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MYCELIAL COMPATIBILITY GROUPS AND MICROSATELLITE MARKERS REVEAL GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS OF SUNFLOWER *SCLEROTINIA SCLEROTIORUM* IN CHINA

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A B S T R A C T

The genetic variability and differentiation among 101 sunflower *Sclerotinia sclerotiorum* isolates collected from four different geographic regions of China were analyzed using mycelial compatibility groupings (MCGs) and microsatellite markers. Twenty three MCGs were identified among all tested isolates. The majority of isolates collected from the same region were grouped in to the same MCGs, indicating less genetic variation of *S. sclerotiorum* within the same region. But there still have exceptions for some isolates. Also microsatellite marker data revealed that all tested isolates from four geographic populations could be divided into three distinct clusters, isolates from Inner Mongolia and Ninxia regions formed cluster I, isolates from Heilongjiang and Xinjiang formed separate clusters II and III . The percentage of variance within and among different geographic populations was 84.54% and 15.46% respectively and both variances were significantly different from each other ($p<0.01$). Meanwhile, association between the microsatellite haplotype and MCGs was observed but not so significant; majority isolates from the same MCG showed the same haplotype, but certain samples showed different haplotypes, although they belonged to the same MCG. Based on the virulence test results, we also found that MCGs not only represent the genetic variation of tested isolates, but also reflect their pathogenic ability to a certain extent.

Keywords: Sunflower *Sclerotinia sclerotiorum*, Mycelial compatibility groups (MCG), Microsatellite hyplotype, Genetic diversity.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a ubiquitous pathogen infecting more than 400 plant species including sunflower (Boland and Hall, 1994). In China, sunflower is mainly planted in the northern parts of China and Inner Mongolia is the largest planting region. Sunflower White Mold caused by *S. sclerotiorum* is the most devastating disease on sunflower in this region. A survery in 2004 showed that 85% of sunflower fields were infected by *S. sclerotiorum* and the average infected rate is around 25%-30% in this region. This disease led to the yield losses around 1125kg -1500 kg per ha per year in Inner Mongolia region (Li, 2004). Studies on the genetic diversity of *S. sclerotiorum* are pivotal for understanding the population genetic structure of this microoganism. Population studies on *S. sclerotiorum* based on mycelia compatibility group combined with fingerprints generated by multicopy repetitive elements suggested that predominantly colonial reproduction and occasional sexual recombination influence its genomics stability (Cubeta *et al.,* 1997; Hambleton *et al.,* 2002; Kohli *et al.*, 1998). Individual isolates were classified into colonial lineages by using different kind of approaches, such as mycelial compatibility groups (MCGs) and DNA fingerprints (Kohn *et al.* 1991; Carbone *et al.,* 1999; Sirjusingh *et al.*, 2001; Hambleton *et al.,* 2002; Auclair *et al.,* 2004). Mycelial compatibility grouping, a phenotypic marker system, was often associated with groups of identical or closely related microsatellite haplotypesis, a popular tool to study the genetic diversity of filamentous fungi (Sexton *et al.*, 2004). Different MCGs are controlled by multiple loci and lead to a nonself-recognition (Glass *et al.*, 2003). When the hyphae of filamentous fungi are different at one or more of these loci,

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compartmentalization and cell lysis occur, leading to a reaction line and inhibition zone appear between the tested isolates. Such isolates are classified as vegetatively incompatible isolates (Kohn *et al.,* 1990). So, mycelial compatibility can be used as an indirect way to evaluate the genetic diversity of filamentous fungi. Microsatellite marker is a powerful tool for studying the population genetic diversity due to their high mutation rates, multi-allelic nature, reproducibility and precision in detecting multiple alleles (Sirjusingh and Kohn, 2001). There have been several reports on the population structure and genetic variability of *S. sclerotiorum* based on the MCGs and microsatellite markers (Carpenter *et al.,* 1999; Hambleton *et al.,* 2002; Sexton *et al.,* 2004 and 2006; Atallah *et al.,* 2004). By using microsatellite markers, Sexton has studied the genetic differentiation among populations of *S. sclerotiorum* collected from canola fields in Australia and found moderate to high levels of genetic differentiation between two different geographic populations (Sexton *et al.*, 2004). Sun *et al.* (2005) compared three *S. sclerotiorum* populations collected from different host plants and countries such as Europe, China and Canada by using RAPD markers, and found significant genetic differentiation among and within different populations. The genetic variability of 40 *S. sclerotiorum* isolates widely distributed in various fields and different host crops of Brazil was analyzed by using RAPD markers and mycelial compatibility groupings (MCGs) (Júnior *et al.,* 2011)**.** The haplotypes obtained provided very characteristic groupings in accordance with MCGs, but did not show any relationship with geographic origin or host type.

