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Allelic database and accession divergence of a Brazilian mango collection based on microsatellite markers

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ABSTRACT. Allelic patterns and genetic distances were examined in a collection of 103 foreign and Brazilian mango (*Mangifera indica*) accessions in order to develop a reference database to support cultivar protection and breeding programs. An UPGMA dendrogram was generated using Jaccard coefficients from a distance matrix based on 50 alleles of 12 microsatellite loci. The base pair number was estimated by the method of inverse mobility. The cophenetic correlation was 0.8. The accessions had a coefficient of similarity of from 30 to 100%, which reflects high genetic variability. Three groups were observed in the UPGMA dendrogram; the first group was formed predominantly by foreign accessions, the second group was formed by Brazilian accessions, and the Dashehari accession was isolated from the others. The 50 microsatellite alleles did not separate all 103 accessions, indicating that there are duplicates in this mango collection. These 12 microsatellites need to be validated in order to establish a reliable set to identify mango cultivars.

Key words: *Mangifera indica*; Variability; Dendrogram; Genotyping

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INTRODUCTION

Total world mango production is of the order of 26 million tons, and it is one of the most important fruits in the world, along with bananas, oranges, grapes, and apples. Countries such as India, China, Thailand, Mexico, Pakistan, and Indonesia are responsible for more than 75% of world mango production, with India constituting approximately 40% of this production (Viruel et al., 2005).

It is probable that mango cultivation started in India, where more than 1000 varieties have already been identified, resulting from selections within cross-pollinated populations. Traders spread cultivation from the center of origin and domestication to other tropical and subtropical regions. The Portuguese introduced the crop to West and East Africa and Brazil. From Brazil, it was probably taken to the Caribbean Islands, from where the Spanish introduced it to Mexico and the Philippines. In the 19th century, mango was introduced to Florida, USA, first from the Caribbean and later from India (Viruel et al., 2005).

DNA markers have been applied to mango principally to identify cultivars and the relationship between them (Krishna and Singh, 2007), probably with a single potential application in the selection of polyembrionic types assisted by random amplification of polymorphic DNA (RAPD) markers (López-Valenzuela et al., 1997). In addition to their utility for improvement and mapping, microsatellites have been the best markers for fingerprinting studies due to their high polymorphism, co-dominance, and reproducibility. Viruel et al. (2005), Duval et al. (2005), Honsho et al. (2005), and Schnell et al. (2006) developed and published around 65 microsatellites for mango.

Singh and Bhat (2009) analyzed 241 mango accessions from 15 different regions of India, identifying the existence of high variability among the accessions studied, as well as substantial gene flow among the accessions from different regions. Gálvez-López et al. (2009) found 2 specific groups of mango native to different Mexican states among 112 accessions from 16 different states analyzed with microsatellite markers and AFLP. Viruel et al. (2005), Duval et al. (2005), Olano et al. (2005), and Schnell et al. (2006) have reported other studies of mango diversity with microsatellites. In Brazil, studies have not yet been carried out with microsatellites in mango. Santos et al. (2008) reported studies with 157 AFLP markers in 104 mango accessions and the existence of high genetic variability among the accessions.

In spite of the availability of microsatellite markers, the minimum number of this type of marker has not yet been proposed for mango to help protect cultivars and settle commercial disputes. This et al. (2004) proposed 6 relevant microsatellite markers to reveal clonal polymorphism in grape cultivars. Leão et al. (2009) used this set of microsatellites to characterize a Brazilian grape collection.

The objectives of the present study were to establish allelic patterns and estimate genetic distances based on microsatellite markers for 103 mango accessions to generate a reference database to support cultivar protection and settle possible commercial disputes as well as to guide breeding programs and genetic resources of the species.

MATERIAL AND METHODS

Plant material and DNA extraction

Young healthy leaves were collected from 103 mango accessions of the Active Germ-

plasm Bank (BAG) of Embrapa Tropical Semi-Arid (Table 1) and maintained in the Mandacaru Experimental Station, Juazeiro, BA. The CTAB method (Doyle and Doyle, 1990) was adopted with minor modifications: a) 6000 and 10,000 rpm for the first and second centrifugation rounds, respectively, and b) addition of 2% 2-mercaptoethanol, and water bath incubation at 60°C for 30 min for all samples. After adding 30 µL Tris-EDTA, the solution was treated with 10% RNase to remove co-extracted RNAs. Quantification and DNA integrity analyses were carried out on 0.8% agarose gel, stained with ethidium bromide. The DNA samples were diluted to 10 ng/µL and stored at -20°C.

Table 1. Origin of 103 mango accessions of the active germplasm bank of Embrapa Semi-Arid evaluated with 50 alleles of 11 microsatellite loci.

