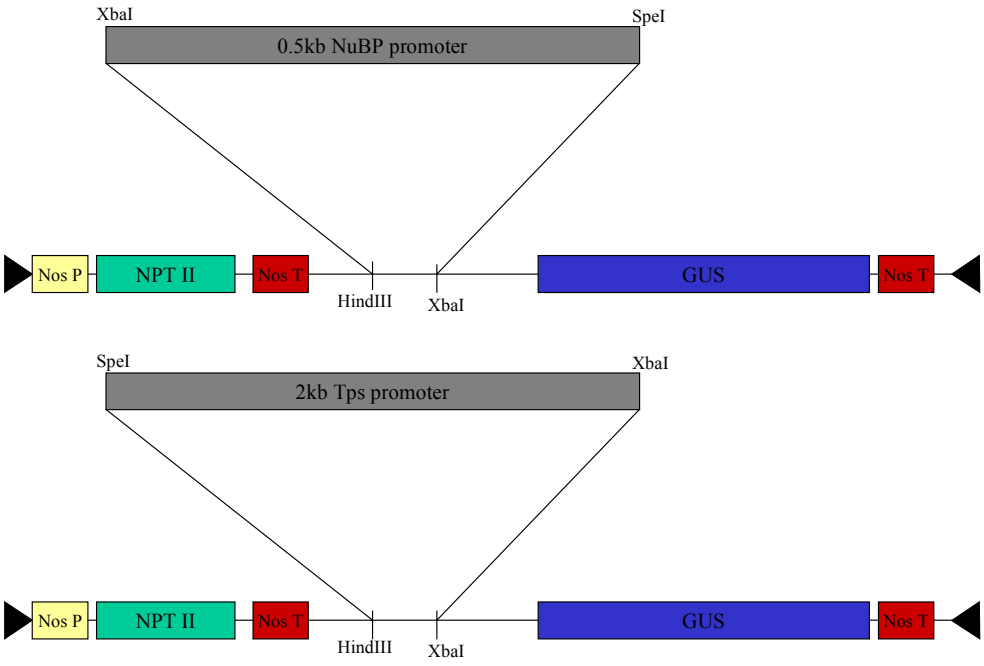

Figure 4-1. Schematic diagram of promoter:*GUS* constructs in pBI 121-derived binary vector that were used to visualize expression of genes identified in *Cypripedium parviflorum* in *Arabidopsis*.
Promoter:GUS fusions were created to analyze expression of genes homologous to *C. parviflorum* in *Arabidopsis*. Homologs of *NuBP* and *Tps* from *C. parviflorum* were identified in *Arabidopsis* through BLAST search at the National Center for Biotechnology Information website. The promoters of both *NuBP* and *Tps* were cloned from *Arabidopsis* genomic DNA using primers designed to amplify the region from the stop codon of the upstream gene to the start codon of either *Tps* or *NuBP*. Promoters were cloned into pGEM T easy and sequenced to confirm the identity of the promoter. The binary vector, pBI 121 was digested with *HindIII*, followed by end-filling to create a blunt end. This was followed with digestion with *XbaI*. This resulted in liberation of the 35S CaMV promoter. *NuBP* promoter was released from pGEM T easy by digestion with *XbaI* followed by end filling and digestion with *SpeI*. *Tps* promoter was released from pGEM T easy by digestion with *SpeI* followed by end filling and digestion with *XbaI*. Promoters were then ligated into pBI 121 in the sense orientation adjacent to the β-glucoronidase (*GUS*) coding region. The *NptII* gene driven by the *Nos* promoter (Nos P) allowed for selection by conferring resistance to kanamycin. Nos T corresponds to the *Nos* terminator.

