Crop Breeding and Applied Biotechnology 9: 164-173, 2009 Brazilian Society of Plant Breeding, Printed in Brazil



Characterization of recommended banana cultivars using morphological and molecular descriptors

Onildo Nunes de Jesus^{1*}, Cláudia Fortes Ferreira², Sebastião de Oliveira e Silva², Terezinha Rangel Câmara³, Taliane Leila Soares⁴, and Kátia Nogueira Pestana⁵

Received 05 October 2008

Accepted 15 May 2009

ABSTRACT - New banana varieties with superior agronomical characteristics have been developed through introduction and/or genetic breeding. In order to guarantee its marketing and intellectual property, these new varieties need to be characterized by efficient inheritable qualitative morphological and molecular descriptors. The aim of the research was to characterize recommended banana varieties using qualitative morphological and molecular descriptors. The aim of the research was to characterize recommended banana varieties using qualitative morphological and molecular descriptors. Twelve genotypes were analyzed using 61 morphological descriptors where 17 were related to the plant, 24 to the bunch and 20 regarding the flower. Eighty-one molecular markers; 47 RAPD primers and 34 SSR primers were used. The morphological and molecular descriptors were efficient in the characterization and identification of specific characteristics for most of the varieties evaluated. Plant and inflorescence descriptors presented the greatest variability of characteristics that can facilitate its use for cultivar protection and registration.

Key words: Banana breeding, variety release, characterization, cultivar protection.

INTRODUCTION

Most banana cultivars used as food originated from the Asian Continent from the inter-specific cross between the *Musa acuminata* Colla (A genome) and *Musa balbisiana* Colla (B genome) species; main reason why they contain common characteristics to both of these species. Bananas are considered the main source of income and food in many developing countries. In 2006, Brazil produced more than 7.09 million tons of bananas, making it the world's second largest banana producer (FAO 2008). Bananas have three ploidy levels in various combinations of the A (M. acuminata) and B (B. balbisiana) genomes, being diploids with 22 chromosomes (2x), triploids with 33 (3x) and tetraploids (4x) (Simmonds 1973, Simmonds and Shephered 1955). Most species with economic potential belong to the last two groups and nowadays there are many varieties being obtained by crosses or by natural mutations that occur within each genomic group.

Genetic identification has received special attention recently, mainly due to the interest of the breeder in terms of protection and also due to the fact

¹ Mestrando em Melhoramento Genético de Plantas, Universidade Federal Rural de Pernambuco, 44380-000, Cruz das Almas, BA, Brazil. *E-mail: onildonunes@yahoo.com.br

² Embrapa Mandioca e Fruticultura Tropical, C.P. 007, 44380-000, Cruz das Almas, BA, Brazil

³ Professora da Universidade Federal Rural de Pernambuco, Depto de Química Vegetal, 52171-900, Recife, PE, Brazil

⁴ Doutoranda pela Universidade Federal do Recôncavo da Bahia, Campus Universitário, 44380-000, Cruz das Almas, BA, Brazil

⁵ Mestranda pela Universidade Federal de Viçosa, 36570-00, Viçosa, MG, Brazil

that the international market is becoming very competitive (Staub and Meglic 1993, Milach 1999), demanding identity and purification certificates of the material being marketed and especially with propagation material. Therefore, safe identification becomes indispensable for cultivar registration or protection.

Although there are morphological, biochemical and molecular descriptors in bananas, whenever the varietal identification demanded by law is considered, only the morphological descriptors have been used to characterize a cultivar (Rocha et al. 2002, Lombard et al. 1999, Priolli et al. 2002). According to Brazilian legislation, cultivar protection depends on the use of phenotypic, easily identifiable descriptors and which are stable through many generations (Rocha et al. 2002). However, difficulties in the identification of closely related genotypes using only morphological descriptors has increased with the development of new varieties, and since these descriptors are influenced by the environment, some can only be evaluated during the late stages of development, demanding time and physical space for their evaluation.

The RAPD (Random Amplified Polimorphic DNA) technique has been used in fingerprinting varieties of many species (Bhat et al. 1995, Howell et al. 1994, Pillay et al. 2000, Yu et al. 2002, Bianchi et al. 2003) to discriminate minimal divergence between species and clones. Varietal discrimination using RAPD, although mentioned in the Brazilian cultivar protection law and being used in some species, still needs to be standardized in order to be used more efficiently, since it has a drawback of lack of reproducibility (Dias et al. 2005).

