The infectivity assays of papaya ringspot virus contained the mutated coat protein cleavage sites

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ABSTRACT

A polyprotein of Papaya ringspot virus (PRSV) is initially synthesized from the vial genome, followed a proteolytic process by three virus-encoded proteinase P1, HC-Pro, and NIa. According to NIa cleavage rule, there were two possible cleavage sites located between NIb and coat protein (CP), and each was 20 amino acid apart. The sequence of the upstream cleavage site is VYHE/SRGTD (named CP1 cut site) and the other cleavage site is VFHQ/SKNE (named CP2 cut site). The double cleavage sites present in PRSV were special in potyviruses. To investigate the possible characteristics of the heterogeneous NIb and CP involved in virus replication and movement , in vitro and in vivo infectious transcripts and six CP mutated viral clone were used in this study. Because the clones with the full-length PRSV genome is about 13Kb, it is possible to produce variated plasmids during bacterial culture processes. To avoid possible mutation, suitable restriction enzymes and nucleotide sequencing analyzes were performed on the CP mutated clones. The CP mutants were then mechanically inoculated into systemic host Carica papaya L. and the local lesion host Chenopodium quinoa. The results revealed the mutated virus with CP1 and CP2 cleavage sites changed at both nucleotide and amino acid levels were unable to infect papaya plants and could not cause local lesion on quinoa. Whereas the CP1 and CP2 mutants with only nucleotide changed but not amino acid changed were infectious. The presence of the viruses in the inoculated papaya plants were further confirmed by western bolt. Our results suggested that the double cleavage sites between NIb and CP of PRSV is important for virus infection in papaya plant and Chenopodium quinoa.

Keywords : Papaya ringspot virus ; proteinase ; coat protein ; western boltting

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