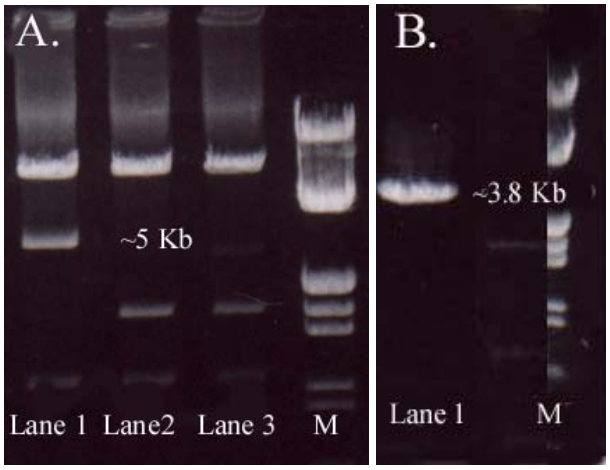

Figure 4-2. Confirmation of *AtTps* promoter:*GUS* fusion in *E. coli*.

A. Colonies of *E. coli* transformed with pBI 121 containing the *AtTps* promoter *GUS* construct that showed resistance to kanamycin were grown overnight in terrific broth. Bacterial cells were pelleted by centrifugation and DNA extracted by alkaline lysis. Resultant DNA was digested with *BamHI* and *SphI* restriction enzymes, separated on a 1% agarose gel and visualized with ethidium bromide and UV light. The restriction enzymes were chosen to liberate a fragment of ~5 Kb (Lane 1) containing the *Tps* promoter, *Nos* terminator and most of the *NptII* gene. Lanes 2 and 3 represent colonies that did not show presence of the promoter in pBI 121. M: molecular size marker.

B. The presence of the *AtTps* promoter:*GUS* construct in the colony identified in Fig 4-2 A. Lane 1 was confirmed by PCR. DNA, extracted as above, was used as template in PCR with an *AtTps* forward primer and a *GUS* reverse primer. Expected band of ~3.8 Kb (Lane 1) was produced. M: molecular size marker.

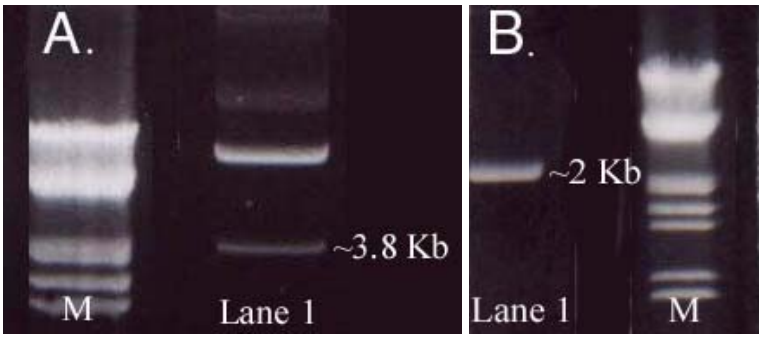

Figure 4-3. Confirmation of *AtNuBP* promoter:*GUS* fusion in *E. coli*.

A. A single colony of *E. coli* transformed with pBI 121 containing the *AtNuBP* promoter *GUS* construct that showed resistance to kanamycin was grown overnight in terrific broth. Bacterial cells were pelleted by centrifugation and DNA extracted by alkaline lysis. Resultant DNA was digested with *BamHI* and *SphI* (Lane 1) restriction enzymes, separated on a 1% agarose gel and visualized with ethidium bromide and UV light. The restriction enzymes were chosen to release a fragment of ~3.8 Kb (Lane 1) containing the *NuBP* promoter, *Nos* terminator and most of the *NptII* gene. M: molecular size marker.

B. The presence of the *AtNuBP* promoter:*GUS* construct in the colony identified in Fig 4-3 A. was confirmed by PCR. DNA extracted as above was used as template in PCR with an *AtNuBP* forward primer and a *GUS* reverse primer. Expected band of ~2 Kb (Lane 1) was produced. M: molecular size marker.