The use of techniques that present high reproducibility and that are stable, such as microsatellite markers, can become an indispensable tool in the varietal identification creating molecular profiles for variety protection (Ghislain et al. 2000). These markers have been employed in the characterization of many economically important species such as bananas (Creste et al. 2003), corn (Padilha et al. 2003), potatoes (Ghislain et al. 2000), grapes (Narváez et al. 2001) and soybeans (Song et al. 1999).

The objective of the present work was to characterize and create a fingerprint of elite banana varieties that are recommended by Embrapa Cassava and Tropical Fruits using qualitative morphological and molecular (RAPD and SSR) descriptors.

MATERIALAND METHODS

Plant material

Twelve banana genotypes were characterized: Caipira, Thap Maeo, Tropical, FHIA-18, FHIA-21, FHIA-01, Pacovan Ken, PA42-44, Nam, Bucaneiro, Preciosa and Garantida (Table 1).

Morphological descriptors

Sixty-one qualitative descriptors were evaluated in three plant parts: 17 plant vegetative characteristics, 24 from the bunch and 20 from heart and male flowers. The descriptors evaluated are summarized in the previous work of Jesus (2006). The details of the evaluations as well as the illustrations were all based in the work of morphological characterization carried out earlier (Silva et al. 1999, IPIGRI 1996). Three plants were evaluated in the first production cycle and the scores for the qualitative characteristics were given by three different evaluators and were assumed by common consensus.

Molecular descriptors

The DNA extraction phase was carried out at the Virology and Molecular Biology Laboratory at Embrapa Cassava and Tropical Fruits. The DNA was extracted from young banana leaves of fourteen banana varieties (Table1), according to Doyle and Doyle (1990). DNA samples were diluted to the final concentration of 5 ng μ L⁻¹.

RAPD markers

The amplification reactions were carried out according to Williams et al. (1990). The reactions were completed to a final volume of 25 µL, containing the following reagents: KCl 50 mM, Tris-HCl 10 mM (pH 8.3), MgCl₂ 2.4 mM, 100 µM of each of the dNTPs (dATP, dTTP, dGTP, dCTP), 0.2 µM of primer (Operon Technologies, Alameda, CA, EUA), 25 ng of DNA and one unit of Taq polymerase (Biossystems). The amplifications were carried out in the Gene amp PCR System 9600 thermocycler with the following steps: initial denaturing step of the DNA strand at 95 °C for 1 minute, followed by 45 cycles, each one consisting of denaturation at 94 °C for 1 minute; primer annealing at 35 °C for one minute and extension of the DNA fragment by the Taq polimerase at 72 °C for two minutes. The amplified fragments were separated in 1.5% agarose gels in 1X

ON Jesus et al.

TBE (EDTA 2 mM and Tris-borate 90 mM), containing ethydium bromide, $0.5 \,\mu$ l mL⁻¹. The samples were submitted to 90 V for approximately 3.5 hours. Forty-seven RAPD primers from the Operon Technologies series (Alameda, CA, USA) were tested (Jesus et al. 2006).

Microsatellite markers (SSR)

Thirty-four SSR primers were tested (Jesus et al. 2006). The amplification reactions were completed to a final volume of 25 mL, containing: KCl 50 mM, Tris-HCl 10 mM (pH 8,3), MgCl₂ 2.4 mM, 100 mM of each of the dNTPs (dATP, dTTP, dGTP, dCTP), 0.2 mM of the primer, 50 ng of DNA and one unit of *Taq* polimerase (Pharmacia Biotech, EUA). The amplifications were carried out in the BioRAD My-Cycler Thermo cycler and each primer was tested according to the annealing temperature suggested by the manufacturer, followed by a touchdown program with the following steps: One cycle at 94 °C for 3 minutes, 10 cycles at 94 °C for 40 seconds, 40 seconds in a touchdown of 65 °C with a decrease of 1 °C at each cycle, 72 °C for one minute, 24 cycles at 94 °C for 40 seconds, 55 °C for 40

seconds, one cycle of 72 °C for 4 minutes and one cycle at 4 °C until conclusion. The eletrophoresis was carried out in polyacrilamide gels (6%) according to Creste et al. (2001).

Data analysis

In order to compare the descriptors, the multicategoric data of the morphological descriptors were transformed into binary data (Cruz 2001) resulting in 46, 61, 60 and 167 markers for plant characteristics, bunch, flowers and general, respectively. SSR and RAPD data were computed as absence (0) and presence (1) due to the poliploid nature of the species. The genetic dissimilarity between all the genotypes was calculated by the Jaccard dissimilarity coefficient, using the GENES program (Cruz 2001). Clusters were generated by UPGMA (unweighted pair group method with arithmetic mean) expressed in the form of a dendrogram using the STATISTICA (Statistica 2002) software which requires the distance matrix. The consistency of the nodes in the dendrogram was verified by bootstrap analysis (Felsenstein 1985) with 1000 permutations.

