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Title of Thesis : **Molecular characterization of *cotton leaf curl virus* (CLCuV) promoter**

Abstract

Cotton leaf curl virus belongs to the genus *Begomovirus* of family *Geminiviridae*. The begomoviruses have single-stranded circular DNA genome of 2.7 kb, which are either monopartite or bipartite, and transmitted by whitefly (*Bemisia tabaci*) insect vector. The genome of monopartite begomovirus is organized into six open reading frames (ORFs). The coat protein gene (*V1*) and *V2* are expressed from the viral sense strand while *Rep* (*C1*), *C2*, *C3* and *C4* genes are expressed from the complimentary sense strand. The intergenic region of monopartite and common region of bipartite begomovirus possess bidirectional promoter. Bidirectional promoters possess the capability of expressing two genes simultaneously, thereby making them superior and functionally more efficient than the normal unidirectional promoter. Insufficient/scarce availability of such promoters arises a need and demand for designing and developing functional bidirectional promoters for use in plant molecular biology and biotechnology. During the course of my Ph.D., bidirectional promoter representing complementary sense and virion sense gene of *Cotton leaf curl Burewala virus* (CLCuBuV) were characterized at molecular level. The nucleotide sequence of bidirectional promoter was initially analyzed using PlanatCARE, PLACE, Cister and PlantPAN databases. Analysis of the promoter sequence of CLCuBuV revealed the presence of several putative *cis* elements such as G-box, stem-loop motif, TATA boxes, a GC-rich region (GTGGGCCCTACC) and a conserved late element. The transcription strength of these promoters was assayed both in stable and transient expression systems in tobacco (*Nicotiana benthamiana* and *N. tabacum*) as well as cotton (*Gossypium hirsutum*) plants. It was compared with that of 35S promoter of *Cauliflower mosaic virus* (CaMV) which is more frequently used in plant genetic engineering. The bidirectional promoter of CLCuBuV and CaMV 35S promoter were fused with *GUS* and *GFP* reporter genes and was quantified using fluorometric GUS

assay, reverse transcription quantitative real-time PCR and confocal laser scanning microscopy (CLSM). Notably, the expression level of GUS driven by CLCuBuV complementary sense promoter in the transformed *N. tabacum* plants was shown to be 4 fold higher than that of CaMV 35S promoter, while the expression by CLCuBuV virion sense promoter was slightly lower than that of CaMV 35S promoter. Further, the expression of GFP was monitored and compared in agroinfiltrated leaves of *N. benthamiana*, *N. tabacum* and *G. hirsutum* plants using CLSM. CLCuBuV complementary sense promoter showed strong consistent transient expression in *N. benthamiana*, *N. tabacum* and *G. hirsutum* leaves as compared to CaMV 35S promoter. The strong constitutive CLCuBuV bidirectional promoter developed in this study could be very useful for high level constitutive expression of transgenes in a wide variety of plant cells.