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Pathogenicity and Genetic Diversity of *Fusarium oxysporum* Causing Soybean Root Rot in Northeast China

Yonggang Li[1,2], Tongxue Zhao[1], Gia Khuong Hoang Hua[2], Lankun Xu[1], Jinxin Liu[1], Shuxian Li[3], Hanwen Huang[4] & Pingsheng Ji[2]

[1] Agricultural College, Northeast Agricultural University, China

[2] Department of Plant Pathology, University of Georgia, Tifton, GA, USA

[3] USDA, Agricultural Research Service, Crop Genetics Research Unit, Stoneville, MS, USA

[4] Department of Epidemiology and Biostatistics, University of Georgia, Athens, GA, USA

Correspondence: Yonggang Li, Key Laboratory of Cold Crop Breeding Improvement and Physiological Ecology in Heilongjiang Province, Agricultural College, Northeast Agricultural University, Harbin 150030, China. Tel: 086-0451-55191064. E-mail: neaulyg@126.com

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Abstract

Soybean is an important edible legume cultivated around the world. However, soybean production is seriously impacted by the widespread of root rot disease. In this study, genetic diversity and pathogenicity of *Fusarium oxysporum* associated with root rot of soybean in Heilongjiang province, China, were examined. A total of 50 *F. oxysporum* strains were isolated from diseased soybean plants grown in Harbin, Heihe, Jixi, Jiamusi and Qiqihar of Heilongjiang province. Pathogenicity study indicated that all *F. oxysporum* strains were able to induce root rot disease on soybean in which 28% of the isolates were highly aggressive, 42% were moderately aggressive, and 30% were weakly aggressive. Aggressiveness of the isolates did not appear to be associated with geographic location or plant age of isolation. Genomic DNA of the isolates was analyzed by polymerase chain reaction using eight amplified fragment length polymorphism (AFLP) primers that generated 1728 bands, of which 99% were polymorphic. Cluster analysis using UPGMA showed that the similarity values ranged from 0.15 to 0.47. At a similarity coefficient of 0.2, the isolates were separated into 7 groups. Analysis of molecular variance indicated that about 92% of the genetic variation resided within populations. No correlation was found between genetic diversity and aggressiveness or the geographic origin of the isolates. Results of the study indicate that pathogenic *F. oxysporum* are commonly associated with root rot of soybean with various aggressiveness and they are genetically diverse.

Keywords: AFLP, soil borne pathogen, virulence, correlation

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important oilseed crop and a valuable source of vegetable proteins (Loganathan et al., 2010). Approximately 7 million tons of soybean seeds were produced in 69 countries in the world (FAO, 2016). With the production of 693 thousand tons of soybean seeds, China is considered as one of the world's leading soybean producers (FAO, 2016). Total areas planted to soybean in China have been increasing, and the largest soybean producing province in the country is Heilongjiang (Li et al., 2013; Shurtleff & Aoyagi, 2016). Heilongjiang is located in northeast China with 4.3 million hectares of soybean produced in the province (Shurtleff & Aoyagi, 2016).

Soybean production is hampered by the occurrence of root rot disease caused by *Fusarium* spp. Fusarium root rot has been a problem in soybean production in many countries worldwide (Sinclair & Backmen, 1989; Arias et al., 2013; Zhang et al., 2013a). Tap and lateral roots infected by the disease often become reddish brown or light to dark brown. The roots become shallow and fibrous and eventually rotted, and plants may be wilted especially under conditions of low moisture and high temperature. Root rot of soybean can be induced by various *Fusarium* species, with *F. oxysporum* being the most common species reported (Nelson, 1999; Shiraishi et al., 2012; Zhang et al., 2013a).

