INTERVENTION OF GEMINIVIRAL DNA REPLICATION THROUGH RIBOZYME MEDIATED DOWN REGULATION OF ITS REP PROTEIN

by

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DNA replication
Ribozyme

Dedicated

To

My Parents

CERTIFICATE

This is to certify that the thesis entitled **"Intervention of Geminiviral DNA replication through ribozyme mediated down regulation of its Rep protein"** *being submitted by* ***Ushasri Chilakamarthi*** *to the Indian Institute of Technology, Delhi, for the award of the degree of* ***Doctor of Philosophy****, is a record of the bonafide research work carried out by her under our supervision and guidance. The research work and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.*

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ABSTRACT

Ribozyme technology has opened up new avenues in the field of gene silencing due to its ability to specifically cleave its target mRNA and suppress the expression of that particular gene. Ribozyme consists of two domains namely substrate recognition domain and catalytic domain. Thus, theoretically, any RNA can be targeted by changing the sequences flanking the conserved catalytic core. We investigated the potential of hammerhead ribozyme in the downregulation of MYMIV (Mungbean Yellow Mosaic Indian Virus) DNA replication by targeting the mRNA of its Replication initiator protein (Rep). MYMIV belongs to *Begomovirus* subfamily of *Geminiviridae* family. Geminiviruses pose serious threat to economically important crops worldwide. They are transmitted by insect vectors and at present insect pest management is known to be the main control measure employed by farmers.

Since, they share common mode of replication and Rep shows high degree of homology among different species of geminiviruses, targeting Rep is likely to confer broad-spectrum resistance. The *rep* mRNA was scanned for the presence of GUC triplets 3' of which is the cleavage site of hammerhead ribozyme. In total 16 GUC triplets were found in this mRNA and the one in helix-2 region was chosen as target site as it is conserved among *rep* of various geminiviruses. Ribozyme designed to that site was validated by computer predicted secondary structure of *rep*-ribozyme complex and by *in vitro* cleavage reactions. When targeted to *rep* mRNA ribozyme showed 40% cleavage after 1 hr under *in vitro* conditions. The kinetic parameters, K_m and K_{cat} values of ribozyme were found to be 49.51nM and 0.216min^{-1}

respectively. It showed reasonable degree of cleavage at physiological pH (7.5) and Mg^{+2} (2 mM) concentrations, which indicated the probable *in vivo* activity of this ribozyme. Efficiency of ribozyme *in vivo* was evaluated in *S. cerevisiae*, a model eukayotic organism. A 30% reduction in colony number was observed after transformation on the selective medium in the case of ribozyme while the antisense control (mutant ribozyme) showed no significant reduction. Ribozyme targeted yeast cells bearing geminiviral *rep* showed decrease in specific growth rate and increase in generation time as revealed by growth curve analysis, suggesting that the ribozyme is also active *in vivo*. RT-PCR analysis also confirmed the catalytic activity of ribozyme and there was 50% reduction in the *rep* mRNA level compared to antisense control. As expected, ribozyme decreased the accumulation of the plasmid carrying geminiviral DNA in yeast by 65% compared to its antisense control. These results indicated the potential of the ribozyme constructed as antigeminiviral agent and its usefulness in developing MYMIV resistant mungbean varieties.

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