



Supplementary Material

Comprehensive Analysis of Arabinogalactan Protein-Encoding Genes Reveals the Involvement of Three *BrFLA* Genes in Pollen Germination in *Brassica rapa*

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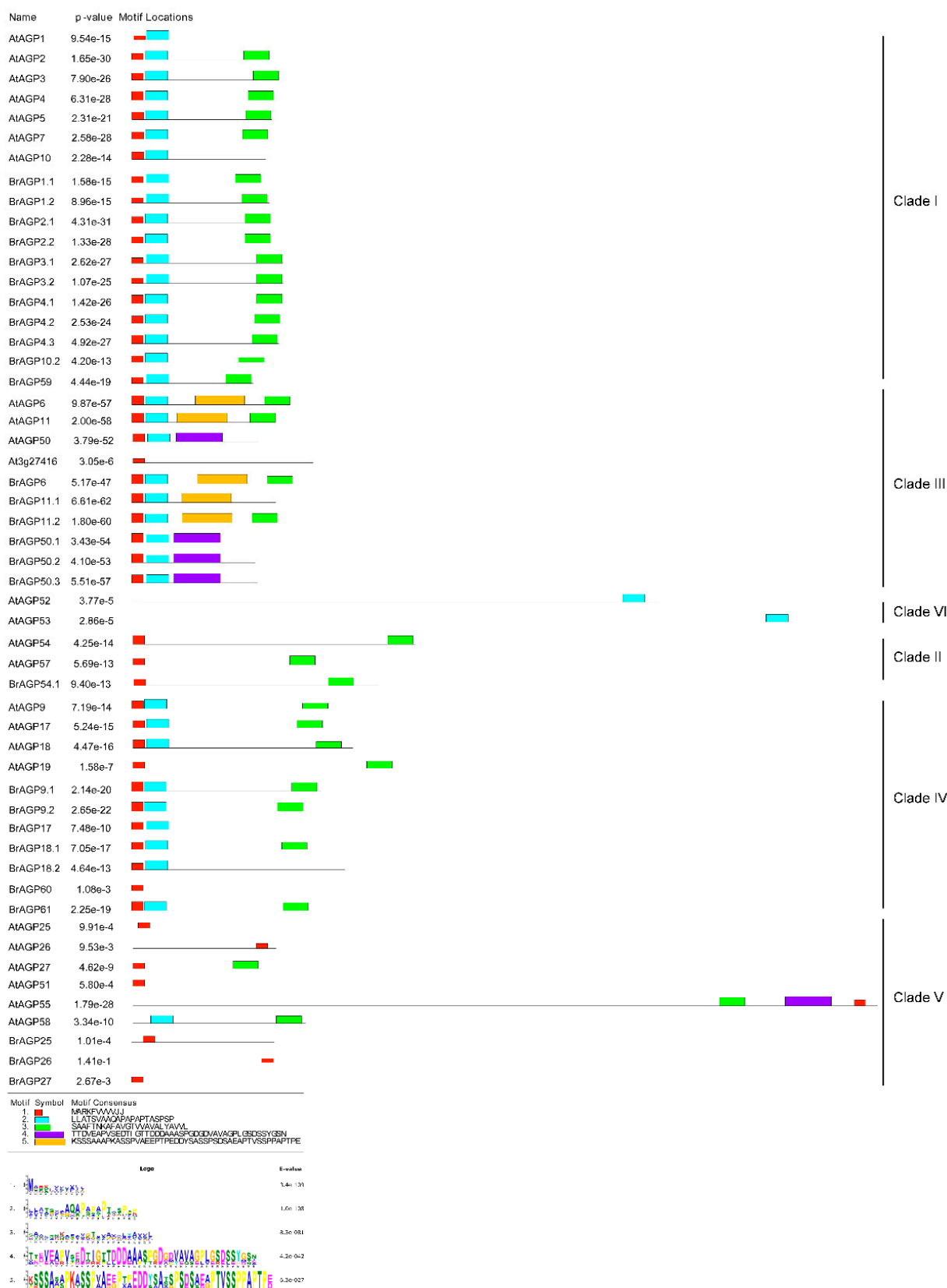


Figure S1. Motif analysis of classical AGPs and Lys-rich AGPs from *Brassica rapa* and *Arabidopsis*. Motif discovery and searching was performed by Multiple Em for Motif Elicitation (MEME) software.

