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Characterization of a *Fusarium poae* world-wide collection by using molecular markers

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Abstract *Fusarium poae* has been considered as a minor species among those that cause Fusarium Head Blight (FHB) disease but in recent years several researchers have documented a high frequency of occurrence of this species. In this study, a total of 173 *F. poae* isolates from Argentina, Belgium, Canada, England, Finland, France, Germany, Hungary, Italy, Luxembourg, Poland, Switzerland and Uruguay were evaluated by using inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP) to evaluate genetic variability within *F. poae* and to amplify MAT idiomorphs as a possible mechanism that could explain part of the variability found in this species. The molecular analysis obtained from both molecular markers showed a high intraspecific variability. However, a partial clustering between *F. poae* isolates and their geographic origin was obtained by ISSR markers while AFLP showed isolates from different geographic locations distributed throughout the dendrogram. Moreover, ISSR grouped all the *F. poae* isolates into a different cluster from the *F. langsethiae* and *F. sporotrichioides* isolates used as outgroups compared with the dendrogram obtained using AFLP markers. Analysis of molecular variance (AMOVA) indicated a high genetic variability in the *F. poae* collection, with most of the genetic variability resulting from differences within, rather than between, American and European populations by using both molecular markers. Regarding MAT idiomorphs, for most *F. poae* isolates both MAT-1 and MAT-2 were present from each isolate.

Keywords AFLP · *Fusarium poae* · Genetic variability · ISSR · MAT

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Introduction

Fusarium Head Blight (FHB) is a disease of small grain cereals that reduces production and quality of barley, wheat and oat. FHB is especially important because of the ability of *Fusarium* species to produce mycotoxins harmful to human and animal health.

The most frequent *Fusarium* species isolated from grain exhibiting FHB symptoms are *Fusarium graminearum*, *F. poae*, *F. avenaceum* and *F. culmorum* (Nicholson et al. 2003). *F. poae* has been considered as a minor species among the FHB disease complex but in recent years several researchers have documented a high frequency of occurrence (Audenaert et al. 2009; Lindblad et al. 2013; Stenglein et al. 2012). However, there have been few reports of diversity of *F. poae* populations.

Basic knowledge of the population structure of plant pathogens is needed to know fungal adaptability sources, to understand the behaviour of the pathogen in response to new agriculture practices and to elucidate differences according to geographic origin or mycotoxin production (Chandra Nayaka et al. 2011). Several molecular markers have been used to genetically characterize *Fusarium* populations such as inter simple sequence repeats (ISSR) in *F. culmorum* and *F. poae* (Dinolfo et al. 2010; Mishra et al. 2003); amplified fragment length polymorphism (AFLP) in *F. poae, F. graminearum* and *F. asiaticum* (Akinsanmi et al. 2008; Astolfi et al. 2011; Qu et al. 2008; Somma et al. 2010); simple sequence repeats (SSR) in *F. poae* (Vogelgsang et al. 2010); random amplification of polymorphic DNA (RAPD) in *F. graminearum* and *F. poae* (Kerényi et al. 1997; Ouellet and Seifert 1993).

ISSR is a useful tool that, like AFLP, requires no prior DNA sequence knowledge for PCR amplification. For this reason these types of molecular markers are known as anonymous DNA markers or sequence non-specific techniques (Agarwal et al. 2008; Leslie et al. 2007). ISSR is a simple and robust technique that allows the identification of genome regions between microsatellite loci with the potential for producing high levels of genomic information (Chandra Nayaka et al. 2011; Su et al. 2008). AFLP combines restriction enzyme digestion with PCR technology (Vos et al. 1995). This technique generates fingerprints with multiple fragments derived from throughout the genome, resulting in a high degree of discrimination and identification of *Fusarium* species (Akinsanmi et al. 2008; Alvarez et al. 2011; Astolfi et al. 2011).

Genetic maps of phytopathogenic fungi have been built with the help of DNA markers and genomic sequence information, including the mating-type locus (MAT) which determines sexual compatibility of heterothallic fungi (Manzo-Sánchez et al. 2007). The two idiomorphs at this locus share no sequence similarity: the product of the MAT-1 idiomorph has a conserved α-domain whereas the product of MAT-2 has a conserved HMG domain (high mobility group) indicating they encode transcription factors (Conde-Ferráez 2007). However, the homothallic *Fusarium graminearum* species contains both MAT-1 and MAT-2 loci in the genome (Yun et al. 2000). Kerényi et al. (2004) used these regions to design primers in order to determine MAT-1 and MAT-2 not only in *Fusarium* species known to have sexual stages, but also in *Fusarium* species in which sexual reproduction is unknown such as *F. poae*, showing that *F. poae* is presumably capable of heterothallic mating.

One of the aims of the present study was to evaluate the genetic variability in a world-wide collection of *Fusarium poae* isolates by using ISSR and AFLP markers and, as studies on the potential for mating could increase the knowledge of *Fusarium* species to maintain their diversity (Kerényi et al. 2004) another objective was to amplify MAT idiomorphs as a possible mechanism that could help to explain the variability found in this species.

