Agronomical and molecular characterization of banana germplasm

Lorenna Alves Mattos[1], Edson Perito Amorim[2], Vanusia Batista de Oliveira Amorim[2], Kelly de Oliveira Cohen[3], Carlos Alberto da Silva Ledo[2] e Sebastião de Oliveira e Silva[2]

[1]Universidade Estadual de Feira de Santana, Avenida Transnordestina, Cep 44036–900 Feira de Santana, BA, Brazil. E-mail: lorennamattos@yahoo.com.br. [2]Embrapa Mandioca e Fruticultura Tropical, Rua Embrapa, s/nº, Cep 44380-000 Cruz das Almas, BA, Brazil. E-mail: edson@cnpmf.embrapa.br, vanusia@cnpmf.embrapa.br, ledo@cnpmf.embrapa.br, ssilva@cnpmf.embrapa.br [3]Embrapa Cerrados, BR 020, Km 18, Cep 73310-970 Planaltina, DF, Brazil. E-mail: kelly.cohen@cpac.embrapa.br

Abstract – The objective of the present work was to characterize banana accessions from the Germplasm Bank at Embrapa Mandioca e Fruticultura Tropical (Brazil), using agronomical, physical and physicochemical characteristics of fruit and simple sequence repeats (SSR) markers. Twenty-six accessions were analyzed, in which high genetic variability was found, especially for the agronomical characters number of fruit and weight of bunch. Accessions with high contents of carotenoids (diploid 'Jaran'), polyphenols (triploid 'Caipira' and tetraploid 'Teparod') and vitamin C (diploid 'Tuugia' and an unknown triploid AAA) in the fruit were identified. Thirteen microsatellite primers revealed an average of 7.23 alleles, which showed high variability. A dendrogram was prepared using the Gower algorithm for the distance matrices obtained from the agronomical, physical and physicolchemical analysis of fruit and SSR markers. Adopting the average genetic divergence as the cut-off point, three clusters were found: G1, formed by the diploids 'Jaran', 028003-01 and M-48; G2, by the diploids 'Malbut' and 'Ido 110'; and G3, by 21 tri-and tetraploid accessions, including one diploid, 'Tuugia'. The triploids with the B genome 'Thap Maeo', 'Walha', 'Pacha Nadan' and 'Champa Madras' were grouped in G2. Results from this work can be used for breeding hybrids with good agronomical traits and fruit quality.

Index terms: *Musa*, functional properties, fruit quality, hybrids, yield.

Caracterização agronômica e molecular de germoplasma de bananeira

Resumo – O objetivo deste trabalho foi caracterizar acessos de bananeira do Banco de Germoplasma da Embrapa Mandioca e Fruticultura Tropical, por meio de características agronômicas, físicas e físico-químicas dos frutos e por marcadores "Simple sequence repeats" (SSR). Foram analisados 26 acessos, nos quais observou-se ampla variabilidade genética, em especial para número de frutos e peso de cacho. Foram identificados acessos com altos teores de carotenoides (diploide 'Jaran'), polifenóis (tetraploide 'Teparod') e vitamina C (diploide 'Tuugia' e um triploide AAA desconhecido). Os 13 iniciadores microssatélites testados apresentaram uma média de 7,23 alelos, que apresentaram alta variabilidade. Com uso do algoritmo de Gower, foi elaborado um dendrograma com as matrizes de distância obtidas por meio das análises agronômicas, físicas e físico-químicas dos frutos e análises moleculares. Com a divergência genética média usada como ponto de corte, foram identificados três agrupamentos: G1, formado pelos diploides 'Jaran', 028003-01 e M-48; G2, pelos diploides 'Malbut' e 'Ido 110'; e G3, pelos 21 acessos tri- e tetraploides, incluindo-se um diploide 'Tuugia'. Os triploides com genoma B 'Thap Maeo', 'Walha', 'Pacha Nadan' e 'Champa Madras' agruparam-se no G2. Os resultados obtidos podem ser utilizados no melhoramento genético, para o desenvolvimento de híbridos com boas características agronômicas e qualidade de frutos.

Termos para indexação: *Musa*, propriedades funcionais, qualidade de frutos, híbridos, produtividade.

Introduction

Banana production is usually based on tri- and tetraploid cultivars; however, diploid genotypes are important sources of alleles for resistance or tolerance to biotic and abiotic factors. Banana breeding programs have generated promising tetraploid hybrids from crosses between triploids and improved wild diploids with important agronomic traits, including reduced height, disease and pest resistance and fruit quality (Silva et al., 2002).

Agronomical, physical and physicochemical characterization of fruit, allied with estimates of genetic variability assessed using molecular markers,

is important for the selection of progenitors, in order to explore heterosis and develop better cultivars.

Many molecular markers, especially those associated with PCR (polymerase chain reaction) based methods, including RFLP (restriction fragment lenght polymorphism), RAPD (random amplified polymorphic DNA) and microsatellites or SSR (simple sequence repeats), have been broadly used to estimate genetic variability and phylogenetic studies in banana (Creste et al., 2004; Amorim et al., 2008; Amorim et al., 2009a, 2009b). Of these, microsatellite technique has shown higher potential use in banana because it permits the detection of greater polymorphism and of co-dominant inheritance, and offers high reproducibility and easy interpretation.

The objective of the present work was to characterize banana accessions from the Germplasm Bank at Embrapa Mandioca e Fruticultura Tropical using agronomical, physical and physicochemical characters of fruit and simple sequence repeats (SSR) markers.

Materials and Methods

Twenty-six accessions, from the Banana Germplasm Bank (BGB) at Embrapa Mandioca e Fruticultura Tropical, originated by tissue culture, including improved and wild diploids, triploids and tetraploids, were used (Table 2). Five plants per accession were evaluated in a complete randomized block design with a 3x2 m spacing. The plants were irrigated by aspersion, and the management was carried out according to technical recommendations for all genotypes (Alves & Oliveira, 1999). The soil was classified as a Latossolo Amarelo distrófico (Xanthic Haplustox). The climate type in Cruz das Almas is AW (Köppen classification), with an annual mean temperature of 24.5°C and rainfall of about 1,500 mm.

