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Full Length Research Paper

Genetic variation of *Fusarium oxysporum* f. sp. *lycopersici* isolated from tomatoes in Thailand using pathogenicity and AFLP markers

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Genetic variation among 25 isolates of *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt was determined using pathogenicity test and Amplified Fragment Length Polymorphisms (AFLP) markers. The isolates were collected from 8 provinces in Thailand. Based on the pathogenecity result, all isolates were divided into two groups as pathogenic and non-pathogeni isolates. Cluster analysis based on AFLP also grouped the pathogenic isolates into 3 subgroups as low, moderate and high virulence. A dendrogram resulting from a cluster analysis showed two main distinct groups: group 1, non-pathogenic isolates; and group 2, pathogenic isolates rooting from outgroup. Eighty one polymorphic bands were analyzed using computer software. The results showed that genetic differentiation occurred among populations (*Gst* = 0.5898). However, the populations in the same geographical areas, Khonkaen and Nong khai, Tak and Phechaboon were more closely related genetically than another populations based on Nei's genetic distance, indicating the movement of the fungal conidia between these areas. This work provided new information on the formae speciales of *F. oxysproum* f sp *lycopersici* NKSC01 and NKSC02, of high virulent which could be classified as race 2 causing wilt of tomato var. Cheery.

Key words: Fragment length polymorphisms (AFLP) marker, tomato, pathogenecity, *Fusarium oxysporum* f. sp. *Lycopersici.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated, popular and important vegetable crops in the world. It is usually infected by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen causing wilt in lowland cultivation that can result to economic losses. The disease management is very difficult due to its endophytic growth and persistence in soil (Agrios, 1997). It has become one of the most damaging diseases wherever tomatoes are grown intensively due to the pathogen persistence in the infested soils (Silva and Bettiol, 2005). Most of *Fusarium* spp. is known as plant pathogenic strain, that cause many diseases such as wilt, root rot and crown rot diseases on a various variety of crops (Nelson et al., 1981). Many researches on *Fusarium* spp. have been focused on studying plant pathogenic isolates (Mohammadi et al., 2004; Pasquali et al., 2004). However, the nonpathogenic groups represent a significant proportionality of the isolates found and keep most genetic diversity within these complex species (Bao et al., 2002). There is a large deal of genetic relationship between pathogenic and non-pathogenic *F. oxysporum* isolates (Baayen et al., 2000). Skovgaard et al. (2002) suggested that particular pathogenic isolates might germinate from non pathogenic strains by mutations affecting a few loci. Some nonpathogenic isolates have been studied to change from pathogenic isolates through loss of virulence (Skovgaard et al., 2002). James et al. (2000) reported that some isolates of *Fusarium oxysproum* were highly

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virulent, whereas others were nonpathogenic fungi. Moreover, both highly virulent isolates and nonpathogenic isolates are not different based on morphological study. Therefore, methods are needed and are important for identifying and quantifying population of highly virulence of *F. oxysporum.* Baayen et al. (2000) and Mayek et al. (2001) stated that molecular markers have been used to study genetic relationships for pathogenicity in many groups of fungi. Histone-H3 encoding gene and amplified fragment length polymorphisms (AFLPs) could be used for studying genetic differences between highly virulence, low virulence and nonpathogenic isolates of *F. oxysporum*. These previous results suggested that molecular marker can be used to separate these two phenotypes and compare the phylogenetic relationships of highly virulent *Fusarium* spp. (Donaldson et al., 1995). AFLP is a powerful technique in molecular marker for studying relationships among isolates of fungi between population and species levels (Cunningham, 1997; Kauserud and Schumacher, 2003; Nelson et al., 1983; Skovgaard et al., 2003). Moreover, AFLP analysis has been used to investigate genetic variation within and between different *Fusarium* spp. (Adb-Elsalam et al., 2002; Kiprop et al., 2002; Sivaramakrishnan et al., 2002).

The objectives of this study are to determine the genetic variation and differentiation of *F. oxysporum* f. sp. *lycopersici* populations isolated from tomato wilt and to find out the correlation among pathogenic isolates (low, moderate, high virulence) and non-pathogenic isolates of *Fusarium* spp. using AFLP and pathogenicity markers. Moreover, genetic differentiation among population of geographical areas was analyzed.

