Journal of Genetic Engineering and Biotechnology (2012) **10**, 47–53

Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Morphological and molecular characterization of somaclonal variations in tissue culture-derived banana plants

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Received 11 December 2011; revised 20 April 2012; accepted 5 May 2012
Available online 6 June 2012

KEYWORDS

Somaclonal variation;
RAPD marker;
Cluster analysis;
PCOORDA;
Morphological traits

Abstract In this study, 40 000 tissue culture-derived banana plants (vitroplants) at different growth stages, i.e. acclimatization, nursery and open field of banana (*Musa* spp.) cultivar 'Grand Naine' were screened for somaclonal variations using morphological investigations and molecular characterization. The total detected variants were grouped into 25 off-types (two of them died) in addition to the normal plant. Random Amplified Polymorphic DNA (RAPD) was carried out to study the differences among the normal cultivar 'Grand Naine' and its 23 variants using 17 arbitrary primers. Cluster analysis results revealed that 'winged petiole' and 'deformed lamina' were more related to the normal plant. However, 'Giant plant' and 'weak plant' related to each other and clustered with normal plant. According to principal coordinate analysis, most of the variants were aggregated nearly, whereas 'variegated plant' was separated apart from the other variants. This may reflect the genetic difference between 'variegated plant' and the other variants. The results obtained from both molecular and morphological analyses were in contiguous with better resolution when using

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Peer review under National Research Center, Egypt.
http://dx.doi.org/10.1016/j.jgeb.2012.05.002

the PCOORDA analysis than cluster analysis. Thus, it can be said that molecular markers can be used to eliminate the undesirable somaclonal variants from the lab without additional culture of the vitroplants in the field in order to save time and efforts.

1. Introduction

Banana (*Musa* spp.) is one of the most important members of the *Musaceae* family. Bananas are grown in 128 countries with a total cultivated area of 4.92 million hectares and total world production of 97.38 million metric tons. India ranked first all over the world in banana production, which produces 27 million metric tons [20]. Plants which have been propagated by *in vitro* tissue culture techniques are known to exhibit a wide array of genetic and epigenetic variation which is known as somaclonal variation [7]. Although the causes of genetic instability are poorly understood, chromosome instability is believed to be one of the most common causes of tissue culture-induced variation [15].

Characterization of induced mutations and somaclonal variations between induced mutant 'GN60A' and its original variety 'Grand Naine' of genomic DNA using arbitrary primers was performed by Fernandez et al. [4]. In addition Pancholi et al. [12] stated that a Random Amplified Polymorphic DNA (RAPD) marker based protocol was developed to screen for somaclonal variation in bananas in tissue culture, using Cachaco Enano (AAB), Yangambi (AAA) and Pisang Awak (ABB) plants. They reported that 17% of the plants were found to be variants and the variation was genotype-dependent. They found also that variability increased with an increase in the copy number of genome A, but it decreased with an increase in the copy number of genome B. Their results indicated that RAPD markers could be used to monitor the levels of somaclonal variation. Rajamanickam and Rajmohan [13] reported that, out of the 41-decamer primers screened for banana RAPD analysis, 34 could produce amplification. Twenty-five primers showed high level of polymorphism and six of the most promising primers (OPA-01, OPA-03, OPA-13, OPB-04, OPB-10 and OPB-12) were used for RAPD analysis. Recently, Saifullah et al. [17] reported that 13 varieties of the cultivated banana, procured from INIBAP, Belgium, were screened using RAPD–DNA markers. Only three RAPD primers (among 20 tested) were chosen as producing polymorphic DNA bands differentiating the investigated cultivars. Based on those identity markers, the genetic fidelity between various subculture levels were determined.

AFLP markers were used in conjunction with morphological descriptors, isoezymes, agronomic traits and Random Amplified Polymorphic DNA (RAPD) markers to characterize the *Musa* accessions in the gene bank [18]. Microsatellite markers were also used to characterize banana genotypes [8,2]. Creste et al. [2] reported that phenetic analysis of microsatellite marker based on Jaccard similarity index derived from presence or absence of the alleles agreed with the morphological classification.

The main objective of this study was to characterize the produced banana vitroplants for both morphological and molecular (RAPD) markers and to compare the results of both marker types.

