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Population structure and genetic relatedness in Brazilian Bermudagrass from sugarcane plantations

J.C. Rocha[1], C.P. de Almeida[1], F.C. Reis[2], R.V. Filho[3], V.L. Tornisielo[2], M.I. Zucchi[4] and L.L. Benchimol-Reis[1]

[1] Instituto Agronômico, Centro de Pesquisa em Recursos Genéticos Vegetais – Campinas, SP, Brasil
[2] Universidade de São Paulo, Centro de Energia Nuclear na Agricultura, Piracicaba, SP, Brasil
[3] Universidade de São Paulo, Departamento de Produção Vegetal – ESALQ/USP, Piracicaba, SP, Brasil
[4] Agência Paulista de Tecnologia dos Agronegócios – APTA, Piracicaba, SP, Brasil

Corresponding author: L.L. Benchimol-Reis
E-mail: luciana.reis@sp.gov.br

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ABSTRACT. *Cynodon dactylon* (Bermudagrass) ise used for forage in Brazilian pastures, as well as in lawns, parks, and sports fields. However, in sugarcane fields, it is a difficult to control weed due to its rapid adaptation and growth mechanisms. It competes with sugarcane plants negatively affecting crop yield. Bermudagrass has polyploidy and easy hybridization, which promote high polymorphism, generating varying responses to the environment. Consequently, understanding the structure and variability of Bermudagrass becomes important for the development of strategies for its management as a weed. We examined the levels of genetic variability and structure of five populations (SP1 to SP5) of Bermudagrass, collected from sugarcane fields in the state of São Paulo (Brazil). Thirteen microsatellites were used. PCoA demonstrated STRUCTURE results (K=4) showing a mixture of SP1 and SP3 populations, with SP2, SP4 and SP5 being the most distant. DAPC also confirmed low genetic differentiation for SP1 and SP3. Genetic variability was found to be greater among than within populations, due to

the predominance of vegetative growth of the species, which promotes low diversity, and due to geographic distances, which reduce gene flow or even make it unfeasible. The SSRs showed high resolution in characterizing the genetic diversity and structure of the five populations of Bermudagrass. The findings of this study may help to establish biological control methods against Bermudagrass in sugarcane fields.

Key words: *Cynodon dactylon*; Polymorphism; Genetic structuring; Population analysis

INTRODUCTION

Cynodon dactylon, popularly known as Bermudagrass, Bahamagrass, fine hierba, and silkgrass, among other names (FAO, 2020), is a C4 perennial grass from the Poaceae family found throughout nearly all parts of the world, from tropical to subtropical and temperate coastal areas (Taliaferro, 1995). Currently, 13 species are described and accepted within the *Cynodon* genus (The Plant List, 2013).

Southeast Africa is most likely the center of origin of the species. However, its current geographic distribution ranges from 45° N to 45° S, extending to about 53° N in Europe (Wu et al., 2004). The high colonizing capacity of the species, mainly due to the mechanisms of vegetative propagation through rhizomes and stolons, has led to its distribution throughout Brazil. In addition, the species is highly adaptable to new environments and to a wide variety of soils, from sandy to highly clayey, preferentially with good moisture and good drainage (Faria Júnior, 2012). The natural hardiness of the species, promoted by its underground rhizome, is another factor that ensures tolerance to pH variation, drought, and burning (Athayde et al., 2005). It can be propagated by seed, but due to short seed viability, vegetative propagation is much more common (Kissmann, 1997).

As natural and artificial hybridization easily occurs (Costa et al., 2013), the species has different levels of ploidy, and populations with 2n = 18, 27, 30, 40 are observed (Chaves et al., 2018). Variation in the ploidy level is one of the main sources of genetic and morphological polymorphisms for the species (Kissmann, 1997; Wang et al., 2020). According to Dors (2010), the effect of polyploidy and the genetic variability among individuals of the species lead to different responses to external factors of control and care of the plant compared to diploid species, due to genetic redundancy and genome heterozygosity.