MATERIALS AND METHODS

Isolation and pathogenicity test

Pure cultures of *F. oxysproum* were isolated from the root samples of tomato wilt disease by tissue transplanting technique from Bangkok, Phechaboon, Tak, Burirum, Khonkaen, Nongkhai, Nakhonratchasima and Sakon Nakhon Provinces in Thailand. The diseased root samples were cut into 1 cm long, and then surface disinfected by 10% sodium hypochlorite for 3 min and washed through sterilized distilled water before moving to water agar (WA). The hyphal tip was sub-cultured onto potato dextrose agar (PDA) to get pure culture. Single spore isolation was carried out for each of the pure culture isolate and mainteined on PDA slants and deposited at the Biocontrol Research Unit and Mycology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

All isolates were tested on tomato seedlings of Cherry variety for pathogenicity using Koch's postulates. Tomato seedlings at 20-day-old were washed under running sterilized water and cut at five points on the root tips before dipping the roots into a 20 ml spore suspension (1×10^7 spores/ml) for 15 min. A control was performed by dipping seedling roots into sterile distilled water. The seedlings were then potted in sterilized soil. After 15 days, symptoms of disease were recorded using the Disease Severity Index (DSI) and rated according to Sibounnavong et al. (2009, 2010) as follows: 1 = no symptoms, 2 = 1-20% of leaves yellow and wilted, 3 = 21-40% of leaves yellow and wilted, 4 = 41-60% leaves yellow and wilted, 5 = 61-80% of leaves yellow and wilted, and 6 = 81-100% of leaves yellow and wilted. The experiment was conducted using a completely randomized design (CRD) with six replications in each treatment. The experiment was repeated twice. Pathogenic isolates or non-pathogenic isolates were recorded. The non-pathogenic isolates were categorized as avirulence (A) and pathogenic isolates were categorized as degree of virulence according to the DSI, following the method of Charoenporn et al. (2010) with little modification: avirulence (DSI =1), low virulence (DSI ≤3.50), moderate virulence (DSI > 3.50-4.50), and high virulence (DSI >4.50).

Deoxyribonucleic acid (DNA) extraction

50 mg of grounded fungal biomass was used for genomic DNA extraction with 0.5 ml of extraction buffer (50 mM Tris-HCl, 850 mM NaCl, 100 mM EDTA, and 1% SDS) and incubated at 65°C for 30 min; and then added with Phenol ($^1/_2$ vol) and Chloroform:IAA (24:1) ($^1/_2$ vol). After centrifugation at 13000 rmp for 10 min, the upper aqueous phase was deproteinized by additional 1 vol of Chloroform:IAA (24:1). After centrifugation at 13000 rmp for 10 min, the DNA molecules were added with 2 vol of absolute ethanol and incubated at -20°C for 1 h. After centrifugation at 13000 rmp for 10 min, the DNA molecules were washed by 70% ethanol and centrifugation at 13000 rpm for 10 min twice. The end products of DNA molecules were dissolved in 100 µl of TE (10 mM Tris HCl 8.0, 1 mM EDTA). The DNA concentration was measured using 1% agarose gel electrophoresis.

Fingerprinting analysis using AFLP marker

The AFLP reactions were performed as described by Vos et al. (1995) with the following modifications: Genomic DNA (500 ng.) was digested with a combination of restriction enzymes *Eco* RI (50 Units) and *Tru* 9I (*Mse* I) (10 uints) in a mix of 10x ligase buffer, 0.5 M Nacl and BSA. The digested DNA fragments were ligated to their respective adapter pair of both enzymes in a reaction of T4 DNA ligase (1µ) and T4 DNA ligase buffer (1x) and incubated at 37°C for 3 h. Later, the restriction-ligation products were diluted 10 fold with TE buffer (10 mM tris, 0.1 mM EDTA,pH 8.0). The first amplifications were carried out with 1 selective nucleotide at 3' end of each primer in volume of 25 µl of PCR buffer containing PCR buffer (1x), dNTP (0.2 mM), each primer (E+A/M+G, E+G/M+A, E+C/M+G and E+G/M+C) 5pmole, $MgCl_2$ (2.5 mM), Taq polymerase (0.5u). This pre-amplification was carried out in a thermal cycler programmed for 20 cycles of 30 s at 94°C; 60 s at 56°C; 60 s at 72°C and held 16°C for 15 min. The selective amplifications were performed using selected combinations of primers with two or three selective nucleotides (Table 1). All seventeen combination primers were screened to investigate the most suitable primers. They were carried out in volumes of 20 µl of PCR buffer containing 5 µl diluted preamplified DNA, PCR buffer (1x), dNTP (0.2 mM), each primer 5 pmole, MgCl2 (2.5 mM) and Taq polymerase (1µ). The PCR amplifications were performed with an initial denaturation at 94°C for 30 s followed by 12 cycles of 94°C for 30 s, annealing at 65°C each cycle was reduced by 1°C for 30 s and extension step at 72°C for 60 s. In each of the following 10 cycles, the annealing temperature was reduced by 1°C. The next 30 PCR cycles continued at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s.

