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BIOLOGICAL RELATIONSHIPS AMONG *Fusarium graminearum s.l.* ISOLATES FROM DIVERSE HOSTS AND ENVIRONMENTS OF ARGENTINA

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ABSTRACT

***Fusarium graminearum* (teleomorph *Gibberella zeae*) is an important plant pathogen that causes diseases on roots, stems, fruits, and seeds of diverse plant families. The fungus obtains nutrients through saprotrophic and parasitic relationships. Mycotoxins produced by *F. graminearum*, which can be found in contaminated grains and flours, constitute a threat to human and animal health. The aim of this study was to infer biological relationships among isolates of *F. graminearum*, obtained from different botanic hosts and locations of the central region of Argentina, by assessment of biodiversity from morphological and molecular markers. *F. graminearum* (local isolates and a foreign control isolate) established closed morphological and molecular (genetic) relationships beyond the diversity of origin. The identity of the collection was validated as *F. graminearum -sensu lato-* and it was the predominant specific-clade in the analyzed population. Furthermore, the capacity of *F. graminearum* to adapt to diverse plant substrates (*Fabaceae*, *Poaceae*, *Asteraceae* and *Rosaceae* families) and ecologic environments (between 30 ° and 36 ° south latitude) observed in this study revealed the large plasticity of this fungus in Argentina.**

Keywords: biomarkers, *Fusarium graminearum -sensu lato-*, geographic origin, molecular markers, plant hosts.

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INTRODUCTION

The genus *Fusarium* includes pathogenic and opportunistic species associated with plants, organic residues and soil, being an interesting example of biodiversity and highlighting their capacity of survival in different environmental conditions (Castañares et al., 2016). According to Mat Razali et al. (2019), these fungal microorganisms have transposable elements in their genome, which is associated with this ability to adapt to different environments and occupy novel niches, known as plasticity.

F. graminearum Schwabe (teleomorph*: Gibberella zeae*) is a saprophytic and soil fungus with a plant-pathogenic phase in its biological cycle. The fungus causes diseases on stems, fruits, and seeds of several crops of the Poaceae family such as rice, barley, wheat, and maize (Incremona et al., 2014; Martínez et al., 2020), the Fabaceae species (Pioli et al., 1997), as well as fruits and seeds of native forest species (Peruzzo and Pioli, 2017). The first pathogenic association between *F. graminearum* and soybean was reported by Pioli et al. (2004). The wide host plant range of *Fusarium* has promoted its distribution and survival in diverse agro-ecological areas, increasing the inoculum sources from the soil and plant debris (Abdel-Azeem et al., 2019).

The biologically active metabolites produced by *F. graminearum* are zearalenones and mainly trichothecenes (Peruzzo and Pioli, 2017), which include deoxynivalenol (DON) and its acetylated derivatives 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) (Aoki et al., 2012; Castañares et al., 2014; Munkvold, 2017; Martínez et al., 2020). The infection of crops by toxicogenic strains can decrease the physiological and sanitary quality of seeds (Pioli et al., 1997) and contaminate grains and flours of cereal, oleaginous and their feeding derivates (Peruzzo et al., 2015). Therefore, *Fusarium* mycotoxins may constitute an important risk for plants as well as human and animal health (Peruzzo and Pioli, 2016; Martínez et al., 2020).

Sixteen phylogenetically distinct species were identified in the *Fusarium graminearum* species complex (FGSC). However, only six *Fusarium* species were identified by morphological species recognition (MSR) based on macro and micro- morphology, while three species were from the FGSC: *F. cerealis* (Cooke) Sacc, syn *F. crookewellense* (Burgess, Nelson and Toussoun) and *F. pseudograminearum* (Aoki and O'Donnell), which were segregated from *F. graminearum* as morphologically and phylogenetically distinct species (Aoki et al., 2012; Leslie and Burgues, 2006).

F. graminearum was previously recognized as Group 1 or the putatively heterothallic population of *F. graminearum.* The diagnosis morphologic key to separate both groups was the presence of abundant homothallic perithecia of *Giberella zeae* from monosporic cultures of *F. graminearum*, meanwhile this was not observed in isolates of *F. pseudograminearum* (McMullen et al., 2012). Both species were reported as producers of deoxynivalenol and zearalenone, which contribute to the virulence of *F. graminearum* in wheat (Malbrán et al., 2012; Torres et al., 2019). In fact, FGSC is considered as the most important cause of Fusarium Head Blight in wheat and barley in many regions of the world (Umpiérrez-Failache et al., 2013), being *F. graminearum* the most aggressive species and particularly dominant isolate from wheat in Europe and America (Aoky et al., 2012; Del Ponte et al.,2015; Khaledi et al., 2017; Torres et al., 2019).