2. Material and methods

2.1. Plant material and morphological traits

Healthy and uniform banana offshoots of cultivar 'Grand Naine' were selected from a farm at Ahmed Oraby Village, Badr City, Beheira Governorate, Egypt in August 2008. The offshoots were proliferated at the Plant Tissue Culture Laboratory, Plant Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University, Egypt. Twenty-five off types were produced throughout the proliferation and the evaluation of the produced vitroplants. The off types were considered as somaclonal variants [5]. All produced banana somaclonal variants and normal vitroplants were grown in greenhouses and open field during the period from 2009 until 2011 in a farm at Ahmed Oraby Village, Badr City, Beheira Governorate, Egypt in order to be evaluated.

Table 1 The banana cultivar 'Grand Nain' somaclonal variants and their appearance stages.

No.	Phenotype case	Appearance stage		
		Acclimatization	Nursery	Field
1	Normal	+		
2	Spear shape lamina	+		
3	Leathery lamina	+		
4	Winged petiole	+		
5	Asymmetric lamina	+		
6	Lamina deformation	+		
7	Half variegated lamina	+		
8	Variegated plant	+		
9	Stripped lamina	+		
10	Malformed plant	+		
11	Fan shape plant	+		
12	Dwarf plant	+		
13	Sprocket lamina		+	
14	Default lamina		+	
15	Reddish lamina		+	
16	Long petiole		+	
17	Pale green pseudo stem		+	
18	Elephant ear shape		+	
19	Erected leaf		+	
20	Blackened pseudo stem			+
21	Shattered punch			+
22	Giant plant			+
23	Vigor plant			+
24	Weak plant			+

Morphological identification was made from the period after 6 weeks of acclimatization until the fruit harvesting in the open field (Table 1). Screening of all vitroplants (40 000) was achieved to identify the off-type plants and classified them according to their type. The morphological data were recorded during all the growth stages from the nursery stage until the fruit stage [5]. A total of 22 morphological traits were recorded during the different stages of growth. Five traits have been measured at the acclimatization stage (plant height (cm), leaf width (cm), leaf area (cm^2), leaf orientation and plant coloration); six traits have been measured at the nursery stage (pseudostem height (cm), leaf length (cm), leaf width (cm), leaf area (cm^2), leaf orientation and plant pigmentation) and eleven traits have been measured in the open field (pseudostem height (cm), leaf length (cm), leaf width (cm), leaf area (cm^2), leaf orientation, plant pigmentation, bunch weight, bunch length, bunch circumference, number of hand per bunch and number of fingers per hand) [5]. The morphological data were used to construct dendrogram and 3-D principal coordinate plot to study and characterize the similarities and differences among the banana cultivar 'Grand Nain' and its tissue-culture derived somaclones.

2.2. DNA isolation

Fresh white cigar leaves of the 'Grand Naine' banana cultivar and its 23 off types (Table 1), which were derived from the tissue culture propagation, were harvested and bulked, immersed in liquid nitrogen and stored at −70 °C until DNA extraction. Total genomic DNA was isolated from the stored leaves using modified standard CTAB method [16] and then preserved at −20 °C until used.

2.3. Random Amplified Polymorphic DNA (RAPD) analysis

RAPD–PCR was performed using 17 10-mer random primers selected from the Operon kit (Table 2). PCR reactions were carried out in 25 µl volumes containing 75 ng of template DNA, 1× reaction buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 1.5 µM of the primer and 1 U of the *Taq* DNA polymerase (Promega). PCR amplification was performed using Biometra gradient Thermolcycler for 35 cycles at 94 °C for 1 min, 30 °C for 1 min and 72 °C for 1 min. The program was preceded by a denaturation step at 94 °C for 7 min and followed by an elongation step at 72 °C for 7 min. The PCR products were separated on 1.5% ethidium bromide stained agarose gels and were photographed on gel documentation system (UVP, Doc-It system).

2.4. Data analysis

Gels of the RAPD analysis were scored as 0/1 for absence/presence of DNA bands, respectively. The total number of band and the number of polymorphic bands were calculated as well as the polymorphic information content (PIC) which was calculated according to Anderson et al. [1] using the following simplified formula:

$$\mathrm{PIC}_i = 1 - \sum p_{ij}^2$$

where p_{ij} is the frequency of the jth allele for marker i summed across all alleles for the locus. The morphological data were standardized and then the similarity matrix was calculated using the simple matching coefficient. Similarity matrix was calculated for the RAPD data using Jaccard coefficient [6]. Dendrograms were constructed for both morphological and RAPD data based on the UPGMA clustering method using NTSYSpc software version 2.0 (Applied Biostatistics, Setauket, New York, USA) [14].

