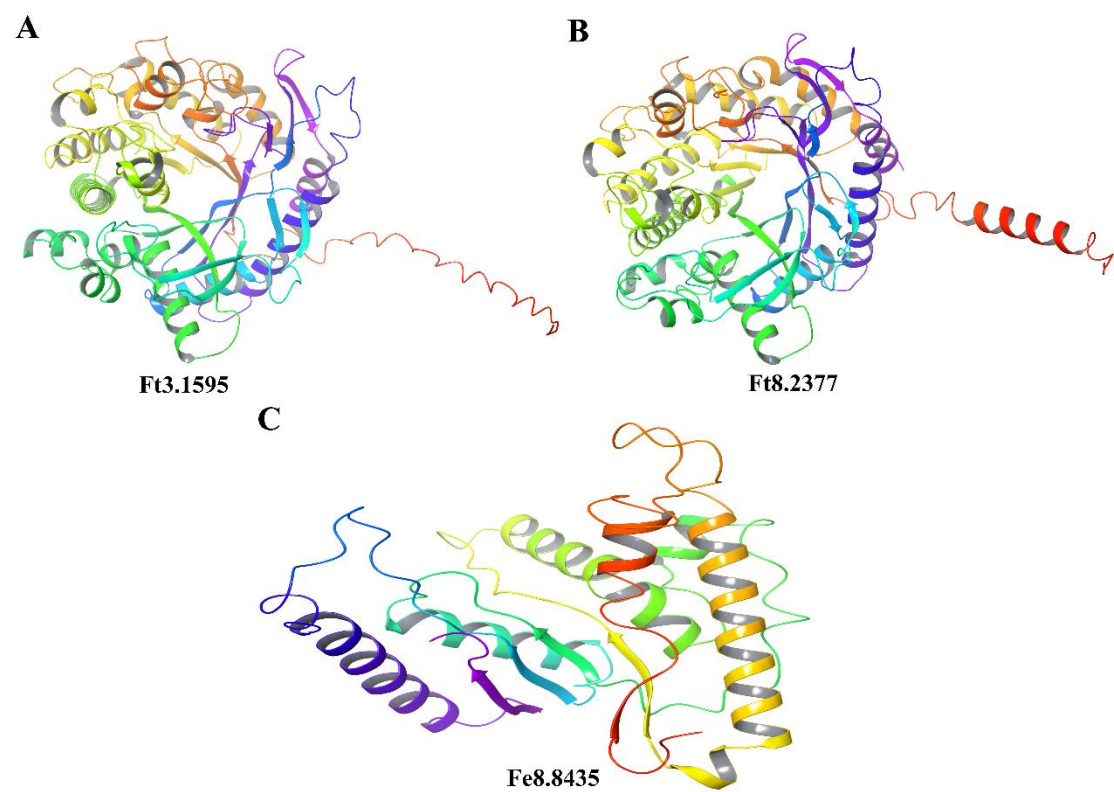
Supplemental Figure 27. KEGG enrichment of METurquoise module genes.



