Population genetic variation analysis of bitter melon wilt caused by *Fusarium oxysporum* f. sp. *momordicae* in China using inter simple sequence repeats (ISSR) molecular markers

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Abstract

Bitter melon wilt caused by *Fusarium oxysporum* f. sp. *momordicae* (FOM) is a devastating crop disease in China. A total of 173 isolates characteristic of typical *Fusarium oxysporum* with abundant microconidia and macroconidia on white or ruby colonies were obtained from diseased plant tissues. BLASTn analysis of the rDNA-ITS of the isolates showed 99% identity with *F. oxysporum* species. Among the tested isolates, three were infectious toward bitter melon and five were pathogenic to bitter melon. However, all of the isolates were pathogenic to bitter melon. For genetic differences analysis, 40 ISSR primers were screened and 11 primers were used for ISSR-PCR amplification. In total, 127 loci were detected, of which 76 were polymorphic at a rate of 59.84%. POPGENE analysis showed that Nei's gene diversity index (H) and Shannon's information index (I) were 0.09 and 0.15, respectively, which indicated that the genetic diversity of the 173 isolates was low. The coefficient of gene differentiation ($G_{ST} = 0.33 > 0.15$) indicated that genetic differentiation was mainly among populations. The strength of gene flow ($N_m = 1.01 > 1.0$) was weak, indicating that the population differentiation caused by gene drift was blocked to some degree. The dendrogram based on ISSR markers showed that the nine geographical populations were clustered into two groups at the threshold of genetic similarity coefficient of 0.96. The Shandong and Henan populations were clustered into Group I, while the Guangdong, Hainan, Guangxi, Fujian, Jiangxi, and Hubei populations constituted Group II. Results of the genetic variation analysis showed that the Hunan and Guangxi populations had the highest degree of genetic differentiation, while the Hubei population had the lowest genetic differentiation. Our findings enrich the knowledge of the genetic variation characteristics of FOM populations with the goal of developing effective disease-management programs and resistance breeding programs.

Keyword Bitter melon wilt · *Fusarium oxysporum* f. sp. *momordicae* · rDNA-ITS · ISSR-PCR · Population differentiation

Introduction

Bitter melon (*Momordica charantia* L.) wilt caused by *Fusarium oxysporum* f. sp. *momordicae* Sun & Huang (FOM) is one of the most damaging crop diseases in China (Chen et al. 2014), Japan (Imamura et al. 2007), India (Rakholiya et al. 2003), and the Philippines (Cumagun et al. 2008, 2010). Sun and Huang first reported the occurrence of bitter melon wilt disease in Taiwan (Sun and Huang 1983), where it has become prevalent since 1996 (Lin et al. 1996). In the past 20 years,

bitter melon wilt has been identified in Zhejiang, Fujian, Guangxi, Guangdong, Hunan, and Jiangxi provinces (Rao and Li 1999; Yang et al. 2010; Zhu and Qi 1998). In recent years, the consumption of bitter melon, a very popular vegetable in China, has increased rapidly and led to an increase in planted areas. However, effective disease control has not been easily achieved even with effective chemical use (Chen et al. 2015; Cumagun et al. 2008). Bitter melon wilt affects the quality and yield of bitter melon and has become a major factor limiting the production of this crop (Chen et al. 2015).

Soil-borne pathogens always invade the plant root hair epidermis via wounds and then extend into the vascular tissue. FOM colonizes the xylem vessels and produces masses of mycelia and conidia to block water transportation. Therefore, typical bitter melon wilt symptoms are characterized by the initial wilting of

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leaves, followed by leaf yellowing and withering, and the darkening and thinning of stems closest to the ground. Eventually, the entire plant wilts and dies (Guo and Mo 2007). No commercially available bitter melon cultivars are immune to FOM (Zhu and Qi 1998). To control this disease, bitter melons are grafted onto rootstocks of *Cucurbita ficifolia* and *C. moschata*, which are resistant to FOM (Li et al. 2008). To some degree, the disease is controlled by this grafting technique; however, this method is time-consuming and labor-intensive. Breeding disease-resistant cultivars are still the most economical and effective strategy to control this disease (Chen et al. 2015). An understanding of the genetic diversity and population structure of FOM is a crucial prerequisite for developing resistant host genotypes as well as effectively deploying available resistant cultivars. However, details of the genetic diversity of FOM remain largely unclear.