Principal coordinate analysis (PCOORDA) was carried out for the standardized decentered morphological and RAPD data. Eigen values and Eigen vectors were calculated for the transformed interval data and the three-dimensional PCO-ORDA plot was constructed using the NTSYSpc software.

Table 2 Total and polymorphic bands number and the polymorphic information content (PIC) generated by 17 RAPD primers from banana cultivar 'Grand Nain' and its somaclonal off-types.

Primer	Sequence (5′–3′)	Total bands	Polymorphic bands	% Polymorphism	PIC
OPA-01	CAGGCCCTTC	8	7	87.5	0.88
OPA-03	AGTCAGCCAC	12	9	75	0.88
OPA-13	CAGCACCCAC	7	7	100	0.86
OPB-10	CTGCTGGGAC	9	7	77.8	0.84
OPB-12	CCTTGACGCA	9	8	88.9	0.89
OPC-07	GTCCCGACGA	5	4	80	0.80
OPC-15	GACGGATCAG	7	7	100	0.86
OPD-02	GGACCCAACC	9	7	77.8	0.84
OPE-15	ACGCACAACC	5	3	60	0.72
OPH-17	CACTCTCCTC	9	9	100	0.89
OPM-16	GTAACCAGCC	9	8	88.9	0.89
OPM-20	AGGTCTTGGG	5	3	60	0.72
OPN-03	GGTACTCCCC	2	1	50	0.50
OPN-09	TGCCGGCTTG	9	9	100	0.89
OPN-10	ACAACTGGGG	8	6	75	0.84
OPW-07	CTGGACGTCA	8	6	75	0.84
OPR-02	CACAGCTGCC	8	8	100	0.78

3. Results and discussion

3.1. Polymorphism and polymorphic information content (PIC)

The total number of bands and the number of polymorphic bands of 'Grand Naine' banana cultivar and its variants were calculated for each RAPD primer as well as the polymorphic information content (PIC) (Table 2). The 17 RAPD primers produced in total 129 bands, 109 from which were polymorphic (84.5% polymorphism) (Table 2). The total number of bands for each primer ranged from two bands for the primer OPN-03-12 for the primer OPA-03 while the number of polymorphic bands ranged from one band for the primer OPN-03 to nine bands for the primers OPA-03, OPH-17 and OPN-09 (Table 2). The polymorphism percentage for the RAPD primers ranged from 50% for the primer OPN-03 to 100% for the primers OPA-13, OPC-15, OPH-17, OPN-09 and OPR-02 (Table 2). The polymorphic information content (PIC) for RAPD primers was high in general and ranged from 0.50 for the primer OPN-03 to 0.89 for the primers OPB-12, OPH-17, OPM-16 and OPN-09 (Table 2). Thus, it seems that very high percentages of polymorphisms as well as PIC (82% as average overall the primers) were generated from the RAPD primers used in this study. These results could support their use in characterization studies to differentiate the variants from the original parents. In contrast, Zaffari and Kerbauy [21] stated that The Random Amplified Polymorphic DNA (RAPD) analysis was carried out using 20 primers, having only two of them (10%) shown polymorphism among the 'dwarf' and 'variegated plants' in relation to the normal plants.

DNA fingerprinting is widely used to detect somaclonal variation and to assess the genetic identity and stability in banana vitroplants [10]. In the present investigation, the Random Amplified Polymorphic DNA (RAPD) technique has been used to detect the differences among 'Grand Naine' banana cultivar and its variants. Similarity matrix of the normal 'Grand Naine' banana cultivar and its variants was calculated based on RAPD data using the Jaccard similarity coefficient to obtain the similarity correlation coefficient between each pair of the used genotypes (Table 3). The highest relationship was observed between the normal plant and 'winged leaf' type (0.84), as well as between 'deformed lamina' and 'winged petiole' (0.84), followed by between 'spear shape lamina' and normal plant (0.82) and then between 'deformed lamina' and normal plant (0.82). In the contrary the lowest similarity was observed between 'blackened pseudo stem' and 'variegated plant' (0.40), 'pale green pseudo stem' and 'variegated plant' (0.41) and between 'giant plant' and 'variegated plant' (0.42, Table 3). These results support the previous results using the morphological traits [5] when they reported that both 'winged leaf' and 'deformed lamina' types were not differed from the 'Grand Nain' normal type.

