RESEARCH

Diversity in Apomictic Populations of *Paspalum simplex* Morong

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ABSTRACT

Paspalum is considered a genetic model for studying the sources of genotypic variability and breeding techniques in apomictic plants. *Paspalum simplex* is a warm-season forage grass that well represents the genus since it contains different ploidy levels and apomixis is linked to polyploidy. The objective was to evaluate the diversity present within and among apomictic polyploid populations of *P. simplex*. Germplasm was collected from 17 sites covering the species' region of natural distribution. The diversity present at the molecular level within and among populations was evaluated using ISSR markers. Variability for agronomic traits was also evaluated by cultivating all populations into the field. The 17 analyzed populations were all polyploid, including 13 pure tetraploid, and 4 mixed tetraploid-hexaploid with predominance of the tetraploid cytotype. Most of the diversity was present among polyploid populations (85% of the total variation), and there was not a correlation between genetic and geographical distances. The within-population diversity was low for most populations with the exception of one of them. Each genotype was restricted to a single location. Variability for initial growth, spring and fall growth, and the extent of the vegetative phase was observed within and among polyploid populations. The within-population variation for these phenotypic traits was mainly due to the presence of one or a few off-type plants. A highly genotype-specific colonization of new sites appears to occur in *P. simplex*, and then apomixis mediates the formation of uniform populations.

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; ISSR, inter-simple sequence repeat; PCR, polymerase chain reaction; TAE, tris-sodium acetate-ethylenediaminetetraacetic acid; UPGMA, unweighted pair-group method arithmetical average; UV, ultraviolet.

Many important forage grasses reproduce by apomixis (Vogel and Burson, 2004), which is a type of asexual reproduction through seeds. Among them, *Brachiaria decumbens*, *B. brizanta*, *Cenchrus ciliaris*, *Panicum maximum*, *Paspalum dilatatum*, and *P. notatum* are extensively cultivated around the world (Skerman and Riveros, 1994). Although different breeding techniques have been developed for the genetic improvement of apomictic species (Hanna, 1995; Vogel and Burson, 2004; Miles, 2007), most commercially available cultivars have been the result of direct selection of natural variants collected at different locations (Vogel and Burson, 2004; Blount and Acuña, 2009). These ecotypes are evaluated in the target environment and the superior ones are released as cultivars. Another commonly used method consists on the generation of novel apomictic hybrids by crossing induced sexual tetraploid plants and natural apomictic genotypes (Hanna, 1995). This method also depends on the variability contained in natural apomictic plants since the number of induced sexual plants is always limited. Thus, a broad genotypic diversity is essential for the genetic improvement of apomictic species.

Published in Crop Sci. 54:1656–1664 (2014).
doi: 10.2135/cropsci2013.11.0780

Although apomixis is responsible for the creation of genetically uniform populations, apomictic species usually contain a rich diversity. Some examples are *Ranunculus carpaticola* (Paun et al., 2006), *Brachiaria humidicola* (Jungmann et al., 2010), *Paspalum notatum* (Espinoza et al., 2006; Cidade et al., 2008; Reyno et al., 2012), and *Panicum maximum* (Sousa et al., 2011). Little is known about how this diversity is generated in apomictic species and how polyploidy and apomixis contribute to the adaptation of these species to different environments. This information would indicate the evolutionary patterns for each species and the potential sites for germplasm collection. Most of the available information comes from population studies that have been done with nongrass species, such as, species of *Hieracium*, *Antennaria*, *Taraxacum*, *Ranunculus*, and *Erigeron* (Hörandl and Paun, 2007). These studies have shown that apomictic populations are generally composed by a single or a few genotypes. The exceptions are those apomictic populations that live in proximity to sexual populations of the same or a closely related species (Bayer, 1990; Menken et al., 1995). However, residual sexuality or mutations can occur, increasing the variability of isolated apomictic populations (Houliston and Chapman, 2004).

A few studies have attempted to elucidate how new apomicts are generated in grasses and how this genotypic diversity is widespread in the species' area of natural distribution. One important case of study is the genus *Paspalum*, which contains species with different ploidy levels and linked reproductive characteristics. As in many other genera, apomixis is always linked to polyploidy (Ortiz et al., 2013). The genus has approximately 330 species (Zuloaga and Morrone, 2005), and many of the characterized species are apomictic (Ortiz et al., 2013). *Paspalum notatum* and *P. simplex* have been the best characterized species of the genus since they share genetic characteristics that represent a high proportion of the *Paspalum* species. They both are also considered good forages by the local ranchers, and *P. notatum* is also cultivated in several countries. These two species have different ploidy levels, including diploid and polyploid plants (Urbani et al., 2002; Gates et al., 2004). The diploid cytotype is sexual and cross-pollinated, while the polyploid plants are apomictic. While the tetraploid cytotype predominates in both species, the diploid is restricted to small areas within the region of natural distribution of each species. Most importantly, tetraploid populations living in proximity to diploid populations are highly variable and share most of the contained variability with the neighboring 2*x* population (Daurelio et al., 2004; Brugnoli et al., 2013). Moreover, the level of within-population diversity in 4*x* populations being allopatric to 2*x* populations has been estimated to be low for *P. notatum* (Daurelio et al., 2004) and *P. denticulatum* (Sartor et al., 2013). In contrast, similar levels of within- and among-population diversity were observed for *P. notatum* (Reyno et al., 2012) and *P. nicorae* (Sartor et al., 2013). Moreover, variation for the within-population diversity has been observed among three allopatric populations of *P. simplex* (Brugnoli et al., 2013). Thus, further research is needed to understand the allocation of the diversity among and within populations to better define the evolutionary processes involved and identify potential collection sites for breeding purposes. This can be accomplished by analyzing a higher number of tetraploid populations of *P. simplex*, representing its area of natural adaptation.