Due to the high phenotypic plasticity and rapid ground covering ability of the species, several cultivars were developed for pasture and landscaping (Horowitz, 1996). However, Bermudagrass, despite also being used as forage, is an important and worrying weed in several cultures, especially in sugarcane. The perennial nature of sugarcane and slow early season growth, combined with wide row spacing, provide a favorable environment for Bermudagrass growth (Holm et al., 1977). In Brazil, the control of Bermudagrass during sugarcane cultivation is usually carried out before planting with the application of herbicides, which can occur after mechanical control, preferably in the dry season. It is important that the pre-planting weed control be effective, as the plant quickly spreads through stolons on the soil surface with scaly and branched rhizomes below the surface (Horowitz, 1996), competing with the crop until the cane field is renewed, which occurs about five years after planting.

The state of São Paulo is the largest producer of sugarcane in Brazil, accounting for about 50% of the national production (CONAB, 2021). There are several factors that can influence the productivity of this crop, and competition with weeds for light, water and nutrients are the most important. Bermudagrass may also harbor organisms harmful to plant health such disease-causing viruses, bacteria, fungi, and nematodes (Kissmann, 1997).

Molecular markers have been a useful tool for understanding the genetic diversity of Bermudagrass. The various molecular markers include microsatellites (Simple Sequence Repeats – SSRs), which are combinations of 1 to 6 bases repeated in tandem (Zhiyong et al., 2013). Tan et al. (2012) demonstrated that EST sequences and sorghum SSR primers were useful sources for the development of Bermudagrass SSRs. There was higher transferability from sorghum genomic SSR primers to *C. transvaalensis*, indicating that this species may share more conserved sequences with sorghum compared to *C. dactylon*. Another study using heterologous EST-SSRs (Khanal et al., 2017) showed that sugarcane EST-SSRs could be used for identifying polymorphism (transferability) among *Cynodon* species (*C. dactylon* and *C. transvaalensis*) and hybrids. A small set of informative SSRs differentiated the cultivar groups and identified potentially mischaracterized cultivars.

Guo et al. (2017) developed more than 1000 SSRs and found the dinucleotide motif (AT/TA) to be the most common. Zhang et al. (2019) estimated genetic diversity across 153 EST-SSR *loci* from *C. dactylon* populations collected in a latitudinal gradient across China and genetic diversity between different ploidy levels. The authors reported the remarkable mixing structure existing between populations across latitudes and the increase in genetic diversity with the level of ploidy. They also reported that environments with higher temperatures and high precipitation rates could favor higher rates of variability within populations; but if there is a low gene flow rate, this variability could be exhausted over time. Zhang et al. (2021), using 105 EST-SSRs, reported low gene flow, with rich genetic differentiation, among populations of *C. dactylon* along longitude gradients. The authors stated there was no clear linear trend of intra-population genetic diversity along longitude, and the intra-population genetic diversity was not related to climate.

Currently, there are not any studies accessing the genetic diversity through microsatellites of Brazilian Bermudagrass weeds growing in sugarcane plantations at the state of São Paulo. Thus, it is important to understand the genetic variability present in the species and the genetic structuring of natural populations found to be able to develop management strategies. The goals of this study were to understand the levels of genetic variability among *C. dactylon* populations collected in different sugarcane fields in the state of São Paulo and to discover the population structure of each population through microsatellite markers.

MATERIAL AND METHODS

Plant material, DNA extraction, and genotyping

Five sugarcane regions throughout the state of São Paulo with a high rate of infestation with *C. dactylon* were selected for collection of natural weed populations of the species (SP1 to SP5). Population is defined as individuals of the same species that occupy the same space during a particular time interval. Therefore, the samples collected in the same city and in the same sugarcane field were representative of the same population. The highest sugarcane production

areas with high weed infestation within the state were chosen. To represent the different populations, seven random samples were collected in each location, for a total of thirty-five plant samples (Table 1). The selected samples were grown in a greenhouse at the Agronomic Institute (Campinas, SP, Brazil) in 3-liter pots filled with a 1:1 mixture of clay and plant substrate.

Table 1. List of characteristics of the regions where the 35 samples of Bermudagrass (*Cynodon dactylon*) from the state of São Paulo were collected. M. Tp. - mean temperature; M. R.- mean rainfall. The climate of the regions is according to the climatic classifications of Köppen and Thornthwaite: Cfa (humid subtropical climate, with hot summer); Cwb (subtropical highland climate, with dry winter and mild summer); Aw (tropical climate with dry winter).

