Genetic Diversity among Isolates of *Phytophthora sojae* in Anhui Province of China based on ISSR-PCR Markers

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ABSTRACT. To explore genetic differentiation and the genetic relationships of *Phytophthora sojae* in Anhui Province, the inter-simple sequence repeat (ISSR) technique was used to analyze the genetic diversity of *P. sojae*. One hundred and sixty ISSR fragments were observed, including 129 (80.6%) polymorphic bands. This suggested that abundant genetic diversity existed among *P. sojae* in Anhui Province. The pairwise genetic similarity coefficients among the 62 strains ranged from 0.72 to 0.96, with a mean value of 0.85, indicating that there was a high level of genetic variation. *Phytophthora sojae* strains were divided into five clusters based on neighbor-joining (NJ) analysis, and the clustering was not related to geographic source, according to Mantel's test ($r=0.3938$). In addition, the clustering of strains from the same geographical source had little relationship to the year of collection. Analysis of molecular variance (AMOVA) showed that 16.65% of the genetic variation was derived from the collection area and 83.35% of the genetic variation was within-population variation. The genetic flow between different geographical sources ranged from 0.623 to 2.773, with a mean of 1.325, suggesting that gene exchange was frequent. Genetic distance and the genetic differentiation coefficient were not related to spatial distance.

Phytophthora sojae is a destructive soilborne oomycete pathogen of soybean (*Glycine max*). Soybean phytophthora root and stem rot incited by *P. sojae* has been reported in Asia, Africa, Europe, North America, South America, and Oceania as soybean production is increasing around the world (Tyler, 2007). In China, phytophthora root and stem rot on soybean were reported in Fujian, Heilongjiang, and Anhui provinces (Dai et al., 2015; Lamour and Kamoun, 2009). *Phytophthora sojae*, a soilborne pathogen, forms a large number of oospores in the roots and stems of soybean, and these oospores can survive on residual plant material in the soil (Lamour and Kamoun, 2009). In addition, *P. sojae* was the primary infection that led to soybean phytophthora root rot in the next year (Garant et al., 2007). In Anhui Province, soybean phytophthora root rot might spread by soybean straw being returned to the field and inappropriate mechanized farming according to the Regulations for Control of Soybean Phytophthora Blight that was formulated by the Anhui Provincial Quality and Technical Supervision Bureau (Hefei, China). For example, uncleaned soybean combines, which carry *P. sojae* through soybean straw and soil, disperse pathogens among adjacent areas.

Some studies of the genetic diversity of *P. sojae* have been reported (Galley et al., 2007; Mohammadi et al., 2014; Silvina

et al., 2011; Wu et al., 2013, 2017). The first phylogenetic analysis of *P. sojae* was performed; its taxonomy and classification in relation to the other three *Phytophthora* species (*Phytophthora medicaginis*, *Phytophthora trifolii*, and *Phytophthora megasperma*) were evaluated by Hansen and Maxwell (1991). Researchers observed that their mitochondrial DNA content differed based on restriction fragment length polymorphism (RFLP) results (Förster et al., 1994). Meng et al. (1999) used random amplified polymorphic DNA (RAPD) markers to analyze 55 isolates of *P. sojae* from Illinois, Indiana, Iowa, and Minnesota. The results indicated that there was diversity in pathotype (race) classifications and geographic regions. Galley et al. (2007) also evaluated the genetic diversity of 32 isolates of *P. sojae* from South America and found that there was a high level of variability even among isolates in the same geographic region. Simple sequence repeats (SSRs) were identified from transcript and genomic sequences from *P. sojae* and *Phytophthora ramorum* (Garnica et al., 2006). Wang et al. (2007) compared *P. sojae* isolates from China and the United States with RAPD and ISSR to clarify the origin of *P. sojae*. The results did not support the conjecture that *P. sojae* in the United States may be derived from China. Recently, RAPD, SSR, and ISSR were used to assess the genetic diversity of *P. sojae* isolates from China and Iran (Mohammadi et al., 2014; Wu et al., 2014, 2017). In Heilongjiang Province, China, 39 *P. sojae* isolates were collected from soil and soybean and genetic diversity analysis was performed by Wu et al. (2013) using two methods: RAPD and SSR. They found that the similarity values among *P. sojae* isolates indicated they were