3.2. Cluster analysis based on molecular data

According to cluster analysis of the RAPD data, the variants were clustered with different degrees of similarity in relation to the normal plant (Fig. 1). The variants and their normal parent 'Grand Naine' banana cultivar were clustered into five clusters according to the cluster analysis (Fig. 1). The first cluster included two subclusters; the first subcluster contained the normal plant (cultivar 'Grand Naine') and the variants 'winged petiole', 'deformed lamina', 'giant plant' and 'weak plant'. The variants 'winged petiole' and 'deformed lamina' were clustered together along with the normal plant (Fig. 1). The second subcluster contained 'half variegated' lamina, 'vertical stripped' lamina and 'malformed' plant (Fig. 1). The variant 'spear shape lamina' was separated in between the above mentioned two subclusters. The second cluster included 'asymmetric lamina', 'fan shape plant' and 'shattered bunch' variants (Fig. 1). The third cluster included the variants 'long petiole' and 'elephant ear shape lamina' (Fig. 1). The fourth cluster included 'leathery lamina', 'dwarf plant', 'default lamina', 'blackened pseudo stem' and 'reddish lamina' variants. The fifth cluster included 'sprocket lamina' and 'vigor plant' variants, while the 'erected leaf' variant was separated in between the fourth and fifth cluster. Both 'pale green pseudo stem' and 'variegated plant' variants were clustered separately each apart of the above-mentioned clusters (Fig. 1).

These results were in agreement with those previously obtained by the screening along the different stages [5]. Most of the somaclonal variants appeared related to each other according to the cluster analysis. Those variants showed the same trend in the vegetative and reproductive stages for example, the 'winged petiole' variant reverted in the subsequent stage and showed the same behavior as the normal plant, which exactly we can see in the previous cluster analysis. The same trend was observed with the 'deformed lamina' variant and many other somaclonal variants.

3.3. Principal coordinate analysis (PCOORDA) Based on Molecular Data

According to the principal coordinate analysis (PCOORDA) of the RAPD data, the first three principal coordinates (PCs) accounted for 41.4% of the total variation (18% for PC1, 13.4% for PC2 and 9% for PC3) (Fig. 2), which reflect the reliability of the generated 3D graph of the PCOORDA analysis. The variants distributed on the base of the first PC, which represented the highest variance (Fig. 2), whereas the 'variegated plant' variant separated apart from other variants at the most high level of the first PC. It may be indicated that the 'variegated plant' genetically different from other types, while it was aggregated with all variants based on the second PC.

The following variants were aggregated near from the middle of PC1: 'vigor plant', 'sprocket lamina', 'erected leaf' and 'pale green pseudo stem' and were separated from all other variants, which were aggregated along with the normal plant at low level of the first PC (Fig. 2). At the second principal coordinate (PC2), there were no high differences among the variants in relation to their distribution at this level except for 'pale green pseudo stem' variant which was located at the lowest level of this PC (Fig. 2). At the third PC, there were no high differences among the variants in relation to their distribution at this level except for 'vigor plant', 'default lamina' and 'reddish lamina', which were located at very low level of that PC (Fig. 2).

Data obtained by PCOORDA were in consistent with those previously obtained by morphological screening and previous cluster analysis. The 'variegated plant' appeared segregated alone at the edge of the PC1. The latest variant showed different

Table 3 Similarity coefficient correlation among banana cultivar Grand Nain and its somaclones depends upon data generated by RAPD markers.

