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Some Factors Affecting the *In Vitro* Culture of Banana

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Abstract. Factors affecting *in vitro* regeneration of shoots in shoot tip explant cultures of banana cultivar 'Basrai', such as solid and liquid media, growth regulators, vitamins, and antioxidants were studied. Three-quarters strength of MS liquid medium supplemented with 17.75 µM 6-benzyladenine (BA), 11.42 µM indole-3-acetic acid (IAA) and 205 µM adenine sulphate induced the formation of mean number of 12.3 shoots, with the mean length of 3.0 cm, after three weeks of culture. Maximum shoot multiplication (14.33) occurred in liquid medium containing 22.19 µM BA. Addition of 2.0% activated charcoal (AC) to the liquid medium improved quality of the regenerated plants with expanded and glossy leaves, though the number of shoots was reduced (13.66). Profuse formation of roots was characteristically induced by AC. Addition of citric acid (CA) to the medium caused decline in morphogenetic expression of the cultures.

Keywords: activated charcoal, ascorbic acid, citric acid, *in vitro* plant culture, gelling agent, growth regulators, multiple shoots, banana culture

Introduction

Edible banana and plantains are a major staple food in tropical and subtropical parts of Africa and Asia. Most commercial cultivars are triploids and, therefore, essentially seed-sterile. Consequently, these cultivars are propagated only by vegetative means. Previously, efforts have been made to enhance the propagation rates using conventional techniques (Rowe and Richardson, 1975; Hamilton, 1965). However, during the last three decades, several efforts have been made to propagate banana and plantains *in vitro* (Roels *et al.,* 2005; Agrawal *et al.,* 2004; Jalil *et al.,* 2003; Habiba *et al.,* 2002). The shoot tip culture method, obviously, has a great potential for producing specific pathogen-free planting materials in quantity. Applications of *in vitro* culture techniques have significantly improved handling of germplasm of the genus *Musa,* to which also belongs the edible banana. Micropropagation has also played a key role in the plantain and banana improvement programmes using shoot tip culture. Plants can be selected, screened and maintained free from serious diseases and pests in a better manner as compared to conventional techniques (Arias, 1993; Drew *et al.,* 1992). The present investigation reports the effect of various media, supplemented with different growth hormones either in combination or alone, antioxidants and different gelling agents on the micropropagation of banana to produce an efficient and reproducible protocol for rapid multiplication of banana cultivar 'Basrai'.

Materials and Methods

Explants of 2-4 cm shoot apices were isolated from suckers of *Musa paradisica* cultivar 'Basrai'. All roots were removed from the rhizomes and the soil was washed off. The outer leaf sheaths of the pseudo-stem were peeled off, one at a time, until the size of the inner leaf sheaths was minimized to 1 x 2 cm at their base. Using a scalpel, the remaining outer leaf bases were carefully removed under a stereomicroscope, until only one or two young leaf primordia were left. The shoot apices were excised by making four incisions into the rhizome beneath the apices. Excised apices were placed in 30 ml of 10% commercial bleach with 2-3 drops of Tween-20. The apices were allowed to soak for 5 min and swirled occasionally. The bleach was decanted and the apices rinsed five times with sterile distilled water. The final decant was poured through a sterile sieve to collect the apices.

The sterilized apices were shifted from the sieve on to the culture media (Benerjee *et al.,* 1986). Modified MS basal medium (Murashige and Skoog, 1962) was used with different combinations and concentrations of growth regulators, antioxidants and gelling agents to study their effect on shoot proliferation and browning. Media constituents are shown in Table 1. The MS basal salt concentrations were used at the reduced strength of three-quarters throughout the investigations. Additional variations in the nutritional components were made through vitamin supplementations, where thiamine HCl (1.48 µM), pyridoxine HCl (2.43 µM), nicotinic acid (4.0 µM), *myo*-inositol (554.9 µM), and glycine (26.69 µM) were added. Omissions of vitamins were as mentioned (Table 1). 6-Benzyladenine (BA) at a concentration of 22.19 µM or less, with or without 11.42 µM indole-3-acetic acid (IAA) and 205 µM adenine sulphate, occasionally replaced with 15% coconut water (CW), were supplemented to the basal salt composition. The other additives included antioxidants and solidi-

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Table 1. Combinations of three-quarters of salt strength of MS basal medium (Murashige and Skoog, 1962) supplemented with 2.0% sucrose used for the *in vitro* culture of banana