Management of Fusarium root rot of soybean is difficult. Information regarding fungicides effective for suppressing *F. oxysporum* on soybean is limited. In northeast China, growers often abandon production of soybean when the fields get heavy infestation by the pathogen due to lack of effective disease control measures and panic of severe yield loss. Host resistance is a recommended strategy for managing soil borne diseases. In a study conducted in Canada (Zhang et al., 2013a), 70 soybean cultivars were evaluated under field conditions and 17 cultivars with the lowest severity of root rot caused by *F. oxysporum,* ranging from 1.3 to 2.2, were the most resistant. Soybean cultivars with resistance to Fusarium root rot under conditions in northeast China are not known to be available. It is unknown neither if *F. oxysporum* causing root rot of soybean are phenotypically and genetically diverse. It is highly desirable to determine diversity of the pathogen for developing effective disease management programs such as the use of host resistance.

Genomic fingerprinting techniques have been widely used to study genetic variability, population structure, and species phylogeny of *F. oxysporum* (Silva et al., 2013; Chen et al., 2014; Zimmermann et al., 2015). Among the techniques, amplified fragment length polymorphism (AFLP) is well recognized which has great discriminatory power (Vos et al., 1995; Mueller & Wolfenbarger, 1999; Silva et al., 2013). In a study on Fusarium wilt of bitter gourd caused by *F. oxysporum* f. sp. *momordicae*, AFLP analysis differentiated isolates with high virulence from those with low virulence, and pathogenicity of the 48 pathogen isolates was correlated with geographical locations of isolation of the isolates (Chen et al., 2014). In another study on *F. oxysporum* f. sp. *radicis-cucumerinum* from cucumbers, analysis with AFLP markers divided the 30 isolates in two distinct clusters (Tok & Kurt, 2010). All isolates in one cluster belonged to a vegetative compatibility group and isolates in the other cluster belonged to another vegetative compatibility group. These studies indicate that AFLP analysis is a useful tool in assessing genetic diversity of *F. oxysporum* populations.

The objective of this study was to determine pathogenicity of *F. oxysporum* associated with root rot of soybean in northeast China and use AFLP technology to analyze genetic diversity of the pathogen. This study advances our understanding of the etiology of the disease and population genetics of the pathogen, which provides valuable information for developing disease management programs.

2. Materials and Methods

2.1 Sample Collection and Pathogen Identification

Soybean plants showing root rot symptoms were sampled from different regions in Heilongjiang, China, in 2012 (Figure 1). Roots were washed with tap water, cut into pieces of 0.5 cm^3, disinfested with 70% ethanol for 2 s and 0.5% NaOCl for 5 min, and rinsed three times with sterile distilled water. Root tissues were placed on potato dextrose agar (PDA) and incubated at 26 °C. Fungal hyphae grown from the tissues were transferred to fresh PDA plates. The isolates were identified as *F. oxysporum* according to cultural and morphological characteristics (Nelson et al., 1983; Leslie & Summerell, 2006). Single conidium isolates were obtained using the methods reported previously (Leslie & Summerell, 2006; Petkar et al., 2017).

For molecular identification, mycelia from 5-day-old cultures on PDA were used to extract DNA using the methods reported by Zhang et al. (2010) with minor modifications. In brief, mycelia of the isolates were pulverized using liquid nitrogen and homogenized using extraction buffer containing cetyl trimethyl ammonium bromide (CTAB), 0.5% charcoal along with 0.2% β-mercaptoethanol. After incubation at 65 °C for 15 min, homogenates were purified three times with chloroform:isoamyl alcohol (24:1). The upper aqueous phase (400 µL) was transferred to a tube containing 800 µL isopropanol, and DNA pellets were obtained by adding 0.67 volumes of propanol. The pellets were washed with ice-cold ethanol (70%), air dried, dissolved in 50 µL of deionized water, and stored at 4 °C. Polymerase chain reaction (PCR) analysis was performed with *F. oxysporum* species-specific primers FOF1 and FOR1 using the conditions described previously (Zhang et al., 2013b). PCR products were visualized by running 1.5% agarose gel and observed under a gel documentation system.