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very close. The similarity values among *P. sojae* isolates ranged from 49% to 82% and 47% to 85% based on the RAPD and SSR data, respectively. RFLP, amplified fragment length polymorphism, RAPD, ISSR, and SSR were usually used for genetic diversity analysis. Among the molecular markers, ISSR is an easily used and reliable marker for investigating genetic diversity in plants, animals, and fungi (Barth et al., 2002; Budak et al., 2004). Furthermore, cluster analysis was an important focus of genetic diversity analysis. The currently popular dendrogram analysis was carried out by the unweighted pair group with arithmetic mean (UPGMA), NJ, Bayesian, and unweighted parsimony methods (Breton et al., 2008; Geuna et al., 2003; Nei and Takezaki, 1983). Among these, the UPGMA method relies on the assumption that all evolutionary lineages evolve at the same rate, but the NJ method requires no such assumption (Nei and Takezaki, 1983). This assumption cannot always be satisfied, and the UPGMA method is not an exact method in practice. However, some researchers have reported that the Bayesian method requires enormous computational time (Talavera and Castresana, 2007). Some studies showed that the NJ analysis gave the best reflection of the average pairwise combinations in all dendrograms (Geuna et al., 2003).

Although some previous studies have evaluated the genetic diversity of *P. sojae*, little is known about the genetic structure of *P. sojae* in Anhui Province, eastern China. Here, we investigate the genetic structure of *P. sojae* isolates from five cities (Bozhou, Fuyang, Suzhou, Bengbu, and Luan) of Anhui Province. The aim of this study was to better understand the population structure and genetic diversity of *P. sojae* in Anhui Province, through the use of ISSR markers, to further provide useful genetic information that could help to monitor the pathogen in the region and improve control strategies for *P. sojae*.

Materials and Methods

ISOLATE COLLECTION AND DNA EXTRACTION. A total of 62 single-spore *P. sojae* isolates were collected from soil and infected soybeans from five cities, Bozhou (BZ), Fuyang (FY), Suzhou (SZ), Bengbu (BB), and Luan (LA), in Anhui Province from 2008 to 2014 (Table 1). Information about the localities, collection year, cluster, and isolate number is provided in Supplemental Table 1. *Phytophthora sojae* from soil samples was isolated using the soybean leaf baiting method (Schmitthenner et al., 1994). *Phytophthora sojae* from soybean plant samples was isolated according to the method of Tyler (2007). Briefly, diseased tissues from symptomatic stems or roots were washed with sterile water and disinfected with 0.2% sodium hypochlorite. The small tissues were placed on 10% (v/v) V8 solid medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and 20 $\mu\text{g}\cdot\text{mL}^{-1}$ rifampicin and cultured at 25 °C in darkness. *Phytophthora sojae* isolates were

identified based on the morphological characteristics of the pathogen and nuclear ribosomal DNA internal transcribed spacer (ITS) analysis. The ITS region was amplified with specific primers for *P. sojae*, ITS4 and ITS6 (Dai et al., 2015). *Phytophthora sojae* isolates were preserved at 12 °C at the Mycology and Plant Fungal Disease Laboratory of Anhui Agricultural University (Hefei, China). The *P. sojae* strains used in this study were cultured on 10% V₈ medium at 25 °C in the dark for 5 d. Then, six to eight plugs of actively growing mycelia were obtained with a 9-mm cork borer, and *P. sojae* discs were placed into 10% V₈ liquid medium. *Phytophthora sojae* strains were cultured at 25 °C for 3–5 d, and mycelia were harvested for DNA isolation. Mycelia of *P. sojae* strains were ground into powder with liquid nitrogen, and the *P. sojae* genomic DNA was isolated with the CTAB method according to Tyler et al. (1995). All DNA samples were quantified using a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, Waltham, MA) and then diluted to 100 ng· μL^{-1} with double-distilled H₂O (ddH₂O) for inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) amplification.