For gel analysis, the amplification reaction products were mixed with 10 µl of formamide dye (98% formamide, 10 mM EDTA pH 8.0,

Table 1. Primers combinations were used for screening.

EcoRI primer + selective base		MseI primer + selective base	
+A	+AC	+GTA	+GT
+G	+ACG	+ACG	+G
+G	+ACT	+AAC	+G
+G	+AGC	+AGC	+G
+C	+GCG	+GTA	+C
+G	+GTC	+CTA	+C
+G	+CGC	+CGC	+G
+G	+CTG	+CAC	+G
+AG		+GT	

0.3% bromo phenol blue and 0.3% xylene cyanol) and heated at 95°C for 3 min and quickly cooled on ice. Each sample (2 µl) was examined on a 5% polyacrylamide gel plus 7 M urea on a Model S2 sequencing gel electrophoresis apparatus. Electrophoresis was performed at constant power of 50 W for 3 h. After electrophoresis, the gel plate was removed, fixed in 10% acetic acid for 30 min, and washed in distilled water 3 times for 2 min. The gel plate was stained for 30 min in silver solution (1 g of silver nitrate and 1.5 ml of 37% formaldehyde per liter) and rinsed with distilled water. After staining, the gels were developed in a cool developer solution (30 g of sodium carbonate, 1.5 ml of 37% formaldehyde and 0.01 g of sodium thiosulfate) until the bands appeared. The staining was stopped by adding 10% acetic acid (fixed solution) for 1-2 min, rinsed with distilled water for 2 min and dried under fume hood overnight.

Data analysis

The fingerprint patterns were scored for both monomorphic and polymorphic bands as binary data by 1 (present) or 0 (absent). The binary data were analyzed with the computer program NTSYS pc version 2.02 (Rohlf, 1993). An unweighted pair group arithmetic mean method (UPGMA) cluster analysis was performed using the DICE's similarity coefficient. Dendrogram was generated with the tree option (TREE) and a cophenetic value distance matrix was derived from dendrogram with a COPH program in NTSYSpc. The cophenetic value distance matrix was compared for level of correlation with the original matrix with the MXCOMP NTSYS program. Bootstrap values were calculated with 1000 replications by Winboot program (Yap and Nelson, 1996).

All the polymorphic bands were recorded in the GenAlex6 format. A principle coordinate plot based on genetic distances between all pairs of AFLP genotypes was generated in GenAlex6 and was used to generate a two-dimensional principal coordinate analysis based on the population of AFLP genotypes in the PCA plot. Neighbor joining tree based on Nei's (1978) genetic distance was generated using UPGMA modified from neighbor procedure of PHYLIP version 3.5.

RESULTS

Isolation and pathogenicity test

25 isolates of *F. oxysporum* were obtained from Bangkok (BKRS01 and BKRF01): Phetchaboon (PBRS101, PBRS102, PBRS103, PBRS104, PBRS201, PBRS202 and PBRS203), Tak (MSRS01, MSRS01, TRS01 and TRS02); Burirum (BRC03); Khonkaen (KK2 and KSoC02); Nongkai (NKSC01, NKSC02, NKRC02, NKRC04 and NKRC09); Nakhonratchasima (NSC01) and Sakonnakorn (SRC02, SSoC03 and SSoC04). These isolates were pathogenically reconfirmed by Kock's postulate method in 20 days old tomato seedling var. Sida. Result showed that 11 isolates, BKRS01, BKRF01, BRC03, KSoC02, NKRC02, NKRC04, NKRC09, NSC01, SRC02, SSoC03 and SSoC04 were non-pathogenic or avirulent group (DSI = 1). The pathogenic isolates showed 11 isolates were low virulent (L), one isolate was moderate virulent (M) and two isolates were high virulent (H) as shown in Table 2.