Table 1. Selected genotypes from Embrapa Cassava and Tropical Fruits

Genotypes	Genomic group	Subgroup	Cross (Origin)	Reaction to diseases
Tropical	AAAB	Silk	Yangambi n°2 x M53 (Embrapa)	Resistant to yellow Sigatoka Resistente a Sigatoka and tolerant to Fusarium wilt
FHIA-18	AAAB	Prata	'Prata Anã' x 2n (FHIA)'	Moderately resistant to yellow and black Sigatoka
Preciosa	AAAB	Prata	'Pacovan' x M53 (Embrapa)	Resistant to yellow and black Sigatoka and Fusarium wilt
Thap Maeo	AAB	Mysore	Cultivar tipo Mysore (Tailândia)	Resistant to yellow and black Sigatoka and Fusarium wilt
Garantida	AAAB	Prata	Prata São Tomé' X M53 (Embrapa)	Resistant to yellow and black Sigatoka and <i>Fusarium</i> wilt
Caipira	AAA	Street, and	Cultivar (África Ocidental)	Resistant to yellow and black Sigatoka and <i>Fusarium</i> wilt
Bucaneiro	AAAA	Gros Michel	Híbrido High Gate (Jamaica)	Resistant to yellow and black Sigatoka and <i>Fusarium</i> wilt
Pacovan Ken	AAAB	Prata	'Pacovan' x M53 (Embrapa)	Resistant to yellow and black Sigatoka and <i>Fusarium</i> wilt
FHIA-01	AAAB	Prata	'Prata Anã' x 2n (FHIA)	Resistant to black Sigatoka and Fusarium wilt
FHIA-21	AAAB	Тегта	Honduras	Resistant to yellow and black Sigatoka and Fusarium wilt
PA42-44	AAAB	Prata	'Prata Anā' x M53 (Embrapa)	Resistant to yellow Sigatoka and Fusarium wilt
Nam	AAA	-	Tailândia	Resistant to yellow Sigatoka and Fusarium wilt

¹FHIA: Fundación Hondureña de Investigación Agrícola

Crop Breeding and Applied Biotechnology 9: 164-173, 2009

Characterization of recommended banana cultivars using morphological and molecular descriptors

RESULTS AND DISCUSSION

Morphological characteristics

Data regarding the qualitative characteristics evaluated for 12 banana genotypes are listed in Table 2. In general, most genotypes evaluated presented broad variability for the characteristics studied since most classes of descriptors were observed, except almost ripe (CCQ) and ripe fruit (CCM), fruit flavor (SAB) and consumption (CNF), which presented less variability. Regarding the almost ripe fruit (CCQ) characteristic, eight genotypes evaluated had yellow coloration and three presented light green coloration. Similar behavior was observed for the peel color of ripe fruits (CCM), where eight genotypes presented yellow coloration and four a yellow-orange color. The gray, red and brown color classes were not observed in the genotypes evaluated. Such results are in agreement with reports by Silva et al. (1999) for evaluations carried out in the banana germplasm bank at Embrapa Cassava and Tropical Fruits. The low variability regarding the characteristics for flavor (SAB) and consumption (CNF) is perfectly explained by the fact that most of the genotypes evaluated are for in natura consumption, except for FHIA-21, which is consumed after boiling or frying.

The plant and male flower presented a greater number of descriptors capable of differentiating one genotype in particular. As such, the Thap Maeo cultivar presented nine characteristics (three regarding the vegetative aspect of the plant and 6 of the flower) capable of differentiating it from all others (Table 2). However, cultivars FHIA-01, PA42-44 and Pacovan Ken did not present particular morphological characteristics which distinguished them from the remaining genotypes.

The dendrogram of the 17 plant characteristics (Figure 1A) separated the varieties into three main groups. The varieties FHIA-21 (AAAB), PA42-44 (AAAB), FHIA-01 (AAAB) and Bucaneiro (AAAA) were in the first group; Thap Maeo (AAB), Caipira (AAA) and Nam (AAA) in the second and Garantida (AAAB), Pacovan Ken (AAAB), FHIA-18 (AAAB), Preciosa (AAAB) and Tropical (AAAB) (Tabela 1 and Figure 1A), which are varieties with common parental genotypes, in the third group. FHIA-21 can be differentiated due to the continuous presence of anthocyanin in the entire length of the pseudostem (ANT), the more or less closed margin of the petioles

(FMP), the colored strip which confounds with the color of the petiole due to the weak purple spots (CLM) in the leaves of the plantlets (Table 2). The Thap Maeo cultivar presents a dark green (orange) coloration in the pseudostem and the color of the colored strip in the base of the leaf which can be mistaken by the color of the petiole. These characteristics allowed for the identification of these varieties within the groups formed (Table 2 and Figure 1A).

