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SURVEY, COLLECTION AND CHARACTERIZATION OF ELITE (HEAVY BUNCH) SOMACLONAL VARIANTS FROM TISSUE CULTURED 'GRAND NAINE' BANANA (*Musa spp.* AAA) IN FARMERS' FIELDS AROUND BANGALORE

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ABSTRACT : The present study was carried out during the period 2006-07 to characterize the elite (heavy bunch) somaclonal variants of tissue cultured 'Grand Naine' banana from the farmers' fields around Bangalore by visual screening. A total of eleven elite variants were collected and compared with original 'Grand Naine' (control) plants. Of all the elite somaclonal variants collected and evaluated, the variant GNV-04 was found very promising. It had significantly showed higher bunch weight (59.75 kg), bunch length (2.00m), number of hands per bunch (21.01) and number of fingers per hand (20.01). The quality parameters such as TSS, reducing and total sugars were significantly higher, with moderate titratable acidity. The organoleptic evaluation tests significantly favoured the control to a certain extent, but taste and texture were better with the variant GNV-04 compared to control. To confirm the variants at DNA level, RAPD analysis was conducted to identify the difference in the banding patterns. Forty three primers were used for the analysis of which OPF-09 differentiated the variants and the normal Grand Naine bananas. A band size of 320 bp was produced in all the normal samples but was absent in the variants tested. In the present study RAPD markers were proved to be effective and precise to confirm the variants identified using molecular characters. Of the eleven superior variants analysed, variants GNV-04, GNV-08 and GNV-10 showed positive phenotypic characters which could be used in developmental programmes of Grand Naine banana.

Keywords : *Somaclonal variation, Musa, Grand Naine, RAPD marker.*

Banana is an important fruit crop that has replaced the other tropical fruit crops in terms of production and productivity. The genus Musa L. comprises members that are important as food and cash crops in the humid tropics. Its centre of origin is thought to be the Indo-Malaysian axis (Simmonds, 27), but it has spread to most tropical and subtropical regions of the world. *In vitro* propagation of Cavendish group banana (*Musa sp.* 'AAA') is gaining importance in the banana industry. However, the occurrence of somaclonal variants is at present limiting the use of tissue cultures plants in spite of several advantages. Banana being a polyploid and vegetatively propagated crop, somaclonal variants obtained by tissue culture technique also can provide a rapid and reliable approach for plant improvement. Plantains and bananas have become the subjects of intense improvement programmes in which modern biotechnological methods which had contributed significantly to the genetic improvement (Gordian and Philip, 6). Apart from faster multiplication rates in lab conditions, tissue cultured bananas pose many advantages like regular availability, earliness, synchronized blooming and comparatively higher yields. However, the technique has been reported to predispose plant materials to chromosomal instability which does not preclude the genomic instability that ordinarily arises due to cryptic chromosomal rearrangements, somatic crossing over with sister chromatid exchanges, transposable elements, and gene amplification/diminution phenomena (Mantell, 17; Hartwell *et al.,* 7).

But, the awful reports of somaclonal variations among in vitro plants produced by many biofactories are limiting the expansion in the use of tissue-cultured bananas. Unsatisfied farmers have even approached consumer courts seeking compensation for the heavy losses incurred in the fields due to dismal performance of somaclonal variants among tissue cultured plants which are rather higher in cost (Shiddlingeswara *et al.,* 26). The phenomenon of somaclonal variation can be defined as a genetic variability generated during in vitro culture. Several features of the in vitro technique may raise the rate of somaclonal variations observed in plants management and other poor agronomic practices followed by the farmers. By doing so they are denying the contributions of pre-existed genetic variability and over exploited in vitro techniques. Hence

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the present study was conducted to confirm the genetic variability using RAPD in somaclonal variants in order to prove that the somaclonal variations among tissue cultured plantlets are the results of either the pre-existed genetic variability of over exploited in vitro techniques and not due to managerial problems.

In *Musa* sp., somatic mutations (Samson, 24) and somaclonal variations (Vuylsteke *et al.,* 34; Sandoval *et al.,* 25) have been implicated in genome instability. Furthermore, viral particles have been reported to interact with the Musa genome to destabilise the genome, especially under in vitro culture environments. Naturally, changes occur in the genomes of plants, but their rates are slow and natural selection removes deleterious ones from the milieu. However, *in vitro* systems quicken the mutation rate because additional selection pressure is placed on the cultured material manifesting as somaclonal variations. Somaclonal variations, are not altogether undesirable since some may serve as novel raw material for further crop improvement (Larkin and Scowcroft, 14).