Media code	Growth regulators		Adenine sulphate (205 µM)	Vitamin* supplement (µM)	Antioxidants			Gelrite-Sigma (0.25%)
	BA (22.19 µM)	IAA (11.42 µM)			AA (56.72 µM)	CA (520 µM)	AC (2.0%)	
B_1	+	+	+	+	+	-	-	-
B_2	+	+	+	+	+	-	+	-
B_3	+	+	+	+	+	+	-	-
B_4	+	+	+	+	+	+	-	+
B_5	+	+	+	+	+	-	-	+
B_6	less	+	+	-	-	-	-	-
B_7	less	+	+	-	-	-	-	+
B_8	+	-	CW (15%)	+	-	-	-	+
B_9	+	-	+	-	-	-	-	-

BA = 6-benzyladenine; IAA = indole-3-acetic acid; AA = ascorbic acid; CA = citric acid; AC = activated charcoal; CW = coconut water; less = 17.75 µM BA; *vitamin supplement composition = 1.48 µM thiamine HCl + 2.43 µM pyridoxine HCl + 4.0 µM nicotinic acid + 554.9 µM *myo*-inositol + 26.69 µM glycine

fying agents. Among the antioxidants used were 56.72 µM ascorbic acid (AA), 520 µM citric acid (CA) and 2% activated charcoal (AC), which were added or omitted from the media as shown in Table 1, prior to the pH adjustment at 5.7 using 0.1 N HCl or NaOH. Media were autoclaved at 15 psi for 15 min. Cultures were incubated at 29±2 °C under 16 h photoperiod (3000-4000 lux), maintained with cool white florescent lights. Subsequently, regenerated plants were washed with water and transferred to soil.

Results and Discussion

The bases of the apical explants, inoculated on to the media employed by Benerjee *et al.* (1986) grew in normal circular fashion, while the upward growth was due to the elongation of leaves surrounding the apical dome. After 7-10 days, the colour of the 70% explants was restored from pale to fresh green. After 12 days of the initial culture establishment phase, the shoot apices were subcultured on to the different combinations of the media used (Table 1) to induce enhanced multiplication. Browning rapidly occurred in the 0.7% agar solidified media (Fig. 1a). Polyphenolic discharge from the wounds caused senescence. Replacement of agar with gelrite (Sigma) resulted in the inhibition of exudation from the explants (Fig. 1b). It might be due to the purity of the gelling agent.

Table 2 shows the effect of different compositions of the media and concentration of growth regulators, selected vitamins and gelling agents on the induction of shoot primordia and their proliferation. Medium-B_6, having reduced concentration of BA (17.75 µM) and no vitamins, induced maximum number of buds (12.3) as compared to medium-B_9 containing 22.19 µM BA and 205 µM adenine sulphate, and medium-B_8 containg 205 µM BA, 15% CW and the complete vitamin supplement, in which case only 10.6 and 6 shoots were respectively formed (Table 2). Behaviour of cultures in liquid medium-B_6 and gelrite medium-B_7, having the same nutrient composition, was also studied. Incorporation of 0.25% gelrite in medium-B_7 caused significant decrease in the number of shoots (4.0), as compared to 12.3 shoots in the liquid medium-B_7. In all liquid media (B_1, B_2, B_3, B_6 and B_9), the number of shoots increased as compared to gelrite solid medium-B_4, B_5, B_7 and B_9. Hence, liquid cultures were found to be suitable for shoot proliferation.

a b

Fig. 1. Comparative effect of solidifying agents on *Musa paradisica* cultivar 'Basrai' cultures growing *in vitro*: **(a)** cultures grown on medium solidified with 0.7% agar (Sigma) showing extensive browning; **(b)** cultures grown on medium solidified with 0.25% gelrite (Sigma) showing healthy and normal growth.

