Yupa Phokaew 2013: Development of Immunochromatographic Technique for Detection of *Citrus tristeza virus* (CTV) in Rutaceae. Master of Science (Agriculture), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Assistant Professor Kanungnit Reanwarakorn, Ph.D. 127 pages.

Tristeza virus disease caused by *Citrus tristeza virus* (CTV) which is one of the most important citrus disease worldwide, causing yield loss on citrus plants. This work was CTV survey of citrus orchards in 10 provinces. By reverse transcription-polymerase chain reaction (RT-PCR), CTV coat protein (CP) gene was amplified of 700 nucleotides in size. And by multiplex RT-PCR, CTV *p*23 gene was amplified of 400 and 239 nucleotides in size. These both sizes were designed for detection of CTV severe and mild strains, respectively. Comparison of nucleotides and amino acid sequences of 19 CTV Thai isolates and CTV isolates from GenBank database, they were displayed 89-100% and 94-100% similarity, respectively. Base on this work, CTV Thai isolates were severe strains by molecular analysis.

Production of polyclonal antibodies to the recombinant coat protein of CTV in rabbit and chicken eggs. Then, development of detection method was performed with Triple Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (TAS-ELISA). The TAS-ELISA were shown at OD 405 between 1.320-3.299 in disease samples and less than 0.2 in healthy samples. By TAS-ELISA, these polyclonal antibodies were specifically to CTV, but they did not react with healthy plant sap and other tested viruses. To compare with the commercial detection kit, the results were no significance.

Immunochromatographic assay was developed for efficient and quick detection of CTV. Anti-CTV RIgG and anti-CTV ChIgY were prepared by ammonium sulfate precipitation from polyclonal antibody, Anti-CTV RIgG conjugated with colloidal gold particle and immobilized on a conjugate release pad (CRD). A test line and control line were coated with anti-CTV ChIgY and goat anti-rabbit IgG, respectively. Nitrocellulose membrane and buffer tests were found the Prima40 nitrocellulose membrane giving better reaction line which was clearly visualized within 90 seconds and Na_2BO_3 buffer displayed better reaction with control line, clearly visualization, but did not react with test line. Sensitivity test was performed with disease citrus sap at 1:2 to 1:64 dilution, the immunochromatographic strip showed the sensitivity at 1:32 and no cross reaction to healthy sap. Specificity test of the immunochromatographic strip was shown no reaction with other tested viruses.

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