

Figure S1. The exon-intron configurations of 11 male fertility-related CCCH zinc-finger protein genes in *B. campestris* (A). Phylogenetic relationships and multiple sequence alignment of CCCH zinc finger motifs from 11 male fertility-related CCCH zinc-finger proteins (B).



Figure S2. Sequence alignment of BcMF30a and BcMF30c. Both BcMF30a and BcMF30c contain a CCH motif, a LOTUS domain and an RRM domain.

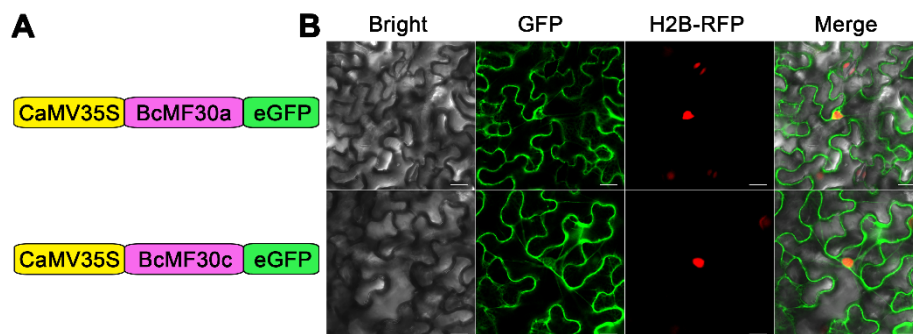


Figure S3. Subcellular localization of BcMF30a-eGFP and BcMF30c-eGFP fusion proteins in tobacco epidermal cells. (A) Constructs used for the subcellular localization analysis. (B) Both BcMF30a-eGFP and BcMF30c-eGFP were located in nucleus and dispersed in the cytoplasm of tobacco epidermal cells. H2B is a marker protein of nucleus. Pictures represent white field images (Bright), epifluorescence (GFP and RFP) and merged images (Merge). Bar = 25 μm.

