

RBP45D –At5g19350

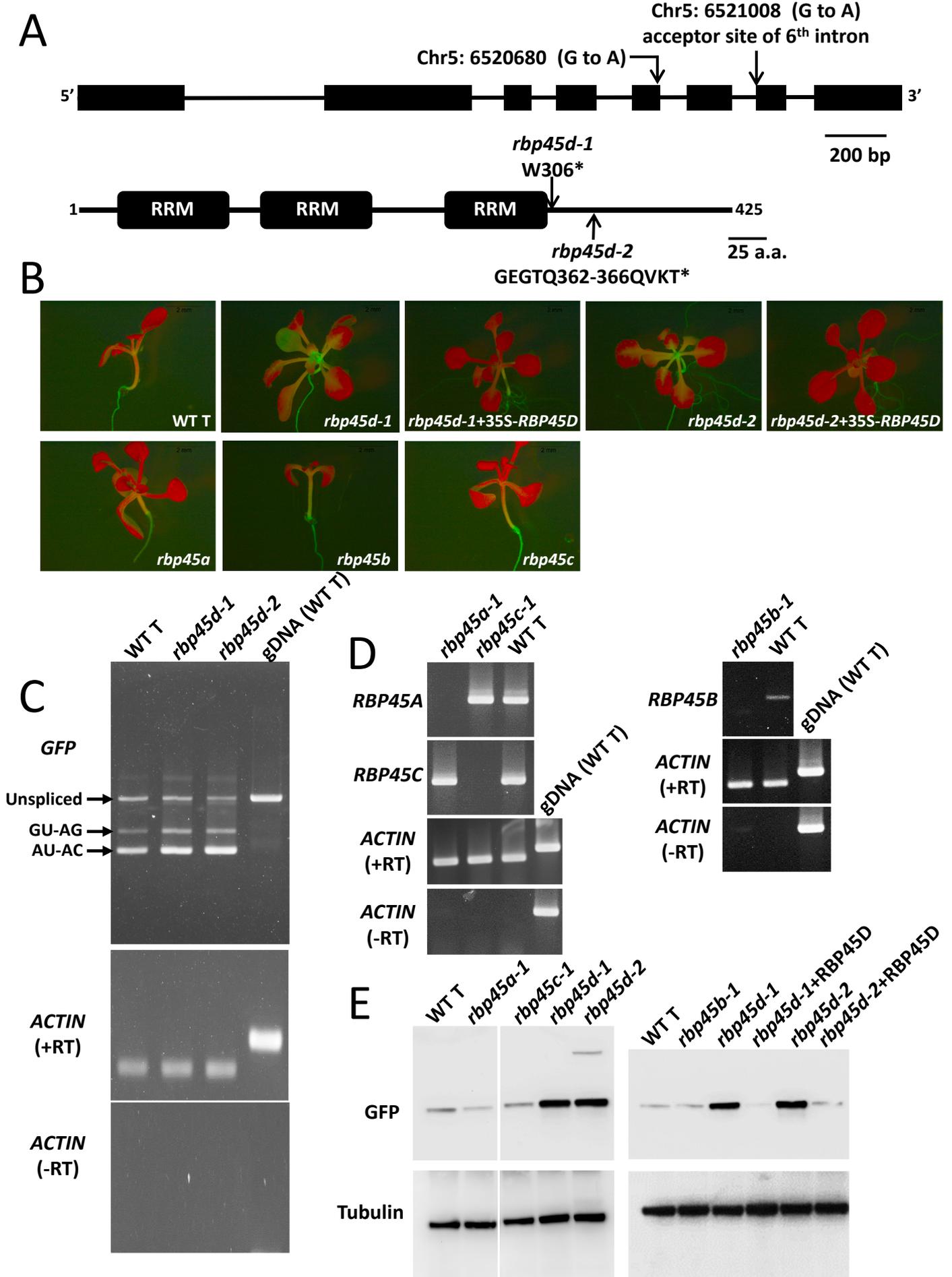


Figure S1: RBP45D

- A. Top: Intron-exon structure of the *RBP45D* gene (At5g19350) and positions of nucleotide changes in mutants. Bottom: Positions of RRM (RNA recognition motif) domains in the RBP45D protein (425 amino acids) and the position of the amino acid substitution in *rbp45d-1* and the consequence of the splice site mutation in *rbp45d-2*.
- B. GFP fluorescence in seedlings with the indicated genotypes. The two *rbp45d* mutants are hyper-GFP relative to the WT T line. The two complemented mutants, which show a return to more WT T levels of GFP, are additionally labeled with '+35S-*RBP45D*' (35S denotes the 35S promoter of cauliflower mosaic virus). The *rbp45a*, *b* and *c* T-DNA insertion mutants all have GFP fluorescence levels similar to the WT T line.
- C. Semi-quantitative RT-PCR indicating levels of the three *GFP* splice variants in the two *rbp45d* mutants. The levels of both the translatable AU-AC transcript and the untranslatable GU-AG transcript are somewhat elevated in the mutants, suggesting increased splicing efficiency of both non-canonical and canonical splice sites in *GFP* pre-mRNA. Actin, with and without reverse transcriptase (RT), was used as a constitutively expressed control. gDNA, genomic DNA control.
- D. RT-PCR to demonstrate loss of the corresponding wild-type *RBP45* transcripts in the *rbp45a-1* and *rbp45c-1* (left) and *rbp45b-1* (right) T-DNA insertion mutants. Actin, with and without reverse transcriptase (RT), was used as a constitutively expressed control.
- E. Western blot indicating no increase in GFP protein levels in the *rbp45a-1* and *rbp45c-1* (left) and *rbp45b-1* (right) mutants relative to the WT T line whereas GFP protein increases in the two *rbp45d* mutants, consistent with their hyper-GFP phenotype. Consistent with the GFP fluorescence levels in seedlings (part B), the elevated levels of GFP protein in the two *rbp45d* mutants return to the wild-type level after introducing an RBP45D construct under the control of the 35S promoter (*rbp45d*+RBP45D). Antibodies to tubulin were used to probe the blot to control for loading levels,