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Propagation of Banana by Shoot Tip Culture

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Abstract

Experiments were conducted to test the effects of concentrations of MS inorganic mineral salts, glucose and growth regulators on growth and development of banana (*Musa* spp.) shoot tips explants derived from *in vitro* grown plantlets. A modified Murashige and Skoog (MS) medium was used as basal medium. Among MS-salts strengths tested, full MS-salt strength (1X) was optimal for all parameters measured compared with other strengths tested. Glucose concentration of 4% and sucrose concentration of 3% (control) were better than the other concentrations glucose tested for growth and development of cultured shoot tips, and there was no significant difference between them The results revealed the importance of inclusion of kinetin (kin) and naphthalene acetic acid (NAA) in nutrient media for banana shoot tip culture at relatively low concentrations, the best results for all parameters measured were obtained on medium containing 0.0mg/litre kin and 0.01 mg/litre NAA. The combination treatment of 0.3 mg /litre kin and 0.03 mg/litre NAA recorded the best results for mean shoot elongation compared with the other combination treatments tested, whereas the greatest mean number of roots was obtained with the combination of kin at 1.0 mg litre and NAA at 0.1mg/litre. The best results for mean root length were, however, obtained on nutrient medium exclusive of growth regulators. Well rooted plantlets were acclimatized and eventually established in soil under lath house conditions with maximum success rate recorded with those obtained on medium containing sucrose. The growth of these plants appeared normal and was vigorous.

Key words: *Musa* spp., shoot proliferation, tissue culture, micro-propagation

Introduction

Banana an arbor-herbaceous plant belongs to the *Musa* spp. AAA genotype group. It is the major fruit crop in the tropics and subtropics. It ranks as the fourth most important staple crop in developing countries, and is the chief source of nourishment for millions of people around the world, particularly in tropical countries. Banana cultivation in Sudan is currently receiving much attention at both public and private sectors. It can be grown under irrigation in most states of the country and fruits can be produced all-year-round providing a steady cash income or

supply of a high nutritious food for growers. A continuing challenge for banana growers with an interest in expanding in banana plantings has been the inaccessibility of planting material of desired cultivars.

Bananas are commonly propagated by vegetative means using suckers of various sizes or by planting whole or small pieces of the large underground rhizome, the true stem. Sucker production is genotype dependent and some banana clones are known to be shy suckers (Vuylsteke and De-Langhe, 1985). Production of a sufficient amount of suitable planting material is often a tedious, impractical and time consuming. The cost of suckers or rhizomes is the largest single factor contributing to total production cost of banana. Because of weather or accidental delay, rough handling, careless packing and transportation for long-distances of suckers usually impair survival and may result in death or poor re-growth upon planting. An additional problem with suckers is that a sucker normally will give only one plant placing limitations on utilization of propagation material.

Tissue culture propagation methods for some *Musa* spp. have been described in the literature (Kodym and Zapata-Arias, 2001; Srangsam and Kanchanapoon, 2003; Sebastian and Mathew, 2004; Anilkumar and Sajeevan, 2005). Not all cultivars will respond in the same way to a generalized medium and/or growing condition. Differences exist among the various genotypes to type and concentrations of the chemical components of the culture medium and incubation condition. Apical meristem (Hwang *et al.,* 1984); shoot tips (Wong, 1986) and floral apices (Sebastian and Mathew, 2004) have been used as explants for *in vitro* culture initiation in banana. Propagation rates achieved by tissue culture techniques are much higher than those reported by conventional methods (Devi and Nayer, 1993). *In vitro* produced banana plants have the advantage of a higher establishment rate at any time of the year (Drew and Smith, 1990) grew vigorously, free of most pests and diseases producing a uniform crop, and have a shorter time to flower and harvest giving a higher yield in the first crop cycle (Robinson and Eckstein, 1993). There is great potential for micro-propagation of banana but problems such as vitrification, successful establishment *ex-vitro* and production of aberrant plantlets (Walduck *et al.,* 1988; Smith and Drew, 1990; Smith and Hamill, 1993; Ramage *et al.,* 2004; Martin *et al*., 2006) remains to be solved.

The need for generating planting stocks in large quantities has stimulated recently a surge of interest in the production of clonal of “Dwarf Cavendish” cultivar, the most widely grown banana cultivar in Sudan, by use of tissue culture techniques. In this study, the chemical components of the nutrient medium were manipulated to determine the optimal concentration of some components of media for shoot proliferation and subsequent growth and development of banana shoot tips under *in vitro* conditions.

