Population Structure and Aggressiveness of *Sclerotinia sclerotiorum* From Rapeseed (*Brassica napus*) in Chongqing City

Yang Yu, Junsong Cai, Linhao Ma, Zhiqiang Huang, Yabo Wang, Anfei Fang, Yuheng Yang, Ling Qing, and Chaowei Bi[†]

College of Plant Protection, Southwest University, Chongqing City, 400715, People's Republic of China

Abstract

Sclerotinia sclerotiorum is one of the most devastating fungal plant pathogens of oilseed *Brassica* and is distributed worldwide. In particular, *Sclerotinia* stem rot has always been a serious threat to rapeseed production in Chongqing City, China. In this study, simple sequence repeat (SSR) markers and mycelial compatibility groups (MCGs) were used to characterize the population structure of 90 geographic isolates of *S. sclerotiorum* collected from rapeseed in nine counties of Chongqing. A total of 52 microsatellite haplotypes were identified, and a few haplotypes were found with high frequency. Gene diversity ranged from 0.1570 to 0.4700 in nine populations. A constructed unweighted pair group with arithmetic mean dendrogram based on Nei genetic distance and a STRUCTURE analysis revealed that the genetic composition of the isolates collected in the five counties located in western Chongqing

are different from those collected in the two eastern counties, suggesting that breed lines should be cultivated in both the western and eastern regions to effectively evaluate resistance levels. A total of 47 MCGs were identified, and 72% of the MCGs was represented by single isolates. Seven of 13 MCGs that included at least two isolates contained isolates from only one county. SSR haplotypes were not correlated with MCGs. A subset of 34 isolates were inoculated on rapeseed stems, and the aggressiveness showed variation. This research revealed the population genetic structure and aggressiveness of this pathogen in Chongqing, and the results will help to develop disease management and resistance screening strategies.

Keywords: aggressiveness, population structure, *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum is a ubiquitous ascomycete plant pathogen that is distributed worldwide (Bolton et al. 2006). This fungus infects more than 500 known plants of diverse phylogenetic origin and is the causal agent of stem rot in rapeseed. *Sclerotinia* stem rot in rapeseed is a serious problem in China and causes yield loss ranging from 5 to 30% every year and $\leq 80\%$ in severely infected fields (Hou and Huang 2006).

S. sclerotiorum produces abundant long-living survival structures called sclerotia, which may germinate myceliogenically to produce hyphae that infect stems of host plants directly or germinate carpogonically and release millions of airborne ascospores to infect petals (Bardin and Huang 2001). Management of *Sclerotinia* diseases is still difficult because sown rapeseed cultivars do not have high levels of resistance, and fungicide-resistant strains are increasingly reported in fields (Bolton et al. 2006; Derbyshire and Denton-Giles 2016; Lehner et al. 2015). A thorough understanding of the genetic diversity and population genetics structure of the fungal pathogen will provide important information for integrated disease management (Attanayake et al. 2019).

Many different molecular marker techniques including simple sequence repeat (SSR), amplified fragment length polymorphism, and sequence-related amplified polymorphism have been applied to investigate the genetic diversity of *S. sclerotiorum* populations (Leyronas et al. 2018; Li et al. 2009; Sharma et al. 2018; Sirjusingh and Kohn 2001). Because of the high mutation rate and multiallelic nature of *S. sclerotiorum*, SSRs are recognized as one of the most efficient molecular markers. Mycelial compatibility groups (MCGs), a macroscopic assay of self/nonself recognition controlled by multiple loci, are also used to

characterize the relationships between *S. sclerotiorum* isolates (Carpenter et al. 1999; Kamvar et al. 2017; Koga et al. 2014; Lehner et al. 2015).

Based on these molecular methods, many studies have been conducted to characterize the population structures of *S. sclerotiorum* isolates from specific hosts and geographic regions. These studies revealed that the population of *S. sclerotiorum* in many regions was predominantly clonal, with infrequent outcrossing (Dunn et al. 2017; Kohli and Kohn 1998; Sexton and Howlett 2004). The formation of clonality for *S. sclerotiorum* is due mainly to the sexual reproduction of millions of ascospores by self-fertilization and asexual reproduction of abundant sclerotia (Kohn 1995). However, some studies still describe the occurrence of outcrossing in some regions, including Brazil, Iran, and the United Kingdom (Gomes et al. 2011; Hemmati et al. 2009; Li et al. 2009). The populations of *S. sclerotiorum* are usually loosely structured over space but not by cultivar, aggressiveness, or field source (Leyronas et al. 2018). In addition, the aggressiveness of the isolates is usually independent of their geographic distribution or host plants (Leyronas et al. 2018; Sharma et al. 2018).