ISSR-PCR AMPLIFICATION. Fifty-four primers were synthesized by Sangon Biotechnology (Shanghai, China) and initially screened on 20 isolates. Thirteen primers (Table 2) that produced clear and reproducible bands were selected for ISSR-PCR amplification. The amplifications were performed in a 25- μL reaction mixture [12.5 μL 2 × Taq Plus PCR Master Mix (KT205–02; Tiangen Biotech Co., Beijing, China), 0.2 μM primer, 9.5 μL ddH₂O, and 0.5 μL template DNA], and the following PCR program was run using a thermal cycler (Arktik TCA0002; Thermo Fisher Scientific): initial denaturation for 3 min at 94 °C, 35 cycles of denaturation at 94 °C for 30 s, annealing at a specific temperature (given in Table 2) for 30 s, and extension at 72 °C for 90 s, followed by a final extension at 72 °C for 10 min. The PCR products were separated on 1.8% agarose gels containing ethidium bromide in Tris–acetate–EDTA buffer at 5 V·cm^{–1} for 3 h. Then, the agarose gels were visualized under an ultraviolet transilluminator in an imaging system (EC3 300; Ultraviolet Products, Upland, CA). All amplifications were repeated three times.

DATA ANALYSIS. The DNA bands from the ISSR-PCR amplification were recorded as present (1) or absent (0) and converted into a binary matrix. Cluster analysis of *P. sojae* was performed on the basis of a genetic distance matrix, and the dendrogram was constructed by NJ with NTSYS-pc 2.1 (Rohlf, 2000). The observed number of alleles (NA), effective number of alleles (NE), Nei's genetic diversity (*h*), Shannon's information index (*I*_s), total genetic diversity (*H*_t), and genetic diversity within groups (*H*_s) of *P. sojae* were computed with POPGENE 1.3.1 software (Yeh et al., 1999). The genetic differentiation coefficient (*G*_{ST}), gene flow coefficient (*N*_m), genetic identity (*I*), and genetic distance (*D*) among geographical groups of *P. sojae* were also calculated with POPGENE

Table 1. Information of 62 isolates *Phytophthora sojae* in five cities in Anhui Province, China.

Sampling site (city)	Isolate no.
Bozhou	BZ1, BZ2, BZ3, BZ4, BZ5, BZ6, BZ7, BZ8, BZ9, BZ10, BZ11, BZ12, BZ13, BZ14, BZ15, BZ16, BZ17, BZ18, BZ19, BZ20, BZ21
Fuyang	FY1, FY2, FY3, FY4, FY5, FY6, FY7, FY8, FY9, FY10, FY11
Suzhou	SZ1, SZ2, SZ3, SZ4, SZ5
Bengbu	BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9, BB10, BB11, BB12, BB13, BB14, BB15, BB16, BB17, BB18
Luan	LA1, LA2, LA3, LA4, LA5, LA6, LA7

Table 2. Properties of ISSR primers used for amplification of *Phytophthora sojae* isolates in this study.

Primer	Sequence 5'-3'	Annealing temp (°C)	Bands amplified (no.)	Polymorphic bands (no.)	Polymorphism (%)	h^z	I_s
ILE35	(AC) ₈ T	55	13	11	84.6	0.2128	0.3388
ILE46	(GA) ₈ CT	54	12	10	83.3	0.3020	0.4523
ILE67	(GA) ₈ CC	56	13	11	84.6	0.2870	0.4324
ILE79	ACA(AC) ₇	55	14	11	78.6	0.1970	0.3134
ILE112	(AAG) ₆ C	55	12	10	83.3	0.2097	0.3420
ILE120	CAC(GA) ₇	54	10	9	90.0	0.2116	0.3382
ILE131	CAC(CA) ₇	53	11	9	81.8	0.3055	0.4445
UBC809	(AG) ₈ G	50	12	9	75.0	0.2040	0.3145
UBC811	(GA) ₈ C	50	11	7	63.6	0.1889	0.2850
UBC835	(AG) ₈ YC	50	15	11	73.3	0.2257	0.3409
UBC841	(GA) ₈ YC	54	13	10	76.9	0.2692	0.4068
UBC866	(CTC) ₆	60	11	9	81.8	0.2648	0.4024
UBC873	(GACA) ₄	54	13	12	92.3	0.3352	0.4973

^z h = Nei's gene diversity; I_s = Shannon's information index as a measure of gene diversity.

software for all pairwise combinations of populations (Nei, 1972; Slatkin and Barton, 1989; Yeh et al., 1999). The Mantel test was used to analyze the significance of the correlation between genetic distance and geographical distance among different geographic groups (Miller, 1997). Analysis of molecular variance within and between *P. sojae* populations was evaluated according to Excoffier et al. (1992).