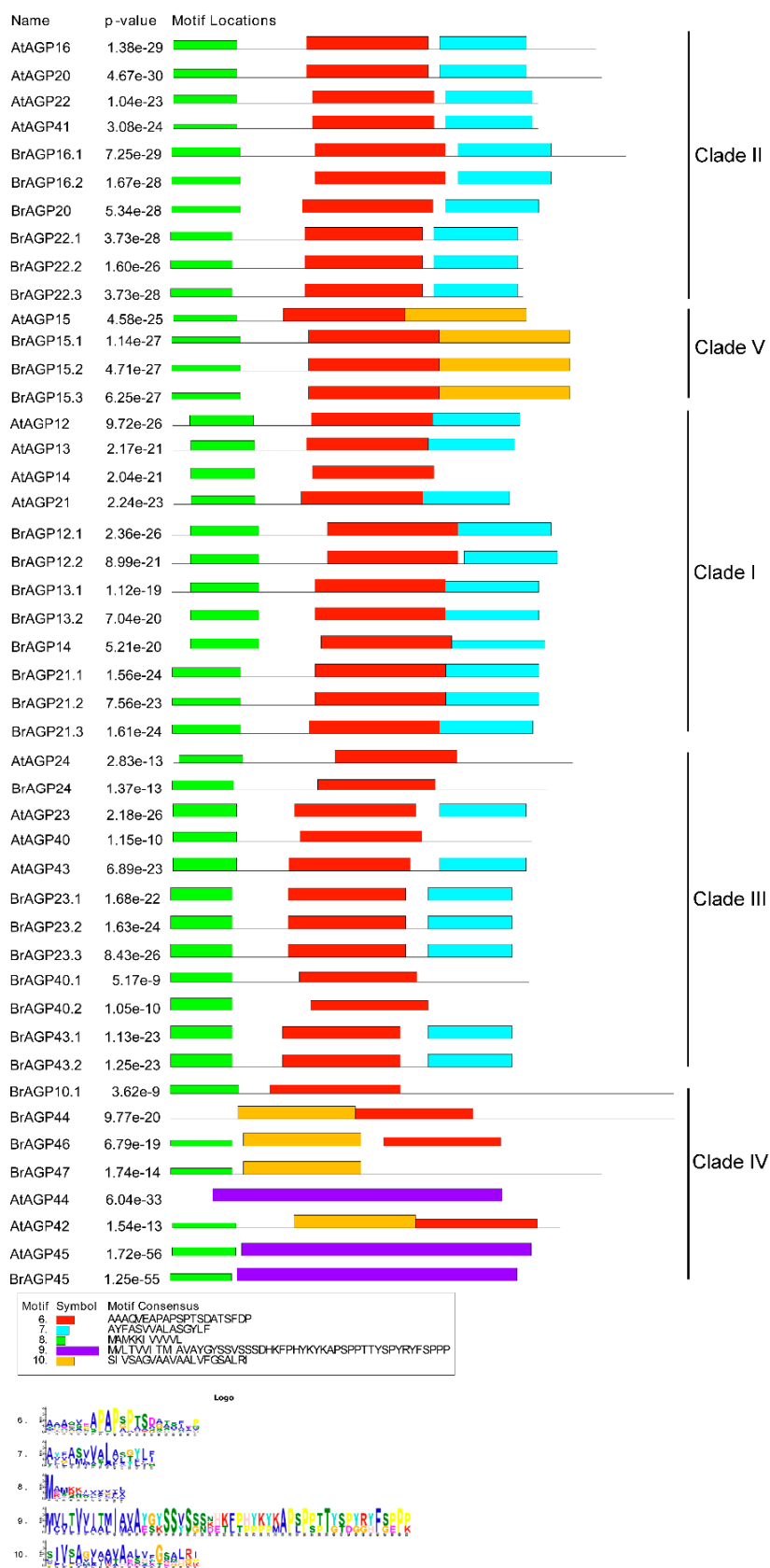


Figure S2. Motif analysis of AG-peptides from *Brassica rapa* and *Arabidopsis*. Motif discovery and searching was performed by Multiple Em for Motif Elicitation (MEME) software.