Accessions	Origin	Accessions	Origin
Alfa	Brazil	Juazeiro VI	Brazil
Alphonso	India	Lita	Brazil
Amarelinha	Brazil	Keitt	EUA
Ametista	Brazil	Kensington	Australia
Amrapali	India	Kent	EUA
Aplle DCG 406	Thailand	Langra	India
Ataulfo	México	M-13269	EUA
Beta	Brazil	Mallindi	India
Black Java	Australia	Maçã	Brazil
Bonita	Brazil	Mallika	India
Bourbon	Brazil	Manga d'água	Brazil
Brazil	Brazil	Manguito	Brazil
Calmon	Brazil	Manila	Filipinas
Carabao	Filipinas	Manzanillo	México
Carlotão	Brazil	Mastruz	Brazil
Caxangá	Brazil	Maya	México
Chené	South Africa	Momi-K	EUA
China	Brazil	Mon Amon	Thailand
Comprida Roxa	Brazil	Morais	Brazil
Coração Magoado	Brazil	Nego não chupa	Brazil
CPR	Brazil	Néldica	South Africa
Dama de Ouro	Brazil	Olour	India
Da Porta	Brazil	Ômega	Brazil
Dashehari	India	Palmer	EUA
Duncan	EUA	Papo de Peru I	Brazil
Edward	EUA	Parwin	EUA
Eldon	EUA	Pêssego DPV	Brazil
Espada	Brazil	Pingo deOuroDPV	Brazil
Espada 35	Brazil	Pingo de Ouro DPV	Brazil
Espada Itaparica	Brazil	Primor Amoreira	Brazil
Espada Manteiga	Brazil	Princesa	Brazil
Espada Vermelha	Brazil	Roxa	Brazil
Espada Ouro	Brazil	Recife	Brazil
Extrema	Brazil	R2E2	Australia
Favo de Mel	Brazil	Rosa	Brazil
Florigon	EUA	Rosary	Brazil
Foice	Brazil	Ruby	EUA
Haden	EUA	Salitre	Brazil
Haden 2H	Brazil	Sta Alexandrina	Brazil
Haden Rosa	Brazil	Scuper Many	EUA
Heidi	South Africa	Simmonds	EUA
Hilda	Brazil	Smith	EUA
Imperial I	Brazil	Surpresa	Brazil
Ipuçaba	Brazil	Tommy Atkins	EUA
Irwin	EUA	Torbet	EUA
Itamaracá	Brazil	Tyler Premier	EUA
Itiúba	Brazil	Ubá	Brazil
Joa	South Africa	Umbu	Brazil
Juazeiro II	Brazil	Winter	EUA
Juazeiro III	Brazil	Van Dyke	EUA
Juazeiro IV	Brazil	Zill	EUA
		65	EUA

DNA PCRs and resolution on polyacrylamide gels

Twenty-eight microsatellites published by Duval et al. (2005) and 15 others published by Schnell et al. (2005) were evaluated in 5 BAG accessions to select those with better polymorphic resolution on polyacrylamide gels. The PCR amplification were carried out in a final volume of 20 µL containing 20 ng DNA, 0.2 µM of each primer, 200 µM of each dNTP, 1.5 mM $MgCl_2$, 1X PCR buffer, and 0.75 U Taq DNA polymerase enzyme. The thermocycler was programmed for the amplifications as follows: an initial cycle of 94°C for 4 min, followed by 32 cycles at 94°C for 45 s, 56°C for 60 s, 72°C for 60 s, and a final cycle at 7°C for 4 min.

Half of the volume of the denaturing buffer of 98% formamide (10 mM EDTA, pH 8.0, 1 mg/mL xylene cyanol, and 1 mg/mL bromophenol blue) was added to the PCR, followed by complete denaturation at 94°C for 5 min in the thermocycler. Amplified PCR products were separated on 6% polyacrylamide gel for approximately 3 h with constant 40-W power. The gels were stained with silver nitrate according to the procedure described by Creste et al. (2001). The 103 accessions were genotyped on 2 polyacrylamide gel plates, 1 plate with 56 accessions and the other with the remaining 47. On the first plate, at least 1 accession representing 1 allelic combination was identified, and was used as a reference allele on the second plate for each microsatellite.

Microsatellite data analysis

The microsatellite loci that presented easily interpreted polymorphisms were selected to genotype the 103 mango accessions. To construct the allelic pattern of each accession, the size estimate in base pairs (bp) for each allele was obtained by the inverse mobility method based on the regression of products of known size of the 50-bp molecular marker (Fermentas Inc., USA), applied in an extra well in the polyacrylamide gel.