Variability of the FGSC remains unclear. Therefore, the combination of morphological, molecular, toxicological, and biological traits is required to achieve an effective characterization and classification (Leslie and Summerell, 2006; Walder et al., 2019). RAPD-PCR analyses combined with studies on phenotypic characters have proven useful to detect differences between strains of *F. graminearum* and other species, while amplified fragment length polymorphism (AFLP) analysis is considered a powerful taxonomic tool to characterize fungal genetic diversity (Davari et al., 2012). In addition, clade specific primers (Nicholson et al. 1998) and sequencing techniques have been applied to identify *Fusarium* species, contributing with major accuracy to the identity validation of ambiguous strains.

Fusarium species are defined in a broader sense, with no differentiation between cryptic species within FGSC. According to the criteria described by Walder et al. (2019), the present study has considered the biological and morphological species concept. Therefore, *Fusarium* species are defined in a broader sense and without differentiating between cryptic species within given species complexes, and thus referred as *F. graminearum sensu lato* (*s.l.*).

The aim of this work was to infer biological relationships among isolates of *F. graminearum*, obtained from different botanic hosts and locations of the central region of Argentina, by assessment of biodiversity from morphological and molecular markers.

MATERIALS AND METHODS

Media and culturing conditions

Fusarium isolates were obtained from symptomatic tissues of different plant species

and locations, and grown in a potato glucose agar (20g L^{-1}) medium acidified with 2 mL lactic acid L^{-1} (APGA) according to Peruzzo and Pioli (2016). Single macro-conidia were obtained from sporodochia formed in the colonies to promote monosporic cultures using the method described by Aoky et al. (2012) and Munkvold (2017). Twenty-eight isolates, obtained from different hosts and environments of Argentina, and the *F. graminearum* isolate (CE143/00, foreign (non-local) control were selected for this study (Table 1). Of the twenty-eight local isolates, ten isolates were obtained from Poaceae; sixteen isolates were obtained from Fabaceae;

Table 1. Origin of isolates of *Fusarium graminearum -sensu lato-* used in this study.

Origin (country, province, locality)	Map coordinates	Substrate	Accession°	Identity validation	Mycotoxin production *in vitro* / *in vivo*
United States of America, Iowa	41°12′24″N 91°32′30″W	corn	1-CE143/00	1, 2, 3, 4	nd / +
Argentina, Santa Fe, Zavalla	33°01′00″S 60°53′00″W	wheat	2-CE135/01	1, 2, 3, 4	nd / +
Argentina, Santa Fe, Barrancas	32°14′00″S 60°59′00″W	soybean	3-CE105/02	1, 2, 3, 4	DON-Zea / +
Argentina, Santa Fe, Va. Amelia	33°11′00″S 60°40′00″W	soybean	4-CE169/02	1, 2, 3, 4	nd / +
Argentina, Buenos Aires, Arrecife	34°04′00″S 60°07′00″W	soybean	5-CE170/02	1, 2, 3, 4	nd / +
Argentina, Santa Fe, Barrancas	32°14′00″S 60°59′00″W	soybean	6-CE171/02	1, 2, 3, 4	nd / +
Argentina, Córdoba, M. Juárez	32°42′00″S 62°06′00″W	wheat	7-FB31	3	nd / nd
Argentina, Córdoba, M. Juárez	32°42′00″S 62°06′00″W	wheat	8-FB32	1, 2, 3	DON / +
Argentina, Santa Fe, San Justo	30°47′00″S 60°35′00″W	soybean	9-FB33	1, 2, 3	nd / +
Argentina, Santa Fe, Álvarez	33°08′00″S 60°48′00″W	lentil	10-FB34	1, 2, 3, 4	ZEA / +
Argentina, Santa Fe, Álvarez	33°08′00″S 60°48′00″W	pea	11-CE111/04	1, 2, 3, 4	ZEA/ +
Argentina, Santa Fe, V. Tuerto	33°45′00″S 61°58′00″W	soybean	12-CE106/04	1, 2, 3, 4	DON-ZEA/ +
Argentina, Santa Fe, Álvarez	33°08′00″S 60°48′00″W	lentil	13-FB37	1, 2, 3, 4	nd/ +
Argentina, Córdoba, M. Juárez	32°42′00″S 62°06′00″W	wheat	14-FB38	3	nd
Argentina, Santa Fe, Álvarez	33°08′00″S 60°48′00″W	faba-bean	15-FB39	1, 2, 3	DON/ +
Argentina, Santa Fe, San Genaro	32°22′00″S 61°19′00″W	soybean	16-FB43	1, 2, 3	nd
Argentina, Santa Fe, San Genaro	32°22′00″S 61°19′00″W	soybean	17-FB44	1, 2, 3	nd/ +
Argentina, Santa Fe, San Genaro	32°22′00″S 61°19′00″W	soybean	18-FB45	1, 2, 3	nd / +

