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Genetic diversity of *Fusarium meridionale, F. austroamericanum*, and *F. graminearum* isolates associated with Fusarium head blight of wheat in Brazil

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Abstract

One of the biggest challenges of wheat production worldwide is to overcome fungal diseases, and among these is Fusarium head blight (FHB) caused mainly by the *Fusarium graminearum* species complex (FGSC). The occurrence of FHB can lead to contamination of grains with mycotoxins, which are harmful to human and animal health. In South America, in addition to *F. graminearum* sensu stricto (*F. graminearum*), which is the most common causal agent of FHB worldwide, *F. meridionale* and *F. austroamericanum* have also been found in wheat fields. Special attention should be paid to *F. meridionale* because it produces the trichothecene mycotoxin nivalenol, which has potentially higher levels of toxicity when compared with that of other trichothecene produced by the FGSC. Knowledge of the diversity and genetic structure of populations of pathogens can assist in understanding pathogen survival in the face of host genetic resistance, crop rotation, and management with fungicides. In this study, we used 10 inter-simple sequence repeat (ISSR) primers to estimate the variability, diversity, and genetic structure of populations of *F. graminearum*, *F. meridionale*, and *F. austroamericanum* co-occurring in wheat fields in Paraná State, Brazil. The population of *F. graminearum* showed the greatest genetic variability according to its polymorphism ($P = 86.61\%$), Nei's genetic diversity ($h = 0.28$), and Shannon diversity ($I = 0.43$) indices as compared with that of the populations of *F. meridionale* ($P = 75.89\%$, $h = 0.23$, $I = 0.36$) and *F. austroamericanum* ($P = 67.86\%$, $h = 0.22$, $I = 0.33$). The dendrogram, principal coordinate analysis, Bayesian analysis ($K = 4$), and the differentiation index ($G_{ST} = 0.27$) showed very high structure and, consequently, high species differentiation. The overall analysis of our data indicates high variability and genetic structure of the studied populations. This genetic condition guarantees high resilience of these species and could make it difficult to obtain wheat cultivars with good resistance to FHB, as well as may cause chemical control to be less effective.

Keywords *Fusarium graminearum* · Molecular markers · ISSR · Mycotoxins · Deoxynivalenol

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Introduction

Wheat (*Triticum aestivum* L.) is the source of 20% of the total calories consumed by humanity and is the second crop in grain production worldwide, surpassed only by corn (Chaves et al. 2013). Maintaining and increasing the production of this cereal is fundamental for world food security (Chaves et al. 2013; Boff et al. 2019). Among the fungal diseases that affect wheat Fusarium head blight (FHB), caused mostly by the members of *Fusarium graminearum* species complex (FGSC), is one of the most relevant (Van Der Lee et al. 2015; Wang et al. 2019; Xia et al. 2020). Losses caused by the occurrence of FHB in wheat in the 1990s were estimated at US $ 2.5 billion and US $ 220 million in the USA and Canada, respectively (Windels 2000), and from 2008 to 2015, there

was a significant increase in FHB epidemics in China (Wang et al. 2019). In South America, several FHB epidemics have been recorded in recent decades (Del Ponte et al. 2009; Palazzini et al. 2017). The importance of FHB in world agriculture is substantial enough that *F. graminearum*, the species most commonly causing FHB, was ranked as the fourth most important fungus in the world (Dean et al. 2012). The level of concern regarding FHB results not only from its devastating impact in the field but also from the frequent contamination of the harvested grains with mycotoxins, mainly trichothecenes, making unsafe their use as human food or animal feed unsafe (Schothorst and Van Egmond 2004).

Among the more than 150 trichothecene mycotoxin types, deoxynivalenol (DON) is a common contaminant of wheat grains, and wheat-based products worldwide (Sobrova et al. 2010). Deoxynivalenol, its acetylated derivatives 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV) are the most common trichothecenes produced by the members of FGSC that cause FHB (Bertero et al. 2018). Even at low concentrations, these trichothecenes are harmful to human and animal health (Varga et al. 2015; Bertero et al. 2018).

Since 2004, 15 phylogenetic species have been identified within the FGSC (O'Donnell et al. 2000; O'Donnell et al. 2004; Starkey et al. 2007; O'Donnell et al. 2008; Yli-Mattila et al. 2009; Sarver et al. 2011). The most widely geographically distributed member of FGSC causing FHB in wheat is *F. graminearum sensu stricto*. This species is predominant in wheat fields in the Americas (North and South), Europe, and Asia (Laday et al. 2004; Zeller et al. 2003; Zeller et al. 2004; Ramirez et al. 2007; Scoz et al. 2009; Van Der Lee et al. 2015). Despite the great prevalence of *F. graminearum* in wheat fields around the world, other species predominate in specific regions. For example, in both China and Japan, in the northern wheat fields, there is a predominance of the species *F. graminearum*, whereas to the south *F. asiaticum* prevails (Suga et al. 2008; Zhang et al. 2012). Likewise, in Ethiopia, the most common species in wheat is *F. aethiopicum* (O'Donnell et al. 2008). Another FHB aspect that deserves to be highlighted is the frequent co-occurrence of more than one FGSC member with differing trichothecene genotypes.

