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Genetic Similarity Among Accessions of *Paspalum notatum* Flügge (Poaceae): a Potential to Parental Selection

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HIGHLIGHTS

- Low genetic similarity in *Paspalum notatum* accessions.
- High genetic distance among diploid accessions.
- The accessions have good potential to breeding program.

Abstract: *Paspalum notatum* is an important forage grass contributing significantly to the coverage of the natural fields of Southern Brazil. Simple sequence repeat (SSR) markers were used to evaluate the genetic similarity of strains within a *P. notatum* collection. Genomic DNA was extracted in bulk from young leaves of five plants from each accession obtained from the USDA. In the molecular analysis, the eight SSR markers evaluated formed seven distinct groups, and two isolated genotypes, with an average similarity index of 0.29, ranging from zero to 0.83. All the loci were polymorphic and the polymorphism information content ranging from 0.41 to 0.69. The results evidenced a low genetic similarity, which can be explored via parental selection in a breeding program.

Keywords: breeding; forage grass; heterosis; SSR markers.

INTRODUCTION

Paspalum notatum Flügge (Poaceae) is a grass species of great economic importance. It is responsible for most of the coverage of the natural fields of southern Brazil [1] and has excellent forage potential [2,3]. However, genetic improvement of this species has been limited to identification of promising material traits, without the possibility of performing crosses to obtain new varieties, due to the apomictic mode of reproduction of most of its accessions [4-7]. *Paspalum* species exhibit ploidy-dependent apomixes. Diploid accessions generally have sexual reproduction, while tetraploid accessions are apomictic [8,9].

Several studies of *Paspalum* species have aimed to find diploid accessions that can be used to perform intra or inter-specific crosses to obtain new cultivars [1,10-13]. *P. notatum,* cultivar Pensacola, is a diploid, sexually reproducing cultivar that has been used, after chromosome doubling by colchicine, to perform crosses with apomictic parents [4,7,14-16]. Other diploid species of *Paspalum* have also been used as sexual parents, after chromosome doubling, to obtain interspecific hybrids [4,17]. Although, chromosome doubling of sexual diploid plants to create tetraploid plants can also result in apomictic plants [15,16,18], several crosses have had satisfactory results [16,17,19].

The discovery of new wild diploid accessions of *P. notatum* has great importance for breeding programs, since they can increase the number of possible crosses with apomictic tetraploids after chromosome doubling. Four wild diploid accessions of *Paspalum notatum* have recently been identified [20]. These accessions exhibited higher dry matter production and greater persistence in winter conditions than Pensacola [2], and morphological traits that allow them to be differentiated from Pensacola [21].

Parental selection is the critical step in the development of new cultivars [22] and can be directed to facilitate exploitation of maximum genetic variability and production of superior recombinant genotypes [23]. Parental selection decisions must be carefully made, because populations with reduced genetic potential may waste time and money. Thus, individuals featuring high performance, wide adaptability and yield stability must be considered when choosing parental genotypes [23]. Studies that quantitatively assess genetic diversity provide useful information for identification of parents that allow exploitation of heterotic effects and generation of segregating populations with greater variability [24].

Methods used to quantify genetic distance include morphological (syn. Phenotypic) traits [25], molecular markers, and pedigree information [26,27]. Molecular markers have the advantage of providing genome assessments that are not influenced by gene-environment (G x E) interactions and are not limited in number, as is true for morphological data [28]. Based on these considerations the objective of this study was to characterize a collection of *P. notatum* accessions through SSR markers to contribute to the identification of future favorable parental combinations.

MATERIAL AND METHODS

A total of 53 accessions of *Paspalum notatum* were obtained from the USDA for molecular characterization. These accessions have been previously evaluated at under field conditions [2]. The samples consisted of a mixture (bulk) of young and healthy leaves from five plants in each accession. DNA extraction was performed according to the CTAB method [29] with minor modifications.

