

ABSTRACT

The plant hormone auxin in maternal tissues plays a major role in promoting seed growth. However, whether auxin affects seed growth in maternal tissues in or outside the seed is unclear. In the process of investigating the roles of the SKP1-CULLIN-F-BOX (SCF) ubiquitin ligases in Arabidopsis reproductive development, using protein extracts from inflorescence tissues, we found that, among the six auxin receptors, only AUXIN SIGNALING F-BOX1 (AFB1) and 5 (AFB5) were consistently co-immunoprecipitated with the ARABIDOPSIS SKP1-LIKE1 protein. This result suggests that AFB1 and 5 play major roles in reproductive development. We then found that seeds from *afb1* and 5 mutants exhibited reduced sizes (weights). The largest reduction in seed weight occurred in *afb5-6*; the average weight/1000 dry seeds of *afb5-6* was approximately 76% of that of the wild type. The seed weights were further investigated in *AFB1* and *AFB5* transgenic plants with the transgenes driven by their respective promoters or the *ASK1* promoter. The results from the T₂ seeds and homozygous T₃ and T₄ seeds suggest that the wild-type levels of the two transcripts are not limiting for seed growth and farther increasing their levels adversely affects seed growth. Histochemical studies of transgenic plants harboring the *AFB1:GUS* or *AFB5:GUS* transgene revealed that *AFB1* and *AFB5* are not expressed in the seed but in the vascular tissue in the fruit wall and the funiculus. Taken together, these results show that AFB1 and AFB5 promote seed growth in maternal tissues outside the seed.

INTRODUCTION

Maternal auxin signaling has been implicated in regulating seed growth, but it is unknown which auxin receptors and where they act to affect seed growth. Furthermore, it is unclear whether the level of maternal auxin signaling in wild type plants is a limiting factor for seed growth. A better understanding of the role of auxin signaling in seed growth regulation is not only important to the knowledge of seed biology, but it may also impact biotechnology development for altering crop seed (grain) sizes.

RESULTS

AFB1 and AFB5 are likely the most predominant AFBs in reproductive tissues

Table 1. SCF components in inflorescence identified by Co-IP (anti-FLAG antibody and protein extracts from *ASK1:ASK1-FLAG* plants) and LC-MS/MS in four independent experiments

Protein identified	Times identified	Biological function
ASK1	4	Many functions
CUL1 (CULLIN1)	3	Many functions
F-box proteins		
AFB1	4	Auxin signaling
AFB5	4	Auxin signaling
SKIP16	4	Possibly in carbohydrate transport and metabolism
COI1	3	Wound-/jasmonate-induced transcriptional regulation
At5g06550	2	Unknown function; with a JmjC domain
At4g00755	2	Unknown function; with a conserved domain in plants
ATPP2-B11	1	Carbohydrate binding, phloem protein
SKIP22	1	Unknown function, homologous to yeast Met30p
AFB2	1	Auxin signaling
At1g27540	1	Unknown function
A11g25141	1	Unknown function
At1g67190	1	Unknown function
At1g13570	1	Unknown function
At1g25150 ^a	1	Unknown function
At1g64840	1	Unknown function; with a conserved domain in plants

^a[At1g25150](#) is identical in amino acid sequence to other four proteins encoded by closely situated genes [At1g25055](#), [At1g24881](#), [At1g25211](#), and [At1g24800](#) on chromosome 1.