In South America, in Argentina, Brazil, and Uruguay, where the areas of larger wheat production are located, *F. graminearum* genotype 15-ADON is predominant, but several other species and genotypes have been identified as co-occurring. In Uruguay, Umpiérrez-Failache et al. (2013) reported the presence of five FGSC members of three genotypes in wheat fields [*F. graminearum* (15-ADON and 3-ADON), *F. asiaticum* (NIV), *F. cortaderiae* (NIV), *F. brasilicum* (NIV), and *F. austroamericanum* (3-ADON)], with. *F. graminearum* (15-ADON) being the most common. In Argentina, *F. graminearum* 15-ADON producer also prevail (Ramírez et al. 2006; Fernández Pinto et al. 2008; Alvarez

et al. 2009; Reynoso et al. 2011; Malbrán et al. 2014; Ortega et al. 2016). Recently, Palacios et al. (2017) evaluated isolates sampled from *Triticum turgidum* L. var. *durum* and identified, in addition to *F. graminearum* with the 15-ADON genotype, *F. cerealis* with the NIV genotype.

In Brazil, the evaluation of 671 isolates sampled from 200 wheat fields over a 3-year period (2009 to 2011) identified the occurrence of five FGSC members of three trichothecene genotypes [(*F. graminearum* 15-ADON (83%), *F. meridionale* NIV (12.8%), *F. asiaticum* NIV (0.4%), *F. cortaderiae* NIV or 3-ADON (2.5%), and *F. austroamericanum* NIV or 3-ADON (0.9%)] (Del Ponte et al. 2015). Among these species, *F. asiaticum* and *F. cortaderiae* were isolated only from wheat fields located in the extreme southern area (state of Rio Grande do Sul) of the region under study. It is interesting to note that in South America, *F. meridionale* (genotype NIV) was observed in wheat fields only in Brazil, whereas *F. austroamericanum* (genotype NIV or 3-ADON) was found in Brazil and Uruguay. In the state of Paraná, Brazil, the largest wheat producer in the country, *F. meridionale* (NIV producer) is of great importance as it is responsible for approximately 30% of cases of FHB (Del Ponte et al. 2015). *Fusarium meridionale* has been described as endemic to Latin America (Aoki et al. 2012), but its presence has also been reported in wheat fields in Australia (Amarasinghe et al. 2019), China (Zhang et al. 2014), Nepal (Desjardins and Proctor 2011), South Africa (Boutigny et al. 2011), and Taiwan (Wang and Cheng 2017). Contrarily, *F. austroamericanum* has been detected in wheat only in South America (O'Donnell et al. 2008; Umpiérrez-Failache et al. 2013; Del Ponte et al. 2015).

Knowledge of variability, diversity, and genetic structure of populations of pathogens can aid in the prediction of their behavior against resistant cultivars, application of fungicides, and climatic changes. This prediction is based on the premise that the higher the genetic diversity and variability of a species, the higher will be its resilience in the face of situations that affect its survival (Nevo 1978; Barrett and Schluter 2008). For a few members of the FGSC, population-genetic data are available, with *F. graminearum* being the most studied. For this species, high levels of genetic diversity have been observed (Mishra et al. 2004; Astolfi et al. 2011; Albayrak et al. 2016; Pan et al. 2016; Palacios et al. 2017; Malbrán et al. 2019; Yerkovich et al. 2020) suggesting that, although the species is homothallic and self-fertile, sexual reproduction is probably occurring. For *F. meridionale* and *F. austroamericanum*, there are no data available on genetic variability, diversity, or genetic structure of populations. Population-genetic data is an important step in understanding the dynamics of *F. meridionale* and *F. austroamericanum* in the region where these occur. The use of inter-simple sequence repeat (ISSR) DNA markers (Zietkiewicz et al. 1994) has already been shown to be effective to reveal

population-genetic data in species of *Fusarium* (Arif et al. 2008; Dinolfo et al. 2010; Divakara et al. 2013; Albayrak et al. 2016; Santillán-Mendoza et al. 2019). Thus, the goals of this study were to estimate genetic variability, diversity, and structure of populations among isolates of *F. graminearum sensu stricto*, *F. meridionale*, and *F. austroamericanum* sampled in wheat fields in southern Paraná State, Brazil.

Material and methods

Populations, DNA extraction, and quantification

In this study, 49 fungal isolates belonging to three populations of *F. graminearum* ($n = 19$), *F. meridionale* ($n = 16$), and *F. austroamericanum* ($n = 14$) were evaluated (Fig. 1). These isolates were sampled between the years 2012 and 2016 in several wheat fields of southern Paraná State, Brazil, and belong to the fungal collection of the Plant Pathology Laboratory of the Universidade Estadual de Maringá (UEM), Paraná, Brazil. They were previously identified at the Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, USDA, Peoria, Illinois, USA, using the multilocus genotyping approach developed by Ward et al. (2008). The population molecular analysis was done at the Central Laboratory of the Cooperativa Agrária Agroindustrial and in the Plant Molecular Biology Laboratory of the Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brazil.

