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Genetic diversity among crabgrass weed ecotypes (*Digitaria* spp.) occurring in field crops in Rio Grande do Sul, Brazil

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Abstract

High genetic diversity is one of the main characteristics that ensures the adaptability and competitiveness of weeds in agricultural crops; therefore, knowledge of such diversity may be useful for outlining weed control strategies both more effectively and with less impact on the environment. The aim of the study was to use amplified fragment length polymorphism (AFLP) to evaluate the genetic similarity among ecotypes of crabgrass (*Digitaria* spp.) from areas cultivated with rice and soybeans from different agricultural regions of the State of Rio Grande do Sul (RS), Brazil. Samples were collected from crabgrass populations (*Digitaria* spp.) from February to March 2009, covering areas of rice and soybean production in 19 districts belonging to different regions of the RS. Three locations in each district were visited, and georeferences were determined at each of the three sampling points, resulting in 171 crabgrass accessions. Of the 171 ecotypes that were listed and classified by morphological traits, 34 were selected based on the geographic distribution criteria, and species diversity was assessed by AFLP. Genetic similarity among individuals was estimated using simple matching (SM) coefficients, and a dendrogram was constructed based on these data. The similarity among all ecotypes, including six different species, was 83%. When the species were analyzed separately, we found 86% similarity among the ecotypes of *D. ciliaris* and 89% among those of *D. sanguinalis*. Due to geographic distance, the lowest genetic similarity was found between the ecotypes *D. tenata* from Cachoeira do Sul and *D. eriostachya* from Itaqui. The ecotypes from upland areas were more similar than those from paddy fields. For *D. ciliaris*, the lowest genetic similarity was found between the ecotypes originating from Itaqui and Viamão. Higher genetic similarity of *D. sanguinalis* ecotypes was observed between Arroio Grande and Dom Pedrito. Knowledge of the genetic diversity of weed populations is essential for the management and control of weeds.

Keywords: AFLP; *Digitaria* sp.; genetic similarity; weeds; weed biology.
Abbreviations: AFLP Amplified Fragment Length Polymorphism; ALS acetolactate synthase; PCoord principal coordinate analysis; SAHN sequential, agglomerative, hierarchical and nested; UPGMA unweighted pair group method with arithmetic means; AS Average Similarity, RS Rio Grande do Sul.

Introduction

Crabgrass belongs to the genus *Digitaria*, in the subfamily Panicoideae of the family Poaceae. This genus includes approximately 300 species that are distributed in tropical and subtropical regions of both hemispheres, and the visual differentiation of these species in the field is difficult due to their high morphological similarity (Kissmann, 1997). Important elements for species classification include the spikelets, based on the form of insertion and the hairiness of the glumes, and published drawings of different species are also used to aid in classification. Correct identification is fundamental to the management of these weeds because different species of crabgrass show differences in herbicide tolerance (Dias et al., 2007). One of the inherent traits of this weed is its great genetic diversity, which is a result of the natural evolution of species, mainly involving Mendelian variation, interspecific hybridization and polyploidy. This variability can affect management practices through the selection of resistant biotypes and by influencing the effectiveness of chemical and biological management strategies (Sterling et al., 2004). Therefore, knowledge of the genetic diversity of weeds can provide useful information for the development of new control strategies (Slotta, 2008). Among the alternatives for accessing the genetic variability of this weed, we highlight the use of molecular genetic markers, aiming to provide information on invasion, heritability of traits, taxonomic relationships, point of origin and gene flow. A wide variety of molecular methods are available for studying genetic diversity, and the choice of the marker technique to be used depends on the goals of the study, the genetic diversity and knowledge of the genomes of the study populations, in addition to the availability of laboratory facilities and the cost (O 'hanlon et al., 1999). Despite permitting the identification of only one allele at each locus, specifically the dominant allele, the amplified fragment length polymorphism (AFLP) approach stands out because of its reproducibility, the large number of markers generated, its considerable power to detect genetic variability and the robustness of this assay compared with those of other dominant markers (Vos et al., 1995). Further, this assay has been demonstrated to produce consistent results in various

situations, similar to microsatellite markers (Meudt and Clarke, 2007). AFLP band profiles result from variations in the restriction sites of the enzymes that are used and the selective amplification of the obtained DNA fragments (Vos et al., 1995; Spooner et al., 2005). Another advantage of this type of molecular marker is that it can be used in organisms for which no prior genetic information is available (Bonin et al., 2007; Vuylsteke et al., 2007), as is the case for most weeds.

Within this context, the present study aimed to use an AFLP approach to evaluate the genetic diversity among crabgrass ecotypes from areas cultivated with rice and soybeans in different agricultural regions of the State of Rio Grande do Sul (RS), Brazil.

