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RESEARCH NOTE

Comparative analysis of regenerants between single and naked meristem (scalps) of *Musa acuminata* var. Berangan

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Abstract. The regeneration capacity of banana meristem *in vitro* was compared between single meristem and scalp methods. Scalps were induced on p4 media containing MS (Murashige and Skoog, 1962) components supplemented with coconut water and high concentration of benzyl amino purine (BAP) (75μM). The number of regenerated shoots produced from scalps was six-fold higher than that generated from single meristems.

A comparison of the growth response upon gibberelic acid (GA_3) treatment was made between plantlets derived from both methods to detect dwarf off-types. The difference in height and the number of leaves between plantlets from both sources was statistically insignificant. On the other hand, RAPD analysis using 40 random primers displayed approximately 26% variation in single meristem while naked meristem exhibited 74% variation.

Key words: Micropropagation, somaclonal variation, RAPD analysis, banana, tissue culture

INTRODUCTION

Banana is a popular fruit crop with significant global sales reaching up to 5 billion tons production per annum. It is consumed as a staple food in the tropical and African countries. Conventionally, introduction of new varieties depend on natural mutations which happens by chance. Its characteristics such as low fertility and various levels of ploidy has hampered the generation of genetic variability (Gregory *et al.*, 1995). Tissue culture has played a role in plantain and banana improvement program world wide (Rowe and Rosales 1996; Vulysteke *et al.*, 1997). New varieties have been introduced and commercialized using somaclonal variation and mutation breeding techniques. In this project, regenerants from scalps were assessed in the laboratory to verify the suitability of this explant for somaclonal variation studies. To date there is no report on the assessment of plantlets derived from scalps both in the laboratory and in the field. Although Schoofs (1997) has developed scalps as an intermediate stage for somatic embryogenesis using corm tissues as explants, no studies were conducted on the regenerants from the scalps.

MATERIALS AND METHODS

Tissue culture studies. Experiments were conducted using *Musa acuminata* cv. Berangan (AAA) meristems. Two single meristems were used for both propagation methods derived from the same mother plant. Meristems were cultured on MS media supplemented with different concentrations of BAP depending on their propagation methods. For propagation of single meristems, MS media supplemented with 33 μM BAP, 1μM IAA, 1μM d-biotin, 56μM asid ascorbic, 87μM sucrose, 2g/L gelrite and 150ml/L coconut water (p5) was used. Scalps were induced on similar media except for a different BAP concentration (75 μM) (p4).

Berangan cultivar was subcultured in p4 media five times in order to eliminate *in vitro* apical dominance. The number of shoots produced were counted after shoot buds were elongated on RM1 media (MS supplemented with 4.75 μM BAP) whereas the number of shoots for single meristems were counted at every stage of subculture. All regenerants were rooted in MS media with 1g/L activated charcoal for about 3 weeks before transferring to the nursery.

GA_3 treatments for dwarfism. This treatment was used by Reuveni *et al.* (1990). Two different concentrations of GA_3 were used for the treatment (5mg/L and 10mg/L). A total of 90 regenerants from scalps and single meristems each were used for every treatment. Regenerants were arranged in a completely randomized design with 15 replicates for each treatment. Mutant dwarf Cavendish var. Novaria were used as negative control. The data collected were analysed using MSTAT computer with LSD program.

Assesment of variation using RAPD analysis. Total

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Table 1. Number of buds and shoots produced from single meristem explants.

Stages	Total no. of clean explants	Total no. of buds and shoots	Total no. of contaminated explants	Multiplication rates (MR)
S1	1	0	0	0.0 ± 0.00
S2	3	3	0	1.00 ± 2.12
S3	10	7	0	0.70 ± 0.70
S4	35	25	1	0.71 ± 1.32
S5	76	41	6	0.54 ± 0.10
S6	146	70	21	0.48 ± 0.46
S7	498	352	118	0.70 ± 0.76
S8	830	332	230	0.40 ± 0.27

were analysed using MSTAT computer with LSD program.