For DNA extraction, each isolate was plated in potato dextrose agar medium and kept for 7 days in a growth-chamber room (25 ± 2 °C). After this period, the mycelium was scraped with a platinum loop, dipped in liquid nitrogen, and crushed until a fine powder was obtained. The crushed fungal tissue was stored in a microtube at -20 °C until DNA extraction. The DNA was extracted according to the protocol proposed by Doyle and Doyle (1987). For each isolate, 1 mL of extraction buffer (20 mM EDTA, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, 0.4% β-mercaptoethanol) was added to 100 mg of crushed tissue and incubated in a water bath at 65 °C for 45 min. The DNA was separated from the solution by precipitation using chloroform: isoamyl alcohol (24:1 v/v) followed by centrifugation. To obtain genetic material of high purity, successive washes with ethanol were performed. After extraction, the DNA was resuspended in TE (10 mM Tris-HCl; pH 8.0; 1 mM EDTA), treated with RNAse at 37 °C for 1 h and stored at -20 °C until use. The quantification of DNA and confirmation of its integrity was conducted using electrophoresis on 0.9% agarose gel stained with ethidium bromide and visualized using UV light. To determine the DNA concentration, after electrophoresis, each sample was compared with known amounts (100, 200, and 400 ng) of intact DNA from the Lambda phage (Thermo Fisher Scientific) run in different wells of the same gel as the samples.

PCR, electrophoresis, and gel analysis

The DNA of each isolate was amplified using 10 ISSR primers developed by the University of British Columbia
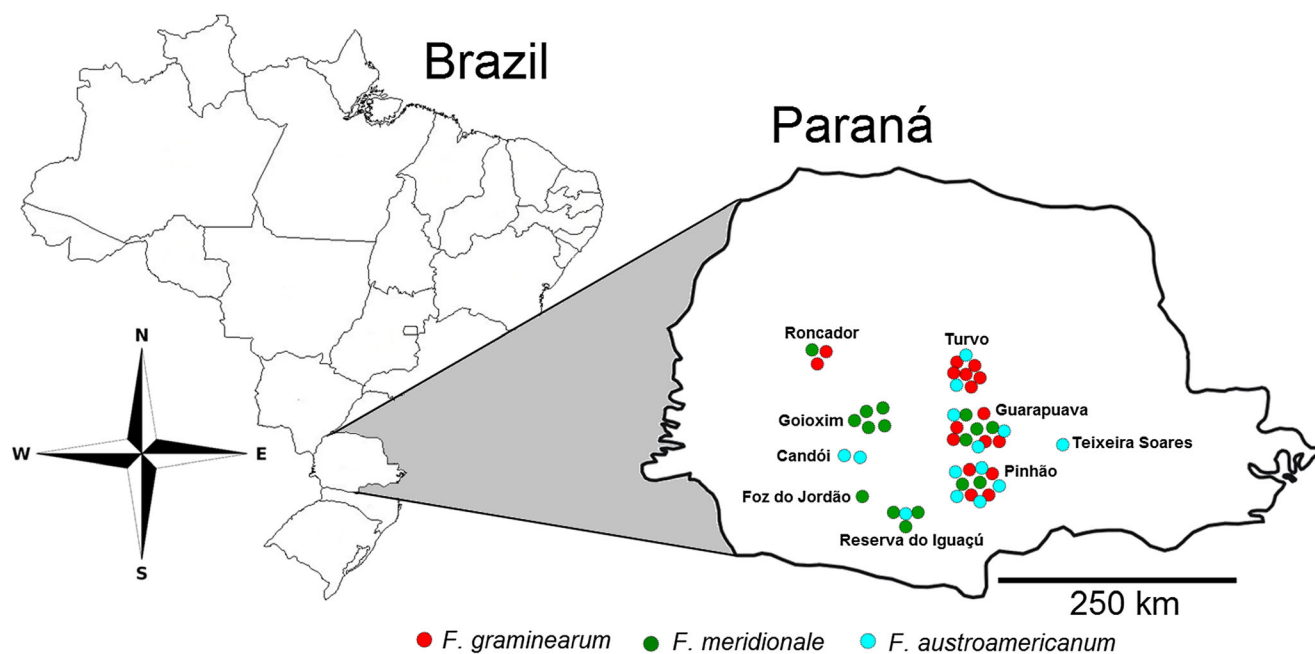

Fig. 1 County of sampling of the isolates belonging to the populations of *Fusarium* species evaluated in this study. Two isolates of *F. graminearum* are not represented on the map due to unknown origin

(UBC), Vancouver, Canada (Table 1). The DNA amplification reactions were conducted in a final volume of 12.5 μL containing 20 ng of DNA, 0.2 μM of primer, 200 μM of each dNTP, 1.5 mM of MgCl$_2$, 1 U of Taq DNA polymerase, and 1X PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]. All reagents and enzymes used in PCR were from Invitrogen. For amplification, the thermocycler (Veriti, Applied Biosystems) was programmed with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 90 °C for 45 s, and annealing temperature of the primer for 30 s (Table 1) and 72 °C for 60 s. At the end of the cycles, a step of 10 min at 72 °C was added for the final extension of the fragments. The amplification products were separated by electrophoresis on 1.8% agarose gel stained with ethidium bromide (0.5 μg mL^{-1}), with a 4 h run at 110 V. To determine the size of the amplified fragments, a molecular weight marker 100 bp DNA ladder (Invitrogen) was used. The electrophoresis result was visualized in UV light and photo-documented with a digital system (L-PIX EX, Loccus do Brasil).

