

Paweena Kasemsin 2007: Screening for *Papaya ringspot virus* (PRSV) Resistance and Analysis of Transgenes in Transgenic Papayas. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Mrs. Kanokwan Romyanon, Ph.D. 83 pages.

Since the production of papaya in Thailand was lost by the aphid transmissible disease caused by *Papaya ringspot virus* (PRSV). Consequently, engineered papaya resistant to viral disease was constructed using transformation of PRSV coat protein (*CP*) or polymerase (*NiB*) genes into papaya genome. In this study, 41 putative transgenic papaya lines, Khak Dum cultivar, derived from *Agrobacterium*-mediated transformation were determined for the presence of *CP* or *NiB* transgenes of PRSV, Suphan Buri (PRSV-SB) and Nakhon Pathom (PRSV-NP) isolates. Among these lines, 13 transgenic papaya lines represented *CP* transgene, 20 transgenic lines represented *CP* gene linked with inverted repeat of *CP* gene (*CP-IR*) and 5 transgenic lines represented *NiB* transgene. These transgenic papaya lines containing either *CP* or *NiB* transgene were challenged with PRSV-SB or PRSV-NP under glasshouse condition. Thirty-four transgenic papaya lines derived from all transgenes were resistant to the challenged PRSV isolates. From Southern blot hybridization, it was found that the insertion number of transgenes in the genomes were ranged from 1 to 3 copies. The insertion sequence of an enhanced 35S CaMV promoter through the *CP* gene from the resistant line (A45), containing one insertion, was identified. From the preliminary result, it was found that PRSV derived transgene was completely integrated into the papaya's genome. Interestingly, all transgenic lines derived from *CP-IR* transgene were the promising transgenic papaya resistant to PRSV. Moreover, transgenic papaya R<sub>1</sub> lines derived from the transgenic resistant lines, A44 and A6, were revealed transgene segregation, about 74% and 83%, respectively. The accumulation of CP transcript was investigated in eight selected uninoculated transgenic resistant lines which contained *CP* gene (A18, A19, A20, A23) and *CP-IR* gene (A25, A28, A35, A37). High expression of CP transcript was found in the transgenic resistant lines, A18, A19, A28 and A37, while lower expression of CP transcript were detected in line A20, A23, A25 and A35. Similar result was also observed in the uninoculated transgenic resistant lines, A11, A12, A14, A16, and A17 which contained *NiB* gene. High expression was found in resistant lines, A11, A16 and A17 while lower expression was detected in resistant lines, A12 and A14. In comparison, CP gene expression before and after PRSV inoculations did not differ during PRSV infection and the level of CP expression was low in the transgenic resistant lines, A20 and A23. However, in high CP expression lines, A18, A19, A28 and A37, transgenic papaya lines resistant to PRSV did not express coat protein, 32 kDa in size. These results suggested that the post-transcriptional gene silencing (PTGS) possibly involves in viral resistance of transgenic papaya.

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Thesis Advisor's signature

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