The infectivity assays of papaya ringspot virus contained the mutation at the dual coat protein cleavage sites

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ABSTRACT

The genome of Papaya ringspot virus (PRSV) contains 10,326 nucleotides that encode a 381 kDa protein processed by three viral proteinase P1, HC-Pro, and NIa. NIa is responsible for the processing of at least six cleavage sites in the C-terminal part of the polyprotein. According to the cleavage rule of NIa, there were two consensus cleavage sequences located between NIb and coat protein (CP), each represented as CP1 and CP2 cut site, and as a results, two in-frame heterologous Nterminal CP would be produced. The double cleavage sites between NIb and CP is only found in PRSV among Potyvirus. The purpose of our study is to characterize the role of the dual cutting sites in PRSV during virus infection. By using the approach of PCR mutagenesis, nine PRSV CP mutants were constructed, eight of them contained the replaced amino acids at CP1 and/or CP2 sites, including HA-GFPCP1QS,HA-GFP-CP1GS, HA-GFP-CP1MS, HA-GFP-CP2ES, HA-GFP-CP2GS,HA-GFP-CP2MS, HA-GFP-CP12MS/GS, and HA-GFP-CP12QS/ES, and the other mutant denoted as HA-GFP-CPdel had 20-amine acid deletion between CP1 and CP2.All constructs were check by restriction enzyme HindIII and EcoR Ⅰ digestion and then confirmed by nucleotide sequencing. Because all the constructs of the CP mutants contained a GFP gene, it facilitates the observation of viral replication and movement in host plants. The plasmids of wild type virus and nine PRSV CP mutants were inoculated into systemic host Carica papaya and local lesion host Chenopodium quinoa for observing symptom expression. Papaya plants inoculated by HA-GFP-CP1QS and HA-GFP-CP1GS showed symptoms at 18 d.p.i. and 20 d.p.i., respectively, while the other mutants were unable to caused symptoms. In order to detect the activity of the virus on the inoculated papaya leaves, Western blot analyses using the antiserum against PRSV were further conducted. It was found that only those leaves inoculated by HA-GFP-CP1QS, HA-GFP-CP1GS and HAGFP-CPdel were able to detect the existence of viruses. As compared with papaya -viiplants inoculated with wild type viruses, the symptoms showed up about one week slower. When all the 9 PRSV CP mutants were inoculated into a local-lesion host quinoa, all mutants were able to caused local lesions. Our preliminary results suggested that the mutations in CP1 and CP2 cut site did hamper the ability of virus infection in host plants. The role of the larger CP derived from CP1 cutting site may involve in the movement of virus and have less impact on virus replication.

Keywords : Papaya ringspot virus, NIa proteinase, Coat protein, Western bloting

Table of Contents

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