To date, several techniques have been developed to characterize the genetic variability of different *Fusarium oxysporum* *forma specialis* isolates, such as vegetative compatibility groups (VCG), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) (Altinok and Can 2010; Assigbetes et al. 1994; Balmas et al. 2005; Duan et al. 2008). Cumagun et al. (2010) clustered 21 FOM isolates into four VCGs, while the VCG diversity ratio was much lower. It was even presumed that most *F. oxysporum* isolates infecting bitter melon should belong to a single VCG. Chen et al. (2015) revealed that 48 FOM isolates were classified into twelve AFLP groups with distinct genetic variation. Owing to the contradiction in results from previous studies, the genetic variation in FOM populations is still not clearly understood.

The inter simple sequence repeat (ISSR) amplifies intermicrosatellite sequences at multiple loci throughout the genome with a single 16–18 bp primer used for PCR reactions (Zietkiewicz et al. 1994). The amplified DNA fragments are often polymorphic between different individuals owing to strict annealing temperatures (McCall et al. 2004). Additionally, the cost of the ISSR analysis is relatively lower compared to that of AFLP, and ISSR produces good reproducibility (Esselman et al. 1999). This technique has therefore been extensively used in population genetics studies of different fungal species, such as *Fusarium oxysporum* (Mohannadi et al. 2011), *Verticillium dahliae* Kleb (Liu et al. 2010), *Botryosphaeria dothidea* (Moug.) Ces & De not (Feng et al. 2017), *Valsa mali* Miyabe et Yamada (Zang et al. 2012), and *Exserohilum turcicum* (Pass.) Leonard et Suggs (Gu et al. 2008).

Therefore, the objectives of this study were to (1) detect FOM pathogenicity in different bitter melon varieties and other cucurbit hosts and (2) to calculate and analyze the genetic variation and population construction within and among different populations using ISSR analysis.

Materials and methods

Sample collection and pathogen isolation

Diseased bitter melon samples were collected from the Fujian, Guangdong, Guangxi, Hainan, Hubei, Henan, Hunan, Jiangxi, and Shandong provinces from 2016 to 2017. The diseased stem tissue was washed with tap water and cut into small pieces. The tissue was immersed in 75% ethanol for 1.5 min, followed by two washes with sterile water. Lastly, tissue pieces were placed on the surface of potato dextrose agar (PDA) plates amended with cefotaxime sodium. The plates were incubated in the dark at 28 °C for three days. Tips of mycelia were cut from the colony margin, transferred to a new plate, and continually incubated in darkness at 28 °C until the fungal colony fully covered the plates. Small pieces (2 × 2 mm) from the colony were placed into 2-ml cryogenic vials containing 1.6 ml of 20% glycerinum and stored at -80 °C.

Pathogenicity and *formae speciales* determination

The pathogenicity of the isolates was tested on seedlings of three bitter melon cultivars (cv. Changlv, Ruyu33, and Ruyu41) and seven cucurbit crops, including muskmelon, cucumber, watermelon, tower melon, bottle melon, pumpkin, and cantaloupe. The pathogen isolates from bitter melon plants were cultured on PDA plates for 7 days at 28 °C. Conidia were obtained from 7-day-old cultures grown on PDA at 28 °C, filtered through two layers of cheesecloth and re-suspended in sterile distilled water at 1×10^6 conidia/ml. The roots of 7-day-old bitter melon plants were dipped into 500 mL of the 1×10^6 conidial suspension for 30 min, transplanted to plastic pots (diameter = 10 cm) containing organic substrate, and kept in an illumination incubator (28 °C, RH = 80%, light:darkness ratio = 12:12). Control plants were treated with sterile distilled water. Disease severity was calculated based on the disease severity standard described by Chen et al. (2015).

DNA extraction and purification

DNA extraction and purification were performed as described by Raeder and Broda (1985) and Nel et al. (2006). Briefly, the mycelia of 5-day liquid culture in 50-ml flasks at 28 °C was collected using double-layer cheesecloth, followed by rinsing with distilled water and removing the extra water using filter paper. The mycelial pads were ground in an atmosphere of liquid nitrogen for DNA extraction using the CTAB protocol. RNA was removed from the DNA sample with RNase (100 µg/µL). The purified genomic DNA was separated on a 1% agarose gel (w/v) stained with

ethidium bromide and photographed under UV illumination. DNA concentration and quality were measured using an ND-1000 spectrophotometer (Thermo Scientific Inc, Grand Island, NY). The DNA working concentration was adjusted to 100 ng/μL and stored at -20 °C.