Bunch descriptors separated the cultivars into three main groups where FHIA-21, Thap Maeo and Nam were in the first group and PA42-44, Preciosa, FHIA-01, Garantida, Pacovan Ken, FHIA-18 in the second group, which presented genotypes with a certain degree of relationship among them and Caipira, Bucaneiro and Tropical varieties in the last group. The closest genotypes were Preciosa and PA42-44, with 80% similarity, indicating that these descriptors were insufficient to differentiate these two varieties by their bunch characteristics. The FHIA-21 cultivar, although being a tetraploid and used for either boiled or fried consumption, was grouped along with the triploid varieties, Thap Maeo and Nam, indicating that plantains are morphologically close to these varieties for bunch characteristics despite great differences in other characteristics (Figure 1A). On the other hand, considering that FHIA-21 is the only variety in the plantain group, it is justifiable that it is separated from the other tetraploids (AAAB). The separation of plantains from other types of bananas based on morphological characteristics was also reported by Ortiz (1997).

When heart (inflorescence) and male flower characteristics are taken into consideration, the precise formation of groups is not observed (Figure 1A). As expected, 'Thap Maco' is highlighted as the most divergent genotype. Among the characteristics that allow its separation from the other cultivars, is the rosy color of the base of the perigone (CBP), the presence of anthocyanin distributed along the perigone (CVP), with red on the anthers (CAT), a lack of apiculate (FAT) and the lack of pollen (POL) (Table 2). These descriptors were not efficient in the discrimination of the Garantida and Pacovan Ken cultivars (with 21% dissimilarity) and of the PA42-44 and Preciosa cultivars (with 30% dissimilarity) once related cultivars and those inside the same group presented similar inflorescence morphology (Table 1). Similar results were obtained by Ortiz et al. (1998) in studies with related cultivars.

				1			12		Plant	nt veg	vegetative		charactertistics1	stics ¹	-		-			
232523		-	ROS	TCV	CME	E DME		FME	ANT	ALF	FMP	P CMP	IP FFP		TFC N	NSC N	NIC N	NIA CLM	M IGB	FOB
Tropical			2	2	3	2		2	0	3	3	4	4		5				1	1
FHIA-18			1	0	3	4		3	0	3	(1)	4							I	-
NAM			1	2	3	4		3	2	-	3	2	I		1		4	4 3	2	3
Thap Maeo			1	4*	3	2		2	2	1	3	2	2*						-	-
Pacovan Ken			0	2	1	0		3	0	3	3	0	1		-				-	-
Bucaneiro			I	0	I	4		3	2	0	1	2	-						1	-
Caipira			-	0	3	2		2	0	T	3	0	1		-				2	3
FHIA-21			2	0	I	2		3	1	0	5*	0	2		1				1	1
Garantida			0	3*	1	2		1*	4*	3	ŝ	4	5		5			4 3	1	1
FHIA-01			-	0	1	4		3	2	0	3	2	1						-	-
Preciosa			0	2	3	4		3	3*	2	3	4	5						-	-
PA-42-44			-	2	3	4		3	1	3	Ι	2	1		1	2	2	4 3	-	-
N° Classes			0	4	4	9		4	4	3	4	4	5		5	3	4 4	4 3	3	3
N° of observed classes	S		0	6	3	3		3	4	3	3	2	5		4	0	3	3 3	2	2
Genotypes									Bu	inch c	Bunch characteristics	cristics	15							
CUU PI	PUB FLX		SEC TAP	FAP	FES FRE	LE CCQ	Q CPQ	Q CCM	A ECA	ADE	FRA	CPM (CPO /	ARO S	SAB A	CD	CNF A'	ATI FCA	A RBP	RFP
Tropical I	2 1	3	Н	I			2	-	2		2		2	1	2	1	-	2	4	S
FHIA-18 1	2 2	0	2*	I	4 3	3 3	I	2	1	2	1	2*	2	1	2	3	-	1 1	-	-
NAM I	2 3*	3	-	5					0	2	3	9	5	I	2	3	1 4	* 33	4	3
Thap Maco 1		3	T	0					5	2	3	9	5	2	5	1	1	1 3	4	5
cen 1	4 2	-	Ι	-					I	0	1	4	5	2	2	3	1		4	5
iro 2		0	-	-					I	T	3	-	2	I	2	1	1 3		4	4
Caipira 3*		3	F	0					2	1	e	4	I	2	2	3	1		4	4
FHIA-21 I		-	Ι	-					2	1	3	9	2	5	2	1	3		4	5
Garantida I		1		-					1	2	2	9	I	2	2	1	1		4	5
FHIA-01 1	2 2	1		-					1	2	3	9	2	3	2	3	1		4	4
Preciosa I		-	-	1					Ι	1	2	4	2	2	5	3	1		4	5
PA-42-44 I	3 2	-	1	T					1	1	5	4	2	2	2	3	I	3	-	-
N° Classes 3	5 3	3	3	4	4 3	9		7	3	2	3	9	3	3	4	3	4 4		4	5
N° of 3	3 3	3	0	5	2 3	2	3	2	2	5	6	4	2	3	1	2	2 3		2	4
observed classes																	*			

