SUMMARY

The present work has been undertaken mainly to monitor the expression of GUS gene under *Arabidopsis thaliana* 2S3 promoter (At2S3p), when introduced into tobacco, yeast and *Agrobacterium* separately. Though there are innumerable reports of expression of different genes under various plant promoters, very little efforts have been given to define a seed specific promoter in terms of its relative strength, efficiency and expression in different systems. Moreover, the very nature of specificity of promoters has also been questioned in the above perspective. Under this backdrop, attempts have been made to elucidate the following points.

1) The full length (1.5 Kb) At2S3 promoter with reporter gene GUS fused to it has been constructed and introduced into tobacco plant and it has been found that the integration of the desired construct in the genome of the transformed plant does take place.

2) The GUS gene has been found to be expressed only at a particular developmental stage of the plant viz. seed development. Its expression level was found to be negligible in other organs of the plant.

3) In contrast to its dependence at a particular period of seed development in plant, the At2S3 promoter has been found to be constitutively active in *Agrobacterium* and yeast.

4) The level of activity of At2S3 promoter in *Agrobacterium* was comparable to that in the tobacco plant while in yeast it was about one third to that in tobacco plant as is evident from fluorometric studies.

5) The expression of the GUS gene has been found proportional to its transcription as evidenced from the northern analysis.

6) A common transacting factor (~16KDa) has been detected in Southwestern blot analysis in all the three systems. In tobacco and *Agrobacterium*, in addition to the band around 16KDa region, other bands of higher molecular weights were also detected.

7) The possibility of 16KDa factor as a transacting factor has been stemmed from the observation that if the leaves of the transformed tobacco plant was pretreated with abscisic acid (ABA), Southwestern experiment with At2S3 promoter indicated appearance of 16KDa band and under this condition the At2S3 promoter could be shown to behave as an active promoter in the leaves. The efficiency of At2S3 promoter in the ABA treated leaves was reflected at the concomitant appearance of GUS gene product as determined fluorometrically.

8) The appearance of this 16KDa protein in the ABA treated leaves was due to the de novo synthesis of this protein has been demonstrated by the observation that pretreatment of the leaves with cycloheximide inhibited the reappearance of this 16KDa protein.

9) Though it has been possible to augment the level of GUS activity in the leaves pretreated with ABA, it was not possible to increase the level as observed in flowering stages of the plant. The level of GUS gene transcript was much higher in case of flowering stages of the plant than in leaves pretreated with ABA.

10) The efficiency of AT2S3 promoter has also been compared with CaMV35S promoter and pyruvate kinase promoter in yeast. It has been found that pyruvate kinase promoter (an endogenous yeast promoter) was 45 fold more active than At2S3p which in turn was 32 fold more active than CaMV35S promoter.

11) From primer extension analysis, it has been found that transcription start point (tsp) of GUS gene transcript under At2S3p in yeast was initiated at a point which was 36 nucleotides upstream from A of the initiation codon ATG of At2S3p, which is also the tsp of GUS gene under the same promoter in plant.

12) In case of CaMV35Sp, one major and several minor tsp were found, none of which is similar to that reported for plant.

13) The 940 bp At2S3, so far sequenced has been found to contain most of the conserved sequence of a typical eukaryotic promoter in general and seed specific promoter in particular.

14) The G box motif along with its context sequences upstream of putative translation start point of At2S3p has been found to bind with nuclear protein extract of yeast, flowering stages of tobacco plant as well as cell extract of *Agrobacterium tumefaciens*.

15) Gel mobility shift assay with the same labelled G box sequence in case of nuclear extract of flowering stages of the plant as well as cell extract of *Agrobacterium tumefaciens* showed the presence of multiple complex retarded at different positions but again the position of the least retarded complex in

both the cases matches with the complex formed in case of yeast nuclear extract.

16) The specificity of the DNA protein complex has been demonstrated by the observation that an excess of homologous cold competitor abolished the complex formation significantly. On the other hand increasing concentration of non homologous competitor poly d(I)d(C) failed to abolish the complex formation.

17) The failure of RNase to abolish DNA protein complex formation ruled out possibility of small nuclear RNA being involved in the formation of the complex.

18) The addition of proteinase K abolished the complex formation significantly implying that protein is involved as a major component in the formation of the DNA protein complex.

19) Gel mobility shift assay in case of yeast nuclear extract with the mutated G box, where the CG of the core sequence was changed to AT fails to form the complex.

20) The At2S3 promoter sequence contains CT block region in concert with CAAG, typical of strong yeast promoter, though the nucleotide position varies.

21) The At2S3 promoter is recognised by *Agrobacterium* in a constitutive manner.

22) In the present study, nopaline synthase terminator has been used in all the constructs and the nopaline synthase terminator sequence is recognised by yeast.

23) Thus the seed specific At2S3 promoter and nopaline terminator sequence have been shown to be recognised by *Agrobacterium tumefaciens*,

Saccharomyces cerevisiae and plant systems implying that At2S3 promoter is not so specific as envisaged before.