rDNA-ITS region amplification

The rDNA-ITS region was amplified with the universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATAT-3'). The PCR reactions were carried out using a 25-μL mixture containing 1X *Taq* buffer (75 mM Tris-HCl [pH 8.8], 20 mM (NH₄)₂SO₄, and

0.01% Tween 20), 10 mM each of deoxyribonucleotide triphosphates (dNTPs), 25 mM MgCl₂, each primer at 0.4 μM, 1 U *Taq*, and approximately 10 ng DNA as template. The amplification conditions were as follows: 94 °C for 4 min; followed by 35 cycles of 94 °C for 30 s, 51 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The above reaction without the DNA template served as a negative control.

Five microliter aliquots of PCR products were separated on a 1.5% agarose gel stained with ethidium bromide. PCR products that produced bright and distinct bands were sequenced independently along both strands by Songon Biotech Co. Ltd. (Shanghai). The sequences were aligned on the NCBI website using BLASTn.

Fig. 1 The morphology of *Fusarium oxysporum* f. sp. *momordicae* isolates on PDA plates **a, b, c, g, h**: the colony frontal view of GuX10, HuN16, HuN15, GuX11, GuX3; **d, e, f, j, k**: the colony opposite view of isolates GuX10, HuN16, HuN15, GuX11, GuX3; **i**: Microconidia of HeN15; **l**: Macroconidia of HeN15. The bar of **i** and **l** was 10 μm

ISSR PCR primer selection and ISSR-PCR amplification

To select primers that generated more polymorphic bands, a total of 40 ISSR primers were screened using genomic DNA from six representative isolates of FOM from different locations. These primers consist of di- or trinucleotide repeats. The screened polymorphic primers were used to characterize the 173 FOM isolates. The 25- μ L mixture contained 2 μ L 10 $\times$ PCR buffer, 1.8 mM MgCl₂, 0.1 mM dNTPs, each primer at 100 μ M, 50 ng template DNA, 1.5 U *Taq* polymerase (TaKaRa Biomedical Technology Co. Ltd, China), and double-distilled water. Amplifications were performed using a thermal cycler (Eppendorf, Hamburg, Germany) with the following PCR program: 5 min of initial denaturation at 94 °C; 30 cycles of 94 °C for 30 s, 30 s for annealing at the primer-specific melting temperature, and 72 °C for 3 min; followed by a final extension of 10 min at 72 °C. The above reactions performed without template DNA served as a negative control. The PCR products were separated at 120 V for 1 h on a 2% agarose gel pre-stained with ethidium bromide. The gel was visualized under UV light and photographed using the InGeniusLHR gel imaging system (Syngene, Frederick, MD). All PCR amplifications were performed at least twice for each isolate. A 3000-bp DNA ladder was used as a size marker.

Data analysis

The repeatable, clearly visible bands generated when using ISSR primers were converted into a binary data set with 0 and 1, where 1 and 0 represent the presence and absence of bands, respectively. Population genetic diversity, Nei's genetic diversity, Shannon's diversity index, and genetic variation were analyzed using POPGENE version 1.32. The dendrogram relationship of different geographical populations was analyzed using the unweighted pair-group mean average (UPGMA) method in the SHAM module based on genetic distance data in NYSTS Version 2.10. Meanwhile, the neighbor-joining (NJ) dendrogram was constructed using the NJ function in the R package of phangorn version 2.5.5. STRUCTURE (version 2.3.4) software was used to obtain the hierarchical organization of the genetic structure of the nine geographical populations.

Results

Pathogen isolation and identification

A total of 173 isolates were obtained from diseased bitter melon plants. The shape of spores and colony characteristics were initially used to identify the isolates. The isolates showed typical *Fusarium oxysporum* characteristics with abundant microconidia and macroconidia on white or ruby colonies (Fig. 1). A single

Fig. 2 Pathogenicity of *Fusarium oxysporum* f. sp. *momordicae* isolates HeN15 on cv. Ruyu41 CK: inoculated with sterile distilled water; IN: inoculated with HeN15 conidial suspensions; CK-Leaf: The healthy leaf from the CK treatment; IN-Leaf: the diseased leaf from IN treatment

Table 1 The pathogenicity of *F. oxysporum* f. sp. *momordicae* from diseased bitter gourd to different cucurbit hosts