Regarding sexual recombination*,* the *S. sclerotiorum* populations from oilseed rape in Canakkale province of Turkey showed a high rate of out-crossing events (Mert-Türk *et al.,* 2007). Li *et al.* (2009) also revealed that there were significant differences in radial growth, aggressiveness and production ability of oxalic acid among different MCGs of sunflower *S. sclerotiorum,* regardless of their geographic origins (Li *et al.*, 2009). Also, population studies on *S. sclerotiorum* demonstrated a strong association between MCGs and microsatellite markers (Carpenter *et al.,* 1999; Hambleton *et al.,* 2002; Sexton *et al.,* 2004 and 2006), although there are reports declaimed that there is no correlation between MCGs and microsatellite markers among *S. sclerotiorum* isolates collected from potato fields (Atallah *et al.,* 2004).

In this study, we used microsatellite markers developed by Sirjusingh and Kohn (2001) combined with mycelial compatibility group (MCG) to analyze the genetic diversity and population structures of 101 *S. sclerotiorum* isolates collected from different sunflower planting regions of China. Meanwhile, the correlation among MCGs, microsatellite haplotype and virulence of sunflower *S. sclerotiorum* isolates were also determined. Our results will provide the basic information of genetic structures and variation of *S. sclerotiorum* isolates from different sunflower planting regions in China.

MATERIALS AND METHODS

Sampling and Culturing of *S. sclerotiorum* isolates: A total 101 *S. sclerotiorum* isolates were collected in 2009 and 2010 (Table 1) from four populations based on their geographic origin. The samples were distributed as follows in Fig.1. All isolates were obtained from sclerotia collected from single infected plant.

For isolation, sclerotia were surface-sterilized using 10% commercial bleach (0.5% NaClO) for 3 min, washed 3 times in sterile water, then placed on potato dextrose agar (PDA) and incubated for 72 h at room temperature (20～24°C). The mycelial tips of each isolate were cut and re-cultured 3 times to obtain pure cultures for MCG study and DNA extraction.

Mycelial Compatibility Groups: Mycelial compatibility grouping was determined by using method described by Li *et al.* (2009) and Kohn *et al.* (1990). Three isolates were placed triangularly on a PDA plate amended with 50 mg/L bromophenol blue (BPB), which was added to enhance the visibility of the incompatible reaction lines. Each isolate was paired with itself as a control for compatibility. Pairing plates were incubated in dark for one week at room temperature. Isolates were listed sequentially according to collection locations, each isolate compared with the two other isolates and then any compatible pairs were used as a basis for expanding the compatibility group. Isolates classified into one MCG group were tested for compatibility with a minimum of two other isolates within the same group. Finally, multiple isolates from each group were tested against isolates from all mycelia pairings and scored as incompatible group based on a clear yellow barrier line or a yellow to green line between colonies of paired isolates were observed. Compatibility occurred when no reaction line appeared and two colonies grew together. Each assay was repeated three times.

Figure 1. Map showing four geographic regions where *sclerotinia sclrotiorum* isolates was collected

Table 1. The Mycelia Compatibility Groups and Geographic Region information of all tested Sunflower *S. sclerotorium* isolates in this study

MCGs	Isolates	Geographic Region
MCG1	1, 3, 6, 8, 13, 18, 41, 43, 53, 57	IM
MCG2	2, 45, 50	IM
MCG3	7	IM
MCG4	4, 21, 22, 47, 48, 55	IM
MCG5	16, 17, 20, 23, 24, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 46, 49, 51, 52, 54	IM
MCG6	19, 25, 44	IM
MCG7	9, 10, 11	IM
MCG8	26	IM
MCG9	5, 12, 14, 15, 56, 58, 59, 62, (89, 95, 96)[a]	IM, (Heilongjiang)
MCG10	60, 61	IM
MCG11	63	IM
MCG12	64, 65,(66)[b]	IM, (Ningxia)
MCG13	67, 69, 70, 72, 73, 75, 76	Ningxia
MCG14	68, 71, 74	Ningxia
MCG15	94, 97	Heilongjiang
MCG16	85	Heilongjiang
MCG17	78	Xinjiang
MCG18	80, 82, 86	Xinjiang
MCG19	79, 81, 83	Xinjiang
MCG20	84	Xinjiang
MCG21	87, 91, 92, 93	Heilongjiang
MCG22	77, 88, 90, 98, 99, 101	Heilongjiang
MCG23	100	Heilongjiang

a: Isolates 89, 95, 96 collected from Heilongjiang; b: Isolate 66 collected from Ningxia. IM: Inner Mongolia

DNA extraction and Microsatellite amplification: Total DNA from each isolate of *S. sclerotiorum* was extracted using CTAB method as described by Simpson *et al.* (2004) with modifications. All tested strains were cultured for 2 days in potato dextrose broth (PDB) medium; then mycelia were filtered and rinsed with sterile water followed by grounding quickly in liquid nitrogen. 600µL TES buffer was added to mix with mycelial powder, followed by addition of 140µL 5M NaCI and 70µL pre-warmed 10% CTAB. Suspensions were vortexed and incubated at 65°C for 30 min. and 600 µl chloroform: isoamyl alcohol (24:1) was then added to remove proteins. After centrifugation, the aqueous layer was transferred to a clean tube and 80µL 5M NaCl and 1,000 µL 100% cold ethanol were added to precipitate DNA. After centrifugation for 5 min at 13,000 rpm, the supernatant was decanted and the pellet was washed twice with 200 µL 80% ice cold ethanol, dried and resuspended in 100µL warmed (65°C) sterile distilled water. All DNA were quantified using a spectrophotometer and diluted to 5 ng μL^{-1} for further PCR amplification.