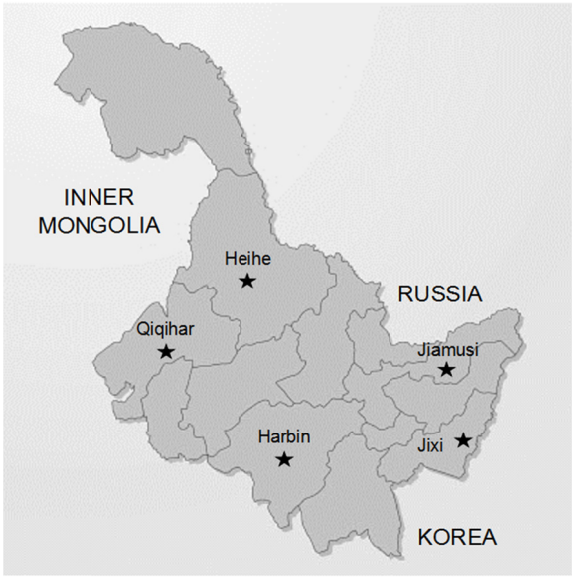

Figure 1. Map showing sampling locations of *Fusarium oxysporum* in Heilongjiang province, China

2.2 Pathogenicity Tests

To prepare inoculum of the isolates, flasks containing 125 g of sorghum seeds and 50 ml of distilled water were autoclaved at 121 °C for 40 min in two consecutive days. A mycelial plug (7 mm diameter) taken from 7-day-old cultures of the isolates was transferred to each flask. The flasks were incubated at 26 °C for a week and mixed by hand every 3 days. Ten sorghum seeds fully colonized by *F. oxysporum* were uniformly distributed on the top of vermiculite in a plastic pot (8 × 8 cm), and then covered with a 0.5 cm layer of sterile vermiculite. In the non-inoculated control, equal numbers of sterilized sorghum seeds were used. Twelve soybean seeds (cv. Hefeng 25) were surface-disinfested with 1.5% NaOCl for 5 min, washed three times with sterile distilled water, and sown in each pot. A randomized complete block design was used with three replicates and two pots for each isolate in each replicate. The pots were kept in a greenhouse at 25±3 °C, and seedlings in each pot were thinned to 10 after emergence. The seedlings were watered daily using overhead irrigation to maintain soil moisture.

To evaluate disease severity, seedlings were removed from the pots 10 days after emergence and roots were washed under running tap water. Disease was rated using a 0-to-7 scale previously described by Li et al. (2013) where 0 = healthy plant; 1 = slightly darkening fibrous root, the aboveground portion grew well; 3 = slightly darkening taproot, the aboveground portion grew well; 5 = severe darkening taproot or hypocotyls erosion, the aboveground portion grew poorly; and 7 = root necrotized and infected plant dead. The experiment was conducted twice under similar conditions. Disease data were expressed as Percent Disease Index (PDI) (Wheeler, 1969). Isolates were defined as highly aggressive if PDI ≥ 50%, median aggressiveness if 50% > PDI ≥ 25%, and low aggressiveness with PDI < 25%. Diseased plants were sampled for isolation and identification of the causal agent as described above.

2.3 Genetic Diversity of F. oxysporum Isolates

Genomic DNA of 50 isolates was extracted as described above. Molecular fingerprinting of the isolates was conducted using AFLP markers with *Eco*RI and *Mes*I as digestion enzymes. Adapters and primers used for AFLP analysis were designed as reported by Vuylsteke et al. (2007) and are listed in Table 1.

