Nutchanan Wiboonchotikorn 2012: Molecular Characterization of Nucleocapsid and Non-Structural Genes and Proteins of Melon yellow spot virus Infecting Melon in Thailand. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Pissawan Chiemsombat, Dr.Agr. 71 pages.

Melon yellow spot virus (MYSV) is a distinct plant virus species in the genus *Tospovirus* which infects cucurbitaceous plants. The virus causes low yield and low quality of the produces. In Thailand there have been several antisera raised against tospoviruses but most of them cross-reacted to the related tospovirus species. This research aimed to investigate MYSV nucleocapsid (N) and non-structural (NSs) genes and proteins, and the in vitro-expressed NSs protein would be applied to produce antibody for specific MYSV diagnosis. Symptomatic plant samples showing yellow spots on leaves were collected in Nakhonpathom, Nakhonratchasima and Phetchaburi provinces. MYSV was detected from 111 out of 321 samples by using ELISA. Twenty nine plant samples were collected from Kanchanaburi and Nakhonratchasima provinces and 20 melon samples were found to be infected. Isolate SUT from melon was selected to study on MYSV N and NSs genes and proteins. Primers were designed for viral genes amplification by RT-PCR and gene sequences were analyzed. The obtained N gene comprised 840 nucleotides encoded for 280 amino acid polypeptide MW of 31.159 kDa. The N protein sequence was similar to Thai MYSV-W3 from watermelon, MYSV-Ph112 from Physalis plant, including Tospo-melo isolates from Japan at 100% identity. The NSs gene contained 1410 nucleotides which coded for 470 amino acid residues of MW53.169 kDa polypeptide. The NSs protein sequence showed the highest identity to MYSV-TW isolate from watermelon in Taiwan. Subsequently, MYSV-SUT NSs gene was cloned into the expression vector in pQE expression system and the recombinant 6xHis-NSs protein synthesized in vitro was obtained. The purified NSs protein has MW of 54.764 kDa and was used at conc.1.6 mg/ml for rabbit immunization. Polyclonal antibody against recombinant NSs protein (PAb-MYSV-NSs) revealed titer ranges of 800-3200 by DAC-ELISA, and specifically reacted to MYSV-infected cucumber leaf sap. By using Dot immunobinding assay, PAb-MYSV-NSs can detect MYSV in cucumber and melon leaf sap without cross-reaction to diseased plant sap of *Watermelon silver mottle virus* (WSMoV), Capsicum chlorosis virus (CaCV), or Tomato necrotic ring spot virus (TNRV).

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