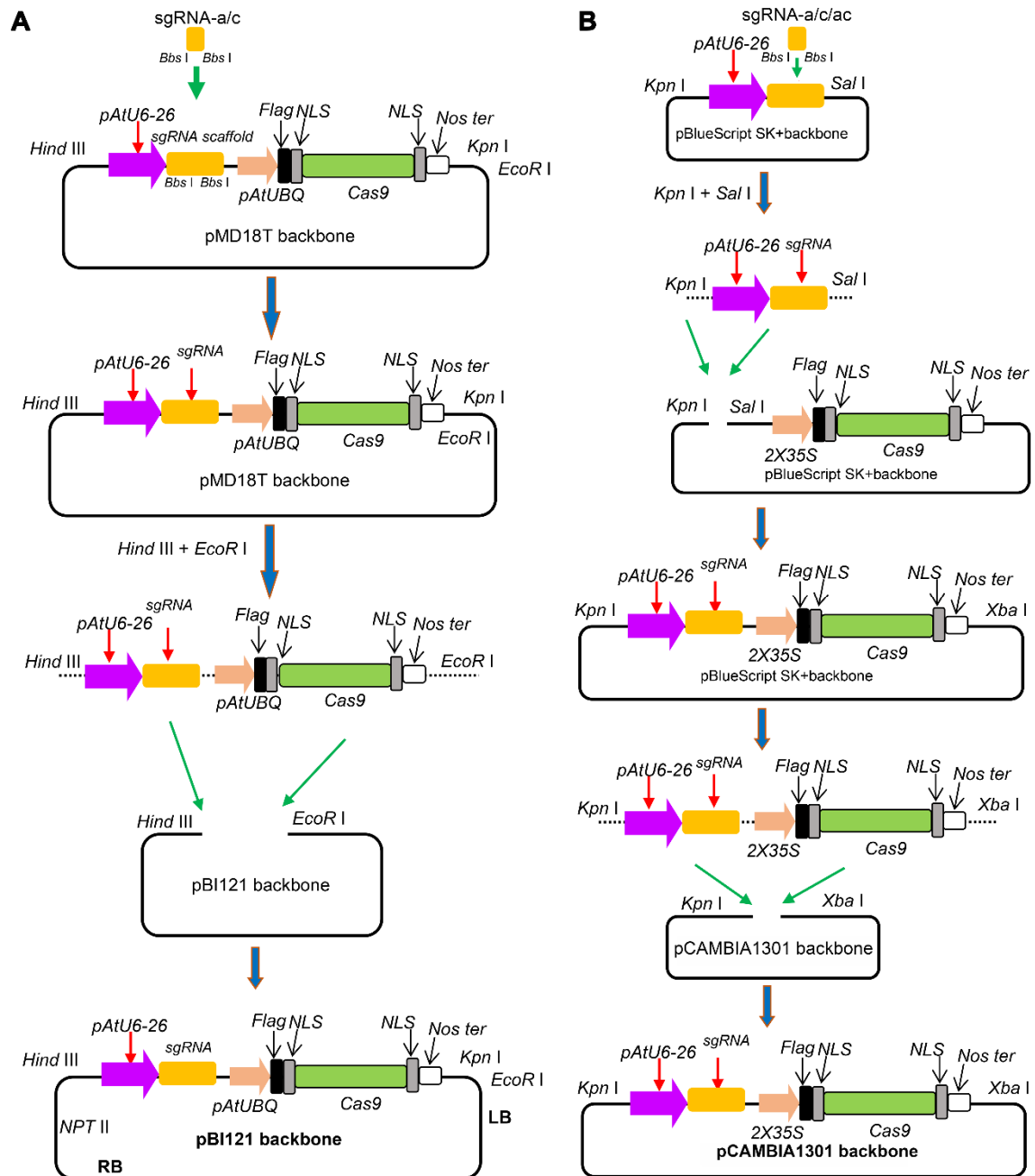


Figure S4. Flow chart of constructing CRISPR-Cas9 knockout vectors. (A) pBI121 as vector backbone, and *Cas9* gene is activated by promoter of *AtUBQ*. (B) pCAMBIA1301 as vector backbone, and *Cas9* gene is activated by 2× *CaMV35S*.

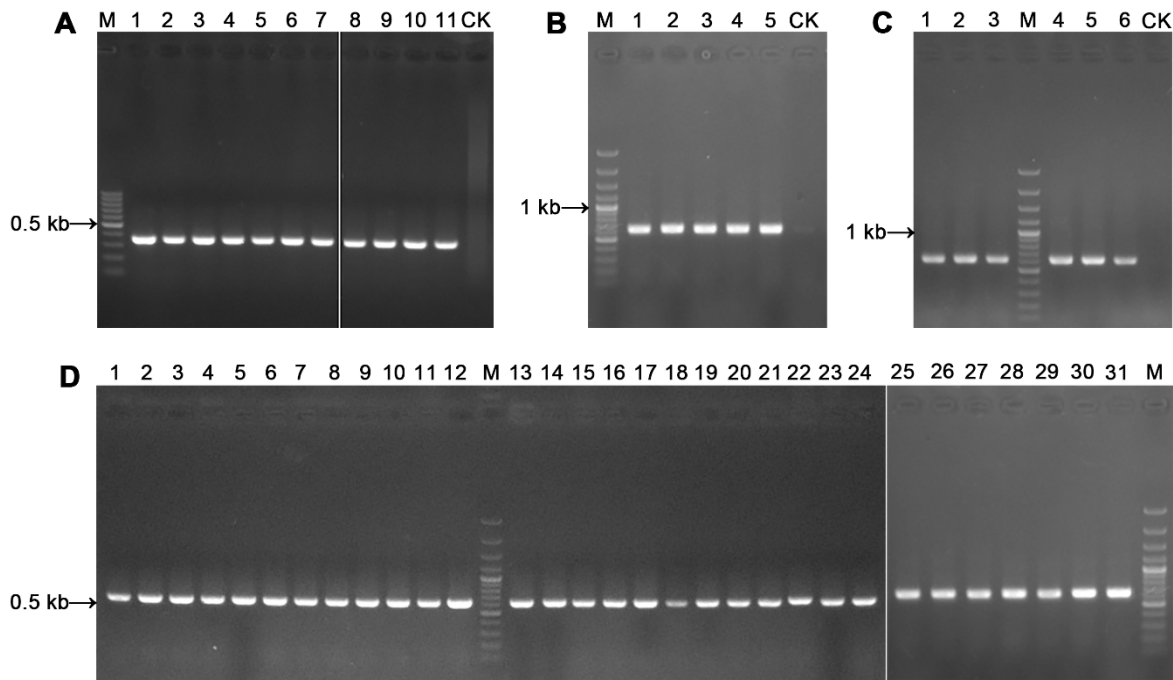


Figure S5. PCR analyses of transgenic *T₀* lines transformed with CRISPR/Cas9 knockout vectors of *BcMF30a* and *BcMF30c*. Transgenic plants transformed with pBI-sgRNA-a (A, lanes 1 to 7), pBI-sgRNA-c (A, lanes 8 to 11), pCA-sgRNA-a (B, lanes 1 to 5), pCA-sgRNA-c (C, lanes 1 to 6), pCA-sgRNA-ac (D, lanes 1 to 31). Lanes CK indicated the negative control. Lanes M indicated DNA molecular marker.

