Available online at www.sciencedirect.com
SCIENCE DIRECT
Scientia Horticulturae 108 (2006) 347–351
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Mus*a AAA cv. '*Zelig*')

Michael W. Bairu, Catherine W. Fennell, Johannes van Staden *

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Received 4 November 2004; received in revised form 28 November 2005; accepted 10 January 2006

Abstract

The effect of the type and concentration of plant growth regulators and sub-culturing on somaclonal variation were studied in Cavendish banana cv. '*Zelig*' obtained from African Biotechnologies Ltd., South Africa. In vitro grown plants at the fourth multiplication cycle were used for the investigation. Auxins (IAA, IBA and NAA) and cytokinins (BA and TDZ) were used to multiply shoots for 10 generations. Bands generated through RAPD-PCR were scored according to whether they were present (1) or absent (0) to determine the extent of somaclonal variation. Results were then analyzed using cluster analysis. The relationship between multiplication rate and somaclonal variation was assessed using correlation analysis. Results indicated that treatments with higher multiplication rates produced more variants; sometimes as high as 72%. Dwarf off-types accounted for 88% of the variation. A dwarf-specific band, about 1500 kb in size, was amplified by the primer OPC-15. The band appeared consistently in normal plants but was absent in all dwarf plants.

Keywords: Cavendish banana; RAPD; Somaclonal variation

1. Introduction

Banana is one of the most important fruit crops in the world in terms of production and consumption. Annual production for the year 2003, for example, was 69,286 (1000 Mt) putting it second only to oranges (FAO, 2003). Cavendish bananas, with about 47% of global banana production, are the most important of all bananas (Arias et al., 2003).

Tissue culture offers considerable potential for the production of economically important plants. In spite of its massive use, the use of shoot tips for in vitro culture of banana cultivars often results in severe genetic defects (Hwang and Ko, 1987; Stover, 1987). These genetic defects result in the production of large numbers of off-types or variants referred to as somaclonal variants (Larkin and Scowcroft, 1981).

Understanding the cause(s) of somaclonal variation and eliminating off-types before field establishment, preferably at the in vitro stage, has great significance for in vitro banana propagation. Although there are different factors (culture medium composition, rate of multiplication, primary explant origin, formation of adventitious shoots, increased duration in culture, and certain genotypes) reported to cause somaclonal variation in banana (Cote et al., 1993), the reports on effects of plant growth regulators seem to be inconsistent. Some authors report that these factors have no effect (Reuveni et al., 1993) while others report that they affect the rate of somaclonal variation both directly (Stover, 1987) and indirectly (Karp, 1994; Damasco et al., 1998). Still others say not enough work has been done in this regard to reach a logical conclusion (Zaffari et al., 2000). Therefore, the effect of plant growth regulators on somaclonal variation was studied in greater depth.

Visual detection of off-types in micropropagated bananas is time consuming, laborious and expensive since it is done 3–4 months after field establishment (Israeli et al., 1991). Earlier detection of off-types in the nursery by inspecting individual plants is also possible but it is again laborious and needs optimal and uniform growth conditions for all plants (Smith and Hamill, 1993). The application of GA_3 to detect dwarf off-types was attempted. However, misclassification occurred in 5–10% of the cases even when the screening was applied under the most stringent conditions (Damasco et al., 1996a). Therefore, the development and use of efficient and reliable methods for

* Corresponding author. Tel.: +27 33 2605130.
E-mail address: vanstadenj@ukzn.ac.za (J. van Staden).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2006.01.039

detecting off-types is of prime importance to the banana industry. Detecting off-types by molecular analysis may offer a better alternative in this regard (Damasco et al., 1996b)

2. Materials and methods

2.1. Plant material and culture condition

Tissue cultured 'Giant Cavendish' banana (*Musa* AAA cv. '*Zelig*') obtained from African Biotechnologies Pty. Ltd., South Africa, were used in all the experiments. All experimental plants were of the same age and genetically uniform. The plants received had been sub-cultured for three cycles (20 week old in culture), at intervals of 4 weeks. Murashige and Skoog (1962) macro- and microelements supplemented with sodium dihydrogen orthophosphate (0.38 g l^{-1}), ascorbic acid (0.18 g l^{-1}), adenine sulphate (0.2 g l^{-1}), sucrose (30 g l^{-1}), Gelrite (2 g l^{-1}), and plant growth regulators, as stated below, were used. After adjusting the pH to 5.8 the media were autoclaved at 121 °C and 103 kPa for 20 min. Filter sterilized ascorbic acid was added when the media were just above the solidification temperature (about 50 °C). Cultures were then incubated in a growth room having 16 h light/8 h dark conditions and irradiance of 43 μmol m^{-2} s^{-1} at a temperature of 26 ± 1 °C.