The problem, however, is that generating somaclonal variants is unpredictable since the type and extent of variation or even synergistic processes forming them are random events. Osuji *et al.* (14) noted that the instability at the genotype level of *Musa* compromises the conventional idea of using phenotypic characters for molecular marking of Musa material. Gordian and Philip (6) worked on tagging useful chromosomal changes in Musa sp. Several research efforts have looked into unravelling the genotypic constitution of *Musa* plants, relying on molecular cytogenetic techniques (Kosina and Heslop-Harrison, 10; Osuji *et al.,* 19 & 20). Consequently, the present effort was aimed at screening the normal regenerants and somaclonal variants of tissue cultured *Musa* cultivars.

One difficulty in dealing with somaclonal variation is identification of any genetic variation with the regenerated plants. A number of different molecular techniques are currently available to detect sequence variation between closely related genomes such as those between source plants and somaclones. Representational difference analysis (RDA) has been applied to detect variation in a limited number of plant species (Cullis and Kunert, 3; Donnison *et al.,* 4; Oh and Cullis 18; Vorster *et al.,* 33; Zoldos *et al.,* 37). Random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) (Labra *et al.,* 13; Linacero *et al.,* 15). Both the techniques are useful in comparing the DNA from any number of different samples for the differentiation of plants because of sequence variation by identifying random polymorphisms. Because RAPD polymorphisms result from either a nucleotide base change that alters the primer binding sites or from an insertion or deletion within the amplified region (Williams *et al.,* 1993), polymorphisms usually result in the presence or absence of an amplification product from a single locus (Tingey and Tufo, 32). The products of these amplifications can be polymorphic and are useful as genetic markers (Hu and Quiros, 8). In this report, we used the RAPD technique to characterize the somaclonal variant for predictive marking of the Musa lines that may be used for incorporation into improvement programmes.

MATERIALS AND METHODS

Morphological Data

Eleven superior variants with four controls were selected from the orchards growing tissue cultured Grand Naine bananas around Bangalore, India. Various growth parameters as pseudostem height, pseudostem circumference, number of leaves per plant, basal leaf length, basal leaf breadth and basal leaf area, yield and reproductive parameters as bunch weight, bunch length, number of hands per bunch, number of fingers per hand, finger weight, finger length, finger diameter and peel thickness and quality parameters as total soluble solids, reducing sugars, total sugars, titratable acidity and organoleptic evaluation for physical and pulp attributes were taken for main crop at bunch harvesting stage. The mean of four normal plants was used as a control for the morphological characters. Analysis, correlation and interpretations were made by employing the RCBD method for field studies and CRD method for laboratory studies as suggested by Fischer and Yates (5) and Sunderraj *et al.* (30), respectively.

DNA isolation

50g of young leaves were collected, pre-treated by washing with distilled water, wiped with 70% (v/v) ethanol, then air dried prior to storage in sealed plastic bags at 4°C. DNA was extracted according to a modified Cetyl trimethyl ammonium bromide (CTAB) method followed by Simon *et al.* (28). 2g of leaf sample was powdered in liquid nitrogen to extract DNA. The powder was mixed with 10 ml extraction buffer, preheated to 65°C, and containing 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 3% (w/v) CTAB, 2% Polyvinyl pyrrolidone and 1% â-mercaptoethanol, then incubated at 65°C for 1 h. The mixture was cooled to room temperature, 6 ml cold 24:1 (v/v) chloroform :

isoamylalcohol was added, and the contents were mixed well. After centrifugation at 6,500/x/g for 8 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform:isoamylalcohol step was repeated until a clear supernatant was obtained. 5 M NaCl was added to the supernatant (0.5 v/v) and mixed gently followed by addition of 1 volume of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C, and then centrifuged at 7,500/x/g for 15 min. The resulting pellet was washed with 70% (v/v) ethanol, air dried, and dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Two µg RNase (Bovine pancreatic ribonuclease, Bangalore Genei, Bangalore, India) was added to each sample which was incubated for 3 h at 37°C, mixed with an equal volume of phenol and centrifuged at 7,500/x/g for 10 min at room temperature. This step was followed by a washing with an equal volume of 1:1 (v/v) phenol:chloroform then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol cold isopropanol, and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

