

Mutation of the N terminus of papaya ringspot virus coat protein disrupt viral systemic infection

吳宗哲、江主惠

E-mail: 345466@mail.dyu.edu.tw

ABSTRACT

Papaya ringspot virus (PRSV) is a RNA virus which encodes a large polyprotein. P1 and helper component-proteinase (HC-Pro) cleave only at their respective C terminus in cis while The NIa protease 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, two consensus cleavage sequences (VYHE/S and VFHQ/S) exist in the region between NIb protein and coat protein (CP) and resulted in the formation of two in-frame heterologous N-terminal CP with 20-amino-acid apart. The purpose of our study is to characterize the roles of the two CP in PRSV during virus infection. By PCR mutagenesis, twelve PRSV CP mutants were constructed and 8 of them with the amino acid replacement at the CP cleavage site. The resulting clones were denoted as CP1QS, CP1MS, CP1GS, CP2ES, CP2MS, CP2GS, CP12QS/ES and CP12MS/GS. Four mutants, each had 5, 10, 15 and 20 amino acids deletion at the N terminus of the coat protein, were denoted as CP 5, CP 10, CP 15 and CP 20, respectively. Plants inoculated by the plasmids of wild type virus 35S-HA and 35S-HA-GFP showed typical PRSV symptoms at 10 and 14 d.p.i., respectively, , while plants inoculated by CP1QS, CP1GS, and CP 10 expressed symptoms at 21 d.p.i. and the other mutants were unable to cause any symptoms. RT-PCR, Northern blotting, Western blotting, and ELISA analyzed the upper leaves of inoculated plants showed that only CP1QS, CP1GS, CP 5 and CP 10 were detectable in the systemic leaves. Taken together, our results indicated that mutations in the cleavage sites of CP1 and CP2 would affect the ability of PRSV infection. The N terminus of the larger CP play a critical role in viral systemic infection and the 11-15 amino acids (SYNTH) in the N terminus of CP may play an important role on viral systemic movement, while the processing of CP2 cleavage site is required for PRSV infection into plants.

Keywords : Papaya ringspot virus、NIa proteinase、Coat protein、Enzyme-linked immunosorbent assay、RT-PCR

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