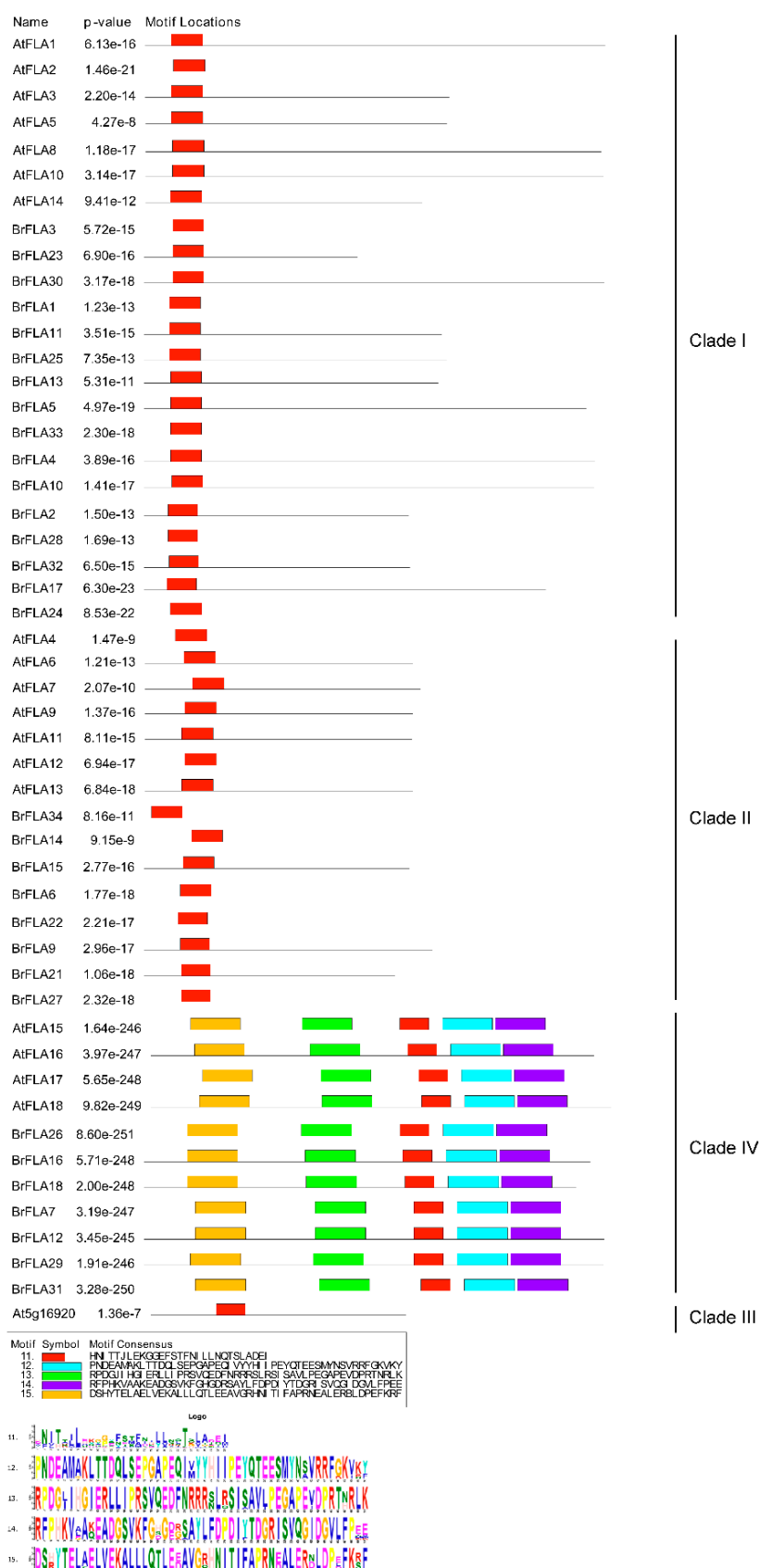
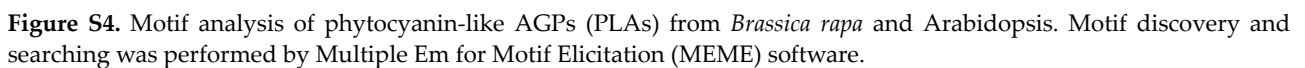