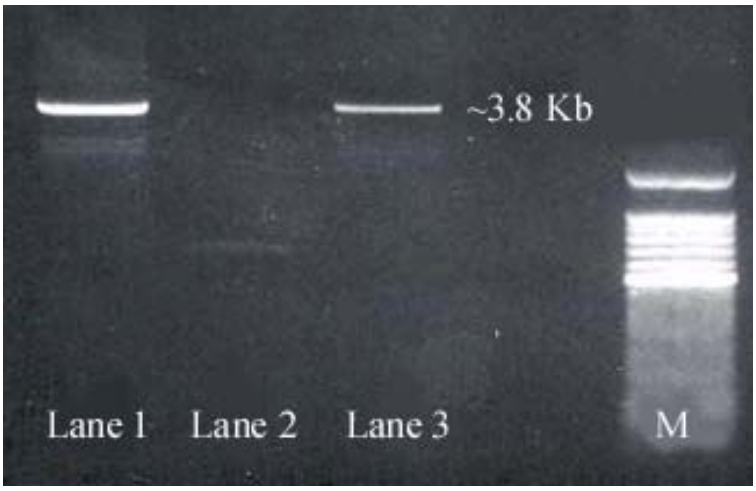

Figure 4-4. Confirmation of presence of *AtTps* promoter:*GUS* fusion transgene in *Arabidopsis* plants using PCR.
Three plants of T1 generation *Arabidopsis* were identified as showing *GUS* expression. DNA was isolated from leaves of the T2 generation of the three *Arabidopsis* lines showing GUS expression. PCR was performed with the *GUS* reverse primer and the forward primer for *AtTps* promoter, which should generate a fragment of approximately 3.8 Kb. PCR products were separated on a 1% agarose gel and visualized with ethidium bromide and UV light. The three lines of transformed *Arabidopsis* are represented by Lanes 1, 2 and 3 respectively. Lanes 1 and 3 show the expected fragment of ~3.8 Kb. M: molecular size marker.

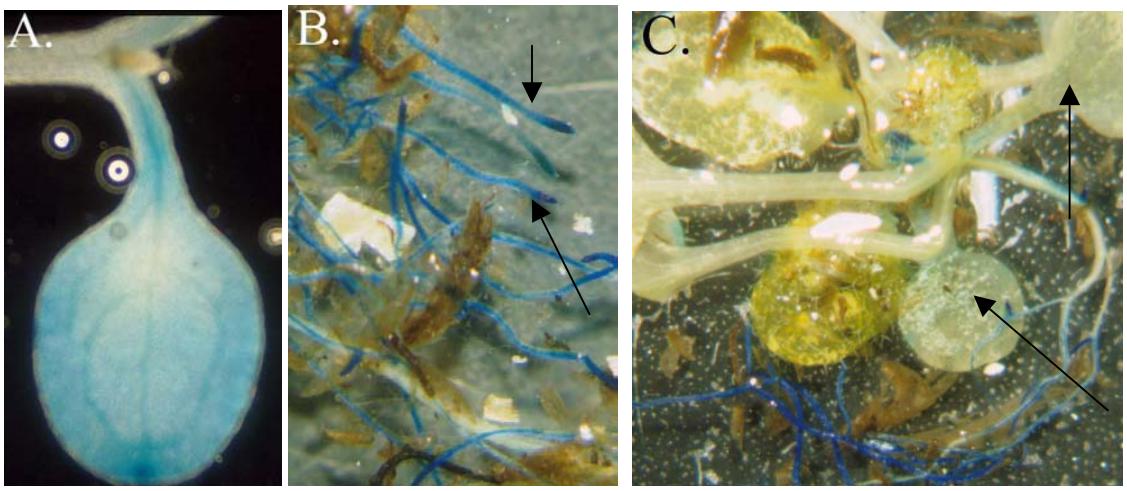

Figure 4-5. *AtTps* promoter-driven *GUS* expression in *Arabidopsis* seedlings and plants. Seeds of transformed *Arabidopsis* were sown on germination media (A) or in potting medium (B and C). After germination and at various times during plant growth, seedlings or plants were removed from the growing medium. Seedlings or plants were immersed in *GUS* staining solution, which was vacuum infiltrated. The *GUS* staining solution contains 5-bromo-4-chloro-3-indoyl-β-d-glucoride (X-gluc) which when cleaved by *GUS* emits a blue color. Seedlings or plants were then incubated at 37°C for 24 h and photographed under a dissecting microscope. Seedlings showed expression (blue color in cotyledons (A). Plants showed expression in roots (B, arrows) but not in mature leaves (C, arows).