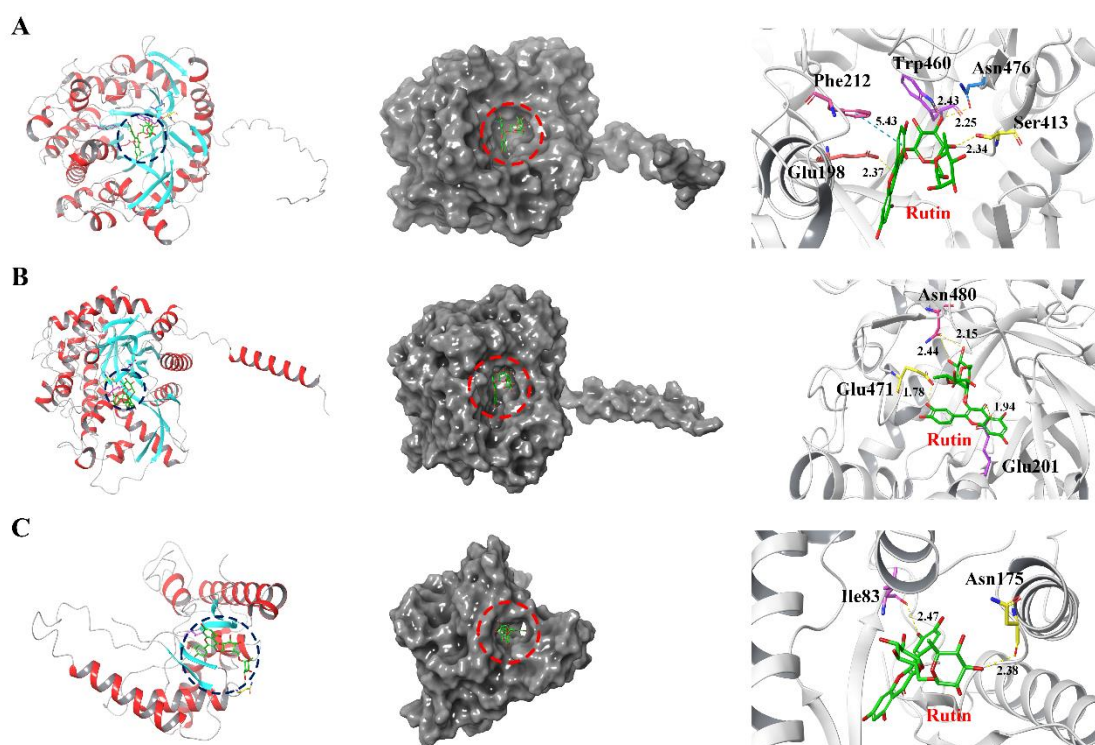
Supplemental Figure 28. The expression analysis of genes in METurquoise module in common and Tartary buckwheat. A: Expression patterns of genes in the METurquoise module. B: