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Research Article

Evaluation of Karyotype Status of *Musa* L. Somaclonal Variants (Musaceae: Zingiberales)

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Abstract: Tissue culture regenerated materials of *Musa* L. spp. (banana and plantain) along with their somaclonal variants were assessed for karyotype abnormalities to be used as indicators for rapid cytological marking. The results show that although slight variations were noted in karyomorphometric characters, like chromosome number, arm length, total chromosome length, and total chromosome volume, these were not significant ($P \leq 0.05$). Therefore, chromosomal abnormalities may not be accurate markers for somaclonal variation in these species. The need to not overemphasise chromosomal instability resulting from culture techniques was highlighted. Other possible causes of somaclonal variations were proposed to explain this phenomenon in *Musa* spp.

Key Words: Tissue culture, somaclonal variants, karyotype, *Musa*, Zingiberales

Introduction

The genus *Musa* L. comprises members that are important as food and cash crops in the humid tropics (FAO, 1975). Its centre of origin is thought to be the Indo-Malaysian axis (Simmonds, 1960), but it has spread to most tropical and subtropical regions of the world. Plantains and bananas (*Musa* spp.) have become the subjects of intense improvement programmes in which modern biotechnological methods have played significant roles. Some of these techniques, however, have been reported to predispose plant materials to chromosomal instability. This 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, 1985; Hartwell et al., 2000).

In *Musa* spp., somatic mutations (Samson, 1982) and somaclonal variations (Vuylsteke et al., 1988; Sandoval et al., 1991) have been implicated in genome instability. Furthermore, viral particles have been reported to interact with the *Musa* genome (Glyn et al., 1991) 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, sensu stricto, are not altogether undesirable since some may serve as novel raw material (genetic diversity) for further crop improvement (Larkin & Scowcroft, 1981).

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. (1997) noted that the instability at the genotype level of *Musa* compromises the conventional idea of using phenotypic characters for molecular marking of *Musa* material. Several research efforts have looked into unravelling the genotypic constitution of *Musa* plants, relying on molecular cytogenetic techniques (Kosina & Heslop-Harrison, 1996; Osuji et al., 1997, 1998). Although this approach is accurate and quite promising, it is still necessary to develop a rapid and inexpensive method of tagging useful chromosomal changes in *Musa* spp.

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Consequently, the present effort was aimed at screening the karyotypes of normal regenerants and somaclonal variants of tissue cultured *Musa* cultivars for predictive marking of the *Musa* lines that may be used for incorporation into improvement programmes.

Materials and Methods

Sword suckers from tissue culture regenerated mother plants of plantain TMP59 (Agbagba cultivar) and desert banana TMB75 (Giant Cavendish cultivar) were used as a source of root tips for chromosome preparation. The suckers were obtained from the field gene bank of the International Institute of Tropical Agriculture (IITA), Onne, near Port Harcourt, Nigeria (GPS data: lat 4°51'N, long 7°3'E; 5 m above sea level). These were trimmed and transferred to pots and watered as required to produce a good crop of roots. Root tips were harvested from robust and actively growing secondary roots (ca. 1 cm) and placed in phosphate buffer (stock solution A = 3.50 g of KH_2PO_4 in 500 ml of water; stock solution B = 3.40 g of K_2HPO_4 mixed 3:2 parts) treated with 400 µl of 0.2% (w/v) β-mercaptoethanol, pH 7. This was to prevent oxidation, which results in discoloration of the roots, while the buffer solution ensured cell division until fixation was carried out. An aqueous solution of 0.02% (w/v) 8-hydroxyquinoline was used to pre-treat the root tips for 45-60 min in the dark since this chemical is photosensitive. A solution of absolute ethanol and glacial acetic acid in the ratio of 3:1 (v/v) was used to fix the root tips for at least 24 h at room temperature. Fixed roots were then stored in 70% ethanol until used for slide preparation.

