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Molecular variability among isolates of *Mycosphaerella graminicola*, the causal agent of septoria tritici blotch, in Argentina

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Abstract The genetic structure and diversity of *Mycosphaerella graminicola* population were studied with ISSR molecular markers, using isolates from several locations of the Argentinean wheat region: subregion IV (SE of Buenos Aires Province) and II South (central part of Buenos Aires Province). Samples were taken from different bread wheat (*Triticum aestivum*) cultivars. A total of 126 isolates were subjected to molecular analysis to compare the genetic structure of the isolates from both wheat subregions. Ten ISSR primers were used: $(GACA)_4$; $(AAC)_7$; $(ATC)_7$; $(AC)_9$; $(AAG)_7$; $(AG)_9$; $(AGC)_5$; $(CAG)_5$, $(GTG)_5$ and $(GACAC)_3$. Eighty-four bands ranging from 200 bp to 8.000 were amplified. Eighty-one distinct haplotypes were identified and 43 isolates did not generate any amplification products. The highest number of polymorphic DNA fragments were produced using ISSR primers $(ATC)_7$ and $(GTG)_5$, which detected bands in 38 isolates. The molecular analysis revealed the existence of 81 different haplotypes among the 126 isolates studied. These results revealed a high degree of genetic diversity in the *M. graminicola* population in Argentina.

Keywords Dendrogram · Genetic diversity · Haplotypes · ISSR · Population

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Introduction

Bread wheat (*Triticum aestivum* L.) is the most widely grown and consumed food crop in the world. It is the staple food of nearly 35% of the world's population, and its demand will grow faster than for any other major crop (Rajaram 1999). *Mycosphaerella graminicola* (Fuckel) Schroeter in Cohn (anamorph *Septoria tritici* Rob. ex Desm.), which is a haploid, heterothallic ascomycete (Kema *et al*. 1996) and one of the most destructive fungal pathogens of wheat (King *et al*. 1983), causes septoria tritici blotch. Septoria tritici blotch causes economically significant yield losses in most of the wheat-growing areas of the world (31% to 54% in climates prone to disease development (Eyal *et al*. 1985)). In Argentina, Annone *et al*. (1991, 1993) reported yield losses from 20% to 50% and Simon *et al*. (1996) found reductions of 3% to 13% in the 1,000-kernel weight. Although several control methods, including the use of fungicides and other cultural practices, may

reduce the effect of septoria tritici blotch on yield, genetic resistance is the most cost-effective and environmentally safe technique for managing the disease (Simon *et al*. 2002, 2003).

The genetic structure of *M. graminicola* populations has been studied for over a decade (*e.g.* Schnieder *et al*. 2001; Zhan *et al.* 2003) and several molecular methods, such as RAPD, AFLP, SSRs and ISSR, have been used to analyze the diversity of this pathogenic fungus (Meng and Chen 2001; Sartorato 2004; Wagara *et al*. 2004). Microsatellites or SSR (Simple Sequence Repeats) are tandem repeat motifs composed of one to six nucleotides, which are ubiquitous, abundant and highly polymorphic in most eukaryotic genomes (Tautz and Renz 1984). ISSR (Intersimple Sequence Repeat) technique consists in the amplification of DNA sequences between SSR by means of anchored or non-anchored SSR homologous primers (Zietkiewicz *et al*. 1994). Unlike SSR, ISSR does not require previous knowledge of the sequence and generates specific and reproducible patterns due to the highly stringent conditions of the reaction (Bornet and Branchard 2001). The first studies with ISSR markers demonstrated the hypervariable nature of these markers (Wolfe and Liston 1998). The ISSR techniques are nearly identical to the RAPD techniques, except for the fact that ISSR primer sequences are designed from microsatellite regions and the annealing temperatures used are higher than those used for RAPD markers. Based on the published and in-progress studies conducted using ISSR markers, it is clear that these markers have great potential for studying natural populations (Wolfe *et al*. 1998).