Crop Breeding and Applied Biotechnology 9: 164-173, 2009

ON Jesus et al.

168

renotypes					4	Flowers	(heart and		male f]	e flower c	charac	characteristics) ¹	ics) ¹					
	FVC	IMB	APB	TIM	MAI	PNC	CBP	CVP	CID	E	RTA	FAT (VOD	CAT D	O aU	1 1		
Tropical FHIA-18 NAM Thap Maeo Pacovan Ken Bucaneiro Caipira FHIA-21 Garantida FHIA-01 Preciosa Preciosa	$-\infty$ ∞ $^{+}_{*}$ ω $-\infty$	∞ − ∞ ∞ ∞ ∞ ∞ 4 ∞ 0 ∞ −	4 v - v 4 ^c v - 4 v 4 4	<pre></pre>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	* 	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~					0-0 [*] 00000-	0 0 - * 0 - 0 - 0 0 0 0			10 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	ANE - 0 0 0 0 0	ANO
N° Classes	5	4	5	3	7	5	4	4	4	4	4	3	3	7				- 0
N° of observed classes 4 4	4	4	4	2	3	4	2	2	2	3	3	3	3				n e	1 0

Combined analysis of the 60 qualitative characteristics showed that cluster of the cultivars presented behavior similar to the one presented when bunch characteristics were analyzed, separating them into three major groups (Figure 1A). The first group was made up by the AAA varieties represented by Caipira and Nam and the second group by AAAB varieties (PA42-44, Preciosa and FHIA-18) and the third group by FHIA-21, Thap Maeo, Bucaneiro, FHIA-01, Garantida, Pacovan Ken and Tropical varieties.

In general, a low dissimilarity between the Preciosa, Pacovan Ken and Garantida cultivars and Pacovan hybrids with the M53 (diploid), was observed and also between the PA42-44, FHIA-01 cultivars and FHIA-18 Prata Anã hybrids; a fact already expected since these genotypes have a common background. This tendency is in agreement with the findings of Priolli et al. (2002) which reported greater difficulty in distinguishing varieties from a common ancestor needing other descriptors besides the morphological to complement the characterization.

Molecular characterization

The cluster analysis for both techniques (RAPD e SSR) is presented in Figure 1A. In general there were not many differences in the clustering generated by both markers when analyzed separately, which was already expected, once both sample variables reached the DNA level. The dendrograms clearly demonstrate the separation between the triploid and tetraploid groups and the reliability of the data is presented by the consistency of the high values of the nodes (bootstrapping). Both techniques presented high bootstrap values (above 50%) for most clusters, therefore being superior to those observed for the morphological descriptors. In general, the greater consistency can be observed in the SSR analysis, thus reinforcing their reliability.

The RAPD markers were able to clearly separate the varieties according to their genomic groups. The first group is comprised of all the genotypes carrying the A genome, and the second group of the genotypes carrying the AB genomes, including all the tetraploid hybrids evaluated. The first group included the Nam (AAA), Caipira (AAA) and Bucaneiro (AAAA) varieties. The second group included the Thap Maeo (AAB) variety and the tetraploid hybrids (AAAB) represented by the cultivars FHIA-01, FHIA-18, FHIA-21, Garantida, Preciosa, Pacovan Ken, Tropical and the PA42-44 hybrid (Figure 1A).

Crop Breeding and Applied Biotechnology 9: 164-173, 2009

Table 2. Cont.

ON Jesus et al.