Assesment of variation using RAPD analysis. Total genomic DNA was isolated from fresh leaves using modified CTAB-method (Doyle & Doyle, 1990). Random decamer primers (Operon Technologies. Inc.) were dissolved in ddH_2O at a concentration of 50ng/µl. Forty primers belonging to Operon kit OPJ and OPU were used for Polymerase Chain Reaction amplifications. A 25µl amplification reaction containing 10mM 10x PCR buffer, 100µM of dNTP's, 1.5mM $MgCl_2$, 50pMol 10-mer primer, 0.3 unit Taq DNA polymerase (Fermentas) and 50ng of genomic DNA were set up for each sample. PCR was carried out on the Mastercycler Gradient 5331 Eppendorf ver. 2.1 using the following steps as described by Williams *et al.*, (1990) with modifications by Weising *et al.*, (1995). Hot start; 1 cycle 94°C for 4 min. (initial denaturing step), followed by 45 cycles consisting of; 15 sec. at 94°C (denaturing), 45 sec. at 36°C (annealing), 90 sec. at 72°C (elongation), final elongation at 72°C for 4 min. After amplification, the reactions were stored at 4°C. The amplification products were analysed by agarose gel electrophoresis (1.4%) (Promega., Madison, WI, USA) containing ethidium bromide (0.25µg/ml). The amplification products were visualized under UV light (302nm) and photographed using 667 polariod film (Polariod (UK) Ltd, Hertfordshire, England).

RESULTS AND DISCUSSION

Regeneration frequency between single meristem and naked meristem. The total number of regenerated primordial shoots from single meristem is shown in Table 1. The regeneration capacity increased from stage 1 to 8 after culture. The total number of shoots after the eight subculture were approximately 830.

Table 2. Number of buds and shoots produced from induced meristem/scalp. Data is a mean value of 7 clumps from different types of clumps with 5-7mm diameter.

Type of clumps	No. of shoots and buds produced
1	16 1.41
2	6.29 ± 0.49
3	2.14 ± 1.46

The total number of shoots produced 4691 from 3587 different types of clumps.

Naked meristems produced 3 types of clumps with different regeneration capacity (Table 2). The mean diameter of the clumps was 5-7mm and they produced an average of 16 plantlets per clump. The total number of shoots observed at the end of stage 8 from all types of clumps were 4691, i.e. six-fold higher than that obtained from single meristem.

This difference in regeneration capacity occurred because in the latter technique, apical dominance was overcame by the use of high concentration of BAP (75 µM). However, in some species high concenteration cytokinin may cause abnormal effects in regenerated plants (Meredith and Carlson, 1978). Thus this study was conducted to see its effect on *in vitro* banana plants.

In vitro *assesment of dwarfism by GA_3 treatment.* Instead of observing this phenotype in the field, *in vitro* testing with GA_3 was carried out. Two concentrations of GA_3 (5 and 10mg/L) were used. No dwarfism was observed in both cases. This result also suggested that a high BAP concentration used in this experiment did not have any effect on the dwarf trait of plants was based on plant height and number of leaves (Table 3).