Statistical analysis

The gels were visually evaluated for the presence (1) or absence (0) of bands in each amplified locus. Only polymorphic bands with good definition were used to construct the binary matrix used in the statistical analysis. The genetic relationship among the genotypes was estimated by Nei's genetic distance (Nei 1972) using the NTSYS 2.2 software (Rohlf 2000) and a dendrogram was obtained by the neighbor-joining method also using the NTSYS. *Fusarium proliferatum* was used as an outgroup and the tree obtained was not rooted so as not to lose the genetic distance scale. The reliability of branches in

the tree was assessed by bootstrapping with 1000 replicates using the FreeTree software version 0.9.1.50 (Hampl et al. 2001). The percentage of polymorphism (P), Shannon diversity index (I), Nei's genetic diversity (h), and the coefficient of genetic differentiation (G_{ST}) were calculated using the POPGENE software version 1.31 (Yeh et al. 1999).

A principal coordinate analysis (PCoA) was performed using GenAlEx software version 6.501 (Peakall and Smouse 2012). The Bayesian analysis was conducted to determine the number of clusters (genetic groups) that best explained the observed genetic variability, using STRUCTURE program version 2.3 (Pritchard et al. 2010). To determine the best-supported number of clusters (K), simulations were made based on the principle that it is possible to obtain any number of clusters between 1 and 10, with 10 repetitions for each simulation. For this analysis, the ancestry admixture model was used, and the allelic frequencies were correlated using 500,000 burn-in and 1,000,000 MCMC (Markov Chain Monte Carlo) steps after the burn-in. To define the K (number of clusters - genetic groups) most likely in relation to those proposed by the analysis, the criteria suggested by Evanno et al. (2005) were used with the help of the Structure Harvester program version 0.9.94 (Earl and VonHold 2012). For all analyses, each group of isolates belonging to the same species was considered a population.

Results

The 10 ISSR primers amplified 128 bands and of them, 112 (87%) were polymorphic and were used in population analysis (Table 1).

The isolates of *F. graminearum* showed the highest polymorphism and were more genetically diverse according to both Nei's genetic diversity and the Shannon diversity index (Table 2). The percentage of polymorphism was above 67% when considering each species individually and 100% considering all species (Table 2). The genetic differentiation index ($G_{ST} = 0.27$) showed that populations were highly structured. The dendrogram obtained based on Nei's genetic distance (Fig. 2) showed that

Table 1 List of the 10 ISSR (inter-simple sequence repeat) primers used to assess the diversity and genetic structure of *Fusarium graminearum*, *F. meridionale*, and *F. austroamericanum* populations. Annealing temperature in °C (TA°C), the total number of amplified fragments (NAF), and the percentage of polymorphism (P) for each primer are provided

Primer	Sequence*	TA °C	NAF	P (%)
UBC 807	(AG)$_8$T	52	14	71.42
UBC 808	(AG)$_8$C	50	15	73.33
UBC 809	(AT)$_8$T	55	13	92.30
UBC 811	(GA)$_8$C	53	8	87.50
UBC 827	(AC)$_8$G	53	15	100.0
UBC 835	(AG)$_8$YC	54	13	92.30
UBC 848	(CA)$_8$RG	55	11	72.72
UBC 861	(ACC)$_6$	52	14	92.85
UBC 868	(GGA)$_6$	50	12	100.0
UBC 873	(GACA)$_4$	50	13	92.30

*Y = (C, T); R = (A, G)

Table 2 Genetic diversity and polymorphism observed in *Fusarium* species sampled in southern Brazil according to Shannon genetic diversity (I); Nei's genetic diversity (h); and percentage of polymorphism (P)

Species	I	h	P (%)
F. graminearum	0.43	0.28	86.61
F. meridionale	0.36	0.23	75.89
F. austroamericanum	0.33	0.22	67.86
Total	0.52	0.35	100

F. meridionale and *F. austroamericanum* were more genetically related and that the *F. graminearum* was the most basal. One isolate of *F. austroamericanum* and three of *F. meridionale* were genetically more similar to *F. meridionale* and *F. austroamericanum*, respectively (Fig. 2). The PCoA reduced the dimensionality of the data and allowed the observation of the spatial structure of the isolates and their contribution to the diversity of the species (Fig. 3). Coordinates 1, 2, and 3 explained 21.31%, 13.07%, and 10.62% of the observed genetic variability, respectively. The cumulative explanation of the observed genetic diversity by the three coordinates was 45.00% (Fig. 3). Additionally, the PCoA allowed us to observe that most isolates were structured according to the species they belonged to, in accordance with the clusters observed in the dendrogram (Fig. 2).

In the simulations made by the STRUCTURE program using Bayesian inference, the highest probabilities were for the number of Ks (clusters, genetic groups) of two and four (Fig. 4). In $K = 2$, the species *F. meridionale* and *F. austroamericanum* were predominant in the genetic group represented by green, whereas *F. graminearum* was predominant in the genetic group represented by red (Fig. 5). At $K = 4$, each species exhibited predominance in a separate genetic group. The magenta-colored genetic group is the one distributed among the three species studied (Fig. 5).