Materials and Methods

Shoot tip explants, 0.5-1.0 cm long, obtained, from *in vitro* established banana plantlets of the "Dwarf Cavendish" cultivar, were used as explant throughout this study. On the basis of previous work in our laboratory, a basal medium was used for banana shoot tips culture. The basal medium used in this study was a modified Murashige and Skoog (MS) medium

(Murashige and Skoog, 1962) and (per litre): 100 mg, myo-inositol; 0.4 mg, thiamine-HCl; 10 mg, ascorbic acid; 5 mg, benzyl adenine (BA); 30 g, sucrose, and 2.5 g, phytagel. The pH was adjusted to 5.7±0.1 with 1N NaOH or 1N HCl before adding the phytagel. The medium was heated on a hot plate stirrer to melt the phytagel, dispensed in 25 ml aliquots into 25 x 150-mm glass culture tubes, capped with polypropylene Bellco Kaput closures, sterilized for 15 min at 121°C and 1.04 kg /cm^2 and left to cool as slant at room temperature.

Banana proliferating shoot culture was established by repeatedly sub-culturing the original shoot tips every 6 weeks to a freshly prepared basal medium after each harvest of newly formed roots to generate additional *in vitro* stock plantlets for experimentations. The basal medium was modified according to experiments needs. In the first experiment the medium was modified so that the inorganic test solution contained either of ¼X, ½X, 1X or 2X MS inorganic salt strength with 1X being the control. The second experiment examined the effects of four concentrations of glucose (1%, 2%, 3%, or 4%), on *in vitro* growth and development of banana shoot tips, with 3% sucrose as control. A completely randomized design was used with 10 single explants replicates for each treatment. Each tube was considered a replicate and each experiment was repeated three times.

In experiment 3, a 5X5 factorial experiment with all possible combinations of five concentrations of kin (in mg/litre) (0.0, 0.1, 0.3, 1.0, and 3.0) and NAA (0.0, 0.01, 0.03, 0.1, and 0.3 mg/l) each, was conducted to determine the optimum growth regulators combinations for shoot proliferation and elongation of excised shoot tips of banana. All explants and cultures were maintained slanted in 4X10 stainless steel racks and incubated at 25±°C with 16-h daily exposure to 1000 lux illumination provided by cool-white florescent lamps. Cultures were evaluated after 6 weeks of incubation for average number and length of shoots and average number and length of roots. Hand sections of new shoots were examined microscopically to evaluate axillary or adventitious origin. Data were subjected to analysis of variance procedures of the SAS Institute (SAS, 1990) and Duncan's multiple range test was used to separate treatment means.

Results

The effects of different MS-salt strengths on shoot and root formation on banana shoot tips cultured *in vitro* are shown in Table 1. The data revealed that the highest number of shoots was obtained on the medium containing 1X MS-salts strength with significant difference from the lowest salt strength tested (¼X). No significant difference in shoot number between ½X, 1X and 2X was found. The data also indicated that shoot length increased with increasing MS-salts strength. The longest shoots were recorded on the medium containing 2X MS-salts strength giving significantly higher value than ¼X and ½X MS-salts strength treatment but was not significantly different from the values obtained on the medium containing 1X MS-salts strength.

Table 1: Effect of different concentrations of MS-salts strength on growth and development of banana shoot tips cultured *in vitro* after 6 weeks of incubation period

MS-salt conc.(X)	No. of shoots	Shoot length (cm)	No. of roots	Root length (cm)
¼	5.70 b	4.30 b	6.10 a	2.70 b
½	7.90 a	4.50 b	5.00 ab	4.00 b
1	8.90 a	5.70 a	4.00 b	4.50 a
2	8.10 a	6.00 a	0.00 c	0.00 c

Means with the same letter(s) in the same column are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Root formation progressively decreases with increasing MS-salts strengths up to 2X MS-salts strength treatment where rooting was repressed. The highest number of roots was obtained with the lowest MS-salt concentration tested (¼X) which was, however, not significantly different from the number of roots produced with the ½X MS-salt strength treatment. Root length increased with increasing MS-salt strength up to the 1X MS-salt strength where significantly high values of root length were obtained compared with the other MS-salts strengths tested.

Growth differences between sugars concentration treatments were evident for all parameters measured (Table 2). Average number of roots was found to be non- significantly higher with 4%, 3% and 2% glucose and 3% sucrose treatments but significantly different from that at 1% glucose treatment. On the other hand, shoot length increased with increasing glucose concentrations. The longest shoots were recorded at 4% glucose treatment followed by 3% and 2% glucose treatment with a non-significant difference from 3% sucrose treatment.

