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Full Length Research Paper

Effects of nutrient media constituents on growth and development of banana (*Musa* spp.) shoot tips cultured *in vitro*

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The objective of this research was to evaluate the effects of nutrient media constituents on growth and development of banana plantlets produced *in vitro*. Apical buds were excised, disinfected, and cultured in Murashige and Skoog (MS) nutrient medium supplemented with organic compounds at three culture stages. In stage 1, the role of sucrose (15 to 60%), inositol (0 to 1000 mg/l) and white's organics (×0.25 to 1.25) on banana plantlets growth was assessed. At the second stage, studies were done on the role of 2 to 5 mg/l of benzyl adenine (BA), longitudinal splitting of apical buds, and the physiological age (1 to 3 months) of banana plantlets. At the rooting phase, indole-3-acetic acid (IAA) (1 mg/l) was supplemented in the presence or absence of BA (7 mg/l). Initially, sucrose concentration at 3.0% significantly promoted plantlet growth. During bud proliferation stage, the addition of inositol and white's organics was non-significantly effective. BA concentration at 7.0 mg/l significantly promoted bud proliferation both at complete and splitted plantlets. However, splitted plantlets were higher in bud proliferation.

Key words: Banana, nutrient medium, *in vitro* culture, buds, splitting techniques.

INTRODUCTION

Musa spp. is considered one of the top ten food crops in the world (Vuylsteke et al., 1993). It represents an essential source of nutrients for millions of people, particularly in tropical and subtropical regions, as well as a cash crop in many developing countries. The crop is a staple food for at least 400 million people, and it comprises about 10% of more than 80 million tons of food annually produced (Sharrock and Frison, 1998; FAO, 1999). Banana plants are usually propagated vegetatively by suckers which grow from lateral buds originating from corms, and suckers are separated for production of individual plants. In some instances, complete or splitted corms with one or several buds are used (Vuylsteke et al., 1993). Conventional vegetative multiplication of banana has been found to express several negative impacts including transmission of diseases, low production and poor preservation of original plant genetic material. Hence, *in vitro* culture technology is considered as an alternative for the production of large numbers of plants in shorter time, lesser space, disease-free plants (Hwang et al., 1984) and adequate germplasm preservation (Vuylsteke, 1989). Tissue culture approaches would also allow for off-season production of fast growing plants compared to conventionally propagated ones (Eckstein and Robinson, 1995).

The history of banana meristems culture goes back to early 1970s (Ma and Shii, 1972) when decapitated shoot tip technique was used in an attempt to reduce apical dominance, thus, increase apical bud proliferation and radiation studies were carried out to attain genetic mutations (De Guzman et al., 1976). Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha, producing 95.6 million tonnes (Singh et al., 2011). Since the first report of banana *in vitro* clonal propagation in 1960s, the tissue culture technology in banana has undergone significant improvement, and is now used widely in banana production worldwide (Agustin and Molina, 2002). Banana and plantains (*Musa* spp.) are some of the earliest crop plants having been domesticated by humans. The

Table 1. Effect of sucrose concentration on banana plantlet growth/establishment, four weeks after *in vitro* culture on the Murashige and Skoog (MS) basal medium.

Concentration	Fresh weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
15	1.75[b]	4.02[b]	28.96[c]	4.63[b]	6.00[a]
30	2.74[a]	5.21[a]	57.09[a]	6.42[a]	5.75[a]
45	2.55[a]	4.96[a]	46.09[b]	5.54[b]	5.79[a]
60	2.58[a]	4.79[a]	39.30[b]	5.25[b]	5.42[a]

Values with similar superscript letter(s) in a column are non-significantly different at $P < 0.05$.

crop is globally ranked fourth, next to rice, wheat and maize in terms of gross value of production (Singh et al., 2011).

In the starting phase of banana genetic manipulation, research groups have focused both on the use of banana meristems (May et al., 1995) and embryogenic cells or protoplasts (Sági et al., 1995a, b; Swennen et al., 1998). Application of micropropagation in banana has several advantages. These include rapid multiplication, limited mother stock, product uniformity and season-independent production. Agronomic advantages are manifested in uniformity of growth and maturity. It has also been possible to develop planting material which is free from sucker-borne diseases and pests. Production of plants in test tubes facilitates safe movement and easy handling of germplasm between laboratories, within and across countries. The yield and returns are expectedly higher. The success of plant tissue culture is mainly dependent on the use of suitable nutrient media (Huang and Murashige, 1976). Murashige and Skoog (MS) (1962) is the most widely used plant culture medium (Vuylsteke, 1989). Several media formulations have been reported for banana shoot tip culture with slight modifications of MS media (Brown et al., 1995). Other popular media include B5 (Gamborg et al., 1968), SH (Schenk and Hildebrant, 1972), N6 (Chu et al., 1975) and Linsmaier and Skoog (LS) (Linsmaier and Skoog, 1975) media.