Eight microsatellite loci selected from those developed by Sirjusingh and Kohn (2001) gave the most consistent amplification of polymorphic products among tested samples (Table 2). Each PCR reaction was carried out in a Thermal Cycler PTC100 (MJ Research Inc., Waltham, MA, USA) and the procedure as follows: 8 min at 95 °C followed by 35 cycles of 1 min at 95°C, 45 s at 55 °C, and 45s at 72 °C, with a final extension at 72 °C for 5 min. The PCR products were separated by vertical electrophoresis on 6% polyacrylamide gel in 1x TBE buffer (Tris-Borate-EDTA: 0.89 mol/L Tris, 0.02 mol/L EDTA-$Na_2.H_2O$, 0.89 mol/L Boric acid), then stained with 20% Silver Nitrate, and visualized using a digital gel documentation system (Scion Image, Frederick, MD, USA). A DNA ladder marker (GeneRulerTM, Hanover, MD, USA) was used to determine the PCR fragment size.

Analysis of microsatellite data: Amplified fragments were scored with binary characters (1 represents the present band, 0 represent the absent band). The bands amplified in each reaction were scored and used for analysis. The number and size of bands for multilocus enotypes (unique arrays of amplified bands) were calculated for each geographical population. The average number of alleles *na*, the number of effective alleles *ne*, Nei's gene diversity *h*, Shannon's index of diversity *I* (Lewontin, 1972), the coefficient for gene divergence (*Gst*), and gene flow (*Nm*) were calculated using POPGENE 1.32 software (Yeh *et al.,* 1997). Dendrograms were generated from the similarity coefficient matrix based on the unweighed pair group mean analysis (UPGMA) method (Nei *et al.,* 1979) using the NTSYS 2.1 software package. Bootstrap sampling (1,000 replicates) was performed for statistical support of branches of the constructed phenogram (Felsenstein *et al,*, 1985). The genetic differentiation within and among populations was estimated by analysis of molecular variance (AMOVA) using the WINAMOVA 1.04 program (Excoffier *et al.,* 1992). The AMOVA analyses were based on pairwise squared euclidian distances among molecular phenotypes.

Virulence testing of different MCGs: The virulence tests of 43 isolates from 6 MCGs (the selected MCG should contain more than three samples) were performed with detached leaves of sunflower cultivar LD5009. The leaves were collected at three to four leaf stages and were placed on a filter paper soaked with sterile water in a sterile plate (30 cm diam.). The agar plug (3 mm diam.) of mycelium was cut from the edge of minimal medium after culturing 3 days and was placed upside down onto the leaf surface (Godoy *et al.,* 1990). The inoculated leaves were incubated at room temperature (RT) and lesion diameters were cross measured 48 hours post inoculation (hpi).

The virulence test of each isolate was performed on five detached leaves. The average size of the lesion diameters on five detached leaves represented the virulence of each isolate. Statistically significant differences of lesion sizes were analyzed using Student's T-test and a two-tailed *P*-value of less than 0.05. The virulence test was repeated three times for each isolate.

RESULTS

Determination MCGs of *S. sclerotorium* isolates: Mycelial pairings of all the isolates on PDA medium produced either an incompatible reaction in which a reaction line between the two isolates was observed in the interaction zone, or a compatible reaction in which no reaction line developed (Fig. 2). The 101 tested isolates, all self-compatible, were classified into 23 different MCGs (Table 1). Six MCGs each contained only one isolate such as MCG3, MCG8, MCG11 *ect.* MCG5, the largest MCG, included 25 isolates, 22 samples from the western part of Inner Mongolia and 3 from the eastern part. Majority MCGs contained isolates collected from the same or close sunflower planting regions such as

MCG7, MCG10, MCG13, MCG14, MCG15, MCG16 *ect.* However, some isolates from rather further sunflower planting regions were also grouped together, such as MCG1 which contained 10 isolates, 5 of them from eastern parts of Inner Mongolia, 4 isolates from western parts and one from the center region. The distance from east to west Inner Mongolia is around 1500 km. MCG12, which contained 3 isolates, one from Ninxia region and the others from eastern part of Inner Mongolia, is around 2000 km apart from Ninxia region. MCG9 contained isolate 62 which was collected from the west part of Inner Mongolia region, however, the other isolates in this group were collected from the east part of Inner Mongolia and also its neighbor region Heilongjiang province. Interestingly, some isolates collected from the same or rather closed regions, but were classified into two different MCGs (MCG18 and MCG19).

