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Molecular Diversity and Genetic Structure of Guineagrass (*Panicum maximum* Jacq.), a Tropical Pasture Grass

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Abstract Guineagrass (*Panicum maximum* Jacq.) is a forage grass found in tropical and subtropical regions. It is an apomictic and tetraploid species from Africa. The objective of this study was to evaluate the genetic diversity of guineagrass accessions sampled from its regions of origin, which is in Tanzania and Kenya. In this study, a total of 396 accessions were analyzed, and a collection of reproducible and informative microsatellites was developed. Thirty microsatellites were employed to characterize these accessions. A total of 576 clones were sequenced from microsatellite-enriched libraries. Flanking primers were designed for 116 microsatellite loci and screened using a sample of 25 guineagrass accessions. The thirty selected polymorphic microsatellites employed in this study produced a total of 192 bands when evaluated in the 396 *P. maximum* accessions, with an average of 6.4 bands per microsatellite. Four genetic clusters were identified in the collection using STRUCTURE analysis, and these results were confirmed using AMOVA. The largest genetic variation was found within clusters (65.38%). This study revealed that the collection of accessions from the *P. maximum* region of origin was a rich source of genetic variability. The geographical distances and genetic similarities among accessions did not indicate a significant association between genetic and geographical variation, supporting the natural interspecific crossing between *P. maximum, P. infestum* and *P. trichocladum* as the origin of the high genetic variability and the existence of an agamic complex formed by these three species.

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Abbreviations

AMOVA	analysis of molecular variance
CTAB	cetyltrimethylammonium bromide
D	discriminating power
DNA	deoxyribonucleic acid
EMBRAPA	Brazilian Agricultural Research Corporation
IPTG	isopropyl β-D-1-thiogalactopyranoside
MCMC	Markov Chain Monte Carlo
NJ	neighbor joining
ORSTOM	Institut Français de Recherche Scientifique pour le Développement en Coopération

PCA	principle component analysis
PCR	polymerase chain reaction
PIC	polymorphism information content
SSRIT	simple sequence repeat identification tool

Introduction

Guineagrass (*Panicum maximum* Jacq., *Megathyrsus maximus* Jacq. Simon BK and Jacobs SWL) is an important tropical forage grass native to Africa, where high genetic diversity is found (Burton et al. 1973). Because of its high yield and nutritional content and wide adaptability to diverse ecological niches, guineagrass has been widely introduced and exploited in most tropical and subtropical countries, including Brazil, Japan, the USA and Australia (Nakajima 1978; Smith 1979; Savidan 1982; Duke 1983). Guineagrass belongs to the family Poaceae, the subfamily Panicoideae and the tribe Paniceae. The species is considered apomictic (of the gametophytic aposporous type) (Savidan 2000; Jain et al. 2006), and its apomictic accessions are autotetraploid (2n=4x=32) (Combes and Pernès 1970; Bogdan 1977); however, sexual plants in nature have been observed and identified as diploid (2n=2x=16) (Nakajima et al. 1979). *P. maximum* forms an agamic complex with the botanical species *P. infestum* Anders and *P. trichocladum* K. Schum (Muir and Jank 2004). Because these three species possess the same chromosome number (2n=4x=32) and intercross freely, intermediate or hybrid accessions may be found in the natural grass populations in East Africa (Savidan and Pernès 1982). In the natural population, 7% of accessions are diploid and reproduce sexually (Pernès 1975). It is thought that these diploid sexual forms intercross with *P. maximum, P. infestum* and *P. trichocladum* through spontaneous haploidization and recurrent tetraploidization (Savidan and Pernès 1982). Natural grass populations can be divided into three types: monomorphic, polymorphic discontinuous and polymorphic continuous. The polymorphic discontinuous populations include well-differentiated biotypes, while the polymorphic continuous populations include mixed populations representing diploid sexual and tetraploid apomictic biotypes. Prior studies have suggested the possibility that the polymorphic continuous population originated from the crossing of a population with the diploid sexual pool (Pernès 1975).

The *P. maximum* germplasm, available at the Brazilian Agricultural Research Corporation—Embrapa Beef Cattle (Mato Grosso do Sul, Campo Grande, Brazil), was introduced in 1982 by a cooperative agreement with the former Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) in France (known today as the Institut de Recherche pour le Développement—IRD). The germplasm includes tetraploid apomictic and tetraploid artificially induced sexual plants and may be considered representative of the existing natural variability of the species due to the eco-geographical scope of the collecting expeditions (Savidan et al. 1989).

The *P. maximum* germplasm comprises a high level of phenotypic variability (Jank et al. 1994; Jank et al. 1997). However, the genetic diversity of the germplasm has not been evaluated at the molecular level. Elucidating the genetic variability of the germplasm is important for the development of breeding strategies, the selection of accessions and the conservation of these genetic resources. With the development of molecular marker technologies, scientific tools have become available to efficiently describe the structure of the genetic diversity present in the germplasm or within cultivars and the diversity among populations without the need for phenotyping (Bolaric et al. 2005). Among the available molecular markers, microsatellites are the most promising marker for genomic applications and are highly informative (Gupta and Varshney 2000). Microsatellite markers consist of short (1–6 bp) tandem repeat DNA sequences randomly dispersed throughout the genome. These sequences are locus-specific, polymorphic and exhibit a co-dominant segregation pattern (Gaitán-Solís et al. 2003). The allelic diversity at microsatellite loci, caused by variations in the number of repeats in the core sequence, is likely caused by polymerase slippage and deficient DNA repair during DNA replication (Field and Wills 1996). Microsatellites have been proven effective for estimating genetic diversity and genetic relationships and for predicting both the genetic value of selected accessions derived from intraspecific crosses and the performance of their hybrid progenies (Varshney et al. 2005; Ebina et al. 2007; Chandra and Tiwari 2010). This paper presents the genetic diversity of 396 *P. maximum* accessions from the Embrapa Beef Cattle germplasm bank using 30 microsatellite loci.

Results and Discussion

Sequence Analysis and Microsatellite Loci Polymorphism

The library enriched for dinucleotide repeat motifs (CT_8 and GT_8) exhibited high levels of microsatellite enrichment. A total of 576 clones were isolated and sequenced. Of these clones, 323 sequences (61%) contained microsatellites, and 236 (48.7%) were suitable for designing primers. Redundant sequences accounted for 23% of the microsatellite-containing clones. The size of the inserts varied between 360 and 900 bp, with an average size of 500 bp. Screening of the library indicated that 80% of the microsatellites consisted of simple dinucleotide motifs (perfect and imperfect), while 20% were composed of compound motifs. The maximum number of

repeats among the microsatellites was 23 (perfect GA). Trinucleotides were less frequent (15%) and exhibited less repeat units (three to five units). Tetranucleotides (3%) and hexanucleotides (2%) were also observed as simple repeats. The most common repeat motif was the $(TG)_n/(CA)_n$ group, which represented over 79.5% of all microsatellites.

Ultimately, a total of 116 primer pairs were designed and tested using PCR amplification. Previously, 20 microsatellite markers had been characterized among 25 *P. maximum* accessions selected from the germplasm bank to investigate polymorphism (Sousa et al. 2011). A total 96 newly developed microsatellites were tested, of which 66 produced a product of the expected size. In total, 55 microsatellites were polymorphic, and 11 were monomorphic (Table 1). Of the 66 microsatellites evaluated, 46 consisted of dinucleotide repeats, 1 was a trinucleotide repeat and 9 were composed of compound repeats. No correlation was observed between the types or length of repeats and monomorphic marker behavior.

A total of 318 bands were obtained from the 55 polymorphic microsatellite markers. The number of bands from each microsatellite locus ranged from 3 to 16, with an average of 5.8 bands per locus. This finding confirms the high polymorphism of the markers. The number of bands detected in each accession ranged from one to four (Fig. 1). These results confirm the autotetraploid nature of *P. maximum* (Combes 1975). In autotetraploids, there are four copies of each homologous chromosome. The resulting meiotic combination events may include quadrivalents, trivalents, bivalents and univalents. In nature, *P. maximum* exhibits diploid-tetraploid-haploid cycles (Savidan and Pernès 1982) in crosses between apomictic accessions and sexual plants. At each locus, the number of bands and the number of patterns were used to calculate the degree of polymorphism. The polymorphism information content (PIC) values were calculated to assess marker informativeness (Mateescu et al. 2005), and the discriminating power (D) of each locus was estimated to compare the efficiency of markers in varietal identification (Tessier et al. 1999). The PIC values ranged from 0.19 to 0.89, with an average of 0.56. The D values ranged from 0.34 to 0.99, with an average of 0.68. The highest PIC and D values were observed at the locus 2PMc217, which contained 16 bands. Of the investigated loci, 70% exhibited more than a 50% probability of discriminating between two accessions. The analysis of the D values indicated that the efficiency of a given marker did not depend solely on the number of patterns it generated, as was reported by Tessier et al. (1999). For example, the loci 2PMc428, 2PMc40.1, 2PMc194, 1PMc39.b and 1PMc55 each produced the same numbers of patterns and bands (6 and 5, respectively), but they demonstrated different discriminatory powers. In contrast, the loci 2PMc52 and 2PMc103, each generating different numbers of patterns (7 and 6, respectively), exhibited similar discriminatory powers. Based on the estimates of PIC and D, 30 microsatellite loci were selected to help characterize the *P. maximum* germplasm bank, which is composed of 396 accessions. The 30 selected microsatellite markers are depicted in Table 1.

