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# Genetic diversity of *Phytophthora capsici* (Pythiaceae) isolates in Anhui Province of China based on ISSR-PCR markers

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**ABSTRACT.** *Phytophthora capsici* is a plant pathogenic oomycete that damages numerous crops worldwide. Consequently, interest in research on the genetic structure of this species has grown in recent decades. However, there is little information about *P. capsici* in eastern China. We investigated the genetic diversity of *P. capsici* isolates from three large regions of Anhui Province in eastern China based on ISSR-PCR technology. Thirteen random primers were screened and used to amplify DNA from 51 samples. We obtained 158 reproducible ISSR fragments, of which 90% were polymorphic, revealing a high degree of polymorphism among the isolates. Genetic similarity coefficients among all the isolates ranged from 0.56 to 0.94, with a mean of 0.84 based on the ISSR data, indicating a high level of genetic variation in these *P. capsici* isolates. Cluster analysis using UPGMA indicated that the Anhui isolates were divided into seven groups according to the DNA fingerprints, although there was no correlation between the ISSR group and geographic origin. Isolates from the same location showed no clustering based on the year of sampling. AMOVA partitioned variability among (13.6%) and within populations (86.4%). The gene flow among populations ranged from 2.804 to 4.937, with a mean of 3.545, indicating highly frequent gene exchange. Genetic distances

 

and genetic differentiation were negatively correlated with geographic distances. These results lead us to suggest that this pathogen has considerable evolutionary potential, which will enable it to adapt to and overcome management strategies over time.

**Key words:** *Phytophthora capsici*; Genetic diversity; ISSR-PCR; Anhui

## INTRODUCTION

*Phytophthora capsici* is an important soilborne oomycete pathogen of pepper (*Capsicum annuum* L.), which was first identified by Leonian in New Mexico with large host ranges including not only pepper but also tomato, cucumber, cucurbit, and other Solanaceae plants (Leonian, 1922; Hausbeck and Lamour, 2004). Generally, the pathogen penetrates the plant through the root and the stem and then causes necrosis of infected areas. Meanwhile, the plant withers, resulting in obstruction in the vascular system. The infected tissues dry up, shrink and turn black. It can infect all parts of the plant at any growth stage, causing damping-off, seedling blight, foliar blight, and fruit rot (Latin and Rane, 1999). Recently, the incidence of *Phytophthora* foliar and fruit rot has increased worldwide (Hwang and Kim, 1995; Ristaino and Johnston, 1999). It has also been reported that the disease had occurred in mainland China and become one of the serious threats to farmers (Ho and Lu, 1997).

*P. capsici* belongs to the phytopathogenic oomycetes that produce both zoospores and oospores for colonizing plant tissues and overwintering, and can be dispersed by surface water, irrigation water and air currents. To date, management of *P. capsici* mainly relies on rotation, sanitation and the use of fungicides, but there is no effective method to provide enough protection against this disease during its epidemic period (Babadoost and Islam, 2001). As known, the genetic structure of pathogen populations reflects its evolutionary history and its potential to evolve. Population genetic studies of pathogens are helpful in the better management of disease in agricultural ecosystems and may also serve as a guide in pepper cultivation (McDonald, 1997). For instance, by knowing the genetic variation maintained within a species, indicating the speed at which a pathogen evolves, measures such as the use of resistant cultivars or fungicides could be adopted. Therefore, knowledge of the genetic variation of *P. capsici* isolates is essential for developing effective measures to control the diseases caused by the pathogenic oomycete in crop fields.

DNA markers have now become a popular means for the identification and authentication of plant, animal and microbial species. At present, this technology has been widely used in the identification of germplasm resources and determination of genetic diversity in populations (Zhang et al., 2007). During the last decade, a number of molecular markers have been widely used to study the genetic diversity of different *Phytophthora* spp (Linde et al., 1999; Ochwo et al., 2002; Yang et al., 2008). Using molecular methods to investigate the genetic structure of *P. capsici* still drew a blank until the late 1980s and early 1990s (Förster et al., 1989; Oudemans and Coffey, 1991; Mchau and Coffey, 1995). Recently, molecular genetic markers, such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP), have been broadly used to learn the genetic structure of *P. capsici* (Lamour and Hausbeck, 2001; Silvar et al., 2006; Wang et al., 2009). Among the molecular markers, inter-simple sequence repeat (ISSR) is the easily usable and reliable one for

 

investigating genetic diversity in plants, animals and fungi, and some studies have also shown that ISSR could provide high genomic polymorphism compared to restriction fragment length polymorphism, SSR and RAPD (Blair et al., 1999; Gilbert et al., 1999; Wang et al., 2009). However, little is known about the genetic structure of *P. capsici* in Anhui Province in eastern China.