Results

PRIMER SCREENING AND POLYMORPHISM OF ISSR-PCR PRODUCTS. In the preliminary test, 54 primers were screened against 20 isolates, and 13 primers that produced clear and reproducible bands were selected for ISSR-PCR amplification to detect genetic characteristics of *P. sojae* strains in Anhui Province (Table 2). The annealing temperatures of 13 primers ranged from 50 to 60 °C (Table 2). An example of the DNA banding profiles of primer ILE79 is shown in Supplemental Fig. 1. The minimum and maximum number of bands generated by any primer were 10 (ILE120) and 15 (UBC835), respectively. The average number of bands was 12.2. The percentage of polymorphism ranged from 63.6% (UBC811) to 92.3% (UBC873).

As shown in Table 2, the h values ranged from 0.1889 to 0.3352. I_s ranged from 0.2850 to 0.4973. The values of h and I_s indicated that these 13 primers were appropriate for distinguishing diverse isolates of *P. sojae*. The genetic diversity of different populations was compared and is shown in Table 3. A total of 13 primers produced 160 bands, of which 129 were polymorphic, accounting for a polymorphism of 80.6%. In Bozhou City, ISSR primers generated 145 bands, which included 84 polymorphic bands, and the percentage of polymorphism was $\approx 57.9\%$. The percentages of polymorphism in the Fuyang, Suzhou, Bengbu, and Luan areas were 51.8%, 34.8%, 65.8%, and 51.1%, respectively (Table 3). These results suggested that polymorphism was abundant among different *P. sojae* isolates in Anhui Province. According to POPGENE analysis results, the H_t of the 62 isolates was 0.2211, and the H_s was 0.1647. The results of AMOVA indicated that 16.65% of the total variation was attributable to population divergence between the populations and 83.35% was attributable to individual differences within populations ($P < 0.001$). There were significant genetic differences within populations.

Table 3. Genetic variations and polymorphic features among isolates of *Phytophthora sojae*.

	TB			NE			
Population ^z	(no.) ^y	PB (no.)	PPB (%)	NA (no.)	(no.)	h	I_s
BZ	145	84	57.9	1.58	1.34	0.20	0.30
FY	143	74	51.8	1.52	1.31	0.18	0.27
SZ	138	48	34.8	1.35	1.24	0.13	0.19
BB	149	98	65.8	1.66	1.39	0.23	0.34
LA	139	71	51.1	1.51	1.32	0.18	0.27
Summary	160	129	80.6	1.81	1.41	0.25	0.38

^zBZ = Bozhou; FY = Fuyang; SZ = Suzhou; BB = Bengbu; LA = Luan.

^yTB = total bands; PB = number of polymorphic bands; PPB = percentage of polymorphic bands; NA = number of alleles (counts the number of alleles with nonzero frequency); NE = effective number of alleles (estimates the reciprocal of homozygosity); h = Nei's gene diversity; I_s = Shannon's information index as a measure of gene diversity.

ISSR CLUSTER ANALYSIS. Neighbor-joining dendrograms were constructed based on genetic distances. The results indicated that 62 isolates of *P. sojae* could be divided into five groups (Fig. 1). Clusters i, ii, iii, and iv included 5, 2, 6, and 13 isolates of *P. sojae*, respectively. The other 36 isolates of *P. sojae* formed group v. In Fig. 1, Pop BZ was distributed into five clusters (i, ii, iii, iv, and v), and Pop FY was also distributed into two clusters (iv and v). All the isolates of Pop SZ and Pop LA were in group v, whereas Pop BB was distributed into clusters iii and v. The reason that the isolates from different locations were distributed across the dendrogram may have been the exchange of the pathogen among locations (Lamour and Kamoun, 2009). Some agricultural practices in northern Anhui Province, such as using soybean stalks for livestock feed and organic fertilizer without proper treatment, probably resulted in exchanging of the pathogen between farms (Wen, 2003). In these areas, soybean stalks were often used for livestock feed and organic fertilizer, and they were traded and transported in these cities. Soybean phytophthora root rot also might be spread by inappropriate mechanized farming. For example, renting an untreated crop harvester, which would carry *P. sojae* through soybean straw and soil, can disperse the pathogen among adjacent areas. Most of the isolates from the same region grouped together, but isolates from the same location showed no clustering based on the year of sampling.

