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Scientia Horticulturae 86 (2000) 13–21

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HORTICULTURÆ

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Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars

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Accepted 22 December 1999

Abstract

Shoot-tips (3 mm) of banana (*Musa* spp.) cultivars Kibuzi (AAA-EA), Bwara (AAA-EA) and Ndiziwemiti (ABB) were cultured on modified Murashige and Skoog nutrient salts. The modified medium was supplemented with various equimolar concentrations (16.8, 20.8, 24.8 and 28.8 μM) of BAP, TDZ, ZN, 2-iP and KN to determine suitable concentration ranges of the cytokinins for micropropagation of banana cultivars. Three sub-culture cycles were used and after each sub-culture, the shoots per explant were counted. To select a suitable and cost effective cytokinin, the optimum concentration, its cost in US\$ and its corresponding proliferation rate were determined. The results showed that shoot proliferation was significantly ($p \leq 0.001$) dependent on cytokinin type, its concentration and the banana cultivar. The responses of cultivars to BAP were significantly ($p \leq 0.05$) better than other adenine-based cytokinins (ZN, KN and 2-iP). The TDZ showed high cytokinin activity and its diluted concentration of 0.045, 0.23, 1.14, 5.68, 6.81 and 9.1 μM showed, in case of Ndiziwemiti, progressively increased proliferation with increasing concentrations up to 9.5 shoots per explant. Ndiziwemiti was recalcitrant to the protocol of Talengera et al. (Talengera, D., Magambo, M.J.S., Rubaihayo, P.R., 1994. African Crop Sci. J. 2, 17–21). In the case of Kibuzi the proliferation rate increased from 2 to 5.4 for 0.045 to 5.68 before suddenly falling to 1.2 shoots per explant. The results demonstrated that cultivars differed significantly ($p \leq 0.05$) in their shoot proliferation responses to different TDZ concentrations and that TDZ is more economical than adenine-based cytokinins. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Proliferation rate; Cytokinin; Recalcitrant; Bananas

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1. Introduction

Bananas and plantains (*Musa* spp.) are crops of great economic importance in Uganda. They provide a major source of food, income and employment to many Ugandans (Rubaihayo, 1991). Conventionally they are propagated by suckers but this method is limited by its dissemination of weevils, nematodes and fungal pathogens (Sagi et al., 1998) and the low number of suckers (Vuylsteke, 1989).

In vitro propagation through shoot-tip culture does overcome these problems. Different in vitro micropropagation protocols, using different adenine-based cytokinins, have been used in several *Musa* spp. of divergent genomic constitution and ploidy (Vuylsteke, 1989). The cytokinins, that have been commonly used, include benzylaminopurine (BAP) (Cronauer and Krikorian, 1984; Vuylsteke, 1989); isopentenyladenine (2iP) (Dore Swamy et al., 1983); zeatin (ZN) (Vuylsteke and De Langhe, 1985) and kinetin (KN) (Cronauer and Krikorian, 1984). However, only BAP has been tested for AAA-EA bananas (Sagi et al., 1998). The proportion of auxin–cytokinin is a determinant for meristem formation (George, 1993) and the hormone balance that becomes established between growth regulators determines the type of buds induced (Trijillo and Garcia, 1996). A BAP concentration range of 8.9–22.2 μM is recommended for *Musa* in vitro propagation (Crouch et al., 1998). The use of rates beyond this range induces increased rates of somaclonal variants (Trijillo and Garcia, 1996).

Diphenyl urea derivatives (DPU) appear not to have been used in *Musa* spp. as no reported use was found in the literature. This paper reports the influences of different types and concentrations of adenine-based and diphenyl urea derived *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron, TDZ) cytokinins on shoot proliferation rates in Kibuzi and Bwara both AAA-EA cultivars and Ndiziwemiti (ABB).

2. Materials and methods

The shoot apices were excised from in vitro propagated cultures of the three banana (*Musa* spp.) cultivars; Ndiziwemiti which was reported to be recalcitrant to the protocols employed by Talengera et al. (1994) with a proliferation rate of 1.5; Kibuzi (AAA-EA) with a mean proliferation rate of 3 and Bwara (AAA-EA) with a proliferation rate of 6. Shoot-tips (3–4 mm) were isolated aseptically and inoculated onto differently modified semi-solid nutrient media. Fifteen explants per treatment were arranged in a completely randomized design with five replicates. The basal medium (MB1) contained Murashige and Skoog macro and microelements (Murashige and Skoog, 1962) supplemented with 0.4 mg l⁻¹ thiamin HCL, 0.5 mg l⁻¹ nicotinic acid, 0.5 mg l⁻¹ pyridoxine HCL, 100 mg l⁻¹ myo-inositol and ascorbic, and variously modified with equimolar concentrations

of the different cytokinins. The cytokinins included BAP, TDZ, ZN, 2iP and KN each at: 16.8, 20.8, 24.8 and 28.8 μM . The concentrations of TDZ were later diluted to : 0.045, 0.23, 1.14, 5.68 and 6.81 μM . Cultures were incubated at $27\pm 2^\circ\text{C}$ under 16 h photoperiod of $26.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ measured with a portable photosynthesis system (model CIRAS-1, Combined Infrared Gas Analyser System Hitchin, Herts SG5 1RT, UK), equipped with a PLC (B) leaf chamber.