The expression of homologous genes of METurquoise module in Tartary buckwheat.



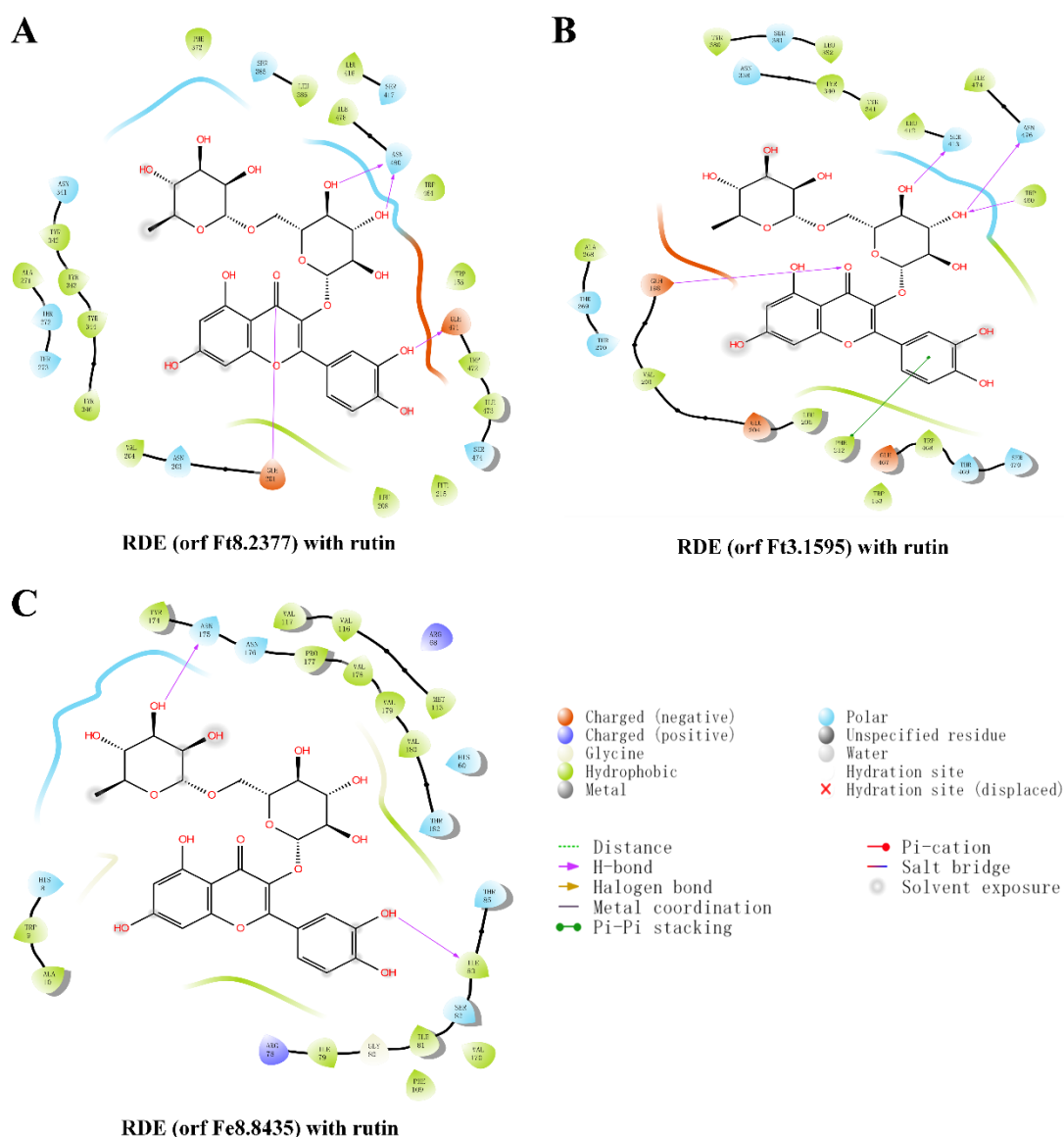
Supplemental Figure 29. The number and expression of rutin biosynthesis-related genes.