Procedures for somatic embryogenesis in banana are numerous, including the use of vegetative tissues such as rhizome fragments and leaf bases (Novak et al., 1989; Ganapathi and Higgs, 1999), proliferating meristem cultures (Dhed'a et al., 1991; Schoofs et al., 1998) and immature male and female flowers (Escalant et al., 1994; Grapin et al., 1998). Though, for most of the banana genome groups, embryogenic cell suspensions were obtained, and plants could be regenerated from there (Schoofs et al., 1998). The objective of this research was to identify an appropriate nutrient medium for *in vitro* culture of banana shoot tips as expressed by several growth parameters.

MATERIALS AND METHODS

Shoot tips from lateral buds were isolated from banana suckers and corms at the Plant Tissue Culture Laboratory in Khartoum. Buds were thoroughly rinsed by tap water for 5 min, immersed in 100 mg/l ascorbic acid for 1 h, after which they were rinsed several times with sterile distilled water. Surface disinfection of buds was carried out in a laminar flow cabinet using clorox (20% v/v) with few drops of tween 20 and shaken for 15 min. Buds were then rinsed and shaken by sterile distilled water for 15 min. Apical buds of 2 to 5 mm were isolated after removal of external leaves and were then cultured in (15 × 2.5 cm) test tubes.

The culture media contained MS basal salts, supplemented with 30 g/l sucrose, 100 mg/l inositol, 7 g/l agar, and × mg/l white's organics according to Mullin et al. (1974) consisting of thiamine, pyridoxine, nicotinic acid and glysene. Sucrose, plant growth regulators and other additives were tested, as stated below. All media were autoclaved at 121°C and 1.05 kg/cm^3, for 15 to 20 min and the pH was adjusted to 5.7 before and after autoclaving. Firstly, sucrose was tested at a range of concentrations of 0.0, 15, 30 or 60 g/l, myo-inositol at concentrations of 0.0, 50, 100, 200, 400 or 1000 mg/l and white's organics at the levels of 0.0, × 0.25, × 0.5, × 1 and × 1.25 of the normal concentration. Several factors were assessed during bud proliferation. These include the concentration of benzyl adenine (0, 1, 3, 5, 7, 9 mg/l). The bud splitting technique was also applied by cutting banana plantlets longitudinally into halves that were cultured at several concentrations of BA as explained earlier, and the response of plantlet age (1 to 4 leaves old) was studied.

At the rooting stage, banana plantlets were cultured in a culture medium supplemented with naphthalene acetic acid (NAA) (0.0 to 1.2) mg/l with or without BA (0.2 and 0.4 mg/l), activated charcoal (0.0 to 2.0) and additional phosphate (0.0 to 255.0). Each experiment was conducted using a completely randomized design. Significant variations were further analyzed using the Duncan's Multiple Range Test. Parameters measured include fresh weight, height, number of roots, root length and number of leaves.

RESULTS

The effects of sucrose, inositol and white's organics were assessed four weeks after culture on MS basal medium. It was evident that sucrose, at 30 mg/l, significantly increased plantlet fresh weight compared to other sucrose concentrations. On the other hand, inositol and white's organics showed a non-significant effect on the measured parameters, with the exception of the significant effect of white's organics at ×1.25 concentration which gave longer plantlets (Tables 2 and 3).

The results of the second phase showed that the increase in BA concentration significantly enhanced bud proliferation and multiplication, particularly with splitted plantlets (Table 4). The best bud proliferation rate of complete plantlets was obtained at 7 mg/l BA and with splitted plantlets at 3 mg/l BA. It was also evident that

Table 2. Effect of inositol concentration on banana plantlet growth/establishment, four weeks after *in vitro* culture on the Murashige and Skoog (MS) basal medium.

Concentration	Fresh weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
0	1.89^{a}	4.29^{a}	36.25^{ab}	5.96^{a}	5.23^{a}
100	1.74^{a}	3.75^{a}	31.90^{ab}	5.24^{abc}	4.93^{a}
200	1.61^{a}	3.71^{a}	27.21^{ab}	4.83^{bc}	5.25^{a}
400	1.98^{a}	4.31^{a}	39.59^{a}	5.65^{ab}	5.47^{a}

Values with similar superscript letter(s) in a column are non-significantly different at P < 0.05.

Table 3. Effect of white's organic concentration on banana plantlet/establishment, four weeks after *in vitro* culture on the Murashige and Skoog (MS) basal medium.