Sample	Population (City)	Date of collection	M Tp.	M. R.	Soil	Climate	Coordinates
Sp1Pl1 Sp1Pl2 Sp1Pl7 Sp1Pl10 Sp1Pl11 Sp1Pl14 Sp1P15	Boituva	05 Aug 2017	18.4ºC	26 mm	Argisoil-Nitosoil	Cfa	23°16'28.8"S 47°37'26.6"W
Sp2Pl1 Sp2Pl3 Sp2Pl5 Sp2Pl9 Sp2Pl11 Sp2Pl17 Sp2Pl18	Ribeirão Preto	30 Aug 2017	19.5 ºC	18 mm	Oxisol	Cwb	20°45'21.1"S 47°36'44.0"W
Sp3Pl2 Sp3Pl3 Sp3Pl7 Sp3Pl12 Sp3Pl15 Sp3Pl16 Sp3Pl18	Piracicaba	02 Sep 2017	19.8ºC	55mm	Argisoil-Nitosoil	Cfa	22°35'37.5"S 47°40'44.5"W
Sp4Pl2 Sp4Pl8 Sp4Pl11 Sp4Pl12 Sp4Pl13 Sp4Pl15 Sp4Pl18	Lençóis Paulista	22 Sep 2017	19.7ºC	62mm	Oxisol	Cfa	22°36'24.6"S 48°44'39.8"W
Sp5Pl1 Sp5Pl2 Sp5Pl4 Sp5Pl8 Sp5Pl11 Sp5Pl12 Sp5Pl18	Araçatuba	04 Out 2017	23.1ºC	118mm	Oxisol	Aw	21°24'22.7"S 50°55'44.0"W

For genomic DNA extraction, new leaves from the apical region of the stolons of each sample were collected and identified, followed by maceration in liquid nitrogen. Due to the great difficulty encountered in obtaining high quality genomic DNA, various protocols were tested: PT1, Doyle and Doyle (1987); PT2, Dellaporta et al. (1983); and PT3, Doyle and Doyle (1987) with modifications. For PT3, the final NaCl concentration was reduced to 1.25 M and 2-

mercaptoethanol was excluded. The concentration of PVP40 (Polyvinylpyrrolidone) was doubled to allow that even without 2-mercaptoethanol there was no oxidation of the samples. The genomic DNA of the 35 samples was extracted using the PT3 protocol and diluted to 10 ng / µl.

A total of 13 SSR markers (Guo et al., 2017) were used for genotyping populations (Table 2). The amplifications (PCR) were performed in a BioRad thermocycler (MyCycler™) with Mix 5x Firepol® Master Mix 12.5 mM in a final volume of 15 µL. The amplification conditions were carried out as described by Wang et al. (2013).

The quality of the amplifications was verified by electrophoresis on a 2.5% agarose gel stained with GelRed™. The amplification products were separated using high-precision capillary electrophoresis by an automated 96-capillary Fragment Analyzer™ system (Advanced Analytical Technologies, Ames, IA, USA) using the DNF-905 Kit with a degree of separation from 1 to 500 bp.

Table 2. Microsatellite molecular markers (SSRs) used for genotyping the 35 Bermudagrass (*Cynodon dactylon*) samples collected from five sugarcane growing regions of the state of São Paulo.