Results and Discussion

Power of the markers

Of the 12 tested combinations of pairs of initializing oligonucleotide (primers), only five showed consistent amplification, generating 280 banding profiles, all of which were polymorphic. The total number of bands generated by each primer combination ranged from 38 to 82, with an average of 56 polymorphic bands per primer combination. The two combinations of primers that generated the largest number of polymorphic bands were M-CAC/E-ACC (59) and M-CAC/E-ACA (82). The polymorphisms detected by AFLP analysis result from several types of mutations, leading to the loss or gain of restriction sites at each locus. These restriction sites are recognized by enzymes used for digestion plus selective amplification, as determined by arbitrary nucleotides located at the 3' ends of primers (Cavalli, 2003). High polymorphism rates, similar to those that were identified in this study, have been detected in *Ranunculus acris* using the same technique by Odat et al. (2004), who observed a polymorphism rate of 79.5%. Further, Monte-Corvo et al. (2000), who studied *Pyrus* cultivars, calculated a polymorphism rate of 87%. However, Goulão et al. (2001) found a rate of only 57.2% in analysis of the genetic similarity among cultivars of *Malus* spp. in association with a higher degree of relatedness among a large number of woody fruit species. Cultivated plants, which are crossed and backcrossed, show patterns different from those that occur by natural selection and dispersion, as in weeds.

Genetic diversity among accessions

Clustering detection

The average genetic similarity among the 34 ecotypes was 83%, resulting in a total of six subgroups. The Mantel correlation coefficient obtained from a comparison between the cophenetic and similarity matrices was high (r = 0.96), indicating that the properly produced dendrogram accounted for the similarity matrix generated from the polymorphic AFLP bands of the 34 *Digitaria* spp. accessions (Fig. 2). Bootstrapping analysis demonstrated that the major clusters had values ranging from 60.4 to 100%, with the highest value obtained for the G3 subgroup and the lowest for the G5 and G4 clusters. The nodes where there were no bootstrapping values had values of less than 60.4%. The ΔK results from Evanno et al. (2005) statistical analysis showed that the most informative *K*s are 2 and 3 (Fig. 3). The summarization of replicates of each *K* showed a good similarity index, very close to the maximum similarity level (Fig. 2). The results that were obtained by the Bayesian clustering approach corroborated the results obtained by hierarchical dendrogram analysis, enabling the identification of five major allelic clusters with high agreement regarding the grouping of species as a function of the sampling sites (Fig. 2).

Genetic relationship among accessions inside clusters

The clusters that were formed by hierarchical analysis of the dendrogram along with the Bayesian approach were G1 (ecotype 5), G2 (ecotype 19), G3 (ecotypes 22 and 27), G4 (ecotypes 6 and 47), G5 (ecotypes 87 and 102) and G6 (other ecotypes) (Fig. 2). The clusters G1 and G2 were formed by the species *D. ternata* and *D. eriostachya*, respectively, with 61% similarity between the two groups, and, as expected, these genotypes were the least similar to the other ecotypes. This finding was due to the fact that these ecotypes belong to different species and come from the geographically distant regions of Cachoeira do Sul (central) and Itaqui (western), respectively.

Environmental changes often create environmental barriers against the flow of genes and thus increase the genetic differentiation between populations over time (Linhart and Grant, 1996). In addition to these changes, the genetic diversity of plants can be influenced by other processes, such as population size, and current events, such as modification of the environment through agricultural practices or gene flow influenced by humans or animals. In this study, we observed the effects of these factors on the variability among populations, such as the similarity between the G3 and G4 clusters, as well as the gene flow between the species in subgroup G5 and the allelic pool 'D' (Fig. 2).

The clusters G3 and G4 were formed by ecotypes of the *D. ciliaris* species, and the similarity among the ecotypes of these clusters was close to 85%, indicating high similarity among the four ecotypes from the same species. Nevertheless, there was a clear separation between these two clusters. G3 was formed by two ecotypes collected in Itaqui, with 88.5% similarity, suggesting a relatively distinct allelic pool compared to the ecotypes of cluster G4, which was corroborated using the Bayesian clustering approach, in which ecotypes 22 and 27 were clustered in the allelic pool 'E'. In contrast, the G4 was clustered closest to G5, which was formed by two distinct species (ecotypes 87 and 102) with 84% similarity (Fig. 2). This high similarity among the ecotypes of clusters G4 and G5 may result from management practices and the proximity of the sites, whereas the ecotypes of each cluster are from nearby rice-growing areas, which are exposed to the same environment; therefore, the similarity that was observed between the ecotypes of *D. ciliaris* (87) and *D. sanguinalis* (102) may be associated with the gene flow between these species. The last cluster (G6) included four distinct species, and the greatest similarity was found between the ecotypes belonging to the species *D. sanguinalis* and *D. ciliaris*. In general, genetic similarity of up to 95% was found the between ecotypes 31, 101, 50, 118, 130, 83, 39, 71, 113, 163, 154, 32 and 35. This similarity can be attributed to gene flow between species from nearby locations that may occur due to the outcrossing rate in the genus *Digitaria* (Huangfu et al., 2009). Gene flow tends to result in the homogenization of populations, thereby reducing divergence, and further studies of the ecology of pollination and seed dispersal and of species demographics are needed to elucidate the determinants of gene flow and the distance of connectivity between populations (Perecin et al., 2004). Although similarity analysis indicated low variability among the ecotypes of the cluster G6, the clustering obtained by the Bayesian approach revealed the existence of two major allelic

Table 1. Botanical identification and geographical locations of crabgrass (*Digitaria* spp.) accessions that were collected from agricultural areas in several districts of the State of Rio Grande do Sul, Brazil.

