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RESEARCH PAPERS

Assessment of genotypic diversity among *Fusarium culmorum* populations on wheat in Iran

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Summary. *Fusarium culmorum* is one of the most important causal agents of crown rot of wheat in the north and northwest of Iran. Eight pairs of single-locus microsatellite primers were used to analyze genomic DNA of 88 isolates of *F. culmorum* collected from wheat fields in five provinces of Iran. Thirty-three alleles were detected among eight simple sequence repeat (SSR) loci, ranging from 2–6 alleles (with an average of 4.125 alleles/locus). Eighty-four multilocus genotypes were detected among 88 isolates of *F. culmorum*, indicating a high level of genetic diversity among isolates. Nei's gene diversity of populations ranged from 0.355 to 0.561 with an average of 0.47. The greatest genetic similarity (0.8409) was detected between the Qazvin-East Azarbaijan and Golestan populations, the greatest genetic distance was between the Tehran and Golestan populations. These results show that SSR is a convenient and rapid technique for the analysis of genetic diversity and strain differentiation of *F. culmorum*.

Key words: simple sequence repeat, population genetics, asexual recombination, molecular variance.

Introduction

Fusarium culmorum (W.G. Smith) Sacc. is one of the most important fungal pathogens of wheat, causing two diseases: crown and foot rot (CFR) and Fusarium head blight (FHB) (Aleandri *et al.*, 2007). *Fusarium culmorum* can produce trichothecene mycotoxins and zearalenone, and has been associated with FHB outbreaks in North America and Europe (Desjardins, 2006). This fungus is most common in temperate regions, particularly in cooler parts of Europe and the Canadian prairies (Fernandez and Chen, 2005; Leslie and Summerell, 2006).

Common root and crown rots of wheat have been reported from different wheat growing areas of Iran (Darvishnia *et al.*, 2006). Crown rot, in particular, is the most common wheat disease, and often appears as white heads in wheat fields after the flowering stage. The disease causes economical yield losses in most wheat growing areas of Iran, especially in the Fars (Ravanlou and Banihashemi, 1999), Golestan, East Azarbaijan, Ardabil, Tehran, Zanjan (Saremi *et al.*, 2007; Pouzeshimiab *et al.*, 2012), Kermanshah and Lorestan provinces (Safaee *et al.*, 2012). *Fusarium graminearum* and *F. culmorum* are, respectively, the most common and important fungal pathogens in cereals in Europe and Australia (Miedaner *et al.*, 2008). For *F. culmorum*, no sexual stage is known (Bowden and Leslie, 1999). FHB is a disease that has re-emerged as a significant, though sporadic, threat to global wheat production (Goswami and Kistler, 2004). FHB epidemics are correlated with severe economic losses resulting from reduced grain quality and quantity (Scott and Chakraborty, 2010).

Fusarium culmorum can show high levels of phenotypic variability among isolates in culture, including colony morphology, pigmentation and sporulation (Puhalla, 1981). Variation for aggressiveness and the type and amount of mycotoxin production has been found among isolates collected from vari-

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ous geographic locations (Gang *et al.*, 1998). Despite its importance as a plant pathogen and mycotoxin producer, however, there is inadequate information on the population structure and genetic diversity of this pathogen. De Nijs *et al*. (1997) used random amplified polymorphic DNA (RAPD) markers, to estimate genetic variability among 18 isolates of *F. culmorum* collected from different parts of the Netherlands. They found low genetic variation among the studied isolates, and only two RAPD genotypes were detected. However, Miedaner *et al.* (2001) identified 23 multilocus haplotypes among 41 isolates from Russia. Mishra *et al.* (2002) conducted PCR-based RFLP analysis of nrDNA IGS and 28S gene, and found substantially high intraspecific variation (47.7%) within global populations of *F. culmorum.* Mishra *et al.* (2003) used ISSR markers to study genetic diversity of 75 isolates of *F. culmorum*, and found that 59 different genotypes of the fungus were clustered into seven distinct clades. Using Rep-PCR analysis, Gürel *et al.* (2010) found high genetic diversity (52.3%) among 14 *F.culmorum* isolates collected from different parts of Turkey. Giraud *et al.* (2002) developed eight microsatellite markers in *F. culmorum*, and reported polymorphism among *F. culmorum* and *F. graminearum* populations collected from different regions in France.