The objective of this research was to estimate the within- and among-population diversity of a group of allopatric populations of *P. simplex* based on DNA polymorphisms and agronomic characteristics.

MATERIALS AND METHODS

Plant Material

The germplasm for this research was collected from 17 locations during summer 1998 and 1999 across the area of natural distribution of *Paspalum simplex* (Fig. 1). Seed from each population was collected from an area of approximately 2000 m^2. In each site, 12 subsamples of approximately 2 g each were collected, keeping a distance of 10 m among collection points. Each gram of seed of *P. simplex* contains approximately 1690 seeds (average across populations). Seed in each subsample came from a group of plants at each point. The seed sample for each population resulted from bulking the 12 subsamples. Attempting to gather only mature seed, only the seed that shattered when seed heads were struck against the hand was collected.

This collected seed was stored in a refrigerator with low humidity in the germplasm bank at the Instituto de Botánica del Nordeste located in Corrientes, Argentina. Approximately 200 seeds from each population were sown in trays with sterile soil in a greenhouse in August 2009. In all cases, more than 80% of the seed germinated. The seedlings were transplanted to pots during the end of spring 2009. Twenty-five plants were kept to establish a random sample from each population.

Ploidy Level Determination

Flow cytometry was used to determine the ploidy level of each population using a random subsample of between 11 and 15 plants from the sample of 25 plants previously taken from each population. Samples of young leaf blades were removed from each plant. Leaves were placed in a petri dish with a similar amount of tissue of the standard (a plant of the same species for which the chromosome number was established by chromosome counts in root tips). The tissue was chopped with a sharp razor blade after adding 0.5 mL of extraction buffer (Partec P kit CyStain UV). Then, samples were incubated for 2 min and were filtered through a 50 μm nylon mesh directly into the sample tube, to which a 1.5 mL stain solution (Partec P kit CyStain UV) was added. The mixture was incubated for 5 min at room temperature and analyzed with a Partec PA II (Ploidy Analyzer II, Münster, Germany) flow cytometer. The ploidy level of each plant was determined by comparing the peak registered for each plant with the peak of the control plant using Partec FloMax software.

Figure 1. Natural distribution of the 19 collected populations of *Paspalum simplex*. Only the 17 pure polyploid populations were used in this research. The diploid and mixed diploid-tetraploid populations were previously reported in Crop Science (Brugnoli et al., 2013).

Molecular Characterization

The same plants characterized for ploidy levels were also used for the DNA extraction and their further molecular analysis.

Total genomic DNA was isolated using 50 mg of young leaves. Leaves were macerated with the help of a plastic fuse drill and 700 μL of extraction buffer cetyl trimethylammonium bromide (CTAB) 2% (100 mM Tris-HCl pH 7.5; 50 mM ethylenediaminetetraacetic acid [EDTA] pH 8; 700 mM NaCl; 140 mM β-mercaptoethanol) in a tube of 1.5 mL. The samples were incubated at 65°C for 30 min. Then, 500 μL chloroform was added and the mixture was stirred for 5 min and then centrifuged for 10 min. The aqueous phase was recovered and transferred to another tube. The nucleic acids were precipitated with 500 μL of cold 2-propanol. The tubes were then kept in a freezer at −20°C for approximately 30 min. Then, the samples were centrifuged at 4°C for 20 min. The supernatant was discarded and the pellet was washed with a washing solution (EtOH 70° + 0.2M NaOAc) and centrifuged again for 10 min. After centrifugation, the supernatant was discarded and the pellet was suspended in 25 μL of sterile , tris-ethylenediaminetetraacetic acid (TE) buffer (10 mM Tris-HC1 pH 8; 1 mM EDTA pH 8) and kept in a refrigerator. The genomic DNA was quantified by visual comparison to a known patron by electrophoresis in agarose 1% gels in 1 × TAE buffer (40 mM Tris-HCl; 5 mM NaOAc; 0.77 mM EDTA; pH 8.0) at 40 V for 2 h. Genomic DNA was visualized under ultraviolet (UV) light and photographed with GelDoc-It Imaging System (UVP LLC), after staining with ethidium bromide (10 mg mL^{-1}). Each DNA samples was adjusted to 10 ng μL^{-1} for their use in polymerase chain reaction (PCR) amplifications.

A total of ten primers of ISSR were used for PCR amplifications. The ISSR markers were generated according to the methodology described by Cidade et al. (2008). Polymerase chain reaction products were separated by electrophoresis in 2% agarose gels in 1 × tris-sodium acetate-ethylenediaminetetraacetic acid (TAE) buffer, at 70 V for 3 h and stained with ethidium bromide (10 mg mL^{-1}). The molecular profiles were visualized under UV light, photographed, and stored for further analysis with GelDoc-It Imaging System.

Agronomic Characterization

Twenty-five plants from each population were used for the agronomic characterization. Each plant was vegetatively divided in two and transplanted into the field on 1-m centers

following a randomized complete block design with two replications. Important differences were present between blocks, especially the soil type, but the environment within blocks was highly uniform. The soil type in one block was classified as Udipsamment alfico, and as Argiudol for the other block. The experiment was planted near the city of Corrientes, Argentina at the end of January 2010. Plants were fertilized with 3 g of 15-15-15 (N-P-K) per plant in February 2010. The initial growth was estimated on 11 May using a 1 to 5 scale, where 1 represented the plants exhibiting the lowest amount of aboveground biomass, and 5 represented the plants with the highest amount of biomass. Plants were defoliated to approximately 10 cm above the soil level on 12 September. Spring regrowth was estimated using the same scale described above on 25 October. The extent of the vegetative phase was determined by recording the presence of inflorescences in each plant every week and was calculated by counting the number of days between 21 September and day of appearance of the first inflorescence. Growth habit was estimated by measuring plant diameter and height. Plant diameter was determined using the average between the longest and shortest diameter of a given plant while plant height was measured from the base of the plant to the top of the canopy. These two variables were measured during April 2011. All plants were cut to approximately 10 cm above the soil level on 31 Mar. 2011. Fall regrowth was estimated using a 1 to 5 scale as described above between 4 and 11 May 2011.

