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Population genetic structure of *Sclerotinia sclerotiorum* on canola in Iran

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Abstract The genetic structure of 276 *Sclerotinia sclerotiorum* isolates representing 37 field populations from four provinces in northern Iran were analysed with six polymorphic microsatellite loci. In total, 80 haplotypes were detected with 19 haplotypes (23.7%) shared amongst at least two regional populations. None of the haplotypes were shared among all four regional populations. Of the 80 haplotypes, 32 haplotypes (40%) occurred in low frequencies represented by only one isolate. Moderate levels of gene diversity (H=0.51 to 0.61) and genotypic diversity ($\hat{G}$=12.0 to 22.0; clonal fraction = 0.39 to 0.67) for regional populations were observed. Genotypic diversities ($\hat{G}$) did not differ significantly among populations. All regional populations were in linkage equilibrium indicating the occurrence of outcrossing. Low to moderate levels of population subdivision (0.03 to 0.07), were observed among regional populations. Only one large panmictic population was inferred by STRUCTURE, indicating no significant population structure. A Mantel test showed no significant isolation by distance (r=−0.43; P=0.18), indicating anthropogenic movement of inoculum. The results demonstrated that *S. sclerotiorum* populations in northern Iran, are randomly mating and have a number of shared haplotypes among regional populations; this possibly represents recent founder populations and/or a high occurrence of anthropogenic migration of infected plant material among populations.

Keywords Evolutionary potential · Linkage disequilibrium · Microsatellite loci · Population structure

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Introduction

In Iran, canola (*Brassica napus* var. *oleifera*) is the most commonly cultivated oilseed crop. It has been cultivated since 1996 (Fernando et al. 2007) and there is a growing interest in canola production. Sclerotinia stem rot is the most destructive and harmful disease of canola, especially in favourable climatic conditions such as those found on the northern flats of the Caspian Sea (Pakdaman and Mohammadi Goltapeh 2007). Incidence of this disease in canola fields of Iran has ranged from 3% to 50% (Afshari-Azad 2001).

Sclerotinia sclerotiorum is a homothallic ascomycete fungus with a wide host range and geographical distribution. It is one of the most omnivorous plant pathogens (Purdy 1979). The pathogen has been recorded on 408 host species belonging to 278 genera and 75 families; most of them are herbaceous plants from the subclass Dicotyledonae of the Angiospermae but several hosts occur in the subclass Monocotyledonae (Boland and Hall 1994).

Sclerotia of *S. sclerotiorum* can survive for many years in soil (Bourdôt et al. 2001). Soilborne sclerotia produce apothecia at the time when canola flowers, to produce airborne ascospores as primary inocula, which infect petals. Resistance sources to *S. sclerotiorum* in several crops has been identified (Lefol et al. 1997; Hoffman et al. 2002; Zhao and Meng 2003; Zhao et al. 2006) and many studies continue to investigate resistance of canola cultivars as well as other crops against *S. sclerotiorum* (Bradley et al. 2006; Chen and Wang 2005; Rönicke et al. 2005; Kull et al. 2004; Sexton and Howlett 2004; Zhao et al. 2004; Yanar and Miller 2003; Wegulo et al. 1998). In Iran, the evaluation of tolerance in different lines and cultivars of rapeseed to sclerotinia stem rot has shown different levels of tolerance to the disease (Dalili et al. 2002).

In plant-pathogen interactions, development of new pathogenic races and the breaking down of resistance are limiting factors in resistance deployment against plant diseases. The pathogen's life-history characteristics and evolutionary potential are major factors leading to the pathogen overcoming host resistance (García-Arenal and McDonald 2003; Coletta-Filho and Machado 2002; McDonald and Linde 2002). Therefore knowledge of the population genetic structure and evolutionary potential of the pathogen will provide insight into the most suitable breeding strategy for durable resistance (McDonald and Linde 2002). The pathogens with higher evolutionary potential pose a greater risk of defeating resistance genes or counteracting other control methods such as applications of fungicides (McDonald and Linde 2002). Two important factors for pathogen evolution are the reproductive/mating system and gene/genotype flow. The reproduction system will affect the distribution of alleles in a population. Sexual reproduction in out-crossing organisms will result in recombined genotypes, whereas in-breeding and clonal reproduction will fix the alleles within a clone. Out-crossing and recombination combine alleles from different sources into the same genetic background, resulting in different genotypes that may have a higher fitness (McDonald and Linde 2002). Furthermore, high levels of gene/genotype flow may facilitate the exchanging of genetic resources including isolates with novel virulence across a large geographical area (McDonald and Linde 2002).