Map	District	Ecotype	Species	Latitude	Longitude	Elevation[1]
1	Cachoeira do Sul[2]	5	*D. ternata*	-30.197442	-52.916741	43
		6	*D. ciliaris*	-30.200864	-52.920639	46
2	São Sepé[2]	10	*D. ciliaris*	-29.964169	-53.694287	52
3	Itaqui[2]	19	*D. eriostachya*	-29.165362	-56.403396	91
		22	*D. ciliaris*	-29.340978	-56.632149	59
		27	*D. ciliaris*	-29.331569	-56.651647	57
4	Uruguaiana[2]	31	*D. sanguinalis*	-29.751423	-57.019350	54
		32	*D. ciliaris*	-29.751676	-57.019320	59
		35	*D. ciliaris*	-29.796283	-56.981064	77
5	Rosário do Sul[2]	38	*D. ciliaris*	-30.283720	-54.892779	97
		39	*D. aequiglumis*	-30.283632	-54.892788	96
6	Dom Pedrito[2]	46	*D. sanguinalis*	-30.797976	-55.002466	108
		47	*D. ciliaris*	-30.798134	-55.002518	110
		50	*D. ciliaris*	-30.796159	-55.007940	128
		54	*D. sanguinalis*	-30.933376	-54.752505	130
7	Arroio Grande[2]	55	*D. sanguinalis*	-32.281812	-53.075652	38
		62	*D. ciliaris*	-32.235539	-53.047969	35
8	Camaquã[2]	66	*D. ciliaris*	-30.903028	-51.703441	29
		71	*D. aequiglumis*	-30.895978	-51.703359	14
9	Tapes[2]	73	*D. ciliaris*	-30.657912	-51.460523	14
10	Santa Vitória do Palmar[2]	83	*D. aequiglumis*	-32.909343	-52.697922	6
		87	*D. ciliaris*	-32.901353	-52.725523	13
		90	*D. sanguinalis*	-32.880119	-52.707348	11
11	Viamão[2]	91	*D. ciliaris*	-30.109845	-50.694427	3
12	Mostardas[2]	101	*D. ciliaris*	-30.503781	-50.563941	10
		102	*D. sanguinalis*	-30.504060	-50.564876	6
13	Lagoa Vermelha[3]	113	*D. ciliaris*	-28.221020	-51.596503	756
14	Passo Fundo[3]	118	*D. bicornis*	-28.248022	-52.276402	733
		119	*D. ciliaris*	-28.248057	-52.276350	733
15	Carazinho[3]	130	*D. ciliaris*	-28.318571	-52.822838	566
16	Santa Bárbara do Sul[3]	139	*D. ciliaris*	-28.382476	-53.331235	507
17	Ijuí[3]	145	*D. ciliaris*	-28.433638	-53.899608	361
18	Cruz Alta[3]	154	*D. ciliaris*	-28.622449	-53.689633	382
19	Tupanciretã[3]	163	*D. ciliaris*	-29.037818	-53.674218	418

[1]Elevation (m); [2]collected from rice fields; [3]collected from soybean fields.

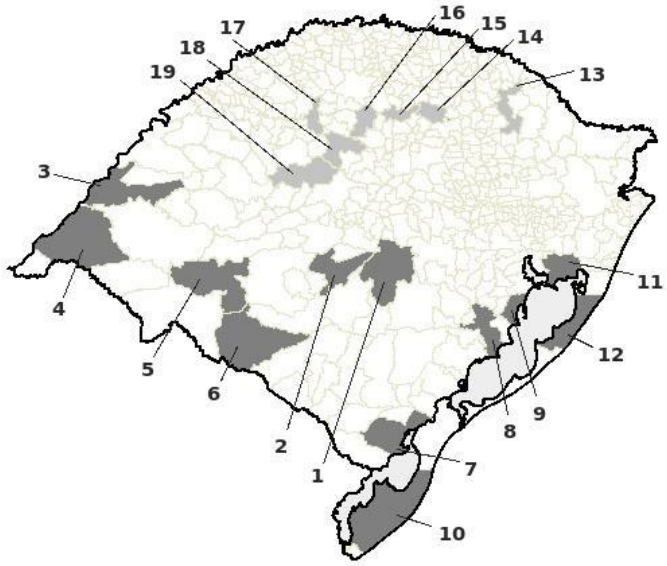

Fig 1. Districts where *Digitaria spp*. were collected in the State of Rio Grande do Sul, Brazil.

pools, and the allelic pool 'D' was formed from four *D. ciliaris* ecotypes that were more genetically similar. Ecotypes 66 and 91 as well as 62 and 73 exhibited genetic relationships that can be attributed to the proximities of the collection sites. Within this allelic pool, another four ecotypes of *D. sanguinalis* were found with the same type of relationship as that described above, i.e., ecotypes 46 and 54 were collected in Dom Pedrito, and ecotypes 55 and 90 were collected in Arroio Grande and Santa Vitória do Palmar, respectively. Therefore, these results indicated that data analysis based on different clustering approaches was more effective for determining the molecular genetic relationships between ecotypes. Although originating from nearby regions, the lowest observed genetic similarity was 61% among ecotypes 5 (Itaqui) and 19 (Cachoeira do Sul), belonging to the species *D. ternata* and *D. eriostachya*, respectively. In populations of *Euphorbia heterophylla* L. (wild poinsettia) from different regions of Brazil, a low average genetic similarity of approximately 40% (Winkler et al., 2003) was also recorded. These results demonstrate that, in general, weed species present high genetic variability, possibly because of the adverse conditions in hostile environments (Lamego et al., 2006) or because of selection pressure conditions imposed by agricultural management practices. This situation has been demonstrated in *Bidens* sp., which exhibit resistance to acetolactate synthase ALS-inhibiting herbicides and have been reported to possess average similarities of 27% (Vidal et al., 2006) and 37% (Lamego et al., 2006), thus demonstrating the existence of high variability within the same population.