No.	*PRIMER*	*FORWARD*	*REVERSE*	*SIZE (bp)*	*REF.*
1	A4	CTGTATCTTATGCTGGTTTG	CGTGCTCTGCCTCTGCTA	185-202	Wang et al. (2013)
2	CDCA5 467/468	GCAATACTAGGGGCCAAGAG	CGGCAGTCAAGTTCAGTAGC	150	Guo et al (2017)
3	CDGA8 1783/ 1784	CCTCCTCCCAACATCTTCTG	GAAATGCATGTTCCTTGCAC	204	Guo et al. (2017)
4	CDGA1 807/808	CAGGAAAACGAGACGAGAGA	CCGGGACGTCAGATATTTTT	326	Guo et al. (2017)
5	CDGA5 1459/ 1460	TTCTGGCATCACTCTCAACG	TGCCTACTCTGTTGAGGACG	165	Guo et al (2017)
6	CDGA8 1807/ 1808	CCTCAACTCCAGTGCTGAAA	TGTTAACCGGGGTTCAGATT	207	Guo et al. (2017)
7	CDCA5 469/470	CGAACATGAACCTCAGGCTA	AAAATACACATGCTGCTGGGG	245	Guo et al. (2017)
8	CDGA4 1301/1302	TGACACAACAGCCACCTTCT	TGCTTTACAAAGGTCAGCCA	145	Guo et al. (2017)
9	CDCA7 641/642	GGATATAAAGGCAGTCGGCA	TTCACAATGTGGCACACAAC	321	Guo et al. (2017)
10	CDCA8 749/750	CCAGCAAGAGGGAAGAAAAG	CTTCCGTGGTTTGGAAAGTT	204	Guo et al. (2017)
11	CDGA1 841/842	ATGCGTATGGAGTTGCATGT	AAGATCTCCATTCTCCACCG	287	Guo et al. (2017)
12	CDGA1 927/928	AAAACACTGATGTTCGCAGC	CGGCCTATACCTCATTCAGT	330	Guo et al. (2017)
13	CDGA2 985/986	CTTTCAAGTTTCAGGCAGACC	GTTGGTTTCAATGCTGGTTG	348	Guo et al. (2017)

Analysis of genetic diversity

Genotyping was performed using the PROSize™ 2.0 program, treating the *loci* as dominant to avoid problems regarding the variable polyploidy observed in the species. Number of effective alleles (Ne), expected heterozygosity (He), unbiased expected heterozygosity (uHe), Shannon's Information Index (I), and percentage of polymorphic *loci* (%P) were estimated using GenAlEx 6.5 (Peakall and Smouse, 2012). Genetic similarity was calculated for samples and populations using Jaccard´s similarity coefficient, and clustering was performed by Neighbor-

Joining with the VEGAN package (Oksanen et al., 2019). From the genetic similarity matrix, Principal Coordinate Analysis (PCoA) was estimated using the ADE4 package (Dray et al., 2007).

Analysis of Molecular Variance (AMOVA), with significance tested by 9,999 random permutations, was used to assess differentiation at two levels of genetic partition: population (region) and samples. Furthermore, Discriminant Analysis of Principal Components (DAPC), proposed by Jombart et al. (2010) and implemented in the ADEGENET v2.1.1 package (Jombart and Ahmed 2011), was used to validate population differentiation, using population information as *a priori*.

Population structuring was assessed using the STRUCTURE v2.3.1 program (Pritchard et al., 2000). The admixture ancestry model was used considering the allelic frequency as uncorrelated, with 50,000 Markov chain Monte Carlo (MCMC) simulations and a burn-in of 25,000. K values ranging from 1 to 11 were tested in 10 independent runs. To determine the best number of clusters, the ΔK method proposed by Evanno et al. (2005) was used through the STRUCTURE HARVESTER program (Earl and vonHoldt, 2012). The coefficient of participation of each accession was given by the alignment of the 10 repetitions of the best K through the CLUMPP method (Jakobsson and Rosenberg, 2007) by the CLUMPAK program (Kopelman et al., 2015). The bar chart was generated by the POPHELPER package (Francis, 2016).

RESULTS

SSR genotyping

Comparing the extraction quality of the three protocols tested, PT3 was superior; it was the only protocol that enabled extraction with no DNA degradation in the three plant samples used, allowing the extraction of all other samples (Figure 1). Genotyping results from the Fragment analyzer equipment (Figure 2) were read considering each band as 1 or 0 (dominant marker), which resulted in the identification of a total of 109 *loci*. Among these, 77% were polymorphic for SP1, 48.62% for SP2, 73.39% for SP3, 57.80% for SP4, and 53.21% for SP5, demonstrating a high polymorphic pattern for the markers used (Figure 2). The genotyping analyses using the PROSize and GenAlEx programs showed a high degree of expected heterozygosity (He), as well as a high Shannon index, for the diversity of populations SP3 and SP1, followed by SP4, SP2, and SP5 (Table 3).