Molecular Analysis of the *Panicum maximum* Germplasm

From the 30 microsatellite loci selected, a total of 192 bands were produced, with an average of 6.4 bands per locus. Based on Jaccard's similarity coefficient, the genetic variation among the accessions was estimated. The similarity values among the 396 accessions of *P. maximum* ranged from 0.16 to 0.86, with an average similarity of 0.32 (Supplementary Table 1). Among the 15 sexual plants (S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20 and S21), the mean similarity was 0.63.

A typical STRUCTURE analysis assumes a model in which there are K populations, each of which is characterized by a set of band frequencies at each locus. Accessions are assigned (probabilistically) to a particular population or jointly to two or more populations if their genotypes indicate that they are admixed. STRUCTURE analysis, combined with the computation of Evanno ΔK statistics, suggested a primary partition of the *P. maximum* accessions into four clusters ($K=4$), with a number of accessions exhibiting admixture (Fig. 2a and b). Therefore, the subpopulations from the STRUCTURE analysis were grouped into four clusters (I, II, III and IV) (Fig. 3 and Supplementary Table 2), with bootstrap values ranging from 49% to 99%. Cluster I (red) contained 50 apomictic accessions and cluster II (green) contained 61 accessions, while the other two clusters, III (blue) and IV (yellow), contained, 107 and 178 apomictic and sexual accessions, respectively. This approach successfully discriminated all of the accessions tested, with the exception of duplicate accessions. Confirmed replicates (Table 2) in the germplasm bank were determined based on genetic and morphological analyses (Jank et al. 1997), and the replicates were grouped together in the same cluster. The sexual plants were grouped with apomictic tetraploids in clusters II (S16), C3 (S18, S17, S12, S21 and S8) and C4 (S11, S13, S14, S19, S20, S15, S9, S7 and S10). These sexual plants were selected from crosses between tetraploid-induced sexual plants and apomictic accessions. The mode of sexual reproduction had been previously identified for each accession using embryonic sac analysis with methyl salicylate clearing and Nomarski differential interference contrast microscopy (Young et al. 1979). Accessions classified as sexual plants exhibited exclusively sexual embryonic sacs (consisting of part of the stigma, an egg, polar nuclei and antipodal cells) (Savidan 1982; Nakagawa 1990).

Table 1 Characteristics of the 66 microsatellite loci in 25 *Panicum maximum* Jacq. accessions: locus name, GenBank accession number, primer sequences (F: forward primer, R: reverse primer), repeat motif from sequenced clone, product length in base pairs, melting temperature (T_m), number of bands (N_A), number of banding pattern, polymorphic information content (PIC) and discriminating power (D)

Locus/ GenBank accession no.	Primer sequences (5′-3′)	Repeat motif	Product length (bp)	T_m (°C)	N_A	Banding pattern	PIC	D
2PMc8a[b]	F: GCGTTGCTGCATGCGATACCT	$(TG)_8$	266	60°	6	7	0.66	0.76
FJ039711	R:GGGGACAAATGCGTTGAAATTAAAAATA							
2PMc255	F: GCCGTGAAGACAAAGAGACC	$(CA)_5$	229	60°	4	6	0.51	0.66
FJ039712	R: GGAGAGCGAAGGGAGACATT							
1PMs35.1	F: TACACTACGCCATTTTG	$(TG)_6$	198	51.4°	4	5	0.43	0.59
FJ039713	R: CTAATAGCTTCCTCAGTAATAG							
1PMs43[b]	F: ATGAAGCGGGGCGTGTAGTATT	$(TC)_5$	200	60°	6	6	0.60	0.72
FJ039714	R: TGGTGGGCGGTAAAGAGTAAAG							
2PMc428[b]	F: CTCTCAGTCCCACAGCACAC	$(CA)_{11}$	206	60°	6	5	0.57	0.70
FJ039715	R:TATTTGGGGATTGGGAGTAGTT							
2PMc35	F: AGCACTGTGCACTAACCAAATG	$(GT)_7$	211	58.8°	4	4	0.49	0.63
FJ039716	R: CGTCTCCGTCCACCGATAG							
2PMc39[a]	F:AATGAGCTACCTTCTTG	$(TA)_5$	180	55°	1	–	–	–
HM235410	R:CATTTTAATTTTCCTGTC							
2PMc376[b]	F: CACCCATAACTGTAAAAGAA	$(GT)_4GC(GT)_5AT(GT)_6$	258	51.7°	12	12	0.87	0.98
FJ039717	R: CTGGAGTAGCAAGAGTGTT							
1PMs96	F: ACAAAGATGGGGCGTGAAGAC	$(CA)_5A(CA)_2$	252	60°	4	5	0.46	0.60
FJ039718	R: CTAGGTAGGCCGACAACAATGA							
2PMc28	F: AACCCGCGCATTTACTACA	$(AC)_6$	241	55°	4	5	0.44	0.52
FJ039719	R: ATGGTTGCAGAGAAGAGATGAC							
2PMc52[b]	F: AGAATGGCACCTGGAGATAG	$(TG)_7$	235	55°	6	7	0.67	0.82
FJ039720	R: GGATAGGCCGAAAGAACAT							
2PMc216[a]	F:GGTTCCATATCCCACAC	$(GT)_8$	196	50°	1	–	–	–
HM235411	R:ATCTCCACATTTAGTATCAA							
2PMc168[b]	F: CCTCGCATTTTTCTGGATTTA	$(TG)_5$	213	60°	12	10	0.79	0.86
FJ039721	R: CATAGACGCACGCACACTCAC							
2PMc40[b]	F: ATATTTCCTCGAGATTTGTGTT	$(TG)_5CA(TG)_4$	254	55°	6	6	0.62	0.70
FJ039722	R: AAGCTTTGGGGATTAGTAGAA							
2PMc7.12	F: TAAACTAGAGGACCCGTGTG	$(GT)_7$	269	60°	4	4	0.40	0.55
GU252057	R:TGTAGGCTCAAGAAAGGATT							
2PMc9.9[b]	F: GTGCGCGGCCAAGAAAAAGT	$(GT)_6$	202	58°	7	8	0.66	0.78
GU252058	R: CTCGAGGGGTGGATAGGACAGG							
2PMc9.17[b]	F: ATCAACGCTTTAATCCCTGTCC	$(CA)_5$	230	60°	7	6	0.64	0.75
GU252059	R: CATCGTCGTCCTCATCGTAGTC							
2PMc282[a]	F:CAGGAACATTATGAAAGTAT	$(CT)_{18}$	163	60°	1	–	–	–
HM235417	R:AAAAAGTTGCTCTAAAAAT							
2PMc14[b]	F: CAGCTCCGTCCCGTATCTCTAA	$(GT)_7$	190	60°	4	5	0.55	0.69

Table 1 (continued)