This present study aimed to examine the genetic structure of *F. culmorum* populations collected from different wheat growing areas of Iran using simple sequence repeat (SSR) markers, to elucidate how this genetic variability is distributed, and to determine what evolutionary factors are involved in shaping the population genetic structures.

Material and methods

Regional zones for survey areas and sampling

This study was carried out in five provinces of Iran, including Tehran, East Azarbaijan, Qazvin, Lorestan and Golestan, during the growing seasons of 2010 and 2011. Based on climatic characteristics and natural barriers, the isolates collected from these provinces were considered as four separate populations. The first population contained isolates collected from the Varamin area in the Tehran province. The Varamin area is located in the southern part of the Tehran province very close to the Semnan desert, and has a warm and dry climate. The second population contained isolates collected from the northwestern provinces of Iran including Qazvin and East Azarbaijan. These areas have a cold and temperate climate. The third population contained isolates collected from the cold and temperate regions of the Lorestan province. This province is located in the west of the country and is surrounded by the Zagros mountains. The fourth population contained isolates collected from the Golestan province located in the north of Iran and surrounded by the Caspian Sea and the Alborz mountains. This area has a warm and humid climate (Figure 1).

Figure 1. Map of Iran provinces showing regions from which *Fusarium* isolates were obtained from wheat fields.

In each province, wheat fields of about 1 ha were selected randomly at about 20 km distance from each other. At crop maturity stage at each site, five sampling points were made in a zigzag pattern (Mitra, 1930), each point being 100 m apart from the next. At each point, plants showing symptoms (bleached heads) were collected. In the laboratory, crown and sub-crown tissues of infected plants were cut and stored in paper bags at 4°C until fungal isolation was performed. A total of 138 wheat fields in 42 regions of five provinces were sampled.

Identification of *Fusarium* species

Collected plant samples were washed in tap water for about 1 h to remove soil particles from the crown

and root regions. Then two pieces (approx. 2 cm long) of infected tissues were excised from crowns and roots showing symptoms of the disease. These sections were surface-sterilized with 1% w/v sodium hypochlorite solution for 3 min, rinsed twice in sterile distilled water and dried on sterile filter paper. The tissue sections were placed on potato dextrose agar (PDA; Merck) and peptone PCNB agar (PPA; Nash and Snyder, 1962) in Petri plates, which were incubated at 25°C in the dark for 5–7 d. To purify the cultures, all *Fusarium* colonies were sub-cultured onto PDA, synthetic nutrient-poor agar (SNA; Nirenberg, 1981) and carnation-leaf agar (CLA; Fisher *et al.*, 1982), using a single conidium technique (Leslie and Summerell, 2006). PDA cultures were incubated at 25ºC in darkness, and CLA and SNA cultures were incubated at 25°C under near-UV light for 1–4 weeks. Colony morphology was recorded from cultures grown on PDA. Morphological characters of each isolate, including shape and size of macroconidia, microconidia, conidiogenous cells and chlamydospores, were assessed from cultures grown on SNA and CLA. *Fusarium* species were identified based on morphological characteristics according to Leslie and Summerell (2006). For long-term storage, a single germinating macroconidium of each species was transferred to a fresh quarter-strength PDA plate and maintained at 25°C under ambient light for 14 d, and then 5 mm^2 agar plugs were transferred to 1.5 mL tubes containing 15% glycerol and kept at -20°C (Scott and Chakraborty, 2010). Based on morphological characteristics, 88 isolates were selected for examination of genetic diversity.