In this study, ISSR-polymerase chain reaction (PCR) was used to investigate the genetic structure of *P. capsici* isolates from different geographical origins of Anhui Province. The primary aim of this study was to obtain more information on the population structure of *P. capsici* in Anhui and to explore the genetic diversity in their populations. Knowledge of the genetic diversity of *P. capsici* in Anhui will establish whether populations of *P. capsici* are genetically differentiated between fields and regions in Anhui, if specific genotypes predominate, and what the potential is for developing recombinant populations. This information can be used to improve current control strategies and to design future studies on specific questions with regard to the population structure of the pathogen within fields and across seasons in Anhui.

## MATERIAL AND METHODS

### Isolate collection and mycelium culture

A total of 51 isolates of *P. capsici* were used to study its genetic diversity by ISSR-PCR. All of them were isolated from symptomatic diseased pepper plants collected from eight locations in Anhui Province from 2006 to 2010. Based on the localities, the samples belonged to three large populations: population between south of the Huai River and north of the Yangtze River (Pop 1) with 22 isolates, population in south of the Yangtze River (Pop 2) with 13 isolates, and population north of the Huai River (Pop 3) with 16 isolates (Figure 1, Table 1).

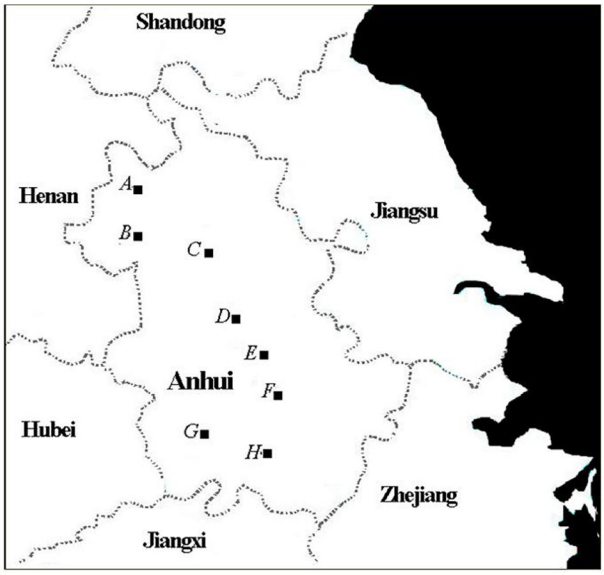


**Figure 1.** Map of locations sampled for *Phytophthora capsici* in Anhui, China, for analysis of genetic diversity: (**A**) Bozhou; (**B**) Fuyang; (**C**) Huainan; (**D**) Hefei; (**E**) Chaohu; (**F**) Tongling; (**G**) Anqing; (**H**) Chizhou.