Table 2: Effect of different concentrations of glucose and 3% sucrose on growth and development of banana shoot tips cultured *in vitro* after 6 weeks of incubation period

Sugar conc. (%)	No. of shoots	Shoot length (cm)	No. of roots	Root length (cm)
Sucrose 3	7.00 ab	7.30 a	11.50 a	5.30 ab
Glucose 1	5.90 b	4.30 b	4.20 c	2.10 d
Glucose 2	7.70 a	6.60 a	8.00 b	3.30 c
Glucose 3	6.90 ab	6.70 a	12.20 a	5.50 a
Glucose 4	7.80 a	7.20 a	12.80 a	6.20 a

Means with the same letter(s) in the same column are not significantly different at P=0.05, according to Duncan Multiple Range Test

The number of roots produced showed higher significant differences among the different glucose concentrations tested. The 3% glucose treatment gave the greatest number of roots per explant than the other glucose concentrations tested with no significant difference from 4% glucose and 3% sucrose treatments. A progressive and significant increase in root length with increasing glucose concentration was evident. The highest values of root length were obtained at 4% glucose with no significant difference from the 3% glucose and 3%

sucrose treatments. The least values for all parameters measured were obtained on the medium containing 1% glucose with a negative significant difference from all other treatments tested. The results of the effect of kin and NAA, individually or in combinations, on growth and development of banana shoot tips is portrayed in Table 3. Significant differences in shoot proliferation between different treatments were obtained. The highest number of shoots (5.6) was obtained on the medium containing 0.0 mg kin/litre and 0.01mg NAA/litre. Total number of shoots per explant in this treatment was significantly greater than all other treatments tested except the 0.3 mg kin /litre and 0.03 mg NAA/litre combination treatments where 5.2 shoots/explants were registered. The longest shoots (11.5cm) were obtained on the medium supplemented with 0.3mg kin /litre and 0.03mg NAA /litre. The second high value of root length (11.2 cm) was obtained on medium devoid of growth regulators.

Table 3: Effect of different concentrations of kin and NAA on growth and development of banana shoot tips cultured *in vitro* after 6 weeks of incubation period

Growth regulators (mg/l)		Parameters measured			
Kin	NAA	No. of shoots	Shoot length (cm)	No. of roots	Root length (cm)
	0.0	3.00 de	11.20 ab	12.20 f	12.00 a
0.0	0.01	5.60 a	11.00 ab	19.20 ab	10.70 ab
	0.03	3.00 de	10.30 abcd	13.50 def	9.00 cd
	0.1	2.10 f	10.70 abc	12.80 ef	9.50 bc
	0.3	2.20 f	10.60 abc	16.00 bcdef	9.50 bc
	0.0	2.90 de	8.30 fg	12.10 f	6.20 gh
0.1	0.01	2.00 f	9.00 defg	13.70 def	5.80 h
	0.03	3.30 d	9.70 bcde	15.60 bcdef	7.80 defg
	0.1	3.10 de	9.90 bcd	16.30 bcdef	6.30 gh
	0.3	3.30 d	10.00 abcd	18.60 abc	5.90 h
	0.0	2.20 f	8.90 defg	14.60 cdef	8.10 cdef
0.3	0.01	5.00 b	11.00 ab	15.60 bcdef	8.80 cde
	0.03	5.20 ab	11.50 a	15.50 bcdef	9.70 bc
	0.1	3.90 c	10.50 abc	15.70 bcdef	7.30 efgh
	0.3	2.00 f	10.60 abc	17.10 abcde	6.20 gh
	0.0	2.10 f	8.90 defg	12.20 f	9.00 cd
1.0	0.01	4.30 c	9.40 cdef	17.90 abcd	6.60 fgh
	0.03	4.00 c	8.90 defg	13.50 def	7.00 fgh
	0.1	2.50 ef	11.00 ab	20.80 a	5.80 h
	0.3	2.90 de	10.90 ab	18.10 ab	6.70 fgh
	0.0	4.00 c	6.60 h	13.20 ef	3.60 i
3.0	0.01	3.00 de	4.90 i	13.30 ef	4.10 i
	0.03	3.20 d	8.40 efg	19.60 ab	3.90 i
	0.1	3.00 de	7.60 gh	14.60 cdef	3.30 i
	0.3	3.20 d	7.80 gh	12.20 f	3.40 i

Means with the same letter(s) in the same column are not significantly different at P =0.05, according to Duncan Multiple Range Test.

All NAA treatments induced rooting of cultured banana shoot tips with varying degrees. The highest number of roots per explants was obtained on the medium containing 1.0 mg kin/litre and 0.1 mg NAA/litre with mean number of roots of 20.8. The second high value for number of roots per explant was recorded on the medium containing 3.0 mg kin /litre and 0.03 mg NAA/litre with mean number of roots of 19.6. Root length was significantly higher on media devoid of growth regulator media but not significantly different from that on medium containing the combination treatment of kin at 0.0 mg/litre and NAA at 0.01 mg/litre.