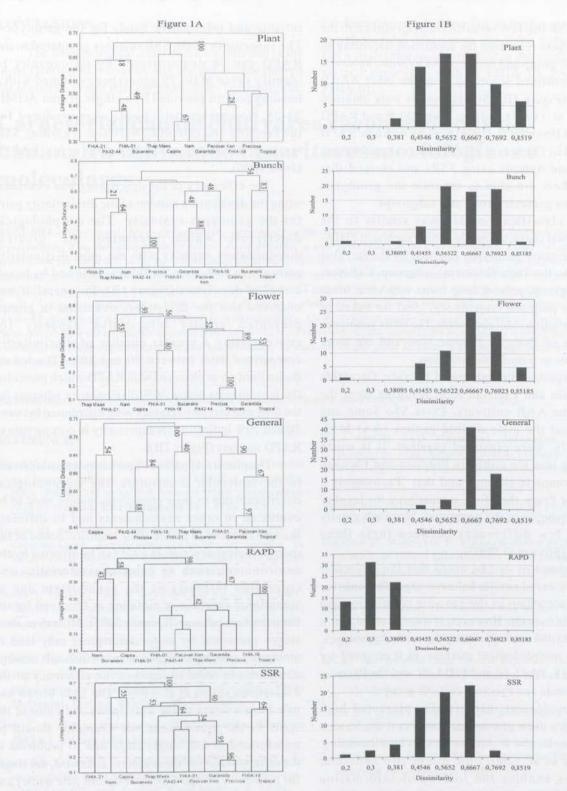


Figure 1. A: Dendrogram of the relationship between cultivars and hybrids from plant, bunch and flower (heart and male flowers) and general morphological qualitative descriptors and molecular descriptors (RAPD and SSRs). B: Classes observed for each pair of values of the dissimilarity matrix in each group of descriptors

Crop Breeding and Applied Biotechnology 9: 164-173, 2009

170

Characterization of recommended banana cultivars using morphological and molecular descriptors

The SSR markers revealed behavior similar to the RAPD markers regarding the separation according to the genomic group and origin of the hybrids; however, FHIA-21 remained separated from the other AAAB genotypes (Figure 1B). Similar results were obtained by Bhat et al. (1995) working with RFLP and RAPD markers and Howell et al. (1994) working with nine RAPD primers and by Creste et al. (2003), that evaluated banana cultivars and hybrids using SSRs and showed that these markers are able to separate the genotypes according to genomic group and subgroups.

This classification also was similar to the morphological characterization, which separated FHIA-21 from the other genotypes justified by the fact that it belongs to the Terra (Plantain) subgroup. Cultivars of this subgroup, present long fruits with clear blunt edges, rosy pulp and consistency, ideal for baked or fried consumption (Moreira 1999). The other genotypes AAAB are of Prata or Pomme type and are more adequate for in natura consumption.

As expected, the tetraploid hybrids: Garantida and Preciosa and Pacovan Ken, resulting from the cross of the AAB cultivars, Prata São Tomé and Pacovan and the male diploid genitor (AA) M53, respectively, were clustered together. It is worth mentioning that the cultivars Preciosa and Pacovan Ken are complete siblings and that 'Pacovan' is a mutation of Prata, therefore, intensifying the level of relationship between the genotypes. Morphologically there are few differences between these three genotypes (Silva et al. 2003).

In general, it can be noted that the molecular markers presented similar behavior regarding cultivar clustering according to the genomic constitution and origin of the hybrids. However, it was observed that it was not possible to discriminate the related genotypes, using only morphological markers, as it occurred for the FHIA-18, PA42-44 and FHIA-01 and the Pacovan Ken, Garantida and Preciosa cultivar groups.

The molecular markers also presented high similarity for these genotypes, however it can be seen that both molecular techniques were complementary. For Rocha et al. (2002) the use of two molecular techniques enables the joining of information generated by the broad coverage of the genome generated by RAPD and the high reproducibility of the SSR bands, responsible for generating important, reliable and informative bands for the genotypes. The superiority of the SSR markers compared to the RAPD can be demonstrated by its capacity to identify seven of the 12 genotypes evaluated with a banding pattern provided by a single primer AGMI-24-25. No characteristic bands were produced for the Preciosa and Garantida varieties regarding SSR markers, however, it was possible using RAPDs (Jesus 2006).

The efficiency of the descriptors was evaluated using the distribution number of the dissimilarity pairs for the genotypes evaluated. The morphological descriptors which presented the greatest discriminatory capacity with the most dissimilarity pairs above 60% were for flower, followed by bunch and plant descriptors (Figure 1B). In general, it was observed that the descriptors evaluated in groups presented greater informative capacity for concentrating a greater number of dissimilarity comparison pairs between 70 and 80%. The lowest dissimilarity was observed with RAPD which presented the most classes below 30% dissimilarity whereas for the SSR markers most classes were distributed between 50 and 70% indicating its superiority in comparison to RAPD markers (Figure 1B).