Argentina, Santa Fe, Clarke	32°30′00″S 61°02′00″W	soy-seeds	19-CE112/05	1, 2, 4	ZEA/ +
Argentina, Santa Fe, Villa Amelia	33°11′00″S 60°40′00″W	soybean	20-CE168/06	3, 4	nd
Argentina, Santa Fe, Soldini	33°02′00″S 60°45′00″W	strawberry	21-FB47	1, 2	DON/ +
Argentina, Buenos Aires, La Emilia	33°21′00″S 60°19′00″W	sorghum	22-CE104/08	1, 2, 4	DON/ +
Argentina, Buenos Aires, La Emilia	33°21′00″S 60°19′00″W	sorghum	23-FB49	1, 2, 3	DON/ +
Argentina, Santa Fe, Casilda	33°03′00″S 61°10′00″W	wheat	24-CE107/10	1, 2, 4	nd
Argentina, Santa Fe, Zavalla	33°01′00″S 60°53′00″W	sunflower	27-FB51	1, 2	nd
Argentina, Santa Fe, Zavalla	33°01′00″S 60°53′00″W	corn	28-FB52	1, 2	nd
Argentina, Santa Fe, Pujato	33°01′01″S 61°02′35″W	wheat	29-FB53	1, 2	nd
Argentina, Santa Fe, Casilda	33°03′00″S 61°10′00″W	wheat	30-FB54	1, 2	nd
Argentina, La Pampa, Gral. Pico	35°40′00″S 63°44′00″W	soybean	31-FB55	1, 2	nd

° Fungal collection obtained through Projects directed by R.N. Pioli. Phytopathology, Laboratory of Plant and Microbial Biodiversity, Faculty of Agronomy Sciences, UNR.
CE: Center of Mycology Reference, Fac. Biochemistry and Pharmacy Sc. UNR.
CE-Code indicates the foreign and 11 local isolates from different hosts and environments, used as control.
[1] Morphological analysis; [2]Molecular analysis by a specific-clade (FgN16) marker to validate *F. graminearum -sensu lato-* identity.[3]Molecular analyses by AFLP; [4] Molecular analyses by RAPD.
°° *In vitro* mycotoxin production in rice substrate. DON=deoxynivalenol; Zea=zearalenone; nd=no detected. *In vivo* mycotoxin transmission to flour from inoculated spikes (wheat) and pods (soybean) (Peruzzo and Pioli, 2016).

one isolate was obtained from Compositae (Asteraceas), and one isolate was obtained from Rosaceae; corresponding to seventeen different agro-ecological environments (30° 47′ 00′′ to 35° 40′ 00′′ S; and 60° 07′ 00′′ to 63° 44′ 00′′ W) of Buenos Aires, Córdoba, La Pampa and Santa Fe provinces, Argentina (Table 1). The isolates deposited in the Center of Reference on Mycology (CEREMIC), Faculty of Biochemistry and Pharmacy, National University of Rosario (FCByF, UNR), Argentina (www.fbioyf.unr.edu.ar), were coded as "CE" and used as identity control. Isolate stock was maintained in tubes with slant APGA medium at 4 °C.

Morphological characterization

Refreshed colonies from *Fusarium graminearum* isolates, obtained from different hosts and localities, were recovered on Petri plates with APGA medium and cultured for 6 days at 26 ± 1 °C in darkness. Spezieller Nährstoffarmer agar (SNA) (Khaledi et al., 2017; Hagerthy et al., 2021) and carnation-leaf agar (CLA) substrates were used to induce production of macro-conidia (anamorphic) and fruiting bodies (perythecia-teleomorphic stage) (Leslie and Summerell, 2006; Peruzzo and Pioli, 2016). Besides, the radial growth rate of colonies (mm per day) was measured to detect variations among *F. graminearum* isolates (Castañares et al., 2014; Munkvold, 2017). The trial was performed in triplicate.