**Table 1.** Number and localities of *Phytophthora capsici* isolates used in this study.

| Isolate No. | Sampling site | Year of collection | No. of bands recorded | Genotype |
|---|---|---|---|---|
| Between south of the Huai River and north of the Yangtze River (Pop 1) | | | | |
| HN1 | Huainan | 2006 | 71 | A |
| HN2 | Huainan | 2006 | 70 | A |
| HN3 | Huainan | 2006 | 80 | A |
| HN4 | Huainan | 2006 | 79 | A |
| HN5 | Huainan | 2006 | 80 | A |
| HN6 | Huainan | 2006 | 83 | A |
| HN7 | Huainan | 2006 | 76 | A |
| HN8 | Huainan | 2006 | 85 | A |
| HF2 | Hefei | 2007 | 54 | E |
| HF3 | Hefei | 2007 | 56 | E |
| HF4 | Hefei | 2007 | 51 | E |
| HF5 | Hefei | 2007 | 64 | A |
| HF6 | Hefei | 2007 | 69 | A |
| HF7 | Hefei | 2008 | 63 | C |
| CH1 | Chaohu | 2009 | 72 | B |
| CH2 | Chaohu | 2009 | 81 | A |
| CH3 | Chaohu | 2009 | 74 | A |
| AQ1 | Anqing | 2008 | 74 | A |
| AQ2 | Anqing | 2008 | 78 | A |
| AQ3 | Anqing | 2008 | 66 | F |
| AQ4 | Anqing | 2008 | 65 | A |
| AQ5 | Anqing | 2008 | 70 | A |
| South of the Yangtze River (Pop 2) | | | | |
| CZ1 | Chizhou | 2009 | 63 | C |
| CZ2 | Chizhou | 2009 | 69 | C |
| TL1 | Tongling | 2010 | 76 | A |
| TL2 | Tongling | 2010 | 82 | C |
| TL3 | Tongling | 2010 | 87 | G |
| TL4 | Tongling | 2010 | 84 | D |
| TL5 | Tongling | 2010 | 81 | D |
| TL6 | Tongling | 2010 | 78 | D |
| TL7 | Tongling | 2010 | 93 | D |
| TL8 | Tongling | 2010 | 65 | D |
| TL9 | Tongling | 2010 | 67 | D |
| TL10 | Tongling | 2010 | 73 | D |
| TL11 | Tongling | 2010 | 63 | D |
| North of the Huai River (Pop 3) | | | | |
| FY1 | Fuyang | 2009 | 80 | A |
| FY2 | Fuyang | 2009 | 80 | A |
| FY3 | Fuyang | 2009 | 78 | C |
| BZ1 | Bozhou | 2009 | 65 | C |
| BZ2 | Bozhou | 2010 | 72 | D |
| BZ3 | Bozhou | 2010 | 73 | B |
| BZ4 | Bozhou | 2010 | 76 | D |
| BZ5 | Bozhou | 2010 | 77 | D |
| BZ6 | Bozhou | 2010 | 71 | D |
| BZ7 | Bozhou | 2010 | 70 | D |
| BZ8 | Bozhou | 2010 | 72 | D |
| BZ9 | Bozhou | 2010 | 79 | D |
| BZ10 | Bozhou | 2010 | 66 | D |
| BZ11 | Bozhou | 2010 | 66 | D |
| BZ12 | Bozhou | 2010 | 69 | D |
| BZ13 | Bozhou | 2010 | 62 | D |

All of the isolates tested were first cultivated on carrot agar for 3 days, and then grown in Plich's liquid medium (0.5 g $KH_2PO_4$, 0.25 g $MgSO_4.7H_2O$, 1 g asparagine, 1 mg thiamine, 0.5 g yeast extract, 25 g glucose, and 1000 mL distilled water) as mycelial plugs (6 x 6 mm), incubated on an orbital shaker (100 rpm) for about 7 days. All mycelia were harvested and stored at -20°C for DNA preparation.

## DNA extraction

Total genomic DNA of each isolate was extracted according to the methods as described by Wang and Zheng (2003), with a few modifications: the air-dried pellet was resuspended in 100 µL Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) and 2 µL RNase (10 mg/mL) was added and the mixture incubated at 37°C for 1 h. The integrity of DNA samples was checked on a 0.8% agarose gel stained with ethidium bromide. All DNA samples were checked for quantity using a spectrophotometer at 260 nm, and then diluted to 50 ng/µL.

## ISSR-PCR amplification

A total of 40 primers synthesized by Sangon Biotechnology Ltd. Co. (Shanghai, China) were initially screened on 10 isolates. Thirteen primers (Table 2) that produced clear and reproducible bands were selected for further analysis. The amplifications were performed in a 25-µL reaction mixture containing 2.5 µL 10X PCR buffer, 200 µM dNTPs, 0.2 µM primer, 0.5 µL template DNA, and 1.25 U Taq DNA polymerase (Takara). Thermal cycling was programmed as follows: initial denaturation for 5 min at 94°C, 35 cycles of denaturation at 94°C for 1 min, annealing at a specific temperature for 1 min, extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The annealing temperatures are given in Table 2. PCR products were separated on 1.8% agarose gels containing ethidium bromide in 10X Tris-borate-EDTA buffer at 5 V/cm for 3 h and visualized under a UV transilluminator. All amplifications were repeated 3 times to ensure their reproducibility.