Materials and methods

Fungal isolates and genomic DNA extraction

A total of 173 monosporic *Fusarium poae* isolates from Argentina, England, Italy, Finland, Switzerland, Poland, Germany, Canada, Belgium, France, Hungary, Luxembourg and Uruguay and two isolates of *F. langsethiae* (CC321) and *F. sporotrichioides* (F95) used as out groups, were evaluated in this study (Table 1). All *Fusarium* isolates were conserved on Spezieller Nährstoffarmer Agar (SNA) slants according to Leslie and Summerell (2006).

Total genomic DNA of all isolates was extracted using the cetyltrimethylammonium bromide (CTAB) method according to Stenglein and Balatti (2006). DNA concentrations were calculated using a fluorometer (Qubit Fluorometer, Invitrogen). All *Fusarium poae* isolates were identified by PCR using species-specific primers according to Parry and Nicholson (1996).

ISSR markers

The ISSR-PCR was performed with six ISSR primers $(CTC(GT)_8$, $(AG)_8$, $(CAC)_5$, $CT(GA)_8$, $AGG(CT)_8$ and $(GCC)_5)$ that were selected based on the results of initial screening of 25 ISSR primers against a set of 30 representative isolates of *Fusarium poae*. PCR was performed in a XP thermal cycler (Bioer Technology Co, Hangzhou, China) according to Dinolfo et al. (2010). The annealing temperature was 48 °C for the first two primers, 53 °C for the following three and 66 °C for the last one listed above. The amplification products were separated by vertical electrophoresis using 4 % polyacrylamide gel with 5 M urea for denaturing DNA. Gel fixing, staining and developing followed to visualize the

Table 1 *Fusarium* isolates used in this study and MAT amplifications

Isolate	Figures isolate code	Host	Geographic origin	City or region	Year of isolation	MAT 1	MAT 2
HSu1a	Fp-Ar1	*Hordeum vulgare* L.	Argentina	Coronel Suarez	2006	−	+
HPu1a	Fp-Ar2	*Hordeum vulgare* L.	Argentina	Puán	2006	+	−
HPu4a	Fp-Ar3	*Hordeum vulgare* L.	Argentina	Puán	2007	+	−
HTa1a	Fp-Ar4	*Hordeum vulgare* L.	Argentina	Tres Arroyos	2007	+	−
HBe1a	Fp-Ar5	*Hordeum vulgare* L.	Argentina	Belloq	2007	+	−
HBe1c	Fp-Ar6	*Hordeum vulgare* L.	Argentina	Belloq	2007	+	+
HBig1a	Fp-Ar7	*Hordeum vulgare* L.	Argentina	Bigand	2007	+	+
TCa1a	Fp-Ar8	*Triticum aestivum* L.	Argentina	Castelar	2005	−	+
TSS1b	Fp-Ar9	*Triticum aestivum* L.	Argentina	Sancti Spiritu	2006	+	+
TSS2a	Fp-Ar10	*Triticum aestivum* L.	Argentina	Sancti Spiritu	2006	+	−
THo1b	Fp-Ar11	*Triticum aestivum* L.	Argentina	Los Hornos	2005	+	+
THo1d	Fp-Ar12	*Triticum aestivum* L.	Argentina	Los Hornos	2005	−	+
TPu1a	Fp-Ar13	*Triticum aestivum* L.	Argentina	Puán	2005	+	−
TPu1c	Fp-Ar14	*Triticum aestivum* L.	Argentina	Puán	2005	+	+
TSa1a	Fp-Ar15	*Triticum aestivum* L.	Argentina	Saladillo	2007	+	−
TSm1a	Fp-Ar16	*Triticum aestivum* L.	Argentina	San Manuel	2007	+	+
TBig1a	Fp-Ar17	*Triticum aestivum* L.	Argentina	Bigand	2007	−	+
TMa1a	Fp-Ar18	*Triticum aestivum* L.	Argentina	25 de Mayo	2007	+	+
TJu1a	Fp-Ar19	*Triticum aestivum* L.	Argentina	Junín	2007	+	−
TPe1a	Fp-Ar20	*Triticum aestivum* L.	Argentina	Pergamino	2007	−	+
LSP1a	Fp-Ar21	*Lycopersicum esculentum* L.	Argentina	San Pedro	2003	+	+
MICA-T-01	Fp-Ar22	*Triticum aestivum* L.	Argentina	Bragado	NN	+	+
MICA-T-04	Fp-Ar23	*Triticum aestivum* L.	Argentina	Arrecifes	NN	+	+
MICA-T-06	Fp-Ar24	*Triticum aestivum* L.	Argentina	San Antonio de Areco	NN	+	+
MICA-T-07	Fp-Ar25	*Triticum aestivum* L.	Argentina	Junín	NN	+	−
MICA-A-01	Fp-Ar26	*Avena sativa* L.	Argentina	Gualeguaychu	NN	+	−
MICA-A-02	Fp-Ar27	*Avena sativa* L.	Argentina	Gualeguaychu	NN	+	+
MICA-A-03	Fp-Ar28	*Avena sativa* L.	Argentina	Urdinarrain	NN	+	+
MICA-A-04	Fp-Ar29	*Avena sativa* L.	Argentina	Urdinarrain	NN	+	+
MICA-A-05	Fp-Ar30	*Avena sativa* L.	Argentina	Basavilbaso	NN	+	+
4/4343/1	Fp-En1	*Triticum aestivum* L.	England	Suffolk	NN	−	+
1021	Fp-En2	*Triticum aestivum* L.	England	Lancashire	2006	+	−
887	Fp-En3	*Triticum aestivum* L.	England	W. Yorshire	2006	+	−
747	Fp-En4	*Triticum aestivum* L.	England	Buckinghamshire	2003	+	+
736	Fp-En5	*Triticum aestivum* L.	England	Norfolk	2003	+	+
731	Fp-En6	*Triticum aestivum* L.	England	Hereford & Worcester	2003	+	+
721	Fp-En7	*Triticum aestivum* L.	England	Humberside	2003	+	−
720	Fp-En8	*Triticum aestivum* L.	England	Merseyside	2003	+	+
718	Fp-En9	*Triticum aestivum* L.	England	Northumberland	2003	+	+
563	Fp-En10	*Triticum aestivum* L.	England	Oxforshire	2002	+	+
552	Fp-En11	*Triticum aestivum* L.	England	Surrey	2002	+	+