Despite its apparent superiority in comparison to the molecular descriptors, the morphological descriptors due to their phenotypic nature need to be evaluated in other environments and in different seasons in order to assure their stability. Some of the morphological descriptors used can be affected by the environment, such as color characteristics and coloration intensity of the pseudostem due to somaclonal variation or mutation as observed for the Pacovan Ken cultivar (Resende 2005). Moreover, most stages presented by some descriptors may lead to ambiguity making evaluations more difficult needing alterations in order to improve the efficiency of the evaluations (Silva et al. 1999). The light brown and pale brown stages of the descriptors and color of the spots in the pseudostem, for example, should be transformed to one single stage due to problems in the differentiation of these colors. Likewise, the stages for pulp color of ripe fruits (white and pale white) and anther color (pale brown and cream) should be transformed into one single stage; white and cream, respectively.

ON Jesus et al.

CONCLUSIONS

The morphological descriptors are efficient in the identification of varieties and should be considered for registration and cultivar protection. The molecular

markers presented clustering behavior similar to the morphological descriptors for some genotypes. The DNA descriptors were efficient in discriminating some related genotypes such as FHIA-18, PA42-44 and FHIA-01.

Caracterização de cultivares recomendadas de bananeira utilizando descritores morfológicos e moleculares

RESUMO – Novas variedades de bananeira com características agronômicas superiores têm sido desenvolvidas mediante a introdução e ou melhoramento genético. Para garantir a sua comercialização e propriedade, essas novas variedades necessitam ser caracterizadas por descritores eficientes e herdáveis como os morfológicos qualitativos ou pelo marcadores moleculares. O objetivo do presente trabalho foi caracterizar variedades recomendadas de bananeira por meio de descritores morfológicos qualitativos e moleculares. Foram avaliados 12 genótipos mediante o emprego de 61 descritores morfológicos sendo 17 relacionados à planta, vinte e quatro do cacho e vinte das flores. Foram empregados 71 marcadores moleculares sendo 47 primers de RAPD e 34 primers de microssatélites. Os descritores morfológicos e moleculares foram eficientes na caracterização e identificação de caracteres específicos para a maioria das variedades avaliadas. Os descritores da planta e da inflorescência apresentaram maior variabilidade de caracteres que pode facilitar o seu emprego para o registro e ou proteção de cultivares.

Palavras chave: melhoramento de bananeira, lançamento de variedade, caracterização, proteção de cultivares.

REFERENCES

- Bhat KV, Jarret RL and Rana RS (1995) DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. Electrophoresis 16: 1736-1745.
- Bianchi VJ, Fachinello JC and Schuch MW (2003) RAPDs na caracterização genético-molecular e no estudo da variabilidade genética de cultivares de ameixeira. Revista Brasileira de Fruticultura 25: 272-274.
- Creste S, Tulmann-Neto A, Silva SO and Figueira A (2003) Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers. Euphytica 132: 259-268.
- Creste S, Tulmann Neto A and Figueira A (2001) Detection of single sequence repeat polymorphism in denaturing polyacrylamide sequencing gels by silver staining. Plant Molecular Biology Reporter 19: 299-306.
- Cruz C D (2001) Programas GENES-versão Windows 2005.6.1. Editora UFV. Viçosa, 642p.
- Dias LAS, Rocha RB and Picoli EAT (2005) Distinctness of cacao cultivars using yield component data and RAPD markers. Crop Breeding and Applied Biotechnology 5: 47-54.
- Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. Eocus 12: 13-15.

- FAO (2008) FAO statistical databases: agricultural production: crops primary: Brazil: bananas. Available at http://apps.fao.org/page/collections. Accessed in June 10, 2008.
- Felsenstein J (1985) Confidence limits of phylogenies: an approach using the bootstrap. Evolution 39: 783-791.
- Ghislain M, Rodriguez F, Villamon-Nuñez J, Waugh R and Bonierbale (2000) Establishment of microsatellites assays for potato genetic identification. In: CIP Program Report. Editora FAO-CIP, Peru, p.167-174.
- Howell EC, Newbury HJ, Swennen RL, Withers LA and Ford-Lloyd BV (1994) The use of RAPD for identifying and classifying *Musa* germplasm. Genome 37: 328-32.
- IPGRI- International Plant Genetic Resources Institute (1996) Descriptors for banana (Musa spp.). Editora FAOP-IPGRI-INIBAP, Rome, 55p.
- Jesus ON (2006) Caracterização morfológica e molecular de cultivares de bananeira. PhD Thesis, Universidade Federal Rural de Pernambuco, Recife, 98p. Available at www.cipedya.com/web/FileDownload.aspx?IDFile=155703. Accessed in October 22, 2008.
- Jesus ON, Camara TR, Ferreira CF, Silva SO, Pestana KN and Soares TL (2006) Diferenciação molecular de cultivares elites de bananeira. **Pesquisa Agropecuária Brasileira**

Crop Breeding and Applied Biotechnology 9: 164-173, 2009

Characterization of recommended banana cultivars using morphological and molecular descriptors

41: 1739-1748.