	Normal	Spear lamina	Leath lamina	Winge. petiole	Asym. lamina	Lamina defor	Half. Var. Lam	Varieg. plant	Vertic. strip. lam	Mal. plant	Fan. plant	Dwarf. plant	Sprock. lam.	Default. lam.	Reddish. lam.	Long. petiole	Pale. g. p. stem	Eleph. ear. lam	Erect. leaf	Black. p. stem	Shatt. punch	Giant. plant	Vigor. plant
Spear. lam.	0.82	1.00																					
Leath. lam.	0.77	0.70	1.00																				
Wing. petiole	**0.84**	0.71	0.71	1.00																			
Asym. lamina	0.70	0.69	0.65	0.67	1.00																		
Lamina. deform	0.82	0.71	0.67	**0.84**	0.69	1.00																	
Half. varieg. lam	0.72	0.68	0.71	0.69	0.61	0.64	1.00																
Varieg. plant	0.47	0.50	0.44	0.45	0.51	0.43	0.41	1.00															
Vertic. strip. lam	0.75	0.72	0.70	0.71	0.61	0.68	0.78	0.43	1.00														
Mal. plant	0.75	0.69	0.67	0.75	0.75	0.74	0.74	0.46	0.79	1.00													
Fan. plant	0.73	0.71	0.70	0.68	0.73	0.68	0.66	0.46	0.67	0.75	1.00												
Dwarf. plant	0.72	0.63	**0.80**	0.70	0.64	0.65	0.77	0.44	0.71	0.71	0.73	1.00											
Sprock. lam.	0.56	0.61	0.53	0.60	0.59	0.60	0.53	0.54	0.61	0.64	0.56	0.60	1.00										
Default. lam.	0.66	0.66	0.69	0.61	0.57	0.60	0.73	0.46	0.74	0.64	0.55	0.67	0.59	1.00									
Reddish. lam.	0.65	0.66	0.79	0.59	0.56	0.60	0.59	0.48	0.60	0.56	0.68	0.68	0.56	0.69	1.00								
Long. petiole	0.71	0.70	0.65	0.64	0.60	0.64	0.71	0.49	0.70	0.68	0.72	0.64	0.58	0.62	0.67	1.00							
Pale. g. p. stem	0.58	0.55	0.55	0.61	0.55	0.64	0.55	**0.41**	0.56	0.57	0.54	0.55	0.51	0.57	0.50	0.45	1.00						
Eleph. ear. lam	0.66	0.64	0.64	0.68	0.64	0.68	0.67	0.49	0.68	0.67	0.61	0.69	0.68	0.68	0.63	0.69	0.50	1.00					
Erect. leaf	0.57	0.57	0.58	0.56	0.67	0.59	0.56	0.52	0.59	0.58	0.59	0.59	0.62	0.55	0.54	0.60	0.43	0.64	1.00				
Black. p. stem	0.67	0.64	0.70	0.64	0.60	0.64	0.71	**0.40**	0.64	0.63	0.62	0.70	0.52	0.71	0.64	0.67	0.51	0.67	0.54	1.00			
Shatt. punch	0.62	0.66	0.57	0.68	0.73	0.69	0.60	0.49	0.66	0.74	0.70	0.65	0.66	0.55	0.54	0.60	0.59	0.68	0.58	0.55	1.00		
Giant. plant	0.77	0.66	0.72	0.77	0.66	0.70	0.74	**0.42**	0.69	0.70	0.68	0.73	0.52	0.69	0.65	0.68	0.59	0.65	0.55	0.70	0.62	1.00	
Vigor. plant	0.54	0.53	0.53	0.51	0.53	0.50	0.54	0.50	0.52	0.58	0.52	0.61	0.64	0.55	0.52	0.54	0.45	0.58	0.54	0.52	0.56	0.52	1.0
Weak. plant	**0.81**	0.73	0.74	0.77	0.70	0.76	0.71	0.50	0.66	0.72	0.72	0.68	0.55	0.64	0.67	0.69	0.60	0.66	0.59	0.67	0.66	**0.79**	0.5

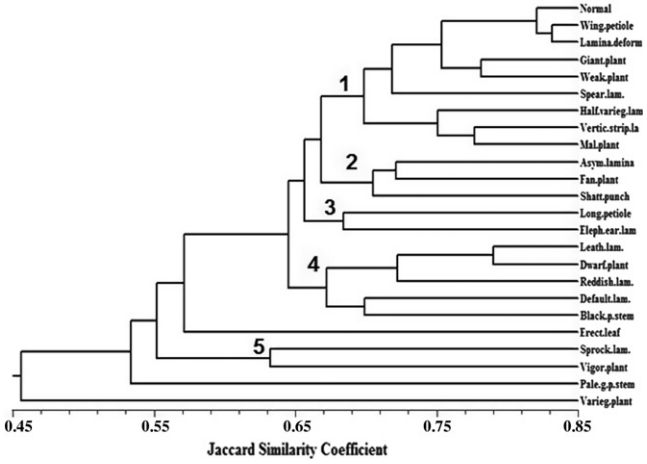

Figure 1 Cluster analysis of banana cultivar 'Grand Nain' and its 23 somaclones generated from RAPD data using Jaccard similarity coefficient and UPGMA clustering method.

