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ARTICLE

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

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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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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			+