Table 1. Sequences of adapters and primers used for AFLP analysis

Restriction enzyme	Type	Code	Sequence
*Eco*RI	Adapter	E1	5'-CTCGTAGACTGCGTACC-3'
		E2	5'-AATTGGTACGCAGTCTAC-3'
	Primers	E00	5'-GACTGCGTACCAATTCA-3'
		E11	5'-GACTGCGTACCAATTCAAC-3'
		E12	5'-GACTGCGTACCAATTCAAG-3'
		E13	5'-GACTGCGTACCAATTCACA-3'
		E14	5'-GACTGCGTACCAATTCACT-3'
		E15	5'-GACTGCGTACCAATTCACC-3'
		E16	5'-GACTGCGTACCAATTCACG-3'
		E17	5'-GACTGCGTACCAATTCAGC-3'
		E18	5'-GACTGCGTACCAATTCAGG-3'
*Mse*I	Adapter	M1	5'-GACGATGAGTCCTGAG-3'
		M2	5'-TACTCAGGACTCAT-3'
	Primers	M00	5'-GATGAGTCCTGAGTAAC-3'
		M11	5'-GATGAGTCCTGAGTAACAA-3'
		M12	5'- GATGAGTCCTGAGTAACAC-3'
		M13	5'-GATGAGTCCTGAGTAACAG-3'
		M14	5'-GATGAGTCCTGAGTAACAT-3'
		M15	5'- GATGAGTCCTGAGTAACTA-3'
		M16	5'-GATGAGTCCTGAGTAACTC-3'
		M17	5'-GATGAGTCCTGAGTAACTG-3'
		M18	5'-GATGAGTCCTGAGTAACTT-3'

2.3.1 Modification of DNA and Template Preparation

AFLP analysis was conducted using the methods of Vos et al. (1995) with minor modifications. To prepare DNA templates, 3 μL genomic DNA (150 ng/μL) was incubated for 4 h at 37 °C with 17 μL of restriction digestion mixture containing 3 U *Eco*RI (15 U/μL), 3 U *Mse*I (10 U/μL), 2.0 μL 10 × PCR buffer with BSA, and 14.5 μL double-distilled water (ddH_2O). Aliquots of 5 μL digested DNA were then analyzed by electrophoresis in a 1.2% agarose gel. In the digestion process, digestion products were ligated with *Eco*RI and *Mse*I adapters at the following conditions: 95 °C for 5 min, 65 °C for 10 min, and 37 °C for 10 min.

2.3.2 Ligation Reaction

Before performing ligation reactions, restriction digestion mixture obtained from the previous step was incubated at 65 °C for 10 min to inactivate restriction enzymes. Each restricted DNA product was, then, incubated with 5 μL of ligation mixture, which consisted of 1.8 μL ATP, 0.5 μL *Eco*RI adapter mix, 1.0 μL *Mse*I adapter mix, 1.0 μL T4 DNA ligase, 0.5 μL T4 DNA ligase buffer, and 0.2 μL ddH_2O, at 37 °C for 12 h. A volume of 5 μL ligated products was loaded on a 1.2% agarose gel to check for complete ligation. DNA templates, which were completely digested and ligated, were used for AFLP reactions.

2.3.3 AFLP Reactions

Pre-amplification was carried out in a volume of 20 μL containing 5 μL DNA template, 2.0 μL 10× PCR buffer (Mg^{2+}), 1.6 μL dNTPs (2.5 mM), 0.6 μL primer E00 (50 ng/μL), 0.6 μL primer M00 (50 ng/μL), 0.1 μL *Taq* DNA polymerase (5.0 U/μL), and 10.1 μL ddH_2O. PCR reactions were performed using the following cycle profile: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s followed by one cycle of 72 °C for 10 min. Amplified products were stored at 4 °C.

Prior to performing selective amplification, pre-selective PCR products were diluted 20 times using TE buffer. Twenty μL PCR reactions were conducted containing 5 μL pre-amplified DNA, 2.0 μL 10× PCR buffer (Mg^{2+}), 1.8 μL dNTPs (2.5 mM), 0.8 μL *Eco*RI primer (50 ng/μL), 0.8 μL *Mse*I primer (50 ng/μL), 0.15 μL *Taq* DNA polymerase (5.0 U/μL), and 9.45 μL ddH_2O. DNA templates were selectively amplified using the following parameters: 95 °C for 50 s, 65 °C for 40 s (with a gradually reduction of 0.7 °C per cycle), 72 °C for 1 min, 31

cycles of 95 °C for 50 s, 56 °C for 40 s, 72 °C for 1 min, and one cycle of 72 °C for 10 min before holding at 4 °C. Finally, 5 μL of PCR products was electrophoresed in a 2% agarose gel.