Polymerase chain reactions (PCR) amplifying Simple Sequence Repeats (SSRs) were adapted to a final volume of 15 µL using: 3 µL of template DNA solution (15 ng/µL), 1.5 µL of 10X PCR buffer (Invitrogen, São Paulo, Brazil), 0.90 µL $MgCl_2$ (50 mM), 0.6 µL of 10 mM dNTP mix containing 2.5 mM of each of the four nucleotides (Invitrogen, São Paulo, Brazil), 1.2 µL primers (100 ng/µL), 0.27 µL Taq DNA polymerase (5 U/µL) (Invitrogen, São Paulo, Brazil) and sterile MilliQ water to complete the volume [30].

Amplification conditions for SSRs are as follows: denaturation at 94 °C for 4 minutes; ten cycles of 94 °C for 1 minute, 50 °C for 30 seconds, and 72 °C for 40 seconds with a decrease of 0.5 °C in the annealing temperature; 35 cycles of 94 °C for 1 minute, 45 °C for 30 seconds, 72 °C for 40 seconds; final extension at 72 °C for 10 minutes [30].

A total of 11 primers were tested based on studies performed with *Lolium multiflorum* L. [31], *Paspalum vaginatum* Sw. [30], *Trifolium repens* L. [32] and *Paspalum urvillei* St. [33] (Table 1). Amplified fragments were electrophoresed in 4% agarose gels containing 0.08 µL/mL ethidium bromide (10 mg/mL) and visualized on an ultraviolet light transilluminator (wavelength 260 nm). Images were captured using a Kodak EDAS (Electrophoresis Documentation and Analysis System) 290. Eight primers were used in the genetic diversity analyses due to satisfactory amplification of the expected DNA fragments (Table 1).

Table 1. SSR primers tested for amplification of 53 *Paspalum notatum* accessions.

Primer	Sequence F (5' – 3') Sequence R (3' – 5')
*Pv-3	TATGGACCGACTGCATGATTCTT CTTACGGAGAGTGGATCGATG
*Pv-11	AGGTTTGTAGGTTGGGTGCAACTGA TAATGGGAGGCGGCGGGTT
Pv-35	TCGAAATCGAAAAAGAAGATCGTTC GATTGGAACATCGACCGCGG
Pv-51	TCCCATCATCAGTTCTTCCAATC TTCTACTACTTATTATCGTGTCCCG
*Pv-53	CTCGGAAACCGCAGCTCA ACCTTATCTCCTCCGCCTCG
*M4-213	CACCTCCCGCTGCATGGCATGT GGAACTGTACAGAACAT
*M15-185	GGTCTGGTAGACATGCCTAC CTTGGACGGACACGACCAT
*M16-B	TGCTGTGGCTCTTGTGAC AGCTCGACTCGGAGCCGA
M4-136	AGAGACCATCACCAAGCC GTTCCTTTAGAAGAAGGTCT
*M2-148	GCAACTTCTATCGAGTTG AGGCACTTCTAGCTCGGAG
*M12-52	CTACAATGCATTCGTGCA TCCCGCGCCCACGGAGAT

*Primers used in genetic similarity analyses.

Amplified SSR DNA fragments were scored for each accession according to a binary matrix: presence (1) or absence (0) characters. The accessions V32 and 87N were excluded from analysis because their DNA extractions were unsatisfactory. The resulting data matrix was analyzed using "Numerical Taxonomy and Multivariate Analysis System" NTSYSpc version 2.1 [34]. Jaccard's coefficient was used to generate a similarity matrix comparing all the accessions. The clustering analysis was performed using the UPGMA (Unweighted Pair-Group Method Using an Arithmetic Average) method to construct a genetic similarity dendrogram.

Number of alleles per locus (A), genotypic and allelic frequencies, polymorphism information content (PIC= 1 - $\sum pi^2$, pi= allele frequency) for each locus, and heterozygosity observed were calculated manually.

RESULTS

Eight of the 11 SSR primers tested were used in the molecular and similarity analyses (Table 1). The markers detected four alleles per locus, for a total of 32 polymorphic DNA fragments in the 53 accessions of *Paspalum notatum* (Table 2). The average allele number was four, and alleles ranged in size from 115 to 383 base pairs (bp) (Table 2).

