ABSTRACT

Begomoviruses are plant-infecting viruses, which are responsible for major crop losses worldwide and are transmitted by the whitefly vector *Bemisia tabaci*. They have a genome of single-stranded DNA that consists of either a single (monopartite) or two components (bipartite) with a component size of approximately 2.7 kb. Many monopartite begomoviruses in the Old World have been found to be associated with betasatellite and alphasatellite molecules, which are about half the size of their helper begomovirus genome. Betasatellites have been shown to be necessary for inducing severe disease symptoms. These DNA β contributes in symptom production and enhance the helper virus DNA accumulation. DNA β encodes the single gene called βC1 on the complementary strand, which is important for pathogenicity and suppression of post transcriptional gene silencing. Tomato is an important Solanaceous plant which ranks second behind potato in terms of production of all vegetable crops. Yield of tomato is a major constrain as it is affected by the various abiotic and biotic factors. Tomato leaf curl disease is one of the major factor which significantly reduces the production of tomato. It is caused by different strains of Tomato leaf curl virus causing upto 100% yield loss in some part of India. Various attempts have been done by the plant breeders through conventional breeding to combat this viral disease. Till now, no successful reports have been highlighted leading to the immunity against this virus. Recent advances in molecular approaches have given us a new hope to generate the resistant/tolerant tomato plants. This includes identification of βC1 promoter element, role of βC1 in post transcriptional gene silencing and transcription regulation of βC1 gene expression. A region of upstream translation start site of βC1 of *Cotton leaf curl Multan betasatellite* (CLCuMB) associated with *Tomato leaf curl New Delhi virus* was tested for its promoter activity. Transcript mapping studies revealed that the transcript had a very short leader sequence of 12 nt and 3′ end was 9 nt downstream of stop codon at 186 nt coordinate position. Transient expression studies in *Nicotiana benthamiana* using the β-glucuronidase reporter gene driven by a βC1 promoter of CLCuMB identified a 169 nt region (between 720 and 869 nt coordinate) upstream of βC1 which is important for βC1 transcription. Histochemical GUS staining of selected solanaceous hosts indicated that βC1 promoter was active in leaves of *N. benthamiana* and *Nicotiana tabacum* and leaves and fruit of tomato. Temporal regulation studies on viral transcript using semi-quantitative PCR showed that the βC1 is a late phase gene and is expressed only 48 h post-inoculation. Virus encoded RNA-silencing suppressors
(RSSs) are the key components evolved by the viruses to counter RNA-silencing defense of plants. Whitefly-transmitted begomoviruses infecting tomato crop code for five different proteins, ORF AC4, ORF AC2 and ORF AV2 in DNA-A component, ORF BV1 in DNA-B and ORF βC1 in satellite DNA β which are predicted to function as silencing suppressors. In the present study suppressor function of ORF βC1 of three betasatellites *Tomato leaf curl Bangalore betasatellite* ToLCBB-[IN:Hess:08], *Cotton leaf curl Multan betasatellite* CLCuMB–[IN:Sri:02] and *Luffa leaf distortion betasatellite* LuLDB-[IN:Lu:04] were examined. Agroinfiltration of GFP-silenced *Nicotiana tabaccum* cv. Xanthi with the cells expressing βC1 protein resulted in reversal of silenced GFP expression. GFP-siRNA level was more than 50-fold lower compared to silenced plants in plants infiltrated with βC1 gene from ToLCBB. However, in the case of 35S-βC1 CLCuMB and 35S-βC1 LuLDB construct, although GFP was expressed, siRNA level was not reduced, indicating that the step at which βC1 interfere in RNA-silencing pathway is different.