Table 1 (continued)

Isolate	Figures isolate code	Host	Geographic origin	City or region	Year of isolation	MAT 1	MAT 2
527	Fp-En12	*Triticum aestivum* L.	England	County Durham	2002	+	+
522	Fp-En13	*Triticum aestivum* L.	England	Cambridgesgire	2002	+	−
506	Fp-En14	*Triticum aestivum* L.	England	Somerset	2002	+	+
444	Fp-En15	*Triticum aestivum* L.	England	Hereford	2002	+	−
303	Fp-En16	*Triticum aestivum* L.	England	Kent	2001	+	+
300	Fp-En17	*Triticum aestivum* L.	England	Nottinghamshire	2001	+	+
295	Fp-En18	*Triticum aestivum* L.	England	Norfolk	2001	+	+
173	Fp-En19	*Triticum aestivum* L.	England	Hampshire	1998	+	+
141	Fp-En20	*Triticum aestivum* L.	England	Wiltshire	1998	+	+
F18	Fp-En21	*Triticum aestivum* L.	England	Cambridge	NN	+	−
F600	Fp-En22	*Triticum aestivum* L.	England	NN	NN	+	+
4/3084/1	Fp-En23	*Triticum aestivum* L.	England	Surrey	NN	+	+
4/3084/2	Fp-En24	*Triticum aestivum* L.	England	Surrey	NN	+	−
4/3084/3	Fp-En25	*Triticum aestivum* L.	England	Surrey	NN	+	+
4/3084/4	Fp-En26	*Triticum aestivum* L.	England	Surrey	NN	+	+
I14-99	Fp-It	*Triticum aestivum* L.	Italy	NN	1999	+	+
6025	Fp-Fi1	*Triticum aestivum* L.	Finland	Ylistaro	2006	+	+
6070	Fp-Fi2	*Avena sativa* L.	Finland	Maaninka	2006	+	+
6101	Fp-Fi3	*Avena sativa* L.	Finland	Rovaniemi	2006	−	+
6107	Fp-Fi4	*Triticum aestivum* L.	Finland	Mikkeli	2006	+	+
6129	Fp-Fi5	*Hordeum vulgare* L.	Finland	Kymenlaakso	2006	−	+
6159	Fp-Fi6	*Avena sativa* L.	Finland	Varsinais-Suomi	2006	−	+
6191	Fp-Fi7	*Avena sativa* L.	Finland	Pirkanmaa	2006	+	+
6273	Fp-Fi8	*Secale cereale* L.	Finland	Piikio	2006	+	+
6326	Fp-Fi9	*Hordeum vulgare* L.	Finland	Satakunta	2006	+	+
6947	Fp-Fi10	*Hordeum vulgare* L.	Finland	Jokionen	2006	−	+
6085	Fp-Fi11	*Triticum aestivum* L.	Finland	Jokionen	2006	−	+
6183	Fp-Fi12	*Hordeum vulgare* L.	Finland	North Osthrobotnia	2006	+	+
Fp0335	Fp-Sw1	*Triticum aestivum* L.	Switzerland	Solothurn	2003	−	+
Fp0338	Fp-Sw2	*Triticum aestivum* L.	Switzerland	Belpberg	2003	+	+
Fp0378	Fp-Sw3	*Triticum aestivum* L.	Switzerland	Seedorf	2003	−	+
Fp0503	Fp-Sw4	*Triticum aestivum* L.	Switzerland	Ruppoldsried	2005	+	+
3107	Fp-Po1	*Triticum aestivum* L.	Poland	NN	2003	+	−
6410	Fp-Po2	*Triticum aestivum* L.	Poland	NN	2006	+	−
6408	Fp-Po3	*Triticum aestivum* L.	Poland	NN	2006	+	−
61401	Fp-Po4	*Triticum aestivum* L.	Poland	NN	2006	+	−
6402	Fp-Po5	*Triticum aestivum* L.	Poland	NN	2006	−	+
60902	Fp-Po6	*Triticum aestivum* L.	Poland	NN	2006	+	−
F730	Fp-Ge1	*Triticum aestivum* L.	Germany	NN	NN	+	−
F732	Fp-Ge2	*Triticum aestivum* L.	Germany	NN	NN	+	−
F733	Fp-Ge3	*Triticum aestivum* L.	Germany	NN	NN	+	−

Table 1 (continued)