There are several published reports on the population genetic structure of *S. sclerotiorum* using different molecular markers (Malvárez et al. 2007; Mert-Türk et al. 2007; Sexton et al. 2006; Sun et al. 2005; Atallah et al. 2004; Kull et al. 2004; Sexton and Howlett 2004; Hambleton et al. 2002; Carbone and Kohn 2001; Carbone et al. 1999; Kohli and Kohn 1998; Cubeta et al. 1997; Kohli et al. 1992). All the markers employed were convergent in identifying the same biological units (Kohn et al. 2008). Also, all studies indicated that populations were genetically diverse and had a predominantly clonal component with occasional recombination within populations, as well as contemporary population diversification at a local scale. What is still lacking is a global scale phylogeography study with multilocus sequence analyses to assess worldwide movement and diversification of genotypes.

The objective of this research was to investigate the genetic structure of *S. sclerotiorum* populations from canola in four provinces of Iran, and assess possible host association in broad bean and tobacco isolates with microsatellite markers. Specifically, we investigated the evolutionary potential of pathogen populations by determining their genetic diversity and reproduction system of regional populations. We aimed to determine whether observed patterns of genetic diversity are consistent with strict clonality or random mating. In order to answer this question, we determined the genotypic diversity of populations and the allelic associations among studied loci by estimating linkage disequilibrium. Furthermore, the presence of population substructure was investigated by estimating population differentiation, the level of admixture and inference on the number of clusters present in the population. The results of this study will allow us to infer the evolutionary potential of *S. sclerotiorum* populations in northern Iran.

Materials and methods

Sclerotinia populations

In total, 252 *S. sclerotiorum* isolates were collected from infected canola stems from four regions in northern Iran that represented the most important canola-growing areas with large, almost continual acreage of canola. These regions included: Ardabil, Guilan, Mazandaran and Golestan Provinces that are mostly located around the Caspian Sea in the north of Iran (Fig. 1). Fields were mostly small and their sizes ranged from 0.5 to a maximum of 3 ha. All fields were sampled in a V-shaped transect. Plants sampled were separated by a minimum distance of 10 m. Depending on the disease severity and size of each field, the number of samples obtained varied from three to nine plants per field from Ardabil, three to 30 plants, two to 28 plants and two to seven plants per field respectively from Guilan, Golestan and Mazandaran from ten, seven, six and 12 fields respectively, representing a total geographical area of about 1,2000 km^2. One sclerotium from each plant was surface-sterilised, cultured on potato dextrose agar (PDA; Merck) and incubated at 21 to 24ºC in darkness for 2 days. Mycelial tips were transferred to new PDA plates resulting in 49 isolates from Ardabil, 84 from Guilan, 62 from Golestan and 57 from Mazandaran. In addition to canola, a small number of isolates were collected from tobacco (Golestan T; n=14) and broad bean (Golestan BB; n = 10) in Golestan. Broad bean plants were located next to one of the infected canola fields, whereas tobacco plants were located at a tobacco research centre, 35 km from the nearest canola field in Golestan (see Appendix 1). For each isolate several sclerotia were dried and stored in 4ºC.

DNA extraction

Isolates were grown on PDA and incubated at 21 to 24ºC in darkness for 5 days. Two plugs of the colony margin were transferred to 250 ml flasks containing 50 ml potato dextrose broth (PDB) and incubated at 21 to 24ºC in darkness for 2 days. Cultures were incubated for a further 4 days at 21 to 24ºC in darkness with shaking at 100 cycles min^{-1} in a rotary shaker. Mycelia were harvested by vacuum filtration, lyophilised and stored at −20ºC. Fifteen to 20 mg of dried mycelia were powdered and transferred to 1.5 Eppendorf tubes for DNA extraction according to Liu et al. (2000) with the following modifications: before precipitation of DNA, an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the supernatant, which was then centrifuged at 12,000 rpm for 10 min. Also, the DNA pellet was washed twice with cold ethanol (70%), and then air-dried at 37ºC for 20 min. The DNA pellet was

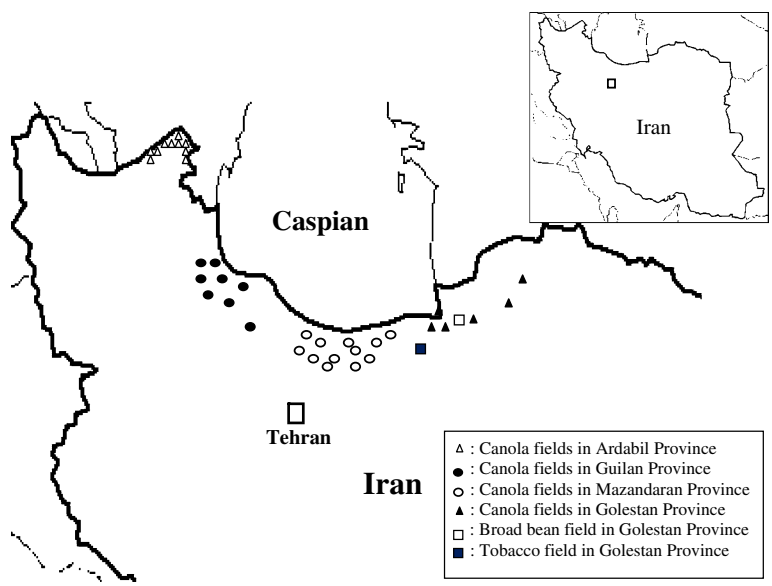

Fig. 1 Distribution of sampled fields of canola, broad bean and tobacco, infected by *Sclerotinia sclerotiorum* in four provinces of Iran

dissolved in 30 µl of 1X TE (10 mM Tris-HCl, 1 mM EDTA) and 2 µl of ribonuclease A (10 mg ml^{-1}) and stored at −20ºC.