Many studies using different molecular markers have shown that there is a high level of genetic diversity within the populations of *M. graminicola* and that the population is composed of many different genotypes. Czembor and Arseniuk (1999) studied different species of *Septoria* (*S. avenae* f.sp. *triticea, S. nodorum* and *S. tritici*) and found that SSR and ISSR markers are the most sensitive ones for the detection of DNA polymorphisms. Extensive population genetic analyses of *M. graminicola* have also been conducted with RFLP markers (Linde *et al*. 2002), and AFLP and RAPD markers have been used to construct a genetic linkage map (Kema *et al*. 2002). Schnieder *et al*. (2001) used AFLP markers to analyze one population of *M. graminicola* from Germany, and observed high within-population diversity and that the significant migration between populations prevented genetic isolation and differentiation of putative geographically separated populations. Working on a total of 90 isolates of *M. graminicola* from western Canada (University of Saskatchewan, Saskatoon) and using RAPD, Razavi and Hughes (2004) detected a high degree of DNA polymorphism with a large number of different molecular phenotypes. Kabbage *et al*. (2008) studied the genetic structure of populations of *M. graminicola* from Kansas at different spatial scales (micro-plot, macro-plot, and statewide) using AFLP and found genetic identities higher than 98% among populations. Tests for population subdivision revealed that 98% of the genetic diversity occurred within populations (Kabbage *et al*. 2008). By using AFLP analysis, Medini and Hamza (2008) revealed a high level of genetic diversity in populations of *M. graminicola* isolates, obtained no clones and found that each isolate showed a unique haplotype. Recently, Goodwin *et al*. (2007) analyzed a database of 30,137 EST (expressed sequence tag) sequences (Kema *et al*. 2003) from *M. graminicola* and identified 38 di- and 71 trinucleotide microsatellites with repeat numbers of six or more. Microsatellites that showed polymorphism between the parents of the *M. graminicola* mapping population were integrated into the existing genetic linkage map (Kema *et al*. 2002). The EST database provided an excellent source of new, highly polymorphic microsatellite markers that can be multiplexed for high-throughput genetic analyses of *M. graminicola* and related species.

In Argentina, *M. graminicola* has been studied with a limited set of isolates of the pathogen from some areas, using RFLP and it has been found that the pathogen has a high virulence degree variation (Cordo *et al*. 2006). Using RFLP, Jürgens *et al*. (2006) compared five populations from Los Hornos, Balcarce and Barrow (all in the Province of Buenos Aires) and determined that the populations from uninoculated fields in Argentina had higher gene and genotype diversities than those from inoculated fields. However, knowledge of the extent of the genetic diversity and of how the pathogen population may vary in different subregions in Argentina is still lacking.

Information about the genetic diversity of *M. graminicola* is very important because it could allow us to determine which genotypes predominate within

a certain geographic area and to evaluate the wheat germplasm resistance of Argentinean cultivars with isolates with high genetic differences. The aim of this study was to extend the study of the genetic structure and diversity of *M. graminicola* population to a large number of isolates from several locations of the wheat region, namely subregion IV (SE of Buenos Aires Province) and II South (central part of Buenos Aires Province), by means of ISSR molecular markers. Our hypotheses were that (*a*) the population of *M. graminicola* has a high genetic diversity and that groups with similar molecular patterns consist mainly of isolates belonging to the same wheat region, and thus, that differences among them are related to the geographic area; and (*b*) it is possible to select within them a huge set of completely different isolates to test the local wheat germplasm.

Materials and methods

Collection and multiplication of isolates Infected leaf tissues were randomly collected from different bread wheat (*Triticum aestivum*) cultivars growing in different locations in Argentina: Necochea and Tres Arroyos (wheat subregion IV) and 9 de Julio and Pla (wheat subregion II South) (Fig. 1). In addition, random samples were taken from the whole set of commercial cultivars grown in the trials of the National Argentinean Net of Cultivars (RET). At each location, the leaves were obtained from four different fields which were approximately 2 km apart from each other. Eight sites (two in each field) were sampled and ten to 15 leaves were collected in each site. All the samples from Necochea and Tres Arroyos were collected in 2005 and the most samples from 9 de Julio and Pla in 2007, except some samples from 9 de Julio which were collected in 2005. Leaves were disinfected using a routine disinfection technique (alcohol 70% for 10 s, 2% sodium hypochlorite commercial, 55 g Cl l^{-1} for 90 s and finally rinsed twice in fresh sterilized distilled water for 90 s each time).