Locus/ GenBank accession no.	Primer sequences (5′-3′)	Repeat motif	Product length (bp)	T_m (°C)	N_A	Banding pattern	PIC	D
GU252060	R: CCGCAGGGAAGCACTATGGT							
2PMc19	F: ATGGTTAAAGATGTTGTGAGTG	$(AC)_9$	248	55°	3	3	0.22	0.37
GU252061	R: GAGGCTGAGTTCTTGGATAG							
2PMc27	F: AAAAGTAGAAGCATTATCCAT	$(CA)_9$	217	60°	4	3	0.33	0.49
GU252062	R: TTGCAAAGTGAAAACATTAG							
2PMc34	F: AGCACTGTGCACTAACCAAATG	$(TG)_7$	211	58.8°	4	4	0.47	0.61
GU252063	R: CGTCTCCGTCCACCGATAG							
2PMc40.1[b]	F: ATATTTCCTCGAGATTTGTGTT	$(GT)_4CA(TG)_5$	254	52°	6	5	0.62	0.72
GU252064	R: AAGGTTTGGGGATTAGTAGAA							
2PMc48.2	F: TTCTTTCTTTCCTGTC	$(CA)_{13}$	220	44°	4	3	0.30	0.44
GU252065	R: TTAGATGCTTGAGTTT							
2PMc51	F: TCAGCAAGAAACATCCTCA	$(GA)_{23}$	244	60°	4	5	0.44	0.61
GU252066	R: TTCCATAACCCAAATCCTG							
2PMc256[a]	F:TGTTCCATTATTGTGTT	$(GA)_9$	215	60°	1	–	–	–
HM235412	R:ACTTTGTTATTGTGAGAA							
2PMc55[b]	F: GGTAGCGCTCTGTCCTCTTG	$(AC)_{10}$	220	60°	6	7	0.67	0.80
GU252067	R: GACGGCCTTTCGCTTATTTC							
2PMc48[b]	F: CCTGTCAAAAACTATGC	$(CA)_{13}$	231	55°	8	9	0.77	0.89
GU252068	R: GGGGAGACCTAACCA							
2PMc60[b]	F: ACAGTTAGCTTAGTGGTTG	$(CA)_8$	237	50°	4	6	0.55	0.71
GU252069	R: TATGAAGGAGTAAAAAGACA							
2PMc62[b]	F: TGCTGTTTCATACTCTCATT	$(AG)_{10}$	228	51.2°	5	6	0.59	0.74
GU252070	R: ACTGTCTGTTGCTTCACTG							
2PMc73	F: TAGTTATGTCATTATTTAGCA	$(CA)_5$	233	40°	4	4	0.31	0.44
GU252071	R: AAGTCTTATTTAGTCATTTTG							
2PMc285[a]	F:ACTTGCATGTTTTTAT	$(GT)_{12}$	175	45°	1	–	–	–
HM235420	R:TTGTTCCATCGTCTAT							
2PMc84	F: GATCTATAAAAGGAGGGAGCAG	$(CA)_{10}$	153	50°	4	4	0.42	0.57
GU252072	R: GGGGGTTACAAGCAGGTC							
2PMc87[b]	F: CCGCTACCTTTTTCTGTCTCCA	$(CT)_5$	248	60°	9	10	0.75	0.86
GU252073	R: CTCGGCGCAAGTTGAAGTTTT							
2PMc90	F: AACGGTAGCTGGTGAAGA	$(CA)_8$	178	53.7°	4	5	0.46	0.55
GU252074	R: ATGTCGATGTGGCAAGTG							
2PMc103[b]	F: GCTACATTGGTCTTG	$(CT)_{16}$	282	60°	8	6	0.67	0.82
GU252075	R: GGCACTTCTTAGGATA							
2PMc143[b]	F: TTGATAGATACAGAGGAACTTG	$(CT)_{10}$	171	60°	10	11	0.79	0.92
GU252076	R: GGTGCCCATTAGATTGAA							
2PMc247[a]	F:GCTCCTTGCTTCACTTTTAT	$(CA)_{17}$	228	45°	1	–	–	–
HM235413	R:ATCCCGTCATTATTCCATT							

Table 1 (continued)

Locus/ GenBank accession no.	Primer sequences (5′-3′)	Repeat motif	Product length (bp)	T_m (°C)	N_A	Banding pattern	PIC	D
2PMc152[b]	F: GGCCCGTCATGTAAGAAC	$(CA)_6$	275	60°	5	6	0.59	0.73
GU252077	R: GAG GCTGAGACCGAGTGG							
2PMc158[b]	F: GGAATAGCCCCAGATA	$(TC)_6(CA)_7$	227	50°	8	7	0.70	0.88
GU252078	R: GGCTACCTTCATTGTTC							
2PMc172	F: AAGCTAGCAGTTTGAT	$(CT)_{20}$	194	45°	4	3	0.22	0.36
GU252101	R: CGTAGGTATTGGAGTG							
2PMc326.1A[a]	F:GAAAGCATGGGCACAC	$(CT)_{15}AT(CT)_3$	244	55°	1	–	–	–
HM235419	R:TCGTCTCAAGGCATCC							
2PMc173	F: AAGGGTATTAGGTTCTGCT	$(GA)_{12}$	258	55°	4	2	0.19	0.34
GU252080	R: CATGACTGACTGGATTAGG							
2PMc175	F: TTCACGGTCAGATTCA	$(CA)_6$	243	45°	4	4	0.44	0.62
GU252081	R: TGCAGCTCATTTGTTT							
2PMc178[b]	F: ACCTGCTTGTTTTGCTTGTTTG	$(CA)_6$	226	60°	12	13	0.85	0.94
GU252082	R: AGGGCTGGCTCTGATTGG							
2PMc194[b]	F: CCACACGTCGCACTGATAAAAA	$(CA)_9$	245	60°	6	5	0.64	0.78
GU252083	R: CCCGAAGGCAGTAGGAGTAGAT							
2PMc198	F: CAGAAAGAAGGAAGGAAAGGAA	$(CT)_7$	255	56.5°	3	5	0.29	0.37
GU252084	R: TCTAGCTGCATGCATAAACACT							
2PMc433[a]	F:GCATGTAGAGCACCAC	(CA)5	180	48.9°	1	–	–	–
HM235414	R:TGTTGAAGTCAGCCTTAT							
2PMc221	F: GCACGATGGGCTAAGG	$(GAA)_5$	198	53.3°	4	4	0.39	0.52
GU252085	R: GCGGCGGAACGATAA							
2PMc217[b]	F: TAACACGGGAGCTGAGGAACAT	$(GA)_{11}$	249	60°	5	6	0.68	0.77
GU252087	R: TGAACATAGCCAGGGAAAGGTC							
2PMc247[b]	F: GCTCCTTGCTTCACTTTTAT	$(CA)_{17}$	228	53.2°	16	14	0.89	0.99
GU252088	R: ATCCCGTCATTATTCCATT							
2PMc302	F: GGCCTTACCCAATCCA	$(GT)_5$	210	51°	4	5	0.44	0.57
GU252089	R: TTCCCTTAACCAAATCACTT							
2PMc326[b]	F: CAATTCGTCCCTCGTCTA	$(CT)_{15}$	254	51°	12	12	0.79	0.90
GU252090	R: GGTTCCATGCACAAATAA							
2PMc340[a]	F:GGAGAATAAGAGAATG	$(GT)_8$	291	53.3°	1	–	–	–
HM235418	R:TAAGTAGGAGGTATGG							
2PMc382[b]	F: ACCCATGATCAGGCAGACAAGA	$(CA)_{10}$	236	60°	6	7	0.76	0.82
GU252091	R: GCAGGCAGGAAAGCAGTAACAC							
2PMc389[b]	F: CAGGTAACATCACAAGTA	$(CA)_9$	177	50°	7	6	0.72	0.84
GU252092	R: CTATAGGTAAAGCCAGTA							
1PMc1.1[b]	F: GGGGGGCGAGAGGGGAGAC	$(GT)_2CT\ (GT)_5$	233	60°	6	6	0.59	0.72
GU252093	R: CGGGCGCAGTTTATGGTTGGT							

Table 1 (continued)

Locus/ GenBank accession no.	Primer sequences (5′-3′)	Repeat motif	Product length (bp)	T_m (°C)	N_A	Banding pattern	PIC	D
2PMc96[a]	F:TCCTCCCCTTCTTTGTA	$(CA)_7$	237	50°	1	–	–	–
HM235415	R:TCTCTTCAGGTCTCCAC							
1PMc13a	F: TCGTCCGCCTGAGCAT	$(GT)_9$	209	57.7°	4	3	0.39	0.47
GU252094	R: ACGGCGCACCACTGAC							
1PMc32	F: AACAGTTTGCAGATGGTAG	$(CA)_2CG(CA)_7$	256	60°	4	5	0.41	0.57
GU252095	R: TTGAGGATTAATGAGAAGTC							
1PMc35.2	F: AATTTTGTTATCCTGCTCCAC	$(GT)_5$	208	60°	3	4	0.33	0.47
GU252096	R: ACCCAAAGATAATTAGAACCTG							
1PMc39.b[b]	F: CCATCACTCGGGTCAG	$(CA)_8$	242	60°	6	5	0.62	0.77
GU252097	R: TTTCGGCAAAACATACA							
1PMc53	F: AAAGGGGGTTACAAGCAGGTC	$(GT)_2GA(GT)_5$	146	60°	4	4	0.45	0.59
GU252098	R: GATCTATAAAAGGAGGGAGCAGA							
2PMc239.1A[a]	F:TAACAAGAGAAATAAACAA	$(GA)_8$	216	50°	1	–	–	–
HM235416	R:GGAGTAAAAGGACCAC							
1PMc55[b]	F: TCCCTCTAGAACCAAGCACA	$(GT)_{13}$	160	60°	6	5	0.60	0.74
GU252099	R: ATCAAGACACATCAAGAACACAT							
1PMc72	F: GAAATCCGCCTCCACCAA	$(CA)_6$	195	60°	4	5	0.57	0.67
GU252100	R: TCCGGCGCCACTTCAT							