Microsatellite genotyping

Microsatellite primers of seven loci described by Sirjusingh and Kohn (2001) were tested for polymorphism among 16 isolates of *S. sclerotiorum*. Locus 13-2 was either monomorphic or displayed null alleles and was therefore excluded from further analyses. The other six microsatellite loci (5-2, 7-2, 9-2, 12-2, 8-3 and 92-4) were polymorphic and were chosen to genotype 276 isolates representing the four regions sampled in Iran. We also included six Australian isolates obtained from Adrienne Sexton (Melbourne University, Australia) to calibrate our allele size assignments. Then we compared the allelic pattern of the mentioned six and other 16 Australian isolates (Sexton et al. 2006) for the six studied loci with the observed allelic pattern of Iranian populations.

For the six polymorphic loci, the forward primers were labelled with a fluorescent dye (G5 dye set: 6-FAM, NED, VIC; Applied Biosystems). Each PCR reaction was performed in a total volume of 10 µl containing 10–50 ng of total DNA, 1 µl of 10X reaction buffer, 2 mM $MgCl_2$ (3 mM for reactions of primer pairs 5-2 and 9-2), 200 µM dNTPs, 0.25 U of *Taq* polymerase (Scientific) and 0.2 µM of each primer (Applied Biosystems). The amplification was conducted in an Eppendorf Mastercycler®, and consisted of an initial denaturation step at 95°C for 5 min, followed by 36 cycles of denaturation at 95°C for 20 s; annealing at 55°C for 30s (except for loci 92-4 and 8-3 where the annealing temperature used was 60°C), and extension at 72°C for 45 s, with a final extension for 5 min at 72°C. A positive control of an isolate with known microsatellite allele sizes, as well as a negative control which did not contain any DNA, were conducted for each PCR plate analysed. After amplification 0.8 to 3 µl of PCR product for six loci were combined and the final mixture was diluted ten times with sterile distilled water. Then 2 µl of the diluted combined PCR products was mixed with 8 µl of Hi-Di formamide and 2 µl of the Gene-Scan 500-LIZ size standard (Applied Biosystems). Fragments were separated on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and analysed using the GENEMAPPER ver. 3.7 (Applied Biosystems). Only one allele was amplified for all loci as expected for a haploid organism. Negative controls did not yield any fragments and positive controls always yielded the correct allele sizes.

Data analysis

Ewens-Watterson tests of neutrality were performed with 1,000 simulations to test the selective neutrality of observed variation in microsatellite loci within populations using POPGENE ver. 3.2. (Yeh et al. 1999). Isolates with the same combination of alleles at all six loci were treated as clonal lineages or haplotypes, the products of asexual reproduction. The occurrence and frequency of haplotypes within a population was expressed as a genotypic diversity ($\hat{G}$) (Stoddart and Taylor 1988). To compare $\hat{G}$ between populations with different sample sizes and compare with previously published genotypic diversity estimates of *S. sclerotiorum*, we divided $\hat{G}$ from each collection by n (sample size). The significance of difference between $\hat{G}$ of four regional populations was calculated using a *t*-test (Chen et al. 1994). Furthermore, a clonal fraction was calculated for each region. The clonal fraction was calculated as the occurrence and frequency of clones within a population, $\frac{n-G}{n}$, where n is the sample size and G is the number of haplotypes (Zhan et al. 2002). Genotypic richness is an estimate of the number of genotypes contained in a population. A simple estimate of richness is the number of unique genotypes within a population (g). When sample sizes are small, increasing sample size will result in increased g, therefore g is not a valid statistic for comparing richness of different populations unless sample sizes are equal (Grünwald et al. 2003). Multilocus genotypic diversity analysis was therefore also conducted with the Shannon—Wiener's index (Shannon and Weaver 1949). To compare evenness of genotypes among populations, E_5 was calculated (Ludwig and Reynolds 1988) for all populations. For all further analyses, only one representative of each haplotype was selected per population to construct clone-corrected data sets.

Genetic variation within populations

To determine the extent of population subdivision among different fields within a region, a null

hypothesis of no genetic differentiation within regional populations was tested by comparing observed θ for each regional population to that for datasets of 1,000 randomisations on clone-corrected datasets of field populations. Corresponding 1-tailed P values were subsequently estimated in MULTILOCUS ver. 1.3 (Agapow and Burt 2001). There were no significant θ values among field populations of each of the four regions (θ=0 to 0.06, P=0.12 to 0.51), allowing field populations to be pooled to represent four regional populations based on geographic proximity, for further analyses (Tables 1 and 2).