Identity of *F. graminearum* (anamorphic stage) was morphologically verified based on 22 features grouped as macro-morphological characteristics (e.g., growth rate in culture, color of aerial mycelium and diffused pigments in the medium) and micro-morphological characteristics (presence of clamydospores, length, shape and septa number of macro-conidia, and the apical and basal cells of macro-conidia) were characterized (Leslie and Summerell, 2006). Morphological characters were observed with stereoscopic (SWIFT) and light (LEICA DM500) microscopes at 40X,100X, 400X and 1000X. Each

morphological feature was scored as present (1) or absent (0) across all genotypes to create a binary matrix, which was used later in a multivariate analysis to detect similarity relationships. A dendrogram was performed from morphological characteristics shared by 25 local isolates (11 codified CE, used as control isolates) and one foreign control isolate (Table 1; Fig. 1).

Genomic DNA extraction

F. graminearum isolates were incubated in Petri dishes with APGA for five days at 26 ± 1 °C in darkness. Genomic DNA was extracted from fungal colonies using a Wizard® Genomic DNA Purification Kit (Promega, Madison, USA) (Mugrabi de Kuppler et al., 2011; Abedi-Tizaki and Zafari, 2016).

PCR specific-clade marker to validate *Fusarium graminearum*

The identity of 26 *F. graminearum* isolates was validated using the clade-specific marker Fg16N-F (5'ACAGAT GACAAGATTCAGCGACA3') and Fg16N-R (5'TTC TTTGACATCTGTTCAACCCA3') developed by Nicholson et al. (1998) and proposed by Mourelos et al. (2014) (Table 1). PCR protocol and resolution of amplification products were performed according to Malbran et al. (2012) and Martínez et al. (2020); the expected band size was ~280 bp compared with a molecular weight marker of 50 bp (Nicholson et al., 1998; Mugrabi de Kuppler et al., 2011; Martínez et al., 2020).

Biological and ecological diversity of *F. graminearum* collection

To infer the biological diversity of the *F. graminearum* collection, the isolates were ordered regarding their origin (different hosts and localities of the central area of Argentina). A matrix was also performed based on the presence/absence of morphological attributes and each band obtained by both molecular profiles (AFLP and RAPD analysis, described below). The origin area of the *F. graminearum* isolates was divided into three sub-regions respect to south latitude: North subregion (30° to 32° SL), Central subregion (32° to 34° SL), and South subregion (34° to 36°SL). The number of no polymorphic and polymorphic bands was analyzed in relation