The microsatellites were observed for the presence (1) versus absence (0) of alleles to construct a similarity matrix of the Jaccard index. A dendrogram for the distances of the cultivars was created by the UPGMA grouping method in the NTSYS program (Rohlf, 1989). The adjustment of the dendrogram phenogram was evaluated by cophenetic correlation. The frequency of the principal alleles, number of genotypes, gene diversity, heterozygosity, and polymorphic information content (PIC) per microsatellite were estimated with the PowerMarker program (Liu and Muse, 2005).

RESULTS

Easily identified polymorphic amplifications were obtained only in the mMiCIR001, mMiCIR003, mMiCIR010, mMiCIR027, mMiCIR028, mMiCIR030, and mMiCIR036 microsatellite loci developed by Duval et al. (2005), and the MiSHRS-1, MiSHRS-4, MiSHRS-29, and MiSHRS-32 microsatellite loci developed by Schnell et al. (2005). Fifty alleles were detected in the 11 microsatellites analyzed, in which the number of alleles per locus varied from 2 to 8, with an average of 4 alleles per microsatellite in the 103 mango accessions genotyped. The largest number of genotypes and the highest gene diversity were observed with the mMiCIR030 microsatellite (Table 2).

Table 2. Genetic parameters estimated for 11 microsatellites in 103 mango accessions of the active germplasm bank of Embrapa Semi-Arid.

Microsatellite	Highest allelic frequency	Genotype number	No. of accessions	No. of alleles	Genetic diversity	Observed heterozygosity	PIC
MiSHRS-1	0.3155	16	103	7	0.7789	0.5146	0.7446
MiSHRS-4	0.5243	9	103	4	0.6348	0.6311	0.5816
MiSHRS-29	0.5243	12	103	5	0.6666	0.7476	0.6320
MiSHRS-32	0.5291	8	103	4	0.5773	0.2816	0.4956
mMiCIR030	0.3058	20	103	8	0.7864	0.8252	0.7543
mMiCIR001	0.9417	4	103	3	0.1102	0.0971	0.1051
mMiCIR003	0.6262	3	103	2	0.4681	0.4757	0.3586
mMiCIR010	0.7233	9	103	4	0.4495	0.4563	0.4204
mMiCIR027	0.8911	3	101	2	0.1941	0.1188	0.1753
mMiCIR028	0.7524	6	103	3	0.3990	0.3495	0.3591
mMiCIR036	0.8689	6	103	4	0.2391	0.2330	0.2302
Average	0.6200	9	103	4	0.4948	0.4500	0.4516

PIC = polymorphic information content.

The averaged observed heterozygosity and PIC of the 11 microsatellite loci was 0.45 and 0.45, respectively, and the MiSHRS-1 and mMiCIR030 loci presented the highest values (Table 2). These two parameters represent the existence of variability because each diploid individual can have up to two alleles per locus (Weir, 1996), in which variability is greater with a higher frequency of heterozygotes and PIC, and the microsatellites that fit this situation are considered the most suitable for diversity studies.

The cophenetic correlation was 0.8, which indicated that the produced dendrogram (Figure 1) presents some inconsistencies in the grouping of the 103 mango accessions with the 50 alleles of the 11 microsatellite loci. This low cophenetic correlation may have been due to the presence of ties in the matrix of similarity, as commented on by Santos et al. (2010) in onion.

The accessions presented a similarity coefficient between 30 and 100%, which reflects the high genetic variability of the collection of mango germplasm studied (Figure 1). Three groups were observed in the dendrogram (Figure 1): group I ranged from Scuper Many to Beta, group II ranged from Rosari to Espada Manteiga, and group III was formed exclusively by Dashehari. Group I was formed predominantly by foreign accessions or the results of crossings between these accessions, such as the Beta and Alfa accession, and by 6 Brazilian accessions as well: Maça, Surpresa, Juazeiro II, Umbu, Ipuçaba, and Pingo de Ouro DPV. Group II was formed by accessions that owe their origin to long adaptation following the introduction of mango to Brazil. Other studies should be carried out to elucidate the isolated position of the Dashehari accession that originated from India, as well as the 6 accessions of notably Brazilian origin in the group of accessions introduced recently from other countries.

The 50 alleles of the 11 SSR loci were not sufficient to separate all 103 mango accessions, suggesting the existence of duplicates in the collection for the following accessions: Haden Rosa and Haden, Kensington and Black Java, Nego não Chupa and Bourbon, Espada and Espada35, Papo de Peru I and Salitre and CPR, and finally, Santa Alexandrina and Itamaracá (Table 3 and Figure 1). Santos et al. (2010) differentiated 44 onion accessions with 13 microsatellite loci; Priolli et al. (2002) differentiated 184 soybean cultivars applying only 12 microsatellite SSR markers. Leão et al. (2009) divided a collection of 221 grape accessions into 4 groups using 7 microsatellite loci as a reference set.

