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TESTING FOR A SUITABLE CULTURE MEDIUM FOR MICROPROPAGATION OF EAST AFRICAN HIGHLAND BANANAS

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ABSTRACT

A study was conducted to find a suitable culture medium for micropropagation of East African highland bananas (*Musa* spp.). Modified Eriksson (ER), Gamborg's B-S (B-S) and Murashige-Skoog (MS) media were tested with a roasting cultivar Gonja-Horn plantain (AAB), a dessert Bogoya-Gros Michel (AAA) and three East African highland cooking (AAA) cultivars Kibuzi, Mbwazirume and Namwezi for *in vitro* proliferation induction. The media were supplemented with 4.5 mg l^{-1} Benzylaminopurine (BAP) and with or without 0.186 mg l^{-1} Naphthalene acetic acid (NAA). Only ER and MS gave proliferation. AAB cultivar proliferated significantly more than the AAAs which did not differ among themselves. The AAA cultivars showed precocious rooting in the absence and presence of 0.186 mg l^{-1} NAA on ER and MS media supplemented with 4.5 mg l^{-1} BAP. However, the presence of auxin NAA significantly lowered shoot proliferation with concomitant significant induction of precocious rooting. This indicated a high level of endogenous auxin in these cultivars and, therefore, the unnecessity of exogenous auxin for their maximum *in vitro* proliferation. It was also found that ammoniacal nitrogen content determined the suitability of a culture medium thus making ER an alternative to the MS salts.

***Key Words*: Auxin, bananas, culture medium, cytokinin, microproliferation.**

RÉSUMÉ

Une étude a été menée en vue de déterminer un milieu de culture adéquat pour la micropropagation du bananier *Musca spp* des hauts plateaux de l'Afrique de l'Est. Les milieux modifiés de Erikson (ER), Gamborg's B-S (B-S) et Marashige-Skooge (MS), ont été testés pour l'induction de la prolifération *in vitro*, avec la variété de plantain Gonya-Horn (AAB), de banane douce Bogoya-GrosMichel (AAA) et 3 variétés faites pour être bouillies (AAA), Kibuzi, Mbwazirume et Namwezi. Les milieux ont été enrichis avec 4,5 mg l^{-1} de Benzylaminopurine (BAP) avec ou sans 0.186 mg d'acide acétique de naphtaléne (NAA). La prolifération a été observée seulement avec ER et MS. La variété AAB a proliféré de maniére plus significative que les AAA, qui en terme de prolifération s'équivalent. Les variétés AAA ont montré un enracinement précoce avec ou sans NAA sur milieu ER et MS enrichis avec du BAP. Cependant la présence d'auxine NAA réduit de maniére significative, la prolifération de bourgeons, et concomitament induit un enracinement précoce. Ceci indique la précence d'une auxine élevée dans ces variétés et l'inopportunité d'un apport exogéne pour maximiser la prolifération *in vitro*. Il a été démontré que la teneur en azote sous forme ammoniacale d'un milieu de culture est déterminante; ainsi le milieu modifié d'Erikson (ER) peut constituer une alternative par rapport au milieu riche en sel Marashige-Skooge (MS).

***Mots Clés*: Auxine, cytokinine, milieu de culture, bananier des hauts plateaux de l'Afrique de l'Est, microprolifération.**

INTRODUCTION

The production of uniform suckers, free from non-obscure pests and pathogens that are transmissible in contaminated planting materials and at a rate relatively higher than that obtainable with the conventional methods of propagation are some of the remarkable advantages of banana micropropagation using the shoot-tip culture technique (Vuylsteke *et al.*, 1981; Vuylsteke, 1989; Swennen and Vuylsteke, 1990). A wide range of *Musa* spp. cultivars of all genomic constitution has been studied for micropropagation (Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985; Bener jee *et al.*, 1986; Mateille and Foncelle, 1988; Vuylsteke, 1989; Cote *et al.*, 1990) . From such a wide study, documentation on the East African highland banana (AAA group) which dominate and is found exclusively in the East African highlands (Sebasigari, 1987) is lacking. This reveals that little attention has been paid to East African Highland bananas.

Although all investigators micropropagating *Musa* spp. have used the Murashige-Skoog salts (Murashige and Skoog, 1962), they have differed in the types and concentrations of the vitamins, amino acids, natural complexes and phytohormones, and even in some of the mineral salts in the culture media (Banerjee and De Langhe, 1985; George *et al.*, 1987; Mateille and Foncelle, 1988; Vuylsteke, 1989). This illustrates lack of consistence in medium composition and possibly cultivar variability. This study was undertaken to determine the proliferation potential of some East African highland cooking banana cultivars and to compare other basal salts with the Murashige and Skoog.