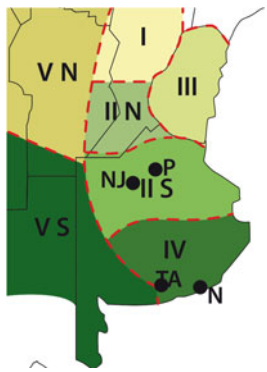

Fig. 1 Map of Argentinean Wheat Region showing the locations where *Mycophaerella graminicola* isolates were collected. *N* = Necochea; *NJ* = 9 de Julio; *P* = Pla; *T* = Tres Arroyos

Forty-two petri dishes with three leaves each were incubated for 2 days at room temperature (18–22°C). Then, one pycnidium was taken from each leaf and placed on a petri dish with yeast malt agar (YMA) broth (Eyal *et al*. 1987) at room temperature for 5–7 days. From single colonies, we obtained a total of 126 monosporic isolates (53 from Necochea, 13 from Tres Arroyos, 54 from 9 de Julio and 6 from Pla). The codes, names and origin of the *M. graminicola* isolates used are indicated in Table 1. The monosporic isolates were grown in yeast sucrose broth liquid media at room temperature (20–22°C) by shaking (150 rpm) for 10 days to obtain the spore suspension for further DNA extraction (McDonald and Martinez 1990). The fungal spores were lyophilized and stored at −20°C until DNA extraction.

DNA extraction, ISSR amplification and electrophoresis DNA was extracted from each isolate using a CTAB extraction protocol (Ferreira and Grattapaglia 1998). The concentration of DNA was measured with a fluorometer (Invitrogen Qubit™ Fluorometer). The stock solution of DNA (5 ng μl^{-1} concentration) was prepared for each isolate and stored at −20°C. DNA was amplified with the ISSR molecular technique. Ten ISSR primers were selected according to previous data (Czembor and Arseniuk 1999): $(GACA)_4$; $(AAC)_7$; $(ATC)_7$; $(AC)_9$; $(AAG)_7$; $(AG)_9$; $(AGC)_5$; $(CAG)_5$; $(GTG)_5$ and $(GACAC)_3$ with different annealing temperatures (Table 2).

Every 20 μl reaction mixture contained: 5× PCR buffer minus Mg^{-2} (Invitrogen, Carslbad, CA, USA), 25 mM $MgCl_2$ (Invitrogen), 200 μM each of dATP, dTTP, dCTP and dGTP (Promega, Madison, WI, USA), 5 mM ISSR primer (Promega), 0.5 U of Taq DNA Polymerase (Invitrogen) and approximately 5 ng of template DNA. Amplification was carried out in a thermocycler with gradient (Thermo Electron Corporation, Milford, MA, USA), programmed first at 95°C for 120 s, then for 40 cycles of denaturation at 94°C for 20 s and the annealing temperature for each ISSR primer for 20 s and elongation at 72°C for

Table 1 Codes, names, origins and year of collection of *Mycosphaerella graminicola* isolates used in this study. Letters following each code indicate the origin of each isolate (N: Necochea; TA: Tres Arroyos; P: Pla; NJ: 9 de Julio)