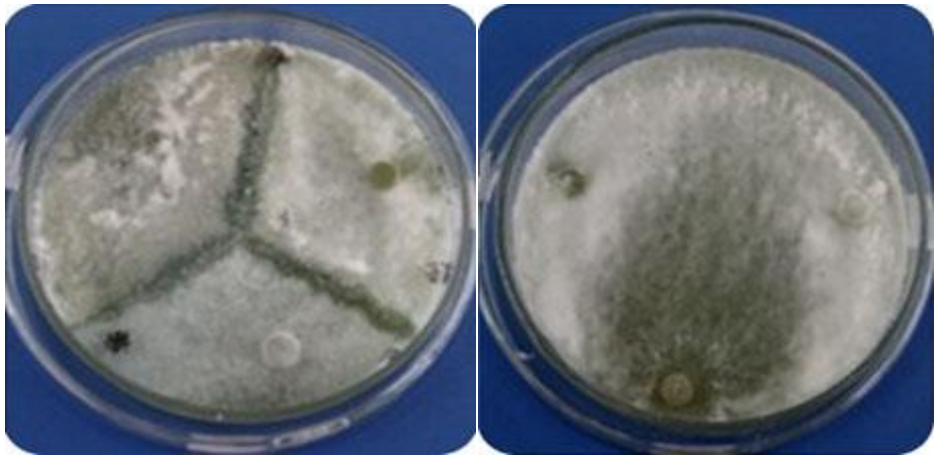
Table 2. List of eight microsatellite loci, their primer sequences, and number of alleles detected in this study

Locus	Primer sequence (5'-3')	Size of range (bp)	Repeat motif
5-2	GTAACACCGAAATGACGGC GTAACACCGAAATGACGGC	318-325	(GT)8
7-2	TTTGCGTATTATGGTGGGC ATGGCGCAACTCTCAATAGG	160-172	(GA)14
9-2	GCCGATATGGACAATGTACACC TCTTCGCAGCTCGACAAGG	358-382	(CA)9(CT)9
12-2	CGATAATTTCCCCTCACTTGC GGAAGTCCTGATATCGTTGAGG	215-225	(CA)9
13-2	TCTACCCAAGCTTCAGTATTCC GAACTGGTTAATTGTCTCGG	284-304	(GTGGT)6
8-3	CACTCGCTTCTCCATCTCC GCTTGATTAGTTGGTTGGCA	251-271	CA12
36-4	GAATCTCTGTCCCACCATCG AGCCCATGTTTGGTTGTACG	415-429	CA6(CGCA)2CAT2
92-4	TCGCCTCAGAAGAATGTGC AGCGGGTTACAAGGAGATGG	374-378	(CT)12

All the isolates collected from Xinjiang province, were grouped into two different MCGs, especially isolates 85 and 86, which were collected form the different sunflower fields of the same town, but still belong to two different MCGs. The same case was for the isolates in MCG21 and MCG22. For 65 isolates collected from Inner mongolia were grouped into 12 MCGs and 15 isolates from Heilongjiang region were classified into 6 different MCGs. We conclude that the genetic variation of tested isolates is rather large and the geographic factor is not the determinate for MCG classification.

Analysis of genetic diversity of *S. sclerotorium* using microsatellite markers: The genetic diversity of each geographical population was evaluated by using Nei's gene diversity and Shannon's index of diversity (Table 3). The percentage of polymorphic bands (*PPB*), the average number of alleles (*na*), and the number of effective alleles (*ne*) of four geographical populations were found different. The population diversity of the isolates collecting from Inner Mongolia region showed the highest value (*PPB* = 100.0, *h* = 0.4582, *I* = 0.6501), however, the diversity of Xinjiang population was the lowest (*PPB* = 85.0; *h* = 0.3403; *I* = 0.4979). The order of genetic diversity from highest to lowest of all tested geographical populations was as follows: Inner Mongolia, Heilongjiang, Ningxia and Xinjiang.

The Euclidean distance matrix was used to analysis the genetic variation of different geographical populations. Our results suggested that 85.5% of the total variation is attributed by the differences within the populations, whereas 15.5% is due to the variation among populations. Both variations were highly significant ($p<0.001$) (Table 4). The coefficient of gene differentiation (*Gst*) and gene flow (*Nm*) were also calculated through POPGENE software. Gene differentiation (*Gst*) among different populations was 0.106, much lower than within the tested population (0.443).



A. Incompatible reaction B. Compatible reaction

Figure 2. The mycelia compatibility group (MCG) test of *S. sclerotiorum.*

Table 3. Molecular variance analysis of four *S. Sclerotiorum* geographic populations

Population	n	g	PPB	Na	Ne	h	I
Inner Mongolia	65	40	100.0	2.0000	1.8549	0.4582	0.6501
Ningxia	12	38	95.0	1.9500	1.6757	0.3784	0.5515
Xinjiang	9	34	85.0	1.8500	1.5974	0.3403	0.4979
Heilongjiang	15	39	97.5	1.9750	1.7170	0.4022	0.5841
Average	25.25	37.75	94.38	1.9437	1.7112	0.3948	0.5709
SD	-	-	-	0.1090	0.1243	0.0383	0.0400

N: population size; g: number of genotypes in populations; PPB: percentage of polymorphic bands; Na: average number of alleles; ne: effective number of alleles; h: Nei' s gene diversity ; I: Shannon's Information index, SD: standard deviation.