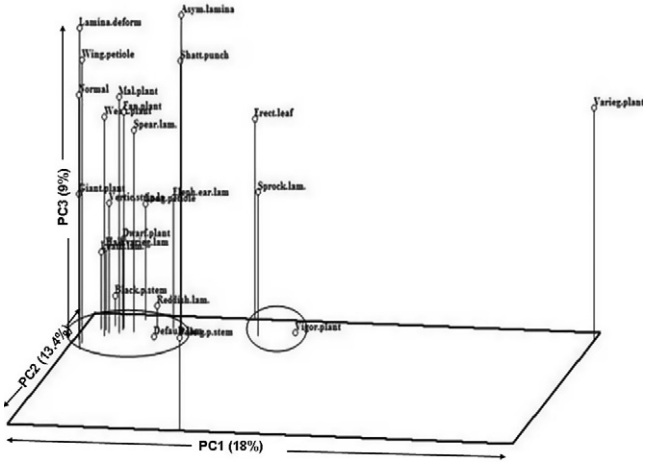

Figure 2 PCOORDA for banana cultivar 'Grand Nain' and 23 of its somaclonal variants based on RAPD analysis.

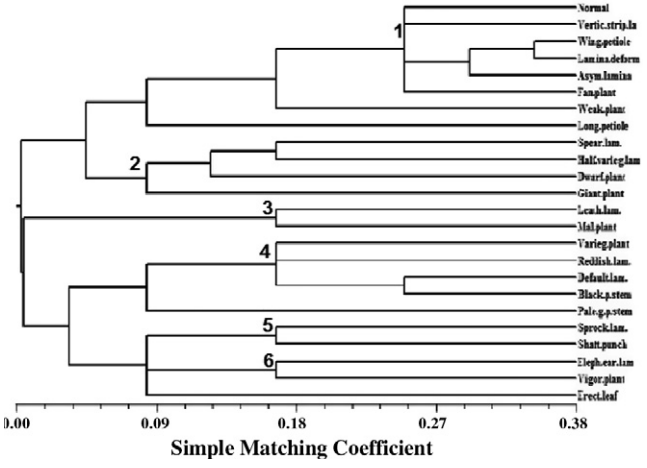

Figure 3 Cluster analysis of similarity tree for banana cultivar 'Grand Nain' and 23 of its somaclonal variants based on morphological parameters.

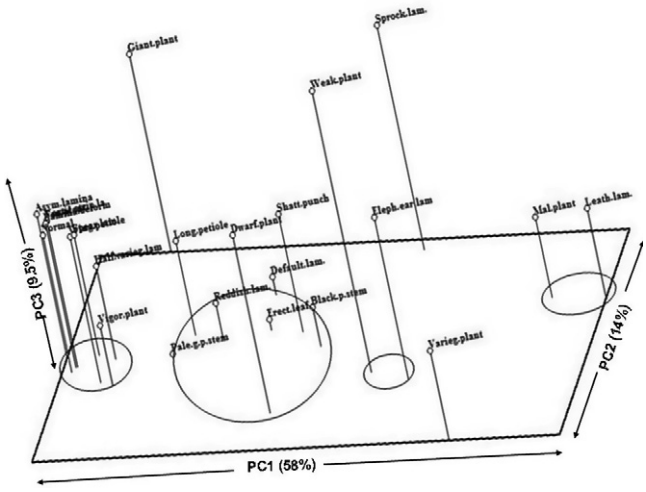

Figure 4 PCOORDA for banana cultivar 'Grand Nain' and 23 of its somaclonal variants based on morphological parameters.

agronomic characteristics from other variants, showed different pattern of DNA fragments, and appeared apart from other variants in the cluster analysis [5]. That was exactly what we obtained from either growth stages or previous cluster analysis. The same thing can be noted about the 'erected leaf' and 'sprocket lamina' and other variants. They gave the same trend all over growth stages, fruiting stage and cluster analysis.