Nei's unbiased measure of gene diversity, H (Nei 1978) and its mean measure for all loci and for all populations over six loci were estimated in GENALEX ver. 6.1 (Peakall and Smouse 2006). Gene diversity estimates for each region, including number of alleles per locus and the number of private alleles were calculated.

Genetic variation among populations

To estimate the distribution of genetic variation at different levels of geographical subdivision: among geographical regions (Ardabil, Guilan, Mazandaran and Golestan), among field populations within regions, and within field populations, a hierarchical analysis of molecular variance (AMOVA) and the significance levels of genetic variations were calculated using a permutation test with 1,000 permutations using GENALEX ver. 6.1. (Peakall and Smouse 2006). To estimate the amount of population differentiation among regions, θ was calculated on clone-corrected datasets for pairwise regional populations and in order to test the null hypothesis of no genetic differentiation among four regional populations, P values were estimated after 1,000 randomisations in MULTILOCUS ver. 1.3 (Agapow and Burt 2001). Nei's unbiased genetic distance was estimated for all pairwise comparisons of regional populations in POPGENE ver. 3.2 (Yeh et al. 1999).

To estimate the level of admixture and infer the number of clusters present in the Iranian *S. sclerotiorum* populations, a Bayesian genotypic clustering analysis was conducted in STRUCTURE ver. 2.2 (Falush et al. 2007, 2003; Pritchard et al. 2000). In the programme STRUCTURE ver. 2.2, an admixture ancestry model-based clustering method with correlated allele frequencies was used. Four independent runs of one to 15 subpopulations (K=1–15) were performed using 100,000 Markov chain steps after a burn-in period of 50,000 steps. We compared the likelihood estimate of each of the K values essayed to determine the number of populations present based on the maximum *ln* K (Pritchard et al. 2000).

A Mantel test was performed to determine if there was a significant correlation between geographic distance (expressed by the logarithm of geographic distance between pairs of populations) and genetic differentiations between all pairs of regional populations. The significance of the correlation was estimated after 1,000 permutations in GENALEX ver. 6.1 (Peakall and Smouse 2006). If the degree of genetic differentiation among populations is affected by geographic distance, a significant negative correlation

Table 1 Population differentiation (θ) among the fields within each regional population as measured in MULTILOCUS ver. 1.3 (Agapow and Burt 2001) and gene diversity (H) as estimated in GENALEX ver. 6.1 (Peakall and Smouse 2006) for four populations of *Sclerotinia sclerotiorum* from canola in Iran

Regional populations	No. of fields	Gene diversity (H)	θ	P^a
Ardabil	10	0.61	0.05	0.12
Guilan	7	0.51	0.00	0.23
Golestan	12	0.58	0.06	0.25
Mazandaran	6	0.59	0.00	0.51

[a] 1-tailed P value refers to θ (population differentiation among fields within each regional population). The null hypothesis is no genetic differentiation among fields of the same region

Table 2 Genetic differentiation (θ) among four regional populations of *Sclerotinia sclerotiorum* in Iran

	Ardabil	Guilan	Golestan	Mazandaran
Ardabil	****			
Guilan	0.06 ($P<$ 0.001)	****		
Golestan	0.04 ($P=$ 0.03)	0.07 ($P<$ 0.001)	****	
Mazandaran	0.04 ($P=$ 0.03)	0.06 ($P=$ 0.002)	0.03 ($P=$ 0.97)	****

1-tailed P values refer to θ (population differentiation among regional populations);

Low P values can reject the null hypothesis of no population differentiation among regional populations

should be evident indicating a natural stepwise pattern of migration.

Random mating

To examine the extent of multilocus gametic disequilibrium among populations, the index of association (I_A) and $\bar{r}_d$ were estimated using MULTILOCUS ver. 1.3 (Agapow and Burt 2001). The index of association (I_A) is a measure of multilocus linkage disequilibrium, with a value of 0 in the presence of random mating (Brown et al. 1980). I_A is usually dependent on the number of loci included in the analysis, which makes comparisons among studies difficult. To avoid this problem, a modified statistic $\bar{r}_d$, was also used which removed the dependency on the number of loci. The proportion of compatible pairs of loci was also calculated in MULTILOCUS ver. 1.3 (Agapow and Burt 2001). Two loci are compatible if all of the observed genotypes are explained by mutation only, and therefore no recombination is inferred (Estabrook and Landrum 1975).

Results

Ewens-Watterson tests of selective neutrality did not detect significant deviations of haplotype distributions from those expected for selectively neutral variation ($P \leq 0.05$) in all studied regional populations, indicating that the observed variation in microsatellite loci was selectively neutral (data not shown). The number of alleles obtained for each locus ranged from three (locus 5-2) to eight with an average of 3.33 over all loci and populations. Nei's unbiased gene diversity (H) for loci over four populations ranged from 0.52 (locus 7-2) to 0.71 (locus 8-3) (data not shown).

