

Transgenic plants are constantly being explored as a possible source to produce recombinant proteins due to low costs involved and easy scalability. There are a large number of reports to demonstrate the potential application of the technology in agriculture and industry. However, lack of high level expression of transgene in a desired tissue has limited this potential for its large application at industrial scale. Therefore, a reliable and versatile transgene expression system in plants is highly desirable as the demand for large scale production of several foreign proteins is steadily increasing. In many instances, post-translational modifications are necessary to obtain an active eukaryotic protein. In this study, we have developed a new expression system for foreign proteins in plants based on the transcription of the transgenes by bacteriophage T7 RNA polymerase that is highly specific for the T7 promoter. In this system, foreign gene expression is totally dependent on the transcription by the introduced RNA polymerase. To enable this process, the T7 RNAP was modified to contain nuclear localization signal.

To regulate the tissue specific expression of foreign genes, the T7 RNAP is expressed under a plant promoter that is highly specific for a particular tissue. To test the regulated tissue specific expression, the T7 RNA polymerase was put under several tissue specific plant promoters. Also for comparison, the GUS gene was cloned under all the tissue specific promoters directly. The *uidA* (GUS) was used to test the expression in tobacco and rice. Ferritin gene was transformed into rice to express in endosperm and HBsAg and Lycopene were transformed into tomato to express in fruit tissues.

Promoters for the small subunit of ribulose-bisphosphate carboxylase (*rbcS-3A*), stress inducible *kin1*, *cor6.6*, phenylalanine ammonia-lyase (*pall*) and *pallΔ* genes that express differentially in different tissues were tested for the expression in tobacco. The pITB228, pITB450, pITB550, pITB650, pITB750 and pITB850 constructs contained *uidA* placed under T7 promoter and terminator sequences and the modified T7 RNAP with NLS was placed under the control of *rbcS-3A*, stress

inducible *kin1*, *cor6.6*, *pal1*, and *pal1Δ* promoters, respectively. For a direct comparison, the *uidA* was also placed directly under *rbcS-3A*, *kin1*, *cor6.6*, *pal1* and *pal1Δ* promoters, in pITB441, pITB541, pITb641, pITB741 and pITB841 constructs, respectively. While *Agrobacterium* mediated transformation method was used to transform tobacco and tomato, particle gun (biolistic) mediated method was used to transform rice. PCR and Southern blot analysis indicated the stable integration of transgenes into the nucleus randomly. Expression of foreign genes was analyzed among 10 – 15 independently transformed transgenic plants. Results from the use of these promoters demonstrated that recombinant protein can be expressed at several fold high (3 – 10 times) as compared to transgene expressed directly under tissue specific promoters directly. Further analysis involving large number of transgenic plants from each group of promoter revealed that variations in expression was low among independently transformed plants under T7 system as compared to large variations observed for transgene expression directly under plant promoters.

The transcript initiation analysis showed that the recognition of the T7 promoter by T7 RNAP was highly specific in plants as well. Primer extension analysis showed that the GUS transcripts initiated from the nucleotide ‘G’, specific for T7 promoter, demonstrating that the T7 RNAP recognize its promoter in the randomly integrated plant genome

To test the wider application of T7-expression system in plants, we have examined the expression of GUS in rice, a monocot plant and a major source of staple food worldwide. Similar to tobacco, GUS activity under CaMV 35S promoter using T7-system was high in leaves and roots of transgenic plants when compared to GUS expressed directly under CaMV 35S promoter. Northern blot analysis suggested that the increased level of GUS expression is due to increase in *uidA* transcription. Quantitative analysis revealed that the GUS expression was 3 – 5 times high in plants that were independently transformed with pITB228 construct when compared to GUS expression in plants that were transformed with

pCAMBIA1301. Transgenic rice plants with ferritin gene under the regulation of GluB1 promoter:T7 RNAP were raised and work is in progress to analyze the transgene expression. Similarly, large number of tomato plants were produced and transferred to greenhouse. These plants will be analyzed for the expression of HBsAg and changes in lycopene content in the fruit tissue.

To test the inducible expression of foreign genes under T7-system, tetracycline inducible expression system in plants was used. Kinetics of de-repression was studied using northern blot analysis, real time polymerase chain reaction and GUS activity. Our results showed that GUS expressed after 12 hours of Tc-treatment with maximum activity at 48 - 72 hours. The co-expression of both T7 RNAP and *uidA* after Tc-treatment coupled with the presence of GUS activity clearly demonstrated inducible expression of GUS under T7-system, extending the application of this powerful tool to various programs in plant biotechnology and to genomic studies.

By applying bacteriophage T7 RNAP system in combination with tissue/organ specific promoters in transgenic tomato and rice we are hopeful to improve amounts of micronutrients as well as vaccine content in transgenic plants to supplement to humans/animal through daily food source. Our results, in principle, proved that the new strategy not only worked in the model plant of tobacco but also worked in a crop plants. These results should pave the way to express several foreign genes that are useful in agriculture and in industry. However, further work is needed to test the final utility of this strategy with several genus and several other plant species.