Table 2. Allele size range (bp), number of alleles (A), polymorphism information content (PIC), and observed heterozygosity (Ho) of each SSR marker.

Primer	Allele size (pb)	A	PIC	Ho
Pv-3	115-364	4	0.41	0.32
Pv-11	135-255	4	0.42	0.46
Pv-53	118-289	4	0.65	0.69
M4-213	121-233	4	0.67	0.73
M15-185	169-330	4	0.56	0.89
M16-B	142-369	4	0.69	0.60
M2-148	144-383	4	0.54	0.72
M12-52	139-358	4	0.60	0.78
Total		32		
Average		4	0.57	0.65
Min-Max	115-383		0.41-0.69	0.32-0.89

In this work, DNA fragments that were not associated with SSR regions (as judged by size) were ignored. These DNA fragments were probably amplified due to the use of heterologous primers for *Paspalum notatum* species. In the gel analyses, only DNA fragments between the 100 and 400 bp markers were considered prior to standardization of analyses to obtain reliable estimates of material diversity.

The PIC values ranged from 0.41 to 0.69, with an average of 0.57 (Table 2). All loci were polymorphic, ranging from zero to four alleles per accession analyzed. This information can be associated with heterozygosis, since the reproduction mode in tetraploids and hexaploids is apomictic, and their place of origin is much diversified. The observed heterozygosity ranged from 0.32 to 0.89, with an average of 0.65 (Table 2). These results reinforce the hypothesis of a high heterosis for the loci evaluated in this work.

The accessions evaluated in this study presented low genetic similarity values, with an average of 0.29 (Jaccard's coefficient), ranging from zero (among several accessions) to 0.83 (between V31 and V66). Based on the genetic similarity values, accessions were separated into seven distinct groups, and two isolated genotypes (Figure 1).