## Data analysis

ISSR-amplified fragments were scored either as present (1) or absent (0) on the basis of the molecular weight (bp) for each isolate, and the data were then converted to a binary matrix. *P. capsici* population genetic parameters were analyzed using POPGENE version 1.3.1 to calculate the number of polymorphic bands, the percentage of polymorphic bands (PPB), observed number of alleles ($N_A$), effective number of alleles ($N_E$), Nei's gene diversity ($h$), Shannon's information index ($I_s$), total genetic diversity ($H_t$), genetic diversity within groups ($H_s$) and the relative magnitude of genetic differentiation between populations ($G_{ST} = (H_t - H_s) / H_t$), gene flow [$N_m = 0.25(1 - G_{ST}) / G_{ST}$], genetic identity ($I$) and genetic distance ($D$) (Yeh et al., 1999). Nei's genetic similarity values, coefficient of genetic differentiation, $N_m$, $I$ and $D$ were determined for all pairwise combinations of populations (Nei, 1972; Slatkin and Barton, 1989). Jaccard's similarity coefficients were calculated via the NTSYS-pc version 2.1 to generate a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA) (Rohlf, 2000).

Analysis of molecular variance (AMOVA) was also applied to partition the components of variance within and between *P. capsici* populations (Excoffier et al., 1992). Input data files for the AMOVA 1.55 programs were generated using DCFA 1.1 (Zhang and Ge, 2002), and the number of permutations was determined at 1000 for significance analysis. AMOVA components were used as estimates of the genetic diversity within and between populations.

## RESULTS

### Primer screening and polymorphism of ISSR-PCR-amplified products

Based on the preliminary experiments, 13 of the 40 ISSR primers producing clear and reproducible fragments were selected for further analysis. An example of the banding profiles of DNA of isolates using primer UBC835 is shown in Figure 2. For each primer (Table 2), the annealing temperature ranged from 48° to 54°C. The minimum and maximum number of bands generated per primer were 8 (UBC809) and 18 (UBC835 and UBC841), respectively, with an average of 12.2 bands per primer. The percentage of polymorphism varied from 50 (UBC809) to 100% (UBC821, UBC841 and UBC873), with an average of 88.5% polymorphism across all individuals. A high level of genetic variation was observed. *h* values ranged from 0.109 to 0.350, and $I_s$ ranged from 0.179 to 0.514 (Table 2), indicating that these 13 primers were appropriate for distinguishing diverse isolates of *P. capsici*. The genetic diversity of different populations was compared as shown in Table 3. Of the 51 isolates, a total of 158 ISSR bands were obtained, of which 142 (89.9%) were polymorphic. Among the three populations, $N_A$ ranged from 1.77 to 1.81, with an average of 1.90; $N_E$ varied from 1.37 to 1.41, with a mean of 1.36; *h* varied from 0.22 to 0.24, with an average of 0.21, and similarly, $I_s$ was 0.34 to 0.37, with a mean of 0.33. Within populations, among individuals of Pop 1 (between south of the Huai River and north of the Yangtze River), 13 ISSR primers generated a total of 125 bands, of which 97 (77.6%) were polymorphic; among individuals of Pop 2 (south of the Yangtze River), 13 ISSR primers generated a total of 140 bands, of which 113 (80.7%) were polymorphic, and similarly, among individuals of Pop 3 (north of the Huai River), 13 ISSR primers generated a total of 118 bands, of which 91 (77.1%) were polymorphic. The results showed that polymorphism existed between different isolates of Anhui Province.