Average seed weight was reduced in *afb1* and 5 mutants

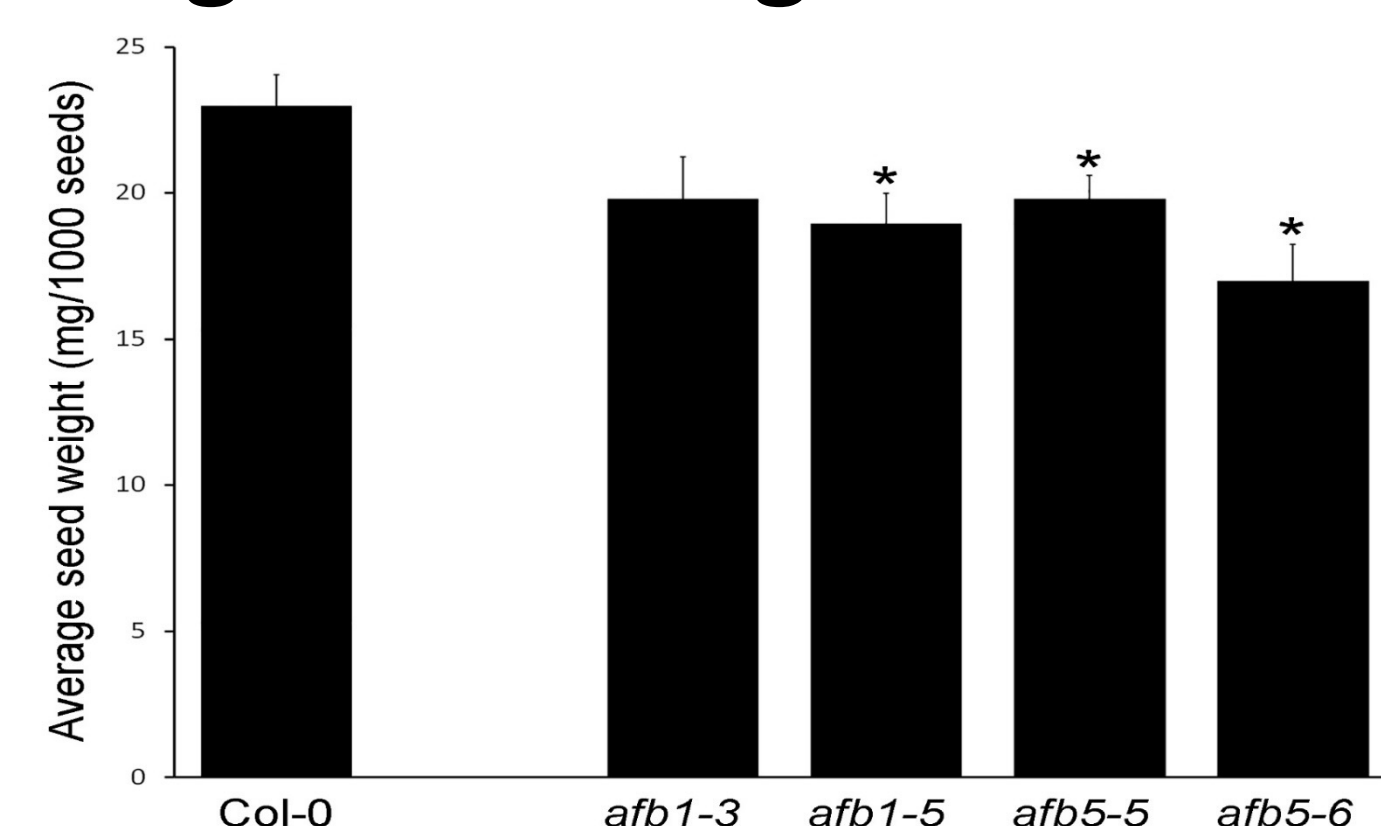


Fig. 1. Average weights/1000 seeds of Col-0 and other genotypes. Standard error bars are shown. Weights are of dry seeds. * Denotes statistically significant differences between the mutants and Col-0 (4-6 lines of each genotype were investigated; t-test, $P < 0.05$).



Fig. 2. Seeds of Col-0 and *afb1* and *afb5* mutants. A) Col-0. B) *afb1-3*. C) *afb1-5*. D) *afb5-5*. E) *afb5-6*. Bar in (A) for all images = 1 mm.

Reduction in average seed weight was more sensitive to the transcriptional reduction of *AFB5* than that of *AFB1* (compare Fig. 1 with Fig. 2)