Fig. 1. Neighbor-joining dendrogram of 62 isolates of *Phytophthora sojae* based on genetic distances; i, ii, iii, iv, and v represent five distinct clusters.

GENE FLOW AND GENETIC DIFFERENTIATION OF THE FIVE LOCATIONS. The values of G_{st} and N_m among the five locations were calculated with POPGENE version 1.3.1 and are shown in Table 4. The average G_{st} was 0.2550, which suggested that the genetic differentiation among the five locations was 25.5%. The coefficient of genetic differentiation was 74.5% in each location and higher than the coefficient of genetic differentiation among the five locations. The low level of G_{st} was caused by a high N_m , according to Wright (1978). N_m was calculated with the following equation: $N_m = 0.25(1 - G_{st})/G_{st}$. The values of N_m ranged from 0.623 to 2.773, with an average of 1.325, which was higher than the threshold value of 1 (Wright, 1978). These results indicated that gene exchange between different locations was abundant, and N_m among the five locations led to differences in G_{st} . For instance, the minimum N_m (0.623) was between Pop SZ and Pop LA; however, the maximum G_{st} was 0.286. By contrast, the maximum N_m (2.773) was between Pop FY and Pop BZ, whereas the G_{st} (0.083) value between these two sites was the lowest. The correlation of population genetic distance and geographic distance was tested using Mantel's test. The results indicated that the correlation between spatial and genetic distance was low ($r = 0.3938$, $P = 0.152$).

GENETIC IDENTITY AND GENETIC DISTANCES AMONG POPULATIONS OF *P. SOJAE*. Matrices of genetic distances and genetic identities (Nei's original measures) among the five locations were established. Genetic identity (I) and genetic distance (D) values were obtained from pairwise comparisons (Table 5), and the results indicated that the highest genetic identity was 0.9701, between Pop BZ and Pop FY, whereas their genetic distance (0.0304) was the smallest. The lowest genetic identity was 0.8871, between Pop SZ and Pop LA, whereas their genetic distance (0.1197) was the largest. These results agreed with the ISSR cluster analysis and could be explained by the N_m .

Discussion

It is necessary to study the genetic structure of plant pathogenic fungi and oomycetes for effective control of disease (Wolfe and Caten, 1987). A large number of studies have shown that molecular markers can detect pathogen population genetic variation among strains to a certain extent, including that derived from pathogenic mutations, strains from different geographic origins and hosts, etc. (Goodwin, and Annis, 1991;

Table 4. Gene flow [N_m (above diagonal)] and genetic differentiation coefficient [G_{st} (below diagonal)] among *Phytophthora sojae* populations.

Location	Bozhou	Fuyang	Suzhou	Bengbu	Luan
Bozhou	—	2.773	0.853	1.766	1.121
Fuyang	0.083	—	1.025	1.458	1.144
Suzhou	0.227	0.196	—	0.910	0.623
Bengbu	0.124	0.146	0.216	—	1.574
Luan	0.182	0.179	0.286	0.137	—

Table 5. Genetic identity [I (above diagonal)] and genetic distances [D (below diagonal)] among *Phytophthora sojae* populations.

Location	Bozhou	Fuyang	Suzhou	Bengbu	Luan
Bozhou	—	0.9701	0.9083	0.9372	0.9186
Fuyang	0.0304	—	0.9338	0.9300	0.9286
Suzhou	0.0962	0.0685	—	0.9045	0.8871
Bengbu	0.0649	0.0726	0.1004	—	0.9390
Luan	0.0849	0.0741	0.1197	0.0629	—

Manulis et al., 1993; Nyasse et al., 1999). In this study, ISSR marker technology was used to analyze the genetic diversity of *P. sojae* in Anhui Province, and clear and reproducible ISSR-PCR amplification bands were observed. The high percentage of polymorphic loci showed that 62 isolates of *P. sojae* had abundant genetic diversity, and the polymorphism rate was 80.6%. The percentages of polymorphic bands in Heilongjiang Province, Fujian Province, Argentina, and the United States were 71%, 60%, 71%, and 84% according to previous reports (Galley et al., 2007; Wu et al., 2013, 2017). Isolates from Anhui Province had higher genotypic diversity than those from Heilongjiang Province, Fujian Province, and Argentina, but this diversity was lower than that of those from the United States. There was no significant difference in genetic diversity between populations, and the difference was mainly due to genetic variation within the population. The same phenomenon was also observed in previous reports (Wang et al., 2007, 2017).