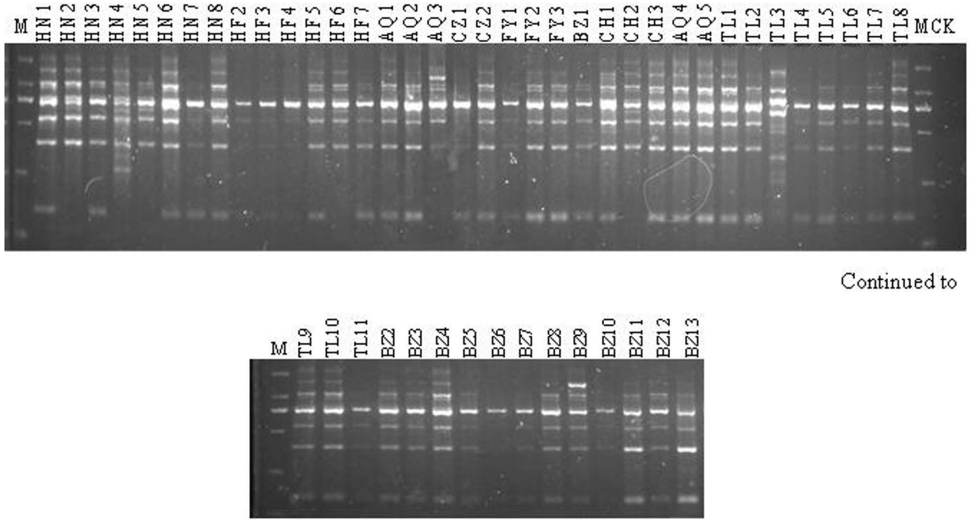


**Figure 2.** Genetic fingerprints of 51 *Phytophthora capsici* isolates with primer UBC835. *Lane M* = DL 2000 marker; CK = no DNA template.

**Table 2.** Properties of ISSR primers used for amplification of *Phytophthora capsici* isolates in a study of genetic diversity in Anhui China.

| Primer | Sequence (5'-3') | Annealing temperature (°C) | Bands amplified | Polymorphic bands | PPB (%) | Mean *h* | Mean $I_s$ |
|---|---|---|---|---|---|---|---|
| ILE120 | $CAC(GA)_7$ | 54 | 12 | 11 | 91.7 | 0.179 | 0.294 |
| P10 | $(GA)_8TG$ | 51 | 13 | 12 | 92.3 | 0.176 | 0.286 |
| UBC807 | $(AG)_8T$ | 52 | 13 | 11 | 84.6 | 0.200 | 0.318 |
| UBC808 | $(AG)_8C$ | 54 | 11 | 9 | 81.8 | 0.188 | 0.288 |
| UBC809 | $(AG)_8G$ | 50 | 8 | 4 | 50.0 | 0.117 | 0.179 |
| UBC811 | $(GA)_8C$ | 50 | 11 | 9 | 81.8 | 0.216 | 0.339 |
| UBC816 | $(CA)_8T$ | 52 | 14 | 13 | 92.9 | 0.245 | 0.379 |
| UBC821 | $(GT)_8T$ | 48 | 9 | 9 | 100.0 | 0.194 | 0.300 |
| UBC835 | $(AG)_8YC$ | 50 | 18 | 17 | 94.4 | 0.218 | 0.331 |
| UBC841 | $(GA)_8YC$ | 54 | 18 | 18 | 100.0 | 0.292 | 0.438 |
| UBC873 | $(GACA)_4$ | 54 | 10 | 10 | 100.0 | 0.350 | 0.514 |
| P15 | $G(ACAG)_3ACAC$ | 54 | 12 | 11 | 91.7 | 0.208 | 0.313 |
| P6 | $(AG)_8S$ | 54 | 9 | 8 | 88.9 | 0.109 | 0.183 |

S = (GC), Y = (CT). PPB = percentage of polymorphic bands; *h* = Nei's (1973) gene diversity; $I_s$ = Shannon's information index, as a measure of gene diversity.

**Table 3.** Genetic variations and polymorphic features among isolates of *Phytophthora capsici* from Anhui, China, by ISSR markers.

| Population | Total bands | Polymorphic bands | PPB (%) | Mean $N_A$ | Mean $N_E$ | Mean *h* | Mean $I_s$ |
|---|---|---|---|---|---|---|---|
| Pop 1 | 125 | 97 | 77.6 | 1.78 | 1.40 | 0.23 | 0.35 |
| Pop 2 | 140 | 113 | 80.7 | 1.81 | 1.37 | 0.22 | 0.34 |
| Pop 3 | 118 | 91 | 77.1 | 1.77 | 1.41 | 0.24 | 0.37 |
| Summary | 158 | 142 | 89.9 | 1.90 | 1.36 | 0.21 | 0.33 |

PPB = percentage of polymorphic bands; $N_A$ = observed number of alleles, counts the number of alleles with non-zero frequency; $N_E$ = effective number of alleles, estimates the reciprocal of homozygosity; *h* = Nei's (1973) gene diversity; $I_s$ = Shannon's information index, as a measure of gene diversity.

