

Vijitra Chokboon 2011: Nucleotide Sequence, Sub-genomic Construction, and Pathogenicity Test of *Papaya ringspot virus* Strain Samut Sakhon (PRSV-SMK). Master of Science (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Mr. Srimek Chowpongpan, Ph.D. 217 pages.

Papaya ringspot disease caused by *Papaya ringspot virus* (PRSV) belongs to *Potyviridae* that consisted of two biotypes, PRSV-W which restricted to cucurbits and PRSV-P infected both papaya and cucurbits. PRSV genome consists of a single-stranded, positive-sense RNA of 10,326 nucleotides. The disease caused up to 100 percent yield losses. The control regimes by rouging infected plants, chemical sprayed to control aphid vectors were unsuccessful, and there was no resistant gene to PRSV in the domestic papaya. Introducing of transgenic papaya was not acceptable at the presence. Applying the mild strain cross protection was ineffective since the variation of the virus strains among the regions. The rapid generation of infectious PRSV clone was suitable for effective control diseases in different regions by means of mild strain cross protection which was compatible with specific regions. Consequently, a full-length genome cloning from PRSV-P Samut Sakhon strain (SMK) was performed by extraction total RNAs using the RNeasy kit (Qiagen) and cDNA was synthesized by Omniscript (Qiagen) primed by a reverse primer T26-3UTR2. The primers were designed from aligned full-length genome from Hawaii (X67673), Taiwan (X97251) and Thailand (AY162218) type-P. Seven subgenomic PCR fragments were generated encompassing the whole genome that ranged from 500 to 2,700 bp and cloned into cloning vector, and submitted for sequencing. The full-length genome sequences of strain Samut Sakhon was 10,323 nucleotides encode for 3,343 deduced amino acid. The comparative analysis of our PRSV isolate with 12 accessions of PRSV genome in GenBank showed the nucleotide homology at 83-89% and amino acids at 90-93%. Construction of infections clone was divide into three subgenome (subgenome 1; P1, HC-Pro, subgenome 2; P3, 6K1, CI, 6K2 and subgenome 3; VPg, NIa, NIb, CP) that each subgenome contained T7 promoter for *in vitro* RNA transcription, 5'UTR, virus genes split into three fragments, 3'UTR. The RNA transcript was co-infected with the PRSV in order to determine the propagation of the subgenome.

---

Student's signature

---

Thesis Advisor's signature