Genetic similarity within and among populations can vary widely between species. In this study, the average genetic similarity among the 34 evaluated ecotypes was 83%, which is very close to that observed in plants of *Andropogon gerardii*, for which the genetic variability has been reported to be 83 and 99% among and within populations, respectively, suggesting the occurrence of a high flux of genes among populations (Gustafson et al., 1999). Natural populations of *M. ilicifolia* from the State of Paraná, Brazil, were evaluated with RAPD markers (Random Amplified Polymorphic DNA), revealing a genetic variability of 85% between individuals from the same population and 15% among populations (Bittencourt, 2000). Similar results were obtained by Perecin et al. (2004) in populations of *M. ilicifolia* and *M. aquifolium*, which were evaluated using isozyme markers. These results demonstrate that regardless of the genetic marker used, there is a greater variability among individuals of the same population, which has also been observed in other plant genera. Geographical location seems to exert a strong influence on the genetic load of ecotypes, resulting in the selection of numerous features. In this study, the lowest genetic similarity due to location within the same species and crop was observed between ecotypes of *D. ciliaris* 27 (Itaqui) and 91 (Viamão), with a similarity of 0.69, which likely resulted from the geographical distance between the two ecotypes. Overall, there was a wide dispersion of ecotypes, such that some ecotypes that were collected in nearby localities were grouped relatively distantly from one another, indicating that genetic similarity is not always directly associated with geographic distance and can be related to evolutionary forces, such as mutation, recombination, migration, genetic drift and selection, which cause changes in the frequencies of genes (alleles) in populations (Perecin et al., 2004), as well as the life histories of species in relation to the taxonomic status, geographic distribution, method of seed dispersal and successional stage (Hamrick and Godt, 1989); in addition, the genetic similarity among species can be affected by human activity through management practices.

This study demonstrated that genetically similar ecotypes from soybean agricultural areas had a coefficient of similarity of greater than 92%, and although they were from different species (Fig. 3), they were all collected in cluster G6. Some ecotypes from the rice-growing areas did not fit into this group because they belonged to the same species. The lowest genetic similarity was 70%, which was recorded between ecotypes 31 and 102, originating from the rice crop region (Uruguaiana and Mostardas, respectively). This finding is explained by the distance between the collection sites, the recent adaptation of these ecotypes to a flood-irrigated environment and changes in the management of these areas resulting from the alternation of irrigated rice with livestock or the agric ultural cultivation of rainfed crops, especially soybean. Analysis involving only ecotypes of the *D. ciliaris* species ranked according to morphological criteria revealed a cophenetic correlation coefficient of 0.98 and an average similarity of 86% (Fig. 4), dividing these ecotypes into seven subgroups, with the genetic similarity remaining high between the analyzed material. The ecotypes 50 (Dom Pedrito) and 130 (Carazinho); 101 (Mostardas) and 130 (Carazinho); 113 (Lagoa Vermelha) and 163 (Tupanciretã); and 130 (Carazinho) and 163 (Tupanciretã) shared 97% genetic similarity, although they originated from separate locations. When only ecotypes of *D. sanguinalis* were evaluated, the results were similar to those observed for *D. ciliaris*, with a cophenetic correlation coefficient of 0.92 and an average similarity of 89% (Fig. 5). These ecotypes formed four clusters, with the greatest similarity (0.94) observed between ecotypes 54 and 55, belonging to Dom Pedrito and Arroio Grande, respectively. In *Eichhornia crassipes* accessions, which propagate mainly vegetatively, the similarity was 90%, which is considered to be low because this form of propagation decreases the possibility of genetic recombination (Cardoso et al., 2002). Although *Digitaria* spp. sexually propagates, in this study, the highest similarity value was 98%, which was recorded between ecotypes 31 and 101 (*D. sanguinalis* and *D. ciliaris*, respectively); however, these ecotypes are of different species, and these two populations may have undergone the process of gene flow due to the proximity of the collection areas.