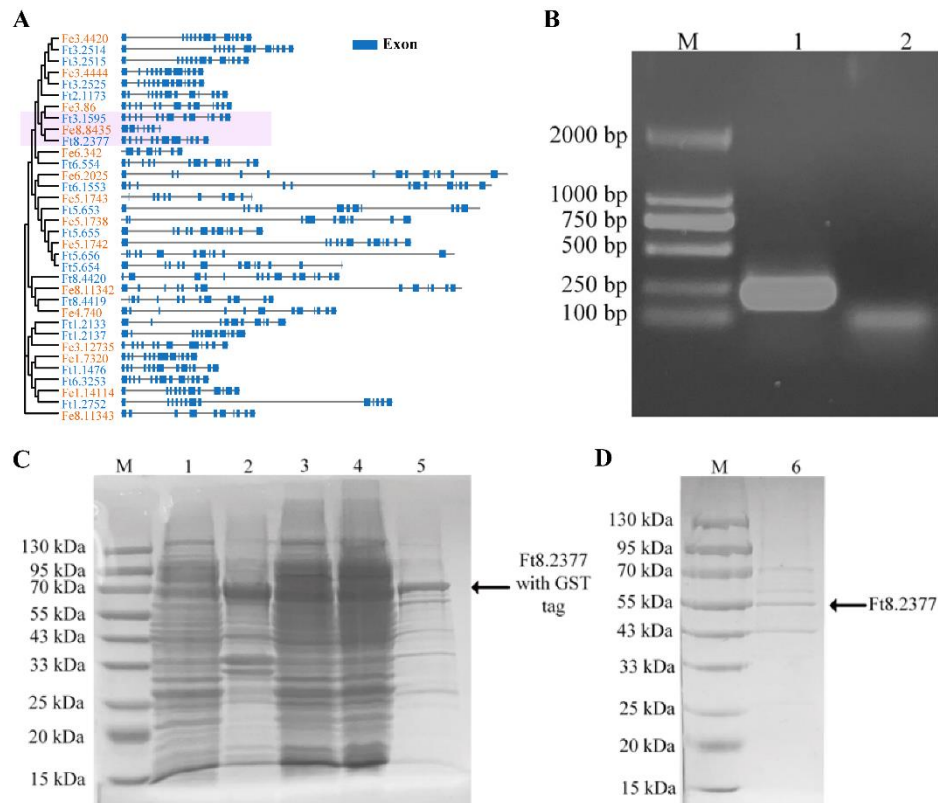
Supplemental Figure 30. Structural prediction of RDE genes. A-C: Structural prediction of Ft3.1595, Ft8.2377 and Fe8.8435, respectively.