Isolates	Cucurbit Hosts									
	Bitter gourd			Muskmelon	Cucumber	Watermelon	Towel gourd	Bottle gourd	Pumpkin	Cantaloupe
	Changlv	Ruyu33	Ruyu41							
GuD1	++	+++	++++	-	-	-	-	-	-	-
GuD2	++	++++	++++	-	-	-	-	-	-	-
GuX1	++	++++	++++	-	-	-	-	-	-	-
GuX2	+	++++	++++	-	-	-	-	-	-	-
HaN1	+++	++++	++++	-	-	-	-	-	-	-
HaN2	+++	++++	++++	-	-	-	-	-	-	-
HeN1	++	++++	++++	-	-	-	-	-	-	-
HeN2	++	++++	++++	-	-	-	-	-	-	-
HuN2	++	+++	++++	-	-	-	-	-	-	-
HuN3	++	++++	++++	-	-	-	-	-	-	-
JiX21	++	++++	++++	-	-	-	-	-	-	-
ShD1	++	+++	++++	-	-	-	-	-	-	-
ShD2	+	++	++++	-	-	-	-	-	-	-
FuJ10	++	++++	++++	-	-	-	-	+	-	-
GuX7	++	++	++++	-	-	-	-	+	-	-
HeN15	++	++++	++++	-	-	-	-	+	-	-
HuN1	++	++++	++++	-	-	-	+	-	-	-
JiX12	+	++	++++	-	-	-	+	-	-	-
JiX15	++	++++	++++	-	-	-	-	+	-	-
ShD6	+++	+++	++++	-	-	-	-	+	-	-
ShD9	+	+++	++++	-	-	-	+	-	-	-

“-” means no symptoms, “+” means slightly symptoms, “++” means moderately symptoms, “+++” means widespread symptoms, “++++” means the plant wilt

DNA fragment of approximately 600 bp was amplified with the universal primers ITS1 and ITS4. Aligning with the published sequences on the NCBI website by BLAST, all isolate sequences tested were similar to those of *F. oxysporum formae speciales* with identities over 99%. Therefore, the tested isolates were identified as *F. oxysporum*.

Pathogenicity and *formae speciales* determination

Nine days after inoculation, typical wilt symptoms were observed on the inoculated bitter gourd seedlings. The inoculated bitter ground showed typical yellowing and wilting symptoms on the lower leaves. The veins between the main veins yellowed,

Fig. 3 The ISSR-PCR amplification products of some FOM isolates with the primer of VCA025. Lanes 1–24 respectively were: ShD1, ShD14, ShD18, HeN5, HeN22, HeN24, HaN7, HaN8, HaN12,

HuN8, HuN10, HuN16, GuX8, GuX28, GuX31, GuD9, GuD18, Gu22, FuJ1, FuJ13, FuJ18, JiX1, JiX8, JiX17 FOM isolates. **M**: 3000bp DNA marker. **CK**: negative control

while the control plants did not show any symptoms (Fig. 2). All isolates could infect the three bitter melon cultivars. The cv. Changlv was relatively resistant to the pathogen, while the other two cultivars, Ruyu33 and Ruyu41, were susceptible. With the exception of eight isolates, FuJ10, GuX7, HeN15, HuN1, JiX12, JiX15, ShD6, and ShD9, which could infect the towel melon and bottle melon, the other isolates were not pathogenic to the seven cucurbit crops tested (Table 1). Thus, the isolates used in this study were identified as *F. oxysporum* f. sp. *momordicae* (FOM).

Primer screening and ISSR polymorphisms

Among the 40 ISSR primers tested, only 11 ISSR primers produced PCR products that displayed distinct and bright polymorphic bands, and almost no bands were observed in the PCR products when the remaining 29 primers were used (Fig. 3). When applied to the 173 FOM isolates, the 11 primers produced 127 DNA fragments, of which 76 polymorphic fragments ranged from 250 to 3000 bp. The percentage of polymorphic bands was 59.84% (Table 2). The number of bands generated from each primer ranged from 8 to 13, with an average of 11.50 per primer. The average number of polymorphic bands was 6.9.