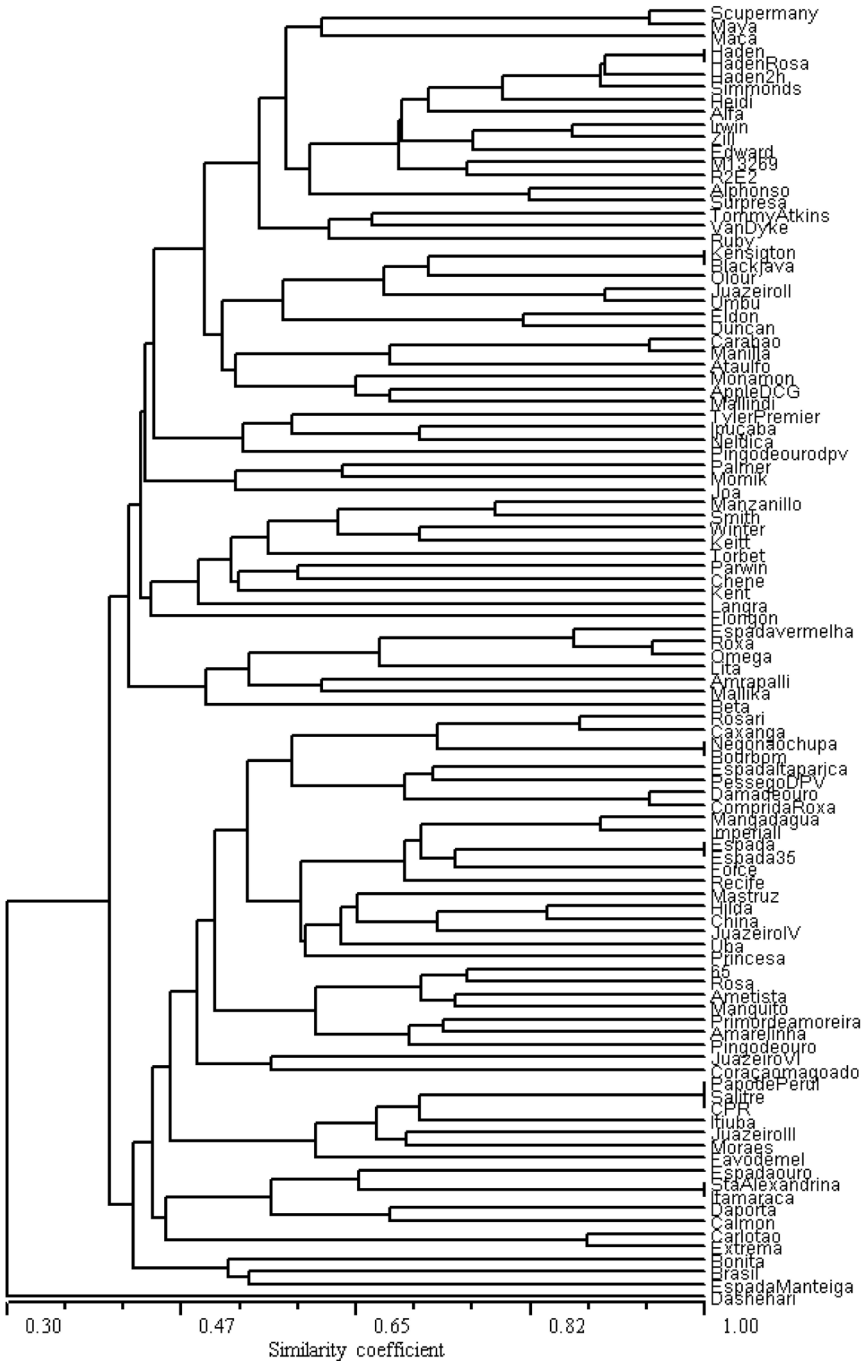

Figure 1. Dendrogram UPGMA of the Jacquard coefficient of similarity of 103 mango accessions genotyped for 50 alleles of 12 microsatellite loci. Cophenetic correlation = 0.8.

Table 3. Allelic pattern and highlighted reference accessions, in base pairs, estimated for 103 mango accessions, genotyped with 11 microsatellite markers.