Dispersion clustering

Multivariate statistical methods, such as hierarchical clustering analysis, including dendrogram approaches, and non-hierarchical approaches, such as principal component analysis (PCA) or principal coordinate analysis (PCoA), have been used in several studies to estimate accurately the molecular genetic variation of a population, and the relationships between groups (clusters) and individuals. PCA and PCoA are very similar approaches. PCoA was applied by Adoukonou-Sagbadja et al. (2007) to classify *Digitaria* spp. genotypes into clusters, using AFLP markers, the use of this approach was efficient to genotype clustering and to separate by species more than hierarchical approach. In the present study, the clusters that were obtained by PCoA were similar to those obtained by the unweighted pair group method with arithmetic means (UPGMA) method (Fig. 6). However, PCoA assigned ecotype 46 to its own cluster, whereas the UPGMA method assigned it to a cluster that included most of the other ecotypes. Examination of morphological features is the optimal method for describing and classifying germplasms; however, although it is inexpensive, this method is disadvantageous because a lot of time is required to analyze

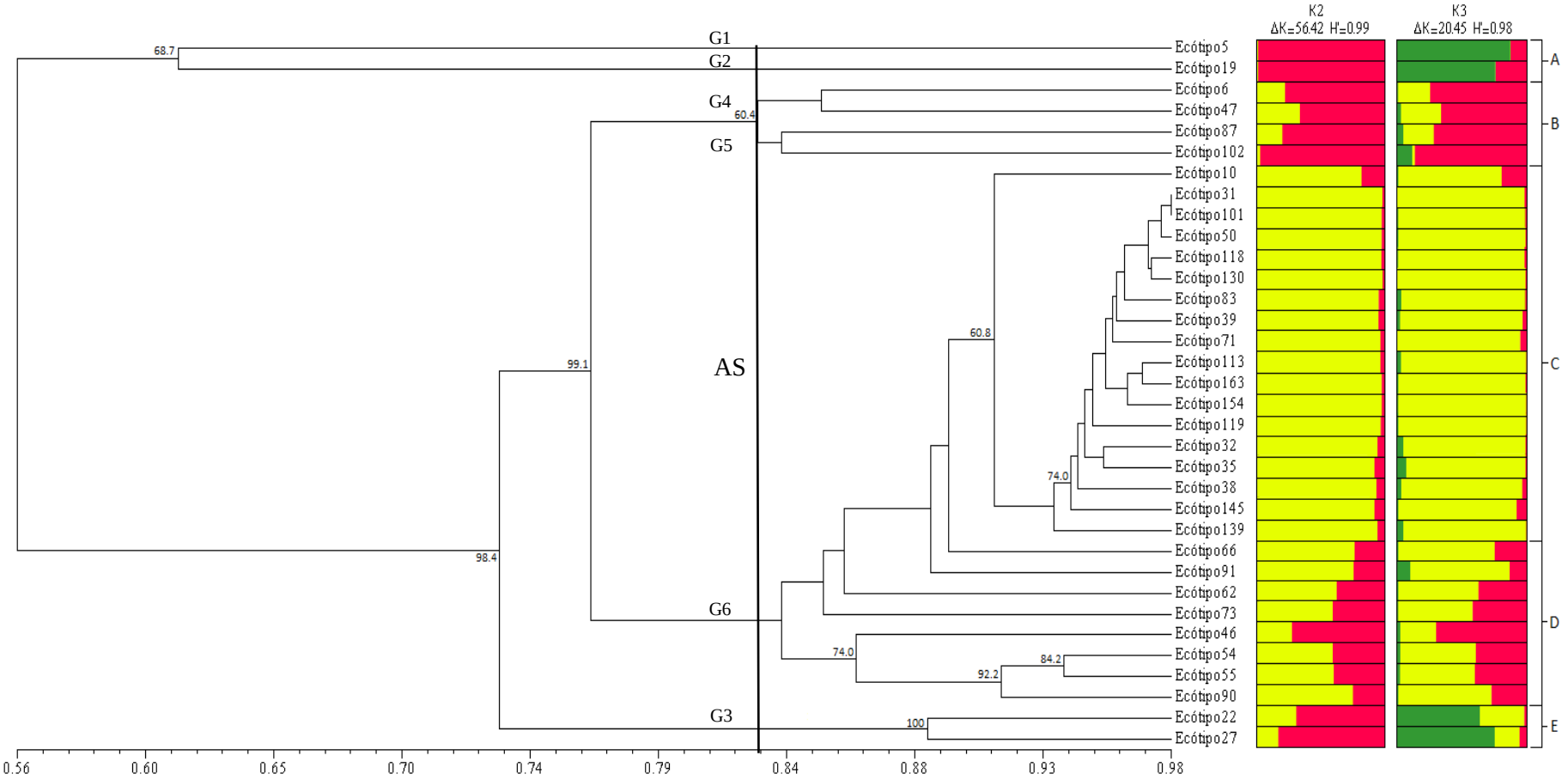

Fig 2. Graphical clustering of the 34 ecotypes of *Digitaria* spp. The UPGMA dendrogram was constructed based on the Simple Matching similarity index, and the color plots were generated using the Bayesian clustering approach of STRUCTURE software. Color plots represent the *K*s (groups) 2 and 3, respectively. Δ*K* was calculated as described by Evanno et al. (2005), and *H*' is the similarity index among the summarized replicates in each *K*. AS=average similarity.