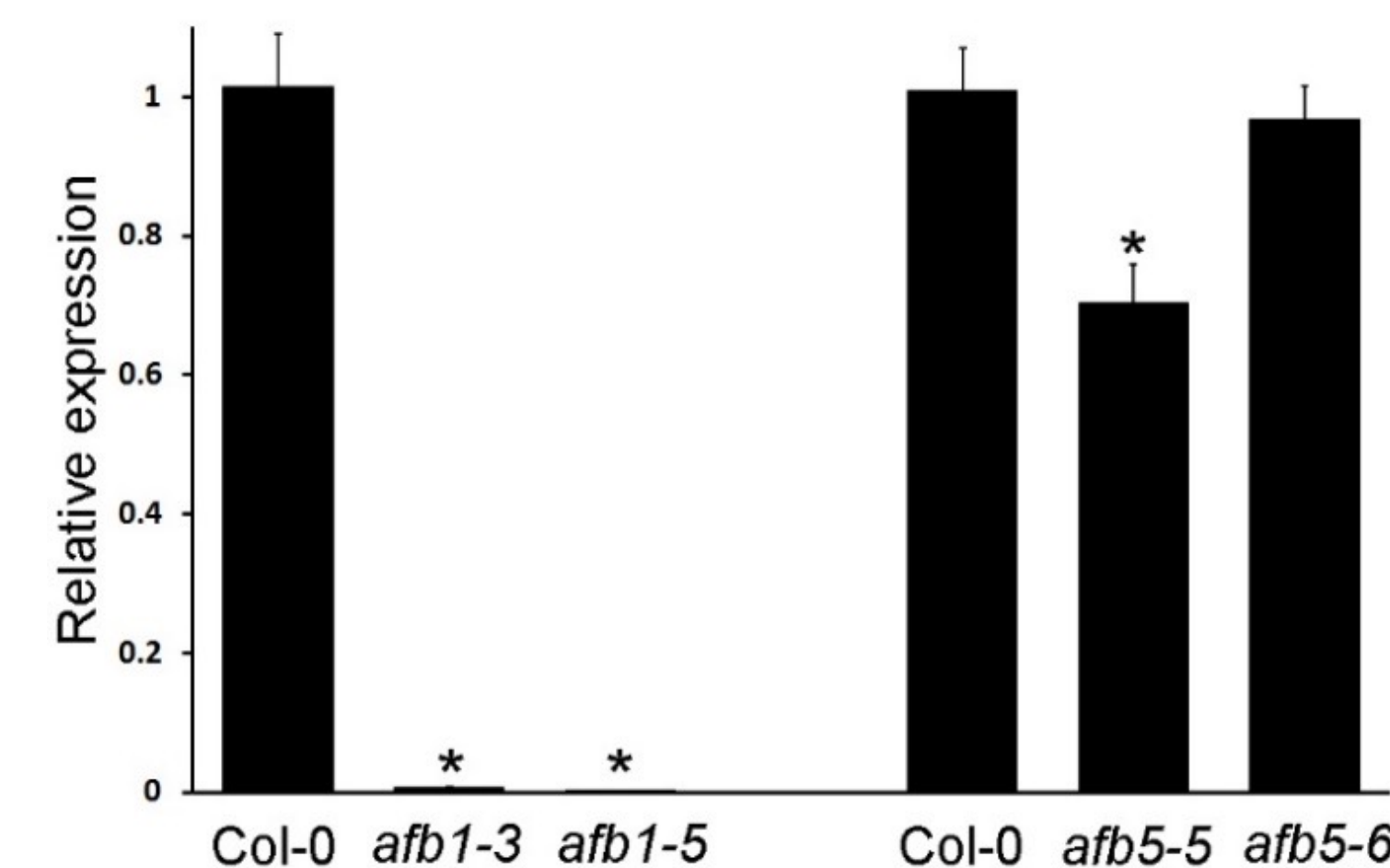


Fig. 3. Relative expression levels of *AFB1* and *AFB5* in Col-0 and their respective mutants. Shown are means \pm standard errors. For each genotype, 3 biological replicates and 3 technical replicates were performed. The expression level in Col-0 is defined as 1. * Denotes statistically significant differences between the mutants and Col-0 (t-test, $P < 0.05$).

Hemizygous T₁ and homozygous T₂ and T₃ *AFB1:AFB1* and *AFB5:AFB5* transgenic plants produced seeds with average weights either similar to or lighter than that of the wild type