Discussion

Genetic variability is the driving factor for evolution because it allows for adaptation (Nevo 1978; Barrett and Schluter 2008). In this sense, the knowledge of the genetic variability in populations of plant pathogens allows the prediction of their adaptive behavior in the face of the genetic resistance of the host and chemical control. The ability to predict a probable evolutionary behavior of a pathogen can assist in the development of management strategies to reduce losses or even prevent epidemics (Burdon et al. 2006). The species *F. graminearum*, *F. meridionale*, and *F. austroamericanum* evaluated in our study showed high molecular variability and genetic diversity, from which a high capacity for adaptation can be inferred. High genetic structuring was also observed in populations, corroborating the concept of phylogenetic species proposed by O'Donnell et al. (2004).

High genetic variability in the species included in this study is evidenced by the percentage of polymorphism (Table 2) and no polytomy in the dendrogram (Fig. 1). For the *F. austroamericanum* and *F. meridionale* species, there was no previous literature information on the percentage of polymorphism. Thus, in comparison with studies conducted with *F. graminearum* can help us understand the variability in these species. For *F. graminearum*, polymorphism has been variable
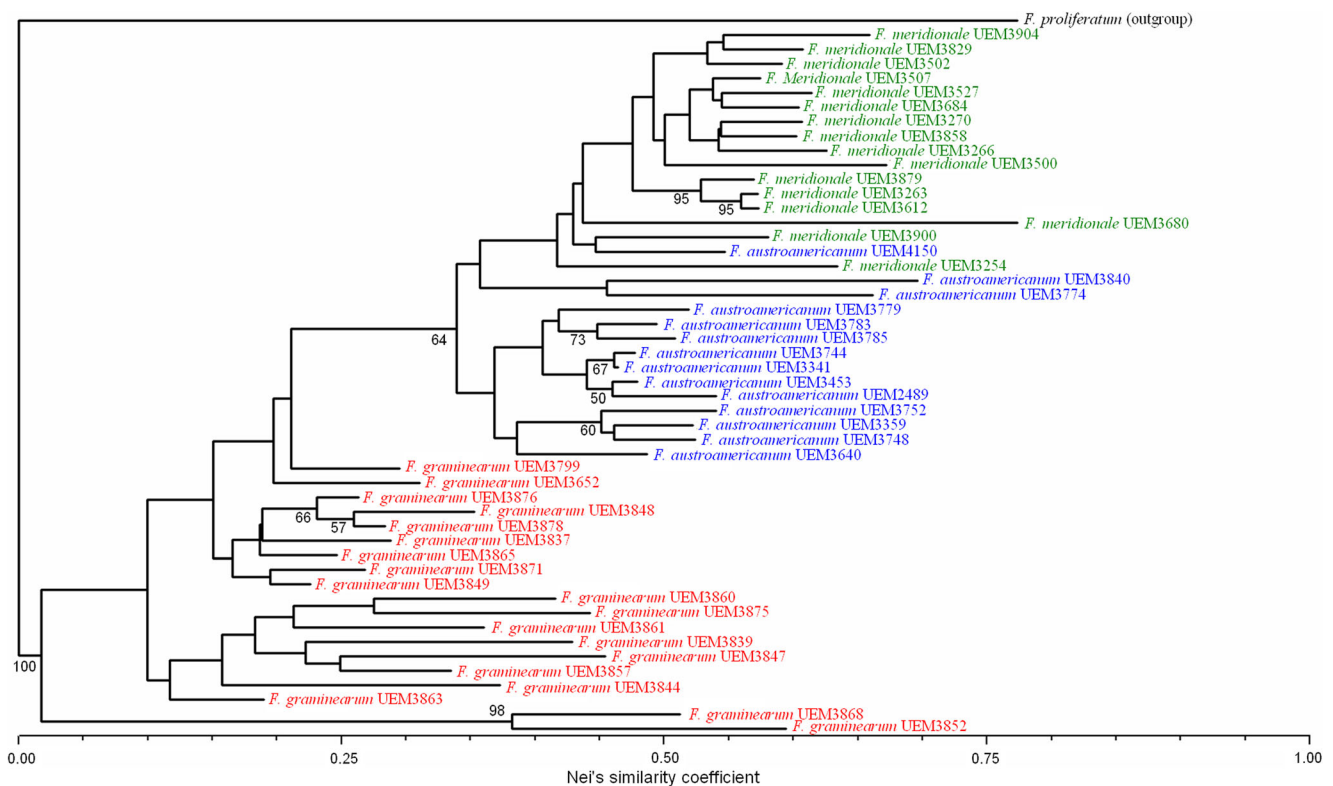