[a] Monomorphic loci

[b] Microsatellite loci selected to characterize the germplasm of *Panicum maximum* Jacq

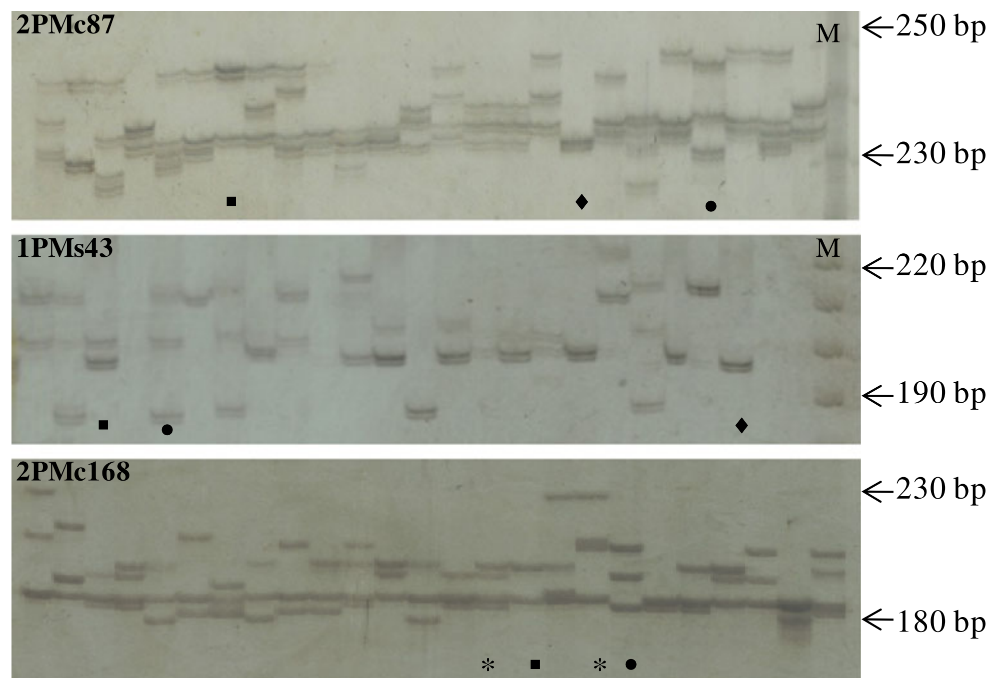

Fig. 1 Allelic variation among 25 *Panicum maximum* accessions detected using silver-stained 6% polyacrylamide gels. M, molecular size marker (10 bp DNA Ladder). ♦ one band; ▪ two bands; • three bands; * four bands

The tetraploid sexual plants were obtained through the use of colchicine to artificially double the chromosomes of diploid sexual plants collected in East Africa for use in breeding programs (Savidan 1982; Nakagawa and Hanna 1992). For practical purposes, crosses between accessions with different chromosomal numbers or with distinct meiotic behavior usually result in infertile progeny. In guineagrass, chromosome duplication using colchicine allowed for the crossing of sexual plants with tetraploid apomictic accessions to obtain fertile hybrids (Combes and Pernès 1970; Pernès et al. 1975).

The original diploid sexual plant K189 was collected in Korogwe, Tanzania, and its doubling is the basis of most tetraploid sexual plants studied. Other plants collected near the collection site of K189, accessions K187B, K190A, K190B, K191, K192 and K193, were all grouped in cluster IV. This genetic background of the sexual progenitor may be the reason why most sexual plants were also grouped in this cluster, despite some of their apomictic progenitors being grouped in other clusters. One of the apomictic progenitors, G3, was grouped in cluster II, and the others, 280 and K211, were grouped in cluster III. The other progenitors for these sexual plants are C1 and G23, which were grouped in cluster IV.

The sexual plants that were grouped in cluster III, were derived from male progenitors C1 and G23, accessions that were grouped in cluster IV, as mentioned above. The exception was S21, which is a tri-cross derived from male progenitors from cluster II and another from cluster III. It is noteworthy that all of the sexual plants in this cluster had admixtures from cluster IV.

The inheritance of apomixis in *P. maximum* is determined by a single dominant gene or a group of genes located close together, which results in sexual and apomictic progenies in a 1:1 ratio (Savidan 1983). Therefore, crosses between sexual and apomictic accessions result in 50% apomictic hybrids and 50% sexual hybrids. This process produces fixed superior accessions that can be multiplied and entered into the selection process, presenting new possibilities for breeding in this species. Thus, the selection of superior accessions may be conducted by selecting the best apomictic accessions from the germplasm bank or by crossing selected sexual and apomictic accessions that exhibit promising characteristics (Savidan 1975, 1982, 1983).

Cluster IV included the Brazilian cultivars Tanzania-1 (ORSTOM T58), Mombaça (ORSTOM K190A) and Atlas as well as the Cuban cultivar Likone (K5829) (ORSTOM G26). The Australian cultivar Green Panic (ORSTOM G15) and the Brazilian cultivars Aries and Massai (ORSTOM T21) were found in cluster III. Despite being unable to separate the accessions in the different clusters according to their morphology, the commercial cultivars included in cluster IV were all tall, wider-leafed plants, while the cultivars included in cluster III were short with narrower leaves. The Massai cultivar is a natural hybrid derived from crosses between *P. maximum* and *P. infestum* that was collected in Dar-Bagamoyo, Tanzania. As a hybrid between the two species cited, the cultivar's inflorescences are intermediate between a panicle typical of *P. maximum* and a raceme typical of *P. infestum*. The inflorescences exhibit primary branches and no secondary branches (Jank 1995;

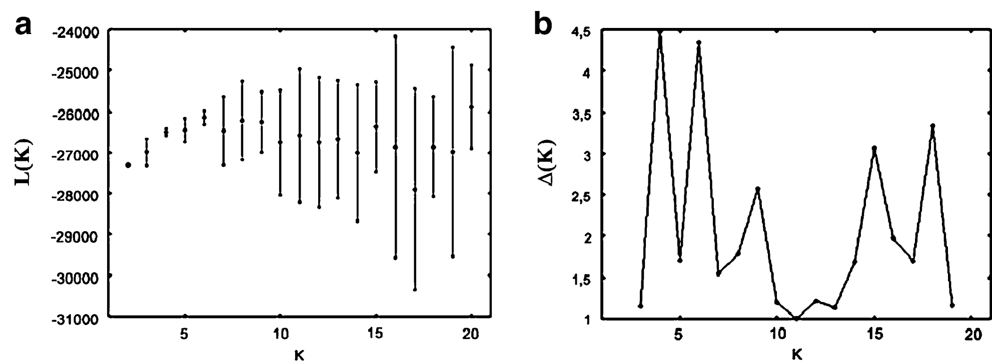

Fig. 2 Determination of K, the most probable number of clusters, using STRUCTURE software for 396 *Panicum maximum* accessions. **a**. Log probability of the data, L(K), as a function of K averaged over 20 replicates, and (**b**). Ad-hoc ΔK statistics as a function of K calculated over 20 replicates

Euclides et al. 2000). In Brazil, cultivars Tanzania-1, Mombaça and Massai are the most widely cultivated. They were selected primarily for the following four characteristics: leaf yield, leaf percentage, the ability to regrow seven days after harvesting and pure seed yield (Jank et al. 1993).

The STRUCTURE analysis indicated that clusters I, II, III and IV possessed mixed-ancestry origins (Kenya and Tanzania). The *P. maximum* accessions that are preceded by T were collected in Tanzania (1969), KK was collected in Kenya (1969), K was collected in Kenya or Tanzania (1967) and G or a number not preceded by a letter were provided by African research institutions as seeds or cuttings, respectively. Cluster I comprised 94% of the accessions from Kenya or Tanzania collected in 1967. Clusters II, III and IV were more diverse because they included accessions collected from the two expeditions in East Africa (1967 and 1969) and African research institutions. The level of genetic diversity within IV (0.69) was higher than that of I (0.39), II (0.41) and III (0.49). Cluster IV exhibited the greatest total number of bands per locus and the highest numbers of bands. This results is justified because cluster IV had the highest number of apomictic accessions (168 accessions) and sexual plants (10 plants). The results of the STRUCTURE analysis for variation within groups were confirmed using AMOVA (Table 3). The largest percentage of variation was determined within groups (65.38%), and a smaller level of variation was observed among groups (34.62%). This result is consistent with the apomictic mode of reproduction of *P. maximum*, in which the intraspecific variability in apomictic species is large.

