

**INTRODUCTION OF HETEROLOGOUS GENE INTO
P1/HC-PRO JUNCTION OF FULL-LENGTH PRSV-W cDNA
CLONE**



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FULL-LENGTH PRSV-W cDNA CLONE

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ABSTRACT

Papaya ringspot virus (PRSV) is a member of genus potyvirus that infects papaya and cucurbit plants. It has a single-stranded (+)RNA genome of approximately 10.3kb. Protein markers have been widely used to study the role of viral-encoded genes and to follow virus systemic invasion of the host plant. This work focused on the cloning of β -glucuronidase (GUS) gene at P1/HC-Pro junction of infectious PRSV-W cDNA to facilitate the tracking of viral replication as well as evaluate stability of the recombinant virus in the host plant. Two cloning sites, *AatII* and *SalI*, were introduced at the 5' terminus of HC-Pro gene in separate plasmids by site-directed mutagenesis. Both clones were able to cause typical PRSV symptoms and the introduced sites were stable in viral progenies as revealed by restriction analyses of RT-PCR products. The GUS gene was successfully inserted at the introduced *AatII* site either fused to HC-Pro or separated by NIa-Pro cleavage sequence. The GUS containing recombinant cDNA clones were inoculated into germinating zucchini seeds. No systemic infection was detected in any plants grown from the inoculated seeds. Infection was also not initiated in *Chenopodium amaranticolor* leaves that had been mechanically inoculated with the recombinant plasmids. On the other hand, the detection of GUS activity in zucchini seeds bombarded with the pSA-HP-GUS3 indicated the successful delivery of cDNA into the nucleus followed by transcription and translation of infectious transcripts. Further investigation is thus necessary to determine the cause of lost systemic infectivity of these GUS recombinant cDNA clones.

KEY WORDS: *Papaya ringspot virus* /P1/HC-PRO JUNCTION / β -GLUCURONIDASE

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