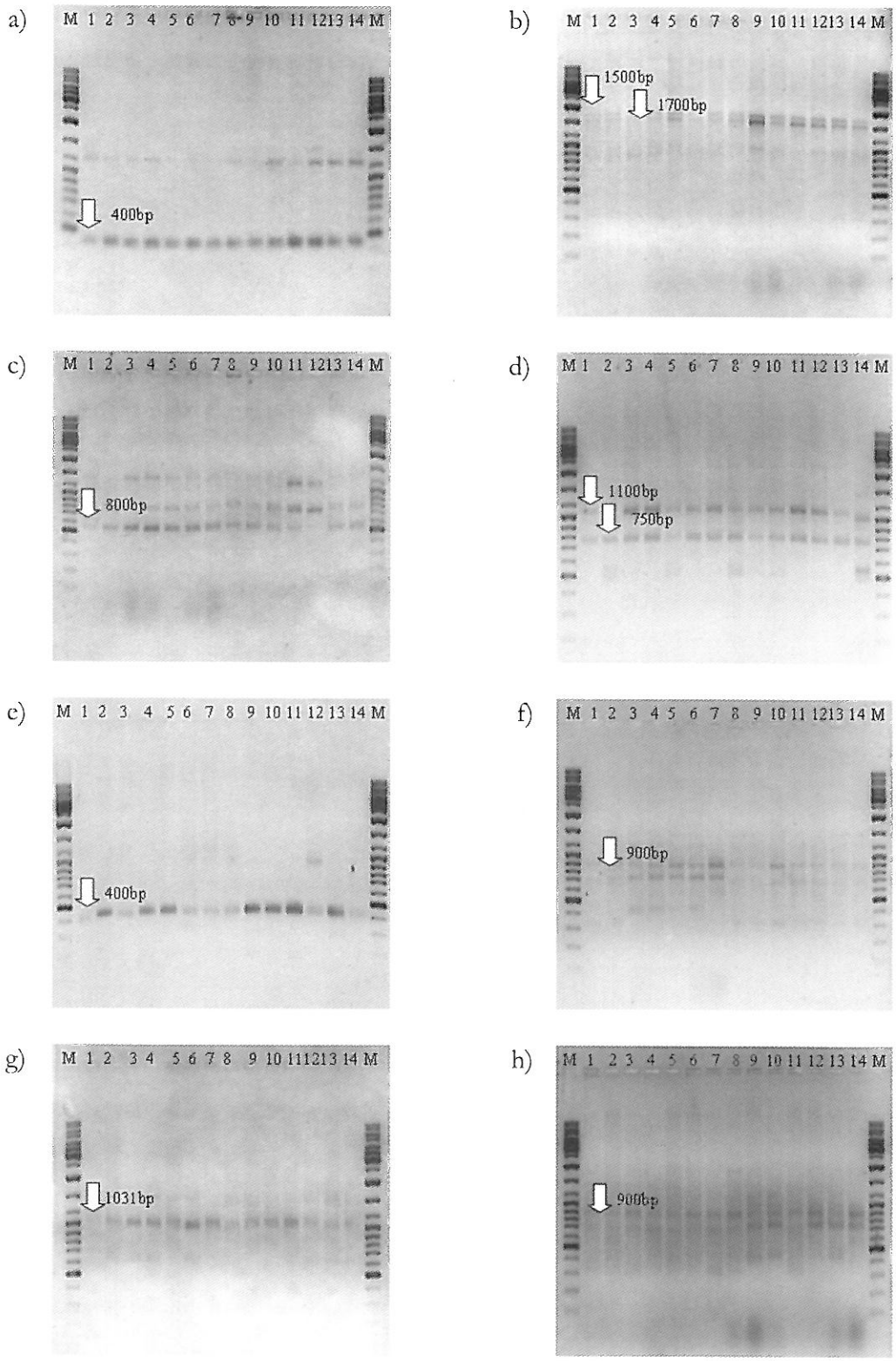

Figure 1. RAPD profiles of micropropagated banana plants obtained with (a) primers OPJ-06; (b) primers OPJ-10; (c) primers OPJ-09; (d) primers OPJ-13; (e) primers OPJ-15; (f) primers OPJ-17; (g) primers OPJ-18; and (h) primers OPJ-19. Lanes 1-7 regenerants from single meristem, 8-14 regenerants from scalp, M= DNA ladder mix. Major band was observed (arrow) in both regenerants.

Table 3. Effects of GA_3 on the height and number of leaves of explants after 9 weeks culture.

Source of explants	Height of shoots at different GA_3 concentrations		No. of leaves produced at different GA_3 concentrations	
	5mg/L	10mg/L	5mg/L	10mg/L
Single meristem	9.67a	9.31a	2.27a	2.13a
Scalps	9.20a	9.67a	2.17a	2.03a

For each element, the values followed by the same letter are not significantly different at $P \leq 0.05$ (Duncan test).

This experiment could be used as preliminary screening for dwarf trait among pisang berangan *in vitro* plants. It has been reported that GA_3 could be used to differentiate dwarf and giant Cavendish banana (Khayat *et al.*, 1999).

RAPD analysis. A total of 182 major scorable amplification bands from forty primers were analysed. Two primers (OPJ-02 and OPJ-08) did not amplify any products (Damasco *et al.*, 1996). Only 10 (5.5%) out of 182 (94.5%) markers were monomorphic shared among the regenerants whereas the remaining 172 markers were polymorphic. The number of scorable RAPD bands generated per primer varied from 1 to 8 with an average 4 markers per primer. The forty primers used exhibited approximately 73.7 % of polymorphism among scalp derived regenerants compared to 39.5 % from single meristem. In general, the amplified DNA fragments ranged from 200-2500 bp. There were about 8 primers (OPJ 06, 09, 10, 13, 15, 17, 18 and 19) generating monomorphic bands among the plantlets (Figure 1).

Polymorphism observed in *in vitro* banana plantlets with somaclonal changes during the tissue culture changes. The results in the present study demonstrate that RAPD analysis could be adopted to screen for variation in the micropropagated banana plantlets.

These results also suggested that induced meristems produced high rates of variation under the influence of a growth hormone. It has been suggested that BAP induces variation by disrupting gene regulation such as DNA methylation (Philips *et al.* 1994).

CONCLUSION

High multiplication rate for scalp-induced regenerants could be an alternative source of explants for plantlets production. Although BAP was used to induce scalps, GA_3 treatment did not display dwarf 'off-types'. However RAPD analysis revealed high frequency of polymorphism. Thus the scalp methods could be used as a source for induction of variants that could be used for selection of useful traits.

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