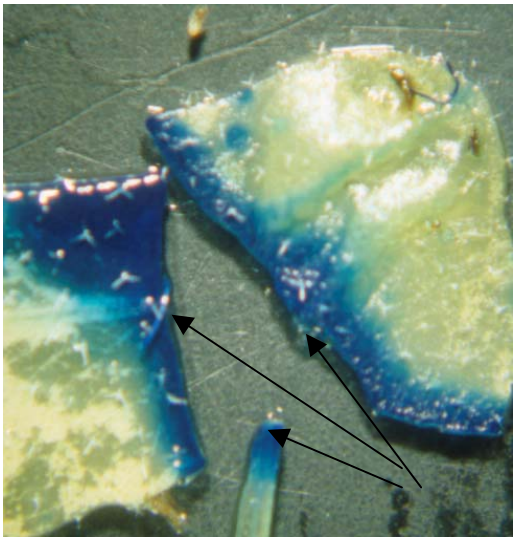

Figure 4-6. Wound induced expression of *AtTps* promoter-driven *GUS* in leaves of *Arabidopsis*.
Plants of transformed *Arabidopsis* were grown in soil-less potting medium. Leaves and stems were cut from plants and immediately placed in *GUS* staining solution (as in Fig. 4-5), which was vacuum infiltrated. Leaves and stems were then incubated at 37°C for 24 h and photographed under a dissecting microscope. GUS expression can be seen as blue color along cut surfaces (arrows)

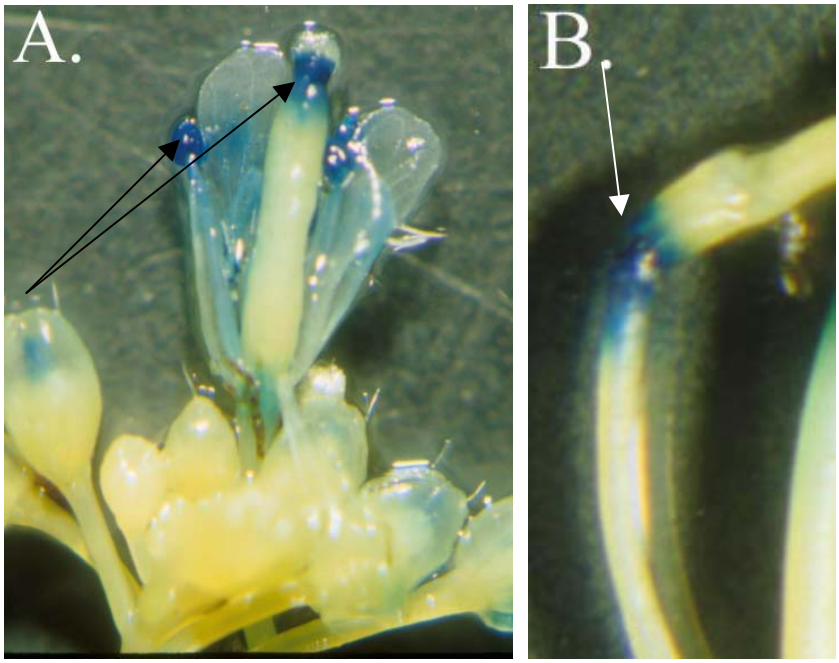

Figure 4-7. *AtTps* promoter-driven *GUS* expression in *Arabidopsis* flowers and fruits. Plants of transformed *Arabidopsis* were grown in soil-less potting medium. Inflorescences were cut from plants and placed in *GUS* staining solution (as in Fig. 4-5), which was vacuum infiltrated. These were incubated at 37°C for 24 h and photographed under a dissecting microscope. Flowers (A) show expression (blue color) in anthers and stigmas (black arrows). Fruits (B) show expression in the silique pedicel junction (white arrow).