Two separate experiments were conducted; one dealing with the type of plant growth regulators used during culture and the other with concentrations. Plants were sub-cultured 10 times for both experiments. Plants from the fifth sub-culture were used as a control for both experiments.

To determine the effect of different types of auxins and cytokinins on somaclonal variation of 'Cavendish' banana, a combination of auxins and cytokinins were tested. These included; auxins (IAA, IBA and NAA at 2 mg l^{-1}) and cytokinins (BA at 5 and TDZ at 3 mg l^{-1}). A total of six (2 × 3) treatments were considered and five explants were used per treatment.

To determine the combined effect of plant growth regulators and sub-culturing, the interaction of sub-culturing and growth regulators were investigated. Plants were sub-cultured 10 times on media with cytokinin concentrations of 2.5, 5 or 7.5 mg l^{-1} BA and an IAA concentration of 2 mg l^{-1}.

2.2. RAPD-PCR analysis

The protocols described by Richards (1997) and Hills and van Staden (2002) with slight modifications (the 3% PVPP was soaked in DNA extraction buffer overnight) were employed to extract total genomic DNA from in vitro grown plantlets. Quantification and purity analysis of DNA was made using spectrophotometry.

DNA amplification reactions were performed in volumes of 25 μl containing reaction mixture as described by Damasco et al. (1996b) with slight modification. These included 2.5 μl PCR Manufacturer's Reaction Buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg ml^{-1} gelatine, pH 8.3; Roche Diagnostics GmbH, Mannheim, Germany), 0.2 mM each of dATP, dCTP, dGTP and dTTP (Roche Diagnostics GmbH, Mannheim, Germany), 0.2 μM of OPC-15 and OPJ-04 random decamer primer (Operon Technologies, Alameda, CA, USA), 54 ng of banana genomic DNA and 1.5 units of Taq DNA Polymerase (Roche Diagnostics GmbH, Mannheim, Germany). Sterile HPLC grade water was used to adjust the final volume to 25 μl and overlaid with 50 μl of paraffin oil.

Amplification was performed in a Hybaid Thermal Reactor (HYBAID Ltd., UK, HYBAID 1991 Model). The amplification reaction was performed at an initial denaturation temperature of 95 °C for 1 min, 36 °C for 20 s and 72 °C 2 min (one cycle) followed by 45 cycles of each 95 °C for 10 s, 36 °C for 20 s and 72 °C for 2 min with a final elongation step (one cycle) of 72 °C for 5 min and 35 °C for 1 min. The amplification products were analyzed by gel electrophoresis in 1.5% agarose (HISPANAGAR, Burgos, Spain) containing 0.25 μg ml^{-1} of ethidium bromide. DNA molecular weight marker XIV (Roche Diagnostics GmbH, Mannheim, Germany) was included on each gel. Visualization of the amplification product was made under UV light, 6 × 8 W-312 nm tube (UVItec Limited, Cambridge, UK). DNA fragments (bands) detected after separation by electrophoresis were scored for the presence (1) or absence (0) with the Molecular Weight Marker XIV ladder on the corresponding fragment size. Data was then analyzed using single linkage cluster analysis (GenStat Sixth Edition). For the 2 sets of experiments, 660 plants in total were considered for analysis. Plants were analyzed after 5 weeks growth in culture.

3. Results

Polymorphic bands produced by primer OPC-15 revealed variability as high as 55%, in a total of 210 plants analyzed (30 plants per treatment). Percentage of variant individuals produced by in vitro culture varied considerably amongst the treatments. Treatments with a high multiplication rate showed greater variation compared to those with a lower multiplication rate (Table 1). A total of 450 plants were considered to assess the effect of multiplication cycle and BA concentration. Generally, somaclonal variation increased with an increase in multiplication cycle and BA concentration. A variation of 72% was observed at the 10th multiplication cycle for the 7.5 mg l^{-1} BA treatment (Fig. 3).

A correlation analysis was performed to assess the statistical significance of the relationship between multiplication rate and

Table 1
Percent somaclonal variation as affected by multiplication rate

Treatments	Variation (%)	Multiplication rate per explant
Control	3.3[a]	–
IAA × BA	40	3.5
IAA × TDZ	20	1.5
IBA × BA	55	3.25
IBA × TDZ	30	1.25
NAA × BA	30	1.8
NAA × TDZ	15	1

Plant growth regulators are applied at concentrations of 2 mg l^{-1}IAA, IBA and NAA; 5 mg l^{-1} BA and 3 mg l^{-1} TDZ.

[a] The control plants were at the fourth level of sub-culture when supplied.