Isolate	Figures isolate code	Host	Geographic origin	City or region	Year of isolation	MAT 1	MAT 2
P1	Fp-Ca1	*Triticum aestivum* L.	Canada	Manitoba	2008	+	−
P2	Fp-Ca2	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P3	Fp-Ca3	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P4	Fp-Ca4	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	−
P5	Fp-Ca5	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P6	Fp-Ca6	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P7	Fp-Ca7	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	+
P8	Fp-Ca8	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P9	Fp-Ca9	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P10	Fp-Ca10	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P11	Fp-Ca11	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	−
P12	Fp-Ca12	*Triticum aestivum* L.	Canada	Saskatchewan	2008	−	+
P13	Fp-Ca13	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	−
P14	Fp-Ca14	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	−
P15	Fp-Ca15	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	+
P16	Fp-Ca16	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	+
P17	Fp-Ca17	*Triticum aestivum* L.	Canada	Saskatchewan	2008	+	+
P18	Fp-Ca18	*Triticum aestivum* L.	Canada	Alberta	2008	+	+
P19	Fp-Ca19	*Triticum aestivum* L.	Canada	Alberta	2008	+	+
P20	Fp-Ca20	*Triticum aestivum* L.	Canada	Alberta	2008	+	+
P21	Fp-Ca21	*Triticum aestivum* L.	Canada	Alberta	2008	+	+
P22	Fp-Ca22	*Triticum aestivum* L.	Canada	Alberta	2008	+	−
P23	Fp-Ca23	*Triticum aestivum* L.	Canada	Ontario	2008	+	+
GE1	Fp-Ge4	*Triticum aestivum* L.	Germany	Dedelow (Uckermarck)	2001	+	+
GE2	Fp-Ge5	NN	Germany	Bonn	2001	−	+
GE3	Fp-Ge6	NN	Germany	Paulineaue (Havelland)	2004	−	+
GE4	Fp-Ge7	NN	Germany	Paulineaue (Havelland)	2004	−	+
GE5	Fp-Ge8	NN	Germany	Paulineaue (Havelland)	2004	−	+
GE6	Fp-Ge9	NN	Germany	Paulineaue (Havelland)	2004	−	+
GE7	Fp-Ge10	NN	Germany	Paulineaue (Havelland)	2004	−	+
GE8	Fp-Ge11	NN	Germany	Paulineaue (Havelland)	2004	+	−
GE9	Fp-Ge12	NN	Germany	Paulineaue (Havelland)	2004	+	−
GE10	Fp-Ge13	NN	Germany	Paulineaue (Havelland)	2004	+	−
GE11	Fp-Ge14	NN	Germany	Hohennauen (Havelland)	2004	+	−
GE12	Fp-Ge15	NN	Germany	Hohennauen (Havelland)	2004	+	−
GE13	Fp-Ge16	NN	Germany	Paulineaue (Havelland)	NN	+	+
Bo101.1	Fp-Be1	NN	Belgium	NN	2008	+	−
Bo104.2	Fp-Be2	NN	Belgium	NN	2008	+	−
Bo203.2	Fp-Be3	NN	Belgium	NN	2008	+	+
B0207.1	Fp-Be4	NN	Belgium	NN	2008	+	−
Ve108.1	Fp-Be5	NN	Belgium	NN	2008	+	+

Table 1 (continued)

Isolate	Figures isolate code	Host	Geographic origin	City or region	Year of isolation	MAT 1	MAT 2
Zw316.2	Fp-Be6	NN	Belgium	NN	2008	+	−
Zw314.1	Fp-Be7	NN	Belgium	NN	2008	+	−
Zw313.1	Fp-Be8	NN	Belgium	NN	2008	+	+
Zw310.2	Fp-Be9	NN	Belgium	NN	2008	+	−
Zw307.2	Fp-Be10	NN	Belgium	NN	2008	+	−
Zw210.1	Fp-Be11	NN	Belgium	NN	2008	+	+
Zw116.1	Fp-Be12	NN	Belgium	NN	2008	+	+
Zw106.1	Fp-Be13	NN	Belgium	NN	2008	+	+
To108.1	Fp-Be14	NN	Belgium	NN	2008	+	+
To302.1	Fp-Be15	NN	Belgium	NN	2008	+	+
182/I468	Fp-Fr1	*Triticum aestivum* L.	France	East	2007	+	−
183/I469	Fp-Fr2	*Triticum aestivum* L.	France	East	2007	+	+
187/I473	Fp-Fr3	*Triticum aestivum* L.	France	East	2007	+	+
188/I474	Fp-Fr4	*Triticum aestivum* L.	France	Center/North	2007	+	+
192/I478	Fp-Fr5	*Triticum aestivum* L.	France	West	2007	+	+
194/I480	Fp-Fr6	*Triticum aestivum* L.	France	East	2007	+	−
195/I481	Fp-Fr7	*Triticum aestivum* L.	France	Center/North	2007	+	+
196/I482	Fp-Fr8	*Triticum aestivum* L.	France	West	2007	+	+
198/I484	Fp-Fr9	*Triticum aestivum* L.	France	Center/North	2007	+	+
199/I485	Fp-Fr10	*Triticum aestivum* L.	France	Center/North	2007	+	+
204/I490	Fp-Fr11	*Triticum aestivum* L.	France	West	2007	+	+
205/I491	Fp-Fr12	*Triticum aestivum* L.	France	West	2007	+	+
206/I492	Fp-Fr13	*Triticum aestivum* L.	France	Center/North	2007	+	+
I71	Fp-Fr14	*Triticum aestivum* L.	France	Southeast	2007	+	+
I72	Fp-Fr15	*Triticum aestivum* L.	France	Southeast	2007	+	+
I109	Fp-Fr16	*Zea mays* L.	France	Southwest	2007	+	+
I110	Fp-Fr17	*Zea mays* L.	France	Southwest	2007	+	+
I111	Fp-Fr18	*Zea mays* L.	France	Center/North	2007	+	+
I475	Fp-Fr19	*Triticum aestivum* L.	France	East	2007	+	−
I476	Fp-Fr20	*Triticum aestivum* L.	France	West	2007	+	−
I477	Fp-Fr21	*Triticum aestivum* L.	France	East	2007	+	+
TAPO21	Fp-Hu1	*Triticum aestivum* L.	Hungary	NN	1992	+	−
TAPO34	Fp-Hu2	*Triticum aestivum* L.	Hungary	NN	1992	−	+
LT03 08(57)	Fp-Lu1	*Brassica oleracea* L.	Luxembourg	Nothum	NN	+	−
LT03 35(80)	Fp-Lu2	*Brassica oleracea* L.	Luxembourg	Nothum	NN	+	−
LT14 05(491)	Fp-Lu3	*Zea mays* L.	Luxembourg	Kayl	NN	+	−
LT14 27(504)	Fp-Lu4	*Zea mays* L.	Luxembourg	Kayl	NN	+	−
LT15 16(525)	Fp-Lu5	*Triticum aestivum* L.	Luxembourg	Dippach	NN	+	+
CA1	Fp-Ar31	*Triticum aestivum* L.	Argentina	Coronel Suarez	2004	+	−
CA2	Fp-Ar32	*Triticum aestivum* L.	Argentina	Necochea	2004	+	−
CA3	Fp-Ar33	*Triticum aestivum* L.	Argentina	25 de Mayo	2004	+	+