A total of 64 primer combinations were tested (8 *Eco*RI primers and 8 *Mse*I primers) in this study and eight combinations, which generated clear bands with high polymorphism and good repeatability, were selected for genetic diversity analysis of the 50 *F. oxysporum* isolates.

2.4 Data Analysis

Selective amplification products were visualized on 4% polyacrylamide gels with an automatic DNA sequencer (ABI PRISM 377 sequencer; Promega, USA). Then, AFLP marker data (70-500 bp) were analyzed using GeneScan version 3.1 (Applied Biosystems, Foster City, CA, USA). The presence/absence of variable bands in the AFLP gel or autoradiogram was scored manually ('1' for present and '0' for absent). Genetic distance was estimated and a dendrogram showing genetic relationship among the *F. oxysporum* isolates was constructed using NTSYSpc version 2.11V (Exeter Software, Setauket, NY) (Rohlf, 2004) based on unweighted pair-group method with arithmetic mean (UPGMA) clustering analysis (Kafkas et al., 2008). Analysis of molecular variance (AMOVA) was conducted using GenAlEx version 6.5 (Peakall & Smouse, 2012).

3. Results

3.1 Pathogenicity of Isolates

Fifty *F. oxysporum* isolates were collected from five different geographic areas of Heilongjiang province, China, including 10 from Harbin, 7 from Heihe, 5 from Jixi, 11 from Jiamusi, and 17 from Qiqihar. The majority of *F. oxysporum* isolates (68%) were obtained from soybean seedlings and the rest of the isolates were from mature soybeans (Table 2). When tested on soybean seedlings, significant variation in aggressiveness was observed among the isolates (Table 2). There were 15 weak pathogenic isolates (PDI < 25%), 21 medium aggressive isolates (25% ≤ PDI < 50%), and 14 highly aggressive isolates (PDI ≥ 50%). The most aggressive isolate was 103 (PDI = 82.9%) obtained from soybean seedling in Heihe. Isolates 12, 93, 33, 147, 163, 167, 125, 90, 91, 166, 150, 137 and 144 had disease index lower than 10%. There was no correlation between aggressiveness of the isolates and their geographic origins, or between aggressiveness of the isolates and plant age (seedling or mature plant) when the fungus was isolated.

Table 2. Pathogenicity of *Fusarium oxysporum* isolates on soybean under greenhouse conditions