PCR amplifications

PCR amplification followed the protocol of Williams *et al.* (36) with minor modifications. Of the 60 primers screened using the pool DNA, 43 clear and distinguishable bands were selected for RAPD-PCR analysis. Reproducibility of the primers was tested by repeating the PCR amplification three times under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 30 ng template DNA, 150 µM each dNTP, 1.5 mM $MgCl_2$, 1.5 units Taq DNA polymerase (Sigma Aldrich Chemicals, Bangalore, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a Corbett Research Thermocycler (Corbett Research Mortlake, New South Wales, Australia), programmed for an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were resolved in a 1.2% (w/v) agarose gel, visualized and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, US). Each reproducible band was visually analysed for the presence and absence between the normal and somaclonal variants. The band sizes were determined by comparing with 500 bp DNA ladder (Genei, Banglore, India), which was run along with the amplified products.

RESULTS AND DISCUSSION

The appearance of somaclonal variants may not be a desirable process in propagation (Cullis, 2). Various types of mutations have been described in somaclonal variants, including point mutations, gene duplication, chromosomal rearrangements, and chromosome number changes (Kaeppler *et al.,* 9; Peschke and Phillips, 21; Phillips *et al.,* 22). Transposable element movement and changes in DNA methylation (Koukalova *et al.,* 11; Kubis *et al.,* 12; Smulders *et al.,* 29), possibly through the function of small interfering RNA (Lippman *et al.,* 16), have also been implicated as potential mechanisms behind somaclonal variations. The trigger for all these types of changes can be described as genomic shock or plasticity, which occurs after the plant has exhausted its ordinary physiological responses to environmental stress (Cullis, 1). This genomic shock response may be a radical, but limited, genomic reorganization, which is an adaptive mechanism and can be activated under stress. The occurrence of hotspots of mutation and recurring menus of alternative alleles is consistent with this response being limited to a sub fraction of the genome.

The clonal propagation of horticultural species and crops, such as bananas and oil palm, is intended to produce elite individuals in mass and negative variations are problematic, where even a low per cent are unacceptable for commercial use (Thomas *et al.,* 31). Significant differences were obtained in the morphological characters between the Grand Naine normal and variants analysed (Table 1). A maximum height and circumference of the pseudostem was obtained in the variants GNV-10 (4.11 m) and GNV-04 (90.00), respectively. Supreme bunch weight of 59.75 kg (GNV-04), 50.75 kg (GNV-10) and 48.21 kg (GNV-08) was obtained when compared with the average weight to normal 36.83 kg. The bunch length varied from 0.80 to 2.00 m among the variants contrasting to the mean length of 1.27 m in the normal plants. The bunch of variants GNV-04 and GNV-10 comprised of 21 and 18 hands with an average of 20.01 and 18.01 fingers, respectively. A maximum finger weight of 157.50 g in GNV-07 and a minimum weight of 136.50 g in GNV-05 were noticed. The quality parameters such as total soluble solids, reducing sugars, total sugars and titratable acidity did not show considerable variations between the normal and

Table 1: Growth and bunch characters of the field identified normal and variants of 'Grand Naine' banana.

Sl. No	Phenotype	Pseudostem		No. of leaves	Basal leaf			Bunch		No. of hands per bunch	No. of fingers per hand
		Height (m)	Circumference (cm)		Length (m)	Breadth (m)	Area ($cm^{2)}$	Weight (Kg)	Length (m)		
1.	GNV-01	2.32	61.50	11.0	1.71	0.61	102.15	40.25	0.80	12.0	16.01
2.	GNV-02	2.22	62.02	12.0	1.81	0.62	111.65	41.24	1.75	10.0	17.01
3.	GNV-03	3.80	84.01	10.0	2.11	0.75	157.50	39.80	1.25	11.0	18.01
4.	GNV-04	4.00	90.00	10.0	3.01	0.68	204.15	59.75	2.00	21.0	20.01
5.	GNV-05	2.31	76.00	09.0	1.90	0.71	134.75	40.50	1.01	14.0	17.01
6.	GNV-06	2.25	69.00	12.0	1.61	0.74	118.50	39.01	0.91	10.0	15.01
7.	GNV-07	3.81	62.50	12.0	1.70	0.71	120.65	42.70	1.01	11.0	18.01
8.	GNV-08	3.21	71.00	11.0	1.50	0.68	102.15	48.21	1.23	13.0	18.00
9.	GNV-09	2.81	65.50	09.0	2.11	0.70	146.50	38.50	0.81	09.0	16.01
10.	GNV-10	4.11	86.01	10.0	3.11	0.61	292.25	50.75	1.75	18.0	18.01
11.	GNV-11	2.65	65.01	12.0	2.80	0.64	182.01	39.71	0.84	10.0	15.00
GN (control)		2.16	61.73	10.76	1.82	0.62	113.82	36.83	1.27	10.77	16.39
CD (P=0.05)		0.019	0.380	0.380	0.010	0.017	0.652	0.105	0.021	0.071	0.391