Three weeks after inoculation, the shoot-tips had formed shoot clusters, which were sub-divided and individual shoot-tips, of comparable sizes, transferred onto fresh media. This procedure was repeated for three successive sub-cultures. At the end of each culture cycle the proliferation responses (shoots outgrowths per explant) were recorded. The data collected was analysed using MSTAT computer program and proliferation rate means separated by LSD. To select a suitable and cost effective cytokinin, the optimum concentration, its cost in US\$ and its corresponding proliferation rate were determined. The cost per unit plantlet regenerated was determined as the quotient of medium cost (cost of basal medium plus the cost of optimum cytokinin rate) and the total number of shoots produced per litre of medium within 4 weeks of culture. Individual shoots were later rooted on MB1 modified with 1.2 μM NAA, weaned and planted into 1:1 sterilized saw dust and soil.

3. Results and discussion

The results of analysis of variance of the shoot proliferation response among the three selected cultivars are presented in Table 1. Their proliferation responses

Table 1

Analysis of variance of proliferation rates of cultivars Bwara, Kibuzi and Ndiziwemiti on MB1 modified with cytokinins^a

Source of variation	Df	MS	F-values
Cytokinin (A)	3	25.40	2793.60 ^b
Cytokinin rate (B)	3	1.70	188.40 ^b
AB	9	1.80	199.48 ^b
Error	32	0.01	
Cultivar (C)	2	51.90	6797.57 ^b
AC	6	9.6	1254.83 ^b
BC	6	0.30	45.45 ^b
ABC	18	0.60	83.90 ^b
Error	64	0.008	

^a Coefficient of variation=2.87%.

^b Significant at $p=0.001$.

were highly significantly ($p \leq 0.001$) influenced by the cytokinin type, cytokinin rate, cultivar and their interactions.

Variation in the activity of different cytokinins can be explained by their different uptake rate reported in different genomes (Blakesley, 1991), varied translocation rates to meristematic regions and metabolic processes, in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Tran Thanh Van and Trinh (1990) and Kaminek (1992). The inherent endogenous auxins and cytokinins levels, must have also played parts in the observed data (Pierik, 1987).

The results of the proliferation response to various equimolar concentrations of individual adenine-based cytokinins are presented in Fig. 1. Shoot proliferation responses of the three cultivars to different BAP concentrations are shown in Fig. 1a. The results indicate that shoot proliferation was cultivar dependent. Cultivar Bwara showed significant ($p \leq 0.05$) increase in shoot proliferation rates with increasing BAP concentration from 5.0 to 8.0 shoots with increase from 16.8 to 28.8 μM .

Cultivar Kibuzi subjected to BAP treatment showed no significant response (2.8 shoots) to concentrations of 16.8 and 20.8 μM but increased to 3.5 shoots at 24.8 μM . At 28.8 μM BAP, Bwara proliferated significantly ($p \leq 0.05$) more than it did on 20.8 μM BAP supplemented medium used by Talengera et al. (1994). It was observed that increasing BAP beyond 16.8 μM did not significantly increase shoot proliferation in Kibuzi or Ndiziwemiti. Vuylsteke (1989) reported similar proliferation behavior in the Nzizi (AAB) cultivar.

Proliferation responses of the three cultivars on medium modified with zeatin at various concentrations are presented in Fig. 1b. Ndiziwemiti did not exhibit a significant response to variation in ZN concentration while Bwara showed depressed shoot proliferation rates. With increasing ZN concentration, Bwaras proliferation response increased from 2.5 to 3.5 before finally falling to 1.5 at 28.8 μM . Reduced shoot proliferation rates in *Musa* spp. on medium supplemented by ZN was earlier reported by Cronauer and Krikorian (1984).

Medium supplementation with 2-iP (Fig. 1c) increased shoot proliferation in Ndiziwemiti from 2.4 to 3.0 at 16.8 and 24.8 μM before falling to 2.0 at 28.8 μM . Kibuzi showed low rates at 16.8 and 20.8 μM and significantly ($p \leq 0.05$) increased rates from 24.8 to 28.8 μM . Bwara proliferated less than it did on 20.8 μM BAP supplemented medium.

Supplementation of the medium with KN progressively increased the shoot proliferation rate from 2.8 to 3.8 in Kibuzi at 16.8 to 24.8 μM before falling to 2.1 at 28.8 μM (Fig. 1d). Bwara proliferated less than it did on 20.8 μM BAP supplemented medium. Higher cytokinin proliferation activity was shown by KN than 2-iP and ZN. 2-iP and ZN have a double bond in their chemical structure which makes them vulnerable to the action of cytokinin oxidases (Kaminek, 1992).

Fig. 1. In vitro rates of Kibuzi, Bwara and Ndiziwemiti on MB1 modified with different cytokinins at equimolar concentrations: (a) shoot proliferation responses to BAP; (b) shoot proliferation responses to zeatin; (c) shoot proliferation responses to 2iP; (d) shoot proliferation responses to kinetin.