Figure S3. Motif analysis of fasciclin-like AGPs (FLAs) from *Brassica rapa* and *Arabidopsis*. Motif discovery and searching was performed by Multiple Em for Motif Elicitation (MEME) software.



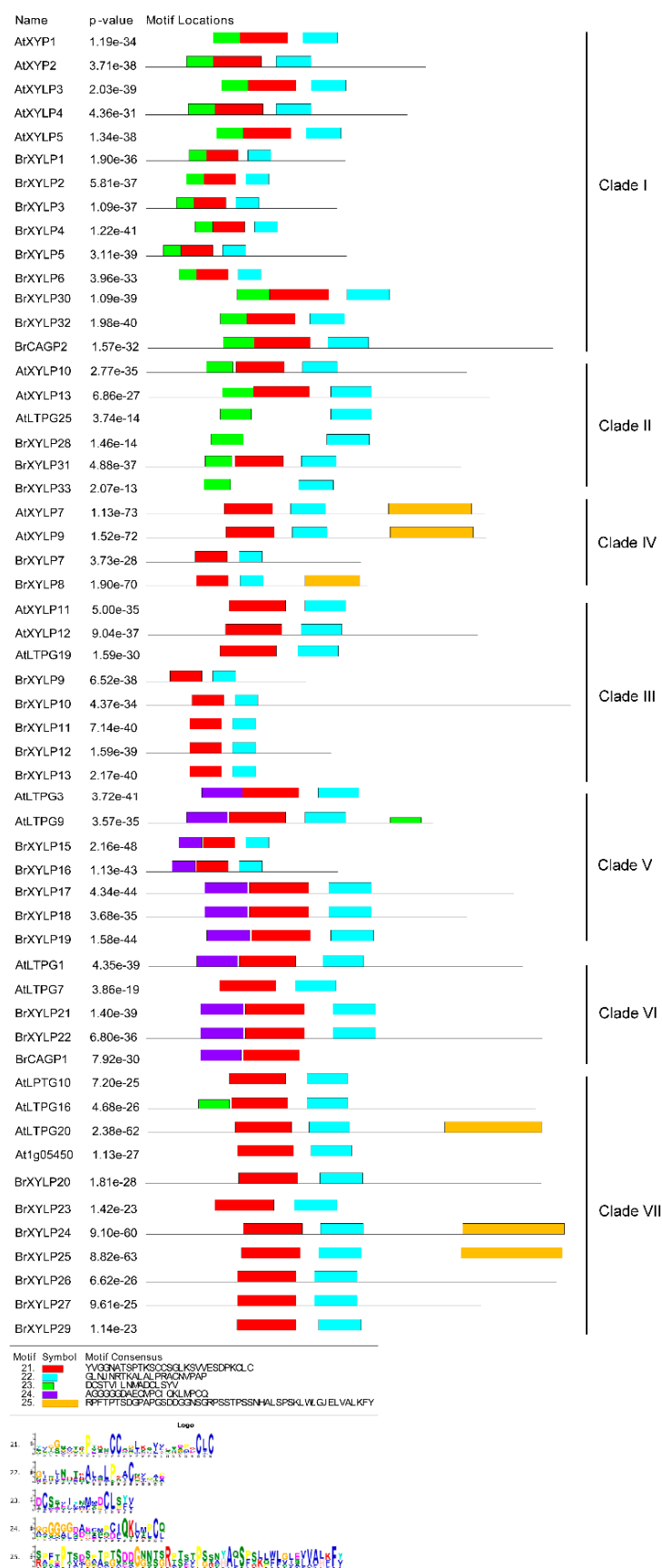


Figure S5. Motif analysis of xylogen-like proteins (XYLPs) from *Brassica rapa* and *Arabidopsis*. Motif discovery and searching was performed by Multiple Em for Motif Elicitation (MEME) software.