Fig. 2 Dendrogram based on Nei's genetic distance of the *Fusarium* isolates sampled in southern Brazil. The dendrogram was obtained using 112 ISSR (inter-simple sequence repeat) loci and by the neighbor-joining grouping method. The numbers on the branches indicate the bootstrapping values obtained with 1000 replicates. Only a bootstrapping value above 50 was shown
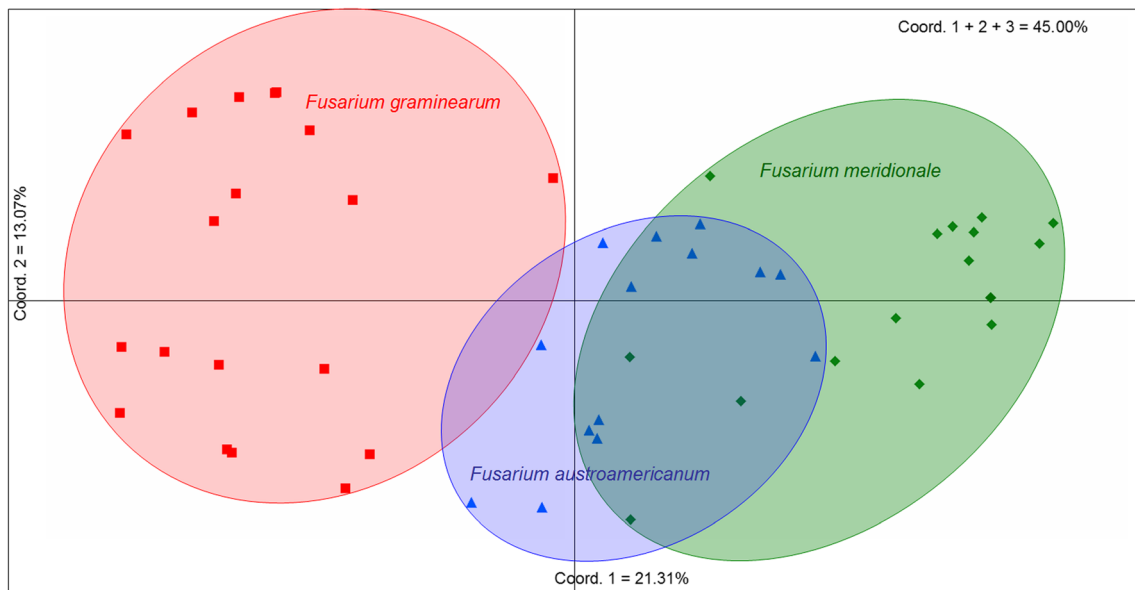
Fig. 3 Principal coordinate analysis of isolates of three *Fusarium* species sampled in southern Brazil based on 112 ISSR (inter-simple sequence repeat) loci

using dominant markers [ISSR and AFLP (amplified fragment length polymorphism)]. Albayrak et al. (2016) evaluated 405 ISSR loci in 43 *F. graminearum* isolates from Turkey and Iran and only 5.62% were polymorphic. Mishra et al. (2004), evaluating eight ISSR primers in 70 *F. graminearum* isolates from Canada, reported polymorphism ranging from 56 to 85%. Malbrán et al. (2019) obtained 71% polymorphism by evaluating 80 ISSR loci in 109 *F. graminearum* isolates sampled in Argentina, whereas Palacios et al. (2017), using 72 ISSR loci in 62 isolates from the same country, obtained 92% polymorphism. Pan et al. (2016), evaluating 172 AFLP loci in 103

F. graminearum isolates from Uruguay, observed 89.5% of polymorphism. In southern Brazil, Astolfi et al. (2011) evaluating populations (111 isolates) from three counties in Rio Grande do Sul using 89 AFLP loci identified polymorphism ranging from 63 to 67%. Cunha et al. (2015) evaluating 54 ISSR loci in 152 isolates of *F. oxysporum f.* sp. *cubense*, sampled in 21 counties in southern Brazil, obtained 81% polymorphism. In this sense, comparing our data with the polymorphism data for *F. graminearum* at a global level and for the *Fusarium* genus at a regional level leads to the conclusion that *F. austroamericanum* and *F. meridionale* populations have

Fig. 4 Determination of the optimal number of *K* (genetic groups) in *Fusarium* by Bayesian inference. The point of intersection between the highest value on the Y-axis and the X-axis indicates the optimal number of *K* (genetic groups)
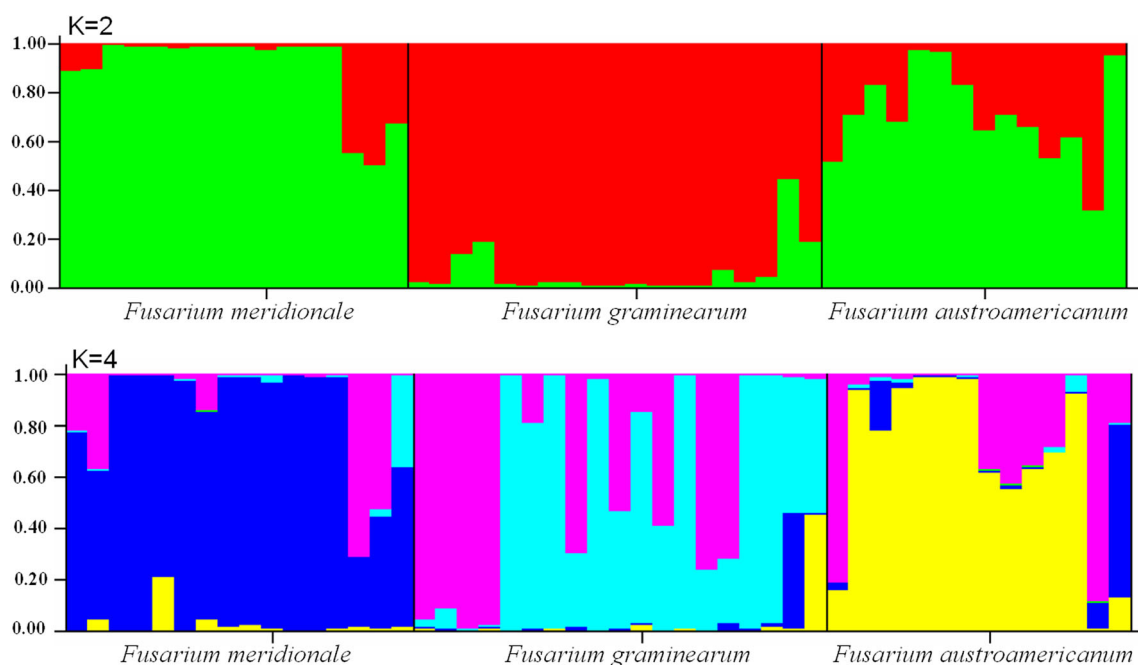