Slide preparation

The stored root tips were rinsed in distilled water for 15 min, hydrolysed in 0.1 N HCl for 5 min, and washed in enzyme buffer (0.01 M critic acid/0.10 M trisodium citrate, pH 4.6) for 5 min. Then, the tips were sliced off and put on a petri dish placed over crushed ice and transferred to 0.01 M citric acid buffer (stock solution A = 1.47 g of trisodium citrate-dihydrate [$Na_3C_6H_50_7.2H_20$]; stock solution B = 1.05 g of citric acid-monohydrate [$C(OH)(COOH)$ ($CH_2COOH.H_2O$)] in 500 ml of water, pH 4.5); this was decanted and replaced with an enzyme mixture for digestion. The enzyme mixture, (1% (w/v) pectolase from *Aspergillus japonicus* Biochemika, Fluka, 1% (w/v) cellulase from *Trichoderma viride* Karlan, 10% (w/v), and pectinase from *Rhizopus* sp. Sigma Chem. Co) were used to digest the root tips at 37 °C for 2 h. The digested material was transferred to a mixture of Sorensen's and Leishman stain buffer for at least 10 min. Each root tip was gently macerated with forceps to remove the root cap and other recalcitrant material. The preparations were covered with cover-slips, cleared in xylene, and mounted in Deepex (DPX).

Slides were observed with an oil immersion objective lens and a Leitz Diaplan phase contrast microscope. Mitotic cells (n = 15) identified with metaphase or prometaphase stages were used for chromosome counting and measurement, both for the normal and somaclonal regenerants for the karyotype information used for assessment of materials. An ocular micrometer scale was used to measure the total length, breadth, and long and short arms of chromosomes for normal regenerants, and their somaclonal variants. Good plates at prometaphase were photomicrographed to reflect chromosome number and morphology.

Total chromosomal volume per treatment was computed as described by Naranjo et al. (1998). Student's t-test was used to test for significance in morphometric mean differences and total complement volume. A Leica Wild MPS 52 microscope camera was used to photograph good plates, using appropriate filters.

Results

Approximately 80%-85% of the chromosome counts showed a count of 3x = 2n = 33 in both the plantain and banana materials. Although there were phenotypically distinguishable descriptors for the somaclonal variants in each case, none were associated with any structural or numerical chromosomal abnormality. Similarly, chromosome morphology was not different in the normal regenerants and their somaclonal variants. Tables 1 and 2 show the karyotype morphometrics of both regular regenerants and somaclonal variants. The slight variations were not significant ($P \leq 0.05$) when subjected to statistical analysis. There were a few cases of variation in number, which were adjudged to be aneuploid cases in both the banana as well as plantain materials. Prometaphase and metaphase chromosomes were similar in both the regular and somaclonal variant chromosome spreads (Figure 1). There were slight differences in total chromosome volume (TCV) (Table 3) within and between the materials used in this study, although these were not significantly different ($P \leq 0.05$).

Table 1. Karyotype morphometrics of normal regenerants of plantain TMP 59 (Agbagba cultivar) and the somaclonal variant genomes.

Chromosome	Plant Source (regenerant)	Sample size n = 15 cells	Breadth X ± SE (µm)	Long arm X ± SE (µm)	Short arm X ± SE (µm)	Total length X ± SE (µm)
1	Normal		0.42 ± 0.01	1.54 ± 0.01	1.40 ± 0.02	2.94 ± 0.01
	Somaclonal		0.40 ± 0.01	1.31 ± 0.02	1.84 ± 0.01	2.15 ± 0.01
2.	Normal		0.39 ± 0.002	2.00 ± 0.01	0.65 ± 0.01	2.65 ± 0.02
	Somaclonal		0.41 ± 0.01	1.55 ± 0.0	1.40 ± 0.02	2.95 ± 0.01
3.	Normal		0.40 ± 0.01	2.00 ± 0.01	0.63 ± 0.01	2.63 ± 0.00
	Somaclonal		0.38 ± 0.10	2.00 ± 0.00	0.70 ± 0.00	2.70 ± 0.01
4.	Normal		0.37 ± 0.02	1.52 ± 0.001	1.00 ± 0.002	2.52 ± 0.02
	Somaclonal		0.37± 0.001	1.37 ± 0.00	0.89 ± 0.001	2.26 ± 0.11
5.	Normal		0.41 ± 0.10	1.30 ± 0.10	1.16 ± 0.03	2.46 ± 0.04
	Somaclonal		0.39 ± 0.00	1.24 ± 0.05	0.36 ± 0.01	1.60 ± 0.02
6.	Normal		0.40 ± 0.02	1.37 ± 0.01	0.76 ± 0.02	2.13 ± 0..02
	Somaclonal		0.35 ± 0.02	1.30 ± 0.01	1.17 ± 0.03	2.47 ± 0.01
7.	Normal		0.36 ± 0.01	1.30 ± 0.01	0.88 ± 0.00	2.18 ± 0.01
	Somaclonal		0.36 ± 0.00	1.22 ± 0.01	0.63 ± 0.01	1.85 ± 0.02
8.	Normal		0.40 ± 0.01	1.21 ± 0.02	0.63 ± 0.03	1.84 ± 0..01
	Somaclonal		0.37 ± 0.01	1.56 ± 0.01	1.42 ± 0.01	2.98 ± 0.01
9.	Normal		0.38 ± 0.00	1.02 ± 0.01	0.71 ± 0.02	1.73 ± 0.01
	Somaclonal		0.40 ± 0.00	1.29 ± 0.01	0.40 ± 0.01	1.69 ± 0.02
10.	Normal		0.36 ± 0.00	1.25 ± 0.01	0.39 ± 0.02	1.64 ± 0.02
	Somaclonal		0.41± 0.01	1.15 ± 0.01	0.36 ± 0.01	1.15 ± 0.01
11.	Normal		0.39 ± 0.01	1.15 ± 0.02	0.35 ± 0.02	1.52 ± 0.02
	Somaclonal		0.39 ± 0.01	1.30 ± 0.01	0.88 ± 0.01	2.18 ± 0.01