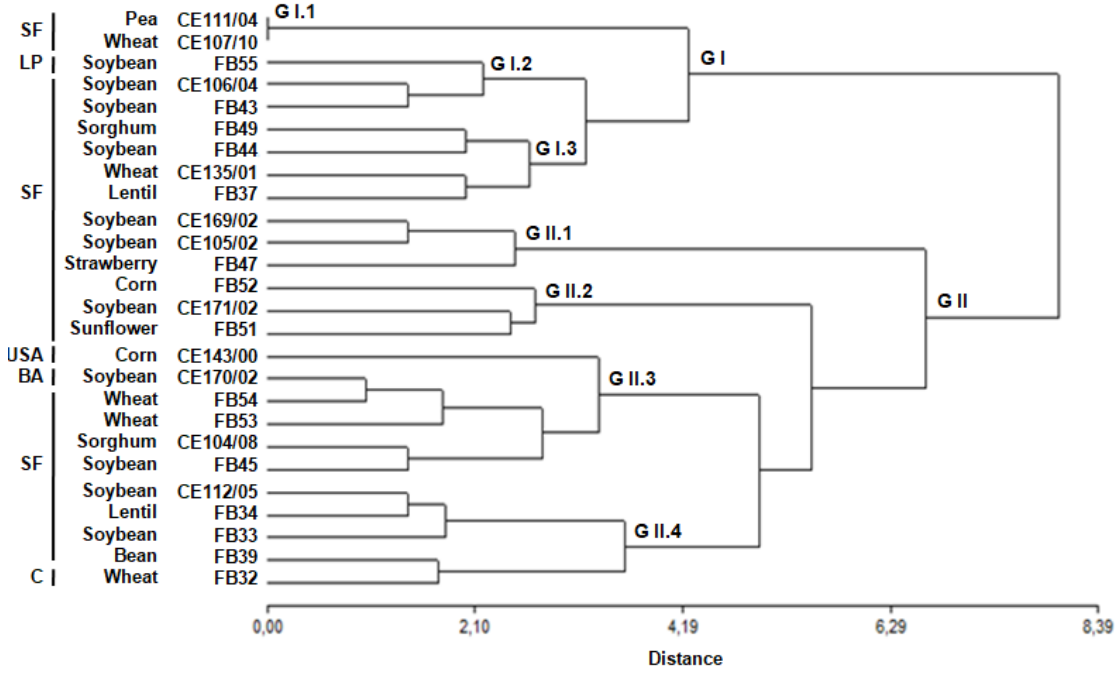

Fig. 1. Dendrogram obtained from morphologic markers, showing the relationships of *F. graminearum -sensu lato-* isolates obtained from different hosts and locations of the central area of Argentina. The distance between individuals was estimated based on unweighted pair-group method using arithmetic averages of Jaccard's similarity coefficient. G, clado/e; BA, Buenos Aires Province; C, Córdoba; LP. La Pampa; SF, Santa Fe; USA, United State of America; CE, Center of Mycology Reference, FCBF.UNR; CE-Code, code of isolates used as control; FB-code, code of novel isolates.

to the total number of bands obtained.

For the AFLP analysis, genomic DNA of 20 *F. graminearum* isolates (Table 1) was digested with two restriction enzymes simultaneously: *Eco*RI (G/AATTC) and *Mse*I (T/TAA). The reaction mixture and ligation procedure were performed according to Qu et al. (2008). The amplification reactions included pre-amplification and selective amplification. The last reaction was based on primers AFLP *Eco*RI/*Mse*I with three selective nucleotides at the 3′ (E+3 and M+3 primers) (Key Gene and synthesized by the Biotechnology Laboratory of the University of British Columbia, Vancouver, Canada). In the E+3 primers, the core sequence E was 5′GACTGCGTACCAATTC3′. In the M+3 primers, the core sequence M was 5′GATGAGTCCTGAGTAA3′. The primer combinations used were E+AAA/M+AAA, E+AAG/M+AAG and E+ACC/M+ACC. The amplification products were separated on denaturing polyacrylamide gels stained with silver nitrate and the corresponding band profiles were analyzed.

The analysis by RAPD was carried out with primers (Operon Technologies, USA): OP-AA01 (5'AGACGGCTCC3') and OP-AA06 (5'GTGGGTGCCA3'), previously used in other fungal characterizations (Table 1). Corresponding dendrograms (morphological and molecular) were performed by hierarchical agglomerative cluster analysis using the estimate Jaccard's similarity coefficient and the arithmetic average of unweighted pair-group method as clustering algorithm. Cophenetic correlation coefficient (r) was calculated for each association, considering that r = 0.8 is a good fit. Multivariate analysis to determine morphological and molecular relationships were made using the program Infostat version 2018 (National University of Córdoba, Argentina) (Hernández et al., 2020).

RESULTS AND DISCUSSION

Morphological characterization

Fungal colonies produced abundant mycelia that varied in color from white to pale pink, reddish and yellow. Cultures also showed diffused pale yellow to red pigments in the agar, while the growth rate was between 4.5 and 13.8 mm d^{-1}. Typical macroconidia of *F. graminearum* (as described by Leslie and Summerell (2006)) were observed on APGA and CLA, but particularly on SNA media. Average length of macroconidia was 43.4 µm (34.3 to 53.1 µm) and usually showed 3.3 to 5.0 septa. Microconidia were absent in all the colonies evaluated.

Dendrogram obtained from morphological markers, showed that *F. graminearum* isolates formed two major groups (GI and GII) with 92 to 100 % of similarity (%S) (Fig. 1). GI included nine isolates with 95.8% S (four CE-control isolates), which were mainly obtained from Fabaceas and locations of Santa Fe province. Likewise, GII included 17 isolates (seven CE-control isolates), from diverse geographic, ecologic and botanic origin with 93.3% S. It is important to point out that all the clades included at least a CE-control isolate, contributing to validate the identity of novel isolates by their macro and micromorphology (Table 1; Fig. 1). Values of similarity higher than 80% among isolates from origins markedly diverse were also reported by Castañares et al. (2014, 2016) and Torres et al. (2019).

The analysis made through a complete morphologic profile of *F. graminearum* isolates demonstrated that these isolates were highly homogeneous (~70%) beyond of host plant range (with only 29% of morphological polymorphism) and environment diversity of origin (with only 31% of morphological polymorphism) (Table 2). Nevertheless, the highest biological diversity was observed among isolates obtained from allogamous species (corn and sorghum), and obtained in the South (50%) (34 to 36° SL) and North (48%) (30 to 32° SL) regions.

During almost two decades, identification was based on macro and micro morphology, and thus the determination of the main fungal genus and more known species was achieved with the support of the CEREMIC (FCByF, UNR).