However, the tendency of gene flow (*Nm*) was opposite to the gene differentiation (*Gst*). The *Nm* value among different populations was 2.547, but within population it was only 0.229. The gene flow (*Nm*) within all tested samples was 1.57. Similarly, the coefficient of gene differentiation (*Gst*) of all tested isolates was 0.2349, which was calculated by population genetic analysis (Table 4), indicating that the gene permutation and interaction do exist within tested isolates. Genetic diversity based on Nei's genetic distance was also analyzed within four geographical populations. The results suggested that Nei's genetic distances of all tested populations is rather low, ranging from 0.0453 to 0.1292; however, the genetic identity is quite high, ranging from 0.8871 to 0.9557. The Inner Mongolia and Ningxia population show the closest genetic distance (0.0453), although Heilongjiang and Ninxia populations had the farthest distance (0.1292). The genetic identity showed the opposite tendency to genetic distance among the tested populations. Based on the matrix of Nei's genetic distances among four geographic populations, the UPGMA cluster analysis was performed and the results suggested that four tested populations could be divided into three distinct clusters (Fig. 3) The first cluster contains populations from Inner Mongolia and Ningxia, indicating that the genetic distance of these two populations is rather close. Xinjiang and Heilongjiang population were grouped as separate clusters. Regarding the relative genetic distance to Inner Mongolia population, the Xijiang population showed closer genetic distance compared with populations from Heijingjiang, although the geographic distance from Inner Mongolia to Xinjing is much further than the distance between Inner Mongolia and Heilongjiang, indicating geographic distance is not the key determinant for the genetic diversity of tested isolates.

The association between MCGs and microsatellite hyplotype: A total 43 *S. sclerotiorum* isolates, which belong to the 6 MCGs and each MCG contained more than three samples, were selected for cluster analyses using NTSYS software (Fig. 4). These isolates were classified into four different clusters when the similarity coefficient value was set on 0.551. Isolates of cluster I exhibited approximately 88% similarity with isolates

from MCG5 and only one isolate from MCG22 in this cluster. Cluster II contains three sub-clusters and the tested isolates predominantly were classified into MCG 5 (8 isolates), MCG22 (5 isolates) and MCG13 (2 isolates). Similarly, cluster III also mainly consists of the isolates from MCG15 (2 isolates) and MCG18 (3 isolates), also 3 single isolate from MCG 5, MCG9 and MCG13 respectively were also included in this cluster. Cluster Ⅳ contained 3 isolates from MCG14, 2 isolates from MCG5. Comparing microsatellite haplotype and MCGs, the association between MCGs and microsatellite haplotypes was observed such as 88% isolates from MCG5 were associated with cluster I, and there still have some isolates from MCG5 associated with three different clusters separately, indicting isolates from one MCG were associated with 4 different haplotypes. Meanwhile, one haplotype associated with more than two MCGs such as MCG5, MCG13 and MCG22 was associated with cluster II, and MCG5, MCG 9, MCG 13, MCG 15 and MCG 18 was associated with cluster III.

Table 4. Molecular variance analysis within and among *S. sclerotiorum* populations (ANOVA)[a]

Source of Variation	DF	Variance Components	Percentage of Variation	P	GST	Nm
Among populations	9.00	3.297	15.46	< 0.001	0.106	2.541
Within populations	87.00	18.493	84.54	< 0.001	0.443	0.627
Total	96.00	21.79			0.2349	1.57

A: significance tests were performed using 1000 permutations. DF: degree of freedom. Gst: coefficient of gene differentiation; Nm: gene flow

Table 5. Genetic identity and genetic distance among four geographic populations of *S. sclerotiorum*

Population	Inner Mongolia	Ningxia	Xinjiang	Heilongjiang
Inner Mongolia	****	0.9557	0.9363	0.9445
Ningxia	0.0453	****	0.8871	0.8788
Xinjiang	0.0659	0.1198	****	0.8957
Heilongjiang	0.0571	0.1292	0.1101	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

The virulence test of different MCGs of sunflower *S. scleortorium:* The results of virulence test of the 43 isolates, which belong to 6 different MCGs and 4 microsatellite clusters (coefficient value was set on 0.551), are presented in Fig. 5. Isolate 86, which is a member of MCG19 and collected from Xinjiang, caused the largest necrotic lesions (4.57cm) on detached leaves 48 hpi, and isolate 16 from Inner Mongolia caused the smallest lesion (0.7cm)($P<0.05$). Based on the lesion size on the detached leaves, we deduced different virulence among the different MCGs. For instance isolates in MCG18 and MCG 22 had relatively higher virulence ability compared with the isolates from MCG5. There were no significant variation in virulence within the same MCG ($p< 0.05$), exempting the isolates 16 and 37 from MCG5 and isolate 77 from MCG 22, indicating the possible existence of genetic mutation on certain gene which encoded pathogenic factor in tested isolates.