Based on Silva et al. (2002), the agronomical traits evaluated were: plant height (PH, m); pseudostem diameter (PD, cm); number of suckers during flowering (NS); number of leaves during harvest (NL); stalk length (SL, cm); stalk diameter (SD, mm); stalk weight (SW, kg); number of hands per bunch (NH); number of fruits (NF); and weight of bunch (WB, kg).

Yellow-Sigatoka was assayed under field conditions. Data was taken during the flowering period, following the methodology proposed by Stover (1972), according to the following grade scale: 0, without symptoms; 1, 1 to 10% of leaf blade with symptoms; 2, from 11 to 30% of leaf blade with symptoms; 3, from 31 to 50% of leaf blade with symptoms; 4, from 51 to 70% of leaf blade with symptoms; 5, more than 70% of leaf blade with symptoms.

The physical characters of fruit evaluated were: length; diameter; fruit weight; pulp weight; peel thickness; pulp diameter and pulp firmness. All analyses were done in triplicate.

Pulp samples from three fruits of each genotype, made up of a central piece and both extremities, were taken out for the physicochemical analyses. Pieces were shredded in a blender, adding water in a 1:2 ratio (pulp:water). The following variables were measured: soluble solids content (SS, ºBrix); pH and titratable acidity (TA), according to the Association of Official Analytical Chemists (1997); vitamin C (VIT, mg $100g^{-1}$), according to Terada et al. (1979); total carotenoids (CTN, µg g^{-1}), according to Rodriguez-Amaya (1999); and total flavonoids (FLA, mg 100 g^{-1}), as described by Rijke et al. (2006). Polyphenol extraction (PLF, mg 100 g^{-1}) was done using a solution of 50% methanol and 70% acetone and quantification was performed with a spectrophotometer using the Folin-Ciocauteau reagent, according to the methodology proposed by Obanda & Owuor (1997). All analyses were done in triplicate.

For the molecular characterization, carried out at the Laboratorio de Virologia e Biologia Molecular of Embrapa Mandioca e Fruticultura Tropical, 13 microsatellite primer pairs were used: three belonging to the Ma series developed by Crouch et al. (1998), four from the AGMI series (Lagoda et al., 1998), five from the MaOCEN series (Creste et al., 2004), and a Mb 1-100 primer described by Oriero et al. (2006) (Table 1). Genomic DNA was extracted from young leaves using the CTAB method (Doyle & Doyle 1990).

Microsatellite amplifying reactions were completed in a final volume of 13 µL containing: KCl 50 mmol L^{-1}, Tris-HCl 10 mmol L^{-1} (pH 8.3), $MgCl_2$ 2.5 mmol L^{-1}, 100 µmol L^{-1} of each dNTPs (dATP, dTTP, dGTP, dCTP), 0.2 µmol L^{-1} of each primer, 50 ng of genomic DNA, and one unit of Taq DNA polymerase (Pharmacia Biotech, Carnaxide, Portugal).

Amplifications were carried out in the 9700 Perkin Elmer Thermocycler (Lincoln Center Drive, Foster City, USA), using the touchdown program: initial cycle of 3 min and 94ºC, followed by 40 s at 94ºC, 40

s at 55ºC, reducing one degree at each cycle, 1 min at 72ºC, totalizing 10 cycles, followed by 25 cycles of 40 s at 94ºC, 40 s at 45ºC and 60 s at 72ºC. Bands were separated in ultrapure 1000 agarose gel (Invitrogen Carlsbad, USA) at 3%, under standard conditions, and the amplification products were stained with ethidium bromide for allele visualization.

Fruit data were analyzed for variance, and the averages grouped by the Scott-Knott (1974) test at 5% of probability. The dissimilarity averages were calculated using the average Euclidian distance. The component analyses were done using the Singh (1981) criteria. All analyses were carried out using the Genes software package (Cruz & Schuster, 2004).

For SSR analyses, the amplified fragments were evaluated as absent (0) or present (1). Genetic similarity was calculated according to Nei & Li (1979).

A combined analysis using the matrices of the agronomical, physical and physicochemical data of fruit and the molecular (SSR) data was carried out using the Gower (1971) algorithm:

$$D_{ij} = \frac{\sum_{k=1}^{p} W_{ijk}\, d_{ijk}}{\sum_{k=1}^{p} W_{ijk}},$$

in which: W_{ijk} is the distribution of the k-i^{th} variable for the total distance between individuals j and I; d_{ijk} corresponds to the weight given to the ijk-i^{th} comparison, with 1 for the valid comparisons and 0 for the invalid ones.

Table 1. Microsatellite SSR locus, repeated motif (F/R), number of alleles and polymorphism information content (PIC).

SSR name	F/R	Alleles	PIC
AGMI 24-25	tttgatgtcacaatggtgttcc/taaaggtgggttagcattagg	5	0.72
AGMI 67-68	atacctttctcccgttcttcttc/tggaaacccaatcattgatc	9	0.89
AGMI 93-94	acaactaggatggtaatgtgtggaa/gatctgaggatggttctg ttggagtg	9	0.82
AGMI 103-103	cagaatcgctaaccctatcctca/ccctttgcgtgcccctaa	11	0.75
Ma 1-17	aggcggggaatcggtaga/ggcgggagacagatggagt	7	0.79
Ma 3-103	tcgcctctctttagctctg/tgttggaggatctgagattg	10	0.86
Ma 1-27	tgaatcccaatttggtcaag/caaaacactgtccccatctc	5	0.84
MAOCEN 01	tctcaggaagggcaatc/ggaccaaagggaaagaaacc	6	0.85
MAOCEN 03	ggaggaaatggaggtcaaca/ttcgggataggaggaggagg	4	0.72
MAOCEN 10	ggaagaaagaagtggagaatgaa/ tgaaatggataaggcagaagaa	7	0.86
MAOCEN 12	gcaagaaagaacgagaaggaaa/ gtggggagggaggcatag	6	0.91
MAOCEN 13	gctgctattttgtccttggtg/cttgatgctgggaatctgg	9	0.84
Mb 1-100	tcggctggctaatagaggaa/tctcgagggatggtgaaaga	6	0.72
Total		94	-
Mean		7.23	0.81

This index was obtained by combining the binary data from the molecular markers and the quantitative data from the morphological characters, giving an estimation of a single similarity index varying from 0 to 1.