Statistical Analyses

Inter-simple sequence repeat products were scored for the presence (1) and absence (0) of homologous DNA bands. Molecular diversity within populations was estimated with number of polymorphic loci and Shannon's information index using the GenAlEx 6 program (Peakall and Smouse, 2006). The Shannon's index varies between 0 and 1; the higher the value, the higher the estimated diversity within populations. Principal coordinates analysis was performed to estimate the diversity within and among populations. The proportion of different genotypes per population was determined using the GenoType and GenoDive software (Meirmans and Van Tienderen, 2004), to detect possible mutation events. Pairwise genetic distances among the populations was estimated for the Jaccard's dissimilarity coefficient (1-S). Also, the dendrogram was constructed using an unweighted pair-group method with arithmetical average (UPGMA) cluster analysis.

Means, medians, quartiles, and coefficient of variations for agronomic traits were calculated. Analysis of variance (ANOVA) and mean separations using the Tukey test were calculated using INFOSTAT software (Di Rienzo et al., 2002). In addition, a multivariate analysis of variance (MANOVA) was performed with the agronomic data to evaluate the existing variability among and within populations.

RESULTS

Ploidy Level

Among the 17 analyzed populations, tetraploid ($2n = 4x = 40$) and hexaploid ($2n = 6x = 60$) plants were found (Table 1). Thirteen populations were composed only of tetraploid plants, and four were mixed 4*x*–6*x* populations.

Table 1. Ploidy level of natural populations of *Paspalum simplex*.

Population	Collection place	Number of plants	Ploidy level	
			4*x*	6*x*
Ibarreta	25°12′ S 59°51′ W	11	11	–
Santa Ana	27°27′ S 58°39′ W	12	7	5
Calchaquí	29°53′ S 60°17′ W	14	13	1
Makallé	27°12′ S 59°17′ W	14	14	–
Alejandra	29°54′ S 59°49′ W	15	15	–
Basail	27°51′ S 59°17′ W	15	15	–
Castelli	25°56′ S 60°36′ W	15	15	–
Corrientes	27°27′ S 58°49′ W	15	15	–
Mercedes†	29°10′ S 58°04′ W	15	15	–
Paso de la Patria	27°18′ S 58°33′ W	15	15	–
Piedras Blancas	31°11′ S 59°57′ W	15	15	–
Pirané†	25°43′ S 59°05′ W	15	13	2
Porto Murtinho	21°42′ S 57°51′ W	15	12	3
Presidente Hayes	23°38′ S 58°42′ W	15	15	–
Puerto Falcón	25°15′ S 57°42′ W	15	15	–
Reconquista†	29°08′ S 59°39′ W	15	15	–
Villa Ana	28°29′ S 59°36′ W	15	15	–

† The ploidy level of the populations from Mercedes, Pirané, and Reconquista was previously reported by Brugnoli et al. (2013).

The proportion of 6*x* plants in each population was variable: 7.1% for the population from Calchaquí, 13.3% for Pirané, 20% for Porto Murtinho, and 41.7% for the population from Santa Ana (Table 1).

Molecular Characterization

A total of 165 ISSR loci were amplified, and 163 ended up being polymorphic. One example of the obtained electrophoresis patterns for two populations can be observed in Fig. 2. Considering the within-population diversity across all polyploid populations included in this research, 9.9% of the analyzed loci were polymorphic. Moreover, the highest proportion of polymorphic loci (PPL) was 27.4% (Calchaquí), and the lowest was 2.8% (Paso de la Patria) (Fig. 3).

The mean proportion of different genotypes (PDG) was 22.7% varying from 0% (only one genotype) to 92.8% among populations (Fig. 3). Each genotype was found to be restricted to a single location. In addition, the Shannon's index varied from 0.011 to 0.125 among populations (Fig. 3). The level of within-population diversity, in general, was low with the exception of the population from Calchaquí (Fig. 4).

Distances among populations varied from 0.73 to 0.94 (Fig. 5), indicating that most populations were not closely related. In addition, the genetic distances among populations were not correlated with the geographical distances ($r = 0.06$; $p = 0.47$). Moreover, the analysis of molecular variance (AMOVA) revealed that 85% of the total variance was the result of the among-population variation, while 15% was related to the within-population variation.