DNA extraction

Isolates were recovered from storage and cultured on PDA in Petri plates, and 100 mg of mycelium was scraped from the surface of each 10-d-old colony. DNA was extracted according to Möller *et al.* (1992) and was stored at -20°C until used.

The identity of putative *F. culmorum* isolates was confirmed by PCR amplification with the *F. culmorum* specific primer pairs Fc01F/Fc01R (Nicholson *et al.*, 1998). Conditions used for PCR amplification followed those of Akinsanmi *et al.* (2004).

Molecular analysis

The 88 selected isolates of *F. culmorum* from different geographical origins were genotyped using a set of eight SSR markers (Table 1; Giraud *et al.*, 2002). PCR reactions were each performed in a final volume of 25 μL containing 0.4 U *Taq* polymerase, 1 × PCR reaction buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP, 0.5 μM of primers (all from CinaGen) 25 ng of template DNA and extent sterile distilled water.

Amplifications were each conducted with an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing for 45 s and extension at 72°C for 2 min, and a final extension phase at 72°C for 7 min. Initially, an annealing temperature of 58°C was used for all SSR primers; however, to obtain a specific single band, the annealing temperature was optimized separately for each pair of primers (Table 1). Electrophoresis of the PCR products were performed on 8% polyacrylamide gels, at a constant 85 W for 2 h, with 5 µL of a 100-bp Ladder included as the molecular mass marker. The gels were subjected to silver staining following the protocol of An *et al.* (2009). The size of the most intensely amplified fragment by each primer pair was estimated using the program GEL version 2/17/89 (Thompson, 1989). Fragments with different molecular weights were considered to be different alleles of a particular locus. Null alleles were assigned to isolates for which no amplification product was produced (Figure 2).

Analysis of genotypic diversity

The amplified products were manually scored for different alleles of each SSR locus. Fragments of the same molecular weight were considered to be the same allele. The data from all entries were combined for statistical analysis.

To determine the number of distinct multilocus genotypes (MLG) within each population the GENCLONE v2.0 software (Arnaud-Haond and Belkhir, 2007) was used. The frequencies of different alleles of each locus were calculated within each location and within the total population. The gene diversity of the population was estimated using Nei's formula (Nei, 1973): $h = 1 - \sum_{i=1}^{n} p_i^2$ where h is the gene diversity of the population and p_i is the frequency of different alleles at a particular locus. In estimating gene diversity of each locus, "nulls" were considered to be the same allele. Haploid diversity of individual loci within fields and across all fields of four populations, mean field haploid

Table 1. Repeat motif, primer sequences, range of fragment size, optimum annealing temperature and number of alleles detected by eight pairs of SSR markers in isolates of *Fusarium culmorum.*

Locus	Repeat motif	Primer sequences (5'– 3')	Size (bp)	Ta (°C)	No. of alleles
F1	(TG)8	GAC AAG CAA GCG ATA GGA AA	175–200	51	3
		CTT GAT AGC ACG GAC CGA CG			
F3	(CA)11	CAT ATT CAA CCG ACC CAC AA	190–210	53	4
		TTG AAT GAT AAG GGC GAC GG			
F4	(GT)11	CTT TTT CCC GGC TCC ATT TT	110–145	53	5
		GCT TTC CCT GCT CGA TCG GG			
F6	(AC)15	TAT TTC GTG CAA GGA CTT GG	105–140	51	4
		CTT GGT CCC TGG ATA TCG AA			
F7	(GT)7	TGA CAA GCA AGC GAT AGG AA	215–245	52	2
		GAG TGG AGT TTC GAT ATC GC			
F9	(AC)13	CGA GCT AAT GGT GGC AGG AT	150–210	50	4
		AAC ACC AAA ACG GCT CAT CG			
F10	(AAG)28	AAG CGC CAA CAG AGA TGA CGA	110–205	55	6
		GAC TGC CGA AAC ACC GAA A			
F11	(GT)9	CAG TCT TGG TCG CTC ATC AG	280–360	52	5
		CAG GTT GGC ACG CTT CTT AA			