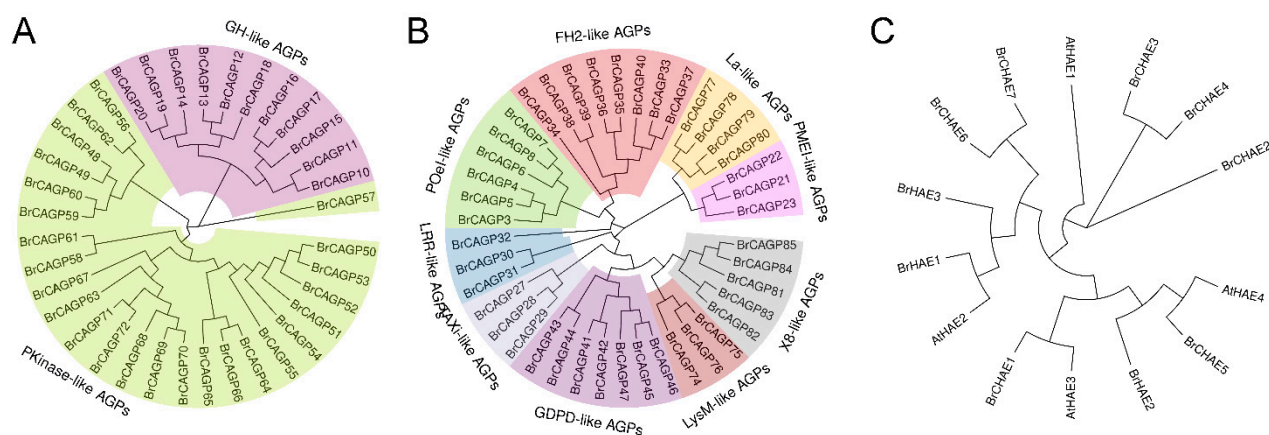


Figure S6. Phylogenetic tree representation of chimeric AGPs (CAGPs) and AGP/extensins hybrids (HAE) from *Brassica rapa* (BrAGPs) and *Arabidopsis* (AtAGPs). (A,B) BrCAGPs with conservative domains. (C) Chimeric HAEs (CHAEs) and HAEs. The Neighbor-Joining tree was constructed using MEGA X software with 1,000 bootstrap. The OmicStudio tools was used to display and manipulate trees.