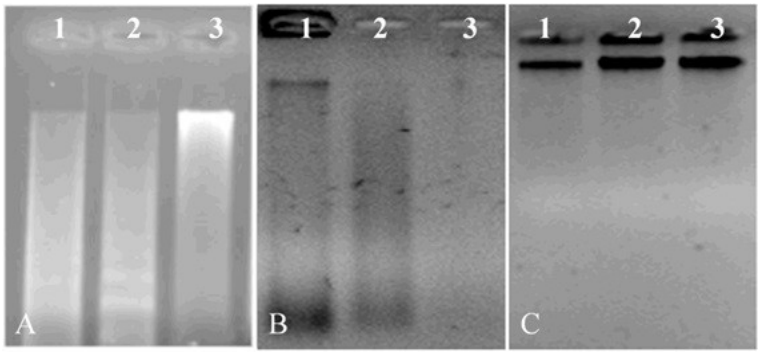

Figure 1. Electrophoretic profile of different DNA samples obtained through three different extraction protocols. On the left is the Dellaporta protocol, in the middle Doyle and Doyle with no modifications, and on the right Doyle and Doyle with modifications.

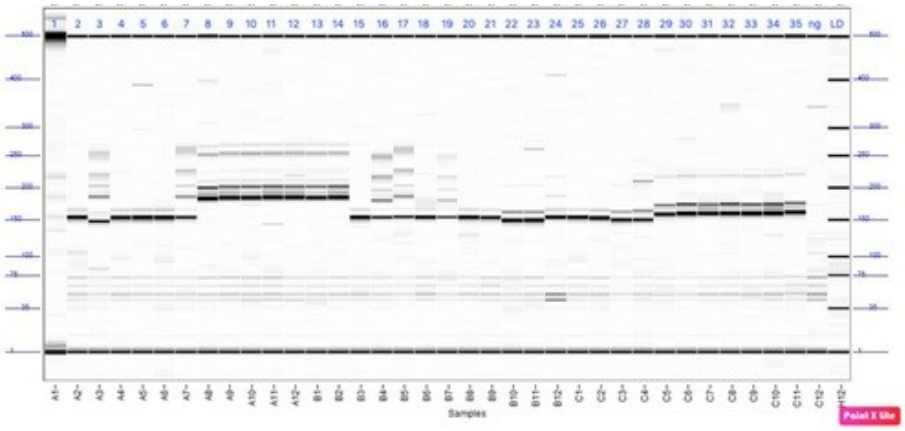

Figure 2. Electrophoretic profile for the amplification of the SSR CDGA5-1459/1450 with 35 samples of *Cynodon dactylon* and 100 bp Ladder, performed by the Fragment Analyzer equipment.

Table 3. Genetic diversity estimated for 35 Bermudagrass (*Cynodon dactylon*) samples with 13 microsatellites. Number of Individuals (No), No. of Effective Alleles (Ne), Shannon's Information Index (I), Expected Heterozygosity (H_E), Unbiased Expected Heterozygosity (uHe), and Percentage of Polymorphic *Loci* (%P).

Populations	No	Ne	I	H_E	uHe	%P
Boituva	7	1.230	0.231	0.147	0.158	51.82%
Ribeirão Preto	7	1.092	0.072	0.050	0.054	12.73%
Piracicaba	7	1.302	0.280	0.183	0.197	57.27%
Lençóis Paulista	7	1.150	0.135	0.089	0.096	26.36%
Araçatuba	7	1.049	0.040	0.027	0.030	7.27%
Mean	7	1.165	0.152	0.099	0.107	31.09%

For STRUCTURE, the data were analyzed for K = 2 (best K according to Evanno et al., 2005, Figure 3a). It was possible to separate the SP5 population from the others, due to the well-defined structure within the set (orange cluster, Figure 3b). However, considering K = 4, the SP4, SP2, and SP5 populations each formed a specific cluster. For K = 5, there was a mixture between the SP1 and SP3 clusters, indicating a high level of interaction between these two populations.