DISCUSSION

S. sclerotiorum isolates collected from four different sunflower planting geographic regions in China were grouped into 23 different MCGs. Our results revealed a high level of genetic variability among these isolates, suggesting in addition to colonel reproduction, there exist sexual recombination. The majority of isolates collected from the same regions could be grouped together, such as MCG6 in which the tested isolates were collected from the Bayannaoer region (Table1); MCG13 mainly contains isolates collected from Ningxia; also majority isolates from Xinjiang region was grouped into MCG19. However, certain isolates, collected from the same geographic region such as from Inner Mongolia (65 isolates) , were grouped into 12 different MCGs. Fifteen isolates from Heilongjiang region were classified into 6 different MCGs, indicting the existence of recombination within the different geographic populations. This result is in agreement with Meter-Turk's reports that there is a high rate of outcrossing as well as evolutionary potential with the population of the *S. sclerotorium* in oilseed rape field in Turkey (Mert-Türk *et al.,* 2007). In our study, some isolates collected from the same or rather close regions, but were grouped into two different MCGs, suggesting the existence of a heterogeneous mix of MCGs in a given location.

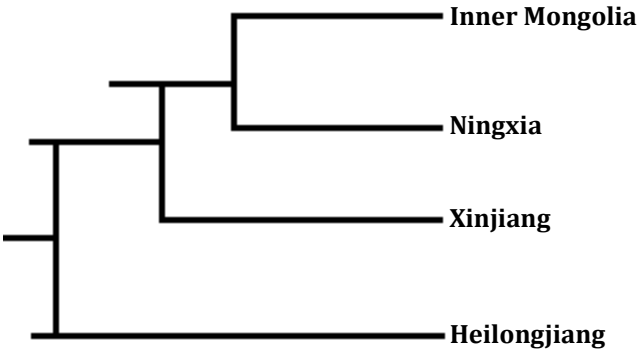


Figure 3. The UPGMA polygenetics map of different *S. sclerotiorum* populations based on Nei's coefficients and group average hierarchical clusters, produced by the NTSYS 2.1 program.

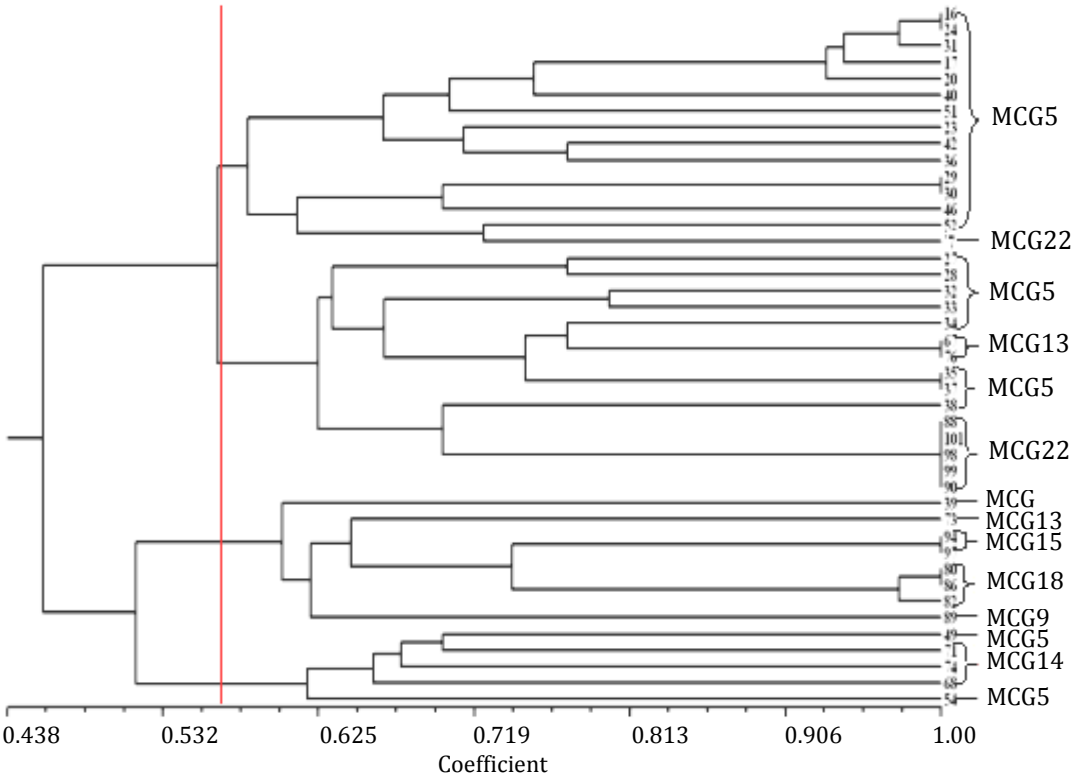


Figure 4. Unweighted pair group mean analysis dendrogram of genetic distance among 43 isolates which belong to 6 different MCGs based on Nei's coefficients.

