Summary and Conclusions
The hepatitis B virus infection is estimated to have 400 million people throughout the globe, making it one of the most common human pathogens. Since immunization is the only method to prevent the disease of the hepatitis B virus, any attempt to reduce its infection requires the availability of large quantities of vaccine the hepatitis B surface antigen HBsAg. In the current study, protocol for making transgenic *Cucumis sativus* that express hepatitis B surface antigen was developed through *Agrobacterium* mediated transformation and for the tissue specific expression of the transgene, fruit specific promoter was isolated. As a conclusion we can say that,

- Our studies revealed that the hepatitis B surface antigen was successfully integrated in to the plant genome using the subcloning vector pRT101 and binary vector pCAMBIA3300.
- The presence of the transgene was primarily analyzed by PCR and the copy number and integration of the gene was confirmed by Southern hybridization. Some of the putative transformants showed more than one copy number of the transgene when compared to the other transformants.
- Even though some of the transformants showed weak signals in Southern, they showed almost same level of expression in the case on Northern hybridization.
- When Western blot was carried out, all the transformed plants produced a functional protein of molecular weight approximately 24kDa. The recombinant protein was expressed in Bl21(DE3) strain of *E.coli* revealed the cloning of the HBsAg was in the right orientation in the pET 32c and the protein profile of the induced sample resulted the recombinant protein having a molecular weight 44kDa including the tag.
- Our result indicates that hepatitis B surface antigen can be cloned and expressed in *Cucumis sativus*, ideal plant available throughout the year.
- In order to confine the transgene expression in tissue level, fruit specific promoter was isolated and sequenced.
- The expression analysis of expansin gene by real time PCR revealed that the gene has its high level of expression in the ripened fruit.
- The isolated 1.5kb promoter region was cloned in to pGEMT easy vector system. The sequence analysis indicated the presence of TATA boxe and several fruit specific motifs. The expansin promoter-GUS construct was
developed for transient expression analysis in the fruit and leaf tissues of *Cucumis sativus*.

- The CaMV 35 S promoter fused to GUS gene uniformly expressed in the leaf and fruit tissues while the expansin promoter expressed only in the fruit slices indicating the specificity.

- In conclusion, the promoter sequence analysis reveals the presence of fruit specific conserved domains such as GCN4 motif, Skn motif, TGTCAC motif etc with significant homology to other reported fruit specific promoters. This fruit specific promoter isolated in the present study can be used in the transgenic research especially when fruit specific expression is essential as in the case of edible vaccine programme.