DNA fingerprint analysis using AFLP marker

Seventeen combination primers were screened on five isolates for investigating suitable primers combination used for the study. Result showed that only three primers combination including EcoRI+G/MseI+ACG, EcoRI+G/MseI+CAC, EcoRI+ACG/MseI+G gave highly number of polymorphic bands when compared with other primer combination which resolved 22, 22.4 and 20.5 polymorphic bands, respectively as shown in Figure 1, Table 3 and 4. The three primers were chosen for further screening on 25 isolates of F. oxysporum f. sp. lycopersici. Then, a total 81 polymorphic bands were amplified using primers combination with EcoRI (E)+3 and MseI (M+1) and EcoRI (E)+1 and MseI (M+3) at the 3' end of the primers on 25 isolates of *F. oxysporum* f sp *lycopersici*. The polymorphic bands were analyzed using NTSYS program. Cluster analysis divided all the isolates into two major groups at 30% Dice' coefficient similarity. Group 1 was described as non-pathogenic isolate group (avirulence) which consisted of KS0C02, BKRF01, SSoC04, SRC02, BKRS01, BRC03, SSoC03, NKRC09, NKRC02, NKRC04 and NSC01. Group 2 was described as pathogenic isolate group which was divided into 3 subgroups as follows:- subgroup 1 was low virulent isolates of MSRS01, MSRS02, PBRS102, PBRS203, TRS01, PBRS201, PBRS103, PBRS101, PBRS104,

Table 2. Isolates of *Fusarium* spp. and their pathogenicity group in tomato var. Cherry.

Provinces	Isolates	DSI[1]	Pathogenic or non-pathogenic isolates
Bangkok	BKRS01	1.00 e[2]	Non-pathogenic
	BKRF01	1.00 e	Non-pathogenic
Phechaboon	PBRS101	2.00 d	Low virulence
	PBRS102	2.00 d	Low virulence
	PBRS103	2.00 d	Low virulence
	PBRS104	2.00 d	Low virulence
	PBRS201	2.00 d	Low virulence
	PBRS202	2.00 d	Low virulence
	PBRS203	2.00 d	Low virulence
Tak	MSRS01	2.00 d	Low virulence
	MSRS02	2.00 d	Low virulence
	TRS01	2.00 d	Low virulence
	TRS02	2.00 d	Low virulence
Burirum	BRC03	1.00 e	Non-pathogenic
Khonkaen	KK2	4.25 c	Moderate virulence
	KSoC02	1.00 e	Non-pathogenic
Nongkhai	NKSC01	4.75 b	High virulence
	NKSC02	5.50 a	High virulence
	NKRC02	1.00 e	Non-pathogenic
	NKRC04	1.00 e	Non-pathogenic
	NKRC09	1.00 e	Non-pathogenic
Nakonratchasima	NSC01	1.00 e	Non-pathogenic
Sakon nakon	SRC02	1.00 e	Non-pathogenic
	SSoC03	1.00 e	Non-pathogenic
	SSoC04	1.00 e	Non-pathogenic

[1]DSI = Disease severity index:- avirulence (DSI =1), low virulence (DSI≤3.50), moderate virulence (DSI>3.50-4.50), and high virulence (DSI>4.50). [2]Average of two repeated experiments from eight replications. Means followed by a common letter were significantly different by DMRT at P=0.01.

TRS02 and PBRS202; subgroup 2 was moderate virulent isolates of KK2, and subgroup 3 was high virulent isolates of NKSC02 and NKSC01. A UPGMA tree resulting from AFLP cluster analysis showed 85.4% bootstrap value of isolates NKSC01 and NKSC02 whose high virulent isolates caused wilt disease of tomato var. Cherry. Among the pathogenic isolates grouped into low virulence (L), AFLP cluster analysis showed over 60% of bootstrap, and 99.5% of bootstrap value for non-pathogenic or avirulent group (Figure 2). Thus, it was clearly demonstrated the relationship between degree of virulence and their genetic relationship. Moreover, It is also clearly shown that the phenetic dendrogram generated by UPGMA on genotypes in 8 populations as pop1:Khonkaen province, pop2:Bangkok province, pop3: Sakorn nakorn province, pop4: Bururum province, pop5: Nongkai province, pop7: Tak province and pop8: Pechaboon province. With this, a principal coordinate analysis (PCA) grouped all of the *Fusarium* spp. isolates into eight major clusters. It is observed that pop 1: Khonkaen and pop 5: Nongkai are located in the Northeast of Thailand with large areas for planting tomatoes, in which these geographical areas were found more moderate and high virulent isolates (Figure 3).

DISCUSSION

A total of 25 isolates of *F. oxysporum* f. sp. *lycopersici* were confirmed morphologically and based on molecular phylogeny. Results of the pathogenicity test and AFLP analysis in this study revealed that 11 isolates were

