CHAPTER 6

SUMMARY AND CONCLUSIONS

Using promoter trap strategy, a promoter region which regulates expression of reporter gene in the anther of Arabidopsis thaliana was isolated. The points of the study are given bellow.

1. The T-DNA tagged mutants generated earlier were characterized preliminarily for kanamycin segregation ratio and GUS expression. The ratio indicated presence of single T-DNA insert in most of the lines.

2. The mutant anth85 was characterized and expression of GUS was detected restricted to the anthers of stage 5-9. Slight GUS expression was also detected in the 1\textsuperscript{st} two leaves of young seedling. The mutant carried insertions at two loci.

3. The T-DNA RB flanking sequences in anth85 were identified by inverse PCR method. One of the insert was located in the upstream region of putative peroxidase gene (At2G24800). Other insertion was found in the putative N-acetyl-gamma-glutamyl-phosphate reductase (At2G19940) and carried vector backbone sequences flanking the RB.

4. Both the inserts in anth85 were segregated from each other by crossing and backcrossing with wild type plants. A homozygous, single insert line, anth85/ 33 was identified by PCR based method and was confirmed by Southern hybridization.
5. The transcription start site of putative peroxidase gene was identified by 5'-RACE, which was mapped at 58 bp upstream of translation start site.

6. The putative peroxidase gene is functionally redundant under normal conditions as homozygous mutant plants show no phenotypic changes.

7. *In silico* analysis of upstream region of putative peroxidase gene revealed presence of several motifs generally found in the promoter region of plant peroxidases or stress inducible genes.

8. The long promoter fragment pDR-XY of putative peroxidase gene regulated GUS expression in young seedling and various plant parts like roots, inflorescence, silique base and stigmatic region etc. The transgenic plants expressed GUS in response to mechanical wounding. Similar pattern of expression of putative peroxidase gene transcript was detected by RT-PCR analysis in wild type plants.

9. Deletion of 120 bp toward the 5'-end results in the loss of GUS activity in the roots and in response to wounding. Deletion of 5'-UTR region results in the anther specific GUS expression. Thus by 5'- and 3'-deletion analysis a smaller promoter fragment pDR-BC1, effectively regulating anthers specific GUS expression was identified.
10. Absence of either 120 bp upstream region or UTR region alone or both results in the loss of activity in the roots and upon wounding. The elements present in these two regions might be having some combinatorial effect.

11. The mutant st9 exhibits all the characteristics similar to those mutants described earlier related to methyl jasmonate pathway.

12. The mutant carried two T-DNA inserts in the segregating population of \(st9/F_2\), which was confirmed by Southern

13. The work has identified a male sterile line (st9) which either might be the one already reported earlier (table 16) or might be a novel and important. Further molecular work has to be undertaken to resolve this issue.