Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 7 (1): 116-118, 2008
ISSN 1682-3974

An Improved System for *in vitro* Propagation of Banana (*Musa acuminate* L.) Cultivars

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Abstract: An efficient medium culture for clonal mass propagation was established for the propagation of two banana (*Musa acuminate* L.) cultivars of Cavendish Dwarf and Valery. The effects of growth regulators were studied on fresh weight, length shoot and shoot proliferation of the meristem explants in Musa cv. Cavendish Dwarf and Valery. The final medium adopted included the salt formulation of Murashige and Skoog, 30 g L^{-1} of sucrose, N-phenyl-N-1, 2, 3-thiadiazol 5-yl Urea (0.5 mg L^{-1}) and Indoleacetic acid (2 mg L^{-1}). Under these conditions, a multiplication rate of 25 plantlets per explant was obtained in 120 days. This system was also effective for the multiplication of the two cultivars, evaluated with a multiplication rate of 10.85-12.88 plantlets after 45 days in culture. Benzyl aminopurine was also effective for the elongation of plantlets.

Key words: Growth factors, micropropagation, multiplication rate, Musa

INTRODUCTION

Banana is one of the most important fruits in the world, both as a staple food as well as a major export commodity for many tropical and sub-tropical countries. The extensive basic work on the *in vitro* propagation of banana (Swennen *et al*., 1991; Kodym and Zapata-Arias, 1999; Nandwani *et al*., 2000) had led to the technological development of *in vitro* mass production of different cultivars. Plant tissue culture techniques can potentially overcome some of the factors limiting traditional approaches to banana improvement. These techniques enable plants to be regenerated from normal and genetically modified cells and tissues in an efficient way under sterile conditions. The objective of present investigation was to develop an micropropagation system for large-scale production of banana in cultivars Dwarf Cavendish and Valery.

MATERIALS AND METHODS

Shoot-tip cultures of two banana (*Musa acuminate* L.) cultivars namely Dwarf Cavendish and Valery were derived from shoot apexes. Explants (ca. 10×10×6 mm) obtained from decapitated shoot apexes of suckers were surface sterilized by 70% ethanol for 20 sec, then incubated in a 5% solution of sodium hypochlorite for 20 min, followed by three rinses in sterile distilled water. The effects of cytokinins [Benzylaminopurine (BAP), Kinetin (KIN) and N- phenyl -N′- 1, 2, 3-thiadiazol 5-yl urea (TDZ)] combined with auxin [Indoleacetic Acid (IAA)] were evaluated on basal Murashige and Skoog (1962) medium. The pH was adjusted to 5.7 with 1 M NaOH before agar and charcoal was added. The cultures were maintained at 25°C with 16 h photoperiod at a photosynthetic photon flux density of 120 μmol m^{-1} sec^{-1}. Subculturing was carried out at 45 day intervals. All treatments were performed on three replications of 10 explants in experiments employing a completely randomized design. The data on shoot number, shoot length and fresh weight of shoot were analyzed by ANOVA followed by Duncan's test.

RESULTS AND DISCUSSION

Induction of multiple shoots in varieties Dwarf Cavendish and Valery were observed on various combinations of growth regulators. Results are shows in Fig. 1-3. In both cultivars, TDZ promotes a higher number of shoots per explant compared to KIN, while, BAP gave intermediary results (Fig. 1a). However, the shoots developed in the presence of TDZ or KIN did not survive upon transferring.

In the absence of cytokinins, the entire shoot died within 2 week. The presence of TDZ significantly ($p<0.05$) reduced the shoot elongation and shoots fresh weight with BAP and KIN. The results are in accordance with those obtained with order banana cultivars by

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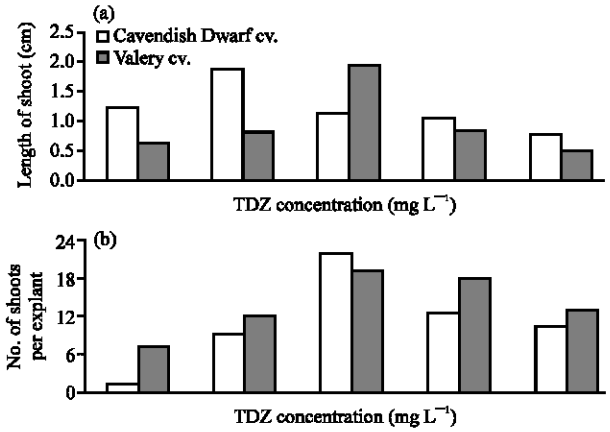

Fig. 1a, b: Effect of TDZ concentration and IAA (2 mg L^{-1}) on multiplication shoot per explant and elongation plantlet in Cavendish Dwarf and Valery cultivars. At least 10 explants were used in each treatment and data were scored after 45 days of growth