The data obtained by the PCoA analysis also showed results parallel to those of STRUCTURE clustering for the most distant populations (SP2, SP4, and SP5), and it showed a mixture within individuals from the SP1 and SP3 populations (Figure 3c and Figure 4c). For SP1 and SP3, three clusters were formed; one composed of individuals 16, 17, and 18, another of 1, 3, and 7, and one more of the rest of the individuals. The results obtained by the PCoA analysis sustained the K = 4 configuration for STRUCTURE analysis (Figure 3b).

The DAPC, as well as the results presented in the PCoA, showed the low genetic differentiation for the SP1 and SP3 regions, through the overlap of the two populations (Figure 4d). However, the analysis showed strong differentiation for the other clusters, mainly for Lençois Paulista and Araçatuba.

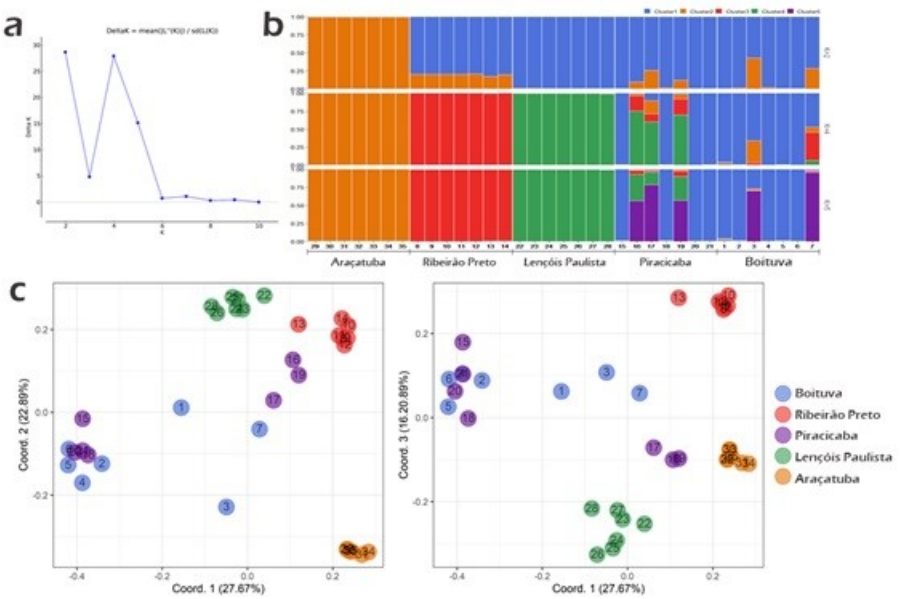

Figure 3. a) Evaluation of the K parameter for characterization of Bermudagrass (*Cynodon dactylon*) populations with 13 microsatellites amplified in 35 samples**. b)** Structure analysis for the genetic variability found for the *C. dactylon* collection regions in the state of São Paulo from K = 2, K = 4, and K = 5. **c)** PCoA matrix for grouping the individuals of *C. dactylon* analyzed.