Genetic diversity within populations

In total, among 276 isolates, 30 polymorphic alleles were detected from six microsatellite loci, and 80 multilocus haplotypes were identified. Six of the 80 haplotypes (7.5%) occurred more than 10 times in the Iranian populations, indicating high levels of clonality. Clones were also identified at the smallest scale of analysis within 27 out of 37 fields analysed (including tobacco and broad bean fields). All field populations, as the smallest scale of the analysis, were composed of more than one clone, indicating diversification within field populations. Number of clones per field and clone frequency within field populations varied depending on sample size. Genotypic diversity ($\hat{G}$) obtained for populations, was moderate and ranged from 12.0 to 22.0 (Table 3). According to a *t*-test, genotypic diversities were not significantly different in pairwise comparisons of the four populations. Clonal fractions were large ranging from 0.39 to 0.67 and haplotypes were distributed evenly within regional populations (E_5=0.77 to 0.86) (Table 3).

Gene diversities for all four populations were high and ranged from 0.51 to 0.61 (Table 1). Two private alleles each were detected in Ardabil, Guilan and Mazandaran. These alleles occurred at a low frequency (0.03 to 0.05) except for one allele from Ardabil which occurred at a frequency of 0.17 and one allele from Mazandaran at a frequency of 0.16 (data not shown).

Genetic diversity among populations

Although a high number of haplotypes were shared among two or three populations (23.7%), none of the 80 haplotypes were shared among all four regions. The highest percentage of shared haplotypes (16.7%) was between Golestan and Mazandaran (Fig. 2).

All five haplotypes from tobacco shared haplotypes with canola isolates from Golestan although

Table 3 Indices of multilocus genotypic diversity for four Iranian populations of *Sclerotinia sclerotiorum*; $\hat{G}$ = Stoddart and Taylor's diversity index (Stoddart and Taylor 1988); $\hat{G}/N$ (%) = Stoddart and Taylor's diversity index scaled by sample size; H′ = Shannon—Weaver's diversity index for multilocus genotypes (Shannon and Weaver 1949); E_5 = index of evenness for multilocus genotypes (Ludwig and Reynolds 1988)

Statistic	Regional populations			
	Ardabil	Guilan	Mazandaran	Golestan
Sample size (n)	49	84	57	62
Number of haplotypes	30	32	19	23
$\hat{G}$	22.0	17.6	12.0	14.6
$\hat{G}/N$(%)	45.0	21.0	21.0	23.5
Clonal fraction	0.39	0.62	0.67	0.63
H′	3.3	3.1	2.7	2.9
E_5	0.86	0.77	0.81	0.82

