

Wareerat Sompratoom 2011: Development of Surface Plasmon Resonance (SPR)

Biosensor Technique for the Detection of *Chilli veinal mottle virus* (ChiVMV).

Master of Science (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Miss Sujin Patarapuwadol, Ph.D. 111 pages

ChiVMV is causing agent of Chili veinal mottle disease and it caused reduction in crop yields up to 50% in pepper. Plate trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) is commonly used for viral detection. However, PTA-ELISA technique has a limitation on ChiVMV detection due to a cross reaction of polyclonal antibody with closely related virus, *Pepper veinal mottle virus* (PVMV). Thus, the development of an alternative method for specific detection of ChiVMV is highly desirable. This thesis was aimed to develop Surface plasmon resonance (SPR) biosensor technique for the detection of ChiVMV.

Rabbit-polyclonal antibody (PAb) against ChiVMV-KPS9 was produced and used through-out the experiments. The SPR disc surface was modified with 11- mercaptoundecanoic acid (11-MUA) for immobilizing ChiVMV-PAb and use as an immunosensor for ChiVMV-KPS9 detection. In comparison with PTA-ELISA, SPR biosensor was able to detect ChiVMV-KPS9 as low as 3.9 μg and 1:4,000 dilution of disease-chili sap samples with clear cut decision whereas PTA-ELISA was able to detect at the level of 1,000 μg and 1:4,000 dilution, respectively. By observing the Kinetic association constant (K_a) value of antigen-antibody reaction, SPR biosensor was found to be highly specific compare to PTA –ELISA. The K_a values of the ChiVMV-PAb binding observed from PTA-ELISA and SPR biosensor were 10^2 - $10^4 \text{ M}^{-1} \text{ S}^{-1}$ and 10^4 - $10^5 \text{ M}^{-1} \text{ S}^{-1}$, respectively. The result demonstrated that SPR biosensor technique can be used for ChiVMV-KPS9 detection. Furthermore, SPR biosensor give a real time result and could be reused the SPR immuosensor disc for ChiVMV-KPS9 detection at least four times. However, the cost of SPR biosensor detection is approximately 95 baht/samples which two times higher than that of PTA-ELISA.

Student's signature

Thesis Advisor's signature