

Figure S1. All homologs of FLS and DFR encoding genes in the R. chingii genome.

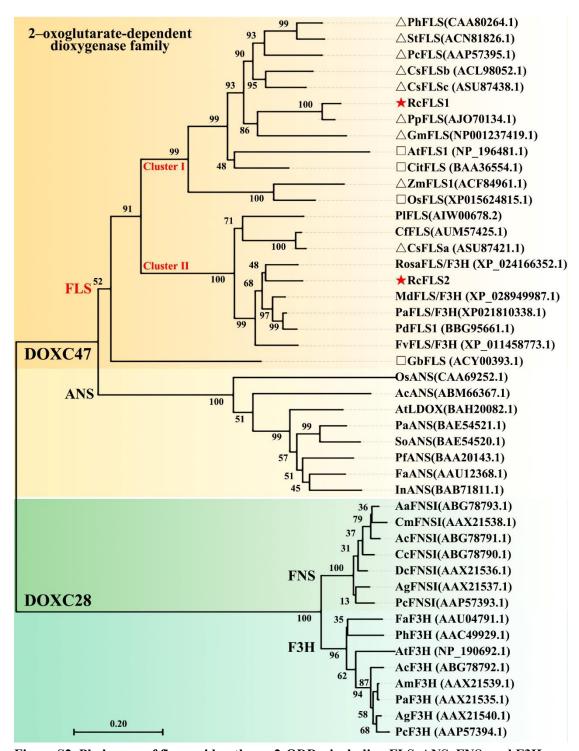


Figure S2. Phylogeny of flavonoid pathway 2-ODDs, including FLS, ANS, FNS, and F3H.

The phylogenetic tree was constructed using the MEGA7 software with neighbor-joining method. The DOXC47 clade is on a yellow background, and the DOXC28 clade is on a green background. The FLS subclade is divided into two clusters, indicated in red font. Triangle-labeled FLS genes were reported to have flavonol synthase (FLS) activity, and square-labeled FLS genes were reported to have both flavonol synthase (FLS) and flavanone 3-hydroxylase (F3H) activities.

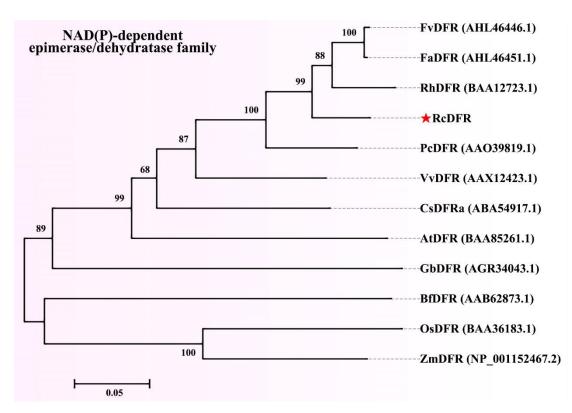


Figure S3. Phylogenetic tree of RcDFR and other DFRs from different plant species.

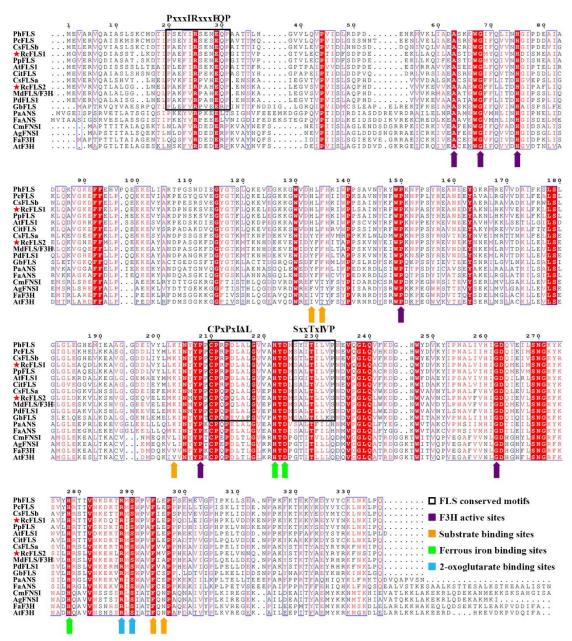


Figure S4. Multiple sequence alignment of RcFLSs with other 2-ODDs involved in the flavonoid biosynthetic pathway.

Multiple sequence alignments were performed using ClustalW. Conserved amino acids and motifs were labeled as follows: the FLS conserved motifs "PxxxIRxxxEQP," "CPxPxLAL," and "SxxTxLVP" are shown in BLACK, while residues involved in the substrate, ferrous iron, and 2-oxoglutarate binding are marked in ORANGE, GREEN, and BLUE, respectively. Residues relevant to F3H activity are shown in PURPLE.

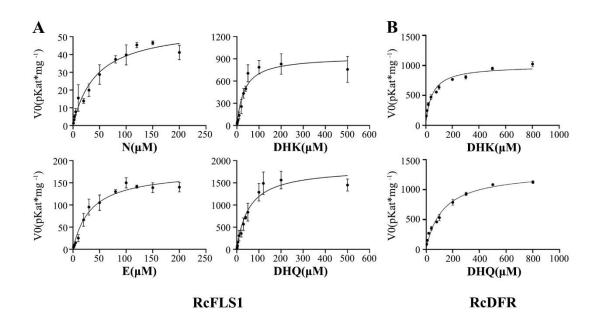


Figure S5. The Michaelis-Menten equation curve of recombinant RcFLS1 and RcDFR proteins.

The graph was plotted using Prism 8 software. The Michaelis-Menten constant was determined by averaging values from fitting three separate data sets.

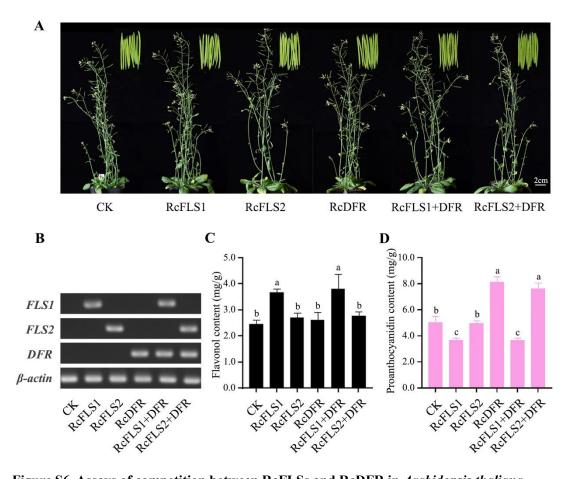


Figure S6. Assays of competition between RcFLSs and RcDFR in Arabidopsis thaliana.

(A) Phenotypes of transgenic *Arabidopsis* plants overexpressing *RcFLSs* and *RcDFR*. *Arabidopsis* green pods are shown in enlarged views. (B) Semiquantitative RT-PCR analysis of the transcription levels of *RcFLSs*, *RcDFR*, and housekeeping gene β -actin. (C) The content of flavonols extracted from transgenic *Arabidopsis* green pods. (D) The content of PA extracted from transgenic *Arabidopsis* green pods. Error bars indicate \pm SD from three repeats. Statistical significance was determined by one-way ANOVA with post hoc Tukey's tests (P < 0.05) and indicated by letters.