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trap lines. Expression of GFP in the male gametophyte of A) GFP-104; B) GFP-670; C) GFP-785 and D) GFP-868 promoter trap lines. Arrow head indicates the region of GFP expression in anther. The pollen of GFP-785 are deformed and collapsed as indicated with arrow heads.

**Fig. 4.4** Analysis of GFP transcripts in the selected promoter trap lines by semi-quantitative RT-PCR.

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**Fig. 4.10** Histochemical GUS assay of transgenic *Arabidopsis* plants showing bi-directional nature of the promoter. A and C, diagrammatic representation of the constructs prepared for expression analysis. Expression construct was prepared by cloning the upstream sequence of *At4g10596* (461 bp) in pORE-R2 reporter vector in sense and reverse orientations in between HindIII and SacI sites. B and D are the GUS expression pattern in the anthers of transgenic *Arabidopsis* plants. GUS localization was observed in mature anthers. Ba, anther; Bb and Bc, closeup view of anther sac and pollen, respectively; Da, inflorescence; Db, anther sac; Dc, closeup view of anther sac. MF and MR: M13 forward and M13 reverse primer binding sites; PENTCUP2: Tobacco cryptic constitutive promoter.

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**Fig. 4.27** Molecular characterization of GFP 785 and SALK_0146328. A) T-DNA insertion in 7th exon of *At5g26290*; B) T-DNA insertion site in SALK_0146328; C) Molecular characterization for T-DNA insertion in GFP 785 with primers (Fp= forward primer and Rp= reverse primer; Ho = homozygous, WT= wild type and Ht= heterozygous); D) and E) Molecular characterization for T-DNA insertion in SALK_0146328 with primers (4EF= 4th exon forward; 5ER= 5th exon reverse and STL= Salk T-DNA left primer) on six individual plants.

**Fig. 4.28** Expression pattern of *At5g26290* in WT *Arabidopsis* plants. A) *in silico* analysis of expression pattern using GENEVESTIGATOR microarray database; B) expression pattern of *AT5G26290* in different tissues of WT Arabidopsis; and C), expression of *At5g26290* gene in floral parts of WT and mutant lines.

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**Fig. 4.33** DIC image of whole mount mature anthers from *Attraf1-1* and *Attraf1-2* *Arabidopsis* mutant lines. Red arrow head indicates the abnormal pollens.

**Fig. 4.34** Alexander’s staining of pollens of *Attraf* mutant lines. The pink stained pollens are viable and differently colored stained pollens are non-viable. In set are enlarged views of pollen. Arrow heads indicates the non-viable pollen.

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**Fig. 4.36** DAPI analysis of the WT and and Attraf1-1 and Attraf1-2 Arabidopsis mutant lines pollen. A) images were taken under UV-light with fluorescence microscope, B) DIC image of the pollens for the same microscopic field. Arrow heads showing the pollens which did not attained tri-nuclear stage.

**Fig. 4.37** DAPI analysis of Attraf mutant lines showing pollen with variable number of nuclei. Data was statistically validated using DMRT where * denotes means of two samples are significantly different at p<0.001

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**Fig. 4.39** SEM analysis of pollens surface architecture of WT and Attraf1-1 and Attraf1-2 Arabidopsis mutant lines. Panel A) resolution at 50 µm and B) resolution at 10 µm.

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**Fig. 4.41** Whole mount DIC images of ovules showing arrestation at different developmental stages at the time of anthesis A) Representative pictures of ovules considered to be at different FG developmental stage; B) Ovules in which embryosac is degenerated; B-I) ovules at FG1 stage and B-II) ovules at FG1 stage in which all the four megaspores are surviving; B-III) shows the results of crosses of Attraf1-2 with different marker lines. C-a) and C-b) results of crosses of Attraf1-2 X ET884, the normal developed ovule shows GUS expression in synergid cell (C-a), but no expression was recorded for ovules with four nuclei (C-b); Cc-f) Results of crosses of Attraf1-2XDD65, the normal developed ovule shows GFP expression in central cell (Cc-d, C-c, DIC image of the ovule and C-d, ovule observed under UV fluorescence), but no expression was recorded for ovules with four nuclei (Ce-f, C-e, DIC image of the ovule and C-f, ovule observed under UV fluorescence).

**Fig. 4.42** Whole mount DIC images of ovules at post-fertilization stage revealing success of either of the two fertilization events leading to development of either endosperm or zygote. A) Ovules in which only endosperms has been developed in Attraf1 mutant of Arabidopsis; B) ovules showing only the zygote formation in mutants. * denotes endosperm nuclei. The area marked with yellow line showing development of embryo after fertilization.

**Fig. 4.43** Preparation of expression construct for complementation. A) Diagramatic representation of T-DNA expression construct in
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Fig. 4.44 Phenotype of Attraf complemented line. A) Silique length and number of ovules per silique; B) whole mount DIC image of mature anther; C) Alexander’s staining of mature anther; D) DAPI staining of pollens; E) SEM analysis and F) ovule at maturity; where a = WT and b= complemented line.

Fig. 4.45 Expression analysis of selected down-stream gametophyte development pathway genes in Attraf mutant.

Fig. 5.1 Depiction of the functional position for AtTRAFl gene in gametophyte developmental pathway based on the results of mutant phenotype and TRAF1 promoter expression as a function of GUS reporter gene.