Table 1 (continued)

Isolate	Figures isolate code	Host	Geographic origin	City or region	Year of isolation	MAT 1	MAT 2
CA4	Fp-Ar34	*Triticum aestivum* L.	Argentina	25 de Mayo	2004	+	−
CA5	Fp-Ar35	*Triticum aestivum* L.	Argentina	Coronel Pringles	2004	+	−
CA6	Fp-Ar36	*Triticum aestivum* L.	Argentina	Guamini	2003	−	+
CA7	Fp-Ar37	*Triticum aestivum* L.	Argentina	Azul	2004	+	+
U1	Fp-Ur1	*Triticum aestivum* L.	Uruguay	Colonia	NN	+	−
U2	Fp-Ur2	*Triticum aestivum* L.	Uruguay	Soriano	NN	−	+
U3	Fp-Ur3	*Triticum aestivum* L.	Uruguay	San José	NN	+	+
U4	Fp-Ur4	*Triticum aestivum* L.	Uruguay	Río Negro	NN	+	−
U5	Fp-Ur5	*Triticum aestivum* L.	Uruguay	Paysandú	NN	+	+
F95	Fs-En	NN	England	NN	NN		
CC321	Fl-En	NN	England	NN	NN		

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Isolates from Brazil were kindly provided by E. Del Ponte, University of Rio Grande do Sul, Porto Alegre, Brazil

Fp Fusarium poae, *Fs Fusarium sporotrichioides*, *Fl Fusarium langsethiae*

DNA fragments by using the silver-staining protocol according to Bassam et al. (1991).

AFLP markers

Genomic DNA was completely digested with *EcoR*I and *Mse*I (New Englands Biolabs, Hitchin, Hertfordshire, UK) and ligated to AFLP adapters at 37 °C for 5 h. The digested and ligated templates were diluted ten-fold with TE (10 mM Tris–HCl pH 8.0 and 1 mM EDTA pH 8.0) 1× buffer before pre-amplification. The pre-amplified products were diluted 20-fold with TE 1× buffer and the PCR-AFLP reaction was performed by using two primer pair combinations (ECORI + AC/MSEI + CC and ECORI + AC/MSEI + CA). The AFLP-PCR was performed according to Vos et al. (1995) modified by Leslie and Summerell (2006). The products were separated and visualized according to the description for ISSR markers. The presence/absence of AFLP fragments ranging from 200 to 400 bp was analyzed.

Data analysis

ISSR and AFLP amplification products were scored manually and separately and recorded in a binary present/absent format. All amplification products were scored including those that were monomorphic. Cluster analysis based on Simple Matching coefficient was performed on the similarity matrix employing the "unweighted pair group method using arithmetic means" (UPGMA) algorithm (Sneath and Sokal 1973).

NTSYSpc version 2.0 was used to perform these analyses (Rohlf 1998). An analysis of molecular variance (AMOVA) was performed to examine hierarchical population structure using distances between haplotypes. The distance chosen was a Euclidean metric equivalent to the number of differences between two individuals in their multilocus profile. Gene diversity (expected heterozygosis, H) (Nei 1987), or the average probability that two randomly chosen alleles at a locus are different, was estimated for the entire population, as well as for the two populations (American and European isolates), with the assumption that the populations are in Hardy–Weinberg equilibrium. Arlequin 2000 was the software used to perform these analyses (Schneider et al. 2000).