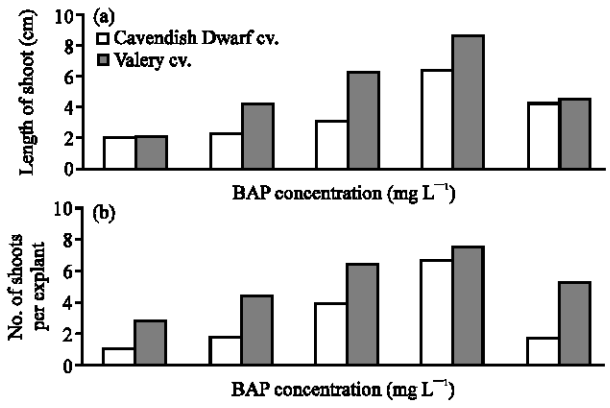

Fig. 2a, b: Effect of BAP concentration and IAA (2 mg L^{-1}) on multiplication shoot per explant and elongation plantlet in Cavendish Dwarf and Valery cultivars. At least 10 explants were used in each treatment and data were scored after 45 days of growth

Alvard *et al.* (1993). The number of shoots significantly increased with increasing concentration of TDZ in the media, but the elongation and fresh weight of shoots decreased significantly (Fig. 1b).

High concentration of TDZ reduced the number of developing shoots, while with 0.15 mg L^{-1} of TDZ; a significantly higher number of shoots per explant were obtained. However, the height and fresh weight of plants were reduced. In 0.25 mg L^{-1} of TDZ, hyperhydricity was

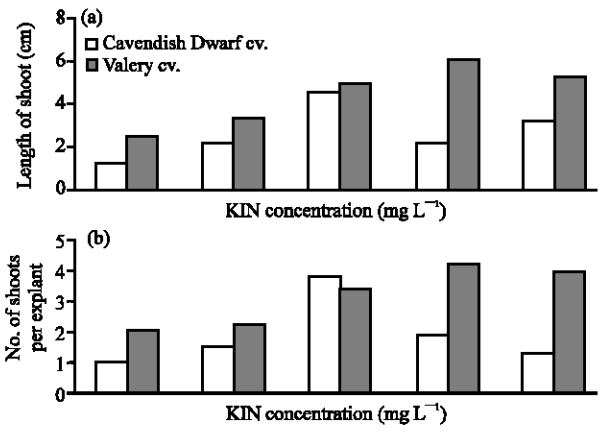

Fig. 3 a, b: Effect of KIN concentration and IAA (2 mg L^{-1}) on multiplication shoot per explant and elongation plantlet in Cavendish Dwarf and Valery cultivars. At least 10 explants were used in each treatment and data were scored after 45 days of growth

observed. The association of 0.15 mg L^{-1} TDZ and 2 mg L^{-1} IAA positively affected the multiplication of the Cavendish Dwarf and Valery compared to 0.2 mg L^{-1} TDZ. TDZ has a strong cytokinin activity and possibly this is the reason for its use in multiplication of banana in tissue culture (Nowak and Miczynski, 2002).

At 2 mg L^{-1} concentration of BAP, the length of shoots and fresh weight of plantlets per explant was increased compared to that of TDZ and KIN (Fig. 2a). The length of shoots and fresh weight increased with increasing concentration of BAP in the media (Fig. 2b). With 2 mg L^{-1} BAP and 1.5 mg L^{-1} KIN, we obtained a significant elongation of shoots and reduction of shoot proliferation and fresh weight (Figs. 3a, b). At high concentration of BAP and KIN the number of shoots was significantly reduced. However, the number of shoots and elongation of plantlets differed from one cultivar to another at different level of TDZ, BAP and KIN. The highest number of shoots was obtained on the medium culture supplemented with TDZ on the medical plant Arnebia euchroma (Royal) explants. Other cytokinins (KIN and BAP) and the auxin were not efficient in inducing proliferation on cotyledons explants (Bo *et al.*, 2005). Similarly, in both cultivars, the survival rate of shoots steadily decreased with the increase in the level of growth factors. In general, medium cultures with optimum concentration of TDZ were better for rapid shoot multiplication and the medium cultures with optimum concentration of BAP were good for elongation of plantlets.

Micropropagation of various cultivars of banana through shoot-tip explants is well documented (Drew *et al*., 1989; Ko *et al*., 1991; Kotecha and Kadam, 1998; Pandey *et al*., 1993; Sudhavani and Reddy, 1997; Nandwani *et al*., 2000). In almost all cases, different combinations of cytokinin and auxin in various concentrations were reported for multiple shoot regeneration. Different workers have reported different media for plantlets regeneration in banana. The technique outlined in this communication can be gainfully employed for pathogen-free and large-scale production of banana plants in var. Dwarf Cavendish and Valery.

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