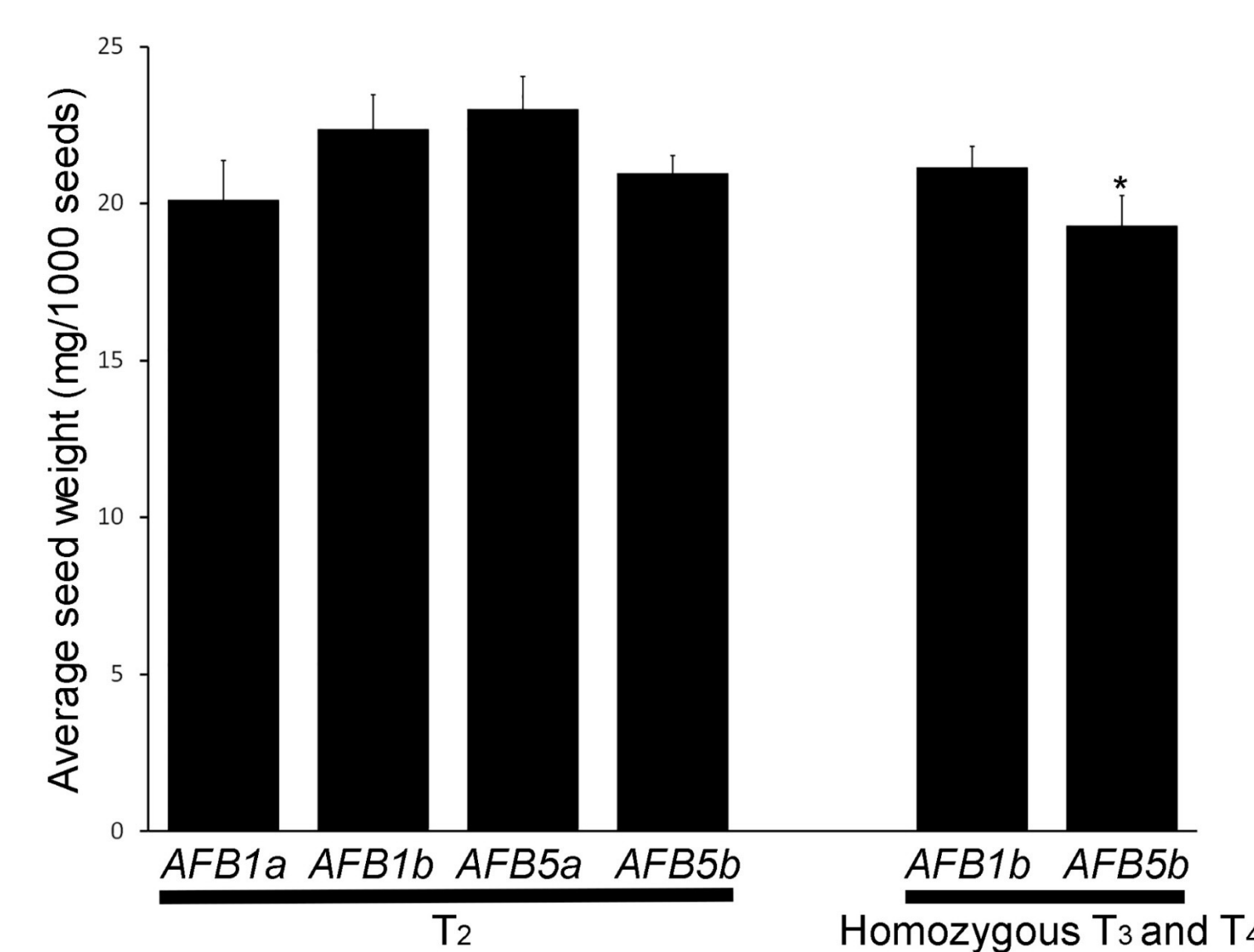


Fig. 4. Average weights/1000 T₂ and homozygous T₃ and T₄ seeds of *AFB1:AFB1* and *AFB5:AFB5* transgenic plants. Standard error bars are shown. Weights are of dry seeds. * Denotes statistically significant difference between *AFB5b* and Col-0 (4-6 lines of each genotype were investigated; t-test, $P < 0.04$). *AFB1:AFB1* and *AFB5:AFB5* were transformed into *afb1-3* and *afb5-5*, respectively. Expression levels of *AFB1* and *AFB5* in the T₂ plants were similar to or somewhat higher than those in Col-0, respectively (RT-qPCR, not shown). *AFB1a*—*AFB1:FLAG-AFB1*, *AFB1b*—*AFB1:AFB1-FLAG*, *AFB5a*—*AFB5:FLAG-AFB5*, and *AFB5b*—*AFB5:AFB5-FLAG*.

ASK1:AFB1 and *ASK1:AFB5* transgenic plants produced seeds with average weights either similar to or lighter than those of seeds produced by the *AFB1:AFB1* and *AFB5:AFB5* transgenic plants