DeltaK = mean(|L''(K)|) / sd(L(K))
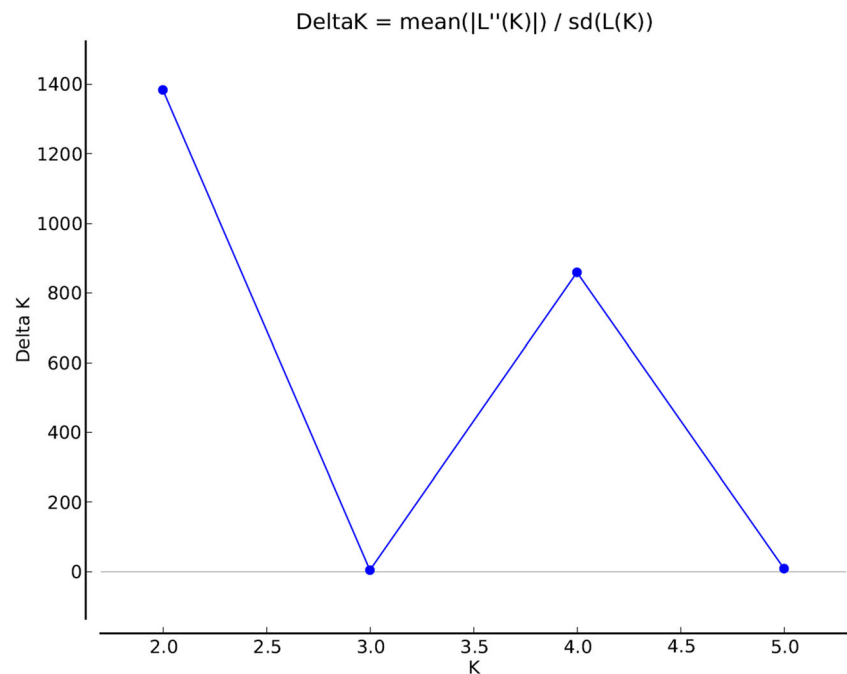
Fig. 5 Genetic structure of *Fusarium* populations evaluated in this study according to the division into two (*K* = 2) and four (*K* = 4) genetic groups obtained by Bayesian Inference

high polymorphism. Nonetheless, it is important to emphasize that the isolates of the two species we studied were from a small geographical area compared with that of other studies in the literature, which further strengthens the finding of high polymorphism in these populations. The same comparison above applied to the *F. graminearum* population evaluated in our study also showed the high polymorphism of this species in the studied region. Polymorphism levels are important information when working with dominant markers because they are one of the parameters that can be used to indicate genetic variability (Fedrigo et al. 2016; Rosa et al. 2017; Mariano et al. 2019). The high polymorphism observed in the populations of *F. graminearum*, *F. meridionale*, and *F. austroamericanum* in southern Brazil implies high genetic variability in these species; thus, presenting a high adaptative potential.

When comparing the values of genetic diversity in the three species studied (Table 2) with data from the literature, we observed that the values we obtained were similar. Mishra et al. (2004) reported Nei's genetic diversity ranging from 0.18 to 0.23 in isolates of *F. graminearum* sampled in Canada. Talas et al. (2011) evaluating *F. graminearum* isolates from Germany using microsatellite markers obtained Nei's genetic diversity ranging from 0.30 to 0.54 and Shannon diversity from 0.53 to 0.96. For the southern region of Brazil, Astolfi et al. (2011), using AFLP markers, obtained Nei's genetic diversity ranging from 0.12 to 0.13 in populations of *F. graminearum* when all polymorphic loci were used. Among the three populations studied by us, *F. graminearum* presented the highest genetic diversity, corroborating the data on genetic variability. For *F. meridionale*

and *F. austroamericanum* species, there is no data on diversity in the literature; however, the comparison with data from other species of the genus *Fusarium* and with consideration for the limited geographical origin and number of our isolates, we can conclude that besides the high genetic variability, these species also have high genetic diversity, which could enhance their adaptive capacity.