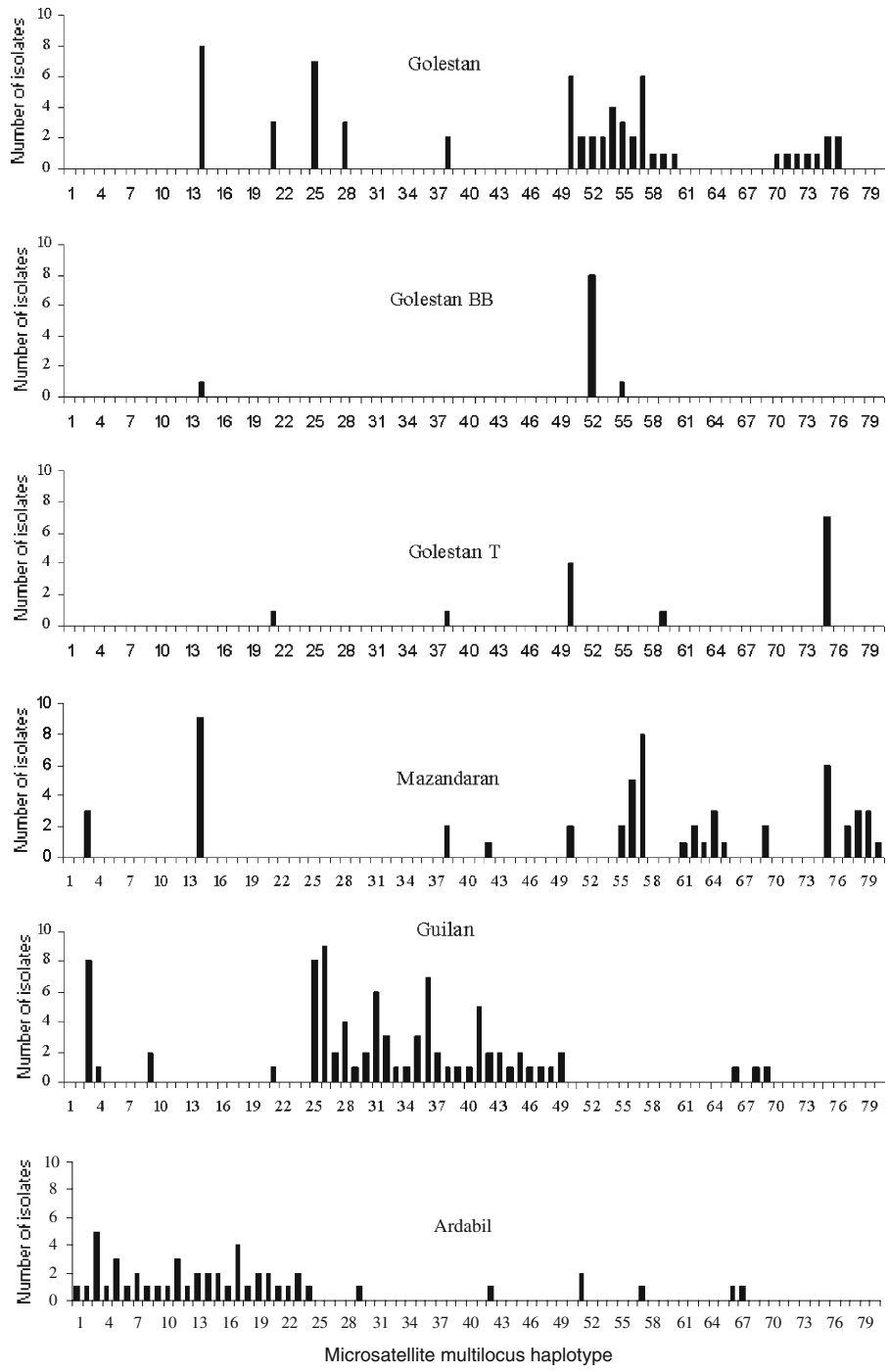

Fig. 2 Frequency distribution of 80 microsatellite haplotypes among 276 isolates of *Sclerotinia sclerotiorum* from canola in Golestan (n=62), tobacco in Golestan (n=14), broad bean in Golestan (n=10), and from canola in Mazandaran (n=57), Guilan (n=84) and Ardabil (n=49)

some tobacco haplotypes were also shared with the other three populations. Similarly, all broad bean haplotypes also occurred in the Golestan population and two isolates also shared haplotypes with Mazandaran and Ardabil populations. No haplotypes on tobacco isolates were shared with broad bean isolates, although sample sizes were limited. Estimates of θ were significant for all pairs of regional populations except between Golestan and Mazandaran (θ=0.03, P=0.97) (Table 2). Only one large panmictic population could be inferred with STRUCTURE ver. 2.2 (ln P(D)=−1548; Var[LnP(D)]=12.1), with a low

within-population differentiation for the populations (F_{ST}=0.066). The probability did not improve with inferring more populations, but remained constant until decreasing when more than 11 clusters were inferred.

The results of AMOVA showed that 96% of the total genetic variation was attributable to genetic differences within regions, whereas only 4% was due to the variation among regional populations (data not shown). Based on the Mantel test, no significant correlation was found between genetic differentiation and the natural logarithm of geographic distance (r=−0.43, P=0.18), indicating that there is no significant isolation by distance.

Random mating

Linkage disequilibrium and proportion of compatible pairs of loci were estimated within each of the four regional populations. In order to test the null hypothesis of complete panmixia, MULTILOCUS compares the observed dataset to datasets obtained by randomly shuffling alleles amongst individuals of the same population (Burt et al. 1996) in 1,000 randomisations. A 1-tailed P value is then generated to determine if the value obtained for the observed data set is significantly different from the randomised data set. Both the index of association (I_A) and $\bar{r}_d$ values obtained were not significant for all regional populations (Table 4). The proportion of compatible pairs of loci, a pairwise measure of association between loci, was also not significant for all of the regional populations (Table 4). These results are consistent with recombination and random mating with frequent sexual reproduction.

Discussion

The genetic diversity of 276 isolates of *S. sclerotiorum* representing four geographic populations from Iran, were studied using six microsatellite loci. These populations were characterised by high levels of gene diversity, moderate levels of genotypic diversity and a number of shared haplotypes among populations. The genotypic diversities obtained within Iranian populations of *S. sclerotiorum* ranged from 21 to 45% which on average is lower than that found in Australian populations ($\hat{G}/N$%=36 to 80%, Sexton and Howlett 2004; $\hat{G}/N$%=28 to 68%, Sexton et al. 2006) by analysing eight microsatellite loci. The genotypic diversity obtained in Turkey (63%) (Mert-Türk et al. 2007) was also higher than that in the current study. However, more loci increases the possibility of assigning genotypes and might account for the higher genotypic diversities found in both Australia and Turkey. Furthermore, a comparison among populations from Turkey is difficult since different microsatellite loci were used and only two of them were included in the present study. Clonal fractions of populations from Iran (ranging from 0.39 to 0.67) and those from Ontario (0.60), California (0.12) and Washington (0.56) (Malvárez et al. 2007) are similar and confirm that most populations have a large clonal fraction. Haplotypes were distributed evenly in Iran (E_5=0.77 to 0.86) indicating no regional influence in haplotype distribution.