Principal components analysis (PCA) was employed to visualize individual accessions in a multivariate space based on values of genetic similarity derived from the proportion of the accessions in the data. This type of graphical representation enables the evaluation of the population structure and geometric distances among all of the accessions in the study. The first two principal coordinates in the PCA accounted for 57.04% and 14.52% of the total variation, respectively. The PCA produced three distinct distributions of accessions (Fig. 4), which are colored in the figure according to the STRUCTURE results. A scattergram of these two axes indicated little origin correlation, particularly in the south of Kenya and north of Tanzania. This result indicates a close relationship between the accessions of *P. maximum*. One advantage of the PCA was that it allowed for the evaluation of the relationships between sets of two accessions, which helped to visualize possible introgression between clusters. Cluster I was the most structured cluster, exhibiting exclusively apomictic accessions, with the exception of the S16 sexual accession. Clusters II and III were more dispersed and shared gene pools.

The genetic relationships among the *P. maximum* accessions did not indicate an association with their geographical distribution. All of the groups exhibited wide geographical distributions in Kenya and Tanzania. Some accessions collected in 1967 and others from African research institutions were closely related genetically (Cluster II, green), but they exhibited a broad geographical distribution, which included regions in the south of Kenya and the north of Tanzania. In the first collecting expedition (1967), one diploid sexual plant was discovered in the region of Korogwe in Tanzania (Combes and Pernès, 1970; Savidan, 1982), which suggests that the center of origin could have been located in Tanzania. The wide distribution of the guineagrass accessions strongly supports intercrossing with diploid sexual plants in natural habitats, which may have preceded dispersal to other regions. Our data support the occurrence of natural interspecific crossings between *P maximum, P infestum* and *P. trichocladum* at the geographical origin of the high genetic variability, and these findings additionally suggest the existence of an agamic complex formed by these three species. This possibility is supported by various apomictic accessions of guineagrass being clustered according to their genetic similarity. The results of the STRUCTURE analysis could represent the sexual crossing events according to the conditions in natural habitats. According to Pernès (1975), natural populations can be divided into three main types: monomorphic, polymorphic discontinuous and polymorphic continuous. Prior studies have suggested that the polymorphic continuous population originated through the crossing of populations from the diploid sexual pool. Our results indicate that the

polymorphic discontinuous population corresponds with cluster I (red), which contains only apomictic accessions. The polymorphic continuous populations corresponded to the accessions included in clusters II (green), III (blue) and IV (yellow), which contain both apomictic accessions and sexual plants.

The accessions that were introduced in Brazil and other regions, specifically, Sri Lanka, Venezuela, Suriname, Australia, Guadalupe, S. Morocco and Vietnam, over the past 400 years were grouped by admixture into four groups. All domesticated accessions exhibited wide geographical distribution, with close genetic relationships within groups of the accessions collected in Kenya and Tanzania, suggesting that these accessions were collected in the region of origin (East Africa). Nevertheless, these accessions are apomictic, and a relative scarcity of polymorphisms in the domesticated accessions prevented the detection of more subtle genetic differences among closely related accessions.

Microsatellites have become one of the most widely employed molecular markers for genetic analysis. The enrichment of DNA fragments through the binding of microsatellite probes is a simple and efficient method for the isolation of microsatellites and has been successfully applied to a number of plant genomes. Molecular marker analysis demonstrated the pattern of the distribution of the genetic diversity and the population structure of the *P. maximum* germplasm. This study found that this germplasm collection was a rich source of genetic variability, providing the necessary raw material for breeding programs. In support of the long-term conservation of germplasms, microsatellite markers may be employed to demonstrate that accessions or cultivars are true to type for the following reasons; to help ensure their proper maintenance; to determine the degree of relatedness among accessions or groups of accessions; to clarify the genetic structure, partitioning or variation among accessions, populations and species; and to help determine the presence of a specific gene or gene complex in particular accessions (Koh et al. 1996). Therefore, the development of these microsatellite markers is an important first step toward the development of a genetic linkage map and a better understanding of the genomic organization of *P. maximum*.

Material and Methods

Plant Material and DNA Extraction

A total of 396 *P. maximum* accessions were analyzed in this study (Table 2). The accessions were obtained from the germplasm bank of the Brazilian Agricultural Research Corporation—Embrapa Beef Cattle at Campo Grande, Mato Grosso do Sul, Brazil. They were collected in Kenya and Tanzania by the former ORSTOM, France (Combes and Pernès 1970). The accessions were introduced into Brazil through a cooperative agreement with Embrapa (Savidan et al. 1989).

Fig. 3 Population structure analysis. Each accession is represented by a thin vertical segment, which can be partitioned into *K* colored segments that represent the individual estimated membership of the *K* cluster. The colors of the bar correspond to one of the four clusters identified using the STRUCTURE program (I = red, II = green, III = blue and IV = yellow) ▶

Genomic DNA was extracted from freeze-dried leaf samples through using cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). DNA samples were quantified through comparison with known quantities of λ-phage DNA on a 1% agarose gel.

Constructing the Library, Screening for Microsatellite Repeat Sequences, DNA Sequencing and Designing Primers

The microsatellite markers used in this study were developed from an enriched genomic library employing the protocol described by Billotte et al. (1999). The extracted DNA (*P. maximum* cv. Tanzania ORSTOM-T58) was digested using the *Rsa*I restriction enzyme (Invitrogen, CA, USA) and ligated to the adapter sequences 5′-CTCTTGCTTACGCGTGGACTA-3′ and 5′-TAGTCCACGCGTAAGCAAGAGCACA-3′. The library was enriched for dinucleotide sequences using $(CT)_8$- and $(GT)_8$-biotinylated microsatellite primers with labeled probes. The selected DNA fragments were recovered using Streptavidin MagneSphere Paramagnetic Particles (Promega, WI, USA) with a biotinylated probe. After the DNA fragments were recovered, magnetic selection was performed according to the manufacturer's specifications. Selected fragments were PCR-amplified using primer sequences complementary to the adapters and then ligated into the pGEM-T vector (Promega, WI, USA). *Escherichia coli* XL-1 Blue cells (Stratagene, CA, USA) were transformed with the recombinant plasmids and cultivated on Luria-Bertani (LB) agar plates containing 100 μg mL^{-1} ampicillin (Sigma, Germany), 50 μg mL^{-1} X-galactosidase and isopropyl β-D-1-thiogalactopyranoside (IPTG) (MBI Fermentas, MD, USA). Single white colonies were transferred to microplates for long-term storage at −80°C. A total of 576 recombinant colonies were selected and sequenced in both directions using T7 and SP6 promoter primers using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, CA, USA) with the BigDye terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). The simple sequence repeat identification tool (SSRIT) (http://www.gramene.org/db/markers/ssrtool) was employed to identify microsatellites present in non-redundant sequences (Temnykh et al. 2001). The sequences containing micro-