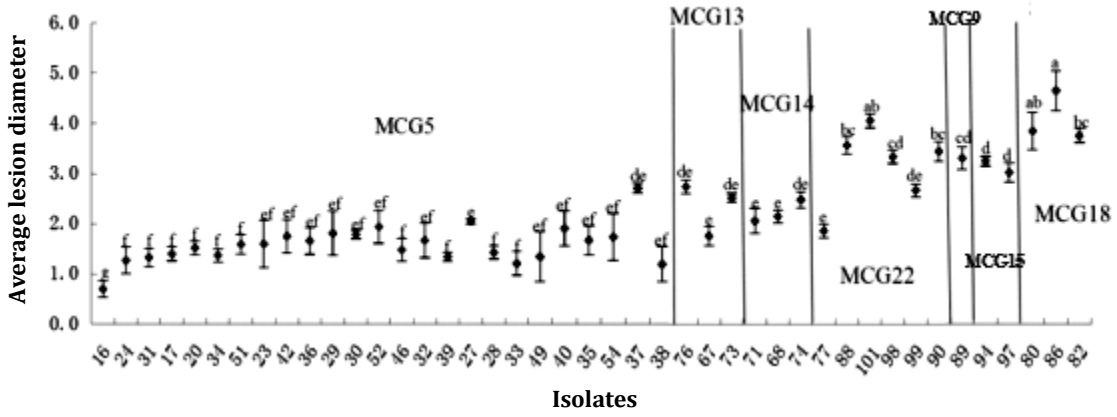


Figure 5. Virulence test results of 43 S. sclerotiorum isolates on detached sunflower leaves.

The values are the means of three repeats. Error lines present standard deviation of samples. Bars with the same letters are not significantly different. The genetic variation of different geographical populations was analyzed through Euclidean distance matrix in our study. The results suggested that 85.5% of the total variation is attributed by the differences within populations, whereas 15.5% is due to the variation among populations. This is in agreement with the findings of Mert-Türk F. *et al*. (2007) who verified that high genetic diversity existed within 32 *S. sclerotiorum* isolates from oilseed rape fields in Turkey Qanakkale provinces and the level of gene polymorphism could up to 63% (Mert-Türk *et al.,* 2007). Meanwhile, Atallah and his colleague also found that 92% of the variability within subpopulations of 167 *S. sclerotiorum* isolates collected from potato fields (Atallah *et al.,* 2004). In Australia, Sexton *et al.* (2004) also found 19% genetic difference of *S. sclerotiorum* from canola fields could be accounted for by variation among populations, while 79.4% genetic difference was contributed by the variation within populations.

We calculated the coefficient of gene differentiation (*Gst*) and gene flow (*Nm*) for different *S. sclerotiorum* geographic populations by POPGENE software, The gene differentiation (*Gst*) among populations was 0.106, but the gene flow (*Nm*) was 2.547, indicating that the gene transfer of *S. Sclerotiorum* among different populations is much more often than within populations. Similarly, the coefficient of gene differentiation (*Gst*) in the tested populations calculated by population genetic analysis was 0.2349, which indicates that genetic variation among tested populations is 23.49%. The gene flow (*Nm*) was 1.57 among all tested samples, which indicates that the gene permutation and interaction among populations is relatively high. This is consistent with findings from *S. sclerotiorum* populations in North America and Australia (Atllah *et al.,* 2004; Cubeta *et al.*, 1997; Kohli *et al.*, 1998; Sexton *et al*., 2006).

We analyzed the association between microsatellite haplotypes and MCG groups and observed it is indeed the case. Such matches have been reported by Carpenter and his colleague that a close association was observed between MCGs and haplotype (Carpenter *et al.,* 1999; Hambleto *et al.,* 2002; Sexton *et al.,* 2004). However, in our study, one MCG was associated with more than one haplotype and one haplotype with two more MCGs also were observed also. This is in consistent with report on genetic differentiation among populations of *S. sclerotiorum* from Australian canola field (Sexton *et al.,* 2004).

Regarding the virulence results, different MCGs represents a certain extent the different average levels of virulence of the isolates within the same MCGs. Although the virulence was variable among all the tested isolates within the same MCG, it still keeps in the level which is obviously different from the level of other MCGs. Also, the same MCGs, such as MCG5, were associated with more than one haplotype (Cluster I, II and III), but the virulence levels were still similar, indicating that virulence maybe controlled by multiple loci instead of by single locus. We did not test the variation of the pathogenicity factors such as OA secretion level and PG enzymes activities among different MCGs, although such information could support our findings.

In conclusion, this study is the first report on the genetic variation within and among the different geographic populations of *S. sclerotiorum* isolates collected from different sunflower planting regions in China. The association between mycelial compatibility populations and the probable occurrence of sexual recombination indicates the potential for the emergence of different genotypes in each area and highlights the need to maintain agricultural practices that restrict gene flow between different geographic populations around the country. MCGs not only represent the genetic diversity, but also predict the virulence variation of the tested isolates. Further investigations on the correlation of virulence of different MCGs and the pathogenicity factors are required. Moreover, the evolution mechanisms of *S. sclerotiorum* under different environmental stress also need to be clarified in the future by using a larger number of isolates from different sunflower planting regions.

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REFERENCES

Atallah, Z.K., B. Larget, X. Chen and D.A. Johnson. 2004. High genetic diversity, phenotypic uniformity, and

evidence of outcrossing in *Sclerotinia sclerotiorum* in the Columbia Basin of Washington State. *Phytopathology*, *94*, 737–742.