The Gower distance matrix was used to cluster the 26 accessions by UPGMA (unweighted pair-group method averages), using the NTSYS-pc software (Rohlf, 2000).

Results and Discussion

The averages for all agronomical characters showed significant differences, except for the number of suckers during harvest (NS) (Table 2). The coefficient of variation varied from 9.12 (PD) to 57.08% (NF), similarly to those observed by Amorim et al. (2009a) for the same variables.

Plant height (PH) varied from 1.44 m for 'Walha' to 3.54 m for 'Ambrosia', with an average of 2.79 m (Table 2). Four clusters were formed with 028003-01 and 'Walha', classified in the last group with the lowest values by the Scott-Knott (1974) test.

There was great variability for plant height, which is a positive fact for breeding, since this make it possible to identify diploid progenitors for crosses aiming at the development of short hybrids.

The mean pseudostem diameter was 17.76 cm, with averages varying between 9.67cm for the diploid 'Idu-110' and 24.56 cm for the tetraploid 'Calipso' (Table 2). This trait is related to vigor and resistance to pseudostem breakage. Genotypes with thicker pseudostems are less susceptible to toppling over (Silva et al., 2002).

The highest value observed for live leaves during harvest was 9.20 for the Tropical and Maravilha cultivars, and the smallest for the Towoolle, with 2 leaves. Fruit filling is directly correlated with number of live leaves during harvest. According to Soto Ballestero (1992), cultivars from the Cavendish subgroup generally need at least eight active leaves per plant for good fruit development.

The shortest mean stalk length was observed in 'Tuugia', with the highest value for 'Champa Madras'. There was a direct relationship between stalk diameter and stalk weight, which had the highest values in 'Ambrosia' and 'Calipso' (Table 2), and the smallest in 'Tuugia'.

The mean number of hands and fruit per bunch was 6 and 83, respectively (Table 2). The highest numbers of fruit were found in 'Thap Maeo', 'Ambrosia', 'Jaran' and 'Calipso'. These two traits are important in banana genetic studies, since the hand constitutes the market unit, and the increase in the number of hands can increase bunch weight, a trait that expresses genotype yield (Silva, 2002).

Five clusters were formed for resistance to yellow Sigatoka during flowering. Most genotypes were resistant, except 'Jaran' and 'Malbut' (Table 2). All the physical and chemical characters of fruit, except titratable acidity, showed significant differences (Table 3). The variation coefficients varied from 0.86% for flavonols to 31.24% for fruit weight.

The mean fruit length was 13.31 cm, with averages varying from 6.78 cm for 'Jaran' to 18.7 for 'Calipso', forming five groups. In general, tetraploids had higher averages than triploids or diploids. Similar behavior was observed for the diameter and average weight of fruit and for pulp weight and diameter, which formed five, four, four and six groups, respectively. The average peel thickness was 0.24 cm, varying from 0.13 for the diploid 'Idu-110' to 0.47 for the triploid 'Bakar'. Through the Scott-Knott (1974) test, three groups were formed (Table 3). The same behavior occurred for pulp firmness (PF), with averages varying from 0.67 Lb ('M-48') to 1.2 Lb ('Terapod').

The average for soluble solids was 19.48 ºBrix, varying from 14.60 ºBrix for 'Towoolle' to 25.70 ºBrix for 'Teparod'. Three groups were formed by the Scott-Knott clustering test. These results are in agreement with Soto Ballestero (1992).

The average of total carotenoid content, among the 26 accessions, was 3.19 μg g^{-1}, varying from 0.98 μg g^{-1} ('Tropical') to 8.23 μg g^{-1} ('Jaran') (Table 3). These results agree with reported data by other authors (Englberger et al., 2003; Amorim et al., 2009b).

Table 2. Averages of the agronomical characters evaluated in 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank[1].

