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Thesis Title: "STUDIES ON THE ROLE OF PRE-COAT PROTEIN GENE (ORF AV2) AND COAT PROTEIN GENE (ORF AV1) OF MUNGBEAN YELLOW MOSAIC INDIA VIRUS (MYMIV) IN PATHOGENESIS"

ABSTRACT

India is one of the major pulse producing countries of the world, with 37% of its total area under pulse cultivation, which accounts for more than 25% of the world's total pulse production. Yellow mosaic disease is one of the most devastating viral diseases of grain legumes in South-East Asia. In India, yield loss due to the disease is estimated to be $300 per anum. There are two virus species causing YMD in grain legumes, they are the species *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV) belonging to the genus *Begomovirus* of the family *Geminivirideae*. Objectives of the present study were-

1) To elucidate the role of *Mungbean yellow mosaic India virus* (MYMIV) pre-coat protein gene (ORF AV2) in pathogenesis with specific reference to replication.

2) To study the role of pre-coat protein gene (ORF AV2) and coat protein gene (ORF AV1) in whitefly transmission of MYMIV.

A series of site directed mutagenesis for ORF AV2 and deletion mutagenesis for ORF AV1 were performed to facilitate the work. Likewise to facilitate the work on replication ,an antisense construct, targeting Rep. gene was constructed. Additionally, since MYMV and MYMIV differ considerably in the ORF AV2, infectivity of both viruses were compared through Agroinoculation.

The salient findings of the study are summarized below :

- In the case of site directed mutagenesis, totally three mutants were used for Bg isolate. They are BgM_1, Bg del-A and BgW_2. Similarly five mutants were used for Cp isolate. They are CpK_{73}, CpC_{86}, CpC_{84-86}, CpG_{44} and $CpH_{15}G_{14}$.

- In the case of deletion mutagenesis, totally six deletions were performed , three for N'terminal (AV1-Δ75, AV1-Δ150, AV1-Δ211) and three for C'terminal (AV1-Δ57, AV1-Δ108, AV1-Δ160) of ORF AV1.
- Symptoms were severe than WT in mutants CpC_{86} and CpG_{44}.
- Coat-protein gene deletion mutants of Bg isolate of MYMIV were made, (BgAV1-cΔ57 for C-terminal and BgAV1-nΔ150 for N-terminal) and Agroinoculation of these mutants on legume hosts produced attenuation of symptoms. Southern blot analysis revealed high lavel of viral replicative forms in tha case of mutant BgAV1-nΔ150.
- As anticipated fully assembled particles were not detected in EM in these two mutants in EM, and whitefly transmission did not result in symptom production using Agroinoculated plants with these mutants as inoculum source.
- From the results it is clear that for MYMIV, coat protein is not required for systemic spread and disease expression in French bean, but is definitely required for cowpea and mungbean.
- The number of geminate particles in the plant leaf samples inoculated with wild types was much higher than the mutants per field of observation. Detection of geminate particles and whitefly transmission of mutants showed that site directed mutagenesis in ORF AV2 did not affect transcription and translation of ORF AV1.
- RNAi construct targeting Replicase gene of MYMIV-[Sb] was made in plant transformation vector, pBinAR and the efficiency of this construct was tested by co-Agroinoculation along with the infectious clones of MYMIV-Sb and the inhibition of the disease development was confirmed.
- Partial tandem repeat (PTR) constructs for DNA A and DNA B components of MYMV [Bg] were made in pBin19. Agroinoculation results showed that the type of symptoms produced with Bg isolate of MYMV and MYMIV were almost similar.
- Maximum infection was seen in French bean, in which the virus produced severe stunting and downward leaf curling of cotyledonary leaves.
- Bg isolate was infectious on cowpea cv.Pusa Komal, produced mild atypical leaf curl symptom and was replication incompetent, viral replicative forms could not be seen in Southern blot analysis.