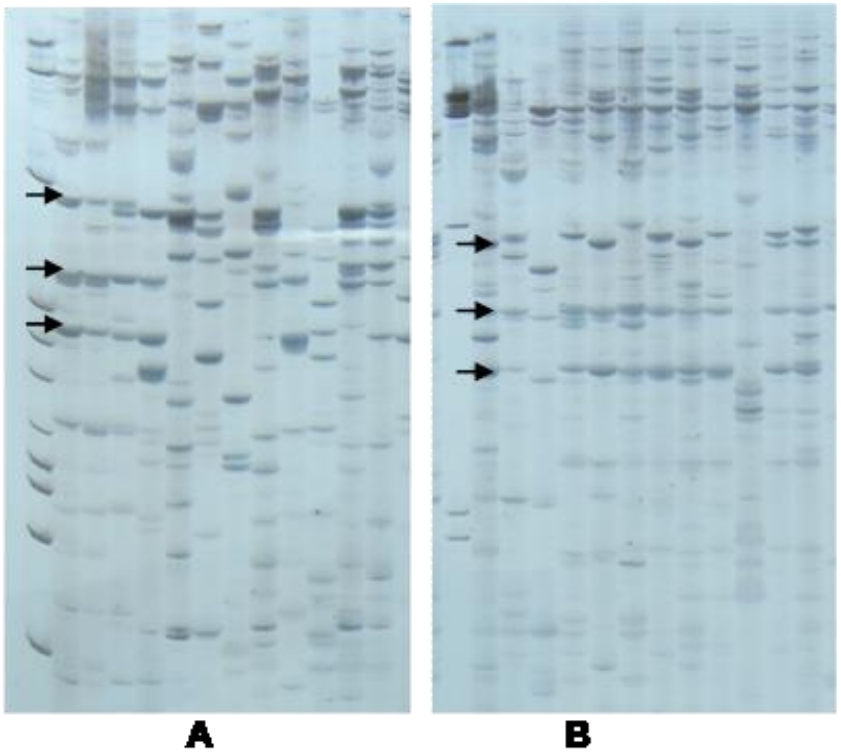

Figure 1. DNA fingerprint of Fusarium oxysporum f. sp. lycopersici by AFLP markers using E+ACG/M+G (A) and E+G/M+CAC (B) primers. The polymorphic bands shown by arrows and lane 1 are 100 bp plus DNA Ladder (Fermentas).

Table 3. Total number of polymorphic bands of screening primer pairs.

EcoRI primer	MseI primer	Number of bands
A	GTA	17
G	ACG	22
G	AAC	12.6
G	AGC	18.8
C	GTA	17
G	CTA	11.2
G	CGC	12.2
G	CAC	22.4
AG	GT	8.2
AC	GT	11.8
ACG	G	20.5
ACT	G	15.8
AGC	G	17
GCG	C	15.2
GTC	C	16
CGC	G	8
CTG	G	11.8

categorized as non-pathogenic or avirulent group and 14 isolates were categorized as pathogenic group which was divided into 3 subgroups of low virulent (L), moderate virulent (M) and high virulent (H). As a result, the isolates of KSoC02, NKRC09, SSoC03 and SSoC04 were shown to be non-pathogenic isolates or avirulence, but Charoenporn et al.

Table 4. Nei's unbiased measure of genetic identity (above diagonal) and genetic distance (below diagonal) of AFLP genotypes of *Fusarium oxysporum* f. sp. *lycopersici* populations in Thailand.

POP ID	1	2	3	4	5	6	7	8
1	****	0.9272	0.9272	0.7791	0.9882	0.6440	0.8757	0.9169
2	0.0756	****	0.8846	0.8511	0.8885	0.6205	0.8618	0.8848
3	0.0734	0.1226	****	0.7726	0.9347	0.6458	0.8526	0.8510
4	0.2496	0.1612	0.2580	****	0.7989	0.5679	0.7151	0.7538
5	0.0119	0.1183	0.0675	0.2245	****	0.7292	0.8978	0.9214
6	0.4401	0.4772	0.4373	0.5658	0.3159	****	0.6721	0.6841
7	0.1327	0.1487	0.1594	0.3353	0.1078	0.3973	****	0.9630
8	0.0869	0.1224	0.1613	0.2826	0.0819	0.3796	0.0377	****