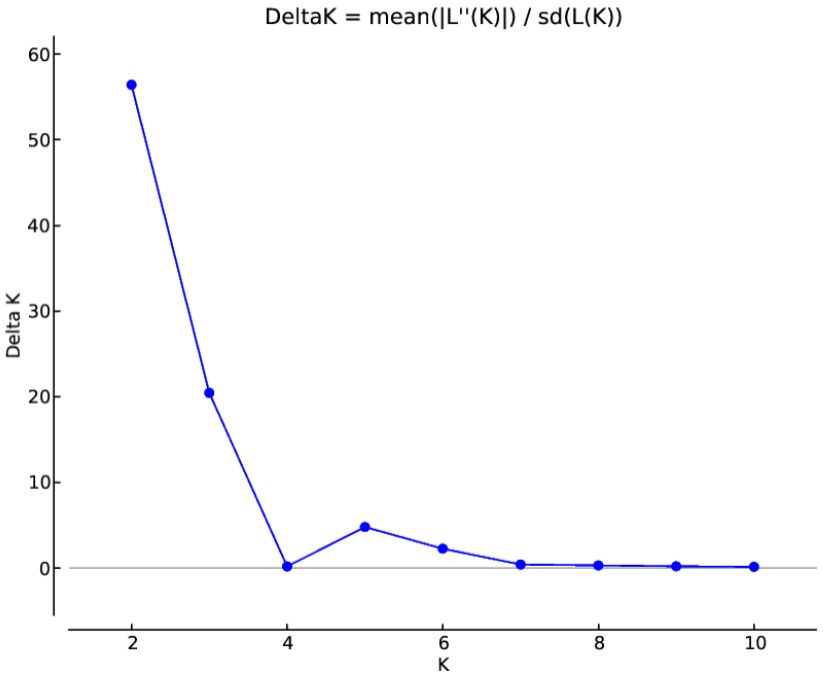

Fig 3. Application of statistical nonparametric analysis of Evanno et al. (2005) to obtain the most informative *K* estimation. The values indicate the information level contained in each *K* of the STRUCTURE Bayesian approach.

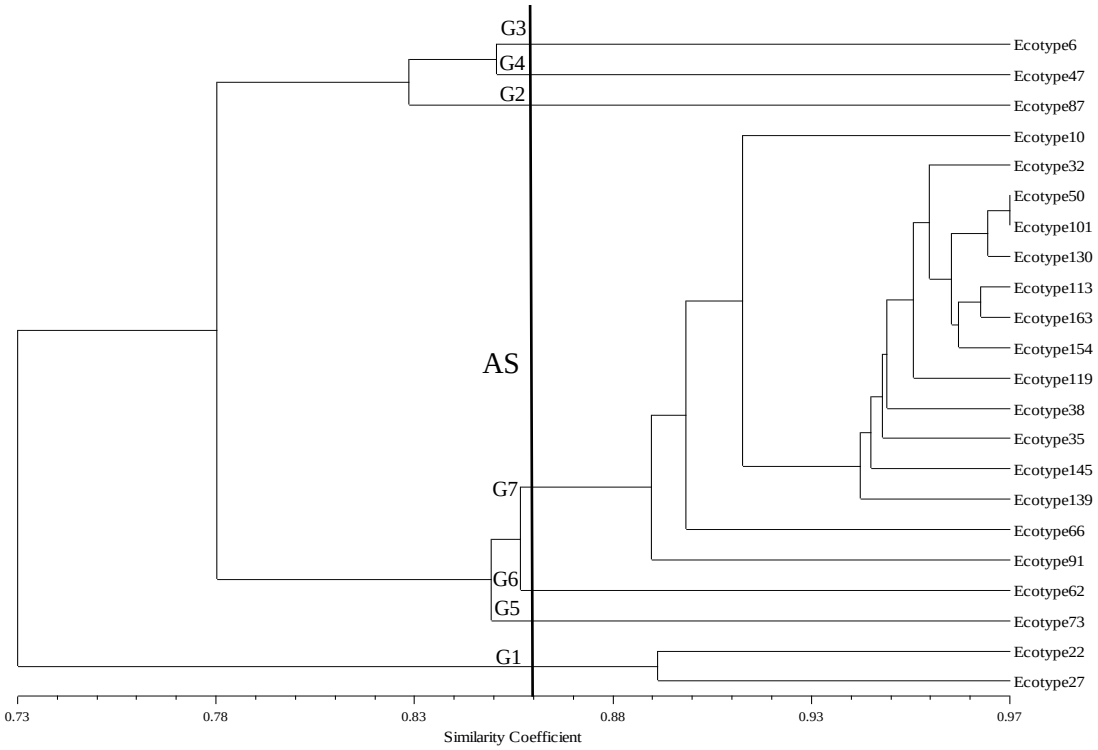

Fig 4. Dendrogram of the genetic similarity between 22 ecotypes of *Digitaria ciliaris*, as determined by the unweighted pair group method with arithmetic means (UPGMA) and calculated using the simple matching similarity coefficient. AS=average similarity.