Comparison of similarity matrix between ISSR and AFLP was performed using the MXCOMP programme of the NTSYS-pc 2.0 (Rohlf 1998).

Mating type analysis

Conserved portions encoding the α and HMG box of the MAT-1 and MAT-2 idiomorphs respectively were amplified for the entire set of isolates according to Kerényi et al. (2004). The primer sequences were fusALPHAfor (5′-CGCCCTCTKAAYGSCTTCATG-3′) and fusALPHArev (5′-GGARTARACYTTAGCAATYAGG GC-3′) to amplify MAT-1 and fusHMGfor (5′-CGACCT CCCAAYGCYTACAT-3′) and fusHMGrev (5′-TGGG CGGTACTGGTARTCRGG-3′) to amplify MAT-2. The PCR conditions were: 95 °C for 2 min, followed by 30 cycles consisting of 30 s at 94 °C, 30 s at 50 °C (for MAT-1) and 52 °C (for MAT-2), 5 min at 72 °C and a final elongation step at 72 °C for 10 min. Moreover, a multiplex PCR was done for the entire collection of isolates at 52 °C with the two set of primers at the same conditions described above. Amplification products were examined by electrophoresis in 1.5 % (w/v) agarose gels containing GelRed™ (Biotium; Hayward) at 80 V in 1× Trisborate-EDTA buffer for 1 h at room temperature and fragment sizes were estimated. To confirm that the amplified fragments correspond to MAT-1 and MAT-2 idiomorphs, amplification products from three isolates were purified by using the *PureLinK* PCR purification kit (Invitrogen, Buenos Aires, Argentina) and sequenced, from both the sense and antisense ends of the amplification products by using Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130). One amplification product was purified from a MAT-1 isolate (MICA-T-06), another from a MAT-2 isolate (MICA-T-07) and two amplification products from an isolate with both MAT-1 and MAT-2 (LPS1a). All the sequences were compared, using BLASTN, with sequences in the NCBI database (Altschul et al. 1990). The MAT-2 sequences were deposited in the NCBI/GenBank database with accession numbers: KF876686-KF876687. The MAT-1 sequences were not submitted to GenBank because these sequences were less than 200 bp in length.

Results

Genetic variability determination

Genetic variability among *Fusarium poae* isolates was analyzed using ISSR and AFLP. Regarding ISSR, 68

amplification products were polymorphic among a total of 121 amplification products obtained from the DNA of *F. poae* isolates. Cluster analysis resolved 169 haplotypes among the 173 isolates analyzed with one cluster that included all the *F. poae* isolates and another cluster with *F. langsethiae* and *F. sporotrichioides,* at an arbitrary level of 44 % similarity (Fig. 1). ISSR partially resolved clusters of *F. poae* isolates based upon their country of origin. At 75 % of similarity, two clusters were resolved: cluster I which was resolved into two sub-clusters that were designated as Ia and Ib, and cluster II, which included 85 % of the total of *F. poae* analyzed.

Sub-cluster Ia contained most of the *F. poae* isolates from Belgium and 11 isolates from France, Canada and Germany while sub-cluster Ib included only isolates from Belgium and Germany. Cluster II was resolved into two sub-clusters designated as sub-cluster IIa and sub-cluster IIb, the latter of which only contained isolates from Uruguay. Sub-cluster IIa was resolved at 82 % of similarity into two sub-clusters designated as sub-cluster IIa1 and IIa2. The first of them included only isolates from Argentina, while sub-cluster IIa2 included most of the *F. poae* isolates obtained from different countries such as Argentina, England, France, Italy, Finland, Switzerland, Germany, Poland, Canada, Luxembourg and Hungary.

The *Fusarium poae* diversity was estimated to be 0.9998 (DS=0.0007). AMOVA results demonstrated that most of the variation resulted from genetic differences within (89.51 %) rather than from differences between American and European populations (10.49 %).

AFLP analysis (amplification products ranging from 200 to 400 bp) revealed that 30 were polymorphic among a total of 32 amplification products obtained from the DNA of *F. poae* isolates. Cluster analysis defined 150 haplotypes among all the *F. poae* isolates analyzed. The AFLP technique was not able to resolve *F. poae*, *F. sporotrichioides* and *F. langsethiae* (Fig. 2). Unlike the dendrogram obtained from ISSR markers, AFLP was not able to partially group *F. poae* isolates according to geographical origin. Notably, cluster I was resolved at 54 % of similarity which included two *F. poae* isolates obtained from France, while cluster II was resolved into two sub-clusters designated as sub-cluster IIa and sub-cluster IIb which contained only two isolates from Belgium. Sub-cluster IIa was resolved into two sub-clusters designated sub-cluster IIa1 which contained the remaining isolates analyzed from different countries plus *F. sporotrichioides* and *F. langsethiae* throughout the dendrogram and sub-cluster IIa2 which contained *F. poae* isolates obtained from Belgium.

The population diversity was estimated to be 0.9982 (DS=0.0009). The most variability was found in differences within American and European isolates (98.58 %), compared with those found between groups (1.42 %), by using AMOVA analysis.

When a comparison of the similarity matrix obtained from ISSR and AFLP analysis was made, there was no correlation among the analyzed markers (r=0.20) and the results were analyzed individually.