(2010) reported that these isolates were low virulent that cause wilt of tomato var. Sida. It can be explained that the different varieties of tomatoes may affect the pathogenicity level of wilt disease infected by same isolate of *F. oxysporum* f. sp. *lycopersici* (Cai, 2003). Isolate KK2 with high virulent to tomato var. Sida as previous reported (Charoenporn et al., 2010) became moderate virulent in tomato var Sida in this study. Bunyatratchata et al. (2005) reported that isolate KK2 isolated from Northeast part of Thailand had been tested, and its pathogenicity caused wilt symptom on tomato var Sida. This is at the same disease level with Banny and UC82-L varieties which are susceptible to standard tested isolate Fol 007 race 2. It was concluded that KK2 was race 2 (Grattidge, 1982; Maiatt et al., 1996). Isolates NKRC02, NKRC04 and SCR02 with moderate virulent to tomato var. Sida in previous report (Charoenporn et al., 2010) became non-pathogenic to tomato var. Cherry in present study. It was observed that those isolates were variable for pathogenicity to different varieties of tomatoes (Cherry and Sida varieties); from low and moderate virulent became non-pathogenic and from high virulent became moderate virulent. This phenomenon may explain that different varieties of tomatoes are affected with isolate of *F. oxysporum* f sp. *lycopersici*, as also stated by Sibounnavong et al. (2009); and continuing subculture of Fusarium may lead to variable and lower degree of pathogenicity (Agrios, 1997). It is interesting that isolates NKSC01 and NKSC02 with high virulent to tomato var Sida as previously reported (Charoenporn et al., 2010), still expressed high virulent in tomato var. Cherry in this experiment. This can explain why the isolates were more stable than the other isolates or these isolates can infect both Cherry and Sida varieties (Sibounnavong et al., 2010). However, Charoenporn et al. (2010) previously reported that isolates KK2, KSoC02, NKSC01, KKSC02, NKRC02, NKRC04, NKRC09, NSC01, SRC02, SSoC03 and SSoC04 were sequenced to confirm identification in species by using ITS sequences with the length of complete ITS1, 5.8S and ITS2 including small portions of 18S rDNA and 28 S rDNA.

In this study, AFLP has been used as a powerful technique in molecular fingerprinting to study the relationship among fungal isolates and their pathogenicity, as also shown by Brown (1996), Janssen et al. (1996) and Majer et al. (1998).

Regarding the result from this study, a total of 81 polymorphic bands were amplified using three primers combination with EcoRI+G/MseI+ACG, EcoRI+G/MseI+CAC, EcoRI+ACG/MseI+G at the 3' end of the primers on 25 isolates of *F. oxysporum* f sp. *lycopersici*. The restriction enzymes, length and composition of selective nucleotides would help to determine complexity of the final AFLP fingerprint as reported by Janssen et al. (1996). Three nucleotides of primers combination for AFLP analysis can help to differentiate *Fusarium* spp causing root rot disease on wheat and give good polymorphic bands (Mohammad et al., 2009). The primer selectivity is related to genome size and good selectivity is found with primers of three selective nucleotides (El-Kazzaz, 2008). However, Gonnalez et al. (1998) reported using two instead of three selective nucleotides in order to generate adequate number of fragments for AFLP analysis of *C. lindemuthianum* isolates. Primer selectivity is also good for primers with one or two selective nucleotides in simple genome such as bacteria, fungi and some plants, although selectivity is still acceptable with primers of three selective nucleotides.

Statistical analysis of AFLP data enabled the classification of *F. oxysproum* f sp. *lycopersici* into two AFLP groups; non-pathogenic or avirulent and pathogenic or virulent groups. With this, the pathogenic group was clearly divided into three subgroups which correlated with the result of pathogenicity.

In this study, 25 isolates of *F. oxysproum* f sp. *lycopersici* were analyzed with primers to determine the distribution of genetic diversity among isolates which represented in different planting areas. Mohmed et al (2003) stated that the high-resolution genotyping method of AFLP analysis was suitable to study the genetic relationships within and between populations of *Fusarium* spp. In the present research however, there was no clear relationship between provinces and distribution of pathogen. This result was similar to those of Charoenporn et al.,