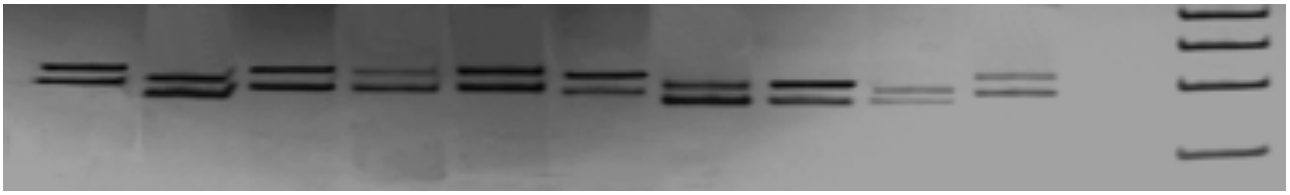

Figure 2. Representative DNA polymorphism among ten selected strains of *Fusarium culmorum* detected by microsatellite primer pair F9. Lanes from left to right: strains 1 and 2 from Lorestan; 3 and 4 from Tehran; 5 and 6 from Golestan; 7, 8, 9, and 10 from Qazvin-East Azarbaijan populations; Co^-, negative control; M, 100-bp Ladder as the DNA size marker.

diversity and Nei's unbiased estimate of genetic identity between fields, number of observed alleles (N_a), number of effective alleles (N_e) and Shannon's information index (I), were calculated for each population using GenALEx v.6.2 software (Peakall and Smouse, 2012).

Analysis of gene diversity in subdivided populations (Nei, 1987) was carried out using POPGENE version 1.31 software (Yeh *et al.*, 2000).

AMOVA

To evaluate the extent of population differentiation, the molecular variance (AMOVA) was analysed and the genetic variation within and between populations was quantified. The Φ_{PT} (analogue of FST fixation index) value for genetic variability and the percentage of polymorphism (% P), were calculated for each population, using GenALEx for

every step. Additionally, AMOVA was repeated through original measurements of Genetic identity and Genetic distance (Nei, 1972) using POPGEN. Isolation by distance was tested using the POPGEN software to discover if there was an association between linearised pairwise Φ_{PT} values i.e. $\Phi_{PT}/(1- \Phi_{PT})$ and geographical distances between the four populations.

Cluster analysis

A similarity matrix was created from the binary data using Nei and Li's coefficient (Nei and Li, 1979), and a dendrogram was generated using the UPGMA method (unweighted pair group method using arithmetic averages) using NTSYSpc 2.0 software (Rohlf, 2000). Principal coordinate analysis (PCoA) was performed using a covariance matrix of individual pairwise allele-sharing distances in the program GenAlEx. This multivariate approach was chosen as a complement to the cluster analysis information, because cluster analysis is more sensitive to closely related individuals, whereas PCoA is more informative regarding distances among major groups (Hauser and Crovello, 1982).

Results

Analysis of genotypic diversity

From a total of 457 fungal isolates, 185 were identified as *F. culmorum* according to morphological and molecular characteristics. Only six isolates belonged to *F. graminearum* and 64 to *F. pseudograminearum*. Of the *F. culmorum* isolates, 88 representatives of different locations of wheat growing provinces in Iran were selected for genotypic diversity analysis (Table 3).

The number of amplified alleles per locus ranged from two (F7) to six (F10) with an average of 4.13 alleles per locus, and the size of the fragments ranged between 105 and 360 base pairs (bp) (Table 1). In total, 33 alleles were detected among eight SSR loci. The percentage of polymorphic loci was 100% in the Tehran, Golestan and East Azarbaijan-Qazvin's populations, and 87.5% in Lorestan. The SSR locus F10 had the greatest haploid diversity across all fields and within individual fields, and the SSR locus F7 was monomorphic in the Lorestan population (Table 2).