Chongqing City is located in the upper portion of the Yangtze River and is one of most important rapeseed production areas in China. *Sclerotinia* stem rot has always been an economically important disease affecting rapeseed in Chongqing. However, the population structures of *S. sclerotiorum* from rapeseed in Chongqing are still unclear. In the present study, we analyzed and characterized the phenotypic and genetic diversity of *S. sclerotiorum* isolates from rapeseed in different geographic locations in Chongqing.

Materials and Methods

Isolate collection. Ninety geographic isolates of *S. sclerotiorum* were obtained from sclerotia that were collected from rapeseed plants with stem rot across nine counties of Chongqing City, China in 2018: Hechuan, Tongliang, Rongchang, Yongchuan, Jiangjin, Qianjiang, Wanzhou, Wuxi, and Wushan (Fig. 1). The sclerotia that were obtained from each plant represent a single isolate. For each county, sclerotia were collected from two or three fields. The distance between sampled plants within a field was ≥ 10 m.

Strain isolation and identification. Sclerotia collected from a diseased plant sample were surface sterilized in a 1% NaOCl solution for 10 s. The sclerotia were then sterilized with 70% ethanol for 60 s and then rinsed in distilled water. The sterilized sclerotia were

[†]Corresponding author: C. W. Bi; chwbi@swu.edu.cn

Funding: This research was funded by the National Key Research and Development Program of China (2018YFD0200900) and Fundamental Research Funds for the Central Universities, China (XDJK2019B034).

The author(s) declare no conflict of interest.

Accepted for publication 19 November 2019.

cultured on potato dextrose agar (PDA) medium at 20°C for 3 d. The tip of the hyphae was cut from the resulting colony edge and transferred to the PDA medium. For each sclerotium, one hyphal-tipped isolate was obtained. The 90 geographic isolates exhibited typical morphologies of *S. sclerotiorum* on PDA medium. The isolates were further identified by amplifying a specific fragment of nuclear ribosomal DNA internal transcribed spacer sequences with the primers SSFED/SSREV (Freeman et al. 2002). All isolates were stored on PDA medium at 4°C.

DNA extraction. The different isolates were cultured on cellophane over PDA medium at 20°C, and mycelia were harvested at 3 d after inoculation. The DNA from different samples were extracted via a cetyltrimethylammonium bromide method according to Sambrook and Russell (2001).

Genotyping. Nine loci including 5-2, 7-2, 8-3, 12-2, 13-2, 17-3, 36-4, 42-4, and 92-4 (Sirjusingh and Kohn 2001) were used to determine the alleles in the 90 isolates by using primers labeled with fluorescein amidite (FAM). PCR mixtures were composed of 10 µl of Taq DNA solution, a concentration of 5 pmol of each primer, 2 µl of genomic DNA template, and enough nuclease-free water to create a final volume of 20 µl. The amplified product was analyzed via capillary electrophoresis. One allele was amplified for every locus in each isolate, which further indicates that *S. sclerotiorum* is a haploid organism. The allele sizes were determined by manually scoring the resulting electropherograms. Each isolate was genotyped at least twice for each microsatellite locus to confirm the reproducibility of the results.

Data analysis. Haplotype diversity and related frequencies were obtained in PowerMarker 3.25 (Liu and Muse 2005). The observed number of alleles (N_a), effective number of alleles (N_e), Nei gene diversity (h), Shannon information index (I), Nei genetic identity, and genetic distance were all calculated with POPGENE version 1.3.2 (Yeh et al. 1999). The index of association I_A and an alternative measure r_d for each population were calculated with Multilocus version 1.3 to determine the linkage disequilibrium of the multilocus (Agapow and Burt 2001). Analysis of molecular variance between and within *S. sclerotiorum* populations was performed in ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Phylogenetic trees were constructed in NTSYSpc version 2.10.

A Bayesian approach was used to infer the population genetic structure in STRUCTURE version 2.3.4 (Falush et al. 2003). The analysis was conducted via the admixture model with a 100,000

burn-in period and 1,000,000 iterations. The number of populations (K) was tested with a range from 1 to 10, with 10 replicates for each. K was deduced from both the maximum of $L(K)$ and the delta log likelihood (Evanno et al. 2005).

Mycelial compatibility. Three mycelial agar plugs from different isolates of *S. sclerotiorum* were placed in an equilateral triangle in 9-cm Petri dishes on PDA medium. The isolates were tested in all possible combinations, with three replicates for each combination. The cultures were inoculated at 20°C for 7 days, and mycelial compatibility and incompatibility interactions were examined as described by Kull et al. (2004).