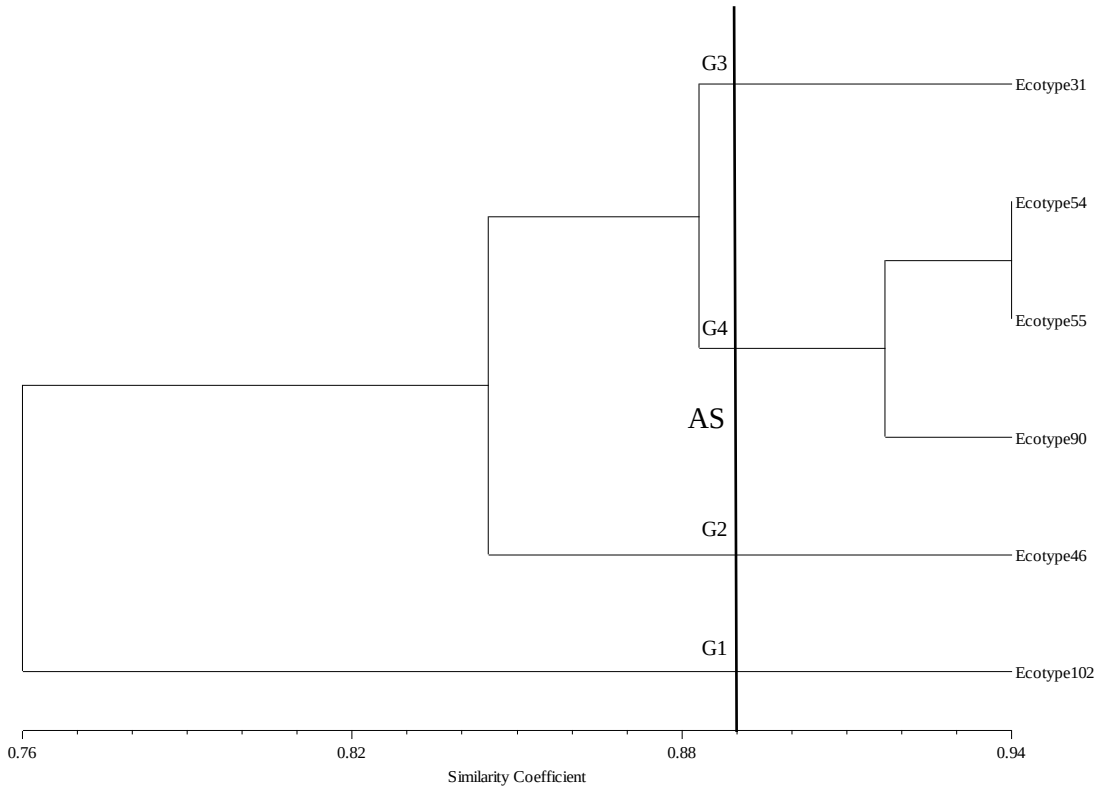

Fig 5. Dendrogram of the genetic similarity among six biotypes of *Digitaria sanguinalis*, as obtained by the unweighted pair group method with arithmetic means (UPGMA) and calculated by the simple matching similarity coefficient. AS=average similarity.

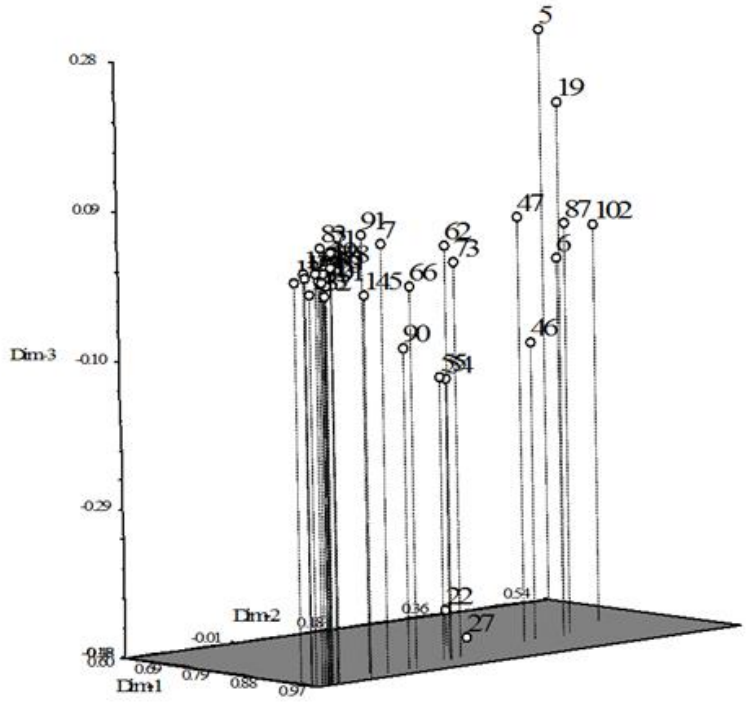

Fig 6. Associations between 34 accessions of *Digitaria* spp., as determined by principal coordinate analysis (PCoord) of the simple matching similarity coefficients calculated from 280 AFLP bands that were generated by five primer combinations. * The circles indicate the grouping of the accessions.

the material and the variability of very similar characteristics, which can lead to mistaken identification. Thus, molecular analysis has emerged as an essential tool for examining the variability within and between genotypes, detecting geographical and environmental effects on genotypes, and establishing correlations with traits of interest, and it is very useful for validating morphology models.