Interestingly, locus 13-2 was either monomorphic or displayed a null allele in the subset of Iranian *S. sclerotiorum* isolates tested, whereas seven alleles were found in 239 isolates from Australia (Sexton and Howlett 2004; Sexton et al. 2006). Similarly two alleles were found for this locus in 167 isolates from North America (Atallah et al. 2004).

In total, we detected 30 alleles for the six loci analysed. For the same six loci, two separate studies have demonstrated 30 and 32 alleles respectively in 134 and 105 isolates from Australia (Sexton and Howlett 2004; Sexton et al. 2006). Among 11 micro-

Table 4 Index of association (I_A), $\bar{r}_d$ and proportion of compatible pairs of loci (PrCompat) for Iranian populations of *Sclerotinia sclerotiorum* from canola

	Ardabil	Guilan	Golestan	Mazandaran
PrCompat	0.00 (P=1.00)[a]	0.07 (P=0.59)	0.20 (P=0.10)	0.20 (P=0.39)
I_A	−0.05 (P=0.75)	−0.18 (P=1.00)	0.06 (P=0.29)	0.01 (P=0.42)
$\bar{r}_d$	0.01 (P=0.75)	−0.04 (P=1.00)	0.01 (P=0.29)	0.00 (P=0.42)

[a] 1-tailed P values as calculated in MULTILOCUS ver. 1.3 (Agapow and Burt 2001) after 1,000 randomisations

satellite loci which were used for a population genetic study of *S. sclerotiorum* in North America (Atallah et al. 2004), four loci were in common with ones used in current study. The number of alleles for these four loci was 18 and 12, respectively for 276 Iranian and 167 American isolates. The lower numbers of alleles in North America is consistent with a smaller sample size used in that study.

Comparison of allelic patterns of 22 Australian isolates (Sexton et al. 2006) with Iranian populations revealed 16 of the 30 alleles as common alleles between Australian and Iranian isolates, although no shared haplotypes were found. Given the small sample size of the comparative—Australian population (n=22), and the large geographic distance between Iran and Australia, as well as strict quarantine conditions in Australia, it is plausible that these two countries do not share haplotypes. Shared haplotypes is more likely to occur between Turkey, a neighbouring country of Iran, through exchange of haplotypes through soil, seeds and other agricultural products. Different microsatellite loci were studied (Mert-Türk et al. 2007) therefore, sharing of haplotypes could not be assessed.

Of the 80 haplotypes identified in this study, 19 haplotypes were shared among two or three of the regions studied. Shared microsatellite haplotypes among populations were also found within Australia and North America (Sexton and Howlett 2004; Sexton et al. 2006; Atallah et al. 2004). Shared haplotypes among populations either indicate exchange of haplotypes among populations and/or the populations represent the same founder population. There are several mechanisms by which haplotypes can be exchanged among neighbouring countries/fields. Transporting contaminated soil and fertilising with manure from animals fed infected plants are two common ways of spreading fungal inocula in the form of sclerotia or mycelium from field to field (Abawi and Grogan 1979). Irrigation is also other way for spreading of *Sclerotinia* species among fields. Sclerotia remained viable for at least ten to 21 days in flowing water (Abawi and Grogan 1979). Infected seeds potentially could be a source of infection, although this method of transmission has not been proven. Migration (exchange of haplotypes) among populations through the movement of sclerotia with soil over large distances, can link different fields into one genetic neighbourhood. Furthermore, airborne ascospores, which are the most common source of infection within fields (Abawi and Grogan 1979), also provide a mechanism for long-distance dispersal of haplotypes among populations.

Shared haplotypes among populations could also be the result of sexual reproduction, i.e. inbreeding or out-crossing among similar genotypes. Resultant ascospores would be genetically similar and appear clonal. Because ascospores can be dispersed over long distances (Cubeta et al. 1997), it is not surprising to find shared clonal genotypes among several regions.

The mating structure of a fungus is an important factor dictating whether the population consists of many clones (asexual), or many haplotypes (sexual). *Sclerotinia sclerotiorum* is a homothallic fungus and thus it is able to produce apothecia (sexual fruiting bodies) from single ascospores. For a homothallic fungus, sexual reproduction may not lead to the recombination of alleles. However, homothallism (self-fertility) does not rule out the possibility of outcrossing which can result from somatic recombination (Ekins et al. 2006). Previous reports provided direct evidence for outcrossing, ie MCG differentiation among sibling ascospores (Atallah et al. 2004; Kohli and Kohn 1998). Furthermore, linkage disequilibrium tests suggested the occurrence of recombination in populations of *S. sclerotiorum* from North America and Australia which provided indirect evidence for outcrossing (Atallah et al. 2004; Malvárez et al. 2007; Sexton et al. 2006; Sexton and Howlett 2004; Kohli and Kohn 1998). The mechanism for the reported outcrossing is, however, still unclear.