Accession	Origin	Ploidy	Character[2]										
			PH	PD	NS	NL	SL	SD	SW	NH	NF	BW	YSF
Jaran	Indonesia	AA	2.83b	15.73d	1.67[ns]	8.67a	52.00b	37.33b	0.33c	8.00b	148.00a	3.20d	2.35a
2803-01	Brazil	AA	1.76d	9.75f	1.50	7.25a	22.25d	31.25b	0.24c	5.00c	67.00b	3.30d	0.71e
Malbut	New Guinea	AA	2.55c	15.00d	2.67	8.33a	25.33d	34.67b	0.20c	6.00c	64.00b	2.93d	2.12a
Idu-110	France	AA	2.33c	9.67f	2.67	5.00b	40.33b	32.00b	0.40c	7.00b	87.00b	3.30d	0.71e
Tuugia	Hawaii	AA	2.53c	12.67e	2.67	6.67a	14.67d	28.67b	0.23c	6.00c	59.00b	2.09d	0.71e
M-48	Equador	AA	2.75b	14.67d	3.33	6.33a	43.00b	42.00b	0.50c	6.00c	84.00b	4.57d	0.71e
Pipit	Indonesia	AAA	2.21c	12.00e	3.50	5.00b	34.00c	31.50b	0.28c	5.00c	92.00b	3.80d	1.14d
Caru Roxo	Brazil	AAA	3.33a	21.00b	1.67	8.00a	48.33b	50.33a	0.80b	5.00c	64.00b	7.00c	1.58c
Wasolay	New Guinea	AAA	2.64c	13.33d	2.00	7.67a	37.33c	34.67b	0.30c	5.00c	51.00b	3.03d	0.88e
Markatooa	New Guinea	AAA	2.45c	17.50c	1.50	7.50a	33.50c	39.00b	0.49c	5.00c	63.00b	4.75d	1.73c
Bakar	Indonesia	AAA	3.33a	18.00c	2.00	9.00a	47.00b	50.00a	0.90b	6.00c	79.00b	9.80c	1.22d
AAA Unk.[3]	New Guinea	AAA	2.90b	16.50d	2.00	6.50a	30.50c	40.50b	0.41c	6.00c	57.00b	6.20c	1.55c
Nam	Thailand	AAA	2.23c	16.50d	1.00	3.00b	41.50b	44.00a	0.90b	6.00c	87.00b	4.33d	1.87b
Towoolle	New Guinea	AAA	2.20c	14.50d	1.50	2.00b	41.50b	40.50b	0.32c	4.00c	42.00b	2.85d	1.40c
Caipira	Honduras	AAA	2.46c	17.17c	1.33	3.00b	37.33c	51.00a	0.80b	7.00b	132.00a	9.67c	0.71e
Thap Maeo	Hawaii	AAB	3.43a	20.60b	4.25	8.00a	44.25b	55.75a	0.93b	10.00a	158.00a	15.03b	0.71e
Walha	India	AAB	1.44d	14.67b	0.67	2.67b	24.00d	33.67b	0.19c	4.00c	30.00b	1.71d	1.90b
Pacha Nadan	France	AAB	3.47a	18.00c	2.50	3.50b	46.00b	51.00a	0.89b	7.00b	88.00b	8.47c	1.58c
C. Madras	Brazil	ABB	3.49a	21.50b	2.00	5.00b	70.00a	45.50a	0.65b	7.00b	94.00b	12.90b	0.71e
Ambrosia	Brazil	AAAA	3.54a	24.42a	1.80	7.40a	38.00c	60.60a	1.82a	9.00a	154.00a	21.26a	0.71e
Calipso	Brazil	AAAA	3.15a	24.56a	1.60	7.00a	36.80c	58.00a	1.60a	8.00b	138.00a	18.62a	0.71e
Tropical	Brazil	AAAB	2.76b	20.40b	1.80	9.20a	45.60b	48.20a	0.80b	6.00c	92.00b	9.96c	0.71e
Maravilha	Brazil	AAAB	2.66c	20.60b	3.00	9.20a	44.00b	48.80a	0.61b	5.00c	53.00b	6.74c	1.58c
Porp	New Guinea	AAAB	2.85b	20.25b	3.00	7.75a	34.50c	46.25a	0.53c	5.00c	51.00b	5.95c	1.22d
Ouro da Mata	Brazil	AAAB	3.22a	20.67b	2.67	8.67a	47.67b	51.33a	0.70b	5.00c	78.00b	6.75c	1.29c
Teparod	Thailand	ABBB	2.93b	18.00c	2.00	5.00b	34.00c	36.67b	0.33c	6.00c	37.00b	3.87d	0.71e
F (Trat.)			12.30*	22.05*	1.38[ns]	2.37*	3.87*	7.97*	8.95*	4.59*	5.97*	14.93*	22.80*
CV (%)			9.77	9.12	57.08	35.28	23.91	13.44	40.22	21.53	32.76	33.77	17.07
Mean			2.79	17.76	2.19	6.77	38.59	44.34	0.67	6.00	83.00	7.78	1.08

[1]Averages followed by the equal letters, in the columns, belong to the same group by the Scott-Knott (1974) test, at 5% probability. [2]PH, plant height (cm); PD, pseudostem diameter (cm); NS, number of suckers during flowering; NL, number of leaves during harvest; SL, stalk length (cm); SD, stalk diameter (mm); SW, stalk weight (kg); NH, number of hands; NF, number of fruits; BW, bunch weight (kg); YSF, yellow Sigatoka during flowering. [3]Unknown triploid. [ns]Nonsignificant. *Significant at 5% of probability.

Total flavonoids had an average of 2.25 mg 100 g^{-1}, varying from 0.85 mg 100 g^{-1}, ('Maravilha') to 6.64 mg 100 g^{-1} ('Teparod'), indicating broad variation in these accessions (Table 3). Among the diploids, the average was 2.67 mg 100 g^{-1}, and M-48 (4.68 mg 100 g^{-1}) stood out. For the triploids, it varied from 1.09 to 4.02 mg 100 g^{-1}; and for the tetraploids, the average was 1.95 mg 100g^{-1}, with 'Teparod' (6.64 mg 100 g^{-1}) standing out, corroborating Lako et al. (2007), who reported variation from 2 mg 100 g^{-1} to 10 mg 100 g^{-1} of flavonoids in different *Musa* genotypes.

The average total polyphenol content was 45.31 mg 100 g^{-1}, varying from 12.84 mg 100 g^{-1} for the Towolle triploid to 257.80 mg 100 g^{-1} for 'Teparod' (Table 3).

The highest vitamin C content was found in 'Teparod' and in the unknown AAA triploid. Results reported in the present work are in agreement with those of Amorim et al. (2007).

Regarding molecular analysis, 94 alleles were found, with an average of 7.23 alleles per primer. The largest number of alleles was identified for primer AGMI 103-103 (11), and the smallest for MAOCEN 01 (Table 1). The average number of alleles per SSR locus is similar to other reports for banana (Amorim et al., 2008; Amorim et al., 2009a).

The polymorphism information content (PIC) varied from 0.72, for AGMI 24-25, MAOCEN 03 and Mb 1-100 primers, to 0.91 for the MAOCEN 12 primer, with an average of 0.81. The correlation between the number of alleles and the PIC value was high ($r = 0.76$, $p \leq 0.005$), indicating that the primers with larger number of alleles had greater discriminatory power.

Re-sampling indicated that 85 alleles were enough for a precise estimation of the genetic divergence between the accessions. Correlation between the matrix considering 80 alleles and the matrix with 94 alleles was 0.98, with deviated squares (SQ_d) of 0.27

Table 3. Average of physical and physicachemicol fruit characters, evaluated for the 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank[(1)].

