Construction of Infectious Transcript of Papaya leaf distortion mosaic virus

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ABSTRACT

The genome of Papaya leaf distortion mosaic virus (PLDMV) Dali (DL) strain has been completely sequenced with 10,153 nucleotides. The DL strain was isolated from papaya plants with symptom similar to that caused by Papaya ringspot virus (PRSV). The host range of DL is different from that of Japan P and C strains. The available of the full-length sequence of PLDMV makes it possible to construct an infectious clone through enzyme digestion and ligation. In addition, through DNA replacement, it is possible to determine whether certain genes in PLDMV would affect the host range of PLDMV. In order to obtain a full-length of PLDMV-DL, an infectious clone of PRSV HA was used as a backbond and the PLDMV DNA fragments were replaced into PRSV step by step into PRSV. Four plasmids that overlapping each other and cover the full-length of PLDMV genome were constructed previously, including p35SPL2711-HA9779Not, PL2680NarI-4361, PL4247-6604, and PL5761-9810. Through properly enzyme digestion and ligation, it is possible to obtain a full-length PLDMV construct. However, in the process of full-length construction, PL2680NarI-6604 was found to have an extra 88 repeated viral nucleotide at the position of 4315. This area was further re-constructed through RT-PCR and the full-length PLDMV-DL plasmid was finally complete. Papaya plants were inoculated by the purified plasmid which contains the full-length PLDMV and were assayed by ELISA and RT-PCR at 14 day-post-inoculation (d.p.i), however, the assay result was negative in virus infection. The full-length PLDMV plasmid was further entirely sequenced and the results shown that 16 nt at position 3900-3915 of PLDMV was deleted. An attempt to replace the mutated region was not success. According to the results of RT-PCR from the total extraction of RNA from cultured bacterium, a viral RNA sequence corresponding to the P3 gene was detected. By BPROM program, a possible cryptic prokaryotic promoter elements in the upper stream of P3 coding region was found. Site-directed mutagenesis was carried and the final PLDMV full-length plasmid △TM8-PLDMV with 16 nt of deletion in P3 region was obtained. Further replacement of the mutated area is require to obtained a complete full-length PLDMV plasmids. To exam if the coat protein gene is the determinant of the host range of PLDMV, a construct with all the sequence from DL strain except the CP gene which was from Japan PLDMV C type was obtain by overlapping PCR. This plasmid pSK + DL4296-polyA (C-CP) will be replaced into PLDMV-DL plasmid once the full-length construct is obtained.

Keywords : Papaya leaf distortion mosaic virus、infectious clone、cryptic prokaryotic promoter elements、coat protein

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