Among the 88 genotyped isolates, 84 different multilocus genotypes (MLGs) and four duplicate genotypes were identified. Of all *F. culmorum* isolates which constituted distinct MLGs, 33 were from Teh-

Table 2. Number of observed alleles and gene diversity of eight simple sequence repeat (SSR) markers among 88 isolates of *Fusarium culmorum* from the Tehran, Golestan, East Azarbaijan- Qazvin and Lorestan populations of Iran.

Locus	All populations		Tehran		Golestan		East Azarbaijan -Qazvin		Lorestan	
	N_a[a]	h[b]	N_a	h	N_a	h	N_a	h	N_a	H
F1	3	0.504	3	0.558	3	0.595	3	0.599	2	0.218
F3	4	0.505	4	0.590	4	0.624	3	0.571	2	0.218
F4	5	0.482	4	0.544	3	0.394	4	0.621	2	0.406
F6	4	0.426	4	0.629	4	0.524	3	0.417	2	0.245
F7	2	0.145	2	0.475	2	0.071	2	0.0907	1	0.000
F9	4	0.455	3	0.24	4	0.475	3	0.541	4	0.563
F10	6	0.682	6	0.758	4	0.647	4	0.694	4	0.719
F11	5	0.559	4	0.697	4	0.675	4	0.479	2	0.469

[a] N_a, number of observed alleles.
[b] h, locus diversity; where p_i is the frequency of the i^{th} allele within a population and, is the sum of the squared population allele frequencies: h = 1-Σpi^2.

Table 3. Summary of genetic diversity statistics describing field isolates of *Fusarium culmorum* from four Iranian populations, including: Mean and SE over Loci for each Pop and Grand Mean and SE over Loci and Pops using software GenAlex.

Pop	N	MLG		% P	N_a	N_e	I	h	Uh
Tehran	31	31	Mean	100	3.750	2.529	1.011	0.561	0.580
			SE		0.412	0.306	0.122	0.056	0.058
Golestan	27	26	Mean	100	3.250	2.221	0.884	0.501	0.520
			SE		0.313	0.236	0.125	0.070	0.073
East Azarbaijan- Qazvin	22	19	Mean	100	3.250	2.207	0.875	0.502	0.526
			SE		0.250	0.230	0.115	0.066	0.069
Lorestan	8	8	Mean	87.50	2.500	1.787	0.619	0.355	0.406
			SE		0.378	0.291	0.150	0.081	0.092
Total	88	84	Mean	96.88	3.188	2.186	0.847	0.480	0.508
			SE	3.13	0.182	0.136	0.066	0.035	0.037

N, No. of isolates within the population; MLG, No. of multilocus genotypes within a population; % P, percentage of polymorphic loci; N_a, No. of observed alleles within the population; N_e, No. of effective alleles = ; I, Shannon's Information Index. h = gene diversity, $-1 * \sum (p_i * \text{Ln}(p_i))$, Uh = Unbiased Diversity = $(N / (N-1)) * h$.

Table 4. Summary of analysis of molecular variance (AMOVA) of *Fusarium culmorum* isolates from wheat fields in four populations from Iran.

Source	df	SS	MS	Est. Var.[a]	%	Stat	Value	Probability[b]
Among populations	3	65.461	21.820	0.730	10%			
Within populations	84	554.164	6.597	6.597	90%			
Total	87	619.625		7.327	100%	Φ_{PT}	0.100	0.001

[a] Estimated Variance.
[a] Probability for Φ_{PT} is based on standard permutation across the full data set. Φ_{PT} = AP / (WP + AP) = AP / TOT (where AP, Est. Var among populations; WP, Est. Var. within populations). Levels of significance are based on 1000 iterations.

ran, and eight were from Lorestan. In the 27 isolates sampled from Golestan, 26 MLGs and one duplicate were detected. In East Azarbaijan-Qazvin population among 22 strains, 19 MLGs and three duplicate genotypes were detected (Table 3).