Based on POPGENE analysis, $H_t$ of the 51 isolates was 0.2056 and $H_s$ was 0.1868 (90.9%). AMOVA yielded significant genetic differences ($P < 0.001$) within populations. The results of AMOVA detected 13.6% of total variation attributable to population divergence between the populations and 86.4% to individual differences within populations.

## ISSR cluster analysis

Based on the data above, the cluster analysis was performed and the ISSR dendrogram was generated by UPGMA. The results showed that the genetic background of each isolate was not absolutely identical and the similarity coefficient ranged from 0.56 to 0.94, with an average of 0.84. All the isolates tested could be divided into 7 clusters at 82% similarity (Figure 3). Meanwhile, Pop 1 was distributed into 5 clusters (A, B, C, E, and F), mostly cluster A with a percentage of 72.7%; Pop 2 was classified into 4 clusters (A, C, D, and G), mainly cluster D with a percentage of 61.5%; Pop 3 was divided into 4 clusters (A, B, C, and D), mainly cluster D with a percentage of 68.8%. Isolate $AQ_3$ from Pop 1 was distant from other isolates of Pop 1, and also the isolate designated $TL_3$ from Pop 2 was quite distance from the other isolates of Pop 2 on the dendrogram. The results indicated that Pop 1 comprised more genotypes than the other populations. Generally speaking, most of the isolates from the same

region grouped together, but not all the isolates from the same region were divided into the same cluster, and isolates from the same location showed no clustering based on the year of sampling in this study.

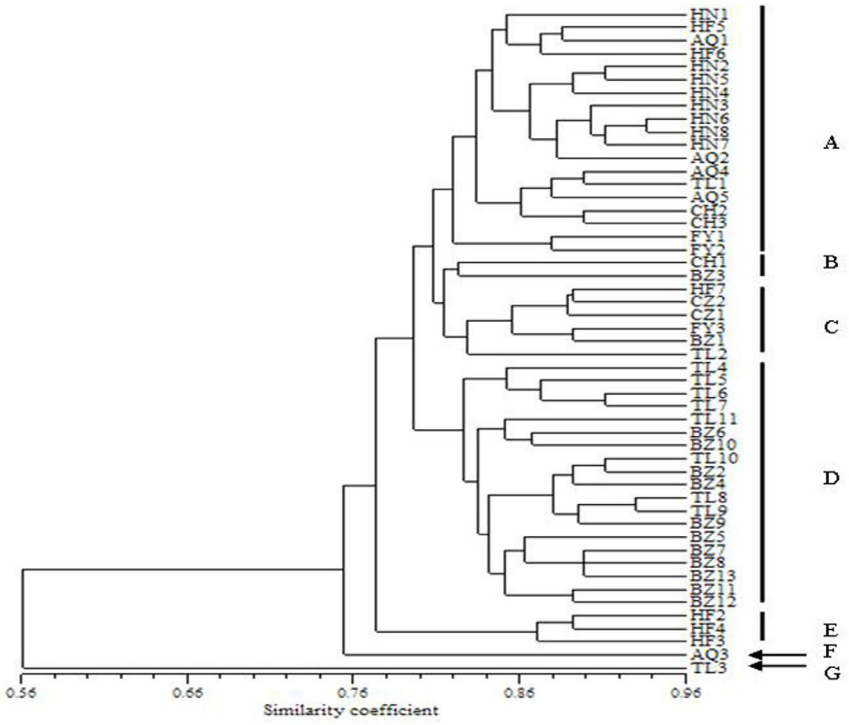


**Figure 3.** UPGMA dendrogram of genetic relationships of 51 isolates of *Phytophthora capsici* based on genetic similarity using ISSR marker.