variant fruits (Table 2). Among the variants GNV-04 showed promising yield characters compared to other variants selected. The bunch, hand and finger characteristics of normal and variant (GNV-04) are shown in Figures 1-4. The organoleptic evaluation for physical and pulp attributes of somaclonal variant GNV-04 is significantly better than control (Table 3).

2g of young leaves preferably cigar leaves, were used to extract DNA, as mature leaves were highly fibrous and, rich in polyphenols and polysaccharides that hindered the extraction of PCR quality DNA. The pre-treatment of the leaves removed dust particles and external microbial contaminations. The CTAB method for DNA extraction was found optimal to release the nucleic acid from the cell and, to remove RNA and

Table 2: Finger and quality characters of the field identified normal and variants of 'Grand Naine' banana

Sl. No	Phenotype	Finger			Peel thickness (mm)	Total Soluble Solids (^{0}Brix)	Reducing Sugars (%)	Total Sugars (%)	Titratable acidity (%)
		Weight (g)	Length (cm)	Circumference (cm)					
1.	GNV-01	150.70	17.30	12.02	1.02	18.01	13.04	16.70	1.04
2.	GNV-02	154.50	18.03	10.70	0.82	19.01	16.33	17.50	1.04
3.	GNV-03	151.00	19.02	10.40	1.02	20.02	15.97	19.50	0.90
4.	GNV-04	141.50	16.65	11.70	1.22	23.55	16.02	21.01	0.85
5.	GNV-05	136.50	16.35	11.25	1.05	19.40	14.21	18.50	0.95
6.	GNV-06	152.25	17.45	12.55	0.97	21.03	16.13	19.01	1.01
7.	GNV-07	157.50	15.70	13.03	1.02	22.00	14.14	19.45	1.01
8.	GNV-08	155.50	16.15	11.90	1.05	20.01	15.83	19.02	1.02
9.	GNV-09	148.45	16.75	10.80	0.92	19.40	13.92	16.25	0.91
10.	GNV-10	151.50	17.70	12.00	0.82	21.72	16.23	20.03	0.80
11.	GNV-11	150.30	17.01	13.30	0.92	19.02	15.13	18.70	0.81
GN (control)		154.13	18.98	13.38	0.93	20.43	15.52	19.38	0.84
CD (P=0.05		0.858	0.371	0.285	0.020	0.177	0.031	0.217	0.020

Figure 1: **Matured bunch of field identified variant (GNV-04)**.

Figure 2: **Ripened bunches of field identified normal and variant (GNV-04) 'Grand Naine' banana.**

Figure 3: **Ripened hands of field identified normal and variant (GNV-04) 'Grand Naine' banana**

Figure 4 : **Ripened fingers of field identified normal and variant (GNV-04) 'Grand Naine' banana**