In the Iranian *S. sclerotiorum* populations, we also found indirect evidence for outcrossing since all tests for random association among different loci showed that populations were in linkage equilibrium. In addition to the results of a genotypic disequilibrium test, non-significant proportions of compatible pairs of loci suggest recombination. Apothecia are commonly found on canola in Iran (pers. observation) and indicate the occurrence of sexual reproduction; however whether those apothecia have been produced by means of outcrossing is unknown. Employing more direct approaches such as analyses of segregation ratios among ascospores within apothecia is necessary to determine the occurrence and extent of in/outbreeding in Iranian populations of this plant pathogen.

A Mantel test did not detect significant isolation by distance among regional populations of *S. sclerotiorum*. This result indicated that the studied populations are not in genetic drift-gene flow equilibrium, thus the populations are either founder populations, or that man-mediated dispersal of *S. sclerotiorum* is occurring through the movement of soil and/or infected plant material. Furthermore, STRUCTURE results suggest that there is no population structure and all studied populations belong to one large panmictic population, irrespective of their geographic origin or host. This pathogen has a wide host range, and haplotypes are shared among populations of different hosts, thus different sources of inocula are likely to be transferred by soil and agricultural products among regions. All 14 isolates from tobacco shared haplotypes with the population from Golestan. Some haplotypes from tobacco were also shared with all other populations. Furthermore, all 10 isolates from broad bean shared haplotypes with Golestan and some haplotypes were shared with the other canola populations. The occurrence of shared haplotypes from broad bean with haplotypes from Golestan is expected since broad bean isolates were sampled from plants next to an infected canola field in Golestan. Shared fungal haplotypes among host populations reiterates the broad host range of the pathogen and little genetic host specialisation.

A large proportion of genetic variance (96%) was attributed to the genetic variation within regional populations, similar to what has been found in North America (Atallah et al. 2004). This is higher than what was found within populations in Australia (79.4% and 90%) (Sexton and Howlett 2004; Sexton et al. 2006).

In conclusion, our results showed that the populations of *S. sclerotiorum* on canola in northern Iran have moderate levels of genetic diversity and a mixed reproduction system, but, on the other hand, have high levels of admixture and represent one large panmictic population. Combined with no isolation by distance, the Iranian populations most likely represent a founder population. Moderate levels of genetic diversity and linkage equilibrium among loci may contribute to higher levels of fitness in pathogens and can contribute to rapid evolution against control measures such as the deployment of resistance genes or fungicide applications.

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Appendix 1. Samples of *Sclerotinia sclerotiorum* in four northern provinces of Iran

Regions	Fields	Host	No. of isolates	Location
Guilan	Gu 1	canola	20	Anzali port
	Gu 2	canola	12	Anzali–Rezvanshahr rd
	Gu 3	canola	6	Anzali–Rezvanshahr rd
	Gu 4	canola	6	Rezvanshahr–Astara rd
	Gu 5	canola	7	Rezvanshahr–Astara rd
	Gu 6	canola	30	Rezvanshahr–Astara rd
	Gu 7	canola	3	Rasht, Institute of rice
Ardabil	Ar 1	canola	5	Sarband–Pars abad rd
	Ar 2	canola	3	Sarband–Pars abad rd
	Ar 3	canola	5	Sarband–Pars abad rd
	Ar 4	canola	5	Sarband–Pars abad rd
	Ar 5	canola	5	Pars abad–Bilesavar rd
	Ar 6	canola	9	Pars abad–Bilesavar rd
	Ar 7	canola	5	Pars abad–Bilesavar rd
	Ar 8	canola	4	Pars abad–Bilesavar rd
	Ar 9	canola	5	Pars abad–Bilesavar rd
	Ar 10	canola	3	Pars abad–Bilesavar rd
Mazandaran	Ma 1	canola	5	Soorak (Behshahr)
	Ma 2	canola	6	Neka
	Ma 3	canola	6	Sari (Dashte naze)
	Ma 4	canola	4	Gharakhil
	Ma 5	canola	6	Esbukola
	Ma 6	canola	4	Baye kola
	Ma 7	canola	5	Jooybar–Babol rd
	Ma 8	canola	4	Jooybar–Babol rd
	Ma 9	canola	7	Babol–Amol rd
	Ma 10	canola	3	Mahmoodabad
	Ma 11	canola	5	Amol
	Ma 12	canola	2	Noor
Golestan	Go 1	canola	6	Azadshahr–Minoodasht rd
	Go 2	canola	2	Aliabad
	Go 3	canola	9	Golestan

Go 4	canola	5	Kord kooy
Go 5	canola	12	Torkaman port
Go 6	canola	28	Ghaz port
Go B	broad bean	10	Kordkooy
Go T	tobacco	14	Tirtash, Tobacco research centre

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