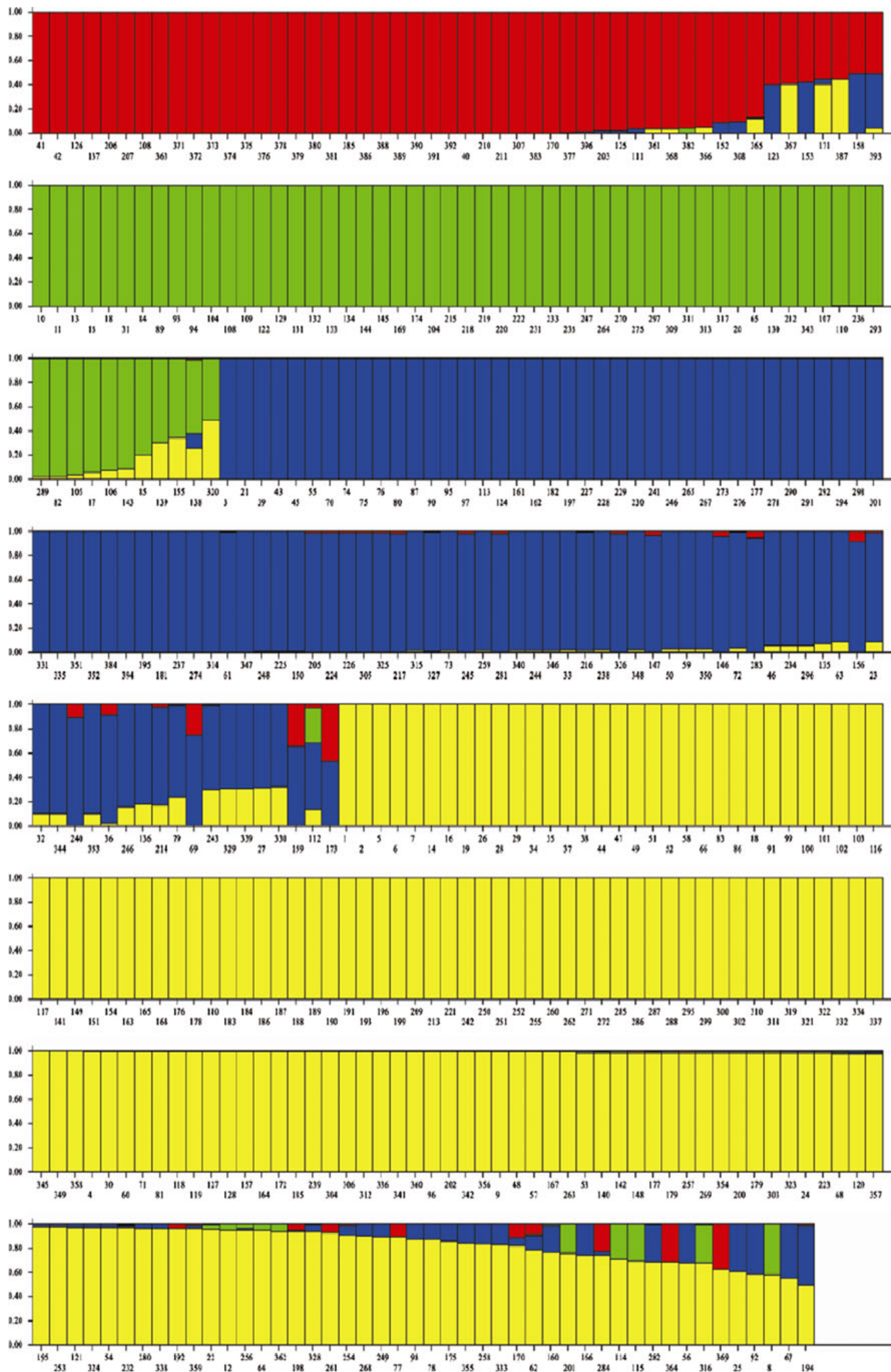

Table 2 Information for the 396 *Panicum maximum* Jacq. accessions

Sample code	Accession ID	Origin	RM	Sample code	Accession ID	Origin	RM
1	K190A[c]	Tanzania, Korogwe	APO	199	K214	Kenya, L. Lunga-Mombasa	APO
2	T58[c]	Tanzania, Korogwe	APO	200	K217	Kenya, L. Lunga-Mombasa	APO
3	3Colorado[a]	Unknown	APO	201	K218	Kenya, Mombasa	APO
4	Natsuyutaka	East Africa	APO	202	K219	Kenya, Mombasa	APO
5	C1	Africa	APO	203	K220	Kenya, Mombasa-Voi	APO
6	60	Congo, Brazzaville	APO	204	K223	Kenya, Mombasa-Voi	APO
7	77	Kenya	APO	205	K224	Kenya, Mombasa-Voi	APO
8	82	Cameroon, Nkol Bisson	APO	206	K225	Kenya, Mombasa-Voi	APO
9	87	Cameroon, Nlohelouem	APO	207	K227	Kenya, Mombasa-Voi	APO
10	93	Introduced from Brazil	APO	208	K228	Kenya, Mombasa-Voi	APO
11	174	Ivory Coast, Daloa	APO	209	K237	Kenya, Voi	APO
12	304	Zaire, Gandajika	APO	210	K241	Kenya, Voi-Machakos	APO
13	B2	Introduced in Brazil	APO	211	K241D	Unknown	APO
14	B6	Introduced in Brazil	APO	212	K244	Kenya, Voi-Machakos	APO
15	B7	Introduced in Brazil	APO	213	K249	Kenya, Nairobi	APO
16	B10	Introduced in Brazil	APO	214	KK7	Kenya, Meru-Embu	APO
17	B12	Introduced in Brazil	APO	215	KK8	Kenya, Meru-Embu	APO
18	B19	Introduced in Brazil	APO	216	KK10	Kenya, Meru-Embu	APO
19	B22	Introduced in Brazil	APO	217	KK12	Kenya, Meru-Embu	APO
20	B26	Introduced in Brazil	APO	218	KK14	Kenya, Meru-Embu	APO
21	78	Introduced in Angola	APO	219	KK14E	Unknown	APO
22	S7	IRD cross	SEX	220	KK15	Kenya, Meru-Embu	APO
23	S8	IRD cross	SEX	221	KK16	Kenya, Meru-Embu	APO
24	S9	IRD cross	SEX	222	KK17	Kenya, Meru-Embu	APO
25	S10	IRD cross	SEX	223	KK18	Kenya, Meru-Embu	APO
26	S11	IRD cross	SEX	224	KK21	Kenya, Meru-Embu	APO
27	S12	IRD cross	SEX	225	KK23	Kenya, Meru-Embu	APO
28	S13	IRD cross	SEX	226	KK23E	Unknown	APO
29	S14	IRD cross	SEX	227	KK25	Kenya, Meru-Embu	APO
30	S15	IRD cross	SEX	228	KK26	Kenya, Meru-Embu	APO
31	S16	IRD cross	SEX	229	KK33	Kenya, Meru-Embu	APO
32	S17	IRD cross	SEX	230	KK34	Kenya, Meru-Embu	APO
33	S18	IRD cross	SEX	231	GI	Gabon, Irat	APO
34	S19	IRD cross	SEX	232	G2	Uganda, Serere	APO
35	S20	IRD cross	SEX	233	G3	Nigeria, Zaria	APO
36	S21	IRD cross	SEX	234	G4	Botswana, Tuli	APO
37	S22	IRD cross	APO	235	G5	Botswana, Nata	APO
38	S23	IRD cross	APO	236	G6	Zimbabwe, Melsetter	APO
39	T4	Tanzania, Dar-Bagamoyo	APO	237	G7	Zimbabwe	APO
40	T7	Tanzania, Dar-Bagamoyo	APO	238	G8	Zimbabwe, Marandellas	APO
41	T11	Tanzania, Dar-Bagamoyo	APO	239	G9	Zimbabwe, Victoria Falls	APO
42	T11D	Unknown	APO	240	G10	Zimbabwe, Marandellas	APO
43	T18	Tanzania, Dar-Bagamoyo	APO	241	G11	Malawi	APO
44	T19	Tanzania, Dar-Bagamoyo	APO	242	G11E	Unknown	APO
45	T21[c]	Tanzania, Dar-Bagamoyo	APO	243	G12	Introduced in Angola	APO
46	T23	Tanzania, Dar-Bagamoyo	APO	244	G13	Zimbabwe, Marandellas	APO
47	T24	Tanzania, Dar-Bagamoyo	APO	245	G14	Zimbabwe, Marandellas	APO
48	T45	Tanzania, Korogwe	APO	246	G15	Zimbabwe, Marandellas	APO
49	T46	Tanzania, Korogwe	APO	247	G16	Introduced in Brazil	APO
50	T60	Tanzania, Korogwe-Kilosa	APO	248	G17	Zimbabwe	APO
51	T62	Tanzania, Korogwe-Mikume	APO	249	G18	Kenya	APO
52	T65	Tanzania, Korogwe-Kilosa	APO	250	G19	Kenya, Machakos	APO
53	T68	Tanzania, Korogwe-Kilosa	APO	251	G20	Kenya	APO

Table 2 (continued)