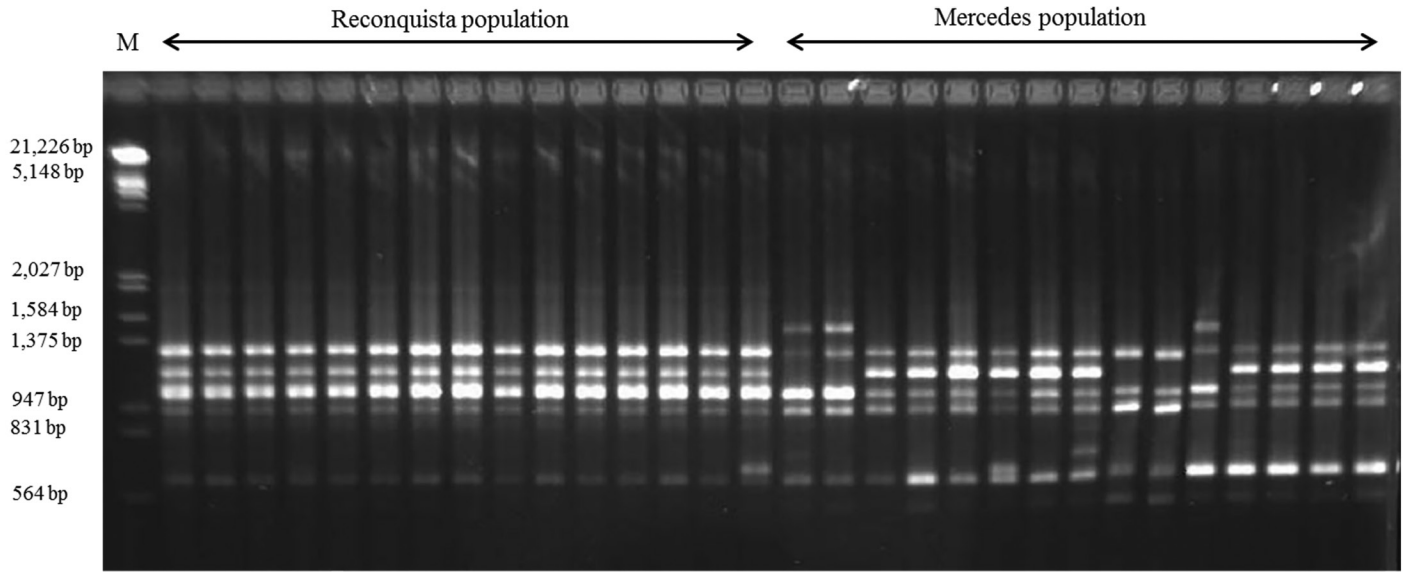

Figure 2. Inter-simple sequence repeat (ISSR) pattern of two populations of *Paspalum simplex*. M: Lambda DNA/*Eco*RI + *Hind*III marker. The size of DNA fragments (in kb) are indicated on the left.

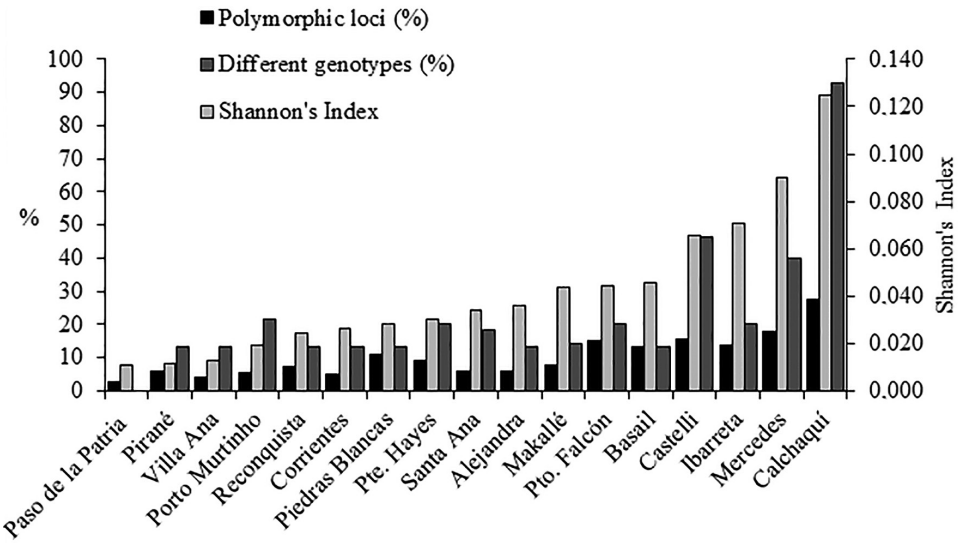

Figure 3. Percentage of polymophic loci, the Shannon' index, and proportion of different genotypes in 17 populations of *Paspalum simplex.*

Agronomic Characterization

Variation among and within polyploid populations of *Paspalum simplex* was observed for all evaluated traits. The most variable traits considering both among- and within-population diversity were the extent of the vegetative phase, initial growth, and spring and fall regrowth (Table 2). The extent of the vegetative phase varied among populations from 36 to 119 d, considering the beginning of the spring as the starting point (Table 2). Several populations started flowering uniformly, such as the populations from Corrientes and Villa Ana, while others exhibited wide variation, such as the populations from Makallé and Santa Ana. The maximum range was observed for the population from Santa Ana, which had one individual that started flowering in early October and another in late January. Furthermore, a positive correlation was observed between initial and spring growth ($r = 0.6$) and between initial and fall growth ($r = 0.5$).

In contrast, low variation was observed for plant height and diameter considering both inter- and intra-specific variation. The phenotype of the hexaploid plants was similar to the predominant phenotype present in each population. However, these hexaploid plants had a lower general vigor, and several did not survive the low winter temperatures.

When the diversity was estimated based on a multivariate data analysis considering all the evaluated traits, no marked differences were observed among populations (Fig. 6). The median for the Euclidean distance estimated in each population varied between 2.3 and 3.3. No correlation was observed between the Euclidean and geographical distances ($r = -0.06$; $p = 0.46$).