The greatest and lowest numbers of effective alleles were, respectively, found in the Tehran (2.529±0.306) and Lorestan populations (1.787±0.291), with an average of 2.186±0.136 over all populations (Table 3). The mean population diversity using the Shannon information index (I) was estimated as 0.847 (SE=0.066). The Tehran population was the most diverse (I=1.011) and the Lorestan population was the least diverse (I=0.619) (Table 3).

The range of gene diversity within a population was between 0.355 (Lorestan) and 0.561 (Tehran) with an average of 0.48 (Table 3).

AMOVA

Molecular analysis of variance showed that 90% of the diversity was distributed within populations and 10% among populations (Table 4). The high global Φ_{PT} value (0.100, P = 0.001) indicates significant genetic variability (Table 4). A sense of genetically significant difference was found in the four populations.

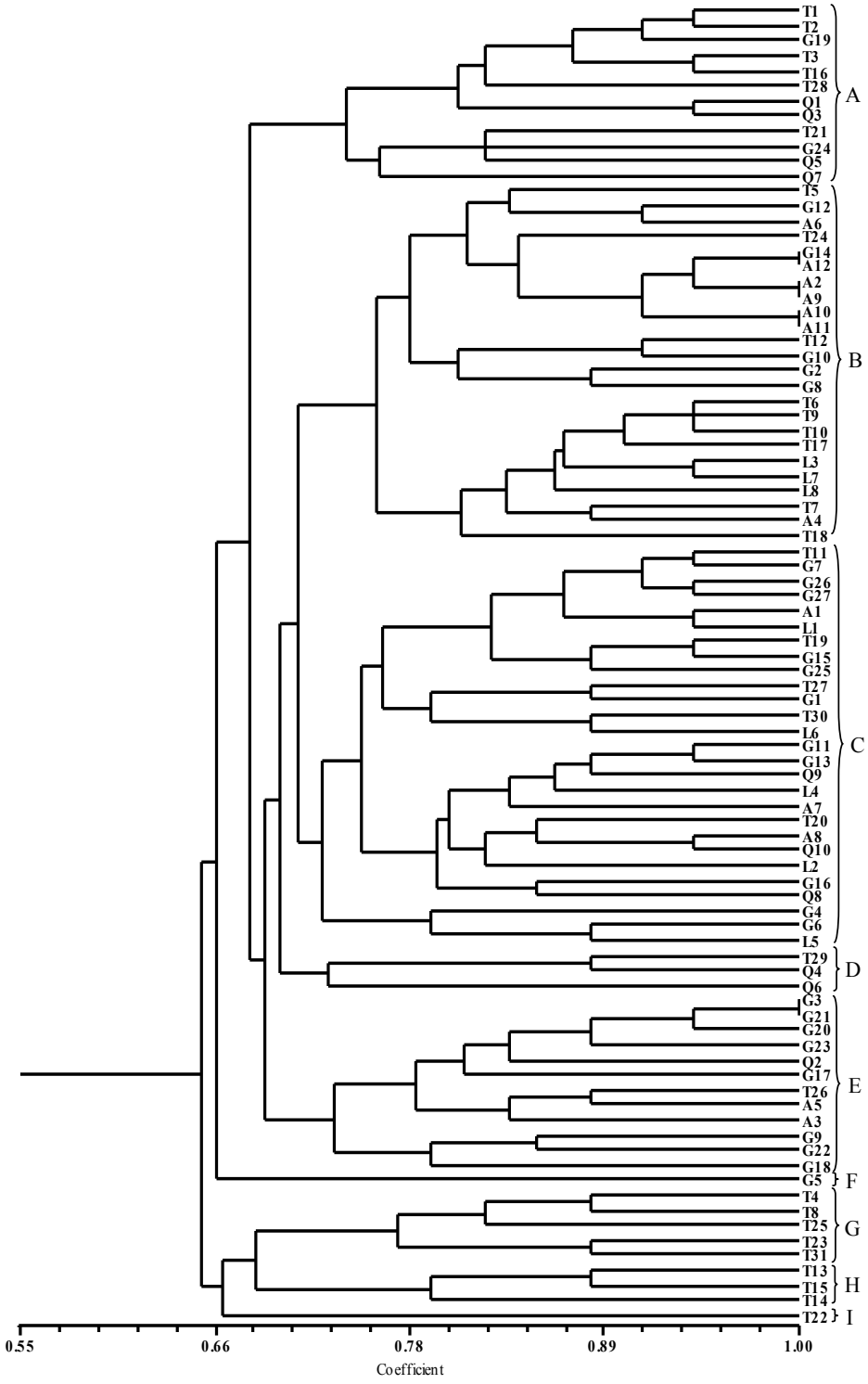

Figure 3. UPGMA analysis of 88 *Fusarium culmorum* isolates based on combined SSR data analysed using Nei and Li's similarity coefficient (Nei and Li, 1979). Isolate numbers contain the province from which they were collected: T (Tehran), G (Golestan), A (East Azarbaijan), Q (Qazvin) and L (Lorestan).

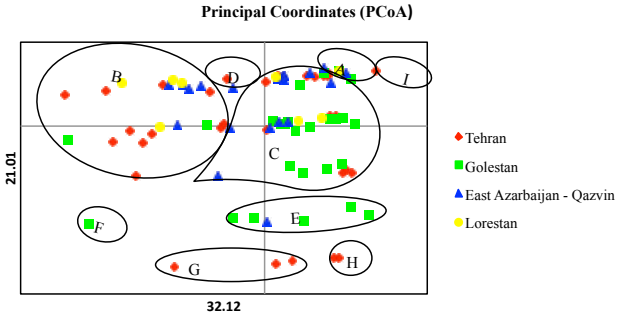

Figure 4. Principal coordinate analysis of 88 isolates among four *Fusarium culmorum* populations, based on SSR markers.

Cluster analysis

The combined data contained 33 SSR fragments, 32 (97%) of which were polymorphic (Table 3). The dendrogram (Figure 3) shows a trend of clustering of regional populations to some extent. The cluster analysis revealed that the 88 sampled isolates of *F. culmorum* clustered into nine distinct groups. The groups A, B, C, D, E, F, G, H, and I contained, respectively, 12, 24, 27, three, 12, one, five, three and 1 isolates (Figure 3). The clustering