Accession	MiSHRS locus				mMiCIR locus						
	1	4	29	32	030	001	003	010	027	028	036
65	492/539	197/197	**330/356**	554/554	295/301	412/412	773/758	749/783	218/243	**694/694**	751/751
Alfa	492/548	197/212	356/362	581/581	308/308	412/412	773/758	776/783	243/243	659/694	693/751
Alphonso	548/548	197/197	343/356	595/595	256/308	412/412	773/758	783/783	243/243	659/659	751/751
Amarelinha	492/492	197/197	343/356	554/554	295/301	412/412	773/758	749/783	218/243	659/659	751/751
Ametista	492/492	197/197	330/375	554/554	295/301	412/412	773/758	783/783	218/243	659/694	751/751
Amrapalli	492/548	204/212	356/362	554/595	301/318	412/412	773/758	783/783	243/243	659/694	693/751
Apple DCG	539/548	197/197	356/356	581/581	318/318	412/428	773/773	755/783	243/243	659/659	751/751
Ataulfo	384/539	197/204	356/356	581/581	256/328	412/412	773/773	755/783	243/243	694/694	751/751
Beta	384/539	192/204	356/356	595/595	295/328	412/412	773/758	783/783	243/243	659/694	693/751
Black Java	548/548	197/197	330/356	581/581	295/328	412/412	773/773	783/783	243/243	659/659	**728/751**
Bonita	492/492	192/197	356/375	554/554	301/328	412/412	773/758	783/783	218/218	659/694	751/751
Bourbom	510/539	197/204	343/356	554/554	295/328	412/412	773/773	783/783	243/243	659/659	751/751
Brasil	492/510	197/204	343/356	554/581	301/308	412/412	773/773	783/783	218/218	659/694	714/751
Calmon	510/510	204/204	330/330	554/581	308/311	412/412	**773/773**	749/783	243/243	659/659	751/751
Carabao	539/548	197/197	343/356	**595/595**	256/328	412/412	773/773	755/783	243/243	659/694	751/751
Carlotão	510/548	197/197	375/375	554/581	295/308	412/428	773/773	783/783	243/243	659/659	751/751
Caxangá	510/510	197/204	343/356	554/554	295/295	412/412	773/773	783/783	243/243	659/659	751/751
Chené	510/548	204/212	356/356	554/581	301/328	**412/423**	758/758	776/783	243/243	659/694	751/751
China	510/539	197/204	343/356	581/581	295/301	412/412	773/773	755/783	243/243	659/694	751/751
C. Roxa	539/539	204/204	356/375	554/554	295/301	412/412	773/758	749/783	243/243	659/659	751/751
C.Magoado	548/548	197/204	330/356	**554/640**	295/301	412/412	773/773	**755/783**	218/243	659/694	**714/751**
CPR	384/548	197/204	356/375	581/581	295/328	412/412	773/758	749/783	243/243	659/659	751/751
Da porta	510/539	204/204	330/375	554/554	301/308	412/412	773/758	749/783	243/243	659/659	714/751
Dama Ouro	492/539	204/204	356/375	554/554	295/301	412/412	773/758	749/783	243/243	659/659	751/751
Dashehari	**529/539**	212/212	343/356	595/595	308/308	412/412	758/758	783/783	243/243	670/670	728/751
Duncan	539/548	197/204	330/356	581/581	295/318	412/412	**758/758**	749/783	243/243	659/670	714/751
Edward	548/548	197/197	356/362	581/581	308/308	412/412	773/758	755/783	243/243	659/659	751/751
Eldon	539/548	197/204	330/356	581/581	295/318	412/412	758/758	755/783	243/243	659/670	**728/751**
Espada	384/510	197/204	330/356	554/581	295/301	412/412	773/773	**749/783**	243/243	659/659	751/751