Genetic diversity among different geographical populations

The genetic parameters of the nine populations were calculated using the PopGene software package. Results indicated that the different populations had different genetic diversity characteristics. The Jiangxi population had the greatest genetic diversity, with Nei's gene diversity index (*H*) and Shannon's information index (*I*) values of 1.07 and 0.06, respectively, while the Hubei population had the lowest

Table 2 The ISSR primers with annealing temperature (*T_m*) used in this study and the ISSR-PCR amplified results

Primers	Sequence (5' to 3')	Annealing temperature (°C)	TB	PB	PPB (%)
VCA02	(GT) ₆ CC	48.50	10	4	25.00
VCA013	(AG) ₈ S	45.30	10	1	10.00
VCA021	(GTC) ₆	46.60	12	8	66.67
VCA022	(GTG) ₆	56.10	15	13	86.67
VCA025	(CAC) ₄ GC	53.40	8	7	87.50
VCA026	(GAG) ₄ GC	56.10	12	8	66.67
VCA029	(AAG) ₆	45.30	13	12	92.31
U01	DDB(CCA) ₅	44.90	14	5	35.71
U02	DHB(CGA) ₅	45.30	13	10	76.92
U03	YHY(GT) ₅ G	46.60	9	7	77.78
U05	NDB(CA) ₇ C	43.50	11	1	9.09
Average			11.50	6.90	
Total			127	76	59.84

TB the number of total bands, *PB* number of polymorphic bands, *PPB* the percentage of polymorphic bands

genetic diversity, with *H* and *I* values of 0.01 and 0.02, respectively. The average value showed that the number of alleles (*N_a*) and the effective number of alleles (*N_e*) were 1.13 and 1.08, respectively, while *H* and *I* were 0.05 and 0.07, respectively, in the nine geographical populations (Table 3).

At the species level, the *N_a*, *N_e*, *H*, and *I* were 1.57, 1.13, 0.09, and 0.15, respectively (Tables 3 and 4). According to the evaluation criterion of population diversity richness, *H* and *I* values should be greater than 0.20 and 0.30, respectively. Therefore, the genetic diversity of the 173 tested isolates from nine geographical populations was relatively low. The values of total heterozygosity (*H_t*), intraspecific heterozygosity (*H_s*), coefficient of gene

Table 3 Summary of genetic variation statistics for all loci of ISSR markers among nine populations of *Fusarium oxysporum* f. sp. *momordicae*

Geographical Populations	No. of isolates	<i>N_a</i>	<i>N_e</i>	<i>H</i>	<i>I</i>	NP	P(%)
Shandong	19	1.20	1.09	0.05	0.08	25.0	16.69
Henan	22	1.20	1.10	0.06	0.10	26.0	20.47
Guangdong	21	1.09	1.06	0.03	0.05	12.0	9.45
Hainan	12	1.13	1.08	0.05	0.07	16.0	12.60
Hunan	17	1.30	1.20	0.12	0.17	38.0	29.92
Guangxi	20	1.28	1.17	0.10	0.14	36.0	28.35
Fujian	20	1.17	1.11	0.06	0.09	22.0	17.32
Jiangxi	22	1.10	1.07	1.07	0.06	13.0	10.24
Hubei	20	1.03	1.02	0.01	0.02	4.0	3.15
Average	19	1.13	1.08	0.05	0.07	21.3	16.47
Species level	173	1.57	1.13	0.09	0.15	72.0	56.69

N_a Observed number of alleles, *N_e* Effective number of alleles, *H* Nei's *s* gene diversity index,

I Shannon's information index, *NP* Number of polymorphic loci, *P* percentage of polymorphic loci

Table 4 The genetic diversity among 173 *F. oxysporum* f. sp. *momordicae* isolates by ISSR markers

	n	Na	Ne	H	I	Ht	Hs	Gst	Nm
Species level	173	1.57	1.13	0.09	0.15	0.09	0.06	0.33	1.01
St. Dev		0.50	0.26	0.14	0.20	0.02	0.01		

n Sample size, *Na* Observed number of alleles, *Ne* Effective number of alleles, *H* Nei's gene diversity index, *I* Shannon's information index, *Ht* total heterozygosity, *Hs* intraspecific heterozygosity,

Gst coefficient of gene differentiation, *Nm*, gene flow, $Nm = 0.5(1 - Gst)/Gst$

differentiation (*Gst*), and gene flow (*Nm*) were 0.09, 0.06, 0.33, and 1.01, respectively. *Gst* indicated that the genetic differentiation among the nine geographical populations was generally high ($Gst = 0.33 > 0.15$). The *Nm* value revealed that the population differentiation caused by gene drift was blocked to some degree ($Nm = 1.01 > 1.0$) (Table 4).

