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Molecular Interaction of Beta-Satellites with Redish Leaf Curl Virus

Radish (*Raphanus Sativa L.*) is a part of very important vegetable family, Brassicaceae. Recently, Radish leaf curl virus with an unknown beta molecule was reported in Pakistan. It is a ssDNA virus with monopartite genome from genus Begomovirus and spread by whitefly (*Bemisia tabaci*). This study was designed to check the molecular interaction of betasatellites with Radish leaf curl virus. For this purpose, in 2021 symptomatic leaves collected from virology lab and then were subjected to genomic DNA extraction by CTAB method. Extracted DNA was amplified by PCR using Beta01 and Beta02 primers and then ligating it in a cloning vector pTZ57R. This cloned viral DNA was subjected to sequencing followed by BLAST analysis. This clone has 1350bp size and was given number 885 molecule. For dimer construction, this 885 clone was double digested using KpnI and SalI restriction enzymes to make its partial clones in pTZ57R vector. The partial clones were of the 2.2kb and 0.6kb and given the number, 885a and 885b respectively. These partial clones were then cloned in pTZ57R by a restriction digestion of both partial clones and vector, by same endonucleases enzyme i.e. KpnI and SalI. Finally, the full length 885 clone was ligated with already shifted 885 and 8885 clones in pTZ57R by restricting them with SalI restriction enzyme and then ligated using T4 DNA ligase to complete the dimer. Hence, the constructed infectious clone or dimer was inoculated in model host plant *Nicotiana banthamiana* via *Agrobacterium* mediated transformation, for infectivity analysis.

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