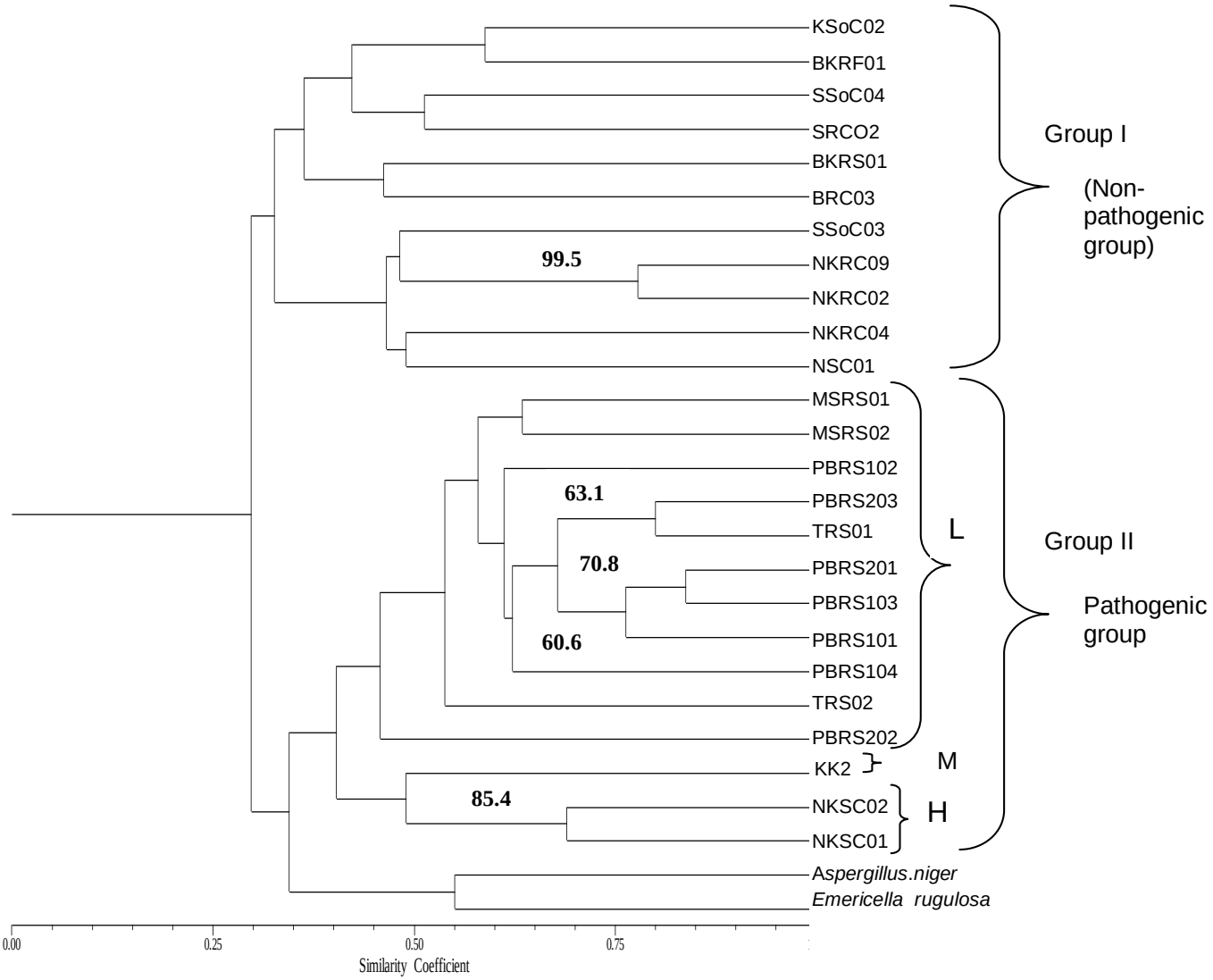

Figure 2. Phenetic dendrogram of Fusarium oxysporum f. sp. lycopersici isolates based on the binary matrix of polymorphic bands, using the UPGMA algorithm and Dice's similarity coefficient (NTSYS program). Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branch.

(2010). In this study, It was shown that the phenetic dendrogram generated by UPGMA was modified from neighbor procedure of PHYLIP version 3.5 based on genotypes in 8 populations as pop1:Khonkaen province, pop2:Bangkok province, pop3: Sakorn nakon province, pop4:Burirum province, pop5: Nongkai province, pop7: Tak province and pop8:Pechaboon province. A principal coordinate analysis (PCA) grouped all of the *Fusarium* spp. isolates into eight major clusters. No clear trend was detected between clustering in the AFLP dendrogram and geographic origin of the tested isolates as similar report of Mohmed et al. (2003). But it is observed that pop 1: Khonkhaen and pop 5: Nomhkai are located in the Northeast of Thailand where majority of the areas are planted tomatoes, in which these geographical areas were found more moderate and high virulent isolates.

In conclusion, the genetic variation among isolates of *F. oxysproum* f sp *lycopersici* was clearly relationship between pathogenicity groups and AFLP groups. But it was not clearly correlated between AFLP and geographical areas. Moreover, this work provided new information on formae specialis of *F. oxysproum* f. sp. *lycopersici* which could be classified as race 2 that can cause wilt to different varieties of tomato, for example Cheery and Sida varieties rather one variety. Bunyatratchata et al. (2005) reported that *F. oxysporum* f sp *lycopersici* race 2 can infect tomato var. Sida in Thailand as compared to standard race testing varieties of Bonny Best, UC82-L. There was a good correlation between AFLP groups and groups from result of pathogenicity test. Regarding the result of the present study, it was demonstrated clearly that the use of AFLP is