Aggressiveness tests. Thirty-four isolates were randomly selected for aggressiveness tests according to Mei et al. (2012). *Brassica napus* cultivar Zhongyou821 was grown in the experimental fields of Southwest University, and stems that were approximately 30 cm in length were cut at the mature stage (approximately 6 months after sowing). The stems were then wounded by a dissecting needle. Each stem was inoculated with two 0.6-cm mycelium plugs from actively growing colony edges at two points with an interval of 10 cm. Each isolate was inoculated onto at least three stems. The inoculated stems were placed in 90% RH at 20°C. The lengths of the lesions were recorded at 4 d after inoculation. Isolates were classified as highly aggressive, aggressive, or mildly aggressive according to the methods of Willbur et al. (2017) with a slight modification. Isolates with a lesion length value not statistically different from the longest lesion length were considered highly aggressive. Isolates with a length value not statistically different from the shortest lesion length were considered mildly aggressive. The other isolates were considered aggressive.

Results

Genetic diversity. Eight of nine microsatellite primer pairs exhibited polymorphisms except primer pair 42-4, which showed monomorphisms. Table 1 shows the amplification results of the eight primer pairs. A total of 24 alleles were detected, and the number of alleles per locus ranged from 2 to 6, with an average of 3 alleles

Table 1. Genetic variation at eight microsatellite loci used to characterize *Sclerotinia sclerotiorum* isolates collected from rapeseed in Chongqing

Locus	Alleles observed	Size range (bp)	Gene diversity ^a
5-2	2	317–319	0.1244
7-2	3	160–173	0.4170
8-3	3	244–252	0.5459
12-2	2	215–221	0.2923
13-2	4	291–345	0.5440
17-3	6	334–365	0.7284
36-4	2	411–417	0.4998
92-4	2	372–374	0.4516

^a Gene diversity calculated via Nei's method (Nei 1973).

Table 2. Estimates of genetic variability for nine *Sclerotinia sclerotiorum* populations from Chongqing

Population	n^a	H^b	$H(\text{unique})$	N_a^c	N_e^d	h^e	I^f
Hechuan	11	9	7	2.2500	1.6663	0.3704	0.5879
Tongliang	10	8	5	2.2500	1.9280	0.4250	0.6608
Rongchang	7	7	4	2.3750	1.8627	0.4184	0.6772
Yongchuan	10	6	2	2.2500	1.9601	0.4700	0.7107
Jiangjin	12	10	4	2.6250	1.9637	0.4497	0.7308
Qianjiang	11	6	4	1.3750	1.2721	0.1570	0.2284
Wanzhou	10	6	4	2.0000	1.5389	0.2900	0.4620
Wuxi	7	6	5	2.0000	1.7396	0.3878	0.5764
Wushan	12	11	9	2.2150	1.6508	0.3177	0.5196

^a Population size.

^b Haplotype.

^c Observed number of alleles.

^d Effective number of alleles (Kimura and Crow 1964).

^e Nei (1973) gene diversity.

^f Shannon information index (Lewontin 1972).

Fig. 1. Map of the nine counties of Chongqing city, obtained from MAP WORLD (www.tianditu.gov.cn). 1, Hechuan; 2, Tongliang; 3, Rongchang; 4, Yongchuan; 5, Jiangjin; 6, Qianjiang; 7, Wanzhou; 8, Wuxi; 9, Wushan.

per locus. The allele size ranged from 160 to 417 bp, and Nei gene diversity ranged from 0.1244 to 0.7284.

A total of 52 haplotypes were identified within 90 isolates from nine *S. sclerotiorum* populations based on microsatellite data. The

Fig. 2. Microsatellite haplotype frequency for nine *Sclerotinia sclerotiorum* populations from rapeseed in Chongqing for 16 haplotypes comprising more than one isolate. HC, Hechuan; JJ, Jiangjin; QJ, Qianjiang; RC, Rongchang; TL, Tongliang; WS, Wushan; WX, Wuxi; WZ, Wanzhou; YC, Yongchuan.

Table 3. Linkage disequilibrium measures for nine *Sclerotinia sclerotiorum* populations from Chongqing^a

Population	I_A	r_d	Clone-corrected dataset	
			I_A	r_d
Hechuan	0.67**	0.04**	0.30, NS	0.02, NS
Tongliang	1.76***	0.11***	0.98***	0.06***
Rongchang	0.65, NS	0.04, NS	0.65, NS	0.04, NS
Yongchuan	4.45***	0.26***	2.44***	0.14***
Jiangjin	1.54***	0.08***	1.12***	0.06***
Qianjiang	0.81***	0.16***	0.33, NS	0.07, NS
Wanzhou	3.52***	0.27***	1.13*	0.09*
Wuxi	2.04***	0.15***	1.61***	0.12***
Wushan	0.56*	0.04*	0.57*	0.04*

^a Values significant at *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$; NS, not significant.