of the isolates based on SSR data exhibited a pattern of genetic variation that was slightly related with the geographical provinces of the isolates. Except for the Groups F and I, which each had only one isolate, and the Groups G and H, which came from Tehran, all the other groups had different isolates from different provinces. Genotypes of groups G and H were distinct from all the other genotypes.

The PCoA coordinated nine groups that were similar to cluster analysis (Figure 4). The first and second coordinates described 32 and 21% of the total variation of the SSR data, respectively, and summed up to 53% (Figure 4). This confirms the suitability of the SSR approach for genetic clustering. Nei's measures of genetic distance (Nei, 1972) (Table 5) indicated that the Tehran and Golestan populations had the least similarity (0.6859), and the East Azarbaijan-Qazvin and Golestan populations were most similar (0.8409) (Table 5).

Discussion

Based on eight polymorphic SSR markers, between 86 and 100% of the *F. culmorum* isolates collected from the four populations constituted distinct MLGs (Table 3). This genotypic diversity may be due to different ecological and genetic forces involved in differentiation of populations.

Some primer pairs did not produce any amplification products in some isolates of *F. culmorum*, possibly due to mutation in the primer binding sites of the genome (Owen *et al.*, 1998).

Genetic variance within and among populations, and gene migration between populations or subpopulations, are essential parameters to provide understanding of population ecology (Hartl and Clark, 2007). Our results show that most of the genetic variation (90%) occurred within populations (Table 4), indicating that field populations of *F. culmorum* in the study areas had high genetic diversity, even among isolates obtained from a single farm.