Concentration	Fresh weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
0.0	7.73^{a}	5.07^{b}	44.60^{a}	5.93^{a}	5.07^{b}
×1/2	2.61^{a}	5.00^{b}	46.00^{a}	5.93^{a}	5.73^{a}
×1	2.42^{a}	5.33^{b}	49.00^{a}	6.40^{a}	5.47^{ab}
×1.25	2.44^{a}	5.37^{a}	53.13^{a}	5.73^{a}	5.33^{ab}

Values with similar superscript letter(s) in a column are non-significantly different at P < 0.05.

Table 4. Effect of Murashige and Skoog (MS) basal medium supplemented with benzyl adenine (BA) on bud proliferation, four weeks after culture *in vitro.*

BA (mg/l)	Rate of bud proliferation	
	Complete plantlet	Splitted plantlet
0.0	1.09^{b}	1.13^{b}
1.0	2.00^{ab}	3.69^{a}
3.0	2.00^{ab}	3.94^{a}
5.0	2.50^{ab}	3.38^{a}
7.0	3.06^{a}	3.34^{a}
9.0	1.64^{ab}	3.19^{a}

Values with similar superscript letter(s) in a column are non-significantly different at P < 0.05.

younger plantlets (one leaf) showed significantly higher bud proliferation, the rate then decreased with plantlet age (2 to 4 leaves). The addition of NAA at 0.4 mg/l gave a significant effect on plantlet fresh weight as well as root length (Figure 5). However, number of roots was significantly higher at 0.6 mg/l NAA. When NAA was supplemented with 0.2 to 0.4 mg/l BA, a significant effect resulted in fresh weight and number of roots compared to that obtained with NAA without BA. Increasing BA from 0.2 to 0.4 mg/l significantly increased plant fresh weight (Tables 6 and 7). The optimum sucrose concentration was 30 gm/l, as reflected by the increase in fresh weight, plant height, as well as number of roots and root length. Increasing sucrose concentration had a negative impact on all parameters measured. Agar concentration was optimum at 6 to 8 gm/l as expressed by the significant increase in plant fresh weight as well as plant height. Younger plantlets (single leaf) gave a significantly higher rate of bud proliferation compared to older plantlets (2 to 4 leaves) which gave a lower bud proliferation rate (Table 8).

DISCUSSION

Plant cells and tissues in the culture medium are heterotrophic and therefore depend on external carbon for energy. Sucrose is cheap, readily available, readily assimilated and relatively stable and is therefore the most commonly used carbon source. Other carbohydrates (such as glucose, maltose, galactose and sorbitol) can also be used, and in specialized circumstances may prove superior to sucrose. Quantitatively, some published research findings argue the reduction of optimum sucrose

Table 5. Effect of Murashige and Skoog (MS) basal medium supplemented with different concentrations of naphthalene acetic acid (NAA) on banana shooting development, four weeks after culture *in vitro*.

NAA (mg/l)	Fresh weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
0.0	1.27^{d}	3.60^{b}	34.07^{c}	5.73^{c}	5.71^{a}
0.2	1.91^{d}	3.70^{b}	37.20^{c}	6.60^{c}	6.07^{a}
0.4	3.59^{ab}	5.00^{a}	57.40^{b}	9.00^{b}	6.27^{a}
0.6	3.54^{ab}	5.00^{a}	66.53^{b}	10.40^{a}	6.53^{a}
0.8	4.17^{a}	5.07^{a}	81.47^{a}	10.80^{a}	6.13^{a}
1.0	2.94^{bc}	4.73^{ab}	30.47^{c}	8.67^{b}	6.27^{a}
1.2	2.60^{bc}	3.53^{c}	36.53^{c}	8.07^{b}	6.53^{a}

Values with similar superscript letter(s) in a column are non-significantly different at $P < 0.05$.

Table 6. Effect of Murashige and Skoog (MS) basal medium supplemented with different concentrations of naphthalene acetic acid (NAA) and 0.2 mg/l benzyl adenine (BA) on banana plantlets growth and development, four weeks after culture *in vitro*.

NAA (mg/l)	Fresh weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
0.0	2.06^{c}	4.50^{a}	43.33^{b}	5.8^{b}	5.67^{b}
0.4	2.84^{ab}	4.07^{a}	45.00^{a}	7.67^{a}	5.63^{b}
0.6	2.63^{bc}	4.27^{a}	38.13^{ab}	6.67^{ab}	6.53^{a}
0.8	3.07^{ab}	4.10^{a}	47.13^{ab}	7.40^{ab}	6.07^{ab}
1.0	3.29^{a}	3.70^{a}	41.27^{a}	8.47^{a}	6.07^{ab}

Values with similar superscript letter(s) in a column are non-significantly different at $P < 0.05$.