Isolate code	Name of isolate	Origin (location)	Year of collection	Argentinean wheat subregion
1N	FALP00105	Necochea	2005	IV
2N	FALP00205	Necochea	2005	IV
3N	FALP00305	Necochea	2005	IV
20N	FALP02005	Necochea	2005	IV
24N	FALP02405	Necochea	2005	IV
26N	FALP02605	Necochea	2005	IV
30N	FALP03005	Necochea	2005	IV
33N	FALP03305	Necochea	2005	IV
35N	FALP03505	Necochea	2005	IV
38N	FALP03805	Necochea	2005	IV
39N	FALP03905	Necochea	2005	IV
43N	FALP04305	Necochea	2005	IV
44N	FALP04405	Necochea	2005	IV
56N	FALP05605	Necochea	2005	IV
70N	FALP07005	Necochea	2005	IV
72N	FALP07205	Necochea	2005	IV
74N	FALP07405	Necochea	2005	IV
75N	FALP07505	Necochea	2005	IV
76N	FALP07605	Necochea	2005	IV
77N	FALP07705	Necochea	2005	IV
78N	FALP07805	Necochea	2005	IV
79N	FALP07905	Necochea	2005	IV
81N	FALP08105	Necochea	2005	IV
82N	FALP08205	Necochea	2005	IV
83N	FALP08305	Necochea	2005	IV
85N	FALP08505	Necochea	2005	IV
87N	FALP08705	Necochea	2005	IV
95N	FALP09505	Necochea	2005	IV
97N	FALP09705	Necochea	2005	IV
98N	FALP09805	Necochea	2005	IV
99N	FALP09905	Necochea	2005	IV
100N	FALP10005	Necochea	2005	IV
102N	FALP10205	Necochea	2005	IV
103N	FALP10305	Necochea	2005	IV
105N	FALP10505	Necochea	2005	IV
106N	FALP10605	Necochea	2005	IV
120N	FALP12005	Necochea	2005	IV
122N	FALP12205	Necochea	2005	IV
129N	FALP12905	Necochea	2005	IV
130N	FALP13005	Necochea	2005	IV
137N	FALP13705	Necochea	2005	IV
142N	FALP14205	Necochea	2005	IV
149N	FALP14905	Necochea	2005	IV
152N	FALP15205	Necochea	2005	IV
47TA	FALP04805	Tres Arroyos	2005	IV
49TA	FALP04905	Tres Arroyos	2005	IV
50TA	FALP05005	Tres Arroyos	2005	IV
52TA	FALP05205	Tres Arroyos	2005	IV
89TA	FALP08905	Tres Arroyos	2005	IV
93TA	FALP09305	Tres Arroyos	2005	IV
203P	FALP20307	Pla	2007	II South
205P	FALP20507	Pla	2007	II South
224P	FALP22407	Pla	2007	II South
201NJ	FALP20107	9 de Julio	2007	II South
202NJ	FALP20207	9 de Julio	2007	II South
208NJ	FALP20807	9 de Julio	2007	II South
214NJ	FALP21407	9 de Julio	2007	II South
215NJ	FALP21507	9 de Julio	2007	II South
217NJ	FALP21707	9 de Julio	2007	II South
225NJ	FALP22507	9 de Julio	2007	II South
226NJ	FALP22607	9 de Julio	2007	II South
227NJ	FALP22707	9 de Julio	2007	II South
228NJ	FALP22807	9 de Julio	2007	II South
229NJ	FALP22907	9 de Julio	2007	II South
230NJ	FALP23007	9 de Julio	2007	II South
232NJ	FALP23207	9 de Julio	2007	II South
236NJ	FALP23607	9 de Julio	2007	II South
237NJ	FALP23707	9 de Julio	2007	II South
238NJ	FALP23807	9 de Julio	2007	II South
239NJ	FALP23907	9 de Julio	2007	II South
244NJ	FALP24407	9 de Julio	2007	II South
247NJ	FALP24707	9 de Julio	2007	II South
248NJ	FALP24807	9 de Julio	2007	II South
249NJ	FALP24907	9 de Julio	2007	II South
250NJ	FALP25007	9 de Julio	2007	II South
253NJ	FALP25307	9 de Julio	2007	II South
254NJ	FALP25405	9 de Julio	2005	II South
255NJ	FALP25505	9 de Julio	2005	II South
260NJ	FALP26005	9 de Julio	2005	II South
265NJ	FALP26505	9 de Julio	2005	II South
291NJ	FALP29105	9 de Julio	2005	II South
292NJ	FALP29205	9 de Julio	2005	II South
293NJ	FALP29305	9 de Julio	2005	II South

120 s. The final primer elongation segment of the run was extended to 12 min at 72°C (Czembor and Arseniuk 1999). Each reaction was performed at least twice.