MATERIALS AND METHODS

The study was conducted in the Plant Tissue Culture Laboratory at Makerere University Agricultural Research Institute, Kabanyolo. A roasting banana type Gonja-Horn plantain (AAB), a dessert type Bogoya-Gros Michel (AAA) and three East African highland cooking (AAA) cultivars Kibuzi, Mbwazirume and Namwezi were used in the investigation. Shoot-tip cubes of about 2 cm^3 were isolated from suckers, surface-sterilized in ethanol (96%) for 20 seconds and in 10% (v/v) laundry grade sodium hypochlorite bleach solution (Reckitt and Colman Industries, Kenya) in which Tween 20 at 0.2% v/v was added, for 20 minutes. The cubes were then rinsed three times with sterile water and aseptically dissected to expose the shoot-tip (5 mm) which were placed onto the culture nutrient medium.

Three basic salt formulations Eriksson (ER), Gamborg's B-5 (B-5), and Murashige-Skoog (MS) were compared. Modification was made in ER salts by substituting the unavailable zinc versanate 15 mg l^{-1} with zinc sulphate at 10.6 mg l^{-1}. The three salts were supplemented with (mg l^{-1}): 100 myo-inositol, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 0.4 thiamine-HCl, 2.0 glycine, 10.0 ascorbic acid and 30,000.0 sucrose. The pH of the media was adjusted to 5.8 and the media solidified with 1.8 g l^{-1} phytagel. Fifteen ml volumes of media were autoclaved for 15 min at 121°C and pressure of 1.05 kg cm^{-2}. Cultures were maintained at 25–29 °C, humidity of 54% and a 14 hr light intensity of 1773 ± 42 lux, and arranged in a completely randomized design on the growth shelves. First transfer of cultures to fresh media was done after 1 week, followed by 4 weeks intervals before the first subculture was done 8 weeks after culture initiation. Subculturing was done at intervals of 4 weeks.

The three media were supplemented with an auxin Naphthalene acetic acid (NAA) at 0.186 mg l^{-1} and a cytokinin benzylaminopurine (BAP) at 4.5 mg l^{-1}. The media were evaluated using Gonja-Horn plantain (AAB), Bogoya-Gros Michel (AAA), and three cooking (AAA) types Kibuzi, Mbwazirume and Namwezi, all replicated five times.

Due to the precocious rooting observed in the AAA cultivars, ER and MS basal media were tried with and without 0.186 mg l^{-1} NAA, while maintaining BAP at 4.5 mg l^{-1}. Triploid A cultivars Bogoya Gros Michel, Kibuzi and Mbwazirume were used with five replicates.

RESULTS AND DISCUSSIONS

Only modified ER and MS basal media gave proliferation in the five cultivars tested (Table 1). On B5, there was excessive explant enlargement and browning at the periphery. On ER and MS, Gonja (AAB) proliferated by production of bud clusters enclosed within one or two leaves. The rest of the cultivars (AAA genomic group) gave rise to shoots and buds.

Eriksson and MS did not significantly ($P \leq 0.05$) differ in promoting proliferation of the five cultivars (Table 1). There was genomic differences

in proliferation, and Gonja-Horn plantain (AAB), proliferated significantly ($P \leq 0.05$) more than the AAA cultivars Bogoya, Kibuzi, Mbwazirume and Namwezi, which did not significantly differ in proliferation among themselves.

All AAA cultivars showed precocious rooting at first subculture with similar root intensity, but no such rooting occurred for Gonja (AAB) on the proliferation media (Table 1). Root numbers per explant varied from 2.8 for Bogoya to 3.6 for Kibuzi on both media. Eriksson and MS basal media did not significantly ($P \leq 0.05$) differ in promoting precocious rooting.

Incorporation of NAA at 0.186 mg l^{-1} in ER and MS basal media supplemented with 4.5 mg l^{-1} BAP significantly ($P \leq 0.05$) lowered the proliferation rate (Table 2) with a significant ($P \leq 0.05$) concomitant precocious root induction (Table 3) in Bogoya-Gros Michel, Kibuzi and Mbwazirume. The two media did not significantly

TABLE 1. Mean proliferation[a] and precocious rooting[b] of five banana cultivars cultured on basal Eriksson (ER) and Murashige-Skoog (MS) media supplemented with 4.5 mg l^{-1} BAP and 0.186 mg l^{-1} NAA.

		Shoot-buds per explant		Roots/ explant	
Cultivars	Genome	ER	MS	ER	MS
Bogoya (Gros Michel)	AAA	2.5 b	2.6 b	3.4 a	2.8 a
Kibuzi	AAA	1.9 b	2.2 b	3.6 a	3.6 a
Mbwazirume	AAA	1.9 b	2.8 b	3.2 a	3.2 a
Namwezi	AAA	2.4 b	2.7 b	3.0 a	3.0 a
Gonja (Horn plantain)	AAB	5.3 a	5.1 a	0.0 b	0.0 b
Medium mean		2.8	3.1	2.6	2.5

Means followed by a similar letter in each column are not significantly different at $P \leq 0.05$. LSD (0.05) between media means = 0.7 shoot-buds.
[a] Mean of initial culture and first subculture.
[b] Mean of first subculture.