Application of a high concentration of TDZ evoked a higher degree of shoot proliferation than adenine-based cytokinins. The shoot proliferation responses of cultivar Ndiziwemiti to various TDZ concentrations are shown in Plate 1. At 16.8 μM equimolar concentration shoot proliferation on TDZ supplemented

Plate 1. Shoot proliferation responses of cultivar Ndiziwemiti on MB1 modified with various TDZ concentrations.

media was manifested by the appearance of numerous fleshy bulbous structures each of them producing several stunted tiny adventitious buds on their surfaces (Plate 1). This high cytokinin activity of TDZ has been reported by several workers (Thomas and Katterman, 1986; Fellman et al., 1987; Mok et al., 1987). In all cultivars the media supplemented with high TDZ concentrations induced suppressed shoot elongation (Plate 1). This behavior is attributed to TDZs ability to accumulate endogenous cytokinins in cultured tissues (Huetteman and Preece, 1993). The concentration rates of TDZ were reduced and the results are presented in Fig. 2. Shoot proliferation in Ndiziwemiti (ABB) progressively increased with increasing TDZ concentration from 1.2 shoots at $0.045 \mu\text{M}$ to 9.0 shoots at $6.81 \mu\text{M}$. This cultivar which was recalcitrant to the protocol employed by Talengera et al. (1994) where it produced only 1.5 shoots per explant was induced by TDZ to proliferate profusely. TDZ is resistant to all cytokinin oxidases and induces the accumulation of endogenous cytokinins (Kaminek, 1992). The proliferation rate of cultivar Bwara increased from 5.0 shoots at $0.045 \mu\text{M}$ concentration to 6.4 at $1.14 \mu\text{M}$ before it decreased with increasing concentration of TDZ. A similar trend was observed with Kibuzi where proliferation increased from 2 shoots at 0.045 to $5.68 \mu\text{M}$ and then showed a sudden decline in proliferation to 1.2 shoots at $6.81 \mu\text{M}$.

Beyond the critical points in Bwara (6.2 shoots) and Kibuzi (5.5 shoots), the explants enlarged with decreased number of shoots. Ndiziwemiti proliferated adventitiously up to $6.81 \mu\text{M}$ when it became difficult for individual shoots to be

Fig. 2. Shoot proliferation rates of Kibuzi, Ndiziwemiti and Bwara cultivars on MB1 medium supplemented with various TDZ concentrations.

distinguished (Plate 1). Crowded and stunted shoots proliferated profusely on TDZ free MB1 medium up to the third cycle where normal growth resumed. High proliferative cultivars (Bwara and Kibuzi) on 20.8 μM BAP supplemented medium, showed a lower shoot proliferation critical point on TDZ supplemented medium while Ndiziwemiti that had the least shoot proliferation on a BAP supplemented medium showed a higher critical point. Huettelman and Preece (1993) reported high proliferation rates at very low TDZ concentrations in woody plant species, which have low proliferation on BAP supplemented medium. This behavior is believed to be due to the ability of TDZ to increase the biosynthesis of endogenous adenine-type cytokinins (Thomas and Katterman, 1986; Huettelman and Preece, 1993), thus making TDZ an effective cytokinin for stimulation of shoot bud proliferation in recalcitrant banana genotypes. These results demonstrated that TDZ was effective against recalcitrance in Ndiziwemiti and increased proliferation rates in non-recalcitrant banana cultivars.

The medium-associated costs in relation to the number of shoots generated per litre of medium supplemented with different cytokinins are presented in Table 2.

The cost of unmodified MB1 was 0.335 US\$ per litre. The cost effectiveness of the modified medium used was indicated by the production cost of individual shoot regenerated on the respective medium (Table 2). The medium supplemented with TDZ was the cheapest, at a cost of 0.66 US cents per shoot regenerated. The cost per plantlet regenerated on BAP modified medium was 0.79 US cents. The ZN supplemented medium was the most expensive at 33.5 US cents per shoot produced. The results showed that it was more economical to use TDZ in banana micropropagation than adenine-based cytokinins.

Table 2

Medium-associated costs for Ndiziwemiti shoot multiplication on MB1 supplemented with different cytokinins

Cytokinin	Optimum rate (μ M)	Cost of cytokinin (\$) ^a	Cost of medium (\$ per litre) ^a	Shoots per litre of medium	Cost/shoot US (cents)
BAP	20.9	0.060	0.395	500	0.79
TDZ	1.13	0.325	0.660	1000	0.66
ZN	21.0	7.700	8.040	240	33.5
KN	20.9	0.131	0.470	420	1.12
2iP	20.7	0.520	0.860	500	1.72

^a Prices are based on SIGMA chemical catalogue 1997 (SIGMA, 1997).

Acknowledgements

The work reported in this paper was supported by Forum grant RF 96008 # 91 of Rockefeller Foundation. The authors are indebted to Mr. G. Mugisha and Mr. S. Ogole for their technical assistance.

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