According to Leslie and Summerell (2006), however, a few morphological attributes can differentiate *F. pseudograminearum* (Fpg) from *F. graminearum* (Fg). Basically, such differences are growth average rate per day (< or > 7 mm per day, for Fpg and Fg, respectively), and production *in vitro* of perithecia by Fg for being a homothallic population. In particular, FB34 FB37 and CE104/08 showed typical characteristics of *F. graminearum* (Table 1) but did not produce perithecia. However, this absence could be circumstantially caused by sterile mycelia. On the other hand, CE170/02, FB32, and FB39 isolates showed both characteristics usually associated with Fpg (Aoky et al., 2012). In any case, the identity of these six peculiar isolates was confirmed as *F graminearum -s.l-* by their molecular characterization (Fig. 2). Therefore, the morphological characterization showed that *F. graminearum* isolates obtained from diverse environments of Argentina and the foreign control shared and preserved the macro and micro-morphology. The discrepancies through morphological characterization of *F. graminearum* populations were evaluated and analyzed based on the clade-specific molecular test (Table 1; Fig. 2).

Table 2. Biological diversity of *F. graminearum* isolates obtained from different host species and geographic origin, based on the polymorphism (%) from morphologic, AFLP and RAPD markers analysis.

	Total Bands			Polymorphic Bands number and (%)			Non Polymorphic number and (%)		
Host	**morph°**	**AFLP**	**RAPD**	**morph°**	**AFLP**	**RAPD**	**morph°**	**AFLP**	**RAPD**
Wheat	17	34	44	3 (18)	4 (12)	23 (52)	14 (82)	30 (88)	21 (48)
Corn	14	29	38	9 (64)	7 (24)	5 (13)	5 (36)	22 (76)	33 (87)
Sorghum	11	0	51	4 (36)	0	41 (80)	7 (64)	0	10 (20)
Phaseoleae	20	30	51	2 (10)	4 (13)	9 (18)	18 (90)	26 (87)	42 (82)
Fabeae	18	37	56	5 (28)	13 (35)	17 (30)	13 (72)	34 (65)	39 (70)
Sub Total	*80*	*130*	*240*	*23 (29)*	*28 (22)*	*95 (40)*	*57 (71)*	*122 (78)*	*145 (60)*
Origin									
North	14		52	6 (48)		41 (79)	8 (57)		11 (21)
Center	20	44	56	0 (0)	13 (30)	6 (11)	20 (100)	31 (70)	50 (89)
South	17	31	51	10 (59)	9 (29)	15 (29)	7 (41)	22 (71)	36 (71)
Sub Total	*51*	*75*	*159*	*16 (31)*	*22 (29)*	*62 (39)*	*35 (69)*	*53 (71)*	*97 (61)*

° morphological.

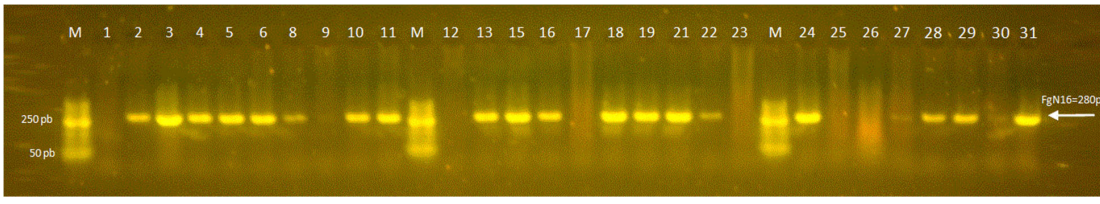

Fig. 2. Agarose gel 2%, stained with SybrSafe, shows the fragments amplified using a PCR assay performed from 8.25 ng/µL of genomic DNA of each fungal isolate. Expected band for *F. graminearum -sensu lato-* isolates (280 bp) was obtained from amplycons of the specific-clade Fg16N-F (5′ - ACA GAT GAC AAG ATT CAG CGA CA - 3′) and Fg16N-R (5′ - TTC TTT GAC ATC TGT TCA ACCCA - 3′) developed by Nicholson et al. (1998). M, molecular weight marker of 50 bp; Number Order (1 to 31) corresponding to the novel and control isolates included in Table 1; Arrow, indicates expected band (~280 bp) for the specific-clade Fg16N marker.