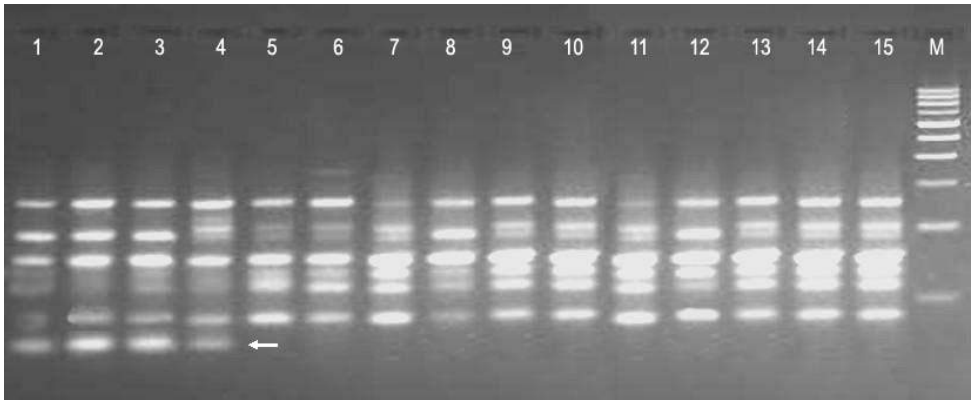

Fig. 5: **Gel profile of normal and somaclonal variants of banana using RAPD-PCR primer OPF-09.**
Normal banana (Lane 1-4): 'GN-1', 'GN-2', GN-3' and 'GN-4'. **Somaclonal variants (Lane 5-15):** 'GNV-1', 'GNV-1', 'GNV-2', 'GNV-3', 'GNV-4', 'GNV-5', 'GNV-6', 'GNV-7', 'GNV-8', 'GNV-9', 'GNV-10' and 'GNV-11'. **Lane M:** 500 bp DNA ladder. **Arrow:** OPF-09_{320}

Table 3: Sensory evaluation for physical and pulp attributes of the field identified normal and variant 'Grand Naine' banana fruits

Sl. No	Sample Code	Physical attributes					Pulp attributes				
		VA	Fruit Colour	Fruit Size	Fruit Shape	OA	Taste	Firmness	Aroma	Grittiness / Texture	OA
1.	GN (control)	3.51	3.00	2.81	2.51	3.24	3.21	2.51	1.80	3.02	3.02
2.	GNV-04	3.24	2.51	3.02	4.01	3.01	4.01	2.01	1.83	3.30	4.01
	CD(P=0.05)	0.048	0.068	0.125	0.091	0.048	0.091	0.091	0.048	0.088	0.125

* = Significant at 5% 6-point hedonic scale
VA - Visual Appearance; OA - Overall Acceptability

proteinaceous contamination rendering the DNA suitable for PCR amplifications. PCR amplification procedure followed was the standard protocol reported by Williams *et al.* (36) with minor modifications, which produced good amplifications with 30 ηg of template DNA. The amplifications using 1.5 units of Taq DNA polymerase and 1.5 mM $MgCl_2$ produced intense and clear banding patterns. A primary screening of 60 RAPD primers resulted in selection of 43 primers that produced clear and reproducible fragment patterns. Screening is essential to save time, cost and to reject primers not informative for the analysis (Prakash *et al.,* 23).

RAPD has proved to be useful in identifying regions of the banana genome that vary between normal and somaclonal variants. The primer OPF-09 proved useful to differentiate the variants and the normal Grand Naine bananas by producing a polymorphic band of size 320 bp found in all the normal samples but was characteristically absent in the somaclonal variants tested (Figure 5). This evidence is consistent with the fact that all the sequences present in the somaclonal variants are also present in the normal plants, but not *vice versa*. The production of a single marker (OPF-09320) differentiating normal and the somaclonal variants suggesting the presence of a limited set of loci being modified during the generation of variation due to in vitro conditions. The region is vulnerable to large number of mutations that arise frequently during in vitro propagation. This variation of the sequence also makes the region a candidate for identifying single-nucleotide polymorphisms as possible markers developed through *in vitro* propagation of Grand Naine bananas. The findings of this study are consistent with the notion that there is a labile fraction of the genome that is modified during the generation of somaclonal variation in banana. The difference in the products reported (OPF-09320) should have all the characteristics of representatives of the susceptible region of the genome.

The primer OPF-09 was tested effectively to distinguish individuals developed from commercial in vitro propagation procedure. Small number of variants was successfully tested based on the amplifications produced by this primer, yet a larger number of individuals are essentially to be evaluated. The phenotype of the identified individuals should be determined to confirm the association of the marker and the specific phenotype. Large number of decamer primers could also be tested to identify the frequency of liable regions within the banana genome. However, the preliminary evidence indicates that primer OPF-09 can be used as a diagnostic key for identifying somaclonal variants in Grand Naine banana plants produced through in vitro propagation. The marker reported here and the primer has the potential of being developed into a robust diagnostic DNA marker to ascertain somaclonal variation. Among the eleven positive variants tested three variants (GNV-04, GNV-08 and GNV-10) showed encouraging morphological characters and could be used in breeding programmes for the development of new banana varieties.

