Pipat Chiampiriyakul 2007: Construction of an Infectious Full-length cDNA Clone of a *Papaya ringspot virus* Type W. Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Supat Attathom, Ph.D. 349 pages.

The purpose of this research was to construct an infectious full-length cDNA of a Thai isolate of *Papaya ringspot virus* type W (PRSV-W) which causes serious problems in cucurbit crops nationwide. The full-length cDNA of PRSV-W was constructed from eight overlapping cDNA clones covering the whole viral genome except the 3'terminal poly(A) nucleotides.

Two full-length PRSV-W cDNA clones under the control of the enhanced CaMV 35S (pDPCwPN7978) or bacteriophage T7 (pDPCwAN0178) promoters were successfully constructed in this study but they were not stable to be propagated in *E. coli* as bacterial host cells. Due to the instability of the full-length cDNA clones, linear-formed constructs corresponding to the two full-length cDNA clones without plasmid vector sequences were developed from overlapping cDNA clones by overlap extension PCR amplification. Linear full-length PRSV-W cDNAs (PCwAH8584) were constructed downstream from the bacteriophage T7 RNA polymerase promoter. The *in vitro* capped transcripts generated from the PCwAH8584 cDNAs were infectious in pumpkin plants. The infectivity of the *in vitro*-transcribed RNA was confirmed by serological detection of the virus coat protein and by observation of virion by electron microscopy.

The nucleotide sequence of the RNA of PRSV-W Thai isolate is 10323 nucleotides in length excluding the 3'terminal poly(A) tail and contains a single open reading frame (ORF) of 10032 nucleotides encoding a large polyprotein of 3343 amino acids and predicted molecular weight of 380.3 kDa with 5'- and 3'- non coding regions (NCRs) consisted of 85 and 206 nucleotides, respectively.

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