Table 2. Karyotype morphometrics of normal dessert banana TMB 75 (Giant Cavendish cultivar) and the somaclonal variant genomes.

Chromosome	Plant Source (regenerant)	Sample size n = 15 cells	Breadth X ± SE (µm)	Long arm X ± SE (µm)	Short arm X ± SE (µm)	Total length X ± SE (µm)
1	Normal		0.35 ± 0.00	2.03 ± 0.01	1.75 ± 0.01	2.78 ± 0.03
	Somaclonal		0.34 ± 0.02	2.01 ± 0.01	0.71 ± 0.01	2.27 ± 0.01
2.	Normal		0.29 ± 0.01	1.99 ± 0.02	0.54 ± 0.01	2.53 ± 0.01
	Somaclonal		0.28 ± 0.00	1.90 ± 0.01	0.50 ± 0.00	2.40 ± 0.02
3.	Normal		0.30 ± 0.02	1.46 ± 0.01	0.65 ± 0.01	2.11 ± 0.02
	Somaclonal		0.36 ± 0.01	1.44 ± 0.01	0.64 ± 0.01	2.08 ± 0.01
4.	Normal		0.34 ± 0.01	1.44 ± 0.01	0.35 ± 0.02	2.09 ± 0.01
	Somaclonal		0.36 ± 0.01	1.58 ± 0.02	1.35 ± 0.01	2.93 ± 0.01
5	Normal		0.31 ± 0.02	1.58 ± 0.01	1.20 ± 0.02	2.78 ± 0.02
	Somaclonal		0.39 ± 0.00	1.24 ± 0.05	0.36 ± 0.01	1.60 ± 0.02
6.	Normal		0.37 ± 0.01	1.84 ± 0.01	0.85 ± 0.01	2.69 ± 0.03
	Somaclonal		0.35 ± 0.01	1.83 ± 0.00	1.02 ± 0.01	2.85 ± 0.02
7.	Normal		0.29 ± 0.01	1.36 ± 0.01	1.21 ± 0.02	2.57 ± 0.02
	Somaclonal		0.28 ± 0.01	1.65 ± 0.01	0.74 ± 0.01	2.39 ± 0.01
8.	Normal		0.31 ± 0.01	1.67 ± 0.01	0.74 ± 0.01	2.41 ± 0.02
	Somaclonal		0.37 ± 0.02	1.44 ± 0.01	0.52 ± 0.01	1.96 ± 0.02
9.	Normal		0.28 ± 0.01	1.80 ± 0.02	0.48 ± 0.02	2.28 ± 0.01
	Somaclonal		0.34 ± 0.02	1.12 ± 0.01	0.66 ± 0.01	1.78 ± 0..01
10.	Normal		0.35 ± 0.01	1.46 ± 0.02	0.54 ± 0.02	2.00 ± 0.01
	Somaclonal		0.32 ± 0.01	1.36 ± 0.02	1.10 ±0.01	2.46 ± 0.02
11.	Normal		0.32 ± 0.02	1.04 ± 0.02	0.77 ± 0.01	1.81 ± 0.01
	Somaclonal		0.31 ± 0.01	1.03 ± 0.00	0.65 ± 0.01	1.68 ± 0.02