AFLP group	Strains	Origin	Sampling stage	DI (%)*
E	11	Qiqihar	Seedling	62.4 H
	16	Qiqihar	Seedling	62.9 H
	14	Qiqihar	Seedling	42.9 M
	15	Qiqihar	Seedling	45.2 M
	19	Qiqihar	Seedling	40.5 M
	30	Heihe	Seedling	71.4 H
	36	Heihe	Adult	68.6 H
F	12	Qiqihar	Seedling	5.7 L
	73	Qiqihar	Adult	46.7 M
	79	Harbin	Adult	39.0 M
	93	Harbin	Adult	2.4 L
A	9	Qiqihar	Seedling	42.4 M
	33	Heihe	Adult	6.7 L
	103	Heihe	Seedling	82.9 H
	84	Qiqihar	Adult	51.4 H
	88	Qiqihar	Seedling	45.7 M
	65	Jixi	Seedling	32.9 M
	60	Jixi	Seedling	52.9 H
	130	Jiamusi	Seedling	44.3 H
	156	Qiqihar	Seedling	71.4 H
	147	Jiamusi	Seedling	4.3 L
	49	Jixi	Seedling	37.1 M
	161	Harbin	Adult	36.7 M
	162	Harbin	Adult	16.7 L
	163	Harbin	Adult	9.5 L
	165	Harbin	Adult	42.9 M
	167	Qiqihar	Seedling	6.2 L
	99	Heihe	Seedling	71.4 H
	102	Heihe	Adult	64.8 H
	122	Jiamusi	Adult	49.5 M
	125	Jiamusi	Seedling	2.4 L
	119	Jiamusi	Seedling	37.6 M
B	10	Qiqihar	Seedling	38.1 M
	90	Qiqihar	Seedling	2.9 L
	91	Qiqihar	Seedling	3.8 L
	46	Heihe	Adult	33.8 M
	76	Harbin	Adult	41.9 M
C	158	Qiqihar	Seedling	29.5 M
	159	Qiqihar	Seedling	55.2 H
	166	Harbin	Adult	7.6 L
	164	Harbin	Adult	27.6 M
D	135	Jiamusi	Seedling	50.0 H
	149	Jiamusi	Seedling	16.2 L
	150	Jiamusi	Seedling	2.4 L
	140	Jiamusi	Seedling	44.3 M
	146	Jiamusi	Seedling	33.3 M
	152	Harbin	Seedling	51.4 H
	145	Jiamusi	Seedling	42.9 M
G	137	Jixi	Seedling	4.8 L
	144	Jixi	Seedling	4.8 L

Note. * DI = Disease index. H = highly aggressive with DI ≥ 50%; M = Median aggressiveness with 25% ≤ DI < 50%; and L = low aggressiveness with DI < 25%.

3.2 AFLP Analysis

Eight primer pairs and their combinations were used to amplify 50 *F. oxysporum* isolates and resulted in 99% polymorphic fragments. Totally, 216 different amplification products with fragment sizes ranging from 70 to 500 bp were produced. The highest number of polymorphic bands was generated with an AFLP primer combination E13/M13, whereas the lowest number of polymorphic bands was generated with primer combinations E11/M11, E15/M15 and E17/M13 (data not shown).

Cluster analysis showed that similarity values ranged from 0.15 to 0.47 (Figure 2). The highest similarity coefficient was measured between isolates 90 and 91 from Qiqihar. At a genetic similarity of 0.20, the 50 isolates could be divided into seven groups. Group A contained 21 isolates from adult and young soybean seedlings with diverse origins including Harbin, Heihe, Jixi, Jiamusi, and Qiqihar. Group B contained 5 isolates, 3 from young seedlings in Qiqihar and 2 from adult plants in Heihe and Harbin. Group C contained 4 isolates from young and adult plants in Qiqihar and Harbin. Group D contained 7 isolates from young seedlings in Jiamusi and Harbin. Group E contained 7 isolates, all from young seedlings except one from an adult plant in Heihe. Group F contained 4 isolates from young and adult plants in Qiqihar and Harbin. Group G contained 2 isolates from young seedlings in Jixi.