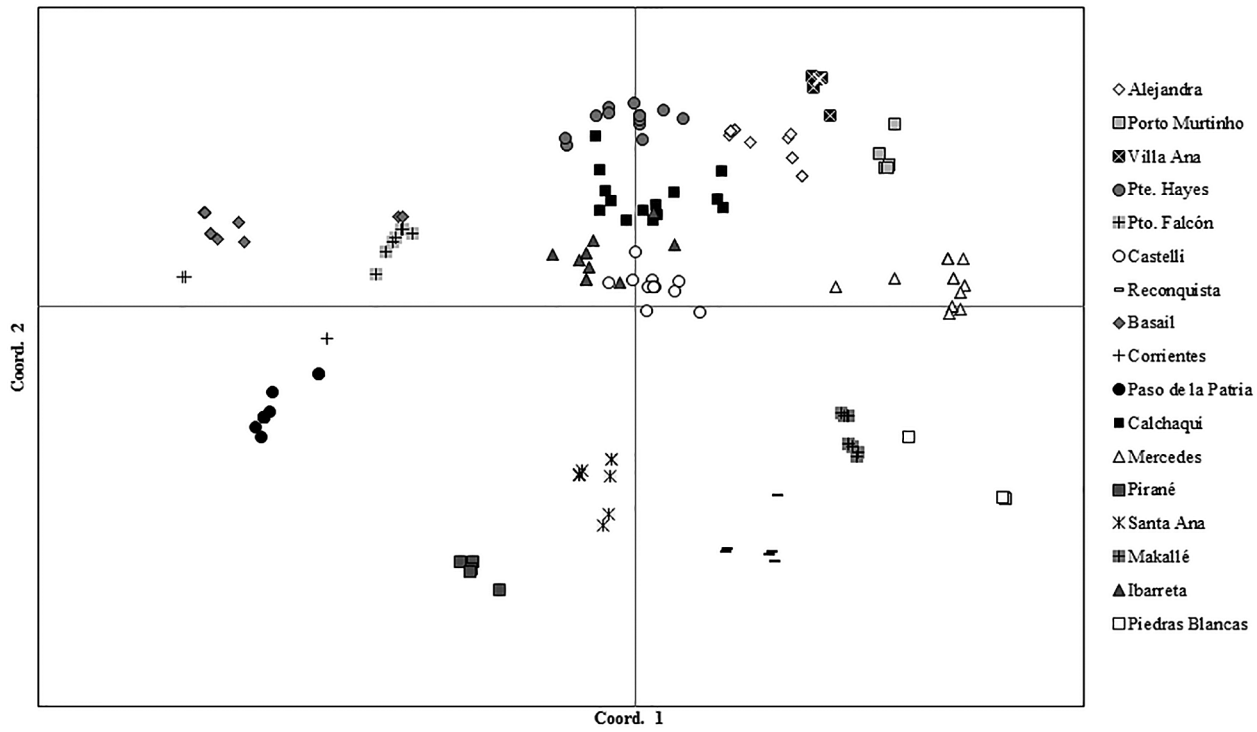

Figure 4. Principal coordinates graph for 17 natural populations of *Paspalum simplex* based on inter-simple sequence repeat (ISSR) data.

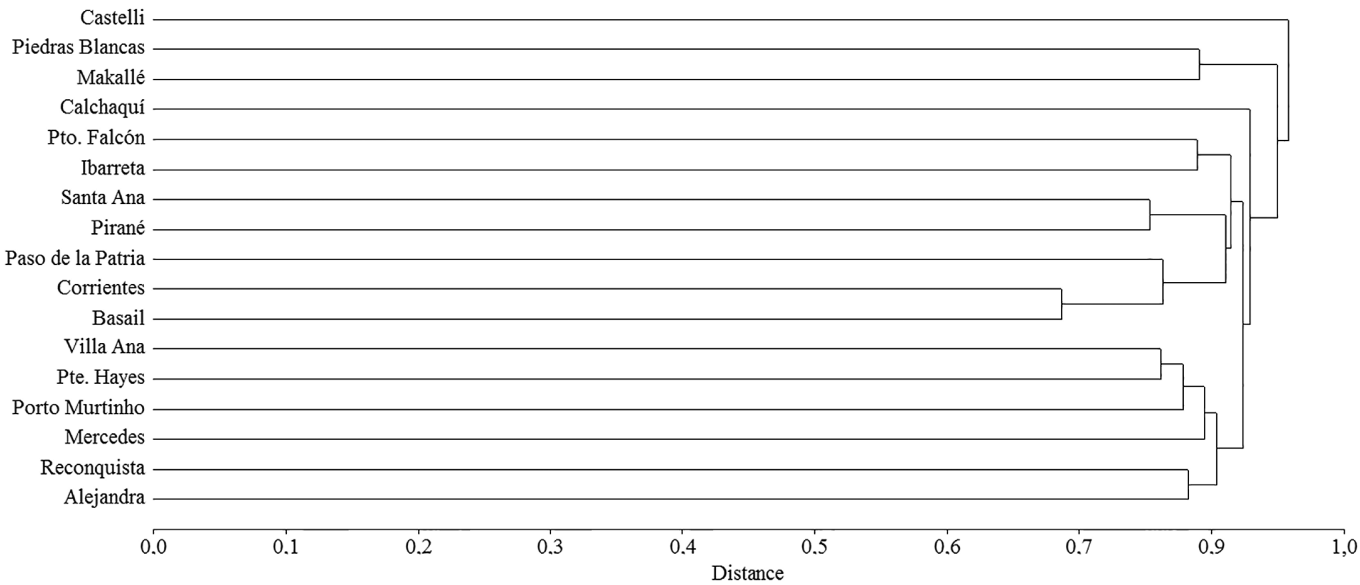

Figure 5. Unweighted pair-group method arithmetical average (UPGMA) dendrograms constructed using the Jaccard´s dissimilarity coefficient (1-S) of natural populations of *Paspalum simplex* based on inter-simple sequence repeat (ISSR) molecular profiles.

The relation between the diversity estimated with the molecular and agronomic characterization was evaluated based on the Mantel's Test. Although a significant correlation was observed between the data obtained based on the two techniques, the correlation was low ($r = 0.2318$; $p = 0.001$).

DISCUSSION

The success of a breeding program depends on the diversity present in the available germplasm for the species of interest. Forage and turf cultivars of apomictic grass species have been the result of direct selection of superior plants from collected germplasm or from a population of hybrids, which are generated by crossing induced sexual plants and apomictic ecotypes (Vogel and Burson, 2004). Thus, current efforts for improving apomictic grass species are highly dependent on the available genotypic variability.

This research has aimed to define how diversity is distributed among and within apomictic polyploid populations of *Paspalum simplex* with the objective of improving the current understanding of how the genotypic diversity

Table 2. Variation observed for agronomic traits among and within populations of *Paspalum simplex*.