Table 4. Analysis of molecular variance of *Sclerotinia sclerotiorum* isolates in nine populations from Chongqing

Source of variation	Degrees of freedom	Sum of squares	Percentage of variation (%)	P value
Between populations	8	31.987	13.00	<0.01
Within populations	81	130.17	87.00	<0.01

Table 5. Nei genetic identity (above diagonal) and genetic distance (below diagonal) among nine populations of *Sclerotinia sclerotiorum* from Chongqing

Population	Hechuan	Tongliang	Rongchang	Yongchuan	Jiangjin	Qianjiang	Wanzhou	Wuxi	Wushan
Hechuan	—	0.9394	0.9524	0.9154	0.9131	0.8006	0.8761	0.8942	0.7998
Tongliang	0.0625	—	0.9109	0.9352	0.9333	0.8194	0.8373	0.8457	0.8299
Rongchang	0.0488	0.0993	—	0.8973	0.9179	0.8184	0.8475	0.8892	0.8646
Yongchuan	0.0884	0.0670	0.1083	—	0.9586	0.7446	0.8579	0.9154	0.8159
Jiangjin	0.0909	0.0691	0.0856	0.0423	—	0.7341	0.7865	0.8998	0.7593
Qianjiang	0.2224	0.1992	0.2004	0.2949	0.3091	—	0.7521	0.7615	0.8890
Wanzhou	0.1323	0.1776	0.1654	0.1533	0.2401	0.2849	—	0.8261	0.8291
Wuxi	0.1119	0.1676	0.1175	0.0884	0.1056	0.2724	0.1911	—	0.8450
Wushan	0.2234	0.1865	0.1455	0.2035	0.2754	0.1176	0.1874	0.1684	—

number of haplotypes in each population ranged from 6 to 11 (Table 2), and 16 haplotypes were shared between two or more isolates (Fig. 2). The most common haplotype was represented by 11 isolates that were collected from four counties: Yongchuan, Jiangjin, Wanzhou, and Wuxi. The second most common haplotype, which differed from the most common haplotype only by allele size at two SSR loci, was represented by seven isolates that were distributed in three counties: Rongchang, Qianjiang, and Wushan. The remaining 36 haplotypes were represented by a single isolate.

The observed and effective numbers of alleles in each regional population ranged from 1.3750 to 2.6250 and 1.2721 to 1.9637, respectively. Nei gene diversity and Shannon information index in each population ranged from 0.1570 to 0.4700 and 0.2284 to 0.7107, respectively. The average of Nei gene diversity for the five western populations (Hechuan, Tongliang, Rongchang, Yongchuan, and Jiangjin) was 0.4267, which is bigger than that for the four eastern populations (Qianjiang, Wanzhou, Wuxi, and Wushan). The Qianjiang population exhibited the lowest gene diversity and Shannon information index values.

When the original dataset was used to test for linkage disequilibrium, the value of the index of association I_A ranged from 0.56 to 4.45, with corresponding r_d values of 0.04 to 0.27 (Table 3). Significance testing showed that almost all populations reject the null hypothesis of random mating except for the Rongchang population. When the clonally corrected dataset was used, I_A and r_d values for Hechuan, Rongchang, and Qianjiang populations were not significantly different ($P = 0.05$) from zero. The remaining six populations still reject the null hypothesis of random mating.

Analysis of molecular variance showed that most genetic variations were found among isolates within the population, which accounted for 87% of the total molecular variation (Table 4). The remaining variance (13%) was caused by the variation between populations. The fixation index for the analyzed isolates was 0.12997, indicating the occurrence of medium variability among the populations of *S. sclerotiorum* in this study.

Pairwise population comparisons of genetic identity values indicated a high degree of similarity between the geographic populations of *S. sclerotiorum* from Chongqing. The values of genetic identity ranged from 0.7341 between the populations from Qianjiang and Jiangjin to 0.9586 between the populations of Jiangjin and Yongchuan (Table 5). An unweighted pair group with arithmetic mean tree was constructed based on genetic distance and showed that the nine populations could be divided into two clusters. The first cluster contained populations of Hechuan, Tongliang, Rongchang, Yongchuan, Wuxi, Jiangjin, and Wanzhou. Within this cluster, the populations of Hechuan, Tongliang, Rongchang, Yongchuan, and Jiangjin were clustered together and were distinct from the Wuxi and Wanzhou populations. The last two populations (Qianjiang and Wushan) were clustered together (Fig. 3).

The population genetic structures for 90 isolates were further analyzed with a Bayesian cluster in STRUCTURE software. Two genetically distinct subpopulations were identified, because the value was associated with the highest value of ΔK . The two genetic populations, shown in green and red in Figure 4, were distributed across all nine counties of Chongqing. Generally, the isolates from the five counties in the western portion of Chongqing (Hechuan, Tongliang,

Rongchang, Yongchuan, and Jiangjin) were largely assigned to the green population, whereas those from Wushan and Qianjiang counties were largely assigned to the red population.