Esp. 35	384/510	197/204	330/356	554/581	295/301	412/412	773/773	749/783	243/243	659/659	751/751
EspItaparica	510/539	197/204	330/375	554/554	295/301	412/412	**773/758**	749/783	243/243	659/659	751/751
Esp.Manteiga	492/510	204/212	356/356	554/554	301/308	412/412	773/758	783/783	243/243	670/694	751/751
Espada Ouro	510/548	197/204	330/375	554/554	301/328	412/412	773/758	749/783	218/243	659/694	751/751
Esp.Vermelha	492/548	197/204	356/356	581/581	308/308	412/412	758/758	783/783	243/243	659/694	693/751
Extrema	510/548	197/197	362/375	554/581	295/295	412/428	773/773	783/783	-	659/659	714/751
Favo de mel	492/510	204/204	356/356	581/581	295/301	412/412	773/758	783/783	243/243	659/659	751/751
Florigon	539/539	192/212	343/356	581/581	295/295	412/412	773/773	776/783	243/243	670/670	751/751
Foice	384/384	197/204	343/356	554/581	295/301	412/412	773/773	749/749	243/243	659/659	751/751
Haden	548/548	**197/212**	**356/362**	581/581	295/308	412/412	773/758	776/783	243/243	659/670	751/751
Haden 2h	539/548	197/212	356/362	581/581	295/308	412/412	773/758	776/783	243/243	659/659	751/751
Haden Rosa	548/548	197/212	356/362	581/581	295/308	412/412	773/758	776/783	243/243	659/670	751/751
Heidi	548/548	197/212	356/356	581/581	308/311	412/412	773/758	776/783	243/243	659/659	751/751
Hilda	510/510	197/204	343/356	581/581	295/295	412/412	773/773	755/783	243/243	659/659	751/751
Imperial I	384/510	197/197	343/356	554/581	295/328	412/412	773/773	749/783	243/243	659/659	751/751
Ipuçaba	548/548	192/197	343/356	554/581	295/328	412/412	773/773	749/749	218/218	659/659	751/751
Irwin	548/548	197/197	343/356	581/581	308/311	412/412	773/758	783/783	243/243	659/659	751/751
Itamaracá	510/548	192/197	330/375	554/554	308/328	**412/428**	773/758	783/783	243/243	659/659	751/751
Itiúba	384/384	192/204	356/375	581/581	301/328	412/412	773/758	783/783	243/243	659/659	751/751
Joa	492/492	204/212	356/362	581/640	308/328	412/412	773/758	749/776	218/243	659/694	714/751
Juazeiro II	**539/548**	197/204	330/356	554/554	295/328	412/412	773/773	783/783	218/243	659/659	728/751
Juazeiro III	384/548	197/204	356/375	581/581	301/308	412/412	773/758	783/783	243/243	659/659	751/751
Juazeiro IV	510/510	197/204	356/375	554/554	295/301	412/412	773/773	755/783	243/243	659/659	751/751
Juazeiro VI	510/510	204/204	330/375	554/554	295/328	412/412	773/773	755/783	243/243	659/694	751/751
Keitt	510/548	192/212	356/362	554/581	295/328	412/412	773/758	776/783	243/243	659/659	751/751
Kensigton	548/548	197/197	330/356	581/581	295/328	412/412	773/773	783/783	243/243	659/659	728/751
Kent	510/510	192/197	356/356	554/581	308/328	412/412	758/758	776/776	243/243	659/694	751/751
Langra	384/384	192/212	356/375	554/554	256/328	412/412	773/758	783/783	243/243	659/659	751/751
Lita	539/539	197/204	356/356	581/581	308/328	412/412	758/758	783/783	243/243	659/694	**693/751**
M13269	548/548	192/197	343/356	581/581	292/308	412/412	773/758	**776/783**	243/243	659/659	751/751