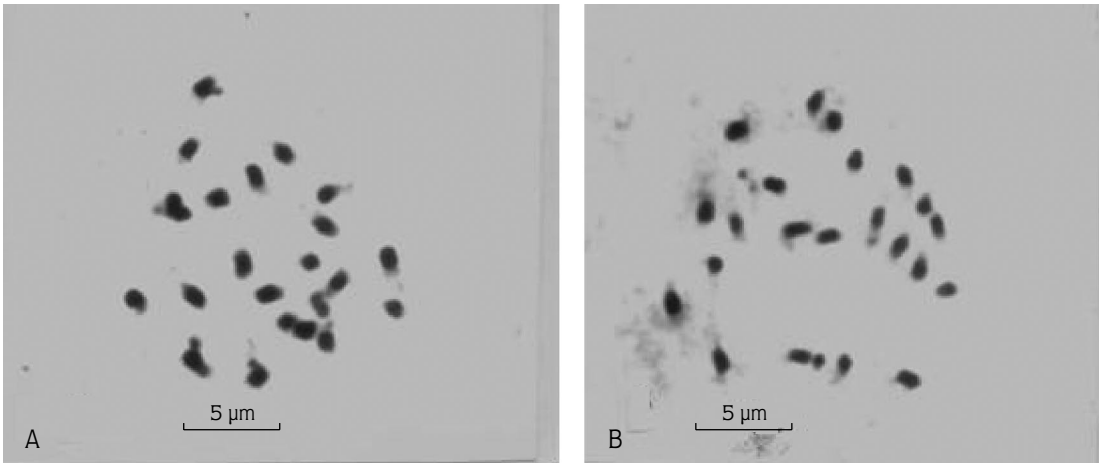

Figure 1. Karyotypes of plantain and banana tissue culture regenerants showing the prometaphase chromosomes of TMP 59 (Agbagba) (A) and prometaphase chromosome spread of TMB 75 (Giant Cavendish) (B).

Table 3. Total chromosome volume for Musa cultivars and their somaclonal variants.

Regenerants	TMP 59 (Agbagba)	TMB 75 (Giant Cavendish)
Normal genome	2.89	2.11
Somaclonal variants	2.76	2.03

Discussion

Against the backdrop of reports of chromosomal instability in the *Musa* genome, the results presented here appear rather unusual. Cell and tissue culture usually impart behavioural changes in telomeric sequences; nevertheless, Wang et al. (1992) noted that re-stabilisation of chromosomes may also come about by addition of telomeric sequences. The report by Vuylsteke et al. (1991) showing that tissue culture leads to somaclonal variation in *Musa* was part of what prompted this current effort; however, our results show that no visible numerical, morphological, or volumetric changes were observed between the normal and somaclonal regenerants of the *Musa* spp. used in this study. It is possible that the length of time the materials remained in culture was too short to induce these abnormalities. This outcome neither confirms nor contradicts the phenomenon in this species, as chromosomal changes may be cryptic rather than grossly structural. Osuji et al. (1997) showed that there are chromosomal changes that may only be deciphered with molecular cytogenetic techniques. Such techniques are used to amass information on repetitive DNA sequences and their applicability to chromosomal or genetic marking. Bearing this in mind, we looked for variation in total chromosomal volume (Table 3) and the slight differences observed were not significant ($P \leq 0.05$). Naranjo et al. (1998) affirmed, however, that variation in DNA sequences leads to karyotype variations as well. Although several researchers have reached similar conclusions (Vanit Hof et al., 1963; Baetcke et al., 1967; Bennet, 1972; Price et al., 1973), our results are not in agreement with these reports. Perhaps, we should look beyond the numerical, morphological, and volumetric differences as indicators of somaclonal variation, and instead look for answers in genic phenomena. Mantell (1985) reported that the presence of somatic crossover events in sister chromatid exchanges, as well as transposable elements, may be implicated in genome instability leading to the formation of somaclonal variants. This view was accented to by Glyn et al. (1999), who added that viral particles may be the culprits in *Musa* spp.

Furthermore, the manner of regenerating cultured material may influence the types of regenerants produced. For instance, there were differences in morphological lines relating to mineral and water characteristics between *Coffea arabica* plantlets regenerated in vitro and those generated by ex vivo methods (Barry-Ettiene et al., 2002). These observations were not linked to chromosomal phenomena in that study.

Since our results (Figure 1 A and B) do not reveal any numerical nor structural evidence to explain variation among supposed isolines, we propose that causes of genome instability in cultured *Musa* material should be searched for at the genic rather than chromosomal level.

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