ISSR clustering results showed that the strains from the same group did not all belong to the same genotype, and the division of the clustering group was not directly correlated with geographic origin (Galley et al., 2007; Wang et al., 2007). There was genetic differentiation between strains, and the possible reasons for this were gene mutation, genetic drift, gene flow, reproduction and mating system, and selection pressure from primary resistant soybean cultivars (Lamour and Kamoun, 2009; McDonald and Linde, 2002). Mutations were the root source of genetic differentiation and could directly change the gene sequences of individuals and generate a new allele within a population. Genetic drift affected the probability of mutation, which might result in an increase in genetic diversity (Silvina et al., 2011). Some studies have shown that gene flow plays an important role in genetic differentiation and species diversity and has a positive effect on the formation and adaptive evolution of species (Garant et al., 2007; Seehausen, 2004). Gene flow describes the movement of alleles and genotypes of toxic mutants between populations from different regions, and it is associated with genetic differentiation. In addition, the evolution of asexual reproduction and heterozygous hybridization of *P. sojae* (Drenth et al., 1996) probably led to genetic differentiation. The selection pressure from soybean cultivars

with primary resistance might drive an increase in the mutation frequency of pathogenic alleles (Meng et al., 1999). Lohnes et al. (1996) and Kyle et al. (1998) evaluated the soybean phytophthora root rot resistance of soybean germplasm resources from central and southern China, which contains multiple physiological races of *P. sojae*. The results showed that the identified resources were generally resistant to soybean phytophthora root rot, especially those from the provinces of Anhui, Jiangsu, Zhejiang, Shandong, and Henan. Therefore, potential selection pressure on *P. sojae* existed in these areas.

Abundant genetic diversity existed among *P. sojae* in Anhui Province. The average N_m was 1.325, which was greater than the threshold value of 1 (Wright, 1978), indicating that gene exchange between different locations was abundant. Inappropriate agricultural practices in Anhui Province might disperse the pathogen. Therefore, for the prevention and control of this disease, it is necessary to strengthen the plant quarantine to cut off the long-distance transmission channels (Parke et al., 2014). We should fully tap the basic ability of the resistant cultivars controlled by minor genes, and at the same time, we should use fungicides more scientifically and in rotation to avoid resistance due to genetic variation (Dorrance, 2018). Research on the genetic diversity of *P. sojae* in Anhui would supply useful information to improve the control strategies for *P. sojae* (Parke et al., 2014). Moreover, because *P. sojae* poses a great risk to soybean cultivation in Anhui Province, Anhui Agricultural University, the Anhui Institute of Standardization (Hefei, China), the Plant Protection Research Institute of Anhui Academy of Agricultural Sciences (Hefei, China), the Plant Protection Station of Anhui Province (Hefei, China) and the Feidong Agricultural Technology Promotion Center of Anhui Province (Hefei, China) jointly drafted two monitoring regulations, rules for investigation and forecasting technology for soybean phytophthora blight (*P. sojae*) and regulations for the control of soybean phytophthora blight (Province of Anhui, 2013a,b). The former regulation stipulated the recording of items and grading indicators of the incidence of soybean phytophthora blight in Anhui Province, the systematic investigation of disease conditions, and the relevant methods for field surveys and forecasting, and it also provides guidance for effective monitoring and investigation of soybean phytophthora blight in Anhui Province. The latter regulation emphasized that prevention and control must be combined to prevent and treat soybean phytophthora blight. First, inspections were conducted and soybeans were quarantined in accordance with laws and regulations to prevent the intrusion and spread of pests. Second, to suppress and eliminate *P. sojae*, it is important to make rational use of disease-resistant cultivars in combination with disease-causing types (Dorrance, 2018). In accordance with the needs of cultivation and management, the use of such cultivars should be combined with agricultural operations to create farmland ecological environments that are conducive to crop growth, and development that is not conducive to the occurrence of diseases should be practiced. Finally, chemical control and biological control should be combined (Dorrance, 2018; Schmitthenner, 1985).

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Supplemental Table 1. Information for isolates *Phytophthora sojae* from Anhui Province, China, used in this study.