	Traits											
	Height		Diameter		Vegetative phase		Initial regrowth‡		Spring regrowth§		Fall regrowth¶	
Populations	Mean	Cv	Mean	Cv	Mean	Cv	Mean	Cv	Mean	Cv	Mean	Cv
	cm		cm		d							
Corrientes	105.7cde†	7.3	21cdef	10.9	36g	0	2.7efg	17	3defg	13.4	2.1i	23.8
Paso de la Patria	112.1bc	5.2	20.7def	11.4	76.4cd	8.3	3.3cde	20.9	3.6abcd	18.9	3.9bcd	19.7
Porto Murtinho	119ab	7.7	20.2def	11.6	40.4efg	21.6	3.8bcd	26.6	3.3cdef	24	4.2b	15.1
Santa Ana	126.3a	3.1	22.8abcd	6.7	67de	44.3	4.7a	9.3	4.5a	11.9	5a	27.2
Villa Ana	100.4de	9.9	20ef	9.2	36.6h	0	2.9defg	26.7	3.7bcd	36.1	3.8bcd	15.6
Reconquista	105cde	7.7	22abcdef	10.2	36.4h	0	4.3ab	18.7	3.3abcd	22.6	3.7bcde	17.6
Alejandra	95.8e	5.5	24.3a	7.73	48.1fgh	34.6	3.1def	20.5	3.4bcde	21.4	3.9bcd	23.4
Pirané	105.3cde	7.2	19.7f	11.9	46.9gh	38.8	2.5fg	21.1	2.2h	20.4	2.5ghi	30.8
Makallé	100.5de	9	23.4abc	8	50.9fg	45.6	4.3ab	14.6	4.1ab	17.5	4bc	14.4
Presidente Hayes	98.6de	11.9	21.7bcdef	7.5	119.4a	3.9	3.1cdef	17.7	3.5bcde	14.8	4bc	20.9
Piedras Blancas	99.3de	10	21.6bcdef	9.2	35.6h	0	2.2g	33.5	2.5gh	17.3	2.5hi	19.9
Castelli	113bc	17.7	22.4abcfe	9.2	108.8ab	9.2	2.5fg	25.8	2.8efgh	19.6	2.8efgh	32.9
Basail	103.3cde	8.3	22abcdef	19.3	38.3gh	34	2.5fg	16.3	2.6fgh	21.2	2.1i	32.5
Ibarreta	107.7bcd	6.6	20.6def	22.2	103.7b	9.6	2.9efg	34.2	2.8efgh	17.8	3.3defg	34.5
Puerto Falcón	106.1cde	12.5	21.6bcdef	10.7	82.5c	22.2	2.6fg	21.8	3.6bcd	19.2	3.1defh	28.6
Mercedes	100.5de	6	24.1ab	11.8	36.7h	0	3.8bc	19.4	3.7gh	19.4	3.3cdef	41.4
Calchaquí	104.1cde	10	19.6f	11.7	60.5ef	39.9	2.5fg	31.9	2.5fgh	23.2	2.8fghi	24.5

† Population means followed by different letters are different ($P \leq 0.05$).

‡ Initial regrowth was estimated on 11 May 2010 using a 1 to 5 scale, where 1 represented the plants exhibiting the lowest amount of above-ground biomass, and 5 represented the plants with the highest amount of biomass.

§ Spring regrowth was estimated on 25 Oct. 2010 (45 d after the plants were defoliated) using a 1 to 5 scale, where 1 represented the plants exhibiting the lowest amount of above-ground biomass, and 5 represented the plants with the highest amount of biomass.

¶ Fall regrowth was estimated on 11 May 2011 (41 d after the plants were defoliated) using a 1 to 5 scale, where 1 represented the plants exhibiting the lowest amount of above-ground biomass, and 5 represented the plants with the highest amount of biomass.

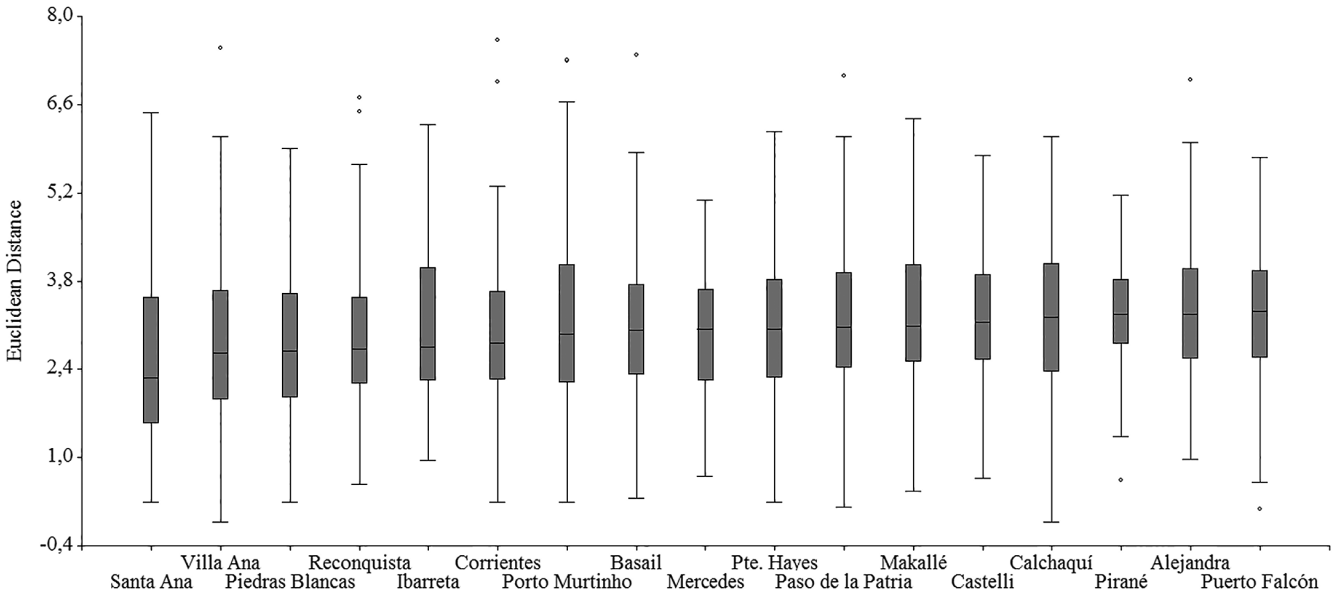

Figure 6. Side-by-side boxplot representing the within-population diversity estimated based on agronomic traits information. Each box plot represents one of the 17 populations of *Paspalum simplex*. Populations are ordered from left to right based on increasing values of the median genetic distance.