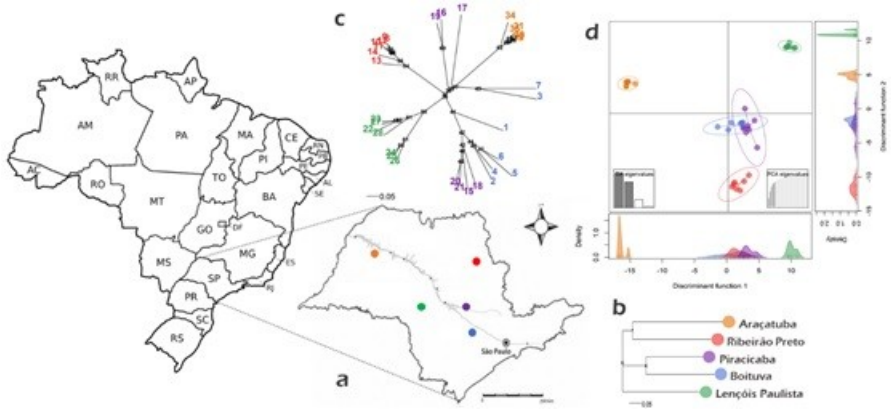

Figure 4. a) Analysis of the geographic distribution of *Cynodon dactylon* populations collected in the state of São Paulo, Brazil. **b)** Dendrogram formed with Jaccard coefficient and Neighbor-Joining cluster for the 5 populations of *C. dactylon* with 13 microsatellites (SSRs). **c)** Structuring of 35 samples of *C. dactylon* through PCoA (Principal Coordinate Analysis) within the studied populations. **d)** Analysis of the DAPC (discriminant analysis of principal components) structure for the 35 populations of *C. dactylon* analyzed with 13 SSRs.

Genetic relationships

The data collected on the populations showed similarity of soil for the regions where the SP1 and SP3 populations were collected, which were characterized by Argisol-

Nitisol. As for the SP2, SP4, and SP5, the constituent soil was Oxisol. Microclimate data for each region indicated that in SP1, SP3, and SP4, the Cfa microclimate (humid subtropical climate; Thorthwaite, 1948) is predominant. The Cwb microclimate (subtropical highland climate with dry winter and mild summer; Thorthwaite, 1948) characterizes SP2 and SP5.

The dendrogram (Figure 4b) indicated a correlation between genetic and geographic distances, which was also observed by Wang at al. (2013). AMOVA results showed greater variance between populations (61%) than within them (39%) (Table 4).

Table 4. Analysis of molecular variance (AMOVA) for the 35 samples of Bermudagrass (*Cynodon dactylon*) from the state of São Paulo, Brazil.

Source of Variance	DF	SS	Variance Components	Variance %
Between Populations	4	369.1	12.098	61%
Within Population	30	228.0	7.601	39%
Total	34	597.1	19.699	100%

DF - degrees of freedom; SS – sum of squares

DISCUSSION

Three DNA extraction protocols were tested, and PT3 was the one that resulted in the best DNA quality (Figure 1). This was due to a 22% decrease in NaCl concentration and the replacement of 2-mercaptoethanol by PVP40, which led to more integral DNA, allowing the inhibition of oxidants and the reduction of particulates that impair the purity of the extracted genetic material.

The polymorphism rates observed in the genotyping analyses proved to be effective in identifying a high polymorphic pattern for the populations studied, as it was also observed by Guo et al. (2017) and Khanal et al. (2017). The analyses showed that among the populations studied, SP1 and SP3 were more diverse than the others, with SP2 having the lowest polymorphic rates (Figure 3). This result was also observed in the DAPC analyses (Figure 4), which showed that SP2, despite being closer to SP1 than to SP4, does not maintain a minimum gene flow with these populations to enable higher rates of transferability of gametes and, consequently, to increase the polymorphism indices in the population.

The differentiation of the SP2 and SP5 populations possibly occurs due to the strong genetic structure of the two populations, their geographic isolation, and their ability to disperse by budding. Analyses carried out using the Weather Spark mapping system (2020) showed that the mean wind directions for the SP2 and SP5 regions may explain the genetic isolation of these populations from the others. Ling et al. (2012) reported that there was a strong correlation between the genetic relationships analyzed by SSRs of wild accessions of Bermudagrass collected from southwest China and growing locations meaning that accessions from the same collection sites tended to be clustered into the same group.

Studies carried out by Rai and Jain (1982) with *Avena barbata* showed that wind direction can modify the mean rates and distances of pollen and seed dispersion between plants and neighboring populations. For Boituva, the average normal wind direction is out of the east and south, enabling gene flow towards SP3 to the north and SP4 to the west.

However, in SP4, due to the distance between the two populations and the presence of mountainous regions close to Boituva, the winds might not be efficient enough to effectively carry out this flow. For Ribeirão Preto, the normal average wind direction during the year is very largely to the east, towards Araçatuba (which, due to the great distance, turns out to be unlikely, although gene flow is possible), and to the south, towards Piracicaba. However, this flow may also not be very efficient, due to geographic barriers between the populations. To confirm this hypothesis, the genetic transferability for *C. dactylon* populations between the regions under study should be analyzed to determine if there is a gradient of genetic material transfer. There were winds from Piracicaba towards Boituva, a factor that could explain the strong genetic relationship between these populations. For L. Paulista and Araçatuba, the main wind directions were out of the north and east; thus, pollen dispersion from SP4 and SP5 ends up not naturally reaching any of the other populations studied here, and the transferability index of these locations to the others is low.