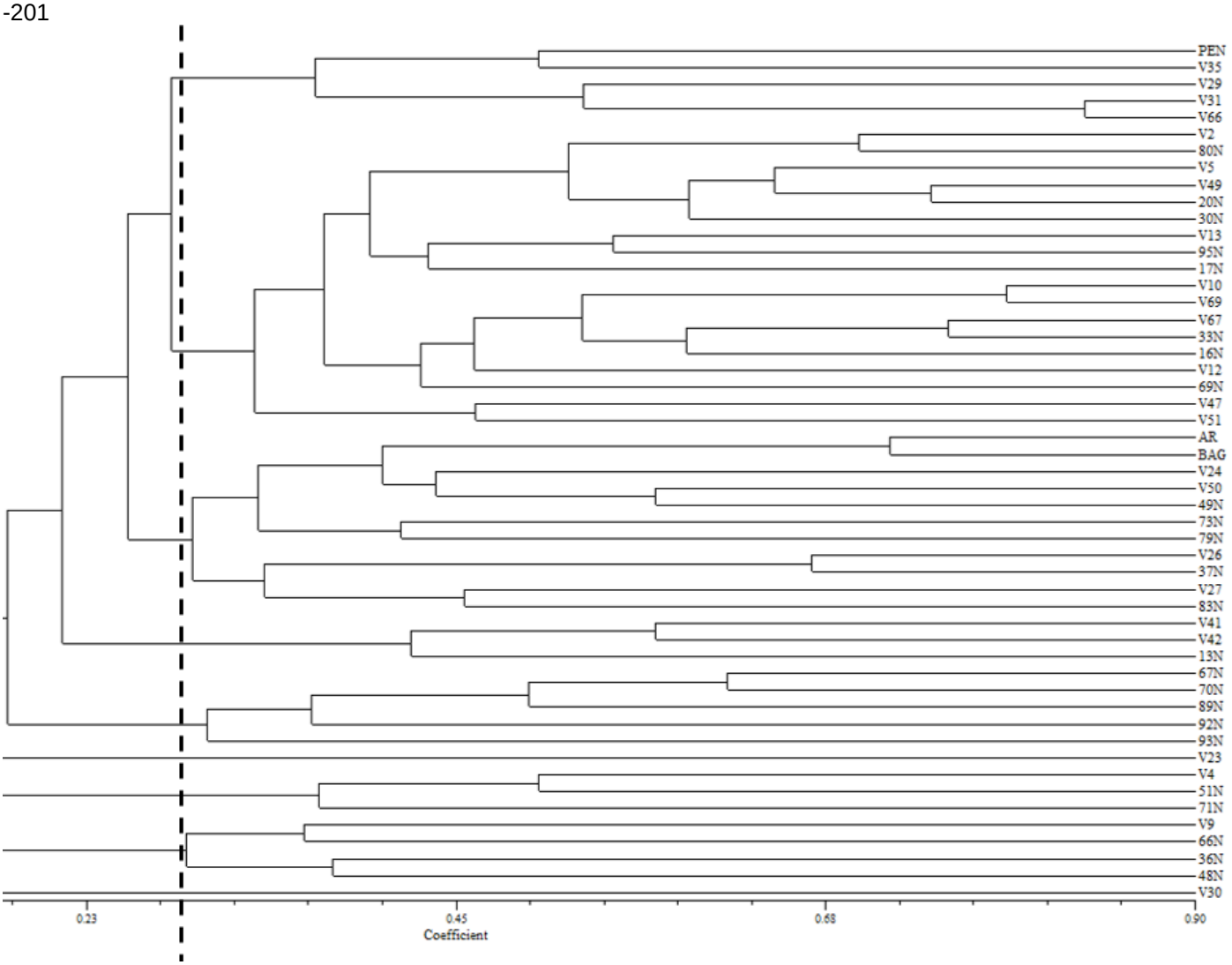

Figure 1. Relationships among *Paspalum notatum* accessions based on molecular markers, and obtained by Jaccard's similarity. The dashed line indicates the mean similarity.

DISCUSSION

Assessment of genetic diversity based on phenotypes has limitations, since most morphological characteristics of economic importance are influenced by environmental factors and plant developmental stage. By contrast, molecular markers based on DNA sequence polymorphisms are independent of environmental conditions, and show a higher degree of polymorphism [35].

The primers used in this study were designed for other species, such as maize (*Zea mays*), rice (*Oriza sativa*) and sorghum (*Sorghum bicolor*). Transferability of these SSR markers was studied to *Paspalum* was

studied, and transfer rates of 67.5, 49.0 and 66.8% were obtained, respectively [30]. Other researchers have also obtained satisfactory data using these markers [31,32]. The use of these same primers to analyze *Paspalum urvillei*, also detected the presence of four alleles per locus, yielding 28 polymorphic DNA fragments [33]. In *Paspalum notatum,* the use of 11 SSR-specific markers identified 7.9 alleles per locus, and the PIC ranged from 0.36 to 0.89 [36]. These values are higher than those obtained in our work, probably due to the use of specific primers.

The results obtained in this study were in accordance with the large morphologic diversity [21] and variability in dry matter production [2] observed in the same group of accessions in field conditions. The observed genetic similarity allowed separation of the 53 accessions of *P. notatum* into seven groups and two isolated genotypes. These groups did not present a clear relation with the region of origin of the accessions, as well as forage production [2], morphologic analysis [21] or ploidy [20]. Low genetic similarity was also observed among the diploid accessions, 66N, 67N, 92N, and Pensacola, although they grouped together (Figure 1). The observed genetic diversity was similar to that described by other authors using dominant markers. A study of 95 accessions of *P. notatum* with ISSR markers detected a wide polymorphism, with only 2.2% of monomorphic DNA fragments, with Jaccard's index ranging from 0.43 to 0.97 (average 0.59) and the formation seven distinct groups, suggesting considerable genetic variation within species [37]. On the other hand, the use of AFLP markers, found low genetic distances ranging from 0.01 to 0.36 [38].