3.4. Cluster analysis based on morphological data

According to cluster analysis of the morphological data, the variants were clustered with different degrees of similarity in relation to the normal plant (Fig. 3). The variants and their normal parent plant ('Grand Naine' banana cultivar) were clustered into six clusters (Fig. 3). The first cluster included the normal plant (cultivar 'Grand Naine') and the variants 'winged petiole', 'asymmetric lamina', 'deformed lamina', 'vertically stripped lamina' and 'fan shape plant'. The second cluster contained 'spear shape lamina', 'half variegated lamina', 'dwarf plant' and 'giant plant' variants. The variants 'weak plant' and 'long petiole' were aligned between the first and the second cluster (Fig. 3). The third cluster consisted of 'leathery lamina' and 'malformed plant' variants, while the fourth cluster contained 'variegated plant', 'reddish plant', 'default lamina' and 'blackened pseudo stem' variants. The fifth cluster included 'sprocket lamina' and 'shattered bunch' types and the last cluster contained 'elephant ear shape lamina' and 'vigor plant' variants. The variant 'pale green pseudo stem' was in the middle space between the fourth and the fifth cluster, while the 'erected leaf' variant was clustered separately from the sixth cluster (Fig. 3).

3.5. Principal coordinate analysis based on morphological data

According to the principal coordinate analysis (PCOORDA), the first three principal coordinates (PCs) represent 81.5% of the total variation (58% for PC1, 14% for PC2 and 9.5% for PC3) (Fig. 4). The variants were distributed based on the first PC with different degrees of variability, whereas the 'var-

iegated plant', 'malformed plant' and 'leathery lamina' variants were distributed at very high level of the first PC. The following variants were aggregated near from the middle of PC1: 'sprocket lamina', 'erected leaf', 'pale green pseudo stem', 'weak plant', 'elephant ear shape lamina', 'blackened pseudo stem', 'default lamina', 'dwarf' plant 'reddish lamina' and 'long petiole'. At the second PC, there were no high differences among the variants in relation to their distribution at this level except for 'sprocket lamina' and 'giant plant' that was located at the most high level of that PC and the 'variegated plant' that was separated at the most low level of PC2 (Fig. 4). At the third PC, there were high differences among the variants in relation to their distribution at this level. 'Pale green pseudo stem' and 'erected leaf' variants were located at very low level of the PC3, while 'sprocket lamina', 'weak plant' and 'giant plant' variants were located at high level of the third PC (Fig. 4).

Most of variants aggregated together in the different PCs, which may indicate that most variants might reflect epigenetic changes, which happened during *in vitro* culture. This point of view was in agreement with Larkin and Scowcroft, [9]. They reported that somaclonal variation could be defined as genetic variability generated during *in vitro* culture. On the other hand, Shailesh et al. [19] stated that the composite data indicated that such off types were somaclonal variation and were not the result of epigenetic factor(s).

In our study, the variants 'variegated plant', 'pale green pseudo stem' and 'vigor plant' were genetically different from the normal plant according to the PCOORDA analysis suggesting that their phenotype is conferred by genetic rather than epigenetic effect. The same results could be obtained from both morphological and RAPD analyses. Concerning the reasons of somaclonal variation, Damasco et al. [3] strongly indicated that adventitious shoot multiplication is the main factor contributing to the formation of 'dwarf' off-types. Adventitious buds were promoted by high concentrations of benzyl amino purine [benzyl adenine], by splitting propagules longitudinally during micropropagation and by preferentially selecting bulbil-like structures as propagules for further multiplication. The inherent instability of the cultivar being micropropagated was another major factor influencing the production of 'dwarf' off-types. Oh et al. [11] reported that the molecular basis of somaclonal variation is not precisely known but both genetic and epigenetic mechanisms have been proposed. The available evidence points toward the existence of labile portions of the genome that can be modulated when the cells undergo the stress of tissue culture.

The early diagnosis of somaclonal variation has been tested using DNA markers whereas James et al. [7] stated that the source of this variation may derive from variation pre-existing in the mother plant or it may be induced *in vitro*. Many factors are known to influence *in vitro* induced variation, however, it has been proposed that hypo- or hypermethylation of DNA, which may trigger genome-wide changes, may be the underlying cause.

It can be concluded that the banana cultivar 'Grand Naine' was more related to 'winged leaf' variant, and both of them were related to 'leathery lamina' variant. It can be noted also that 'blackened pseudo stem' variant was related to the 'giant plant' variant. Most of variants aggregated together in the different PCs, this may indicated that most variants may reflect epigenetic changes which happened during *in vitro* and other may reflect real genetic variation. The variants 'variegated plant', 'pale green pseudo stem' and 'vigor plant' genetically different from the normal plant according to the PCOORDA analysis which may suggested that, their change are not epigenetic. Also, the results obtained from both molecular and morphological analyses were so far in contiguous with better resolution when using the PCOORDA analysis than cluster analysis.

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