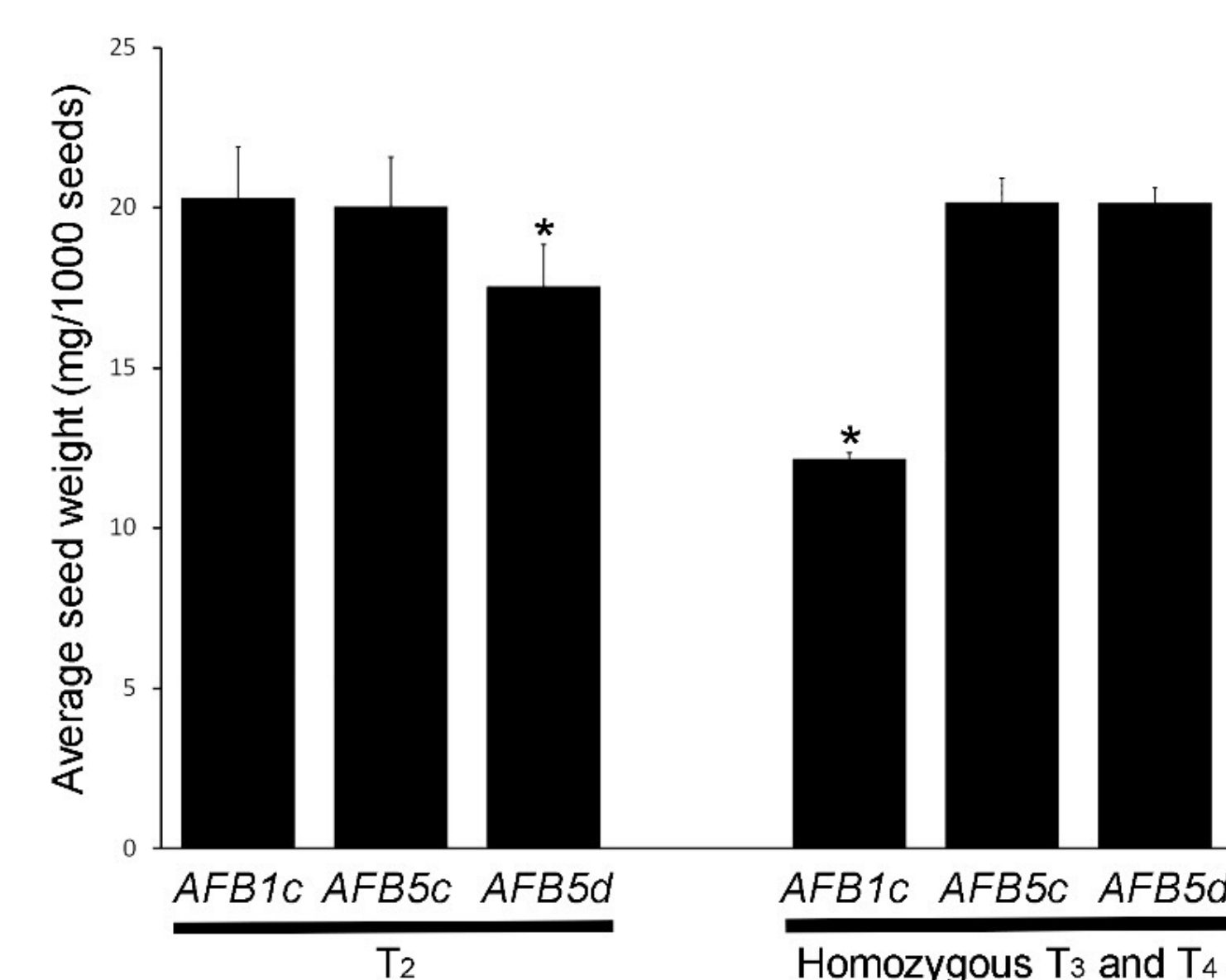


Fig. 5. Average weights/1000 T₂ and homozygous T₃ and T₄ seeds of *ASK1:AFB1* and *ASK1:AFB5* transgenic plants. Standard error bars are shown. Weights are of dry seeds. * Denotes statistically significant differences between these lines and Col-0 (4-6 lines of each genotype were investigated; t-test, $P < 0.04$). *ASK1:AFB1* and *ASK1:AFB5* were transformed into *afb1-3* and *afb5-5*, respectively. Expression levels of *AFB1* and *AFB5* in the T₂ plants were approximately 2-3-fold higher than the transgenic plants in Fig. 3 (RT-qPCR, not shown). *AFB1c*—*ASK1:FLAG-AFB1*, *AFB5c*—*ASK1:FLAG-AFB5*, and *AFB5d*—*ASK1:AFB5-FLAG*.

