4036146 MBMG/M : MAJOR: MOLECULAR GENETICS AND GENETIC ENGINEERING; M. Sc. (MOLECULAR GENETICS AND GENETIC ENGINEERING)
KEY WORDS : PAPAYA RINGSPOT VIRUS / FULL-LENGTH cDNA / *IN VIVO* TRANSCRIPT / CaMV 35S PROMOTER

GULSIRI CHAROENSILP : CONSTRUCTION OF THE CASSETTES OF FULL-LENGTH PAPAYA RINGSPOT VIRUS TYPE P THAI STRAIN FOR *IN VIVO* TRANSCRIPTS AND PRODUCTION IN PAPAYA PLANT. THESIS ADVISORS: MILOSLAV JURICEK, Ph.D., SUNEE KERTBUNDIT, Ph.D.,167 p. ISBN 974-664-396-7

Papaya ringspot virus (PRSV) is a major limiting factor in Thailand's papaya production. The synthesis of biologically functional RNA transcripts from the full-length cDNA clones *via in vitro* or *in vivo* transcription plays a key role in the further research of PRSV at the molecular level. When the molecular biology of PRSV is clearly understood, effective methods for controlling PRSV can be developed. This thesis focuses on development of an effective method that can generate infectious RNA transcripts *in vivo* from Thai isolate of PRSV, type P.

Three plasmid cassettes for *in vivo* and *in vitro* transcription of papaya ringspot virus type P were constructed. The *in vivo* expression cassettes were composed of CaMV 35S promoter or partially duplicated CaMV 35S promoter, the 5′ end combined with 3′ end of PRSV at *Sac*I site, the 127 bp of artificial poly (A) tail and the NOS terminator. The vectors were named pSA1078 (single 35S promoter) and pSA1079 (partially duplicated 35S promoter). The *in vitro* PRSV transcription cassette contains T7 promoter, the full length of papaya ringspot cDNA, the 127 bp of artificial poly(A) tail and the NOS terminator (pSA1110).

The three overlapping fragments of a 10.3-kb full-length of Thai isolate of the PRSV genomic cDNA were obtained by RT-PCR technique. All three fragments were combined by sequential cloning to obtain the full-length cDNA clone of PRSV under the T7 promoter (pSA1100 plasmid). A 9.5 kb *Sac*I fragment from pSA1100 was further cloned into both *in vivo* expression cassettes to obtain the full-length cDNA clones of PRSV under single CaMV 35S promoter (pSA1101) and partially duplicated 35S promoter (pSA1102).

The two full-length cDNA plasmids, pSA1101 and pSA1102 were used to infect papaya plants by mechanical inoculation. Plasmid pSA1110 was used as a negative control while live virus particles were used as positive control. Only 30 % of the positive control plants showed severe symptoms at three weeks post-inoculation while no symptom was so far seen in remaining plants.

These findings suggest that further research in the development of infectious RNA transcripts *in vivo* from Thai isolate of PRSV, type P is required.