The dendrogram (Fig. 2) corroborated the high genetic variability and diversity and indicated no polytomy. Additionally, it allows observing genetic proximity between the *F. meridionale* and *F. austroamericanum* species (Fig. 4). Also, in the dendrogram considering isolates (Fig. 2), it was possible to observe the high structuring of the *F. graminearum* species. The positioning in the dendrogram of three isolates of *F. meridionale* and three of *F. austroamericanum* among the main groups of *F. austroamericanum* and *F. meridionale* may be due to the low number of isolates used or due to the resolving power of the marker used. Albayrak et al. (2016) evaluating 43 isolates of *F. graminearum* and 20 of *F. culmorum* from Turkey also obtained groups in the dendrogram according to the species and with no polytomy; however, the isolates within each group were more genetically similar as compared with those in our study. Dinolfo et al. (2010) evaluating *F. poae* isolates from Argentina and England were unable to separate the isolates according to their geographical origin and also observed polytomy in the dendrogram. The results of these studies show that there are differences in genetic diversity and genetic relationship between isolates both when comparing species and when considering geographic regions. These results justify the need for studies of populations in less geographic coverage to

better understand the dynamics of the variability in *Fusarium* species at the regional scale. This information can assist in the development of more effective strategies for managing the pathogen in regions with a history of FHB epidemics in wheat.

The PCoA results (Fig. 3) corroborated those of the dendrogram and showed that the genetic variation is well distributed among the species and also illustrated genetic proximity between the *F. meridionale* and *F. austroamericanum* species. *Fusarium graminearum* was the species that most contributed to the variation in the group, which was expected because it had greater variability and diversity and also by the number of isolates used. Bayesian inference analysis showed that *F. meridionale* and *F. austroamericanum* were the species with the greatest genetic similarity when considering $K = 2$, corroborating the data from the dendrogram and PCoA. Furthermore, when considering $K = 4$ (which also presented strong support in explaining the genetic variation of populations), the species were more distinguishable, with each one presenting a separate genetic group. One of the genetic groups (represented by the magenta color) is not predominant in any species and seems to be responsible for the genetic relationships among the species (Fig. 5). The number of genetic groups that best explains the genetic variation of populations or species is dependent on the type of data analyzed (species or populations of the same species) and the sample size. For example, Altinok et al. (2018) evaluating 200 isolates from Turkey observed five genetic groups ($K = 5$) in populations of *F. oxysporum f.* sp. *melongenae*. Yerkovich et al. (2020) evaluating 75 *F. graminearum* isolates from Argentina observed two genetic groups. In our study, the limited number of isolates used of each species may be the cause of not obtaining $K = 3$ which would be expected since the number of species evaluated.

In this study, the fixation index ($G_{ST} = 0.27$) indicates a very high genetic structure in the populations, corroborating the proposal by O'Donnell et al. (2004) of phylogenetic species in the FGSC. Additionally, the dendrogram obtained by Nei's genetic distance (Fig. 2) and the structuring data obtained by Bayesian inference (Fig. 5) showed high genetic differentiation among populations, which was expected based on populations of distinct species. Nonetheless, the genetic relationship observed between populations was in agreement with that proposed by O'Donnell et al. (2004), who identified a greater genetic relationship between the species *F. meridionale* and *F. austroamericanum* when compared with *F. graminearum*.

The occurrence of more than one species capable of causing FHB in wheat fields in southern Brazil and the high genetic variability and diversity of the populations of these species may be an aggravating factor in the control of those pathogens. High genetic variability and diversity of populations can result in a failure in using fungicides for the chemical control of the pathogen (Georgopoulos and Skylakakis 1986), as well as of the ineffectiveness of resistant cultivars (McDonald and Linde 2002). This is because pathogen populations with high

variability and genetic diversity have vastly variable genetic constitutions that allow for the rapid selection of genotypes that override chemical control or resistance of cultivars. Feksa et al. (2019) carried out evaluations using a series of fungicides with various active ingredients to prevent or control FHB pathogens in wheat inoculated with a mix of 10 isolates with genotypes 15-ADON, 3-ADON, and NIV. The results suggested that a better strategy to control FHB pathogens and decrease DON production in the humid regions of the state of Paraná should involve multiple applications of fungicides with various modes of action. That study included some isolates used in our work. The high genetic variability observed in the FGSC members causing FHB in the state of Paraná in our study might explain the variation in the FHB severity and DON production found by Feksa et al. (2019). This observation strengthens the hypothesis regarding the difficulty in controlling the pathogens when are present a series of related species with high genetic variability.

The overall analysis of our data together with the literature showed that the high variability found in the populations of *F. graminearum*, *F. austroamericanum*, and *F. meridionale* that cause FHB in southern Brazil is an important factor to be considered in the chemical control of the pathogen with fungicides and through breeding programs aimed at obtaining resistant wheat cultivars. Certainly, selection strategies in breeding programs can be more effective when lineages are selected considering more than one isolate of the causal agent of FHB.

Compliance with ethical standards

Conflict of interest　The authors declare that they have no conflict of interest.

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