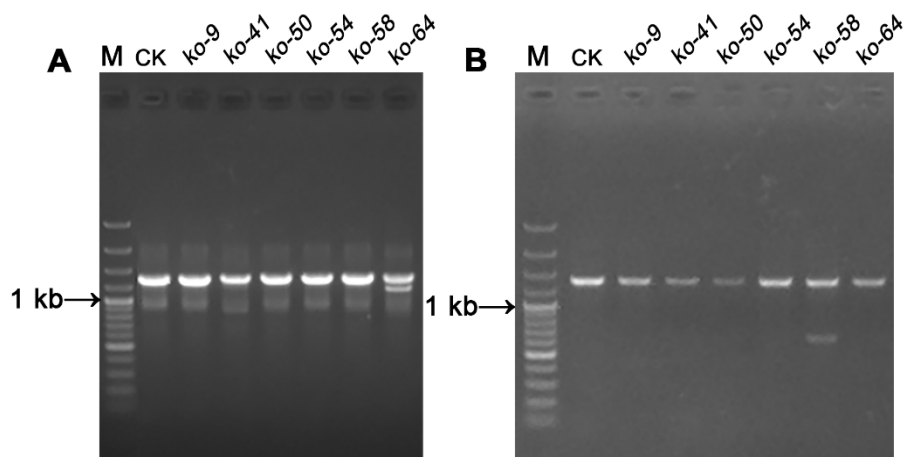


Figure S6. PCR analyses of *BcMF30a* and *BcMF30c* in pCA-sgRNA-ac transgenic *T₀* lines. (A) The amplification results of *BcMF30a*. About 200-bp deletion in *BcMF30a* was occurred in ko-64. (B) The amplification results of *BcMF30c*. About 850-bp deletion in *BcMF30c* was occurred in ko-58.

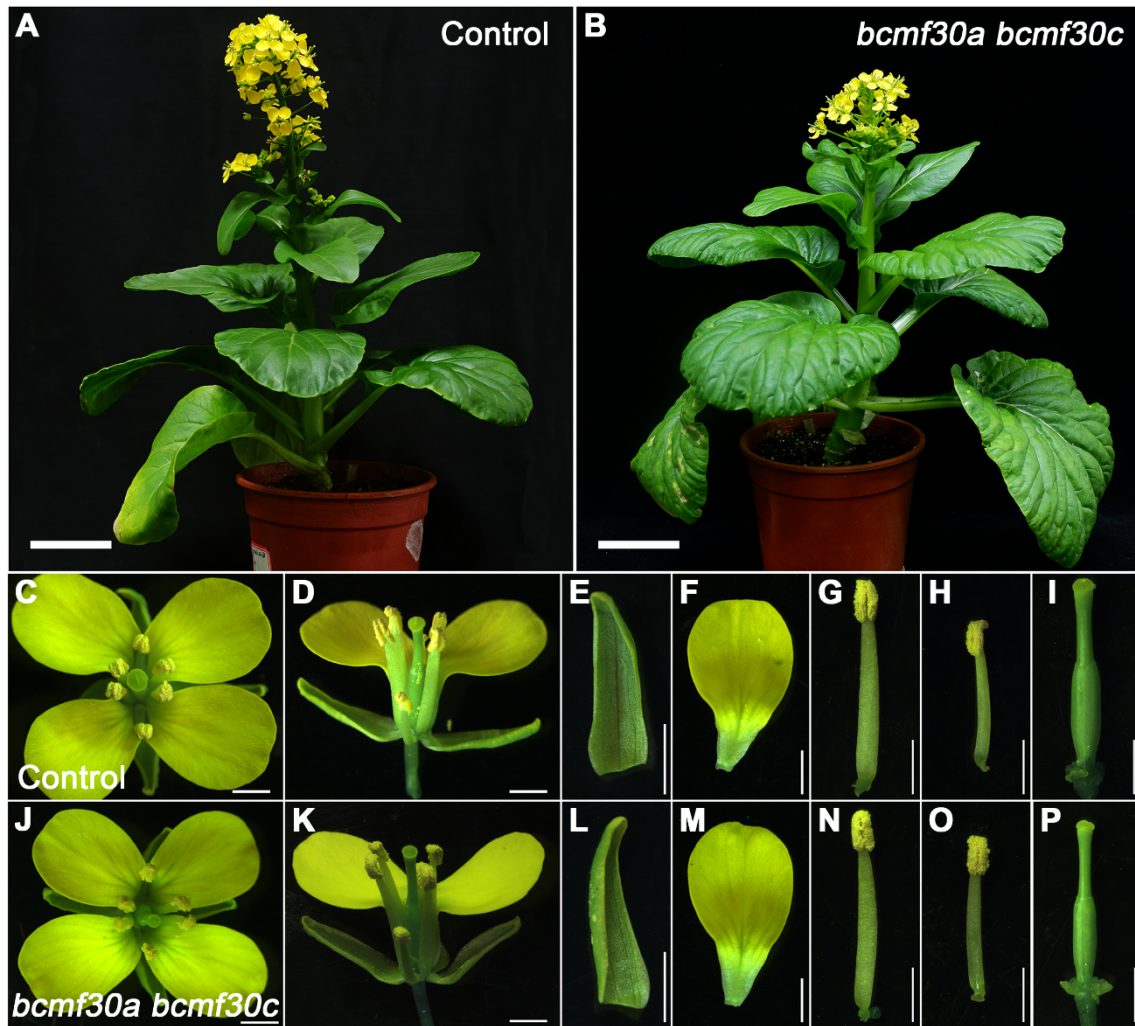


Figure S7. Morphological observation of plants and floral organs of *bcmf30a bcmf30c* plants. (A) and (B) showed the plants of Control and *bcmf30a bcmf30c*, respectively. (C) to (I) and (J) to (P) showed the flower, sepal, petal, stamen and pistil of Control and *bcmf30a bcmf30c*, respectively. Bars = 5 cm in (A) and (B), and 2 mm in (C) to (P).