The genetic identity of the nine geographical populations ranged from 0.947 to 0.986. The maximum genetic identity appeared between the Hunan and Hainan populations. The genetic distances for these populations ranged from 0.017 to 0.055. The maximum genetic distances appeared between the Hubei and the Shandong populations (Table 5).

Based on the genetic distance on the topological structure of the dendrogram, the nine geographical populations were clustered into two distinct groups at 0.962 of the genetic similarity coefficient value (Fig. 4). Group I comprised the Shandong and Henan populations, which were both located in the north of the Qinling Mountain and Huai River. Group II consisted of the Guangdong, Hainan, Fujian, Jiangxi, Hubei, Hunan, and Guangxi populations. In contrast, these populations all lay to the south of the Qinling Mountain and Huai River (Fig. 5). With a genetic similarity coefficient value of 0.968, Group II was divided into three subgroups. Subgroup 1 included the Guangdong, Hainan, Fujian, Jiangxi, and Hubei populations. The Hunan and Guangxi populations formed subgroups 2 and 3, respectively. The topological structure of neighbor-joining (NJ) was similar to the UPGMA dendrogram, which gave strong support in the UPGMA clustering (Fig. 6).

Results from the nine geographical population structure analysis showed that different populations had different levels of genetic differentiation (Fig. 7). The most obviously differentiated populations were that of the Hunan and Guangxi geographical populations. There was a large genetic difference among the isolates in the two geographical populations. The degree of population differentiation of the Hubei geographical population was the lowest. The genetic differences among the isolates within this population were minimal. The Shandong, Henan, Guangdong, Hainan, Fujian, and Jiangxi geographical populations had obvious population genetic differentiation; however, the degree of population genetic differentiation was much weaker than that of the Hunan and Guangxi geographical populations.

Discussion

In this study, morphological characteristics, rDNA-ITS sequencing, and *formae speciales* tests were combined to ensure that all the isolates studied were FOM. In general, *Fusarium* pathogens cannot be identified to the species level with the rDNA-ITS sequence alone; however, *Fusarium oxysporum* appears to be an exception and can be identified specifically using the rDNA-ITS sequence. With the supplement of *formae speciales* test results, all isolates were identified as FOM. The precision and reliability of the ISSR analysis were demonstrated.

Table 5 The Nei's genetic identity and genetic distance of nine populations by ISSR markers

Geographical Populations	Shandong	Henan	Guangdong	Hainan	Hunan	Guangxi	Fujian	Jiangxi	Hubei
Shandong	****	0.976	0.957	0.948	0.954	0.952	0.967	0.952	0.947
Henan	0.025	****	0.970	0.966	0.970	0.958	0.973	0.965	0.954
Guangdong	0.044	0.030	****	0.983	0.966	0.963	0.970	0.978	0.975
Hainan	0.054	0.035	0.017	****	0.986	0.970	0.971	0.972	0.962
Hunan	0.047	0.031	0.034	0.032	****	0.965	0.966	0.967	0.964
Guangxi	0.049	0.043	0.037	0.030	0.036	****	0.970	0.962	0.955
Fujian	0.033	0.027	0.031	0.029	0.035	0.031	****	0.983	0.972
Jiangxi	0.049	0.036	0.023	0.029	0.033	0.039	0.018	****	0.981
Hubei	0.055	0.047	0.025	0.039	0.037	0.046	0.029	0.020	****

The Nei's genetic identity (above diagonal) and the genetic distance (below diagonal).

Fig. 4 The UPGMA dendrogram of nine FOM geographical populations based on the genetic distance

In pathogenicity experiments, we found that the FOM isolates had different aggressivity on Cucurbitaceae crops. Three FOM isolates (HuN1, JiX12, and ShD9) could infect the tower gourd (*Luffa cylindrica*). Five FOM isolates, FuJ10, GuX7, HeN15, JiX15, and ShD6, were pathogenic to bottle gourd (*Lagenaria ciceraria*). Although it has been reported that there is host specificity among the different *formae speciales* of *F. oxysporum*, the cross infectivity of some *formae speciales* has been mentioned in several studies (Bouhot 1981; Kim et al. 1993; McCallum et al. 2001; Snyder and Hansen 1940). Zhu and Qi (1998) showed that FOM from

bitter melon was pathogenic to bottle melon seedlings and club bottle melon seedlings and grown plants. Cumagun et al. (2010) found that some FOM isolates from bitter melon could infect 7-day-old bottle melon seedlings, but not 1-month-old plants. Chen et al. (2015) also reported that some bitter melon FOM isolates produced mild infections in bottle melon (*Lagenaria siceraria* var. *clavata*) seedlings. *F. oxysporum* f. sp. *luffae* was also found to infect bitter melon cultivars (Lv et al. 2000). These findings were identical to our results. However, contradictory results have also been demonstrated in previous reports. *F. oxysporum* f. sp. *momodicae* was first

