

Surasak Khankhum 2007: Cloning and Expression of Non-Structural Protein (NSs) Gene of *Capsicum chlorosis virus* Causing Peanut Bud Necrosis in Thailand. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Pissawan Chiemsombat, Dr. Agr. 88 pages.

A study on the non-structural protein (NSs) gene of *Capsicum chlorosis virus* (CaCV), the tospovirus that caused peanut bud necrosis disease, was conducted. The NSs genes were cloned and NSs protein was *in vitro* expressed. Infected peanut leaf samples collected from Pakthongchai district, Nakhonratchasima province (PPT) and from Muang district, Udon Thani province (PKK 2UD) were diagnosed for tospovirus infection by DAC-ELISA. Primers specific to NSs gene were designed and NSs genes were amplified by RT-PCR using total RNAs extracted from infected peanut leaf tissues as template. The cDNA fragments obtained were identified as NSs gene of CaCV and designated as NSs-PPT and NSs-PKK 2UD. Both NSs genes were composed of 1,320 nucleotides which coded for 439 amino acid residues with molecular weight of 49.6 kDa. Amino acid sequence analysis revealed that both NSs-CaCV from peanuts were 94% identity, and similar to those of CaCV-AIT from tomato in Thailand, CaCV-CP from peanut in China, and *Gloxinia tospovirus* from USA at 85-90% identities. In addition, the nucleoprotein (N) gene of CaCV-PPT was completely cloned and its amino acid sequence was analyzed. The N-PPT gene comprised of 828 nucleotides, coding for 275 amino acid residues with molecular weight of 30.5 kDa. Amino acid sequence analysis indicated that the N protein of CaCV-PPT isolate was similar to those of CaCV-PKK 2UD, CaCV-Pkk, and CaCV-ToK at 97% identities. Moreover, the NSs gene was successfully isolated from thrips vector; *Scirtothrips dorsalis*, collected from peanut field in Pakthongchai district and it showed 96% identity to NSs-PPT by amino acid sequence comparison. The cloned NSs-PPT gene was subsequently expressed *in vitro* by using pQE expression system in *E. coli* cell culture, yielding about 2.98 mg/ml of 50 kDa recombinant 6xHis-NSs polypeptide. The 6xHis-NSs protein was used as immunogen for producing polyclonal antibody in rabbit (PAb-NSs). By using DAC-ELISA technique, the PAb-NSs reacted specifically to CaCV-infected peanut leaf tissue without cross-reactivity to plant tissues infected by *Watermelon silver mottle virus* (WSMoV) or *Melon yellow spot virus* (MYSV). According to its specificity, PAb-NSs produced in this study could be used as immunoprobe for the detection and differentiation of CaCV from other species of tospovirus.



Student's signature



Thesis Advisor's signature

22 / May / 2007