Continued on next page

Table 3. Continued.

Accession	MiSHRS locus				mMiCIR locus						
	1	4	29	32	030	001	003	010	027	028	036
Maçã	510/510	197/197	356/362	581/581	308/328	412/412	773/773	783/783	-	659/659	714/751
Mallika	492/539	204/212	356/362	581/595	301/328	412/412	773/758	783/783	243/243	659/670	693/728
Mallindi	539/539	197/197	356/356	581/581	318/328	412/428	773/758	783/783	243/243	659/694	751/751
Manga d'água	**384/510**	197/197	343/356	554/581	295/328	412/428	773/773	**749/783**	243/243	659/659	751/751
Manguito	492/548	197/197	330/330	554/554	295/301	412/412	773/758	783/783	218/243	**659/694**	751/751
Manilla	539/548	197/197	356/356	595/595	256/328	412/412	773/773	755/783	243/243	659/694	751/751
Manzanillo	492/492	**192/212**	356/362	581/581	295/328	412/412	758/758	776/783	**243/243**	**670/694**	751/751
Mastruz	510/510	**197/204**	330/356	554/581	301/301	412/412	773/773	783/783	**218/243**	659/659	751/751
Maya	384/548	192/197	356/356	581/640	308/328	412/412	773/773	783/783	243/243	659/659	751/751
Momi k	492/492	197/197	343/362	581/581	308/328	412/412	773/758	783/783	243/243	659/659	728/728
Mon Amon	539/600	197/212	356/356	581/581	318/318	412/428	758/758	783/783	243/243	659/659	693/751
Moraes	384/384	204/204	356/356	581/581	295/301	412/412	773/758	749/783	243/243	659/659	751/751
Néldica	548/548	197/212	343/356	554/581	295/328	412/428	773/773	783/783	218/218	659/694	751/751
Nego Chupa	510/539	197/204	343/356	554/554	295/328	412/412	773/773	783/783	243/243	659/659	751/751
Olour	539/548	197/197	356/356	581/581	**295/295**	412/412	773/773	783/783	243/243	659/694	728/751
Ômega	492/548	197/204	356/362	581/595	308/308	412/412	758/758	783/783	243/243	659/659	693/751
Palmer	492/492	197/204	330/362	554/581	308/328	412/412	773/758	776/783	243/243	659/694	751/751
Papo Peru I	384/548	197/204	356/375	581/581	295/328	412/412	773/758	749/783	243/243	659/659	751/751
Parwin	492/492	192/212	330/356	554/581	301/328	412/412	758/758	783/783	243/243	659/659	751/751
Pessego DPV	510/539	197/204	343/343	554/554	295/301	412/412	773/758	749/783	218/243	659/659	751/751
Pingo de ouro	539/539	197/197	343/356	554/554	295/308	412/412	773/758	783/783	243/243	659/659	751/751
PingoO.DPV	510/548	192/204	343/356	554/581	328/328	428/428	773/773	783/783	243/243	659/659	751/751
Pri. amoreira	492/539	197/197	330/356	554/554	295/301	412/412	773/758	783/783	243/243	659/659	751/751
Princesa	510/510	197/197	343/362	554/554	295/301	412/412	773/773	749/783	243/243	659/659	751/751
R2E2	510/548	192/197	356/356	581/581	295/308	412/412	773/758	776/783	243/243	659/694	751/751
Recife	510/510	197/204	343/356	554/581	295/308	412/412	773/773	749/783	243/243	659/694	751/751
Rosa	**492/510**	197/197	330/330	554/554	295/301	412/412	773/773	749/783	218/243	659/694	751/751
Rosari	510/510	197/204	330/356	554/554	295/295	412/412	773/773	783/783	243/243	659/659	751/751
Roxa	492/548	197/204	356/362	581/595	308/308	412/412	758/758	783/783	243/243	659/694	693/751
Ruby	548/548	197/212	343/356	581/640	295/311	412/412	773/758	783/783	243/243	670/694	751/751
Salitre	384/548	197/204	356/375	581/581	295/328	412/412	773/758	749/783	243/243	659/659	751/751
Scuper many	384/548	**192/197**	343/356	**581/640**	308/308	412/412	773/773	783/783	243/243	659/659	751/751
Simmonds	548/548	197/212	356/362	581/581	295/308	412/412	773/758	783/783	243/243	659/659	751/751
Smith	492/492	192/212	356/356	581/581	295/328	412/412	773/758	783/783	243/243	670/670	751/751
SAlexandrina	510/548	192/197	330/375	554/554	308/328	412/428	773/758	783/783	243/243	659/659	751/751
Surpresa	548/548	197/197	356/356	595/595	301/256	412/412	773/758	783/783	243/243	659/659	751/751
T.Atkins	548/548	197/197	330/356	581/581	308/311	412/412	773/758	783/783	243/243	670/694	693/751
Torbet	384/384	212/212	330/356	581/581	295/328	412/412	758/758	755/776	243/243	659/659	751/751
Tyler Premier	510/510	192/197	343/356	581/581	318/328	412/412	773/773	755/755	**218/218**	659/659	751/751
Ubá	510/510	197/197	**356/375**	**554/581**	295/301	412/412	773/773	783/783	243/243	659/659	751/751
Umbu	539/548	197/204	343/356	554/554	295/328	412/412	773/773	783/783	218/243	659/659	728/751
Van Dyke	539/548	197/197	356/362	581/581	308/311	412/412	758/758	776/783	243/243	670/694	751/751
Winter	384/384	192/212	**356/356**	581/581	295/328	**412/412**	773/758	783/783	243/243	659/659	751/751
Zill	548/548	197/197	356/356	581/581	308/308	412/412	773/758	783/783	243/243	659/659	751/751

DISCUSSION

In some situations, the SSR molecular markers can present additional information when morphological descriptors are insufficient to distinguish cultivars of a species with a narrow genetic base (Priolli et al., 2002). Jakse et al. (2005) suggested that additional markers should be used to reveal polymorphisms in situations in which it was not possible to distinguish accessions of a given species with a set of predetermined DNA markers. In addition to those tested in this study, additional microsatellites, or even adjustments to the annealing temperature of the PCR protocol of some of the microsatellites tested here, can be obtained in Viruel et al. (2005).

The Carabao and Manilla accessions were considered genetically identical by Santos et al. (2008) because they had 97% similarity when analyzed with the AFLP marker, and also by López-Valenzuela et al. (1997) when analyzed with RAPD. Although they presented high similarity in the present study (96%), these accessions should not be considered identical because the MiSHRS-29 microsatellite differentiated them (Table 3).