Of the 51 isolates in three populations, 3 of 6 from site D clustered into genotype E, 1 of 5 from site G clustered into genotype F, and 1 of 11 from site F clustered into genotype G. Moreover, all 8 isolates from site C of Pop 1, 2 of 6 from site D of Pop 1, 2 of 3 from site E of Pop 1, 4 of 5 from site G of Pop 1, 1 of 11 from site F of Pop 2 and 2 of 3 from site B of Pop 3 clustered into genotype A; 1 of 3 from site E and 1 of 13 from site A clustered into genotype B; 1 of 6 from site D, 2 of all 2 from site H, 1 of 11 from site F, 1 of 3 from site B and 1 of 13 from site A clustered into genotype C; 8 of 11 from site F and 11 of 13 from site A clustered into genotype D. Therefore, genotypes E, F and G, each originating from one population, were monophyletic while genotypes A and C, from all three populations, and B, from two populations, were polyphyletic.

## Gene flow and genetic differentiation between populations and 8 site locations

The values of $G_{ST}$ and $N_m$ between populations (Table 4) and 8 site locations were calculated using POPGENE version 1.3.1. The average coefficient of genetic differentiation was

0.091, indicating moderate genetic differentiation between populations according to Wright's qualitative guideline (Wright, 1978) that $F_{ST}$ (or $G_{ST}$ in the this case) values above 0.05 indicate moderate genetic heterogeneity.

**Table 4.** Gene flow ($N_m$) and genetic differentiation coefficient ($G_{ST}$) among populations of *Phytophthora capsici*.

| Population | Pop 1 | Pop 2 | Pop 3 |
|---|---|---|---|
| Pop 1 | - | 2.894 | 2.804 |
| Pop 2 | 0.080 | - | 4.937 |
| Pop 3 | 0.082 | 0.048 | - |

Above diagonal was gene flow ($N_m$), below diagonal was genetic differentiation coefficient ($G_{ST}$).

The low level of $G_{ST}$ was caused by a high $N_m$ above the genetic differentiation threshold of 1 according to Wright (1978). $N_m$ was calculated based on the $G_{ST}$ value ($N_m = 0.25 (1 - G_{ST}) / G_{ST}$) and was found to range from 2.804 to 4.937, with an average of 3.545, which was higher than the threshold value of $G_{ST}$. $N_m$ among populations led to the differences in $G_{ST}$. For instance, the minimum value of $N_m$ was between Pop 1 and Pop 3, while the $G_{ST}$ value was maximum, and the genetic exchange between Pop 1 and Pop 3 was relatively less; on the contrary, the maximum value of $N_m$ was between Pop 2 and Pop 3, while the $G_{ST}$ value was the lowest, indicating that the genetic exchange between these two populations was the most active, resulting in a tendency for convergence.

The maximum and minimum genetic differentiation occurred between a shorter (site E and site H; 215 km) and a longer (site A and site F; 479 km) geographical distance, respectively, thus demonstrating the negative correlation between these two factors in the genetic differentiation identified in *P. capsici* isolates.

## Genetic identity and genetic distance between populations of *P. capsici*

Matrices of genetic distances and genetic identities (Nei's original measures) between the three populations were established and calculated with POPGENE version 1.3.1. Genetic identity (*I*) and genetic distance (*D*) values were obtained from pairwise comparisons (Table 5). The results showed that the highest genetic identity was observed between Pop 2 and Pop 3 (0.9765), followed by that between Pop 1 and Pop 3 (0.9604). In contrast, the smallest genetic distance was observed between Pop 2 and Pop 3 (0.0238), followed by Pop 1 and Pop 3 (0.0404). This phenomenon could be explained by the $N_m$ value, and it was in accordance with the results of ISSR cluster analysis.

**Table 5.** Genetic identity and genetic distance among populations of *Phytophthora capsici* isolated in Anhui.

| Population | Pop 1 | Pop 2 | Pop 3 |
|---|---|---|---|
| Pop 1 | - | 0.9596 | 0.9604 |
| Pop 2 | 0.0413 | - | 0.9765 |
| Pop 3 | 0.0404 | 0.0238 | - |

Above diagonal was genetic identity, below diagonal was genetic distance.

