ABSTRACT

A survey of Fusarium wilt of tomato was carried out in 263 locations in Khon Kaen, Kalasin, Nong Khai and Sakol Nakhon provinces. Seven isolates of *Fusarium oxysporum* f. sp. *lycopersici* (Fol), KK1, KK2, KK3, KK4, KK5, KK6 and KS, were isolated from 7 locations. These collected isolates, an isolate of Fol from the Department of Agriculture, Ministry of Agriculture and Cooperatives (PP) and three isolates (CM1, CM2 and CM3) from Chiang Mai University, were evaluated for pathogenicity by root dipping method. Six isolates of Fol (KK1, KK2, KK3, KK4, KK6 and CM2) caused wilt symptoms tomato (Sida variety) but five isolates (KK5, KS, PP, CM1 and CM3) did not. Eleven isolates of Fol were compared by pathogenic reaction with standard cultures (Fol004 race1; Fol007 race 2; Fol029 race 3 and Fol030 race 3) on four differential tomato varieties (Bonny Best, UC82-L, Walter and I3R-1). The results showed that five isolates from Khon Kaen province (KK1, KK2, KK3, KK4 and KK6) were identified as race 2 virulent phenotype while one isolate of Fol from Chiang Mai University (CM2), showed a race 1 virulent phenotype. Race 3 of Fol was not found in this study. In addition, all isolates of Fol were tested for pathogenic reaction with a series of ten Thai tomato varieties, commercial Sida, *Lycopersicon pimpinellifolium* variety, and hybrid varieties (Valentine 183, TW-4, TM 1290, HW 96, HW 98, TML, KKU-I2 and CIN 1463 A x ALE 55 VF). The results indicated that Thai tomato varieties which can be used as differential hosts are commercial Sida and *L. pimpinellifolium* comparable to the standard differential variety Bonny Best. TW-4 and TM 1290 are comparable to Walter and Valentine 183 while HW 96, HW 98, TML, KKU-I2 and CIN 1463 A x ALE 55 VF can be substituted for I3R-1. This study suggests that thai tomato varieties can be used as indigenous differential hosts to support the process of phytosanitary certification.
Eleven isolates of Fol from Thailand, were examined for variation of the ITS1–5.8S–ITS2 region by amplified ribosomal DNA restriction analysis (ARDRA) and genomic DNA by random amplified polymorphic DNA polymerase chain reaction (RAPD–PCR). All isolates could be distinguished from each other by ARDRA analysis, and clustered into four groups (A, B, C and D) by using unweighted pair group method with arithmetic average (UPGMA) analysis. The isolates were grouped into two groups (E and F) by UPGMA analysis based on RAPD–PCR data. There was a similarity between the UPGMA dendrograms of ARDRA and RAPD–PCR analysis. All of the pathogenic isolates were in group A and E. Based on the RAPD–PCR technique, when the genomic DNA is increased using the primer OPE 05, DNA fractions are found with size of about 380 base pairs are only found in the isolates that cause wilt disease. These fractions are not found in the isolates which do not cause disease. Apart from this, from DNA fingerprints it is found that the DNA banding patterns in the isolates which cause disease are clearly different from those from isolates which do not cause disease. Thus the results of the experiment indicate clearly that the RAPD-PCR technique is able to clearly separate between the group that causes disease and the group which does not. This study shows that, at the DNA level, pathogenic isolates are more closely related, whereas non–pathogenic isolates are more diversified.

A total of ninety eight isolates of fungi were collected from the rhizosphere of tomatoes. Based on dual culture test, twenty isolates were screened as antagonistic fungi against *F. oxysporum* f. sp. *lycopersici* (Fol) race 2. Isolates AN9 exhibited strong inhibition by reducing mycelial growth of Fol at 81.11 %. These twenty isolates were tested for controlling tomato wilt in greenhouse. The twenty isolates could significantly reduce the incidence wilt when compared with the control. The most effective antagonistic isolate was AN10, reducing disease by approximately 60 %. All twenty isolates were also evaluated for their semi–quantitative degrading enzyme activities, chitinase, protease and β-1, 3-glucanase, in the potato dextrose broth (PDB). The results showed that *Trichoderma virens* and *T. viride* expressed high chitinase activity with degradation zone diameters of 28.30 and 22.20 mm, respectively. For proteolytic activity, the highly effective isolates were *T. atroviride* and *T. viride* with 39.43 and 27.53 mm of clearing zone, respectively. When the fungi
were grown in Czapek–dox supplemented with fungal cell wall of Fol, no chitinase was excreted. There was only a low level of protease observed on agar plate. The result of quantitative enzyme assays, when the fungi were cultivated into PDB, showed that *T. viride* and *T. atroviride* showed high chitinase activity at 30.39 and 29.04 units/mg protein. High activity of β-1, 3-glucanase of isolates *T. harzianum* and *T. atroviride* were 148.74 and 28.86 unit/mg protein, respectively. For protease activity, *T. atroviride* and *T. viride* expressed high activity at 41.88 and 29.70 units/mg protein, respectively. The enzymes, chitinase, protease and β-1, 3-glucanase were not found when the fungi were grown in Czapek–dox media supplemented with Fol fungal cell wall.

This study suggests that Fol obtained in Thailand could be designated as race 1 and race 2, only and the development of methods for identification races of Fol was able to be investigated by using differential hosts and molecular methods. Moreover, the biological control approach of Fusarium wilt of tomato is potentially alternative.