Table 2 ISSR primers used in this study

Primer code	Primer sequence 5′→3′	Annealing temperature (°C)
(GACA)4	GACAGACAGACAGACA	44
(AAC)7	AACAACAACAACAACAACAAC	48
(ATC)7	ATCATCATCATCATCATCATC	44
(AC)9	ACACACACACACACACAC	54
(AAG)7	AAGAAGAAGAAGAAGAAGAAG	48
(AG)9	AGAGAGAGAGAGAGAGAG	44
(AGC)5	AGCAGCAGCAGCAGC	48
(CAG)5	CAGCAGCAGCAGCAG	48
(GTG)5	GTGGTGGTGGTGGTG	48
(GACAC)3	GACACGACACGACAC	44

PCR products were separated on 2% agarose gels in 1X TBE buffer at 150 V for 4.30 h. Two molecular weight markers (100 bp and 1 kb) were included. The products were stained with SyBr-Green (Invitrogen) (1:100) and DNA banding patterns were visualized under UV light and photographed on a transilluminator.

Data analysis Banding patterns were scored for the presence (1) or absence (0) of bands for each ISSR–primer–isolate combination and data were entered into a binary matrix (similarity matrix). In the similarity matrix, a value of 1 indicated maximum similarity (identical isolates) and a value of 0 indicated minimum similarity (completely different isolates).

All pairs of isolates were then compared applying Dice's coefficient (Nei and Li 1979). The similarity matrix was then used to construct a dendrogram using the unweighted pair group method of arithmetic average (UPGMA) with the NTSyS-pc software (Rohlf 1998) (Fig. 2). Genotype diversity (G) in each population was calculated according to Stoddart and Taylor (1988). The gene flow between the subregions was estimated by the method described by Nei (1972), who estimated the average number of individuals per generation that migrate between the populations.

Results

All primers generated polymorphisms. Eighty-four polymorphic bands ranging from 200 bp to 8.000 were generated by the ten ISSR primers used. From a total of 126 isolates, 83 isolates were amplified and 81 distinct genotypes were identified. Isolates 74N and 250NJ and isolates 205P and 208NJ showed identical patterns. On the other hand, 43 isolates did not generate any amplification products with the ten ISSR primers used. Fourteen of those 43 isolates belonged to Necochea, two to Tres Arroyos (subregion II South), 24 to 9 de Julio and three to Pla (subregion IV). The highest number of polymorphic DNA fragments were produced using ISSR primers $(ATC)_7$ and $(GTG)_5$. These ISSR primers detected 84 polymorphic bands in 38 isolates among the 126 isolates analyzed. On the other hand, the ISSR primer $(CAG)_5$ separated only ten isolates.

The dendrogram generated by the similarity matrix based on Dice's coefficient and the UPGMA clustering algorithm showed that at the 0.20 similarity level, 13 distinct clusters (I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII and XIII) were detected (Fig. 2). The 13 clusters formed in the dendrogram and their respective isolates are shown in Table 3. Cluster I, within which isolates 102N and 103N, and 202NJ and 236NJ were the most related (distance coefficient 0.75), included 50 isolates. Cluster II, within which 74N and 250NJ were identical and 137N and 149N were the most related (distance coefficient 0.75), was composed of 12 isolates. Cluster III included only two isolates from Necochea, separated by a distance coefficient of 0.52. In cluster IV, the isolates were related by a genetic distance of 0.50, and isolates 205P and 208NJ were identical (genetic distance 1.00). Clusters V and IX were formed by two isolates related by 0.34 and 0.23 distance coefficient, respectively, whereas cluster VI was formed by only one isolate. Cluster VIII included isolates 201NJ, 214NJ and 203P and cluster IX was formed by two isolates: 93TA and 254NJ, separated by