Sample code	Accession ID	Origin	RM	Sample code	Accession ID	Origin	RM
54	T72	Tanzania, Korogwe-Kilosa	APO	252	G20E	Unknown	APO
55	T77	Tanzania, Kilosa-Mikume	APO	253	G21	Kenya, Mac Kinnon Road	APO
56	T81	Tanzania, Kilosa-Morogoro	APO	254	G21E	Unknown	APO
57	T84	Tanzania, Kilosa-Morogoro	APO	255	G22	Tanzania, Tengeru	APO
58	T86	Tanzania, Mts. Uruguru	APO	256	G23	Malawi, Nchizi	APO
59	T91	Tanzania, Morogoro	APO	257	G26	Kenya	APO
60	T92	Tanzania, Morogoro	APO	258	G27	Introduced in Angola	APO
61	T95	Tanzania, Morogoro	APO	259	G27E	Unknown	APO
62	T96	Tanzania, Morogoro-Dar	APO	260	G28	South Africa	APO
63	T97	Tanzania, Morogoro-Dar	APO	261	G28A	Unknown	APO
64	T98	Tanzania, Morogoro-Dar	APO	262	G30	Angola	APO
65	T103	Tanzania, Morogoro-Dar	APO	263	G31	Angola	APO
66	T104	Tanzania, Morogoro-Dar	APO	264	G5E	Botswana, Nata	APO
67	T108	Tanzania, Morogoro-Dar	APO	265	G32	Angola	APO
68	T109	Tanzania, Morogoro-Dar	APO	266	G33	Angola	APO
69	T110	Tanzania, Morogoro-Dar	APO	267	G34	South Africa	APO
70	T111	Tanzania, Morogoro-Dar	APO	268	G35	Gabon, Irat	APO
71	T113	Tanzania, Dar	APO	269	G36	Zaire, Kinshasa-Nioki	APO
72	T114	Tanzania, Dar	APO	270	G38	Introduced in Sri-Lanka	APO
73	T116	Tanzania, Dar	APO	271	G39	Introduced in Sri-Lanka	APO
74	T117	Tanzania, Dar	APO	272	G40	Madagascar	APO
75	T200	Tanzania	APO	273	G41	Botswana, Mahalapye	APO
76	T201	Tanzania	APO	274	G42	Botswana, Ngamiland	APO
77	15	Ivory Coast, Binao	APO	275	G43	Botswana, Mahalapye	APO
78	57	Ivory Coast, Daome, Niaouli	APO	276	G45	South Africa	APO
79	58	Central African Republic, Boukoko	APO	277	G46	South Africa	APO
80	64D	Unknown	APO	278	G47	South Africa	APO
81	65	Congo	APO	279	G48	South Africa	APO
82	69	Zimbabwe, Melsetter	APO	280	G50	South Africa	APO
83	73	Introduced in Costa Rica	APO	281	G51	South Africa	APO
84	74	Angola	APO	282	G52	Introduced in S. Vietnam	APO
85	80	Cameroon, Yaounde	APO	283	G54	Introduced in Marocco	APO
86	81	Cameroon, Nkwonvone	APO	284	G54E	Unknown	APO
87	88	Introduced in Brazil	APO	285	G56	Tanzania, Kilosa	APO
88	88A	Unknown	APO	286	G56E	Unknown	APO
89	88[b]	Introduced in Brazil	APO	287	G58	Nigeria, Nsukka	APO
90	89	Introduced in Brazil	APO	288	G58D	Unknown	APO
91	89B	Unknown	APO	289	G59	Nigeria, Nsukka	APO
92	90	Introduced in Brazil	APO	290	G59[b]	Nigeria, Nsukka	APO
93	92	Introduced in Brazil	APO	291	G61	Introduced in Australia	APO
94	92D	Unknown	APO	292	G62	Zimbabwe	APO
95	96	South Africa	APO	293	G64	Kenya, Rumuruti	APO
96	96E	Unknown	APO	294	G68	Introduced in Australia	APO
97	97	South Africa	APO	295	G68A	Unknown	APO
98	97E	Unknown	APO	296	G69	South Africa	APO
99	102	Introduced in Guadalupe	APO	297	G70	Zimbabwe	APO
100	103	Introduced in Guadalupe	APO	298	G71	Introduced in Australia	APO
101	105	Introduced in Guadalupe	APO	299	G71E	Unknown	APO
102	105D[b]	Unknown	APO	300	G73	Introduced in Australia	APO
103	106	Introduced in Guadalupe	APO	301	G74	Introduced in Australia, Brisbane	APO
104	112	South Africa	APO	302	G75	Introduced in Brazil	APO
105	112D	Unknown	APO	303	G76	Introduced in Australia, Brisbane	APO
106	114	South Africa	APO	304	G76D	Unknown	APO

Table 2 (continued)

Sample code	Accession ID	Origin	RM	Sample code	Accession ID	Origin	RM
107	114D	Unknown	APO	305	G77	Introduced in Australia, Brisbane	APO
108	116	Malawi, Lilongwe	APO	306	G77E	Unknown	APO
109	117	Unknown	APO	307	G78	Introduced in Australia, Brisbane	APO
110	118	Unknown	APO	308	G78E	Unknown	APO
111	139	Tanzania, Tengeru-Moshi	APO	309	G85	Introduced in Australia, Canberra	APO
112	172	Nigeria, Lagos	APO	310	G86	Introduced in Australia, Canberra	APO
113	280	Kenya, Nanyuki	APO	311	G88	Central African Republic, Bouar	APO
114	309	Zaire, Gandajika	APO	312	G89	Central African Republic, Bangui	APO
115	353	Togo, Ganave	APO	313	G89E	Unknown	APO
116	354	Togo, Ganave	APO	314	G90	Introduced in Jamaica, Mona	APO
117	S22R[b]	IRD cross	APO	315	G90D	Unknown	APO
118	K4	Kenya, Nairobi	APO	316	G91	Introduced in Jamaica, Mona	APO
119	K8	Kenya, Nairobi	APO	317	G93	Senegal, Dakar	APO
120	K2	Kenya, Nairobi	APO	318	G94	Ivory Coast, Tiantiebe	APO
121	K4	Kenya, Nairobi	APO	319	G95	Introduced in Venezuela	APO
122	K5	Kenya, Nairobi	APO	320	G96	Introduced in Venezuela	APO
123	K15	Kenya, Rumuruti	APO	321	G97	Introduced in Surinam	APO
124	K23	Kenya, Rumuruti-Maralal	APO	322	G98	Introduced in Brazil	APO
125	K28	Kenya, Maralal	APO	323	G99	Burundi, Bujumbura	APO
126	K31	Kenya, Maralal-Rumuruti	APO	324	G100	Introduced in Haiti	APO
127	K32	Kenya, Maralal-Rumuruti	APO	325	G109	Tanzania	APO
128	K35	Kenya, Rumuruti-Nanyuki	APO	326	87	Cameroon, Nlohelouem	APO
129	K36	Kenya, Rumuruti-Nanyuki	APO	327	Aries[c]	Introduced in Brazil	APO
130	K38	Kenya, Rumuruti-Nanyuki	APO	328	Atlas[c]	Introduced in Brazil	APO
131	K39	Kenya, Nanyuki-Nyeri	APO	329	Japa	Unknown	APO
132	K39D	Unknown	APO	330	3697=74[b]	Angola	APO
133	K42	Kenya, Nanyuki-Nyeri	APO	331	3808=89[b]	Introduced in Brazil	APO
134	K42D	Unknown	APO	332	3816=90[b]	Introduced in Brazil	APO
135	K47	Kenya, Meru	APO	333	3859=94[b]	Introduced in Brazil	APO
136	K47D	Kenya, Meru	APO	334	3891=101[b]	Introduced in Guadalupe	APO
137	K48	Kenya, Meru	APO	335	3905=102[b]	Introduced in Guadalupe	APO
138	K59	Kenya, Nkubu	APO	336	3930=105[b]	Introduced in Guadalupe	APO
139	K59E	Unknown	APO	337	3981=114[b]	Introduced in Guadalupe	APO
140	K62	Kenya, Nkubu	APO	338	4120=354[b]	Togo, Ganave	APO
141	K63	Kenya, Nkubu	APO	339	4316=G19[b]	Kenya, Machakos	APO
142	K64	Kenya, Nkubu	APO	340	4316B=G19[b]	Gabon, Irat	APO
143	K65	Kenya, Nkubu	APO	341	4375=G26[b]	Kenya	APO
144	K68	Kenya, Meru-Embu	APO	342	4391=G28[b]	South Africa	APO
145	K71	Kenya, Meru-Embu	APO	343	4405A=G30[b]	Nigeria, Zaria	APO
146	K83	Kenya, Meru-Embu	APO	344	4405B=G30[b]	Nigeria, Zaria	APO
147	K88	Kenya, Meru-Embu	APO	345	4499=G40[b]	Madagascar	APO
148	K89	Kenya, Meru-Embu	APO	346	4464=G36[b]	Zaire, Kinshasa-Nioki	APO
149	K93	Kenya, Embu	APO	347	4502A=G41[b]	Botswana, Tuli	APO
150	K98	Kenya, Forthall	APO	348	4502B=G41[b]	Botswana, Mahalapye	APO
151	K98D	Unknown	APO	349	4618=G58[b]	Nigeria, Nsukka	APO
152	K102	Kenya, Forthall-Nairobi	APO	350	4634=G61[b]	Introduced in Australia	APO
153	K102R[b]	Kenya, Forthall-Nairobi	APO	351	4651=G64[b]	Kenya, Rumuruti	APO
154	K103	Kenya, Forthall-Nairobi	APO	352	4654	Unknown	APO
155	K104	Kenya, Forthall-Embu	APO	353	4669=G68[b]	Introduced in Australia	APO
156	K105	Kenya, Forthall-Nairobi	APO	354	4707=G73[b]	Introduced in Australia	APO
157	K105A	Unknown	APO	355	4723=G75[b]	Introduced in Brazil	APO
158	K106	Kenya, Forthall-Nairobi	APO	356	4731=G76[b]	Introduced in Australia	APO
159	K112	Kenya, Nairobi-Arusha	APO	357	4804=G90[b]	Introduced in Jamaica, Mona	APO

Table 2 (continued)