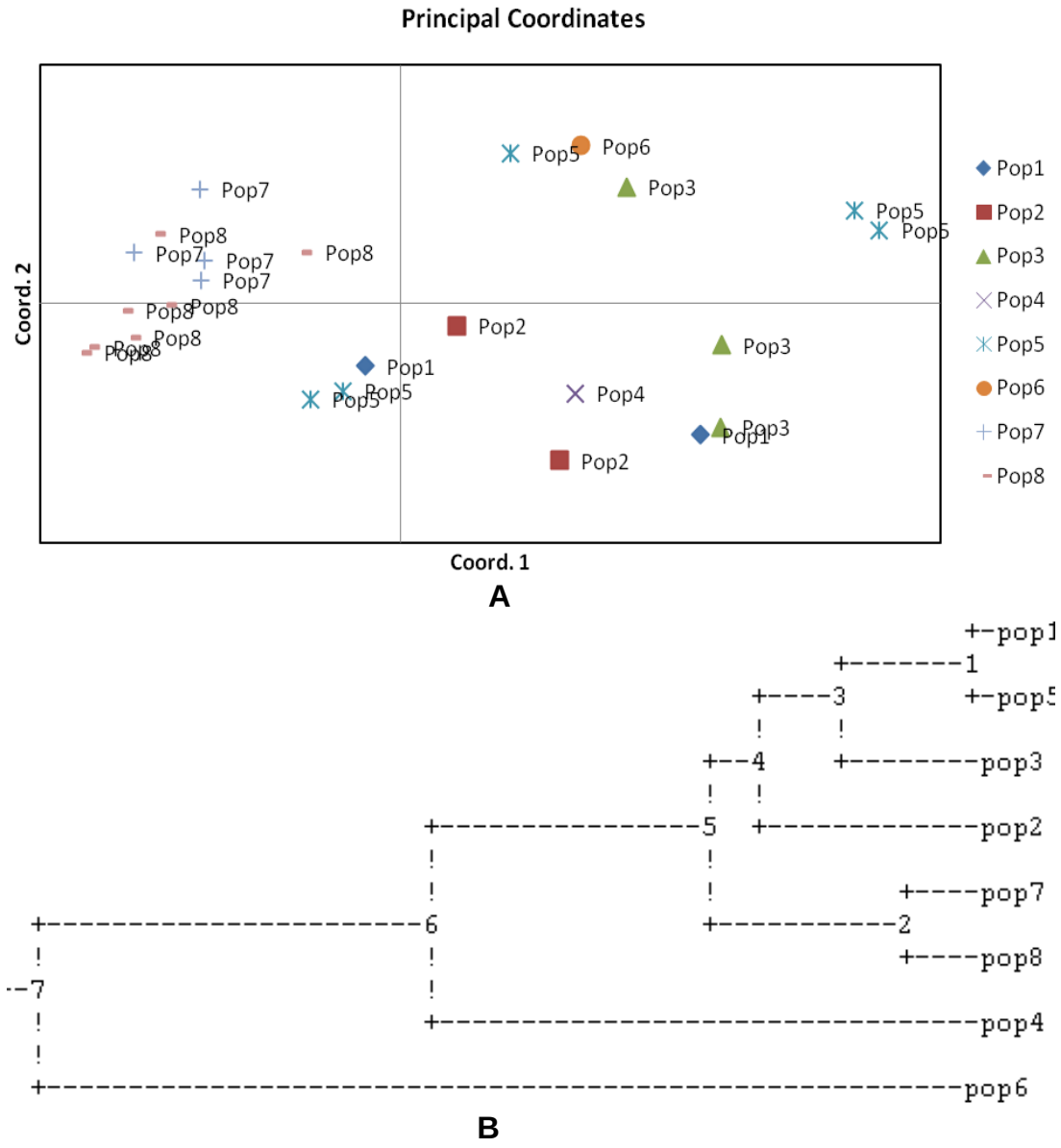

Figure 3. (A) Principle coordinate analysis plot of AFLP genotypes of 25 isolates of Fusarium oxysporum f. sp. lycopersici from Thailand based on genetic Nei's (1978) genetic distance between genotypes. (B) dendrogram based Nei's genetic distance using UPGMA modified from neighbor procedure of PHYLIP version 3.5 based on AFLP genotypes in 8 populations, pop1: Khonkaen; pop2: Bangkok; pop 3: Sakon Nakhon; pop4: Burirum; pop5: Nongkhai; pop6 Nakhon Ratchasima; pop7: Tak and pop8: Phetchaboon.

a powerful, simple and rapid technique for studying the identification and genetic relationship between *F. oxysporum* and their pathogenicity as stated by Majer et al. (1996). AFLP may therefore provide a rich source of molecular markers which are useful for studying genetic variation for specific level.

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