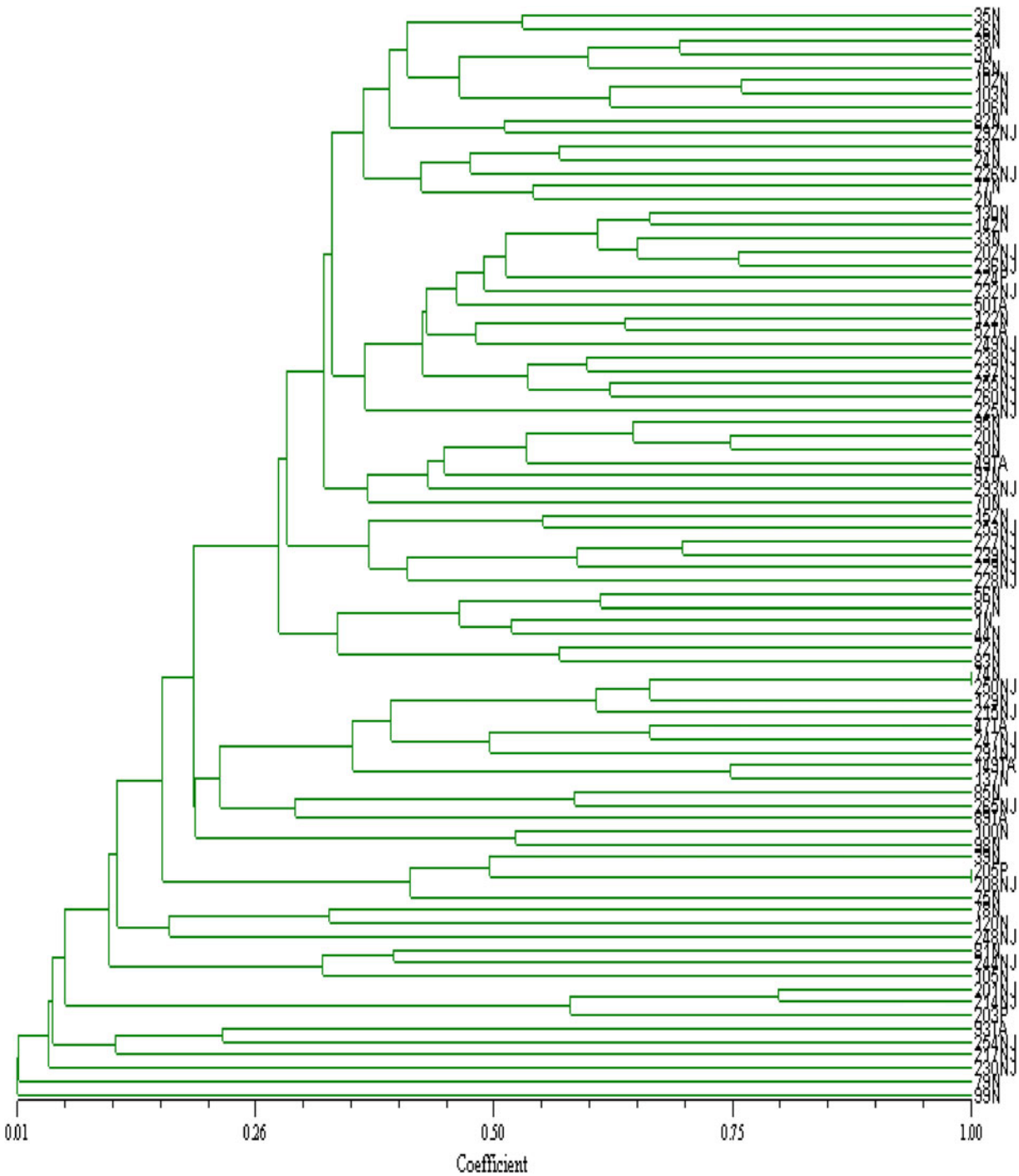

Fig. 2 Cluster analysis dendrogram for 83 isolates of *Mycosphaerella graminicola*, based on ISSR molecular markers using the unweighted pair group method of arithmetic average (UPGMA) with the NTSyS-pc software

a 0.23 distance coefficient. Finally, clusters X, XI, XII and XIII, which were formed by the isolates 217NJ, 230NJ, 79N and 99N, respectively, had the largest genetic distance from the other groups.

The maximum similarity was approximately 100% between isolates 74N and 250NJ and between isolates 205P and 208NJ. Each isolate was considered identical with its pair. The minimum similarity was

Table 3 Cluster analysis and isolates in each cluster

	Isolates	Origin
Cluster I	1, 2, 3, 20, 24, 26, 30, 33, 35, 38, 43, 44, 49, 56, 70, 72, 76, 77, 82, 83, 87, 95, 97, 102, 103, 106, 122, 130, 142, 152, 225, 227, 239, 253 and 293.	Necochea
	50 and 52	Tres Arroyos
	202, 226, 228, 229, 232, 236, 237, 238, 249, 255, 260 and 292	9 de Julio
	224	Pla
Cluster II	74, 85, 137 and 149	Necochea
	215, 247, 250, 265 and 291	9 de Julio
	47 and 89	Tres Arroyos
Cluster III	98 and 100	Necochea
Cluster IV	39 and 75	Necochea
	205	Pla
	208	9 de Julio
Cluster V	78 and 120	Necochea
Cluster VI	248	9 de Julio
Cluster VII	81 and 105	Necochea
	244	9 de Julio
Cluster VIII	201 and 214	9 de Julio
	203	Pla
Cluster IX	93	Tres Arroyos
	254	9 de Julio
Cluster X	217	9 de Julio
Cluster XI	230	9 de Julio
Cluster XII	79	Necochea
Cluster XIII	99	Necochea