Molecular characterization

The ~81 % of the amplified isolates (Table 1) in this study showed the expected band (280 bp) for the FgN16 specific-clade marker of *F. graminearum* (Fig. 1) according to Nicholson et al. (1998) and Mourelos et al. (2014). However, it is very important to indicate that the primer pairs FgN16 F/R (whose sequences have just been indicated) and Fg16F (CTCCGGATATGTTGCGTCAA)/ Fg16R (GGTAGGTATCCGACATGGCAA) (Nicholson et al., 1998; Castañares et al. 2014; Khaledi et al., 2017) have different capacity to separate subgroups or subspecies into/within the FGSC; accordingly, it was duly approached by Qu et al. (2008) and Castañares et al. (2016). Based on the recognition that *F. graminearum* represents a species complex and also considering previous analyses, FgN16 F/R would identify *F. graminearum* as *-sensu lato-* (*s.l*), while Fg16 F/R would determine that *F. graminearum -sensu strictus-*(*s.s*) are possible separate different lineages; among them, *F. graminearum s.s* (lineage 7), *F. meridionale*, and *F. asiaticus* (lineage 6) into of the FGSC (Qu et al., 2008; Del Ponte et al., 2015; Castañares et al., 2016; Machado et al., 2021).

In this context, it is important to consider that those isolates that had showed some morphologically doubtful attributes, such as FB32, FB34, FB37, 39FB, CE 104/08, and CE170/02, were confirmed as *F. graminearum -sensu lato-* (Fig. 1). The clade-specific used in the molecular analysis also validated the identity of 21 isolates that included both the CE-controls from four botanic families of hosts and eight localities of

Santa Fe, Cordoba and Buenos Aires provinces as isolates as *F. graminearum -s.l-*. Among them, nine isolates are producing-mycotoxins *in vitro* or/and transmitting toxins *in vivo* (Table 1; Fig. 2) to the flour from contaminated grains from inoculated spikes and pods of wheat and soybean, respectively (Peruzzo and Pioli, 2016; 2017). Among them, FB32, FB39, FB47, CE104/08 and CE111/04 produced deoxynivalenol (DON); FB34 and CE112/05 produced zearalenone (Zea), and CE105/02 produced both DON and ZEA (Table 1; Fig. 2). Unexpectedly, the isolates obtained from the central area of Argentina as well as Poaceas, Fabacea, and Asteraceae families (Table 1) were capable of infecting spikes, caryopsis, pods and seeds of wheat and soybean, transmitting the mycotoxins to the flour of both crops (Peruzzo and Pioli, 2016).

More in-depth characterization of *F. graminearum s.l* isolates is still required to separate possible genetically-distinct subgroups or clades (Del Ponte et al., 2015; Machado et al., 2021). Nevertheless, the results obtained in the present study are consistent with some reports that have indicated that *F. graminearum -s.s-* was the most frequently isolated species from the FGSC in Iran and many other countries of America (Khaledi et al., 2017; Torres et al., 2019) and that *F. graminearum* strains (DON-type) seem mainly associated with wheat (Van der Lee et al., 2015). Hence, the biological and molecular information obtained as well as inferences about the genetics and epidemiology of this *F. graminearum s.l.* collection could be further explored. The literature has described that the specific pair Fg16F/Fg16R would allow to identify and separate other clades or species inside *F. graminearum s.l.* (Qu et al., 2008; Mugrabi de Kuppler et al., 2011, Castañares et al., 2014; and Machado et al., 2021).Thus, it would be possible to differentiate *F. graminearum s.s.* (that generates a product or band of ≈450 bp) from *F. meridionale* (that generates a band of ≈497 to 500 bp) (Machado et al., 2021), and from *F. asiaticus* (that produces a band of 550 bp) (Qu et al., 2008; Khaledi et al., 2017). Furthermore, it is also important to consider other biological possibilities such as the existence of '*forma especiales*' of *F. graminearum*, or inter-clades or inter-specific relationships in FGSC, such as *F. graminearum* and *F. asiaticus*, because some isolates share until 50% of AFLP identical bands due to the hybridization between them (Qu et al., 2008; Reynoso et al., 2012; Castañares et al., 2016). In this context, the evidence of effective inter-fertile crosses or hybridizations between these phylogenetic species might indicate that both would be clades (intraspecific) belonging of the same species.

Biological diversity of *F. graminearum s.l.* collection

Based on the morphological profile, Table 2 shows that *F. graminearum* isolated from different hosts shared 71% of the total attributes, demonstrating only 29% of polymorphism or biological diversity. Those *F. graminearum* isolates associated with allogamous species showed the highest polymorphic rate, with 36 and 64% for sorghum and corn, respectively. On the other hand, those isolates from autogamous species (wheat, soybean and Fabeae tribu) did not exceed the apolymorphic rate of 28%. Even though those isolates obtained from the North and South sub-regions showed the highest morphological variation, with 48 and 59%, respectively. Likewise, the analysis by AFLP (Table 2) showed that 71% of the total bands were shared by all *F. graminearum*, while only 29% of polymorphisms or biological diversity was observed for the origin. Polymorphism of *F. graminearum* associated with host plant range was lower (22%), and 78% of bands preserved and shared. Those isolates associated with Fabeae (35%) and corn (24%) showed the highest polymorphism and diversity.