- Lombard V, Bari CP, Dubreuil P, Blouet F and Zhang D (1999) Potential use of AFLP markers for the distinction of rapeseed cultivars. In: Wratten N and Salisbury PA Proceedings of X International Rapeseed Congress. Canberra, Australia, GCIRC France Home, Paris, p. 100-103.
- Milach SCK (1999) Disponibilidade de técnicas moleculares para la identificação varietal. In: Pagliano D (ed.) Calidad genética y sanitaria: un instrumento para la competitividad de la cadena Agroindustrial. Editora IICA-PROCISUR, Montevideo, p.39-46.
- Moreira RS (1999) Banana teoria e prática de cultivo. Editora Fundação Cargill, São Paulo (CD-Rom).
- Narvaéz CH, Castro MHP, Valenzuela JB and Hinrichsen PR (2001) Patrones genéticos de los cultivares de vides de vinificación más comúnmente usados en chile basados en marcadores de microsatélites. Agricultura Técnica 61: 249-261.
- Ortiz R (1997) Morphological variation in Musa germplasm. Genetic Resources and Crop Evolution 44: 393-404.
- Ortiz R, Madsen S and Vuylsteke D (1998) Classification of African plantain landraces and banana cultivars using a phenotypic distance index quantitative descriptors. Theoretical Applied Genetics 96: 904-911.
- Padilha L, Guimarães CT and Paiva E (2003) Avaliação da pureza genética em sementes de milho utilizando marcadores microssatélites. Embrapa Milho e Sorgo, Sete Lagoas-MG, 3p. (Circular Técnica 30).
- Pillay M, Nwakanma DC and Tenkouano A (2000) Identification de RAPD markers linked to A and B genome sequences in Musa L. Genome 43: 763-767.
- Priolli RHG, Mendes Junior CT, Arantes NE and Contel EPB (2002) Characterization of Brazilian soybean cultivars using microsatellite markers. Genetics and Molecular Biology 25: 185-193.
- Rocha RB, Muro Abad JI, Pires IE and Araújo EF (2002) Fingerprinting and genetic diversity analysis of *Eucalyptus*

spp. genotypes using RAPD and SSR makers. Scientia Forestalis 62: 24-31.

- Silva SO, Gasparoto, L, Matos AP, Cordeiro ZJM, Ferreira CF, Ramos MM and Jesus ON (2003) Banana Breeding Program in Brazil - Recent Results. Editora CNPMF, Cruz das Almas, 39p. (Documento 122).
- Silva SS, Carvalho PCL, Shephered K, Alves EJ, Oliveira CAP and Carvalho JBS (1999) Catálogo de germoplasma de bananeira (Musa spp.). Editora Embrapa-CNPMF, Cruz das Almas, 152p.
- Simmonds NW and Shephered K (1955) The taxonomy and origin of the cultivated bananas. **The Botany Journal of** Linnean Society of London 55: 302-312.
- Simmonds NW (1973) Los plátanos. Editora Blume, Barcelona, 539p.
- Song QJ, Quigley CV, Nelson RL, Carter TE., Boerma HR., Strachan JL and Cregan P (1999) A selected set of trinucleotide simple sequence repeat markers for soybean cultivar identification. Plant Varieties and Seeds 12: 207-220.
- Statistica (2002) Statistica for Windows v. 6.0: computer program manual. Editora StatSoft Inc Tulsa, UK (CD-Rom)
- Staub JE and Meglic V (1993) Molecular genetic markers and their legal relevance for cultivar discrimination: A case study in cucumber. HortTechnology 3: 291-300.
- Williams JGK, Kubelik AR and Livak KJ (1990) DNA polymorphisma amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.
- Yu Z, Li-Qiong L, Huan L, Jie B, Man-Ye Y, Chien M, Ying-Fan C, Xiao-Lin Q and Fang C (2002) RAPD makers in diversity detection and variety identification of Tibetan hulless barley. Plant Molecular Biology Reporter 20: 369-377.