Mating type analysis

One hundred seventy three *Fusarium poae* isolates were analyzed for the presence of MAT-1 or MAT-2 idiomorphs by single and multiplex PCRs (Fig. 3). A 200 bp fragment corresponding to the MAT-1 idiomorph was amplified from 58 isolates, a 260 bp fragment corresponding to MAT-2 idiomorph was amplified from 31 isolates and from the remaining 84 *F. poae* isolates, both MAT-1 and MAT-2 idiomorphs were amplified (Table 1). No relationship between country of origin and MAT amplifications was observed. The MAT-1 and MAT-2 sequences from selected isolates showed 100 % homology with MAT-1 (GenBank accession AJ535627) and MAT-2 (AJ535631) genes from *Fusarium poae*, respectively.

Discussion

Previous studies using ISSR and AFLP markers were conducted to assess genetic variability of *Fusarium* isolates. When Mishra et al. (2003) used ISSR markers to investigate *F. culmorum* genetic variability of isolates from England, Poland, Ireland, Russia, Netherlands, New Zealand, Australia, Germany, Canada, India, Denmark and USA, a high level of intraspecific variability was observed. Moreover, cluster analysis showed a general relationship between the *F. culmorum* isolates and their geographical origin (Mishra et al. 2003). The same authors then used ISSR to evaluate the genetic structure of *F. graminearum* isolates obtained from Alberta, Saskatchewan and Manitoba, Canada. They observed a high level of genetic variability but were unable to group the isolates according to geographical origins (Mishra et al. 2004).

Fig. 1 Dendrogram obtained by ISSR markers showing haplotype aggrupation of the 173 world-wide collection of *F. poae* isolates plus *F. langsethiae* (Fl En) and *F. sporotrichioides* (Fs En)

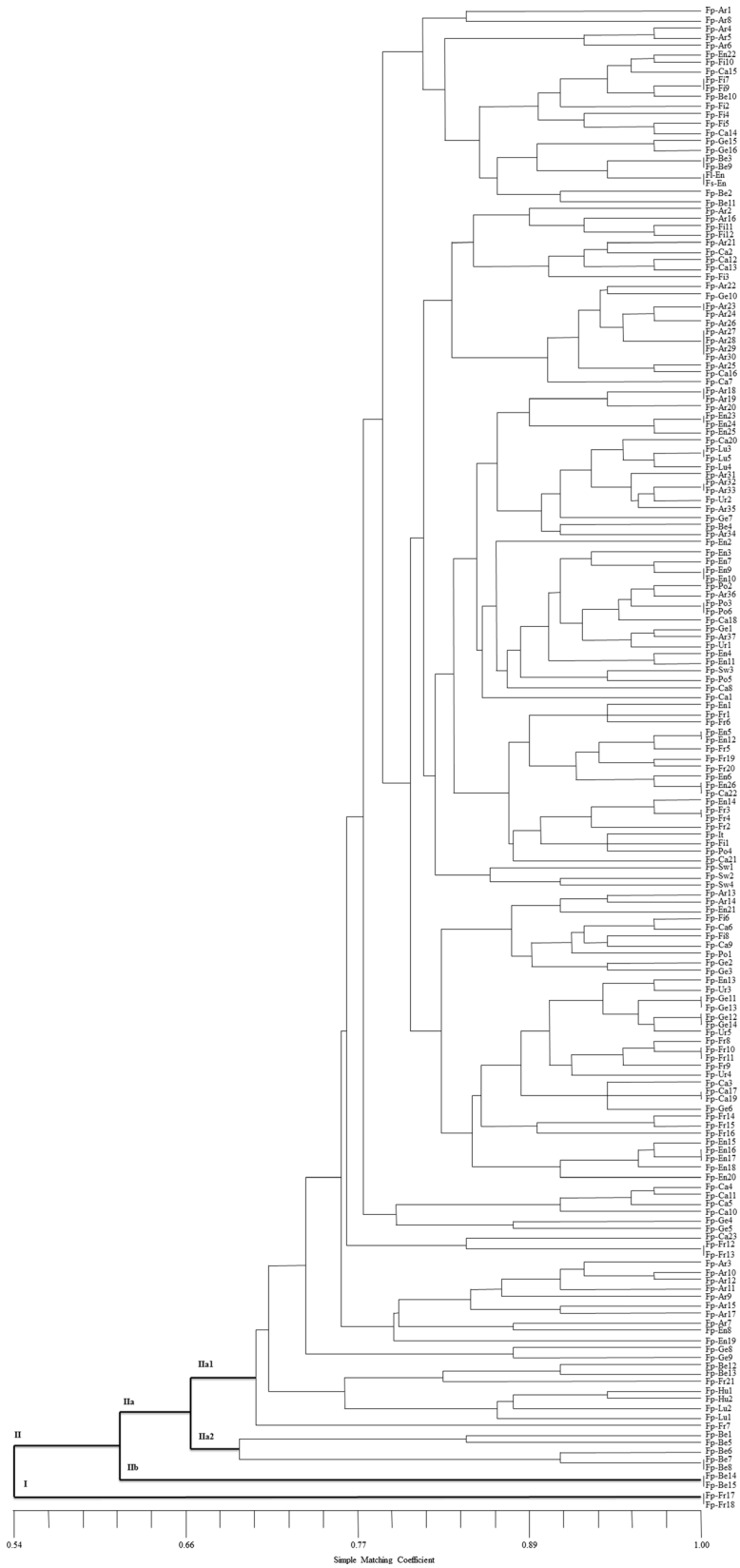

Fig. 2 Dendrogram obtained by AFLP markers showing haplotype aggrupation of the 173 world-wide collection of *F. poae* isolates plus *F. langsethiae* (Fl En) and *F. sporotrichioides* (Fs En)