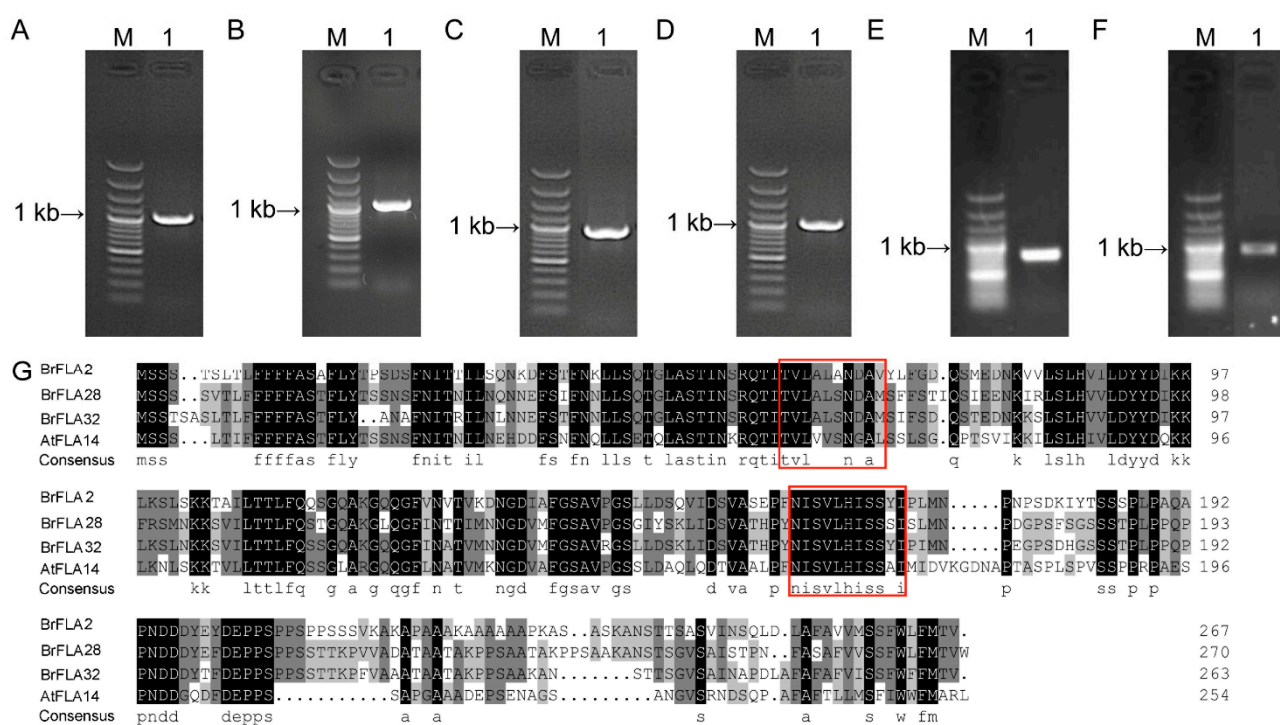


Figure S7. PCR amplification the full-length DNA and cDNA sequences of *BrFLA2*, *BrFLA28* and *BrFLA32*. (A,C,E) Full-length DNA amplification of *BrFLA2* (A), *BrFLA28* (C) and *BrFLA32* (E). (B,D,F) cDNA amplification of *BrFLA2* (B), *BrFLA28* (D) and *BrFLA32* (F). Lane M indicates the molecular marker. (G) Multiple alignments of *BrFLA2*, *BrFLA28*, *BrFLA32* and *AtFLA14* protein sequences. Red squares indicate the conserved motifs H1 and H2 of fasciclin domains.

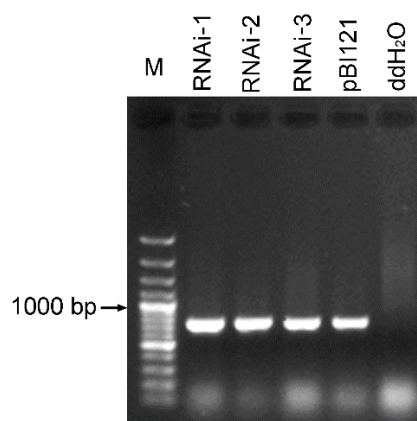


Figure S8. Genomic DNA PCR analysis of *BrFLA2/28/32*-RNAi transgenic plants in *Brassica rapa*. A 740-bp expected band was amplified in three *BrFLA2/28/32*-RNAi transgenic lines and the binary vector pBI121. Lane M indicates the molecular marker.



Figure S9. Morphological features of flowers from *BrFLA2/28/32*-RNAi transgenic plants in *Brassica rapa*. (A–E) A floret (A), a sepal (B), a petal (C), a stamen (D) and a pistil (E) at anthesis stage in a *BrFLA2/28/32*-RNAi plant. (F–J) A floret (F), a sepal (G), a petal (H), a stamen (I) and a pistil (J) at anthesis stage in a control plant. Scale bars = 2 mm.