TABLE 2. Mean effect of naphthalene acetic acid (NAA) in basal Eriksson and Murashige-Skoog media supplemented with 4.5 mg l^{-1} BAP on the proliferation of three (AAA) bananas.

	Number of shoot-buds per explant[a]					
	Eriksson		Murashige-Skoog		Cultivar mean	
	NAA level mg l^{-1}		NAA level mg l^{-1}		NAA level mg l^{-1}	
Cultivar	0.000	0.186	0.000	0.186	0.000	0.186
Bogoya	4.0	2.2	3.9	3.1	4.0	2.7
Kibuzi	3.1	1.6	3.3	1.9	3.2	1.8
Mbwazirume	3.0	1.9	4.5	2.5	3.8	2.2
NAA mean					3.7	2.2
Media mean	2.7		3.2			

LSD (0.05) between NAA level means = 0.5 shoot-buds.
LSD (0.05) between media means = 0.8 shoot-buds.
[a] Mean of initial culture, first and second subculture.

TABLE 3. Mean effect of naphthalene acetic acid (NAA) in basal Eriksson and Murashige-Skoog media supplemented with 4.5 mg l^{-1} BAP on the promotion of precocious rooting of three (AAA) bananas.

	Number of roots per explant[a]					
	Eriksson		Murashige-Skoog		Cultivar mean	
	NAA level mg l^{-1}		NAA level mg l^{-1}		NAA level mg l^{-1}	
Cultivar	0.000	0.186	0.000	0.186	0.000	0.186
Bogoya	1.2	3.2	1.4	4.0	1.3	3.6
Kibuzi	1.0	2.6	1.4	4.6	1.2	3.6
Mbwazirume	1.0	2.8	1.0	2.8	1.0	2.8
NAA mean					1.2	3.3
Media mean	2.0		2.5			

LSD (0.05) between NAA level means = 0.5 roots.
LSD (0.05) between media means = 0.7 roots.
[a] Mean of first and second subculture.

differ in promoting proliferation and precocious rooting.

In presence of NAA precocious rooting started after the first subculture whereas in absence of the auxin it occurred after the second subculture. There was no significant interaction among cultivar, media and NAA.

Failure of Gamborg's B-5 salt formulation to induce proliferation could have been due to its low total nitrogen content and the form in which the nitrogen is incorporated. Banana *in vivo* has a high demand for nitrogen (Purseglove, 1972; Stover and Simmonds, 1987). Yet comparing the three media, B-5 contained 27 mM compared to 49 and 60 mM of nitrogen in ER and MS, respectively. Gamborg *et al.* (1976) reported the repressive effect on *in vitro* growth by the low nitrogen in B-5. In addition to the low nitrogen in B-5 salt formulation, about 93% of the nitrogen is in nitrate with only 7% in ammoniacal form. Since ammoniacal nitrogen was reported to be the most preferred by *in vitro* banana cultures (Marchal, 1990), it is most likely that the lack of easily available nitrogen in B-5 leads to the failure of the medium to promote proliferation. Lack of significant difference in performance of ER and MS media (Tables 1 and 2) despite the low micro-element concentration in the former illustrates the low *in vitro* requirement of these salts. Modified ER basal salts can be an alternative to MS. Due to lack of difference in ER and MS basal media, ER formulation would be preferred considering the cost of the chemicals.

The significantly higher proliferation rates exhibited by Gonja (AAB) over the AAA cultivars could be attributed to the presence of a B genome. Increase in proliferation rate with presence of a B genome was noted by Banerjee and De Langhe (1985) and Vuylsteke and De Langhe (1985). Lack of significant differences in the proliferation of the East African highland (AAA) cooking cultivars reflects their common ancestry reported by Mukasa and Thomas (1970).

The increased proliferation when the auxin was omitted partly suggests the AAA bananas' high level of endogenous auxin as shown by the precocious rooting even on media devoid of the auxin. A high apical dominance reported in banana (Vuylsteke and De Langhe, 1985) could be a function of this high endogenous auxin content, although Vuylsteke and De Langhe (1885) associated this rooting to the exogenous auxin. The incorporation of auxin in the media, therefore, results in increased total auxin level. This high level reduces the effect of the exogenous cytokinin in promoting proliferation since for *in vitro* proliferation a high cytokinin to auxin ratio has to be maintained (Hartmann *et al.*, 1990). The AAA bananas, therefore, require an auxin free medium for maximum proliferation.

Occurrence of precocious rooting could be explained on the basis of rejuvenation. Subculturing was reported by Pierik (1987) as one way of tissue rejuvenation. Pierik (1987) and Hartmann *et al.* (1990) noted the ease of *in vitro* rooting to be more on juvenile tissues.

This study showed that ammoniacal nitrogen content determined the suitability of a culture medium thus making ER an alternative to the MS salts. Exogenous auxin was also found to be unnecessary for maximum *in vitro* proliferation of the East African highland banana cultivars and that the proliferation of these cultivars was comparable.

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