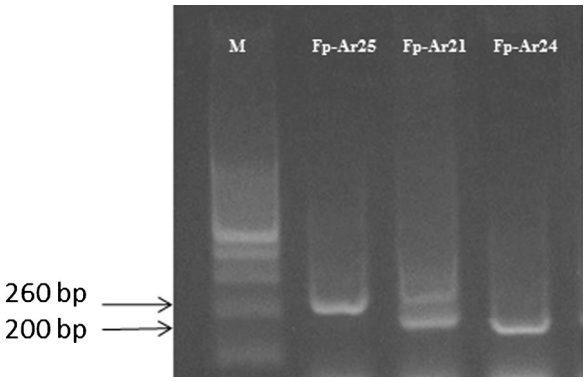

Fig. 3 MAT-1 and MAT-2 amplifications of representative *F. poae* obtained by multiplex PCR. The *arrows* indicate the 200 bp fragments corresponding to MAT-1 and 260 bp corresponding to MAT-2. M = molecular marker

Regarding the current study of genetic variability in *Fusarium poae*, using ISSR markers a high level of genetic variation was detected among *F. poae* isolates and a partial clustering was found between isolates and the country of origin. Moreover, most of the variation resulted from genetic differences within (89.51 %), rather than from differences between American and European populations (10.49 %). Similar results were reported by Kerényi et al. (1997), who determined the genetic relationship among 54 *F. poae* isolates obtained from Canada, New Zealand, South Africa, United Kingdom, Japan, Finland and Hungary by using RAPD. Cluster analysis revealed a moderate level of genetic variability (80 %) among the *F. poae* isolates analyzed. However, RAPD technique was not able to group the isolates according to their geographical origin (Kerényi et al. 1997). Moreover, Dinolfo et al. (2010), using ISSR markers, determined that 99 % of the *F. poae* isolates evaluated from Argentina and England were different and a partial clustering between isolates and geographical origin was found. Most differences were within (83 %) rather than between isolates from Argentina and England (17 %) (Dinolfo et al. 2010).

Another molecular marker widely used to study genetic variability in *Fusarium* species is AFLP, especially *F. graminearum*. Alvarez et al. (2011) and Astolfi et al. (2011) evaluated *F. graminearum* isolated from Argentina and Brazil, respectively grouping *F. graminearum* species complex isolates according to their corresponding lineage.

In our study, AFLP markers revealed a high level of variation among the *Fusarium poae* isolates (99 %). The differences within American and European populations were higher (98.58 %) than those between populations (1.42 %). Moreover, no clustering between isolates and country of origin was found. Interestingly, unlike ISSR, AFLP markers were unable to discriminate between *F. poae* isolates and *F. langsethiae* and *F. sporotrichioides*. Likewise, Somma et al. (2010) determined a high level of genetic variability in 81 *F. poae* isolates obtained from wheat grown in northern Italy by using AFLP. However, no correlation between clusters and mycotoxin production was found (Somma et al. 2010).

Although both molecular markers used in our study detected a high level of diversity within *F. poae*, the correlation between the similarity matrix obtained by ISSR and AFLP was not significant (r=0.20). The genomic regions detected by the two markers and the number of primers used may affect the correlation among different marker systems.

In our study the MAT idiomorphs in different *Fusarium poae* isolates was assessed. Kerényi et al. (2004) demonstrated that MAT-1 or MAT-2 idiomorphs are present and expressed from the genome of eight *F. poae* isolates. Irzykowska et al. (2013) amplified both idiomorphs separately in the genome of 35 *F. culmorum* isolates from stem bases and roots of barley plants originating from different Polish provinces. Kerényi et al. (2004) evaluated the presence and expression of MAT-1 and MAT-2 not only in *F. poae*, but also in different *Fusarium* species with no known sexual stage, suggesting the possibility of a heterothallic condition and that these species may have a cryptic sexual cycle. Interestingly, most *F. poae* isolates (48.5 %) analyzed in our study contained both MAT-1 and MAT-2 idiomorphs; Cepni et al. (2012) reported the same observation in two *F. culmorum* isolates.

In conclusion, both ISSR and AFLP molecular marker systems detected a high level of intraspecific variation among *Fusarium poae* isolates. However, ISSR markers discriminated the *F. poae* population from *F. langsethiae* and *F. sporotrichioides* and produced a partial clustering relating the *F. poae* isolates and their geographical origin.

Different events such as mutations, parasexual reproduction, and migration could be responsible for the high variability found in *Fusarium poae* isolates (Kristler and Miao 1992). *F. poae* isolates with both MAT-1 and MAT-2 idiomorphs, will indicate that these isolates are potentially homothallic. However, more specific studies on *F. poae* MAT idiomorphs are needed to elucidate and to confirm if this result could play a role in *F. poae* variability.

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