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# Characterization of Novel Bi-Directional Promoter from Begomovirus: A Breakthrough in Plant Genomics

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**Abstract :** Begomoviruses belonging to the family Geminiviridae, have single-stranded circular DNA genomes that are monopartite or bipartite. The large intergenic region (LIR) of the monopartite and common region (CR) of bipartite begomoviruses possess promoter activity in their genomes. In this study, we have characterized novel bidirectional promoters from Cotton leaf curl Burewala virus (CLCuBuV) genome using high-throughput software and analyzed with PlantCARE, PLACE, Cister and PlantPAN databases. The promoters (Rep and CP promoters) were assayed both in stable and transient expression systems in tobacco as well as cotton plants. Rep and CP-based promoters from the LIR sequence of CLCuBuV and 35S promoter of Cauliflower mosaic virus (CaMV) were tagged with β-glucuronidase (GUS) and green fluorescent protein (GFP) reporter genes to check the efficacy of the promoters. Histochemical staining of GUS in transformed tobacco (Nicotiana tabacum cv. Xanthi) leaves showed higher GUS expression driven by CLCuBuV Rep (complimentary sense) promoter as compared to conventional CaMV 35S promoter and CLCuBuV CP (virion sense) promoter, respectively. GUS activity in individual plant cells driven by CLCuBuV Rep, CLCuBuV CP, and CaMV 35S promoter were quantified through fluorometric GUS assay and reverse transcription quantitative real-time PCR (RT-qPCR). The expression level of GUS tagged with CLCuBuV Rep promoter in the transformed tobacco plants was obtained 2 to 4 fold higher than CaMV 35S promoter. When CLCuBuV CP promoter was used, lower expression level was monitored than that by CaMV 35S promoter. The expression of GFP-tagged with CLCuBuV promoters was also investigated through agroinfiltration. The CLCuBuV Rep promoters showed stronger consistent transient expression in the leaves of N. benthamiana, N. tabacum and Gossypium hirsutum plants when compared with CaMV 35S and CLCuBuV CP promoter.

**Keywords :** Begmovirus, bidirectional promoter, CaMV 35S promoter, GFP, GUS, qPCR
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