The formation of genetically distant groups favors the selection of genotypes to be used as parents to obtain new cultivars, with the aim of keeping heterosis high. Genetic distance between genotypes is a way to predict genetic variability among hybrid combinations [39]. Examples of molecular markers used in genetic distance studies were reported for several plant species of agronomic importance. A positive correlation between the genetic distance between parents, and heterosis has been reported in maize [40,41], wheat [42], alfalfa [43], rice [44], oilseed rape [45,46] and cacao [47].

CONCLUSION

Taking into consideration the relationship between the groups formed based on SSR markers, on morphological characteristics and dry matter production, could be helpful in selecting progenitors with good forage yields. The combination of desirable morphological characteristics and low genetic similarity increases the probability of obtaining more vigorous progeny. The *P. notatum* accessions possess low genetic similarity, allowing the formation of seven groups and two isolated genotypes. These groups can direct parental selection from genetically distinct accessions. Gathering the several studies carried out with this germplasm collection, it is possible to affirm that these accessions show excellent potential for development of new varieties, because they combine high genetic diversity, good forage production, and persistence in winter conditions, and diploid accessions with higher forage potential than cv. Pensacola.

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REFERENCES

1. Pozzobon MT, Valls JF. Chromosome number in germplasm accessions of *Paspalum notatum* (Gramineae). Rev Bras Genet. 1997;20(1):29-34.
2. Fachinetto JM, Schneider R, Hubber KG, Dall'Agnol M. Avaliação agronômica e análise da persistência em uma coleção de acessos de *Paspalum notatum* Flügge (Poaceae). Agrária. 2012;7(1):189-95.
3. Steiner MG, Dall'Agnol M, Nabinger C, Scheffer-Basso SM, Weiler RL, Simioni C, et al. Forage potential of native ecotypes of *Paspalum notatum* and *P. guenoarum*. An Acad Bras Cienc. 2017;89(3):1753-60.
4. Aguilera PM, Galdeano F, Espinoza F, Quarín CL. Interspecific tetraploid hybrids between two forage grass species: sexual *Paspalum plicatulum* and apomictic *P. guenoarum*. Crop Sci. 2011;51(4):1544-50.
5. Huber KG, Dall'Agnol M, Motta EA, Pereira EA, Ávila MR, Perera MZ, et al. Variabilidade agronômica e seleção de progênies F1 de *Paspalum.* Agrária. 2016;11(4):374-80.
6. Pereira EA, Barros T, Volkmann GK, Battisti GK, Silva JA, Simioni C, et al. Variabilidade genética de caracteres forrageiros em *Paspalum*. Pesqui Agropecu Bras. 2012;47(10):1533-40.