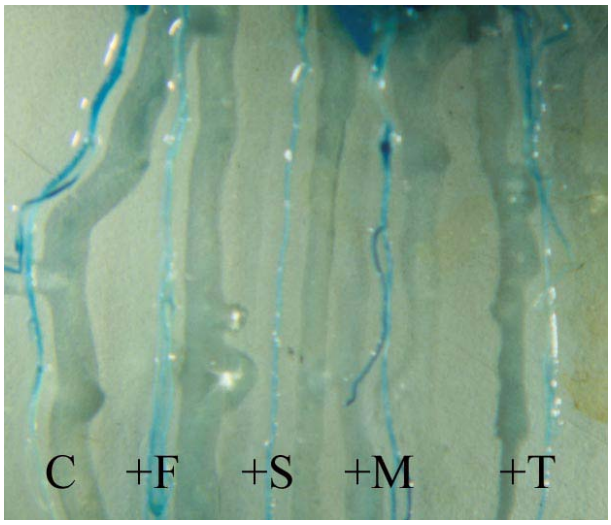

Figure 4-8. Expression of *AtTps* promoter-driven *GUS* in roots of *Arabidopsis* grown with various sugars and a fungus (*Thanatephorus pennatus*).
Seeds of transformed *Arabidopsis* were germinated on GM with 12.5 mM sucrose. After seedlings had developed true leaves they were transferred to oatmeal agar plates overgrown with *Thanatephorus pennatus* (+F) or to GM plates with either 25 mM sucrose (+S), 25 mM mannitol (+M) or 25mM trehalose (+T). Plants were removed from respective treatments after 72 h. The roots were excised and placed in *GUS* staining solution (as in Fig 4-5), which was vacuum infiltrated. These were incubated at 37°C for 24 h and photographed under a dissecting microscope. *GUS* expression (blue color) appears to be consistent across treatments indicating that the various treatments did not affect the *AtTp*s promoter driven expression of *GUS*.

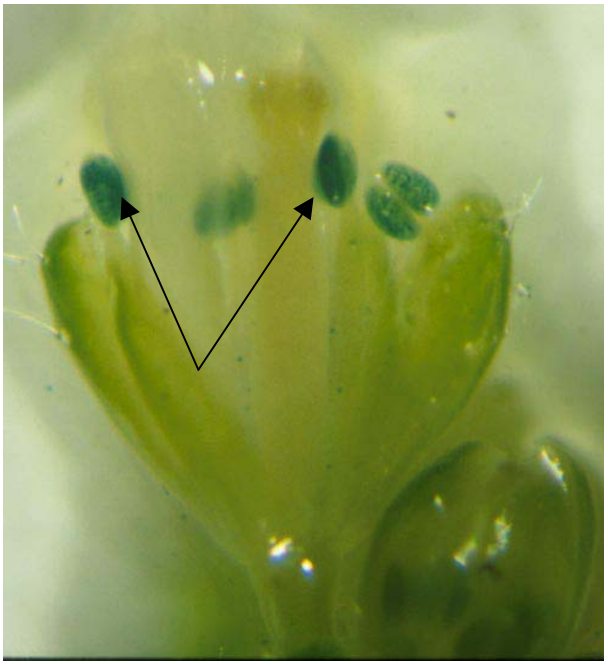

Figure 4-9. *AtNuBP* promoter-driven *GUS* expression in *Arabidopsis* flowers. Plants of the T1 generation of *AtNuBP* transformed *Arabidopsis* were grown in potting medium. Inflorescences were cut from flowering plants placed in *GUS* staining solution (as in Fig. 4-5), which was vacuum infiltrated. These were incubated at 37°C for 24 h and photographed under a dissecting microscope. *GUS* expression (blue color) can be seen within anthers (arrows).