No.	Isolate no.	Sampling site (city)	Source	Yr of collection	Recorded bands (no.)	Clusters ^z
1	BZ1	Bozhou	Plant	2014	101	i
2	BZ2	Bozhou	Plant	2014	103	iii
3	BZ3	Bozhou	Plant	2014	109	iii
4	BZ4	Bozhou	Plant	2014	108	iii
5	BZ5	Bozhou	Plant	2014	109	i
6	BZ6	Bozhou	Plant	2014	105	iii
7	BZ7	Bozhou	Plant	2014	103	iii
8	BZ8	Bozhou	Plant	2014	105	i
9	BZ9	Bozhou	Plant	2014	93	ii
10	BZ10	Bozhou	Plant	2014	101	i
11	BZ11	Bozhou	Soil	2010	102	iv
12	BZ12	Bozhou	Soil	2010	102	iv
13	BZ13	Bozhou	Soil	2010	106	v
14	BZ14	Bozhou	Soil	2010	98	v
15	BZ15	Bozhou	Soil	2010	103	v
16	BZ16	Bozhou	Soil	2010	104	v
17	BZ17	Bozhou	Soil	2010	103	iv
18	BZ18	Bozhou	Soil	2010	106	iv
19	BZ19	Bozhou	Soil	2009	113	iv
20	BZ20	Bozhou	Soil	2009	107	iv
21	BZ21	Bozhou	Soil	2010	103	i
22	FY1	Fuyang	Soil	2008	106	iv
23	FY2	Fuyang	Soil	2008	112	iv
24	FY3	Fuyang	Soil	2010	111	iv
25	FY4	Fuyang	Soil	2010	110	v
26	FY5	Fuyang	Soil	2010	111	v
27	FY6	Fuyang	Soil	2010	111	v
28	FY7	Fuyang	Soil	2010	107	iv
29	FY8	Fuyang	Soil	2010	101	iv
30	FY9	Fuyang	Soil	2010	108	iv
31	FY10	Fuyang	Soil	2010	115	iv
32	FY11	Fuyang	Soil	2010	113	v
33	SZ1	Suzhou	Soil	2008	117	v
34	SZ2	Suzhou	Soil	2008	115	v
35	SZ3	Suzhou	Soil	2009	117	v
36	SZ4	Suzhou	Soil	2009	115	v
37	SZ5	Suzhou	Soil	2009	116	v
38	BB1	Bengbu	Plant	2014	115	v
39	BB2	Bengbu	Soil	2014	115	v
40	BB3	Bengbu	Soil	2014	94	v
41	BB4	Bengbu	Plant	2014	96	v
42	BB5	Bengbu	Soil	2014	103	v
43	BB6	Bengbu	Soil	2010	100	v
44	BB7	Bengbu	Soil	2010	103	v
45	BB8	Bengbu	Soil	2010	109	iii
46	BB9	Bengbu	Soil	2009	107	v
47	BB10	Bengbu	Soil	2008	108	v
48	BB11	Bengbu	Soil	2008	106	v
49	BB12	Bengbu	Soil	2008	94	ii
50	BB13	Bengbu	Soil	2008	109	v
51	BB14	Bengbu	Soil	2008	109	v
52	BB15	Bengbu	Soil	2008	108	v
53	BB16	Bengbu	Soil	2009	108	v
54	BB17	Bengbu	Soil	2009	98	v

Continued next page

Supplemental Table 1. Continued.

No.	Isolate no.	Sampling site (city)	Source	Yr of collection	Recorded bands (no.)	Clusters ^z
55	BB18	Bengbu	Soil	2009	110	v
56	LA1	Luan	Soil	2014	101	v
57	LA2	Luan	Soil	2014	97	v
58	LA3	Luan	Soil	2014	107	v
59	LA4	Luan	Soil	2014	94	v
60	LA5	Luan	Soil	2014	111	v
61	LA6	Luan	Soil	2014	105	v
62	LA7	Luan	Soil	2010	111	v

^zi, ii, iii, iv, and v represent five distinct clusters based on genetic distances.

Supplemental Fig. 1. The genetic fingerprints of 62 *Phytophthora sojae* isolates with primer ILE7: lane M = DL 2000 marker with indicated molecular weight size (bp), lane CK = no DNA template, lanes 1–62 = samples listed in Supplemental Table 1.