Regarding RAPD analysis (Table 2), all *F. graminearum* shared~60% of the total bands and showed 40% of polymorphism or biological diversity. With respect to geographic origin, isolates from the North (79%) and South (29%) were more polymorphic than those obtained from the Central sub-region. The highest diversity or polymorphism was observed in isolates from sorghum (80%) (allogamous) and wheat (52%) (autogamous), both belonging to the *F. Poaceae* family; and autogamous species of *Fabaceae* (30%). Meanwhile, the lowest polymorphism was observed in *F. graminearum* associated with soybean (autogamous) and corn (allogamous), with 18 and 13%, respectively.

The biological diversity analyzed by assessments using AFLP profile showed higher or similar homogeneity than morphological analyses; among *F. graminearum-sensu lato*-isolates, only a 22 and 29% of polymorphism was detected compared to host range and ecological origin, respectively (Table 2). The highest molecular variation was observed in isolates obtained from Fabaceae (autogamous) (35%) and from corn (allogamous) (24%) without distinguishing between North or Central sub-regions. Likewise, RAPD analysis revealed a 60% of homogeneity among *F. graminearum* obtained from diverse botanic species and families, and environments (Table 2). The highest average molecular diversity or heterogeneity (based on % polymorphic bands) was observed among those *F. graminearum* isolates obtained from both allogamous (sorghum) and

autogamous (wheat) species, cultivated mainly in the North sub-region (between 30 and 32° SL) (Table 2).

Morphological and molecular similarity among evaluated isolates from diverse botanic hosts and environments, including the foreign control, would provide evidence of the ecological plasticity, bio-adaptive advantage, and epidemiologic role of *F. graminearum* due to its capacity to infect and survive in different substrates (host plants, weeds, forest, seeds and harvest residues), which is in agreement with Mourelos et al. (2014) and Torres et al. (2019). Other studies have also explained this peculiar characteristic, suggesting that similar genetically organisms from a common point of origin may have been dispersed through their spores to large distances (Aoky et al., 2012); and that these geographically diverse populations are genetically similar and may be part of a larger, randomly mating metapopulation with significant genetic exchange, probably occurring between the various subpopulations (Reynoso et al., 2012).

Discrepancies and different points of view on FGSC require additional non-only molecular studies to distinguish phylogenetic species or subspecies lineages (Qu et al., 2008; Castañares et al., 2014, 2016), or even complementary biological studies to dimension the FGSC and to know the different and effectively fertile species included in it.

There are multiple strategies to manage diseases caused by FGSC on several hosts, including cultural and epidemiology practices (cropping systems, tillage, rotation, alternative cover crops and weed hosts management) (Mourelos et al., 2014; Walder et al., 2019), genetic improvements (planting resistant or less-susceptible cultivars), and chemical and biological controls (Martínez et al., 2020). None of these strategies, however, is completely effective by itself. The results of the present study suggest combining predictive models of favorable conditions (Moschini et al., 2001) with alternative tools and integrated strategies, which could potentially decrease the incidence of the disease and reduce mycotoxins contamination in human food and animal feed chains (Peruzzo and Pioli, 2016; Torres et al., 2019).

CONCLUSIONS

The identity of 90% isolates of the obtained from nine species of four families of host plants (Fabaceae, Poaceae, Rosaceae and Asteraceae) and different localities (Santa Fe, Córdoba and Buenos Aires) was validated as *F. graminearum -sensu lato-* by morphological attributes (or MSR) and the clade specific primer (FN16 F/R), as well as by AFLP and RAPDs. The study allowed the validation of nine *F. graminearum -sensu lato-* mycotoxin producing isolates *in vitro* (rice substrate) and *in vivo* (inoculated plants) of DON, Zea or both. The polymorphism level observed among morphological and molecular profiles (by AFLP and RAPDs) of *F. graminearum*, obtained from different hosts or substrates (bean, lentil, pea, soybean, strawberry, sunflower, corn, sorghum, wheat) and agro-environments (30° to 36° south latitute) of Argentina, provided evidence of the plasticity and adaptative capacity of the fungus.

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Contribution of authors

The first two authors (Peruzzo A.M. and Pioli R.N.), contributed in the same way to the final version of the manuscript.

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