Accession	Ploidy	Character[(21)]													
		FL	FD	FW	PW	PD	PWI	PF	SS	TA	PH	CTN	FLA	PLF	VIT
Jaran	AA	6.78e	2.45d	23.56d	16.59d	1.98f	0.19c	0.88c	17.74d	0.11a	5.13a	8.23a	2.47e	28.76m	17.61k
2803-01	AA	14.22b	2.25e	49.30d	31.49d	1.86f	0.21c	0.86c	18.93d	0.12	4.86b	3.53e	2.88d	38.51h	31.52f
Malbut	AA	9.67d	3.08c	49.39d	36.57d	2.72c	0.16c	0.98b	17.70d	0.14	4.52c	6.88b	2.09f	26.90n	20.42j
Idu-110	AA	10.22c	2.49d	37.11d	28.15d	2.22e	0.13c	0.94c	20.67c	0.13	4.96b	2.86f	2.88d	40.96g	20.10j
Tuugia	AA	12.22c	2.16e	36.48d	24.36d	1.82f	0.14c	0.70d	20.73c	0.13	4.69c	1.41g	1.64g	31.05l	51.10c
M-48	AA	15.11b	2.19e	49.21d	34.30d	1.85f	0.15c	0.67d	15.27e	0.13	4.64c	3.52e	4.08c	41.18g	9.03n
Pipit	AAA	8.67b	2.56d	31.56d	16.89d	2.15f	0.30b	0.78d	17.65d	0.10	5.06a	2.95f	4.68b	61.48e	15.72l
Caru Roxo	AAA	14.67b	3.73b	113.98b	84.41b	3.30b	0.28b	0.82c	20.87c	0.12	4.92b	5.91c	2.16f	33.32j	24.63i
Wasolay	AAA	13.78b	2.68d	63.33c	47.13c	2.30e	0.17c	0.81c	18.00d	0.18	4.20d	3.15e	1.16h	17.51p	14.72l
Markatooa	AAA	13.83b	3.04c	82.60c	60.37c	2.64d	0.19c	0.87c	17.90d	0.14	4.61c	2.29f	1.09h	16.23q	14.41l
Bakar	AAA	15.25b	3.60b	116.08b	67.48b	2.75c	0.47a	1.13a	18.50d	0.10	4.79b	3.99e	1.61g	79.14c	29.43g
AAA Unk.[(3)]	AAA	17.67a	3.93a	144.32a	112.03a	3.57a	0.20c	1.20a	19.70c	0.21	4.44c	2.45f	2.45e	35.48i	54.20b
Nam	AAA	11.58c	3.07c	67.61c	46.31c	2.44d	0.20c	0.90c	20.40c	0.11	5.26a	2.77f	2.75d	31.86k	44.67d
Towoolle	AAA	11.83c	3.15c	72.73c	56.14c	2.92c	0.19c	0.70d	14.60e	0.16	4.62c	2.33f	2.04f	12.84s	10.87m
Caipira	AAA	11.67c	3.37c	68.58c	53.37c	3.01b	0.17c	0.89c	21.40c	0.09	5.03a	1.05g	1.72g	146.31b	11.48m
Thap Maeo	AAB	11.67c	4.00a	95.98b	74.49b	3.55a	0.24c	0.98b	17.07d	0.18	3.84d	3.78e	1.50g	15.71q	37.21e
Walha	AAB	10.61c	2.96c	57.48c	37.14d	2.52d	0.24c	1.03b	18.27d	0.14	5.16a	2.52f	4.02c	43.41f	17.85k
P. Nadan	AAB	13.67b	3.62b	110.88b	73.96b	3.17b	0.28b	0.97b	22.70b	0.16	4.41c	5.83c	1.86g	64.90d	26.85h
C. Madras	ABB	12.75c	4.07a	134.63a	94.34a	3.62a	0.41a	1.21a	17.80d	0.16	4.38c	3.34e	1.76g	27.41n	12.45m
Ambrosia	AAAA	18.11a	3.85b	162.42a	107.70a	3.16b	0.38a	0.93c	18.47d	0.11	4.73b	1.39g	1.38h	27.52n	11.60m
Calipso	AAAA	18.72a	3.76b	150.28a	104.52a	3.16b	0.26c	0.84c	20.13c	0.12	4.85b	1.40g	1.35h	27.12n	9.49n
Tropical	AAAB	15.28b	4.21a	152.37a	113.98a	3.77a	0.30b	1.02b	20.00c	0.15	4.34c	0.98g	1.20h	14.83r	14.68l
Maravilha	AAAB	16.89a	3.78b	131.40a	82.14b	3.02b	0.34a	0.90c	20.73c	0.19	4.46c	1.89g	0.85h	16.03q	9.66n
Porp	AAAB	13.67b	4.30a	137.21a	103.10a	3.78a	0.29b	0.98b	19.80c	0.19	4.29c	2.34f	1.08h	14.77r	13.65l
O. da Mata	AAAB	13.61b	3.31c	94.19b	64.66b	2.92c	0.20c	0.83c	23.73b	0.15	4.43c	4.70d	1.42g	24.56o	19.45j
Teparod	ABBB	13.72b	3.65b	119.78b	73.93b	3.03b	0.30b	1.22a	25.70a	0.07	5.27a	1.44g	6.64a	257.80a	76.83a
F (Trat.)		20.53*	32.03*	19.32*	20.89*	35.60*	10.25*	10.54*	11.64*	13.92[ns]	7.83*	40.79*	105.19*	34,877.64*	1,161.36*
CV (%)		13.64	10.33	31.24	30.47	10.49	30.79	13.72	10.38	18.59	7.84	12.72	8.11	0.86	2.90
Mean		13.31	3.26	89.95	62.98	2.80	0.24	0.91	19.48	0.13	4.68	3.19	2.25	45.31	23.82