## DISCUSSION

Pepper *Phytophthora* blight, caused by *P. capsici*, was first reported in Jiangsu Province, China in 1940, and then, this disease occurred in both the north and south of China, especially heavily in the Yangtze River Basin, Qinghai, Shanxi, Gansu, Yunnan, and Guangdong; it has been one of the most limiting factors to pepper production (Zhang et al., 2006). Genetic structure of plant pathogen populations is needed to implement effective control strategies (McDonald and Linde, 2002). Although more has been learned about the genetic structure of *P. capsici*, so far, little is known about that in Anhui Province.

Many studies have shown that molecular markers could detect genetic variation within populations to a certain extent, including pathogenicity variation, geographical differences and host differences (Goodwin and Annis, 1991; Grajal-Martin et al., 1993; Manulis et al., 1994; Nyasse et al., 1999). In the present study, considerable genetic diversity of *P. capsici* was observed with ISSR markers, based on 51 isolates collected from Anhui Province. The differences in genetic diversity in populations were not obvious, largely due to genetic variation within populations. This was in accordance with the ISSR cluster analysis that not all isolates from the same population clustered into one genotype.

Lamour and Hausbeck (2001) used AFLP marker to investigate the spatiotemporal genetic structure of *P. capsici* in Michigan. They found that population differentiation increased with distance, indicating that the genetic distances between populations correlated positively with geographical distances and that geographical separation posed an obstacle to the possibility and frequency of genetic exchanges between populations. The ISSR analysis of the present study revealed that some isolates from site F (Tongling) were closely related to those from site A (Bozhou), which leads to the controversial conclusion that genetic distances and genetic differentiation are negatively associated with geographic distances. The genetic homogenization between the two locations implied a high level of $N_m$ with each other. Some research has shown that $N_m$ plays a vital role in genetic differentiation and diversity of species and also has a positive effect on the formation and adaptive evolution of species (Garant et al., 2007). The value of $N_m$ between site F and site A was up to 3.396, showing that genetic exchange was tremendously frequent between these two locations. As previously studied, both A1 and A2 mating types were detected in Anhui (Qi et al., 2012). Gene exchange was probably caused by migration of the oomycete along with plant seeds and/or by transmission of the oospores, which was suitable for explaining the close relationship between isolates separated by a vast geographic distance, since such far dispersal of sporangia seemed unlikely.

AMOVA indicated that genetic variation between *P. capsici* populations was about 13.6%, and it was much higher within populations, accounting for 86.4%. Variation in a population may be a product of mutation. Clonal reproduction in many *Phytophthora* species, which led to mutation, is the primary source of new genetic variation. Samen et al. (2003) suggested that there was a considerable level of inherent genetic variability among asexual progeny from the same parental isolate of *P. infestans* with RAPD and AFLP markers. Hao et al. (2003) demonstrated that variation in virulence in *P. sojae* asexual reproduction was frequently present and that the variation could not be inherited steadily after successive reproduction. Mutation in most cases could cause no observable changes in phenotypes, but it is not impossible that a portion of the genetic variation observed with these molecular markers could be due to mutations. Another potential explanation for genetic variation is hybridization be-

tween individuals of different species. *P. capsici* is a heterothallic species and previous studies found both mating types present in the same location. There has been evidence that back and sibling crosses are possible in *P. capsici*, which accounts for the large reservoirs of naturally occurring genetic variation in oomycete pathogens (Hurtado-Gonzales and Lamour, 2009). Furthermore, some researchers found that *Phytophthora* could not only carry out intraspecific hybridization but also introgress by interspecific hybridization. Interspecific hybrids between *P. cinnamomi* and *P. parasitica* have been produced in the laboratory (Boccas and Zentmyer, 1976). Also, progeny obtained from an intercross between *P. capsici* and *P. palmivora* and colony morphology, mating type, protein, and pathogenicity to pepper have been tested. This study showed the phenotype and pathogenicity of some progeny differing from either parent.

The study on the genetic diversity of *P. capsici* in Anhui produced important suggestive information for the management of the disease. The presence of high genotypic diversity, along with large population sizes, suggests that the pathogen has a large evolutionary potential that will enable it to adapt and overcome management strategies over time. The fact that *P. capsici* isolates in Anhui have high genotypic diversity is an important aspect for resistance breeding programs, since multiple isolates collected from several regions would have to be included in resistance screenings.

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