
Thesis Advisors: Assoc.Prof.Dr. Weerasak Saksirirat, Asst.Prof.Dr. Pornthap Thanonkeo

**ABSTRACT**

Eleven isolates of *Fusarium oxysporum* f. sp. *lycopersici* (Fol) were collected from tomato plants in Northeast Thailand. Race identification of the derived Fol isolates and 5 isolates of other Fusarium species, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *cucumerinum*, *F. moniliforme*, *F. poae* and *F. solani* was investigated using random amplified polymorphic DNA (RAPD) technique. The pure cultures of Fol 1 (race 1), Fol 2 (race 2), Fol 3N (race 3), which were provided from the Netherlands and Fol 3A (race 3) from the United States of America, were used as standard races of Fol. DNA fingerprints of all tested Fol isolates regenerated by 8 arbitrary primers (Operon Kit E, 2 primers and Kit H, 6 primers) were examined. The result showed that Fol isolates could be divided into two groups, pathogenic Fol and non-pathogenic Fol. The primers OPE-03 and OPH-20 can be used to discriminate clearly between Fol race 3 and a group of race 1 and 2. However, these primers were not able to discriminate individually among Fol race 1, 2 and 3. Genomic rDNA of ITS1-5.8S-ITS2 region of all Fol was amplified by polymerase chain reaction (PCR) technique using primer ITS1 and ITS4 and characterized by amplified ribosomal DNA restriction analysis (ARDRA) technique. Restriction enzyme *Sau* 3A I and *Rsa* I were used to digest the PCR products. The result showed that enzyme *Sau* 3A I could digest DNA of ITS1-5.8S-ITS2 region rDNA of all Fol isolates. The Fol tested isolates were divided into two groups; group one, twelve isolates of Fol with DNA 320 bp and group two; three isolates of Fol with DNA 250 bp. Enzyme *Rsa* I digested DNA of ITS1-5.8S-ITS2 region rDNA of Fol only two isolates, KK5 and CM1 with DNA 450 bp. However, ARDRA technique using restriction enzyme *Sau* 3A I and *Rsa* I was not suitable to discriminate between Fol race 1, 2 and
3. The PCR products of ITS1-5.8S-ITS2 region rDNA were sequenced and aligned to design 6 specific primers for Fol race 1, race 2 and race 3. Nine isolates of other Fusaria and two isolates of bacteria were used for reference. The primers were tested for their specificity against Fol race 1, 2, 3 and other Fusaria and bacteria. The result showed that the primers were not specific to Fol race 1, 2 and 3. Surprisingly, these primers could amplify genomic DNA of other fungi and some bacteria. However, DNA fingerprint were different in DNA patterns of Fusarium spp. showing only one band and the other showing more bands than Fusarium spp. Genomic DNA of Fol were amplified by using primers FOL1 (forward 5'-CTTGGTTTGCCTGCGCCGTG-3' and reverse 5'-GCAAGCGCGGGTGCGCCAGT-3') and optimized the annealing temperature. The result showed that at 60 °C of annealing temperature it could be discriminated individually among Fol race 1, 2 and 3, based on DNA fingerprints. These selected primers could be further applied for seed certification program for export.