[(1)]Averages followed by equal letters, in the columns, belong to the same group by the Scott & Knott (1974) test at 5% probability. [(2)]FL, fruit length (cm); FD, fruit diameter (cm); FW, fruit weight (g); PW, peel weight (g); PD, pulp diameter (cm); PWI, peel width (cm); PF, pulp firmness (Lb); SS, soluble solids (ºBrix); TA, tritratable acidity; pH; CTN carotenoids (µg g^{-1}); FLA, flavonoids (mg 100 g^{-1}); PLF, polyphenols (mg 100 g^{-1}); VIT, vitamin C (mg 100 g^{-1}). [(3)]Unknown triploid. [ns]Nonsignificant.*Significant at 5% probability.

and stress value (E) of 0.0475. According to Kruskal (1964), a value of E≤ 0.05 is indicative of excellent precision of the estimates. Similar results have been reported by other authors for banana (Creste et al., 2004; Amorim et al., 2008).

Principal component analysis using eleven agronomical variables is presented in Table 4. The first three components explained 74.62% of the total variation. Four clusters were formed (Figure 1 A). However, more divergent accessions can be observed, such as 17 ('Pacha Nadan'), 18 ('Champa Madras') and 3 ('Malbut').

Considering the fruit variables, the first three principal components explained 68.3% of the total variation between the 26 accessions (Table 4). Four groups were formed, with 13 ('Nam'), 17 ('Pacha Nadan'), 24 ('Porp') and 26 ('Teparod') being the most divergent (Figure 1 B). No coincidence was observed between the clusters considering the agronomical and fruit variables, except for 'Pacha Nadan'.

Table 4. Principal component analyses for agronomic and fruit characters in 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank.

Character	Eigenvalue	Total % variation	Cumulative % variation
		Agronomic characters	
Plant height	5.83	48.61	48.61
Pseudostem diameter	1.69	14.11	62.72
Number of suckers	1.42	11.89	74.62
Number of leaves	1.02	8.56	83.18
Yellow Sigatoka during flowering	0.83	6.92	92.11
Stalk weight	0.27	5.97	96.09
Stalk length	0.21	1.75	97.84
Stalk diameter	0.11	0.97	98.82
Bunch weight	0.06	0.54	99.37
Number of hands	0.04	0.35	99.72
Number of fruit	0.03	0.33	100.00
		Fruit characters	
Fruit length	6.6	43.9	43.9
Fruit diameter	2.6	13.7	57.7
Fruit weight	1.6	10.7	68.3
Peel weight	1.1	7.5	75.9
Pulp diameter	1.0	6.9	82.7
Peel thickness	0.8	6.8	89.5
Pulp firmness	0.6	5.3	93.5
Soluble solids	0.4	2.4	94.5
Titratable acidity	0.2	1.5	98.7
pH	0.1	1.0	99.7
Vitamin C	0.0	0.2	99.9
Polyphenols	0.0	0.0	100.0
Flavonoids	0.0	0.0	100.0
Carotenoids	0.0	0.0	100.0

The estimates of the relative contribution of each variable, for expressing genetic diversity using Singh´s (1981) criteria, indicated that for the agronomic variables, the number of fruit contributed with 83.80% of the variation (Table 5), while fruit weight and pulp weight contributed with 59.42% and 30.32% of the variation, respectively, to the fruit variables (Table 5).

Considering the distance matrices for the agronomic, fruit and molecular (SSR) data, a dendrogram was constructed using the Gower (1971) algorithm (Figure 2). The cophenetic value was high (r = 0.88, p<0.0001, 10.000 permutations) and adequate, since rτ 0.56 is considered ideal, reflecting

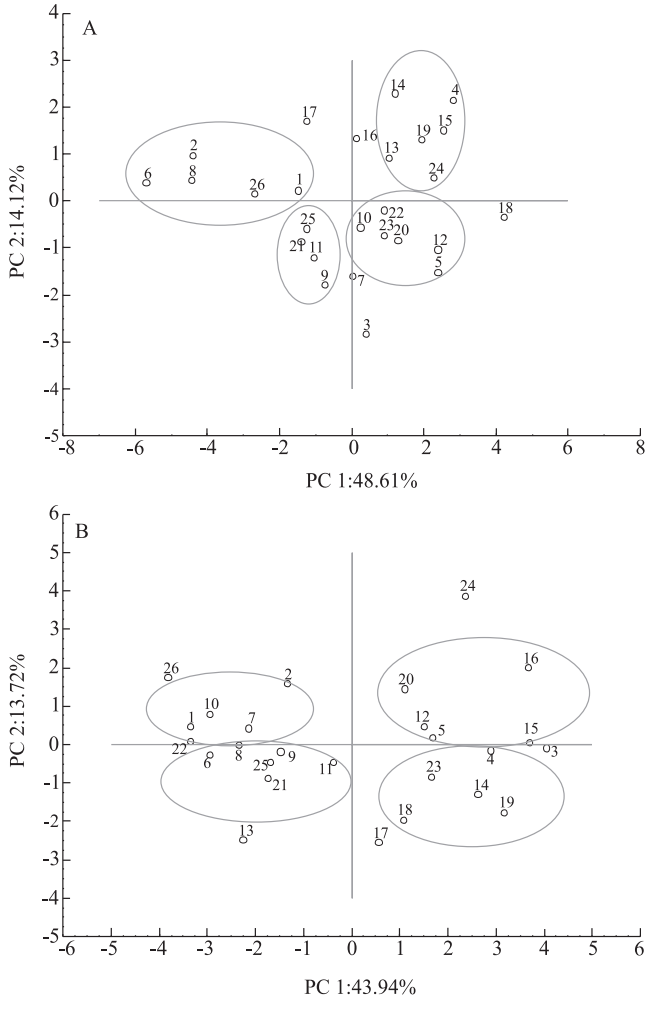

Figure 1. Principal component analyses for agronomic (A) and physical and physicochemical characteristics (B), in 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank.

Table 5. Relative importance (S.j.) of agronomic and fruit characters for the study of genetic diversity, in 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank.