~1% and separated isolate 99N from the rest, indicating that this isolate is the most different from the others. Genotype diversity was always greater in the subregion IV population than in the subregion II South population. Genotype diversity was G=21.73% in the subregion IV population and G=7.86% in the subregion II South population when we considered all the isolates (including those that had no amplification), assuming that those that had no amplification were different from the rest. Differences between populations were significant (test P=0.01). When the isolates that did not amplify were not included, G was 100% for the population from subregion IV and 94.3% for that from subregion II South (Table 4). The amount of gene flow among populations (Nm) across all loci was 7.21 when all the isolates were included, indicating that more than seven individuals moved between subregions every generation. Nm across all loci was 11.37 when we included only the isolates that amplified, indicating that more than 11 individuals moved between subregions every generation.

Discussion

The ISSR markers used in this study revealed a high degree of genetic diversity within the *M. graminicola* population and detected 81 different molecular genotypes among the 83 isolates amplified [Genotype diversity (G=21.73% or 100% (subregion IV) and 7.86% or 94.3% (subregion II South)]. Our results are in agreement with previous works carried out in other countries, where different degrees of genetic diversity among *M. graminicola* populations were found by using different molecular markers (Schnieder *et al*. 2001; Zhan *et al*. 2003). High genotypic diversities have been detected in different populations analyzed using RFLP (Linde *et al*. 2002) and AFLP (Kabbage *et al*. 2008).

Table 4 Information on two populations of *Septoria tritici* from two Argentinean wheat subregions

	Subregion IV	Subregion II South
Total No. of isolates	67	60
Isolates that amplified	50	33
Isolates that did not amplify	17	27
No. of genotypes	50	31
Genotypic diversity (including all isolates)	21.73	7.86
Genotypic diversity (including isolates that did not amplify)	100	94.3

Our analysis showed that there was higher genotype diversity in the population from wheat subregion IV than in that from subregion II South. Our results are in agreement with those of Jürgens *et al*. (2006) and Cordo *et al*. (2006), who found different rates of genotype diversity when comparing populations in Argentina using RFLP. Jürgens *et al*. (2006) studied five populations from three different locations: Los Hornos (ARG1 and ARG5), Balcarce (ARG2) and Barrow (ARG3 and ARG4), and found that 65% of all isolates were unique genotypes. Likewise, Cordo *et al*. (2006) studied the populations from Balcarce (subregion IV) and Los Hornos (subregion II South), and found 39 genotypes among 62 isolates of the pathogen in Balcarce and 35 unique genotypes among a total of 58 isolates in Los Hornos. Genotype diversity was greater in the Balcarce population (G=31.61%) than in the Los Hornos population (G=26.19%). Los Hornos exhibited a high level of clonality because it is not an endemic area for the leaf blotch of wheat and artificial inoculation with the pathogen, which causes a low genotypic diversity, is normally used. Instead, in our study, we took isolates from naturally infected wheat fields from a wider region and found that the level of clonality was low within both populations and that only four genotypes were identical. A high level of diversity in the nuclear genome is typical of field populations of *M. graminicola* (Zhan *et al*. 2003).

In this study, we obtained only four identical genotypes and 81 different genotypes. The cluster analysis showed that the maximum similarity between isolates of *M. graminicola* was 100%. In agreement with our results, Razavi and Hughes (2004), using RAPD molecular markers in a *M. graminicola* population from western Canada, found 100% genotypic diversity among a total of 87 isolates and showed that the maximum similarity between isolates of *M. graminicola* was approximately 81% and that there were no identical isolates.