Similar results were observed in the diversity analyses. The He and I indices showed that there is a high degree of polymorphism for the SP1 and SP3 populations, followed by SP4, SP2, and SP5, consecutively (Table 3). Comparison of the SP1 and SP5 populations shows that the degree of diversity of SP1 is 5.72 times greater; thus, even if SP5 has the most defined population structure, the diversity is low, indicating limited gene flow.

The high level of polymorphism in SP1 and SP3 compared to the other locations can be explained by the high gene flow between individuals and neighboring populations. Cross-pollination, resulting in gene flow among natural populations, may prevent formation of differentiated genetic groups. Likewise, the low polymorphic index of SP5 can be explained by the isolation of the population, about 290 Km to SP4 (the closest population) and 475 Km to SP1 (the farthest population). The degree of gene flow from one plant population to another directly affects their genetic diversity. Levin (1984) describes that in situations where there is migration of individuals to isolated areas, there is an increase in heterogeneity between the original population and the new one, but exchange of material between already established populations tends to increase the diversity within the population and decrease the heterogeneity when they are compared.

The great difference in the degree of diversity observed among populations may also be the result of greater selection pressure in some regions, mainly due to the biotic and abiotic constraints in each location. According to Cargnin (2006), polymorphism leads to higher levels of resistance to external factors. Therefore, populations with greater gene flow between individuals consequently have more accentuated polymorphism, favoring the plant's adaptability to environmental stresses.

Isolation of populations favors inbreeding between individuals, which leads to less diverse populations. STRUCTURE analyses were performed at three levels of K. For K = 2, the SP5 population was most distinct, possibly due to the geographic isolation of this population and its dispersal capacity by budding. As the species depends on pollen exchange for sexual reproduction, which is largely through anemophily, geographic barriers such as the Tietê River (which crosses the state of São Paulo), oscillations in altitude, and geographic distances ended up isolating the SP5 population from the others, reducing the occurrence of cross-fertilization and seed migration (Figure 4a). Martins (1987) contextualizes the gene flow between anemophilous plant populations with the population structure in each environment.

A possible explanation for the population structure and differentiation between populations for K = 2 and K = 4 is the strong reproductive index of the species through rhizomes compared to seeds (Moreira, 1975). This leads to higher proportions of genetically identical individuals (possible clones) and low population differentiation between samples from the same natural population. In contrast, SP1 and SP3 were poorly structured, especially for K = 5, showing a strong relationship between regions favored by proximity and the possibility of gamete exchange. The dendrogram (Figure 4b) also indicated a correlation between genetic and geographic distances which was also observed by Wang et al. (2013) who reported that the most native accessions from similar or adjacent regions were clustered in the same group when using ISSR and SSR markers for accessing *C. dactylon* genetic diversity.

The interactions observed through the analyses were also shown by the dendrogram, where SP1 and SP3 were overlapping clusters and the other locations were distinct branches. Evidence of admixture indicates that some hybridization and introgression must have occurred in the past. As shown by the DAPC, SP2 was the population most distant from the others, probably due to the low genomic conservation within the SSR regions.

Differentiation through AMOVA (Table 4) showed greater variance between populations than within them, which can be understood by the different types of reproduction of this plant in its environment, as well as the possibility that gene flow is affected by geographic barriers between the areas. As noted by Nascimento Filho (2001), populations formed by vegetative growth of individuals have low genetic variance, due to the lack of gamete exchange. This factor may also have contributed to the population structure observed in this study.

The effectiveness of the DNA extraction protocol for obtaining high quality Bermudagrass DNA was an achievement of this study. Our study is the most comprehensive investigation of the genetic diversity and population structure of Bermudagrass as a weed in sugarcane fields at the state of São Paulo to date and provides useful information for improving management of this pest and biological weed control.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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