Mycelial compatibility. A total of 47 MCGs were identified from 90 geographic isolates of *S. sclerotiorum* (Table 6), and most (72%) of the MCGs were represented by a single isolate. Thirteen MCGs contained more than one isolate, and 7 of those MCGs included isolates from only one county. The largest group comprised 12 isolates. Among those, five isolates were collected from Hechuan and seven isolates from Yongchuan. Six isolates collected from Wanzhou and three isolates from Wuxi County made up the second largest MCG group. The third largest groups included six isolates collected from Qianjiang. The number of MCGs among the regional populations of *S. sclerotiorum* ranged from three (Wanzhou) to nine (Jiangjin).

Aggressiveness on rapeseed. The aggressiveness values of a subset of 34 isolates were tested on detached stems of rapeseed. All isolates were able to develop stem lesions after infection. However, the aggressiveness of these isolates exhibited significant variation ($P < 0.01$), with the average stem lesion length ranging from 7.2 ± 1.0 to 40.2 ± 6.8 mm (mean $\pm$ standard deviation). The isolates were classified into three types: highly aggressive (3 isolates), aggressive (16 isolates), and mildly aggressive (15 isolates), based on the variations in lesion length (Fig. 5). The *S. sclerotiorum* collections in Chongqing were divided into two clusters; however, no significant difference in average aggressiveness was found between the two groups.

Discussion

Under natural conditions, the diseases caused by *S. sclerotiorum* are initiated by asexual reproduction of sclerotia or sexual reproduction of ascospores by self-fertilization (Bolton et al. 2006; Kohn 1995). Consistent with this, studies on population structure using molecular markers and MCGs have revealed clonal populations of *S. sclerotiorum* in several regions (Hambleton et al. 2002; Lehner et al. 2015; Winton et al. 2006). In this study, 16 haplotypes were

Fig. 3. Unweighted pair group with arithmetic mean dendrogram of nine populations of *Sclerotinia sclerotiorum* based on Nei genetic distance. HC, Hechuan; JJ, Jiangjin; QJ, Qianjiang; RC, Rongchang; TL, Tongliang; WS, Wushan; WX, Wuxi; WZ, Wanzhou; YC, Yongchuan.

Fig. 4. Assignment of *Sclerotinia sclerotiorum* isolates collected from rapeseed in Chongqing to two subpopulations in STRUCTURE. The Q value indicates the proportion of each isolate belonging to each subpopulation. HC, Hechuan; JJ, Jiangjin; QJ, Qianjiang; RC, Rongchang; TL, Tongliang; WS, Wushan; WX, Wuxi; WZ, Wanzhou; YC, Yongchuan.

Table 6. Mycelial compatibility group (MCG) designations for 90 *Sclerotinia sclerotiorum* isolates from Chongqing

MCG	Isolate code ^a
1	HC-2, HC-9, HC-12, HC-17, HC-24, YC-3, YC-9, YC-11, YC-13, YC-14, YC-15, YC-16
2	WZ-3, WZ-5, WZ-7, WZ-8, WZ-9, WZ-10, WX-1, WX-2, WX-3
3	QJ-2, QJ-5, QJ-6, QJ-9, QJ-10, QJ-11
4	RC-8, RC-11, RC-14, RC-15, HC-16
5	WS-1, WS-10, WS-15, WS-20
6	JJ-6, WZ-1, WZ-2, WZ-6
7	HC-26, RC-9
8	TL-2, TL-3, TL-8
9	JJ-9, JJ-13, JJ-28
10	TL-17, JJ-21
11	JJ-4, JJ-25
12	WX-4, WX-5
13	WS-23, WS-24
14	HC-6
15	HC-11
16	HC-22
17	HC-27
18	TL-10
19	TL-11
20	TL-18
21	TL-19
22	TL-23
23	TL-26
24	RC-1
25	RC-5
26	YC-18
27	YC-19
28	YC-21
29	JJ-3
30	JJ-5
31	JJ-7
32	JJ-23
33	JJ-32
34	QJ-1
35	QJ-7
36	QJ-8
37	QJ-12
38	QJ-15
39	WZ-4
40	WX-6
41	WX-7
42	WS-3
43	WS-5
44	WS-6
45	WS-9
46	WS-18
47	WS-27

^a HC, Hechuan; JJ, Jiangjin; QJ, Qianjiang; RC, Rongchang; TL, Tongliang; WS, Wushan; WX, Wuxi; WZ, Wanzhou; YC, Yongchuan.

sampled at much higher frequency than the others. The two most frequently sampled haplotypes showed differences in allele size at only two SSR loci and were present in seven of nine counties across Chongqing. The most common haplotype was present in isolates from four counties, including Yongchuan and Wuxi, which are >400 km apart. The multilocus index of association tested was applied to the nine geographic populations separately, and the I_A values for most populations were significantly different from zero, which means that the null hypothesis of random mating was rejected. When all nine populations were analyzed together, the I_A value was also significantly different from zero (data not shown). These evidences suggest a clonal population structure of *S. sclerotiorum* from rapeseed in Chongqing.