Histological examinations showed that shoot formation resulted from axillary bud proliferation and not from basal callus tissue. The chance for production of aberrant banana plants (off-types) here would likely be low, because multiplication was obtained on medium containing low concentrations of growth regulators. Also, it has been noticed that roots produced in media containing NAA alone were more vigorous and strong compared to roots obtained on media supplemented with kin alone or in combination with NAA. Rooted shoots from all experiments were transferred to soil with maximum success obtained with plants grown on medium containing sucrose as an energy source (data not presented).

Discussion

The requirement for inorganic salts and total salt strength of the nutrient medium for *in vitro* culture of organs, tissues, cells of many plant species and genotype is rather constant. The selection of a suitable salt formulae and salt-strength for culture and shoot proliferation are a crucial step for tissue culture propagation of plants. The inorganic salt mixture of Murashige and Skoog, (1962) is the most frequently used salt mix in plant tissue culture media for various purposes and served as bases for further modifications. The result of the effect of MS-salt strength on banana shoot tip cultures revealed the superiority of the normal MS-salts strength for shoot proliferation and root induction could be attributed to its high content of nitrates, potassium and ammonium and high ionic salt strength. Similar results were obtained and similar conclusions were reached with banana (Cronauer and Krikorian, 1984) and with a number of various plant species (Murashige, 1974).

The number of shoots and shoot length increased steadily with increasing salt strength up to 4X (the highest salt strength tested). What is noteworthy in this study is the unexpected large number of strong, sturdy and rootless shoots and high values of shoot length obtained on medium containing 2X MS-salt strength. The results are consistent with those reported by others (Suksa *et al.,* 1997; Mamiya and Sakamoto, 2000; Saadalla and Said, 2012) that indicate that notable positive effects on growth and development of *in vitro* culture of some plant species are obtained on media containing higher MS-salt strengths than the normal strength. It is apparent from this study that bananas are plants of high mineral salts requirements for better growth and development under *in vitro* conditions.

The highest number of roots, on the other hand, was obtained on medium containing the lowest total salt strength tested (1/4X), confirming previous studies and observations on other plant species (Anderson, 1980). The promotion of good rooting by reducing the level of mineral salts in the medium was probably due to a reduction in total

salt strength of the medium. Similar speculations and conclusions have been reported by others (Hyndman *et al.*, 1982; Skirvin *et al.*, 1984) who acknowledged reduction of the level of mineral salts in general and nitrogen in particular in nutrient media to promote good rooting in roses. The findings of this study and previous studies (Zeweldu, 1996) indicated that full MS-salt strength is effective in inducing shoots and roots on banana shoot tips in one single step of culture.

Sucrose has been used consistently at the concentration of 3% for various tissue culture purposes of the majority of plant species and varieties (Murashige, 1974). In banana tissue culture sucrose has been and has been commonly used at the concentration of 3% (Ganapathi *et al.*, 1995). However, lower (Mateille and Foncelle, 1988) and higher (Cronauer and Krikorian, 1984) concentrations than 3% have also been used arbitrary for banana tissue culture. Other sugars have been tested in banana tissue culture as possible alternatives to sucrose as energy sources (De Guzman *et al.*, 1980; Folliot and Marchal, 1992). Glucose, a monosaccharide sugar, has been reported to support better growth and development of explants of monocotyledonous plant species cultured *in vitro* than sucrose (Huang and Murashige, 1976). We have not found glucose reported in the literature as an energy source or banana tissue culture. The average number of roots and root length were found to be significantly higher at 4% glucose but not significantly different from that at 3% sucrose indicating the importance of inclusion of relatively high sugar concentration for improving rooting of banana plantlets. The result confirmed preceding findings with banana (Saied, 1999) and with coconut (Fuentes *et al.*, 2005) that indicate that high sucrose concentration in rooting media was found to be important for the induction of high number of long, thick and vigorous roots vital for successful establishment and survival of *in vitro* produced plantlets upon transfer to *ex-vitro* conditions. The results, however, contrasted with those of Mateille and Foncelle, (1988) who advocated reducing the sugar concentration in the rooting medium to half the concentration used in the proliferation medium for better rooting of *in vitro* produced banana shoots. Differences in results were attributed to differences in genotype, composition of nutrient media components, type and source of explants, purpose of culture and incubation conditions.