7. Pereira EA, Dall'Agnol M, Simioni C, Machado JM, Bitencourt MG, Guerra D, et al. Agronomic performance and interspecific hybrids selection of the genus *Paspalum*. Científica. 2015;43(4):388-95.
8. Delgado L, Galeano F, Sartor ME, Quarin CL, Espinoza F, Ortiz JP. Analysis of variation for apomictic reproduction in diploid *Paspalum rufum*. Ann Bot. 2014;113(7):1211-8.
9. Ortiz JP, Quarin CL, Pessino SC, Acunã C, Martínez EJ, Espinoza F, et al. Harnessing apomictic reproduction in grasses: what we have learned from *Paspalum*. Ann Bot. 2013;112(5):767-87.
10. Dahmer N, Schifino-Wittmann MT, Dall'Agnol M, Castro B. Cytogenetic data for *Paspalum notatum* Flügge accessions. Sci Agric. 2008;65(4):381-8.
11. Pozzobon MT, Machado AC, Vaio M, Valls JF, Peñaloza AP, Santos S, et al. Cytogenetic analyses in *Paspalum* L. reveal new diploid species and accessions. Cienc Rural. 2008;38(5):1292-9.
12. Reis CA, Schifino-Wittmann MT, Dall'Agnol M. Chromosome numbers, meiotic behavior and pollen fertility in a collection of *Paspalum nicorae* Parodi accessions. Crop Breed Appl Biotechnol. 2008;8(3):212-8.
13. Sartor ME, Quarin CL, Espinoza F. Mode of reproduction of colchicine-induced *Paspalum plicatulum* tetraploids. Crop Sci. 2009;49(4):1270-76.
14. Machado JM, Dall'Agnol M, Motta EA, Pereira EA, Simioni C, Weiler RL, et al. Agronomic evaluation of *Paspalum notatum* Flügge under the influence of photoperiod. Rev Bras Zootec. 2017;46(1):8-12.
15. Quarin CL, Espinoza F, Martinez EJ, Pessino SC, Bovo OA. A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. Sex Plant Reprod. 2001;13(3):243-9.
16. Weiler RL, Krycki KC, Guerra D, Simioni C, Dall'Agnol M. Chromosome doubling in *Paspalum notatum* var. *saure* (cultivar Pensacola). Crop Breed Appl Biotechnol. 2015;15(2):106-11.
17. Novo PE, Valls JF, Galdeano F, Honfi AI, Espinoza F, Quarin CL. Interspecific hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. Sci Agric. 2016;73(4):356-62.
18. Krycki KC, Simioni C, Dall'Agnol M. Cytoembryological evaluation, meiotic behavior and pollen viability of *Paspalum notatum* tetraploidized plants. Crop Breed Appl Biotechnol. 2016;16(4):282-8.
19. Mota EA, Dall'Agnol M, Nascimento FL, Pereira EA, Machado JM, Barbosa MR, et al. Forage performance of *Paspalum* hybrids from an interspecific cross. Cienc Rural. 2016;46(6):1025-31.
20. Fachinetto J, Dall'Agnol M, Wittmann MT, Rockenbach M. Simioni C. New wild diploids in *Paspalum notatum* Flügge (Poaceae): potential accessions for use in breeding. Crop Breed Appl Biotechnol. 2018;18(4):432-6.
21. Fachinetto JM, Dall'Agnol M, Souza CH, Weiler RL, Simioni C. Genetic diversity of a *Paspalum notatum* Flügge germplasm collection. Rev Bras Zootec. 2017;46(9):714-21.
22. Benin G, Matei G, Oliveira AC, Silva GO, Hagemann TR, Silva CL, et al. Relationships between four measures of genetic distance and breeding behavior in spring wheat. Genet Mol Res. 2012;11(3):2390-400.
23. Bertan I, Carvalho FI, Oliveira AC. Parental Selection Strategies in Plant Breeding Programs. J Crop Sci Biotechnol. 2007;10(4):211-22.
24. Bianchini FG, Balbi RV, Pio R, Bruzi AT, Silva DF. Parents choice and genetic divergence between cambuci fruit tree accessions. Crop Breed Appl Biotechnol. 2017;17(3):214-20.
25. Yadav HK, Shukla S, Singh PS. Genetic divergence in parental genotypes and its relation with heterosis, F1 performance and general combining ability (GCA) in opium poppy (*Papaver somniferum* L.). Euphytica. 2007;157(1): 123-30.
26. Dreisigacker S, Melchinger AE, Zhang P, Ammar K, Flachenecker C, Hoisington D, et al. Hybrid performance and heterosis in spring bread wheat, and their relations to SSR-based genetic distances and coefficients of parentage. Euphytica. 2005;144(3):51-9.
27. Paczos-Grzeda E. Pedigree, RAPD and simplified AFLP-based assessment of genetic relationships among *Avena sativa* L. varieties. Euphytica. 2004;138(1):13-22.
28. Maric S, Bolaric S, Martincic J, Pejic I, Kozumplik V. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breed. 2004;123(4):366-9.
29. Ferreira ME, Grattapaglia D. Introdução ao uso de marcadores moleculares em análise genética. 3th ed. Brasília: Embrapa Cenargem; 1998.
30. 30 - Wang ML, Chen ZB, Barkley NA, Newman ML, Kim W, Raymer P, et al. Characterization of seashore *Paspalum* (*Paspalum vaginatum* Swartz) germplasm by transferred SSRs from wheat, maize and sorghum. Genet Resour Crop Evol. 2006;53(1):779-91.
31. Kubik C, Sawkins M, Meyer WA, Gaut BS. Genetic diversity in seven perennial ryegrass (*Lolium perenne* L.) cultivars based on SSR markers. Crop Sci. 2001;41(5):1565-72.
32. Kölliker R, Jones ES, Drayton MC, Dupal MP, Forster JW. Development and characterization of simple sequence repeat (SSR) markers for white clover (*Trifolium repens* L.). Theor Appl Genet. 2001;102(2):416-24.