Fig. 5 The geographical distribution of nine FOM geographical populations on the Chinese map. The red ellipse stands for the group I and the red circle means the group II, the dashed line in the middle of the map stands for the approximate location of the Qinling Mountain and Huai River, which was the dividing line between north and south China

Fig. 6 The neighbor-joining (NJ) dendrogram of nine FOM geographical populations based on the genetic distance

found on bitter melon in Taiwan in 1983 and at the time was not considered pathogenic to Cucurbitaceae crops (Lin et al. 1996; Snyder and Hansen 1940). Yang et al. (2010) also showed that FOM isolates could specifically infect bitter melon, but did not infect white melon, tower melon, watermelon, and muskmelon. These contradictory results may be attributed to the host age and pathogen-adaptive variation. Gerlagh and Blok (1988) stated that the *formae speciales* specificity of *F. oxysporum* causing seedling wilt in Cucurbitaceae is limited. Bouhot (1981) demonstrated that mutation in one *forma specialis* could be induced to convert to another *forma specialis* with a pathogenic capacity to another host.

Information on population genetic variation and population structure of FOM is essential and vital for understanding the roles of genetic diversity in the future. In this study, 173 FOM isolates from nine geographical populations were studied using the ISSR technique. According to the values of H and I of different geographical populations, the genetic differentiation within populations

was relatively small. This indicates that the genetic homogeneity within the nine populations was high. Cumagun et al. (2008) reported that four VCGs occur in *F. oxysporum* f. sp. *momordicae* isolates. However, it is speculated that FOM isolates may belong to a single VCG because most isolates belonged to VCG1, with the exception of one isolate each from VCG3 and VCG4 and three isolates from VCG2. Chen et al. (2014) also found that the polymorphic bands amplified by RAPD molecular markers from FOM isolates were less; therefore, it was assumed that a high genetic similarity existed in FOM isolates. Later, they showed that the genetic differentiation within the population was lower using the AFLP approach (Chen et al. 2015). The low genetic diversity within the population might be attributed to the strictly asexual reproduction of *F. oxysporum* and limited gene flow between populations.

Gst is an important parameter to measure whether genetic differentiation exists among populations. When the Gst value was greater than 0.15, genetic differentiation was considered large (Wright 1978). Nm is a vital factor

Fig. 7 The hierarchical organization of the genetic structure of nine geographical populations in China. The different colors mean different groups based on the method of Evanno (K=4), the length of the colored segment suggested the estimated membership proportion of each sample in the designed group

affecting genetic differentiation. When the Nm value was less than 1.00, the gene flow was blocked to some degree (Wright 1931). In our study, the G_{st} and Nm values were 0.33 and 1.01, respectively. These data showed that genetic differentiation among geographical populations was high, while gene exchanges between these populations were relatively rare; that is, each geographical population was a relatively independent unit. The most likely reason was the high level of adaptability of pathogens to local climate, soil conditions and host variety. In China, the climate and soil conditions of different bitter gourd-producing regions are quite different. It is hot and rainy in Fujian, Guangdong, Guangxi, Hainan, Hubei, Hunan, and Jiangxi provinces, which are all located to the south of the Qinling Mountain and Huai River, whereas it is arid in Henan and Shandong, which are located in the north of the Qinling Mountain and Huai River. The main cultivated bitter gourd variety and cultivation methods are relatively stable. The same varieties could be used for many years in the same region. Thus, the selection pressures that the pathogen faces will be relatively stable and the pathogen will trend toward homogeneity.

Conclusion

In summary, ISSR is an effective tool for detecting the genetic variation among and within *F. oxysporum* f. sp. *momordicae* populations. The genetic variation within the pathogen populations was relatively low, but was slightly greater among the populations. Gene exchanges between different populations were blocked to some degree. These findings enrich our knowledge of genetic variation characteristics in pathogen populations and may be used for breeding *Fusarium* wilt disease-resistant bitter gourd in the future.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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