is generated in the species and also identifying potential germplasm collection sites. The ploidy levels found in this research are in agreement with previous reports, which have indicated that the tetraploid cytotype is predominant in the species' area of natural distribution (Urbani et al., 2002). The hexaploid cytotype is only present growing within tetraploid populations and seems to have reduced vigor when compared with neighboring tetraploid plants. Although diploid populations were not included in this research, they seem to be the most important source of

variation in the species (Brugnoli et al., 2013). Moreover, the most successful cytotype is the tetraploid, since it has been able to colonize a great variety of environments.

The ISSR markers have been useful for estimating the diversity present within and among populations of *P. simplex*. These results indicate that most of the diversity present in polyploid germplasm is allocated among populations (Fig. 4). Preliminary observations about this phenomenon have been previously reported for *Paspalum notatum* (Daurelio et al., 2004), *P. simplex* (Brugnoli et al., 2013), and *P. denticulatum* (Sartor et al., 2013). These previous studies have indicated that polyploid populations, allopatric to diploid, contained low variability based on a reduced number of analyzed populations. The present study, which has been based on an important number of populations, represents strong evidence that colonization of new microenvironments is highly specific in *P. simplex*. This specificity results in distant genotypes dominating sites located a few kilometers apart. These polyploid genotypes are expected to have originated within the sexual 2*x* populations (Brugnoli et al., 2013) and distributed throughout the rest of the region. The characteristics that make them successful in a particular environment are fixed by apomixis.

Intra-population diversity can be considered low for most analyzed populations, with the exception of the population from Calchaquí (Fig. 3 and 4). This prevalent, low variation may result from the apomictic mode of reproduction present in polyploid cytotypes. However, this observation should not be considered common to all apomictic species since high variation has been observed for other *Paspalum* species, such as *P. nicorae* (Sartor et al., 2013), and unrelated species like *Taraxacum officinale* (Van Der Hulst et al., 2000).

The population from Calchaquí should be further studied considering potential higher residual sexuality, a greater rate of mutations in comparison with the other populations included in this study, or the potential proximity of a diploid population. Single-plant collections at each site would be an appropriate method for germplasm collection. Although all plants of a population should be considered genetically similar, off-types should also be collected to consider potential agronomic applications.

The phenotypic variation observed in this study has not been enough to clearly separate the group of analyzed populations. However, this information is useful for identifying characters that can be improved. There is high variation for earliness of flowering, which is important for generating late flowering lines that can maintain an adequate nutritive value during a longer period (Coleman et al., 2004). Variability for initial growth is also positive considering that a quick establishment is essential for warm-season grasses. Moreover, variability for spring and fall growth would allow selecting for lines with an extended growing period (Acuña et al., 2011). The low variability for plant height and plant diameter is indicative that *P. simplex* is a well-defined bunch grass and that the probability of selecting for different growth habits is very low.

In conclusion, the tetraploid cytotype predominates in *Paspalum simplex* and can be found across its region of natural adaptation. Great diversity is present among polyploid populations of *P. simplex*. This fact is the result of site-specific adaptation of novel polyploid genotypes. Germplasm collections for improving this species should consider the region where cross-pollinated diploid plants live and a large number of sites characterized by the presence of variable ecological conditions to obtain maximum diversity in the collected plant material.

Acknowledgments

The authors would like to thank Dr. Kenneth H. Quesenberry, University of Florida, for reviewing this manuscript and Florencia Galdeano for technical assistance. The work was carried out with contributions from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina, PICT N° 2008-00264, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP 11420100100031, Secretaría General de Ciencia y Técnica (UNNE) PI A001-2009 and PI A005-2010, and Facultad de Ciencias Agrarias, UNNE. E.A. Brugnoli received a fellowship from ANPCYT-UNNE. M.H. Urbani is researcher of UNNE. C.L. Quarin, E.J. Martínez, and C.A. Acuña are career members of CONICET.

References

Acuña, C.A., A.R. Blount, K.H. Quesenberry, K.E. Kenworthy, and W.W. Hanna. 2011. Tetraploid bahiagrass hybrids: Breeding technique, genetic variability and proportion of heterotic hybrids. Euphytica 179:227–235. doi:10.1007/s10681-010-0276-y

Bayer, R. 1990. Patterns of clonal diversity in the *Antennaria rosea* (Asteraceae) polyploid agamic complex. Am. J. Bot. 77:1313–1319. doi:10.2307/2444591

Blount, A.R., and C.A. Acuña. 2009. Bahiagrass. In: R.J. Singh, editor, Genetic resources, chromosome engineering, and crop improvement series: Forage crops. Vol. 5. CRC press, Boca Raton, FL. p. 81–101.