33. Sawasato JT, Dall'Agnol M, Conceição DP, Tafernaberry Jr V, Klafke GB. Utilização de microssatélites e RAPD na caracterização molecular de acessos de *Paspalum urvilei* Steudel. Rev Bras Zootec. 2008;37(8):1366-74.
34. Rohlf FJ. NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.1 [software]. 2001.
35. Dalamu BT, Gaikwad AB, Saxena S, Bharadwaj C, Munshi AD. Morphological and molecular analyses define the genetic diversity of Asian bitter gourd (*Momordica charantia* L.). Aust J Crop Sci. 2012 Feb;6(2):261-7.
36. Cidade FW, Souza-Chies TT, Batista LAR, Dall'Agnol M, Zucchi MI, Jungmann L, et al. Isolation and characterization of microsatellite loci 3 in *Paspalum notatum* Flügge (Poaceae). Conserv Genet. 2009 Feb;10(2):1977–80.
37. Cidade FW, Dall'Agnol M, Bered F, Souza-Chies TT. Genetic diversity of the complex *Paspalum notatum* Flügge (Paniceae: Panicoideae). Genet Resour Crop Evol. 2008 Jun;55(6):235-46.
38. Espinoza F, Daurelio LD, Pessino SC, Valle EM, Quarin CL. Genetic characterization of *Paspalum notatum* accessions by AFLP markers. Plant Syst Evol. 2006 Apr;258(2):147-59.
39. Cruz CD, Regazzi AJ. Modelos biométricos aplicados ao melhoramento genético. 1st ed. Viçosa: Editora UFV; 2001.
40. Betran FJ, Ribaut JM, Beck D, Leon DG. Genetic diversity, specific combining ability and heterosis in tropical maize under stress and nonstress environments. Crop Sci. 2003 May;43(3):797-806.
41. Smith OS, Smith JS, Bowen SL, Tenborgand RA, Wall SJ. Similarities among a group of elite maize inbreeds as measured by pedigree, F1 grain yield, grain yield heterosis and RFLPs. Theor Appl Genet. 1990 Dec;80(6):833-40.
42. Cox TS, Murphy JP. The effect of parental divergence on F2 heterosis in winter wheat crosses. Theor Appl Genet. 1990 Feb;79(2):169-71.
43. Riday H, Brummer EC, Cambell TA, Luth D. Comparison of genetic and morphological distance with heterosis between *Medicago sativa* and subsp. *falcata*. Euphytica. 2003 May;131(1):37-45.
44. Kwon SJ, Ahn SN, Jeong EG, Hwang HG, Choi HC, Moon HP. Relationship between genetic divergence and hybrid performance in japonica rice grown in a cold water-irrigated field. Euphytica, 2002 Dec;128(6):389-96.
45. Diers BW, McVetty PB, Osborn TC. Relationship between heterosis and genetic distance based on RFLP markers in oilseed rape (*Brassica napus* L.). Crop Sci. 1996 Jan;36(1):79-83.
46. Riaz A, Li G, Quresh Z, Swati MS, Quiros CF. Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breed. 2001 Jun;120(5):411-5.
47. Dias LA, Marita J, Damião CD, Fernandes ST. Genetic distance and its association with heterosis in cacao. Braz Arch Biol Technol. 2003 Jun;46(3):339-47.