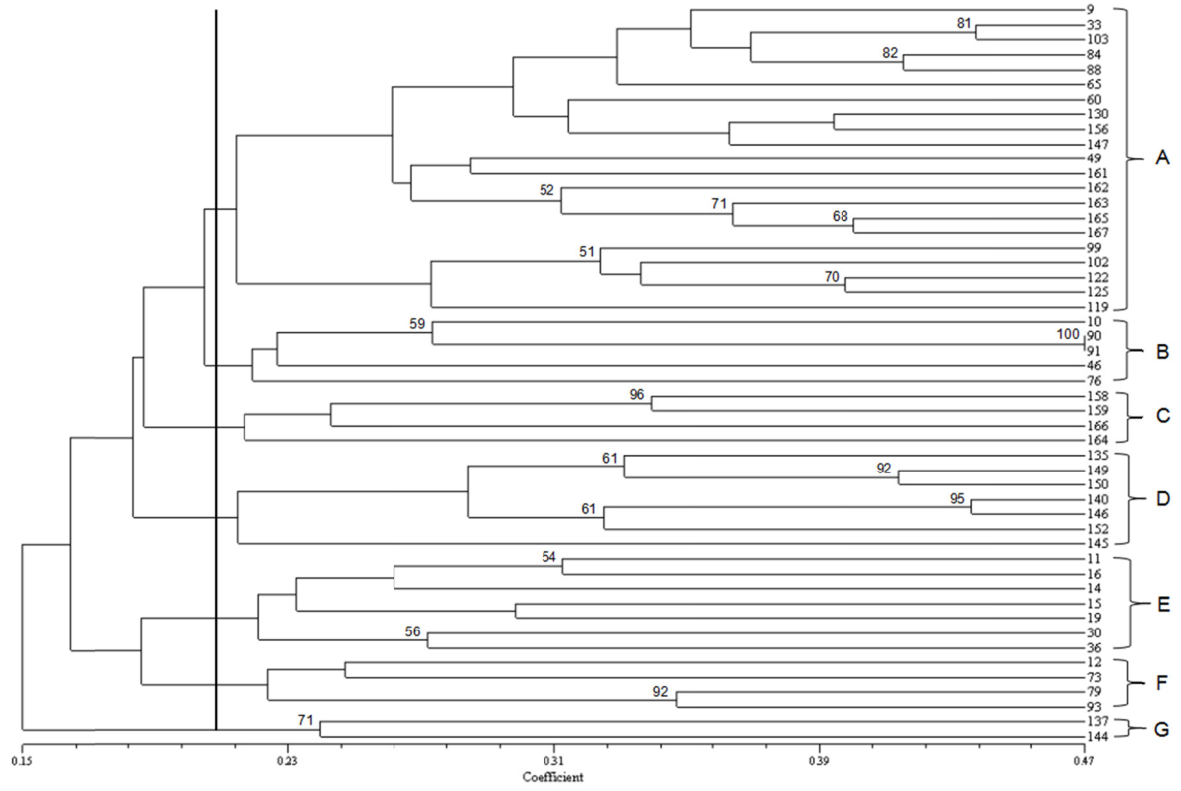

Figure 2. UPGMA tree of 50 *Fusarium oxysporum* isolates sampled from different soybean producing areas of Heilongjiang, China. Bootstrap values ≥ 50% are shown above branch

Highly significant ($P \leq 0.01$) genetic differences were detected when AMOVA analysis was performed to estimate within-group and among-group variations according to AFLP genetic groups or geographic origins of the isolates (Table 3). The fixation index (F_{ST}) is used to estimate the degree of population differentiation. It is suggested that F_{ST} values from 0 to 0.05 represent little genetic differentiation, from 0.05 to 0.15 represent moderate genetic differentiation, from 0.15 to 0.25 represent great genetic differentiation, and F_{ST} larger than 0.25 represent very great genetic differentiation (Hartl & Clark, 1997). There was little genetic differentiation associated with geographic location of isolation, plant age of isolation, and aggressiveness of the isolates with F_{ST} values lower than 0.05. There was moderate genetic differentiation associated with AFLP groups.

Table 3. Hierarchical partitioning of variance among and within population groups of *Fusarium oxysporum* strains based on analysis of molecular variance (AMOVA)

Source of Variation	df	Variance	Total (%)	F_{ST}	*P*
Location					
Among groups	4	3.73	2.3	0.023	0.005
Within groups	45	158.01	97.7		
Plant age					
Among groups	1	0.16	0.1	0.001	0.362
Within groups	48	160.82	99.9		
Genetic group					
Among groups	6	13.01	7.94	0.079	0.001
Within groups	43	150.83	92.06		
Disease					
Among groups	2	0.37	0.23	0.0023	0.234
Within groups	47	160.76	99.77		

