INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Molecular Characterization of Begomoviruses Causing Yellow Mosaic Disease in Mungbean and Disease Management Through Biotechnological Intervention

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SHORT ABSTRACT

Yellow mosaic diseases (YMD), caused by Begomovirus, pose a significant threat to mungbean cultivation in the Indian subcontinent. This study investigates the epidemiology of begomovirus in three YMD hotspot regions, identifying Mungbean Yellow Mosaic Virus (MYMV) in Bihar and Mungbean Yellow Mosaic India Virus (MYMIV) in Assam and Orissa. The study explored the population structure and genetic diversity of MYMV and MYMIV isolates, revealing independent evolution of DNA-A and coevolution of DNA-B. To identify YMD-resistant mungbean genotypes, an agroinoculation-based genotype screening approach was employed. Using prepared infectious clones (MYMV and MYMIV) for screening YMD-resistant and susceptible mungbean genotypes, we identified genotypes highly susceptible to MYMV (cv. ML267) and MYMIV (cv. K851), as well as genotypes immune to MYMV (cv. PDM139, cv. SML668) and MYMIV (cv. Pusa Vishal). The study explores a non-transgenic approach for inducing resistance to YMD in mungbean. Two target regions within the viral genomes were identified for gene silencing using RNAi. We show that out of three intron hpRNAi constructs, namely hpTR-1: AC4/AC1, hpTR-2: AC2/AC3, and hpTR-1+2: AC4/AC1_AC2/AC3 (fusion construct), the hpTR-1+2 construct provided 100% protection, validated through a transient agroinfiltration assay. Subsequently, we show that in vivo synthesized hpRNA of hpTR-1+2 can persist and induce the generation of small interfering RNA (siRNA) in both local and systemic tissues for at least 12 days' post-spray without viral inoculation, validated through semi-reverse transcription-PCR and northern blotting. Our data indicate that the naked hpRNA spray conferred resistance to MYMIV in mungbean, with the most significant inhibition of MYMIV replication observed when plants were treated on the same day, two days, and four days before viral inoculation. Furthermore, the study explored the role of the apoplast in Begomovirus infection. Importantly, we show the presence of genomic components of MYMIV in apoplastic fluid validated by molecular detection of viral genome through RCA and PCR analysis to enhance our understanding of the cell-to-cell movement of begomovirus via

apoplast. Additionally, we have shown that virus infection induces elevated secretion of vesicles into the apoplast. NMR-based metabolomics analysis reveals altered metabolic profiles in both apoplast and symplast in response to MYMIV infection. Citrate downregulation and increased levels of valine, α-β-glucose, and pipecolic acid were observed in both compartments. Phenolic metabolites were absent in the apoplast and downregulated in the symplast, while proline exhibited contrasting levels in MYMIV-infected samples. Additionally, heightened aspartate levels were confined to the symplast. These findings provide insights into metabolites associated with stress and defense mechanisms triggered by MYMIV infection. In conclusion, our findings may help prevent an epidemic of YMD in *Vigna* species, and the study may contribute to enhancing disease management strategies in mungbean cultivation.