In addition, our results indicate differences between isolates from wheat subregion IV and isolates from wheat subregion II South. We assumed that most of the variation can be attributed to a different composition of the population in both subregions. Furthermore, it is necessary to take into account that isolates for both subregions were randomly taken from all the commercial cultivars in different years, which could have added more variation between the subregions. Jürgens *et al*. (2006) also found that the Argentinean populations ARG1, ARG2 and ARG3 differed significantly in genotype diversity.

The consideration of the genetic variation of *M. graminicola* populations is essential to understand the virulence in the different cultivars. Differences around the world could be attributed to factors such as variations in the regular recombination, different migration patterns, and presence and importance of the sexual form. The sexual reproduction of *M. graminicola*, which is known to be present in Argentina (Cordo *et al*. 1990), allows the existence of a large number of genetically diverse isolates. Populations in this fungus are in genetic equilibrium as well as in drift migration equilibrium (Chen and McDonald 1996), attributed to a high rate of sexual recombination (Cordo *et al*. 2006). In most of the wheat areas of the world, the sexual form of *M. graminicola* has been reported as a primary source of inoculum. Regular sexual reproduction provides this pathogen with a mechanism to generate novel allele combinations rapidly, whereas asexual reproduction, gene flow, and natural selection ensure the maintenance and rapid dissemination of allele combinations with highest fitness (Zhan and McDonald 2004).

In the present study 84 different bands, which ranged from 8.000 to 200 bp, were generated. In contrast, Czembor and Arseniuk (1999) reported bands from 2.176 to 154 bp, whereas others reported bands from 2.300 to 200 bp (Boeger *et al*. 1993; Chen *et al*. 1994). Our results showed that the ISSR primers $(ATC)_7$ and $(GTG)_5$ generated the highest polymorphism between isolates and detected polymorphic bands in 38 isolates among the 83 isolates amplified. Czembor and

Arseniuk (1999) reported similar results with the ISSR primer $(ATC)_7$. The results of this study agree with those of Czembor and Arseniuk (1999) about the utility of ISSR molecular markers for polymorphic detection among *M. graminicola* isolates.

The fact that 43 isolates produced no amplification with this set of primers could have been the result of many factors, such as the absence of repeat sequences, and the mispriming due to sequence differences in priming site, etc. Therefore, these isolates should be tested with another set of primers in order to determine whether they are identical isolates. After repeating these isolates at least four times we assumed that they are identical for the set of primers used in this work. We found that most of the isolates from the wheat subregion IV amplified with at least one primer and were located in cluster I, whereas 24 of the 54 isolates from 9 de Julio and three of the six isolates of Pla did not amplify with any of the primers used in this study, indicating that they most likely belong to different clusters. This seems to indicate that the isolates from 9 de Julio and Pla (wheat subregion II South) probably have molecular patterns different from those from Necochea and Tres Arroyos (wheat subregion IV). However, there is an important gene flow (Nm=7.21 or 11.37) between populations from subregion II South and subregion IV. Schnieder *et al*. (2001) also assumed constant gene flow over five populations from Germany. Also, working with Argentinean populations, Jürgens *et al*. (2006) suggested regional gene flow between populations ARG1 (Los Hornos–subregion II South) and ARG2 (Balcarce–subregion IV). The sexual ascospores of *M. graminicola* have the potential to move at least several hundred meters (Shaw and Royle 1989), perhaps over tens of kilometers, indicating their potential as a source of genetic exchange between spatially distant populations.

The work presented here has laid the foundation for future studies by providing valuable information on the genetic diversity of the Argentinean population of *M. graminicola*. The knowledge about the genetic structure of the Argentinean population of *M. graminicola* was expanded to more isolates in different wheat subregions. Also, differences in the genetic structure of the population in some locations of the subregions II South and IV were noted, reinforcing the findings of previous works. The high degree of genetic diversity among isolates compared in this study characterizes a diverse population of *M. graminicola* in some locations of the two Argentinean wheat subregions. Better knowledge of the pathogen population may help in the development of a successful disease management program, particularly in the production of resistant cultivars, effective fungicides and biological control agents (Moreno *et al*. 2008). Thus, the findings of this work may help to select a huge set of completely different isolates with different molecular patterns to test a set of Argentinean cultivars and foreign lines with known resistance.

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