Brugnoli, E.A., M.H. Urbani, C.L. Quarin, E.J. Martínez, and C.A. Acuña. 2013. Diversity in diploid, tetraploid, and mixed diploid-tetraploid populations of *Paspalum simplex* Morong. Crop Sci. 53:1509–1516. doi:10.2135/cropsci2012.08.0497

Cidade, F.W., M. Dall'Agnol, F. Bered, and T.T. De Souza-Chies. 2008. Genetic diversity of the complex *Paspalum notatum* Flügge (Paniceae: Panicoideae). Genet. Resour. Crop Evol. 55:235–246. doi:10.1007/s10722-007-9231-8

Coleman, S.W., J.E. Moore, and J.W. Wilson. 2004. Quality and utilization. In: L.E. Moser et al., editors, Warm season grasses. ASA, CSSA, SSSA, Madison, WI. p. 267–308.

Daurelio, L.D., F. Espinoza, C.L. Quarin, and S.C. Pessino. 2004. Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. Plant Syst. Evol. 244:189–199. doi:10.1007/s00606-003-0070-6

Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada, and C.W. Robledo. 2002. InfoStat, version 2002. Grupo InfoStat, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Córdoba, Argentina.

Espinoza, F., L.D. Daurelio, S.C. Pessino, E.M. Valle, and C.L. Quarin. 2006. Genetic characterization of *Paspalum notatum* accessions by AFLP markers. Plant Syst. Evol. 258:147–159. doi:10.1007/s00606-005-0401-x

Gates, R.N., C.L. Quarin, and C.G.S. Pedreira. 2004. Bahiagrass. In: L.E. Moser et al., editors, Warm-season (C4) grasses. Agron. Monogr. 45. ASA, CSSA, SSSA, Madison, WI. p. 651–680.

Hanna, W.W. 1995. Use of apomixis in cultivar development. Adv. Agron. 54:333–350. doi:10.1016/S0065-2113(08)60903-7

Houliston, G.J., and H.M. Chapman. 2004. Reproductive strategy and population variability in the facultative apomict *Hieracium Pilosella* (Asteraceae). Am. J. Bot. 91:37–44. doi:10.3732/ajb.91.1.37

Hörandl, E., and O. Paun. 2007. Patterns and sources of genetic diversity in apomictic plants: Implications for evolutionary potentials. In: E. Hörandl et al., editors, Apomixis: Evolutions, mechanisms and perspectives. A.R.G. Gantner Verlag, Rugell, Liechtenstein. p. 170–194.

Jungmann, L., B.B. Vigna, K.R. Boldrini, A.C. Sousa, C.B. do Valle, R.M. Resende, et al. 2010. Genetic diversity and population structure analysis of the tropical pasture grass *Brachiaria humidicola* based on microsatellites, cytogenetics, morphological traits, and geographical origin. Genome 53:698–709. doi:10.1139/G10-055

Meirmans, P.G., and P.H. Van Tienderen. 2004. GenoType and GenoDive: Two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4:792–794. doi:10.1111/j.1471-8286.2004.00770.x

Menken, S.B.J., E. Smit, and J.C.M. Den Nijs. 1995. Genetical population structure in plants: Gene flow between diploid sexual and triploid asexual dandelions (*Taraxacum* section *Ruderalia*). Evolution 49:1108–1118. doi:10.2307/2410435

Miles, J.W. 2007. Apomixis for cultivar development in tropical forage grasses. Crop Sci. 47:238–249. doi:10.2135/cropsci2007.04.0016IPBS

Ortiz, J.P.A., C.L. Quarin, S.C. Pessino, C.A. Acuña, E.J. Martínez, F. Espinoza, et al. 2013. Harnessing apomictic reproduction in grasses: What we have learnt from *Paspalum*. Ann. Bot. (Lond.) 112:767–787. doi:10.1093/aob/mct152

Paun, O., J. Greilhuber, E. Temsch, and E. Hörandl. 2006. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). Mol. Ecol. 15:897–910. doi:10.1111/j.1365-294X.2006.02800.x

Peakall, R., and P.E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x

Reyno, R., R. Narancio, P. Speranza, J. Do Canto, B. López Carro, P. Hernández, J. Burgueño, et al. 2012. Molecular and cytogenetic characterization of a collection of bahiagrass (*Paspalum notatum* Flügge) native to Uruguay. Genet. Resour. Crop Evol. 59:1823–1832. doi:10.1007/s10722-012-9806-x

Sartor, M.E., R.N. Rebozzio, C.L. Quarin, and F. Espinoza. 2013. Patterns of genetic diversity in natural populations of *Paspalum* agamic complexes. Plant Syst. Evol. 299:1295–1306. doi:10.1007/s00606-013-0797-7

Skerman, P.J., and F. Riveros. 1994. Gramíneas tropicales. FAO, Roma.

Sousa, A.C.B., L. Jank, T. de Campos, D.A. Sforça, M.I. Zucchi, and A.P. de Souza. 2011. Molecular diversity and genetic structure of guineagrass (*Panicum maximum* Jacq.), a tropical pasture grass. Trop. Plant Biol. 4:185–202. doi:10.1007/s12042-011-9081-6

Urbani, M.H., C.L. Quarin, F. Espinoza, M.I.O. Penteado, and I.F. Rodrigues. 2002. Cytogeography and reproduction of the *Paspalum simplex* polyploid complex. Plant Syst. Evol. 236:99–105. doi:10.1007/s00606-002-0237-6

Van Der Hulst, R.G.M., T.H.M. Mes, J.C.M. Den Nijs, and K. Bachmann. 2000. Amplified fragment length polymorphism (AFLP) markers reveal that population structure of triploid dandelions (*Taraxacum officinale*) exhibits both clonality and recombination. Mol. Ecol. 9:1–8. doi:10.1046/j.1365-294x.2000.00704.x

Vogel, K.P., and B. Burson. 2004. Breeding and genetics. In: L.E. Moser et al., editors, Warm-season (C4) grasses. Agron. Monogr. 45. ASA, CSSA, and SSSA, Madison, WI. p. 51–96.

Zuloaga, F.O., and O. Morrone. 2005. Revisión de las especies de *Paspalum* para América del Sur austral. Ann. Mo. Bot. Gard. 102:1–297.