Madhulatha *et al.* (2004) also studied the effect of 'liquid pulse treatment' of growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). Table 3 shows the effect of antioxidants on the elongation of shoots in both solid and liquid cultures (medium-B_1, B_2, B_3 B_4, B_5). The media contained three-fourths of the basal MS medium, augmened with normal vitamin supplement and growth regulators containing BA (22.19 µM), IAA (11.4 µM) and adenine sulphate (205 µM). Incidence of shoot induction was significantly high in liquid culture medium-B_1 (14.33), as compared to gelrite medium-B_5 (6.33). Similarly, growth of the shoots was also reduced in solid media. Incorporation of 2% activated charcoal (AC) into medium-B_2 caused insignificant decrease in the number (13.66) and length (6 cm) of shoots. However, the leaves were healthier, more glossy and expanded in contrast to those grown on the medium without AC. Besides, the shoots raised in the medium containing AC produced well-developed root system, as shown in Fig. 2a. Roots were almost absent in the media without AC (Fig. 2b). Liquid medium-B_3 and solid medium-B_4, supplemented with 520 µM citric acid (CA) and 56.72 µM ascorbic acid (AA) induced the formation of 9.00 and 7.32 shoots of 2.6 and 3.0 cm length, respectively. The shoot cultures were somewhat abnormal in the presence of these antioxidants added to gelrite (medium-B_4), or liquid medium-B_3. The abnormal shoots were stunted, showing crippled growth (Fig. 3). Initially, the leaf tips darkened and leaf blades curled and eventually the entire cultures collapsed due to rapid onset of browning.

Table 2. Effect of media composition (refer Table 1 for the media code), vitamin supplementation and gelling agents on shoot multiplication and growth after 3 weeks* of the first culture

Media code	Shoot number	Shoot length (cm)
B_6 (liquid)	12.3^{a}	3^{ab}
B_9 (liquid)	10.6^{a}	4^{a}
B_8 (BA+CW)	6.0^{b}	3^{ab}
B_7 (gelled)	4.0^{b}	2^{b}

* = mean separation in columns by Duncan's multiple range test, p = 0.05; BA = 6-benzyladenine; CW(15%) coconut water

Table 3. Effect of antioxidants and gelling agents on shoot multiplication and elongation after 3 weeks* of the first culture

Media code**	Shoot number	Shoot length (cm)
B_1 (liquid)	14.33^{a}	6^{a}
B_2 (liquid)	13.66^{a}	6^{a}
B_3 (liquid)	9.00^{b}	2.6^{b}
B_4 (gelled)	7.32^{bc}	3.0^{b}
B_5 (gelled)	6.33^{c}	1.3_{b}

* = mean separation in columns by Duncan's multiple range test, p = 0.05; ** = refer Table 1 for the composition of each media code

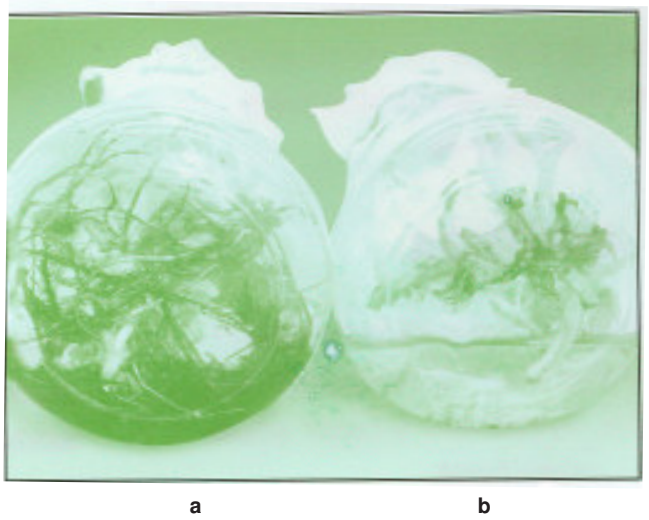

Fig. 2. **(a)** Profuse formation of roots in liquid medium containing 2.0% activated charcoal; **(b)** no root formation occurred in the absence of activated charcoal.

Fig. 3. Abnormal and stunted growth of shoots in the presence of citric acid in liquid medium-B_3 supplemented with growth regulators, adenine sulphate, vitamins and antioxidants but no addition of activated charcoal and gelrite; refer Table 1 for complete composition of the medium.

Significant difference was observed in the liquid culture with AC and addition AA. Gomez Kosky *et al.* (2002) recognized suitability of liquid culture media for the development and secondary multiplication of somatic embryos of banana hybrid cultivar FHIA-18 (AAAB). Lee (1993) reported the use of AC leading to the formation of less number of healthy plantlets. The results presented in this report are consistent with the published works of Lee (2003) in respect of the use of charcoal in liquid medium. Effectiveness of gelrite in solid culture medium for the control of polyphenoles reported by Lee (1993) and Asif *et al*. (2001) also validate our findings.

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