4. Discussion

Fusarium species are common soil borne pathogens that cause destructive diseases on a wide range of hosts (Nelson et al., 1997; El-Kazzaz et al., 2008). *F. oxysporum* is frequently isolated from soybean roots and serious yield impact of root rot associated with *Fusarium* spp. is reported in many soybean producing areas (Wrather et al., 2001; Li & Ma, 2011; Arias et al., 2013). In the present work, *F. oxysporum* was isolated from all the sampling sites in Heilongjiang province, suggesting that this pathogen is one of the most important causal agents of soybean root rot in this region. Our findings are in line with previous studies which showed *F. oxysporum* to be the predominant species recovered from diseased soybean plants in China (Bai et al., 2009; Li & Ma, 2011) and other countries (Leslie et al., 1990; Nelson, 1999; Zhang et al., 2013a).

Variations in aggressiveness among pathogenic strains of *F. oxysporum* from soybean root were reported in a few studies. In a study to assess impact of Fusarium species on soybean yield and growth, significant difference was observed among the *F. oxysporum* strains in their ability to cause root rot and damping-off of soybean (Arias et al., 2013). Ellis et al. (2016) reported that 3 isolates of *F. oxysporum* caused high incidence of vascular discoloration of soybean stems or roots and 25 strains caused low to moderate levels of incidence, and one of the fungal effector genes, *Six6*, was found only in the strains causing high levels of vascular wilt. Of the 50 strains obtained from Heilongjiang province, 14 strains caused severe root rot, 21 strains were moderately aggressive and 15 strains induced low root rot severity on soybean, indicating significant variations in aggressiveness among the strains.

Studies to assess genetic diversity of *F. oxysporum* from soybean root are limited. In studies on *F. oxysporum* from other crops analyzed by AFLP, genetic groups were found to be associated with aggressiveness of *F. oxysporum* f. sp. *momordicae* isolates (Chen et al., 2014) or vegetative compatibility group of *F. oxysporum* f. sp. *radicis-cucumerinum* isolates (Tok & Kurt, 2010). AFLP was used in this study to access genetic diversity of *F. oxysporum* isolates in major soybean producing regions in Heilongjiang. The eight primer pairs used resulted in 99% polymorphic fragments, which is in agreement with previous reports that AFLP generates more polymorphic bands than other DNA-fingerprinting techniques (Vaneechoutte, 1996; Duan et al., 2008). AFLP analysis demonstrated great variability in genetic relatedness among the 50 *F. oxysporum* strains from soybean, with 7 genetic groups identified at a similarity level of 0.2. AMOVA analysis indicated that most of molecular variation was attributed to within populations, suggesting high level of genetic variation and complexity of the *F. oxysporum* isolates. AFLP groups were not correlated with geographic location of isolation, plant age of isolation, or aggressiveness of the isolates, as suggested by low F_{ST} values (< 0.03).

In a study of *Fusarium oxysporum* f. sp. *lentis* isolates from lentil plants, the isolates were clustered in two AFLP groups, and no correlation was found between AFLP grouping and geographical origin or aggressiveness of the isolates (Belabid et al., 2004). AFLP analysis was conducted to study mutation of *F. oxysporum* f. sp. *vasinfectum* isolates associated with cotton, and there was no correlation between the AFLP mutations and increased aggressiveness of the isolates (Wang et al., 2008). Duan et al. (2008) reported that AFLP groups of *F. oxysporum* f. sp. *niveum* isolates were not associated with geographic regions of the isolates but were correlated with their

physiological races. These studies indicate that AFLP grouping may or may not be related to phenotypic traits of *F. oxysporum* isolates, depending on the pathogens and host plants studied.

5. Conclusion

Soybean root rot is a serious disease causing significant yield reduction in soybean production. Results in the study indicate that there is a high level of genetic diversity within *F. oxysporum* strains responsible for soybean root rot in Heilongjiang province, China. The strains differ in aggressiveness that is not associated with their geographic distribution, plant age of isolation, and molecular fingerprinting groups. The information generated in the study advances our knowledge about etiology, pathogenicity, and diversity of soybean root rot pathogen, which facilitates development of effective strategies for managing this major disease.

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