Character	S.J[(1)]	S.J (%)
Agronomic characters		
Plant height	189.62	0.02
Pseudostem diameter	10,367.64	1.01
Number of suckers	438.00	0.04
Number of leaves	3,146.39	0.30
Yellow sigatoka during flowering	1,328.97	0.25
Stalk weight	106.71	0.05
Stalk length	78,005.18	7.65
Stalk diameter	51,985.57	5.09
Bunch weight	16,822.58	1.65
Number of hands	1,464.11	0.14
Number of fruit	85,4305.11	83.80
Fruit characters		
Fruit length	5,330.67	0.26
Fruit diameter	280.74	0.01
Fruit weight	1,191,363.85	59.42
Peel weight	607,896.86	30.32
Pulp diameter	239.52	0.01
Peel thickness	4.86	0.01
Pulp firmness	14.47	0.01
Soluble solids	3,820.95	0.34
Titratable acidity	525.79	0.02
pH	82.3	0.01
Vitamin C	23,213.27	1.15
Polyphenols	2,646.05	0.13
Flavonoids	1,254.77	0.06
Carotenoids	165,132.45	8.23

S.j: contribution of the x variable for the Euclidian distance among i and i' genotypes.

a good fit with genetic similarity (Vaz Patto et al., 2004).

Adopting the average genetic divergence (0.57) as the cut-off point, three groups were formed: G1, formed by the diploids 'Jaran', 028003-01 and M-48; G2, formed by the diploids 'Malbut' and 'Ido 110'; and G3, formed by 21 tri-and tetraploid, including one diploid 'Tuugia' accession. The triploids with the B genome, 'Thap Maeo', 'Walha', 'Pacha Nadan' and 'Champa Madras' were grouped in G2. The results from this work can be used as sources for banana genetic breeding, for the development of tri- and tetraploid hybrids with good agronomic traits.

The most divergent accessions 'Porp' (tetraploid AAAB) and 'Jaran' (diploid AA) also showed divergent concentrations of functional compounds (carotenoids, flavonoids, polyphenols and vitamin C) (Table 3). These accessions can be intercrossed to develop segregating populations for genetic mapping and to identify genes associated with these compounds. Furthermore, other parental combinations can be set up for the development of hybrids with promising agronomic traits and potential use as functional foods.

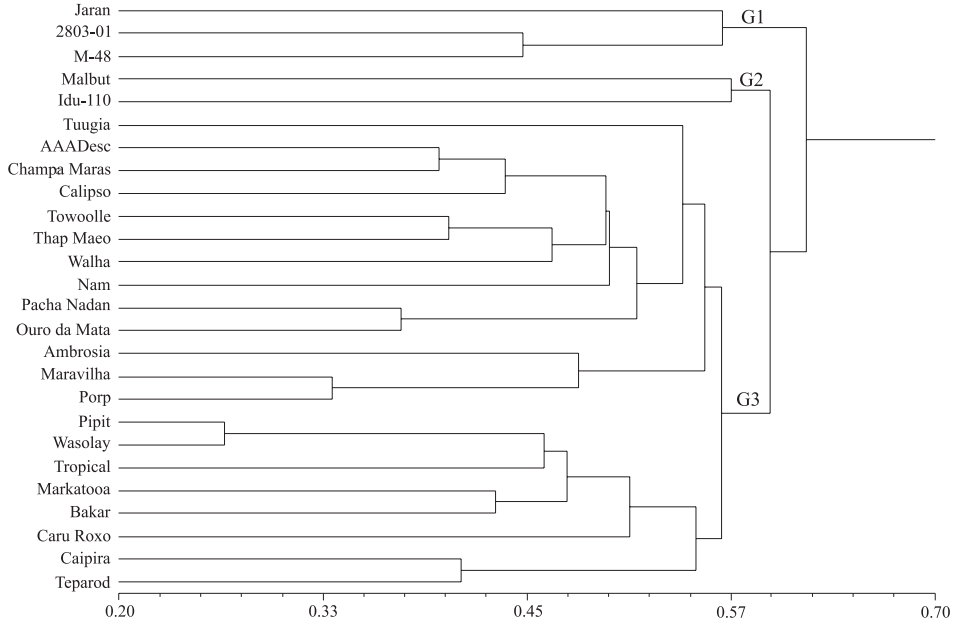

Figure 2. Genetic diversity between 26 banana accessions from the Embrapa Mandioca e Fruticultura Tropical Germplasm Bank integrating agronomic, fruit and molecular data using the Gower (1971) algorithm. Groups 1, 2 and 3 (G1, G2 and G3).

Conclusions

1. There is genetic variability for most agronomic and fruit characters in the 26 banana accessions studied.

2. The number and weight of fruit and pulp weight account for most of the variation found.

3. The Gower algorithm is efficient in quantifying the genetic variability between the 26 banana accessions.

Acknowledgements

To Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb), for the scholarship granted.

References

ALVES, E.J.; OLIVEIRA, M.A. Práticas culturais. In: Alves, E. (Org.). A **cultura da banana**: aspectos técnicos, socioeconômicos e agroindustriais. 2.ed. Brasília: Embrapa-SPI: Embrapa-CNPMF, 1999. p.335-352.

AMORIM, E.P; LESSA, L.S.; LEDO, C.A.S.; AMORIM, V.B. de O.; REIS, R.V. dos ; SANTOS-seREJO, J.A. dos; SILVA, S.O. Caracterização agronômica e molecular de genótipos diplóides melhorados de bananeira. **Revista Brasileira de Fruticultura**, v.31, p.154-161, 2009a.

AMORIM, E.P.; RAMOS, N.P.; UNGARO, M.R.G.; KIIHL, T.A.M. Divergência genética em genótipos de girassol. **Ciência e Agrotecnologia**, v.31, n.6, p.1637-1644, 2007.