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REFERENCES

1. Cullis, C.A. (1999). Environmental stress – a generator of adaptive variation? In: Lerner HR (ed) *Plant Adaptations to Stress Environments.* Marcel Dekker, New York, pp 149–160
2. Cullis, C.A. (2005). Mechanisms and control of rapid genomic changes in flax. *Ann. Bot.,* **95** : 201–206

3. Cullis, C.A. and Kunert, K.J. (2000). Isolation of tissue culture-induced polymorphisms in bananas by representational difference analysis. *Acta Hort.,* **530** : 421–428
4. Donnison, I.S., Siroky, J., Vyskot, B., Saedler, H. and Grant, S.R. (1996). Isolation of y-chromosome-specific sequences from *Silene latifolia* and mapping of male sex-determining genes using representational difference analysis. *Genetics*, **144**: 1893–1901
5. Fischer, R.A. and Yates, M. (1963). *Statistical Tables for Biological, Agricultural and Medicinal Research,* Oliver and Boyd, Edinburg.
6. Gordian, C.O. and Philip, C.A. (2007). Evaluation of karyotype status of *Musa* L. somaclonal variants (Musaceae: Zingiberales). *Turkish J. Bot.,* **31**: 143-147
7. Hartwell, L.H., Hood, L., Goldberg, M.L., Reynolds, A.E., Silver, L.M. and Veres, C.R. (2000). *Genetics: from genes to genomes.* New York: McGraw- Hill.
8. Hu, J. and Quiros, C.F. (1991). Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Repts,* **10**: 505-511
9. Kaeppler, S. M., Kaeppler, H. F. and Rhee , S. (2000). Epigenetic aspects of somaclonal variation in plants. *Plant Mol. Biol.,* **43** : 179–188
10. Kosina, R. and Heslop-Harrison, J. S. (1996). Molecular cytogenetics of an amphiploid trigeneric hybrid between *Triticum durum, Thinopyrum distichumand, Lophopyrum elongatum. Ann. Bot.,* **78**: 583-589
11. Koukalova, B., Fojtova, M., Lim, Y. K., Fulnecek, J., Leitch, A. R. and Kovarik, A. (2005). Dedifferentiation of tobacco cells is associated with ribosomal RNA gene hypomethylation, increased transcription, and chromatin alterations. *Plant Physiol.,* **139** : 275–286
12. Kubis, S. E., Castilho, A.M.M.F., Vershinin, A.V. and Heslop-Harrison, J. S. (2003). Retroelements, transposons and methylation status in the genome of oil palm (Elaeis guineensis) and the relationship to somaclonal variation. *Plant Mol. Biol.,* **52** : 69–79
13. Labra, M., Savini, C., Bracale, M., Pelucchi, N., Colombo, L., Bardini, M. and Sala, F. (2001). Genomic changes in transgenic rice (*Oryza sativa* L.) plants produced by infecting calli with Agrobacterium tumefaciens. *Plant Cell Repts,* **20** : 325–330
14. Larking, T. J. and Scrowcroft, W. R. (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.,* **60** : 197
15. Linacero, R., Alves, E. F. and Vazquez, A.M. (2000). Hot spots of DNA instability revealed through the study of somaclonal variation in Rye. *Theor. Appl. Genet.,* **100** : 506–511
16. Lippman, Z., May, B., Yordan, C., Singer, T. and Martienssen, R. (2003). Distinct mechanisms determine transposon inheritance and methylation *via* small interfering RNA and histone modification. *PLoS Biol.,* **1** : 420–428
17. Mantell, S. H., Matthews, J. A., McKee, R. A. (1985). *Principles of Plant Biotechnology. An Introduction to Genetic Engineering in Plants.* London: Blackwell Scientific Publications.
18. Oh, T. J., Cullis, C. A. (2003). Labile DNA sequences in flax identified by combined sample representational difference analysis (csRDA). *Plant Mol. Biol.,* **52** : 527–536
19. Osuji, J. O., Crouch, J., Harrison, G. and Heslop-Harrison JS (1998). Molecular cytogenetics of Musa species, cultivars and hybrids: Location of 18S-5.8S – 25S and 5S rDNA and Telomere-like sequences. *Ann. Bot.,* **82** : 243-248
20. Osuji, J.O., Harrison, G., Crouch, J. and Heslop-Harrison, J. S. (1997). Identification of the genomic constitution of *Musa* L. lines (Bananas, Plantains, Hybrids) using molecular cytogenetics. *Ann. Bot.,* **80** : 787-793
21. Peschke, V. M. and Phillips, R. L. (1992). Genetic implications of somaclonal variation in plants. *Adv. Genet.,* **30** : 41–75
22. Phillips, R. L., Kaepler, S.M. and Olhoft, P. (1994). Genetic instability of plant tissue cultures: breakdown of normal controls. *Proc. Natl. Acad. Sci.* USA **91** : 5222–5226
23. Prakash, D.P., Narayanaswamy, P. and Sondur, S.N. (2002). Analysis of molecular diversity in guava using RAPD markers. *J. Hortic. Sci. Biotech.,* **77** : 287-293.
24. Samson, J. A. (1982). *Tropical Fruits.* New York: Longman.
25. Sandoval, J., Tapia, A., Muller, L. and Villabobos, V. (1991). Obervaciones sobre la variabilidad encontrada en plantas micropropagades de Musa cv. Falso Cuerno AAB. *Fruits,* **46** : 533-539