Supplemental Figure 31. Docking results for three rutin degrading enzymes: In the first column, overall carton structure of RDE with rutin and the binding site circled in black dash. In the second column, the structure is displayed in Surface and the binding pocket is circled in red. In the third column, the interaction between RDE and rutin, yellow for hydrogen bonds, blue for π - π interactions, and green for π -acton. A: Docking result of Ft3.1595. B: Docking result of Ft8.2377. C: Docking result of Fe8.8435.

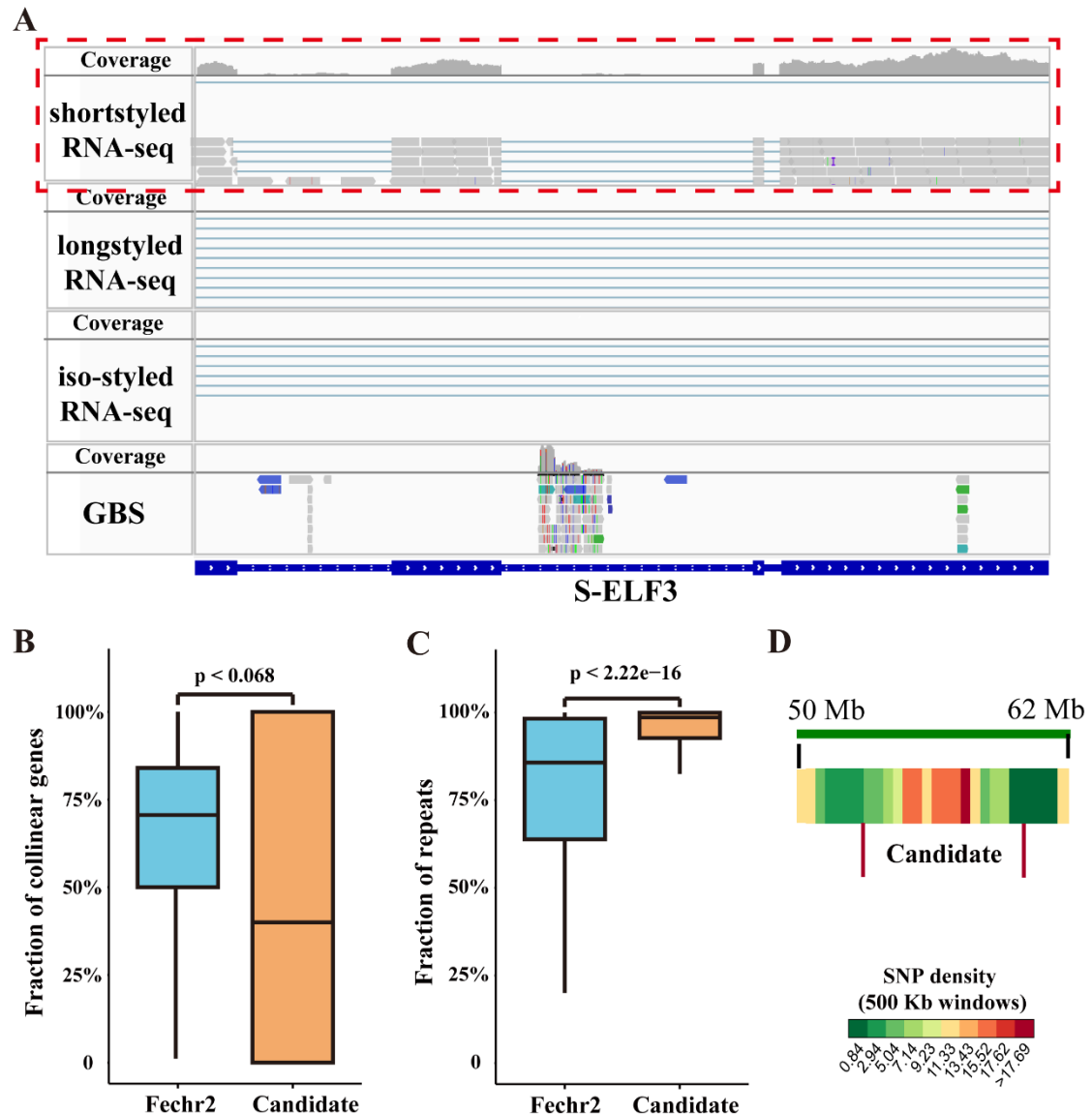


Supplemental Figure 32. Ligand Interaction Diagram of RDEs. A: The 2D plot of the Ft8.2377 binding-pocket residues and their interaction with rutin. B: The 2D plot of the Ft3.1595 binding-pocket residues and their interaction with rutin. C: The 2D plot of the Fe8.8435 binding-pocket residues and their interaction with rutin.

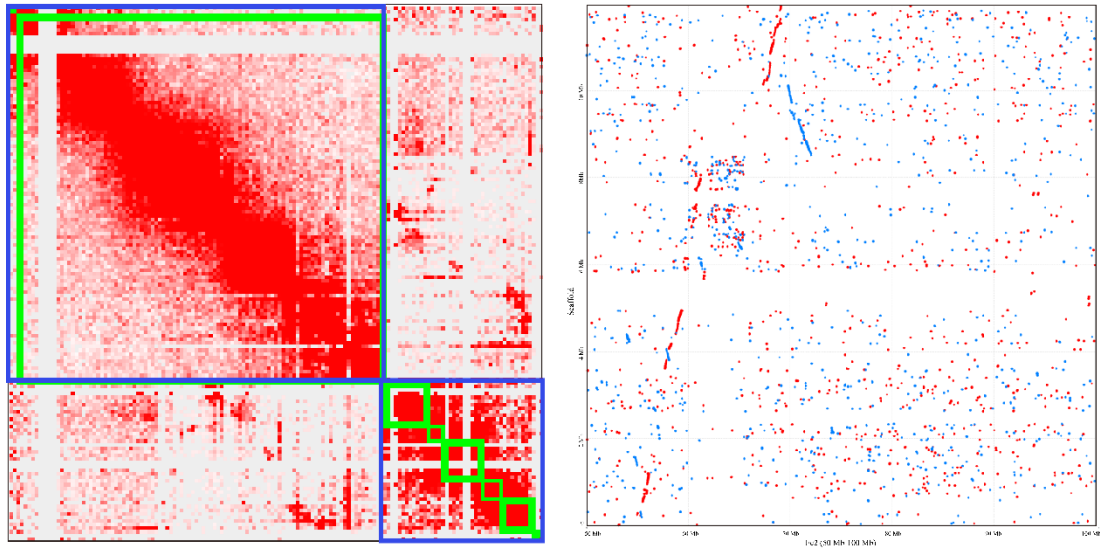


Supplemental Figure 33. The clustering and heterologous expression of RDE genes.