Diversity within populations was greater than between populations, suggesting possible importance of local inoculum sources. The residue-borne fungus *F. culmorum* can spread in the heads of some cereals (e.g., wheat and barley) (Jenkinson and Parry 1994). Macroconidia are an important source of *F. culmorum* inoculum causing FHB (Mitter *et al.*, 2006).

Table 5. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) between populations (Nei, 1972).

Population ID	Tehran	Golestan	East Azarbaijan-Qazvin	Lorestan
Tehran	****	0.6859	0.7591	0.8110
Golestan	0.3771	****	0.8409	0.7561
East Azarbaijan- Qazvin	0.2756	0.1733	****	0.8329
Lorestan	0.2094	0.2796	0.1829	****

Macroconidia do not travel very far in wind tunnel experiments (Fernando *et al.*, 2000) but animals may contribute to their spread.

The presence of straw in the soil generally leads to increased pathogen populations in soils (Bateman *et al.*, 1998). In the field, an increase in fecundity could increase the fitness of a particular *Fusarium* genotype, and in the absence of any negative selective pressure, that genotype could increase in the population (Bec, 2011). Selective factors, like host resistance, act on pathogen diversity causing shifts in the populations (Burdon and Silk, 1997).

In contrast to the 90% within-population genetic diversity, between-population genetic diversity constituted only 10% of the whole diversity (Table 4), the greatest genetic distance occurring between the Tehran and Golestan populations of *F. culmorum*.

The Φ_{PT} value obtained over four populations was 0.142 and highly significant (P<0.001) (Table 4), suggesting structuring among the populations. Although differences between populations are expressed by high Φ_{PT} values, these differences were minor compared to the variation within populations. Moreover, these differences reflect the required time for alleles to diffuse across the distance separating different populations (Zeller *et al.*, 2004).

Significant exchange of genotypes between field populations and⁄or high mutation rates can similarly result in high genetic diversity, especially if selective pressure is absent (Scott and Chakraborty, 2010). The high diversity between genotypes could be due to mutations in priming sites, re-arrangements of chromosomal segments or a recombination process in fungal genomes (Mishra *et al.*, 2003).

Nei's gene diversity and Shannon's index of diversity were the greatest for the isolates originating from the Tehran population. PCoA was consistent with the cluster analysis (Figure 3), confirming the genetic distinctiveness of genotypes G and H (Figure 4). High genetic variability in the Tehran population suggests the appearance of new genotypes in this population. New genotypes could emerge as a consequence of increased cropping of wheat (Hambleton *et al.*, 2002), or movement of *F. culmorum* onto this crop from other host plants. Regular gene flow and random mating between isolates from different populations could result in new genotypes with improved pathological and biological traits (Mishra *et al.*, 2006). Sources of genetic variability in *F. culmorum* populations include mutation, asexual recombination by anastomosis, or gene flow (Miedaner *et al.*, 2008). Which of these accounts for the diversity in *F. culmorum* populations in individual fields is a matter of speculation. Diversity could be explained by sexual recombination by a teleomorph that has not been detected. It is possible that a teleomorph either is undetected or has recently disappeared, a speculation substantiated by the presence of both mating type idiomorphs in *F. culmorum* (Toth *et al.*, 2004).

The ability to track genetic diversity in a pathogen population is an important aid in understanding the role of selective forces in the development of disease epidemics (Bec, 2011). SSR tools are suitable for explaining genetic diversity and population structures.

The frequency of genes that affect host adaptation without preference of particular host genotypes might increase over many cycles of recombination (McDonald *et al.*, 1999). Given the large diversity of *F. graminearum* and *F. culmorum* populations within small spatial scales, several resistance genes of different origin should be incorporated into the same host genotype in the long run (Miedaner *et al.*, 2001). Therefore, the identification of genotype groupings will allow the monitoring of changes in the population structure over time in response to management strategies, and will be useful for developing integrated strategies for disease management and breeding programmes. The success of plant breeding strategies to combat FCR epidemics depends on our ability to monitor and measure the degree of genetic diversity in pathogen populations over time.

Acknowledgements

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