In this study, molecular and morphological analyses were used as complementary tools for the characterization of *Digitaria* spp., and they were found to be effective for identifying the similarity between species. Both of these methods are incomplete because molecular data, unlike phenotypic data, are not required for the identification of cultivars, whereas morphological data currently has very limited use for the identification of polymorphisms; thus, it is necessary to use complementary molecular methods to determine the molecular basis of a trait, considering variations in environmental pressures (D'Império et al., 2011).

Materials and Methods

Plant Materials

Samples from crabgrass populations (*Digitaria* spp.) were collected from field in February and March 2009, covering production areas of rice and soybean from 19 districts belonging to different regions of the State of Rio Grande do Sul, Brazil. Three locations in each municipality were visited, and in each of three sampling points, georeferences were performed, resulting in 171 accessions of crabgrass (Table 1). The following six rice regions in RS (IRGA, 2008) were sampled: West Frontier (Uruguaiana and Itaqui); Campanha (Dom Pedrito and Rosário do Sul); Central Depression (São Sepé and Cachoeira do Sul); Inner Coastal Plain (Tapes and Camaquã); Outer Coastal Plain (Mostardas and Viamão); and South Zone (Arroio Grande and Santa Vitória do Palmar). Carazinho, Cruz Alta, Ijuí, Lagoa Vermelha, Passo Fundo, Santa Bárbara do Sul, and Tupanciretã (Table 1) were chosen to represent soybean field districts with higher production rates in RS (IBGE, 2006).

Traits measured

The ecotypes collected were examined with a magnifying glass and classified according to the morphological traits described by Canto-Dorow (2001). From these classifications, 34 crabgrass ecotypes were selected, using the geographical distribution and morphological diversity of species in these regions as criteria.

Molecular analysis

DNA was extracted from 100 mg of fresh and fully expanded leaves using the protocol described by Doyle and Doyle (1991). AFLP analysis was performed using an AFLP Analysis System I Kit (Life Technologies of Brazil Ltda., São Paulo, SP), according to the manufacturer's instructions, with the restriction enzymes EcoRI / MseI. The PCR products were separated by electrophoresis in 6% polyacrylamide gels at 60 W for two hours. Then, the bands of PCR products were visualized with silver nitrate, according to the protocol of Creste et al. (2001). The primer combinations that resulted in the most consistent amplifications were subjected to repeated PCR and electrophoresis to confirm the polymorphisms.

Statistical analysis

The products that were displayed on the AFLP gel were visually analyzed and designated as "1" for the presence and "0" for the absence of bands. Only the bands that were clearly visualized in the gels were recorded, and based on the obtained data, we constructed a binary matrix from which genetic similarity was estimated using the Simple Matching coefficient (Sokal and Michener, 1958), as recommended by Bonin et al. (2007). From the similarity matrix, cluster analysis was performed in the "sequential, agglomerative, hierarchical and nested clustering methods" (SAHN) module, using the unweighted pair group method with arithmetic means (UPGMA) method, with NTSYS-pc Vers. 2.1 software (Rohlf, 2000). A dendrogram of similarity based on polymorphisms of 34 ecotypes and dendrograms constructed only with polymorphisms associated with the two species with the largest number of representatives were generated. To determine the consistency of the generated clusters in the dendrograms, a matrix of cophenetic similarity was generated to estimate the Mantel correlation coefficient (r) between the similarity matrix and the cophenetic values. Bootstrap analysis was performed using 1000 replicates with Winboot Vers. 1.0 (Yap and Nelson, 1996) to evaluate the robustness of the dendrogram for the 34 ecotypes. Moreover, principal coordinate analysis (PCoA) of similarity was performed using NTSYS-pc Vers. 2.1 software to analyze the non-hierarchical clustering of the 34 ecotypes of *Digitaria* spp.

Non-hierarchical clustering analysis was also conducted using Structure Vers. 2.3.1 software (Pritchard et al., 2000; Falush et al., 2003) to identify groups of individuals. Structure software uses the Bayesian approach, calculating the probability Pr ($X \mid K$) as the data (X) and logPr ($X \mid K$) to determine the probability that an individual belongs to one population or another. This analysis was conducted using the model *Admixture* with correlated allelic frequency, computed with a burnin time of 20^4 followed by 20^5 interactions for each priori information (K), ranging from 1 to 11 with 50 replicates each. The nonparametric statistical analysis of Evanno et al. (2005) was implemented using Structure Harvester Vers. 0.6.93 software (Earl and Von Holdt, 2012) to identify the most informative K. Replicates of the selected Ks were summarized using CLUMPP Vers. 1.1.2 (Jakobsson and Rosemberg, 2007) with the Greedy algorithm. After summarization, each K was plotted using Distruct Vers. 1.1 software (Rosenberg, 2004).

Conclusions

The 34 crabgrass ecotypes from the different evaluated species had a high average genetic similarity. Among the evaluated species and ecotypes of *Digitaria* spp., the lowest genetic similarity was observed between *D. ternata* from Cachoeira do Sul and *D. eriostachya* from Itaqui. The ecotypes from areas that were cultivated with soybean had higher similarity than those derived from rice fields. For *D. ciliaris*, the lowest genetic similarity was found between ecotypes originating from Itaqui and Viamão. The highest level of genetic similarity among the *D. sanguinalis* ecotypes was observed between the Arroio Grande and Dom Pedrito ecotypes.

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