Although the populations of *S. sclerotiorum* in many regions were predominantly clonal, several studies have also shown high genotypic diversity in *S. sclerotiorum* populations (Gomes et al. 2011; Hemmati et al. 2009). In this research, 24 polymorphic loci were obtained in 90 isolates collected in nine counties in Chongqing with eight SSR markers. A total of 52 haplotypes were found from 90 isolates (58%), and 36 haplotypes contained only one isolate. Clarkson et al. (2013) observed 228 SSR haplotypes out of 384 isolates (59%) from the United Kingdom. Lehner et al. (2015) also found the same proportion of haplotypes in Brazil. The proportion of haplotypes in isolates collected from Chongqing is similar to those in Brazil and the United Kingdom, indicating that high genetic diversity exists within the *S. sclerotiorum* populations.

The nine regional populations from Chongqing were clustered into two subgroups. One cluster contained isolates from Hechuan, Tongliang, Rongchang, Yongchuan, Jiangjin, Wuxi, and Wanzhou counties. The other cluster contained isolates from Qianjiang and Wushan. This is not surprising, because Hechuan, Tongliang, Rongchang, Yongchuan, and Jiangjin counties are located in the foothills of western Chongqing and are adjacent to each other, whereas Qianjiang and Wushan counties are located in the eastern portion of Chongqing. The Mantel test also showed a significant correlation between the genetic distance and geographic distance ($R^2 = 0.161$, $P = 0.008$). Our results indicate that the populations of *S. sclerotiorum* are usually loosely structured over space (Kamvar et al. 2017; Leyronas et al. 2018; Li et al. 2009). Although Wanzhou and Wuxi counties are located in the eastern region of Chongqing, these two populations were clustered with the five western populations. One

possible reason for this is the physical geography of Chongqing. Several high mountains run from northeast to southwest and separate the Wanzhou and Wushan counties and thus might block the dispersal of ascospores of *S. sclerotiorum*.

Some studies have reported a loose association between SSR haplotypes and MCGs among *S. sclerotiorum* isolates (Clarkson et al. 2013; Sexton and Howlett 2004). However, Atallah et al. (2004) reported that the *S. sclerotiorum* isolates in Washington State belonging to the same MCG have highly variable microsatellite haplotypes. In this research, 12 of 13 MCGs that comprised more than one isolate had different haplotypes. For example, 12 isolates within the most common MCG had nine microsatellite haplotypes. Six isolates collected from Wanzhou County belonged to the same MCG, and they had five haplotypes. There is only one instance where two isolates (from Wuxi County) belonged to an MCG that had the same haplotype. On the other hand, one haplotype was often associated with more than one MCG. Our research showed that the SSR haplotypes were not correlated with MCGs for *S. sclerotiorum*, indicating an occurrence of recombination in the populations. Actually, the I_A values for three populations were not significantly different from zero when the multilocus index of association tests were applied to the nine populations within Chongqing.

Several studies have been conducted to address the aggressiveness of *S. sclerotiorum* isolates collected from defined geographic areas and indicate a lack of variation in virulence among strains (Atallah et al. 2004; Kull et al. 2004; Sexton and Howlett 2004). Some studies also reported the emergence of isolates with specific virulence (Lehner et al. 2016; Otto-Hanson et al. 2011; Pascual et al. 2010). However, the association between aggressiveness and SSR haplotypes or MCGs for *S. sclerotiorum* is lacking (Attanayake et al. 2013; Irani et al. 2011; Kull et al. 2004; Lehner et al. 2015; Otto-Hanson et al. 2011). In this study, the virulence of a subset of 34 *S. sclerotiorum* isolates were tested with rapeseed stems. These isolates belong to 25 haplotypes based on SSR data. Three isolates were highly aggressive, with average lesion lengths of >30 mm, and these isolates belonged to three haplotypes. Some isolates that belonged to the same haplotype exhibited different aggressiveness values, such as WZ-9 and WX-1. The relationship between molecular markers and the aggressiveness of *S. sclerotiorum* is still an emerging topic. One important reason is that SSR markers are neutral, and aggressiveness is a multigenic trait (Lehner et al. 2015). The study of fungal

Fig. 5. Lesion length of *Sclerotinia sclerotiorum* isolates on detached rapeseed stems. Bars indicate standard deviation. HC, Hechuan; JJ, Jiangjin; QJ, Qianjiang; RC, Rongchang; TL, Tongliang; WS, Wushan; WX, Wuxi; WZ, Wanzhou; YC, Yongchuan.

viruses may provide clues, because some *S. sclerotiorum* isolates contain viruses in hyphae, which might affect the aggressiveness of the fungi (Xie and Jiang 2014).