Auclair, J., G.J. Boland, L.M. Kohn and I. Rajcan. 2004. Genetic interactions between *Glycine max* and *Sclerotinia sclerotiorum* using a straw inoculation method. *Plant Disease*, *88*, 891–895.

Boland, G.J. and R. Hall. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. C*anadian Journal of Plant Pathology*, *16*, 93–108.

Carbone, I., J.B. Anderson and L.M. Kohn. 1999. Patterns of descent in clonal lineages and their multilocus fingerprints are resolved with combined gene genealogies. *Evolution*, *53*, 11–21.

Carpenter, M.A., C. Frampton and A. Stewart. 1999. Genetic variation in New Zealand populations of the plant pathogen *Sclerotinia sclerotiorum*. *New Zeal J Crop Hort*, *27*, 13–21.

Cubeta, M.A., B.R. Cody, Y. Kohli and L.M. Kohn. 1997. Clonality in *Sclerotinia sclerotiorum* on infected cabbage in eastern North Carolina. *Phytopathology*, *87*, 1000–1004.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479-491.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. *Evolution*, 39, 783-791.

Glass, N.L. and I. Kaneko. 2003. Fatal attraction: non-self recognition and heterokaryon incompatibility in filamentous fungi. *Eukaryot Cell*, *2*, 1–8.

Godoy, G., J.R. Steadman, M.B. Dicrman and R. Dam. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. *Physiological and Molecular Plant Pathology*, *37*, 179-191.

Hambleton, S., C. Walker and L.M. Kohn. 2002. Clonal lineages of *Sclerotinia sclerotiorum* previously known from other crops predominate in 1999–2000 samples from Ontario and Quebec soybean. *Canada Journal Plant Pathology*, *24***,** 309–315.

Júnior, C.G.L., E.V. Gomes, M.L. Júnior, L.C.B. Nasser, and S. Petrofeza. 2011. Genetic diversity and mycelial compatibility groups of the plant-pathogenic fungus *Sclerotinia sclerotiorum* in Brazil. *Genetics and Molecular Research*, *10*, 868-877.

Kohli, Y. and L.M. Kohn. 1998. Random association among alleles in clonal populations of *Sclerotinia sclerotiorum*. *Fungal Genet. Biol.*, *23*, 139-149.

Kohli, Y., L.J. Brunner, H. Yoel, M.G. Milgroom, J.B. Anderson, R.A.A. Morrall and L.M. Kohn. 1995. Clonal dispersal and spatial mixing in populations of the plant pathogenic fungus, *Sclerotinia sclerotiorum*. *Moil. Ecol.*, *4*, 69–77.

Kohn, L.M., E. Stasovski, I. Carbone, J. Royer and J.B. Anderson. 1991. Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. *Phytopathol. 81*, 480–485.

Kohn, L.M., I. Carbone and J.B. Anderson. 1990. Mycelial interactions in *Sclerotinia sclerotiorum*. *Exp. Mycol.*, *14*, 255–267.

Kull, L.S., W.L. Pederson, D. Palmquist and G.L. Hartman. 2004. Mycelial compatibility groupings and aggressiveness of *S. sclerotiorum*. *Plant Disease*. *88*, 325-332.

Lewontin, R.C. 1972. The apportionment of human diversity. *Evol. Biol. 6*, 381–398.

Li, Z.Q. 2004. Sunflower diseases and control in Inner Mongolia. *Inner Mongolia Agricultural Science and Technology*. *6*, 63–64 (Chinese, with English abstract).

Li, Z.Q. Y.C. Wang, Y. Chen, J.X. Zhang, and W. G.D. Fernando. 2009. Genetic diversity and differentiation of *Sclerotinia sclerotiorum* populations in sunflower. *Phytoparasitica*. *37*, 77–85.

Mert-Türk, F., M. Ipekm, D. Mermer and P. Nicholson. 2007. Microsatellite and morphological markers reveal genetic variation within a population of *Sclerotinia sclerotiorum* from oilseed rape in the Canakkale Province of Turkey. *Journal of Phytopathology*, *155*, 182–187.

Nei, M. and W.H. Li. 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.*, *76*, 5268–5273.

Sexton, A.C. and B.J. Howlett. 2004. Microsatellite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian canola fields. *Curr. Genet*. *46*, 357–365.

Sexton, A.C., A.R. Whitten and B.J. Howlett. 2006. Population structure of *Sclerotinia sclerotiorum* in

an Australian canola field at flowering and stem-infection stages of the disease cycle. *Genome, 49*, 1408-1415.

Sirjusingh, C. and L.M. Kohn. 2001. Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum*. *Mol. Ecol. Notes*, *1*, 267–269.

Sun, J.M., I. Witold, J. Malgorzata and F.X. Han. 2005. Analysis of the genetic structure of *Sclerotinia sclerotiorum* (Lib.) de Bary populations from different regions and host plants by random amplified polymorphic DNA markers. *Journal of Integrative Plant Biology*, *47*, 385–395.

Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye, and J.X. Mao. 1997. POPGENE V 1.31 (on-line). http://www.ualberta.ca/~fyeh.