AMORIM, E.P.; COHEN, K.O.; AMORIM, V.B.O.; SANTOS-SEREJO, J.A.; SILVA, S.O.; VILARINHOS, A.D.; MONTE, D.C.; PAES, N.S.; REIS, R.V. The genetic diversity of carotenoid-rich bananas measured by diversity arrays technology (DArT). **Genetics and Molecular Biology**, 2009b.

AMORIM, E.P.; REIS, R.V.; AMORIM, V.B.O.; SANTOS-SEREJO, J.A.; SILVA, S.O. Variabilidade genética estimada entre diplóides de banana por meio de marcadores microssatélites. **Pesquisa Agropecuária Brasileira**, v.43, p.1045-1052, 2008.

Association of Official Analytical Chemists. official methods of analysis of Association of **Official Analytical Chemists**. 16 ed. Arlington, 1997.

CRESTE, S.; NETO, A.T.; VENCOVSKY, R.; SILVA, S.O.; FIGUEIRA, A. Genetic diversity of *Musa* diploid and triploid accessions from the Brazilian banana breeding program estimated by microsatellite markers. **Genetic Resources and Crop Evolution**, v.51, n.7, p.723-733, 2004.

CROUCH, H.K.; CROUCH, J.H.; JARRET, R.L.; CREGAN, P.B.; ORTIZ, R. Segregation at microsatellite loci in haploid and diploid gametes of *Musa*. **Crop Science**, v.38, p.211-217, 1998.

CRUZ, C.D.; SCHUSTER, I. **GQMOL**: aplicativo computacional para análise de dados moleculares e de suas associações com caracteres quantitativos. Versão 2.1. Viçosa: UFV, 2004.

CRUZ, C.D.; REGAZZI, A.J. **Modelos biométricos aplicados ao melhoramento genético**. 2. ed. Viçosa: UFV, 1997.

DOYLE, J.J.; DOYLE, J.L. Isolation of plant DNA from fresh tissue. **Focus**, v.12, n.1, p.13-15, 1990.

ENGLBERGER, L.; AALBERSBERG, W.; RAVI, P.; BONNIN, E.; MARKS, G.C.; FITZGERALD, M.H.; ELYMORE, J. Further analyses on Micronesian banana, taro, breadfruit and other foods for provitamin A carotenoids and minerals. **Journal of Food Composition and Analysis**, v.16, n.2, p.219-236, 2003.

FAO. Food and Agriculture Organization of the United Nations. Disponível em: <www.faostat.fao.org/site/340/default.aspx>. Acesso em: 21 jan. 2009.

GOWER, J.C. A general coefficient of similarity and some of its properties. **Biometrics**, v.27, p.857-874, 1971.

KRUSKAL J.B. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. **Psychometrika**. v.29, p.1-27, 1964.

LAGODA, P.J.L.; NOYER, J.L.; DAMBIER, D.; BAURENS, F.C.; GRAPIN, A.; LANAUD, C. Sequence tagged microsatellite site (STMS) markers in the Musaceae. **Molecular Ecology**, v.7, p.659-663, 1998.

LAKO, J.; TRENERRY, V.C.; WAHLQVIST, M.; WATTANAPENPAIBOON, N.; SOTHEESWARAN, S.; PREMIER, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. **Food Chemistry**, v.101, p.1727-1741, 2007.

NEI, M.; LI, W.H. Mathematical model for studying genetic variation in terms of restriction endonucleases. **Procedures of the National Academic Science**, v.76, p.5269-5273, 1979.

OBANDA, M.; OWUOR, P.O. Flavanol composisition and caffeine content of green leaf as quality potencial indicators of Kenyan black teas. **Jornal of the Science of Food and Agriculture**. v.74, p.209-215, 1997.

ORIERO, C.E.; ODUNOLA, O.A.; LOKKO, Y.; INGELBRECHT, I. Analysis of B-genome derived simple sequence repeat (SSR) markers in *Musa* spp. **African Journal of Biotechnology**, v.5, p.126-128, 2006.

RIJKE, E.; OUT, P.; NIESSEN, W.M.A.; ARIESE, F.; GOOIJER, C.; BRINKMAN, U.A.T. Analytical separation and detection methods for flavonoids. **Journal of Chromatography**, v.112, n.1-2, p.31-63, 2006.

RODRIGUEZ-AMAYA, D.B. A **guide to carotenoid analysis in foods**. Washington: ILSI, 1999, 64p.

ROHLF, F.J. **NTSYS pc**: numerical taxonomy and multivariate analysis system. New York: Exeter Software, 2000.

SCOTT, A.J.; KNOTT, M.A. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, v.30, n.3, p.507-512, 1974.

SILVA, S.O.; ALVES, E.J.; LIMA, M.B.; SILVEIRA, J.R.S. Bananeira. In: BRUCKNER, C.H. **Melhoramento de Fruteiras Tropicais**. Viçosa: UFV, p.101-157, 2002.

SINGH, D. The relative importance of characters affecting genetic divergence**. The Indian Journal of Genetics and Plant Breeding**, v.41, n.1, p.237-245, 1981.

SOTO BALLESTERO, M. **Bananos**: cultivo y comercialización. 2. ed. San José: LIL, 1992. 674p.

STOVER, R.H. **Banana, plantain, and abaca diseases**. Kew: Commonwhealth Mycological Institute, 1972. 316p.

TERADA, M.; WATANABE, Y.; KUNITOMA, M.; HAYASHI, E. Differential rapid analysis of ascorbic acid and ascorbic acid 2-sulfate by dinitrophenilhydrazine method. **Analytical Biochemistry**, v.4, n.2, p.604-608, 1979.

VAZ PATTO, M.C.; SATOVIC, Z.; PÊGO, S.; FEVEREIRO, P. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. **Euphytica**, v.137, n.1, p.63-72, 2004.

VIJAYAKUMAR, S.; PRESANNAKUMAR, G.; VIJAYAKUMAR, N.R. Antioxidant activity of banana flavonoids. **Fitoterapia**, v.79, p.279-282, 2008.

Received on November 23, 2009 and accepted on January 20, 2009