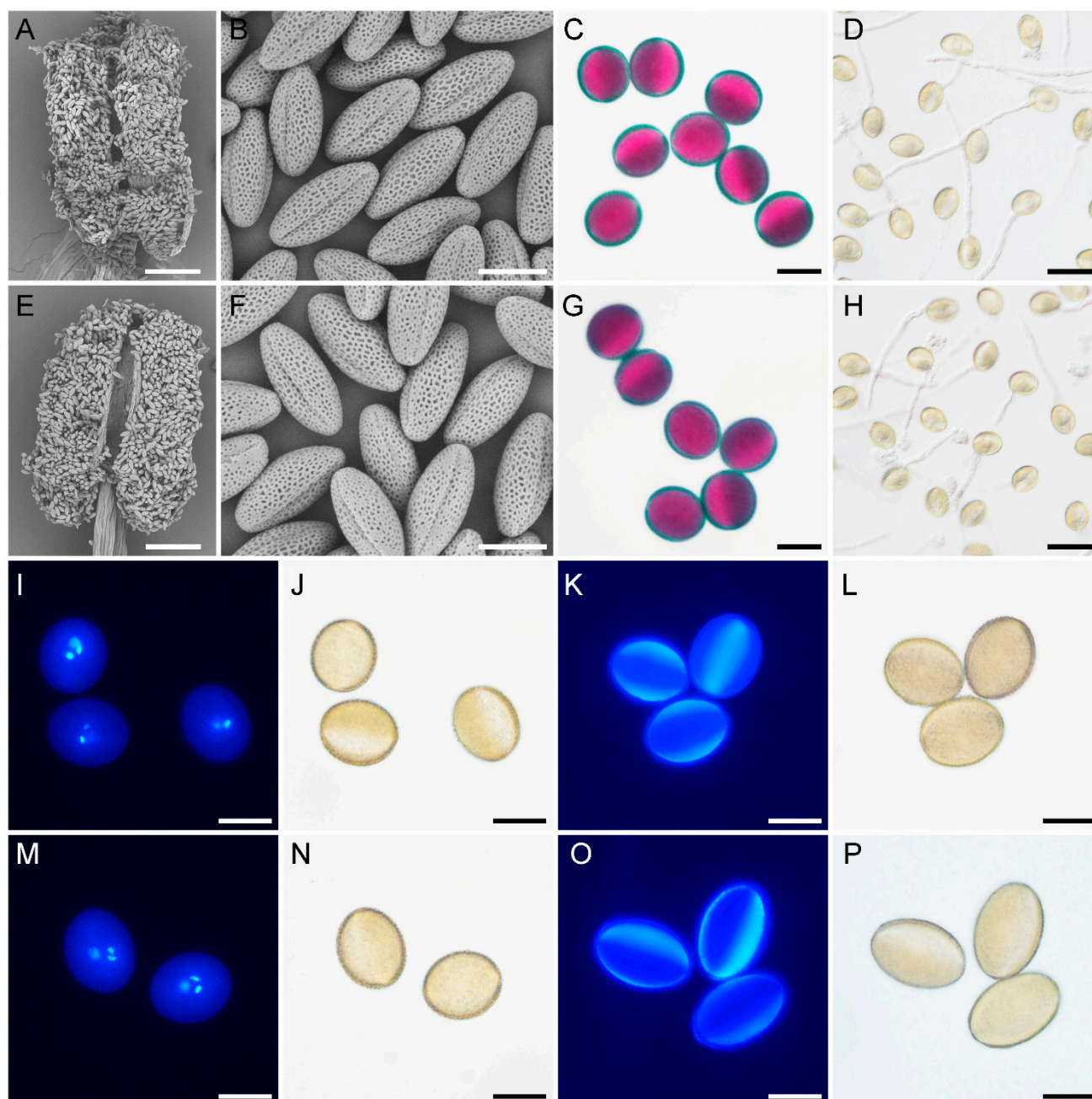


Figure S10. Mature pollen analysis in *BrFLA2/28/32*-RNAi transgenic plants in *Brassica rapa*. (A,B,E,F) Scanning electron microscopy observation of dehiscent anthers (A,E) and mature pollens (B,F) in *BrFLA2/28/32*-RNAi (A,B) and control plants (E,F). (C,G) Alexander staining of mature pollens in *BrFLA2/28/32*-RNAi (C) and control plants (G). (D,H) *BrFLA2/28/32*-RNAi (D) and control (H) pollen grains germinated *in vitro* for 4 h. (I,M) DAPI staining of mature pollens in *BrFLA2/28/32*-RNAi (I) and control plants (M). (J,N) The corresponding bright field images of (I,M). (K,O) Calcofluor fluorescent white staining of mature pollens in *BrFLA2/28/32*-RNAi (K) and control plants (O). (L,P) The corresponding bright field images of (K,O). Scale bars = 200 μ m (A,E); 50 μ m (D,H); 20 μ m (B,C,F,G,I-P).