Sample code	Accession ID	Origin	RM	Sample code	Accession ID	Origin	RM
160	K115	Kenya, Nairobi-Arusha	APO	358	4812=G91[b]	Introduced in Jamaica, Mona	APO
161	K116	Kenya, Nairobi-Arusha	APO	359	4847=G96[b]	Introduced in Venezuela	APO
162	K117	Kenya, Nairobi-Arusha	APO	360	4863=G98[b]	Introduced in Brazil	APO
163	K124	Tanzania, Arusha	APO	361	4928=T93[b]	Tanzania, Morogoro	APO
164	K124D	Unknown	APO	362	K209	Kenya, L. Lunga-Mombasa	APO
165	K124R[b]	Tanzania, Arusha	APO	363	K16	Kenya, Th. Falls-Rumuruti	APO
166	K125	Tanzania, Arusha	APO	364	5274=K39[b]	Kenya, Nanyuki-Nyeri	APO
167	K126	Tanzania, Arusha	APO	365	5282=K42[b]	Kenya, Nanyuki-Nyeri	APO
168	K130	Tanzania, Tengeru	APO	366	5321=K47[b]	Kenya, Meru	APO
169	K138	Tanzania, Tengeru-Moshi	APO	367	5932=K201[b]	Tanzania, Tanga	APO
170	K139	Tanzania, Tengeru-Moshi	APO	368	5461=K130[b]	Tanzania, Tengeru	APO
171	K142	Tanzania, Tengeru-Moshi	APO	369	5568=K145[b]	Tanzania, Tengeru-Moshi	APO
172	K145	Tanzania, Tengeru-Moshi	APO	370	K159	Tanzania, Mts Pare Nord	APO
173	K146	Tanzania, Tengeru-Moshi	APO	371	K160	Tanzania, Mts Pare Nord	APO
174	K146E	Unknown	APO	372	K162	Tanzania, Mts Pare Nord	APO
175	K156	Tanzania, Moshi-Mombo	APO	373	K172	Tanzania, Mts Pare Nord	APO
176	K163	Tanzania, Mts Pare Nord	APO	374	5819A=K175[b]	Tanzania, Mts Pare Nord	APO
177	K164	Tanzania, Mts Pare Nord	APO	375	5819B=K175[b]	Tanzania, Mts Pare Nord	APO
178	K165	Tanzania, Mts Pare Nord	APO	376	K176	Tanzania, Mts Pare Nord	APO
179	K171	Tanzania, Mts Pare Nord	APO	377	K177	Tanzania, Baron`s Falls	APO
180	K173	Tanzania, Mts Pare Nord	APO	378	K17	Kenya, Th. Falls-Rumuruti	APO
181	K174	Tanzania, Mts Pare Nord	APO	379	K25	Kenya, Rumuruti-Maralal	APO
182	K174R[b]	Tanzania, Mts Pare Nord	APO	380	K27	Kenya, Maralal	APO
183	K175	Tanzania, Mts Pare Nord	APO	381	6149=K28[b]	Kenya, Maralal	APO
184	K175D	Unknown	APO	382	K72	Kenya, Meru-Embu	APO
185	K187B	Tanzania, Mombo-Korogwe	APO	383	K74	Kenya, Meru-Embu	APO
186	K190B	Tanzania, Korogwe-Tanga	APO	384	K79	Kenya, Meru-Embu	APO
187	K191	Tanzania, Korogwe-Tanga	APO	385	K93	Kenya, Embu	APO
188	K192	Tanzania, Korogwe-Tanga	APO	386	K95	Kenya, Embu-Forthall	APO
189	K193	Tanzania, Korogwe-Tanga	APO	387	K99	Kenya, Forthall	APO
190	K194	Tanzania, Korogwe-Tanga	APO	388	K230	Kenya, Mombasa-Voi	APO
191	K197	Tanzania, Korogwe-Tanga	APO	390	K238	Kenya, Voi-Machakos	APO
192	K201	Tanzania, Tanga	APO	391	K240	Kenya, Voi-Machakos	APO
193	K204	Tanzania, Mpirani-L. Lunga	APO	392	K243	Kenya, Voi-Machakos	APO
194	K205	Tanzania, Mpirani-L. Lunga	APO	393	T106	Tanzania, Morogoro-Dar	APO
195	K205E	Unknown	APO	394	T115	Tanzania, Dar	APO
196	K206	Tanzania, Mpirani-L. Lunga	APO	395	7676=KK18[b]	Kenya, Meru-Embu	APO
197	K211	Kenya, L. Lunga-Mombasa	APO	396	KK20	Kenya, Meru-Embu	APO
198	K212	Kenya, L. Lunga-Mombasa	APO				

Accession ID ORSTOM: Institut Français de Recherche Scientifique pour le Développement en Coopération; RM: reproductive mode; APO: apomictic (2n=4x=32); SEX: sexual (2n=4x=32); [a] *Panicum coloratum*; [b] Replicates; [c] Brazilian cultivar

Table 3 Molecular analysis of variance (AMOVA) based on STRUCTURE results

Source of variation	Degrees of freedom	Sum of squares	Variance component	Percentage of components	P-value
Among Clusters	3	177.494	3.949Va	34.62	0.0000
Within Clusters	21	537.143	9.354Vb	65.38	–

satellites were edited and clustered using LaserGene v. 5.03 software (DNAStar Inc.). MICROSAT software was utilized to remove the restriction sites in the sequences. PrimerSelect software (DNAStar Inc.) was used to design complementary primer pairs under the following conditions: an expected amplified product size between 150 and 300 bp, GC content between 40% and 60%, melting temperature (*Tm*) between 45° and 60°C, primer length between 18 and 22 bp and with no predicted hairpin or dimer formation.

Fig. 4 Association among 396 *Panicum maximum* accessions revealed using principal components analysis (PCA) based on Jaccard's similarity coefficient calculated from 30 microsatellite loci. Samples are color-coded based on the STRUCTURE results

Polymerase Chain Reaction (PCR) Amplifications and Genotyping

PCR was performed in a total reaction volume of 25 μL containing 0.5 ng of DNA template, 0.8 μM of each of the forward and reverse primers, 100 μM dNTPs (MBI Fermentas, MD, USA), 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl and 0.5 U of Taq DNA polymerase (Invitrogen, CA, USA). All PCR amplifications were performed in a PTC-200 thermal cycler (MJ Research, Waltham, MA,USA) under the following conditions: 94°C for 1 min, 30 cycles of 94°C for 1 min, a specific temperature for 1 min and 72°C for 1 min and a final extension at 72°C for 5 min. Amplification products were genotyped using electrophoresis on 6% denaturing polyacrylamide gels in 1X TBE buffer. A 10 bp ladder (Invitrogen, CA, USA) was used to determine the size of the standards. The DNA fragments were visualized using silver staining according to the method of Creste et al. (2001).

Data Analysis

The PIC values were calculated for estimates of marker informativeness according to the equation of Mateescu et al. (2005);

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

where p^i is the frequency of the ith band, p^j is the frequency of the jth band and the summation extends over n bands. To compare marker efficiencies in varietal identifications, the D was estimated for each primer based on the following formula;

$$D_k = 1 - \sum_{j=1}^{l} p_j \frac{Np_j - 1}{N - 1},$$

where N is the number of individuals and p^j is the frequency of the jth pattern (Tessier et al. 1999).

Molecular data were scored for each accession based on the presence or absence of the band. These data were employed to generate a binary matrix for all pairwise combinations and to calculate Jaccard's similarity coefficient (Jaccard 1908) using NTSYS-pc version 2.1 software (Rohlf 2000). This information was utilized in a principle components analysis (PCA) using DARwin software v. 5.0.157 (Perrier and Jacquemound-Collet 2006). The reliability of the generated cluster was also tested using bootstrap analysis with the BooD program with 1,000 iterations (Coelho 2002). STRUCTURE software version 2.2 (Pritchard et al. 2000) was used to generate a Bayesian inference of the population structure. This method identifies clusters of genetically similar individuals from multilocus genotypes without prior knowledge of their population affinities. The model assumes K genetic clusters, with each cluster having a characteristic set of band frequencies at each locus; a no-admixture model with correlated band frequencies was assumed. As a preliminary step, the analysis was performed for a number of genetic clusters (K) ranging from 2 to 20. Consistent results across runs were obtained using a burn-in period of 100,000 repeats, followed by 200,000 Markov Chain Monte Carlo (MCMC) repeats. The most probable number for K was calculated based on the method of Evanno et al. (2005) using an ad hoc statistic, ΔK, which represents the rate of change in the log probability of the data between successive K values rather than the log probability of the data. An analysis of molecular variance (AMOVA) was conducted using ARLEQUIN 1.1 software (Excoffier et al. 2005). The level of significance for variance component estimates was determined using non-parametric permutation procedures using 1,000 permutations.

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