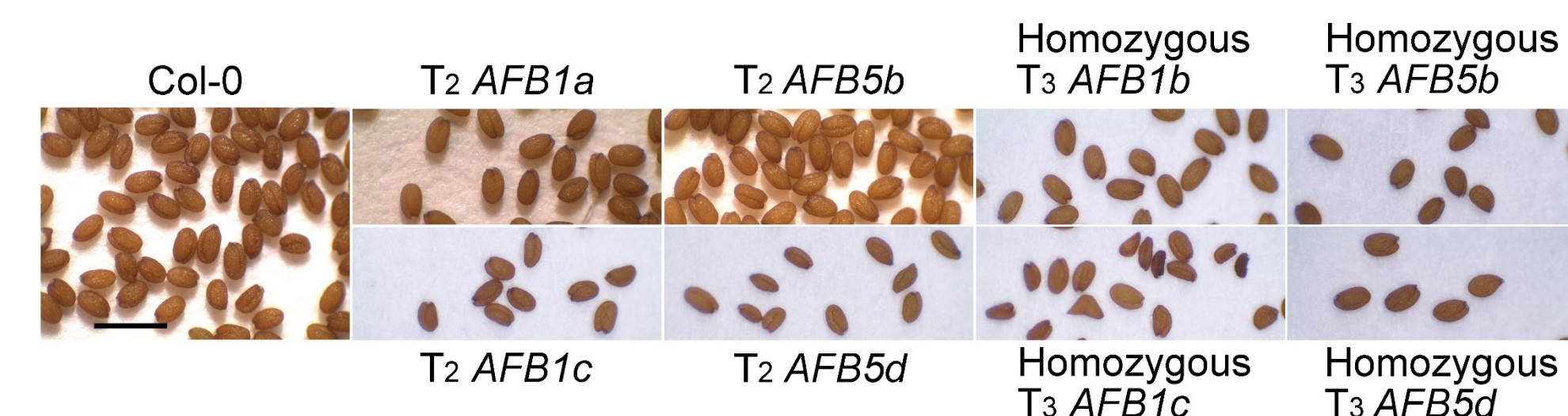


Fig. 6. Seeds of Transgenic plants.

Designations of transgenic plants are the same as in Figs. 4 and 5. Bar in Col-0 is for all images = 1 mm.

AFB1 and *AFB5* are expressed in the fruit wall and funiculus but not in the seed

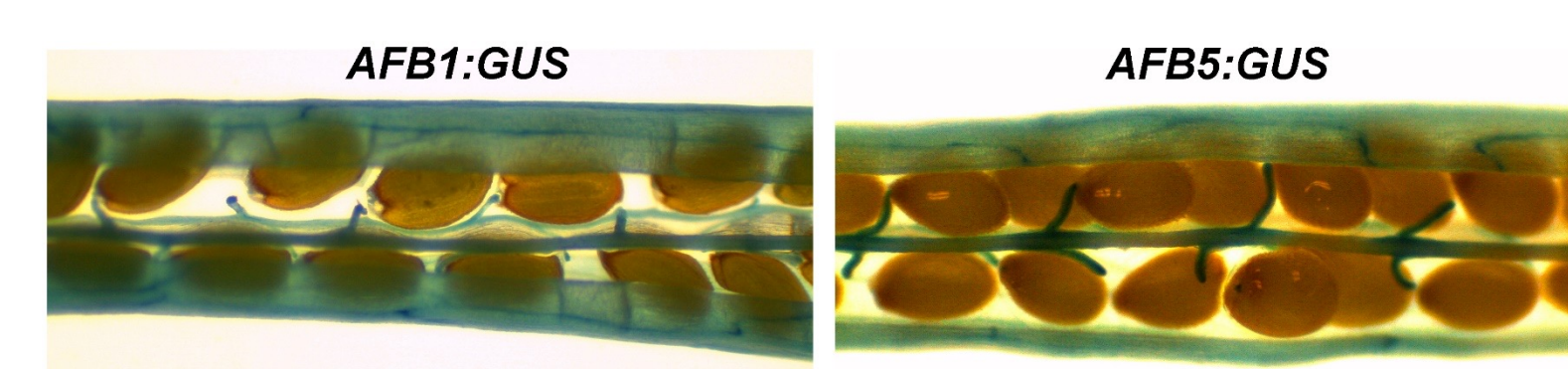


Fig. 7. Representative GUS-staining patterns in siliques of *AFB1:GUS* and *AFB5:GUS* transgenic plants.

CONCLUSIONS

- AFB1 and AFB5 outside the seed promote seed growth.
- AFB1 and AFB5 may act in the vascular tissue in the fruit wall and/or the funiculus to affect seed growth
- The wild-type transcript levels of *AFB1* and *AFB5* are not limiting for seed growth and farther increasing them has an adverse effect on seed growth.
- A posttranscriptional defect in *afb5-6* may be responsible for the reduction in seed weight.