A detailed analysis of the population genetic structure of *S. sclerotiorum* will help develop rapeseed cultivars that are resistant to *Sclerotinia* stem rot. In this research, the *S. sclerotiorum* collected in Chongqing showed high diversity levels and exhibited variance in virulence, suggesting that breeding lines should be inoculated with several different isolates in the regions. Because *S. sclerotiorum* populations that infect rapeseed in the western counties of Chongqing might be genetically distinct from those in the eastern counties, the breed lines should be cultivated in both the western and eastern regions to effectively evaluate resistance levels.

Acknowledgments

We thank anonymous reviewers for their kind suggestions.

Literature Cited

- Agapow, P. M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. *Mol. Ecol. Notes* 1:101-102.
- Atallah, Z. K., Larget, B., Chen, X., and Johnson, D. A. 2004. High genetic diversity, phenotypic uniformity, and evidence of outcrossing in *Sclerotinia sclerotiorum* in the Columbia Basin of Washington State. *Phytopathology* 94: 737-742.
- Attanayake, R. N., Carter, P. A., Jiang, D., del Río-Mendoza, L., and Chen, W. 2013. *Sclerotinia sclerotiorum* populations infecting canola from China and the United States are genetically and phenotypically distinct. *Phytopathology* 103:750-761.
- Attanayake, R. N., Xu, L., and Chen, W. 2019. *Sclerotinia sclerotiorum* populations: clonal or recombining? *Trop. Plant Pathol.* 44:23-31.
- Bardin, S. D., and Huang, H. C. 2001. Research on biology and control of *Sclerotinia* diseases in Canada. *Can. J. Plant Pathol.* 23:88-98.
- Bolton, M. D., Thomma, B. P. H. J., and Nelson, B. D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Mol. Plant Pathol.* 7:1-16.
- Carpenter, M. A., Frampton, C., and Stewart, A. 1999. Genetic variation in New Zealand populations of the plant pathogen *Sclerotinia sclerotiorum*. *N.Z.J. Crop Hortic.* 27:13-21.
- Clarkson, J. P., Coventry, E., Kitchen, J., Carter, H. E., and Whipps, J. M. 2013. Population structure of *Sclerotinia sclerotiorum* in crop and wild hosts in the UK. *Plant Pathol.* 62:309-324.
- Derbyshire, M. C., and Denton-Giles, M. 2016. The control of sclerotinia stem rot on oilseed rape (*Brassica napus*): current practices and future opportunities. *Plant Pathol.* 65:859-877.
- Dunn, A. R., Kikkert, J. R., and Pethybridge, S. J. 2017. Genotypic characteristics in populations of *Sclerotinia sclerotiorum* from New York State, USA. *Ann. Appl. Biol.* 170:219-228.
- Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14:2611-2620.
- Excoffier, L., and Lischer, H. E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10:564-567.
- Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.
- Freeman, J., Ward, E., Calderon, C., and McCartney, A. 2002. A polymerase chain reaction (PCR) assay for the detection of inoculum of *Sclerotinia sclerotiorum*. *Eur. J. Plant Pathol.* 108:877-886.
- Gomes, E. V., Do Nascimento, L. B., De Freitas, M. A., Nasser, L. C. B., and Petrofeza, S. 2011. Microsatellite markers reveal genetic variation within *Sclerotinia sclerotiorum* populations in irrigated dry bean crops in Brazil. *J. Phytopathol.* 159:94-99.
- Hambleton, S., Walker, C., and Kohn, L. M. 2002. Clonal lineages of *Sclerotinia sclerotiorum* previously known from other crops predominate in 1999–2000 samples from Ontario and Quebec soybean. *Can. J. Plant Pathol.* 24:309-315.
- Hemmati, R., Javan-Nikkhah, M., and Linde, C. C. 2009. Population genetic structure of *Sclerotinia sclerotiorum* on canola in Iran. *Eur. J. Plant Pathol.* 125:617-628.
- Hou, M., and Huang, J. 2006. *Sclerotinia* stem rot of oilseed rape. Pages 146-147 in: *Agricultural Plant Pathology*. Science Press, Beijing.
- Irani, H., Heydari, A., and Javan-Nikkhah, M. 2011. Pathogenicity variation and mycelial compatibility groups in *Sclerotinia sclerotiorum*. *J. Plant Prot. Res.* 51:329-336.
- Kamvar, Z. N., Amaradasa, B. S., Jhala, R., McCoy, S., Steadman, J. R., and Everhart, S. E. 2017. Population structure and phenotypic variation of *Sclerotinia sclerotiorum* from dry bean (*Phaseolus vulgaris*) in the United States. *PeerJ* 5:e4152.
- Kimura, M., and Crow, J. F. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49:725-738.
