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WIPAWANEE KASEMWORAPHOOM : CLONING AND EXPRESSION OF  
 HELPER COMPONENT-PROTEINASE GENE FROM PAPAYA RINGSPOT VIRUS,  
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Papaya ringspot virus (PRSV), which is classified to the potyvirus group, causes a serious disease to papaya plantations in Thailand. The helper component-proteinase (HC-Pro) has been shown to be involved in different steps of the potyvirus life cycle. The study of HC-Pro would be useful for understanding the molecular mechanism of virus life cycles, which could lead to the development of strategies for preventing virus infection. In this study, the HC-Pro gene was isolated from papaya ringspot virus of Thai isolate. The HC-Pro gene was cloned and was investigated for its expression in plant by using tobacco as a model.

The DNA fragment containing HC-Pro gene was isolated from PRSV, Thai isolate by RT-PCR technique and was cloned into pUC18 vector. The nucleotide sequences of 5' and 3' end of recombinant DNA was determined to design specific primers for amplification of HC-Pro gene from the recombinant DNA. Moreover, these specific primers were modified to add the start and stop codon for HC-Pro gene expression. The HC-Pro gene was then cloned into 2 types of vectors. First, the HC-Pro gene was cloned to an *E. coli* expression vector for production of fusion and non-fusion HC-Pro proteins, which were used to produce polyclonal antibodies in rabbits and these polyclonal antibodies would be used for checking the HC-Pro gene expression in transgenic plants later. Second, the HC-Pro gene was cloned to a binary vector for introduction of HC-Pro gene to tobacco by *Agrobacterium*-mediated transformation. From analyses of all 7 transgenic tobacco plants available, all of them contained HC-Pro gene as detection with hot start PCR technique and the transcription of HC-Pro gene to mRNA was also detected in all transformed tobaccos by using RT-PCR technique. The translation of HC-Pro gene to HC-Pro protein was detected by western blotting with polyclonal antibodies. The result showed that the introduced HC-Pro gene could express in transgenic plant B as the presentation of remarkable HC-Pro protein band while transgenic plant A did not show the HC-Pro protein band. The HC-Pro protein bands of transgenic plants C1, C2, D, E and F were very faint. In conclusion, it is possible to isolate the HC-Pro gene from PRSV, Thai isolate and express the gene in transgenic tobacco.