The size of the alleles varied from 192 bp in MiSHRS-4 to 758 bp in mMiCIR010 (Table 3), presenting some inconsistency with the size reported by Duval et al. (2005) and Schnell et al. (2005). These discrepancies could be attributed to the method used to estimate fragment size in the present study or the presence of different alleles, since microsatellite markers are multiallelic. In spite of the expected approximate 500-bp size on polyacrylamide gel, Santos and Simon (2004) reported fragment size ranging from 54 to 700 nucleotides when analyzing AFLP amplicons with the same protocol adopted in the present study. The hypothesis of non-specific bands was discarded since a consistent size was observed for all microsatellites across the 103 mango accessions analyzed. This question could only be solved with amplicon gel excision, reamplification, and sequencing of microsatellite alleles differing in size from those reported by Duval et al. (2005) and Schnell et al. (2005), which was not the primary goal of the present study.

The identification of accessions with reference alleles for each microsatellite and their inclusion on the second polyacrylamide gel plate made comparison and correct allelic identification of the remaining accessions possible (Table 3). In the characterization of the grape germplasm bank, Leão et al. (2009) compared the allelic pattern of previous studies and 3 databases of existing microsatellites for the species.

Working with most of the accession analyzed in the present study of the same mango germplasm collection, Santos et al. (2008) reported the formation of 5 groups based on 157 polymorphic AFLP markers: 1) Amrapali, Malika, Embrapa-CPAC hybrids, and some American varieties, 2) predominantly American varieties, with some inclusions of South African and Brazilian hybrids, 3) Brazilian accessions, with some inclusion of Australian, Indian, and American accessions, 4) some accessions of Espada, Rosa, and others of different origins, and 5) *Mangifera foetida* and *M. similis*. This AFLP grouping was different from that reported in the present study. Microsatellite markers, which permit the genotyping of individuals, are considered superior to dominant markers such as the AFLP reported by Santos et al. (2008) in mango, and a bigger contribution to the breeding and management of genetic resources for this important crop would be expected with the present study.

The similarity coefficient between 30 and 100% reflected the high genetic variability of the collection of mango germplasm studied. Santos et al. (2008) reported similarity between 35 and 97% for the same mango collection with 157 polymorphic AFLP bands. Schnell et al. (1995) analyzed 25 cultivar accessions, mostly from Florida (USA), and found similarity greater than 64%, while Viruel et al. (2005) observed similarity greater than 33% in 28 mango accessions from diverse origins, which also demonstrates the high genetic variability of mango.

Gálvez-Lopez et al. (2009) reported high genetic similarity in a study carried out with AFLP and SSR on mango in Mexico, differing from this and previous studies, and which was probably due to constant selection and clonal propagation, which may have reduced genetic diversity. Singh and Bhat (2009) analyzed 18 SSRs in 241 mango genotypes and detected 103 alleles with an average of 5.78 alleles per locus, varying from 3 to 9 alleles per locus. The Jaccard similarity values among the different genotypes varied from 0.024 to 0.808 with an average of 0.258, indicating the presence of high genetic diversity in the germplasm analyzed.

Santos et al. (2010) differentiated 44 onion accessions with 13 microsatellite loci and Priolli et al. (2002) differentiated 184 soybean cultivars with the application of only 12 microsatellite SSR markers. According to Hamilton (2009), human forensic DNA profiles use 10-13 unlinked loci to estimate expected genotype frequencies. The microsatellite loci identified in the present study should be sufficient to allow identification of mango cultivars, a crop propagated clonally for commercial production. This study is the first attempt to use microsatellite markers in cultivar protection for the mango agribusiness in Brazil, and can be used to resolve commercial disputes concerning the certification of mango cultivars used in commercial orchards as well.

The present allelic data (Table 3) include several major mango cultivars that are grown worldwide, such as the Floridian cultivars, and will be very useful as multiconfirmed reference for identification and protection purposes. However, such a database must be confirmed by other laboratories in order to establish a reliable set of microsatellites to identify mango cultivars, as This et al. (2004) have done with grape cultivars. Suspicious alleles, such as those with larger fragment size, should be discarded and the research should be focused on loci within an expected size range, such as that reported by Duval et al. (2005) and Schnell et al. (2005).

As pointed out by Santos et al. (2010) for onion, laboratories that employ systems that use fluorescent initiators and automatic band recording can estimate different numbers of base pairs for the alleles identified in the present study with the expectation that the allelic pattern is maintained if the same protocol for the PCRs and amplifications is adopted. Studies with DNA codominant markers in mango can be important as references for germplasm management studies, controlled hybridizations, and even for the choice of other pollinators of cultivars that present floral abortion. Mango is an important export crop in many developing countries, and collaborative studies should be carried out in order to define a standard microsatellite set for identification and protection purposes that benefit the entire mango community, especially plant breeders and farmers worldwide.

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