- Koga, L. J., Bowen, C. R., Godoy, C. V., Oliveira, M. C. N. D., and Hartman, G. L. 2014. Mycelial compatibility and aggressiveness of *Sclerotinia sclerotiorum* isolates from Brazil and the United States. *Pesqui. Agropecu. Bras.* 49:265-272.
- Kohli, Y., and Kohn, L. M. 1998. Random association among alleles in clonal populations of *Sclerotinia sclerotiorum*. *Fungal Genet. Biol.* 23:139-149.
- Kohn, L. M. 1995. The clonal dynamic in wild and agricultural plant-pathogen populations. *Can. J. Bot.* 73 (S1):1231-1240.
- Kull, L. S., Pedersen, W. L., Palmquist, D., and Hartman, G. L. 2004. Mycelial compatibility grouping and aggressiveness of *Sclerotinia sclerotiorum*. *Plant Dis.* 88:325-332.
- Lehner, M. S., Lima, R. C., Carneiro, J. E. S., Paula Júnior, T. J., Vieira, R. F., and Mizubuti, E. S. G. 2016. Similar aggressiveness of phenotypically and genotypically distinct isolates of *Sclerotinia sclerotiorum*. *Plant Dis.* 100: 360-366.
- Lehner, M. S., Paula Júnior, T. J., Hora Júnior, B. T., Teixeira, H., Vieira, R. F., Carneiro, J. E. S., and Mizubuti, E. S. G. 2015. Low genetic variability in *Sclerotinia sclerotiorum* populations from common bean fields in Minas Gerais State, Brazil, at regional, local and micro-scales. *Plant Pathol.* 64:921-931.
- Lewontin, R. C. 1972. The apportionment of human diversity. Pages 381-398 in: *Evolutionary Biology*. T. Dobzhansky, M. K. Hecht, and W. C. Steere, eds. Springer, New York, NY.
- Leyronas, C., Bardin, M., Berthier, K., Duffaud, M., Troulet, C., Torres, M., Villeneuve, F., and Nicot, P. C. 2018. Assessing the phenotypic and genotypic diversity of *Sclerotinia sclerotiorum* in France. *Eur. J. Plant Pathol.* 152:933-944.
- Li, Z., Wang, Y., Chen, Y., Zhang, J., and Dilantha Fernando, W. G. 2009. Genetic diversity and differentiation of *Sclerotinia sclerotiorum* populations in sunflower. *Phytoparasitica* 37:77-85.
- Liu, K., and Muse, S. 2005. PowerMarker: integrated analysis environment for genetic marker analysis. *Bioinformatics* 21:2128-2129.
- Mei, J., Wei, D., Disi, J. O., Ding, Y., Liu, Y., and Qian, W. 2012. Screening resistance against *Sclerotinia sclerotiorum* in Brassica crops with use of detached stem assay under controlled environment. *Eur. J. Plant Pathol.* 134: 599-604.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* 70:3321-3323.
- Otto-Hanson, L., Steadman, J. R., Higgins, R., and Eskridge, K. M. 2011. Variation in *Sclerotinia sclerotiorum* bean isolates from multisite resistance screening locations. *Plant Dis.* 95:1370-1377.
- Pascual, A., Campa, A., Pérez-Vega, E., Giraldez, R., Miklas, P. N., and Ferreira, J. J. 2010. Screening common bean for resistance to four *Sclerotinia sclerotiorum* isolates collected in northern Spain. *Plant Dis.* 94:885-890.
- Sambrook, J., and Russell, D. W. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sexton, A. C., and Howlett, B. J. 2004. Microsatellite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian canola fields. *Curr. Genet.* 46:357-365.
- Sharma, P., Samkumar, A., Rao, M., Singh, V. V., Prasad, L., Mishra, D. C., Bhattacharya, R., and Gupta, N. C. 2018. Genetic diversity studies based on morphological variability, pathogenicity and molecular phylogeny of the *Sclerotinia sclerotiorum* population from Indian mustard (*Brassica juncea*). *Front. Microbiol.* 9:1169.
- Sirjusingh, C., and Kohn, L. M. 2001. Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum*. *Mol. Ecol. Notes* 1:267-269.
- Willbur, J. F., Ding, S., Marks, M. E., Lucas, H., Grau, C. R., Groves, C. L., Kabbage, M., and Smith, D. L. 2017. Comprehensive *Sclerotinia* stem rot screening of soybean germplasm requires multiple isolates of *Sclerotinia sclerotiorum*. *Plant Dis.* 101:344-353.
- Winton, L. M., Krohn, A. L., and Leiner, R. H. 2006. Genetic diversity of *Sclerotinia* species from Alaskan vegetable crops. *Can. J. Plant Pathol.* 28: 426-434.
- Xie, J., and Jiang, D. 2014. New insights into mycoviruses and exploration for the biological control of crop fungal diseases. *Annu. Rev. Phytopathol.* 52:45-68.
- Yeh, F. C., Yang, R., and Boyle, T. 1999. POPGENE: Microsoft Window-Based Freeware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta, Canada.