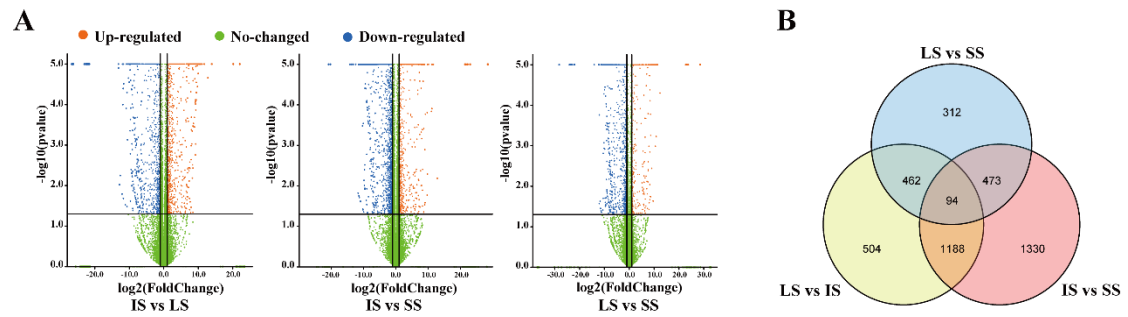
A: The clustering and gene structure analysis of RDEs gene family. Blue rectangle represent exon. B: 1 % agarose gel electrophoresis verification of internal reference gene and Fe8.8435 gene. Lane M: DNA marker; Lane 1: internal reference gene; Lane 2: Fe8.8435 gene. C-D: 12 % SDS-PAGE verification of recombinant protein (Ft8.2377). Lane M: molecular weight marker; Lane 1: supernatant; Lane 2: precipitation; Lanes 3: flow through; Lane 4: washed; Lane 5: RDE with GST tag; Lane 6: purified RDE.



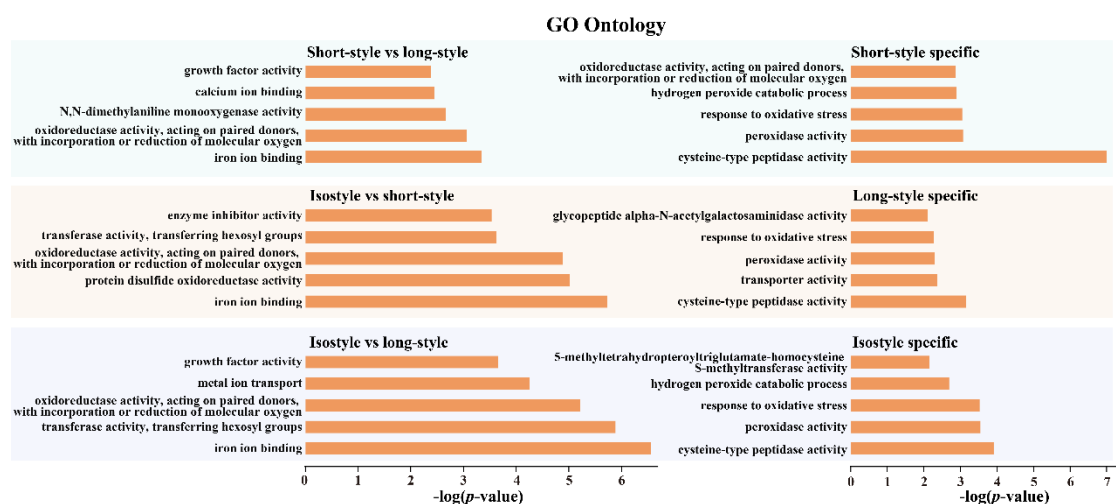
Supplemental Figure 34. Haplotype structure and patterns of molecular evolution at the S-locus. A: The mapping of three floral RNA-seq in S-ELF3 gene region. B: Comparison of the fraction of collinear genes between the S-locus and Chromosome 2 ($n = 100$ kb windows). C: Comparison of the fraction of repetitive sequences between the S-locus and Chromosome 2 ($n = 100$ kb windows). D: SNP density in the S-locus and neighboring loci ($n = 500$ kb windows).



Supplemental Figure 35. Hi-C heatmap of heterozygous regions and genome collinearity.



Supplemental Figure 36. Differentially expressed genes analysis of three flower types. A: Volcano plot of differentially expressed genes. B: Venn diagram showing the overlapping differentially expressed genes among the three flowers.



Supplemental Figure 37. GO enrichment analysis of differentially expressed genes in three flowers.