26. Shiddlingeswara, T., Nachegowda, V., Narayanaswamy, P., Krishnamoorthy, K. B. (2004). Confirmation of field identified somaclonal variants in tissue cultured Cavendish bananas using RAPD markers. Proc. Nat. Sem. on Banana Industry-Present Scenario and Future Strategies, Department of Food Processing Industries and Horticulture, West Bengal.
27. Simmonds, N. W. (1960). Notes on banana taxonomy. 2nd Ed, *Kew Bulletin,* p206-211.
28. Simon, L., Shyamalamma, S. and Narayanaswamy P (2007). Morphological and molecular analysis of genetic diversity in jackfruit. *J. Hortic. Sci. Biotech.,* **82** (5) : 764-768
29. Smulders, M.J.M., Rus-Kortekaas, W. and Vosman, B. (1995). Tissue culture induced DNA methylation polymorphisms in repetitive DNA of tomato calli and regenerated plants. *Theor. Appl. Genetics,* **91** : 1257–1264
30. Sunderraj, N., Nagaraju, S., Venkatarama, H.N. and Jagannath, M.K. (1972). *Design and Analysis of Field Experiments,* University of Agricultural Sciences, Bangalore.
31. Thomas, J. O., Margaret, A. C., Karl, K., Iris, E., Rony, S. and Christopher, A. C. (2007). Genomic changes associated with somaclonal variation in banana (Musa spp.). *Physiol. Plantarum,* **129** : 766–774
32. Tingey, S.V. and Tufo, J. P. D. (1993). Genetic analysis with random amplified polymorphic DNA markers. *Plant Physiol.,* **101** : 349-352
33. Vorster, B.J., Kunert, K.J. and Cullis, C.A. (2002). Use of representational difference analysis for the characterization of sequence differences between date palm varieties. *Plant Cell Repts,* **21** : 271–275
34. Vuylsteke, D., Swennen, R., Wilson, G. F. and De Langhe, E. (1988). Phenotypic variation among in vitro propagated plantains (Musa spp. Cultivar AAB). *Scientia Hort.,* **36** : 79-88
35. Williams, J. G. K., Hanafey, M. K., Rafalski, J. A. and Tingey, S.V. (1993). Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymol.,* **218** : 704-740
36. Williams, J. G. K., Kubelik. A. R., Livak, K. J., Rafalski, J. A., Tingey SV (1990) DNA polymorphism amplified by arbitary primers are useful as genetic markers. *Nucleic Acids Res.,* **18** : 6531–6535.
37. Zoldos, V., Siljak-Yakovlev, S., Papes, D., Sarr, A. and Panaud, O. (2001). Representational difference analysis reveals genomic differences between *Q. robur* and *Q. suber:* implications for the study of genome evolution in the genus Quercus. *Mol. Genet. and Genom.,* **265** : 234–241

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