Table 7. Effect of Murashige and Skoog (MS) basal medium supplemented with different concentrations of naphthalene acetic acid (NAA) and 0.4 mg/l benzyl adenine (BA) on banana plantlets growth and development, four weeks after culture *in vitro*.

NAA (mg/l)	Fresh Weight (g)	Height (cm)	Root length (cm)	Number of root	Number of leaves
0.0	2.47^{b}	5.37^{a}	53.80^{a}	7.47^{b}	5.73^{c}
0.4	3.47^{a}	3.07^{bc}	59.07^{a}	8.60^{ab}	6.27^{abc}
0.6	3.62^{a}	4.17^{b}	58.67^{a}	9.80^{ab}	6.53^{ab}
0.8	3.37^{a}	3.17^{c}	55.07^{a}	10.27^{a}	6.67^{a}
1.0	3.97^{a}	3.83^{b}	49.93^{a}	9.27^{ab}	6.00^{bc}

Values with similar superscript letter(s) in a column are non-significantly different at $P < 0.05$.

Table 8. Effect of physiological age on the rate of bud proliferation of banana plantlets, four weeks after culture in the basal medium supplemented with 7.0 mg/l benzyl adenine (BA).

Plantlet age	Bud proliferation rate
Single leaf plantlet	3.08^{a}
Two leaves plantlet	2.33^{ab}
Three leaves plantlet	1.50^{b}
Four leaves plantlet	1.17^{b}

Values with similar superscript letter(s) in a column are non-significantly different at $P < 0.05$.

concentration to levels lower than 3.0% (Novak et al., 1986; Mateille and Foncelle, 1988). It could strongly be argued that such a decrease in sucrose concentration would negatively affect root growth and development, as well as plant elongation. Supporting findings rather argued for increasing sucrose concentration to 4.5%, to

encourage plant rooting (Harde de Harde and Garcia de Garcia, 1994).

Inositol on the other hand, is a pentose sugar of plant origin, and has been routinely used in a concentration of 100 mg/l in banana tissue culture (Vuylsteke, 1989), and has accordingly been supplemented to MS and B5 at the same concentration (Murashige and Skoog, 1962; Gamborg et al., 1968). In this study, it was evident that the role of inositol was not essential and should not necessarily be included in banana tissue culture as reported in previous studies (Vuylsteke, 1989). Therefore, some carbon sources are plant-specific and their role on *in vitro* culture is likely to be related to tissue type and physiological age. Growth regulators (example auxins, cytokinins and gibberellins plant hormones) play important roles in the growth and differentiation of cultured cells and tissues. Auxins, namely NAA have been reported to encourage plant rooting *in vitro* (Vuylsteke, 1989). Cytokinin usages in plant nutrient media for *in vitro* culture, on the other hand, are dependent on plant tissue growth stage and expected end product. In some studies, banana apical meristems were cultured in media of higher cytokinin concentrations (Krikorian and Cronauer, 1984) or at lower cytokinin at the first stage, and transferred later to a media with higher cytokinin concentration (Jarret et al., 1985; Novak et al., 1986). In our study, evidence was provided that BA was not necessarily required as confirmed by other studies (Hardy de Gomes and Garcia de Garcia, 1994).

As the demand for banana planting material has become a major constraint to the expansion of banana production, the splitting technique proves to be of economic importance. With the application of bud splitting technique, banana plantlets of a younger physiological age (single leaf) significantly promote bud proliferation leading to an important economical value as sub-culturing and time and cost are both minimized.

Conclusion

Sucrose at 3.0% was most optimum for banana shoot tip culture as expressed by better growth vigor at both shoot and root systems. The addition of other carbon sources is therefore not advisable, both for practical and economic reasons. Supplementing culture media with 0.4 mg/l BA was most optimum as expressed by plantlet height, fresh weight, as well as stronger roots. It is therefore recommended that organic additives be excluded from banana culture media as a measure to reduce economic cost as well as likely negative chemical effects on culture media.

Therefore, it is highly recommended that the splitting technique be adopted in banana culture *in vitro.* Such a technique would serve as a substitute to higher concentrations of BA as well as a measure to plantlet genetic deterioration. It is also advised that durations of culture media at the second phase be shortened to three weeks without sub-culturing, as a measure to reach a convenient bud proliferation rate and reduce production cost. It was also evident that this study has improved the nutrient medium constituents, particularly at the third stage where plantlets are encouraged for rooting and acclamation to natural field conditions. The application of splitting technique on younger banana plantlets would lead to better results and overcome physiological barriers that might require complex culture media and increase experimentation and production costs.

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