An increase in number and length of roots was evident at the highest glucose concentration tested. On the other hand, banana plantlets produced on media containing lower glucose concentrations than 3% developed poor root and shoot growth with translucent and fragile leaves. The results were in general agreement with the findings of (Saied, 1999) that the plantlets obtained from glucose are difficult to transfer and establish under *ex-vitro* conditions. A contradictory report by Borkowska *et al.*, (2000) however, showed that strawberry shoots from a glucose containing medium have higher capacity to form roots and consequently better stand establishment than shoots from sucrose containing medium. The current results demonstrated the superiority of 3% sucrose for banana shoot tip culture. Glucose, at 4% concentration, could be considered as a possible substitute for the sucrose in general agreements with earlier reports on strawberry

(Gerdakaneh *et al.*, 2009) and ginger (Mohamed, 2012). However, higher concentrations of glucose merits further investigations.

Growth and development of explants *in vitro* is often controlled by the cytokinin: auxin ratio of the basal culture medium (Murashige, 1974; Huang and Murashige, 1976). Auxins are normally used for rooting and cytokinins are known to promote shoot proliferation and branching in both *in vitro* and *ex-vitro* conditions. The results showed that medium containing low concentrations of NAA and devoid of BA gave significantly high number of shoots and roots. This is in accordance with (Zeweldu, 1996), who found that medium devoid of growth regulators gave high values of shoot and root lengths. One can only speculate on the potential carry-over effect of the growth regulators used in the basal medium. The results of this study, however, deviated from the findings of (Cronauer and Krikorian, 1984; Saied, 1999) who added relatively high concentrations of BA (5.0-10.0 mg/litre) without an auxin for *in vitro* shoot proliferation of banana. The results also disagreed with (Arinaitwe *et al.*, 2000) who found BA to be most effective for banana shoot proliferation compared to other cytokinins tested. Differences in findings were primarily related to explant source, variety, media composition, type and concentration of cytokinin and incubation conditions. Ohki and Sawaki, (1999), however, advocate avoiding the use of BA since it may enhance callus formation, and this, in turn, is prone to production of aberrant plantlets.

Thick, vigorous and strong roots were obtained on medium containing low concentrations of both kin and NAA. These findings are in accord with those of Saied, (1999) who obtained better rooting of banana plantlets by the inclusion of kin and NAA in the rooting medium. In contrast to these results were those reported by Cronauer and Krikorian, (1984) who obtained better rooting of *in vitro* produced banana shoots on medium devoid of growth regulators.

It is worth mentioning that the procedure of sequential subculture of *in vitro* produced shoots onto the same freshly prepared medium would lead; it is hoped, to the production of large numbers of clonal banana plantlets that are disease-and/or pests-free for commercial plantations and foundation plants for nurseries.

It could, therefore, be concluded, that the manipulation of the chemical components of the culture medium can give important contributions to the optimization of shoot proliferation and improvement of the quality of banana plantlets and, as a consequence, of banana production. The importance of the clonal propagation system of banana herein reported may be related to the lack of a callus intermediate step. Plantlets were produced only from the proliferation of pre-existing axillary buds and not from callus. There is good potential for large-scale propagation of banana on a year-round basis from a single shoot tip using this system. Additional research is warranted to improve *in vitro* rooting, to optimize continuous production of planting material and to evaluate the performance of *in vitro* produced plantlets under field conditions before a system for commercial applications can be developed.

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تكاثر الموز بزراعة قمم السيقان

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المستخلص:

اجريت تجارب لإختبار تاثير تركيز املاح وسط "موراشيقى و اسكؤج" غيرالعضوية، سكر الجلوكوز، ومنظمات النمو على نمو وتطور نبات الموز (*Musa* spp.) ، بإستخدام قمم سوق مأخوذه من نبيتات منتجه في الانابيب كأجزاء إستزراع . أستخدم وسط "موراشيقي واسكوج" كوسط اساسي. اوضحت النتائج ان املاح "موراشيقي واسكوج" عند التركيز الكامل هي الامثل لكل القياسات المرصوده مقارنةً بالتراكيز الاخرى التي تم إختبارها، و كان تركيز الجلوكوز 4% و تركيز السكروز 3% (الشاهد) هما الافضل لنمو و تطور القمم المزروعه مقارنةً بالتراكيز الأخرى التي أختبرت و لا توجد بينهما فروقات معنويه. أفادت النتائج باهمية إضافة تراكيز منخفضه نسبياً من الكينتين (Kin) و نافثالين حمض الخليك (NAA) للاوساط الغذائيه لزراعة قمم سيقان الموز، فقد تم الحصول على افضل النتائج لكل القياسات المرصوده على وسط غذائي يحتوي على 0.0 مليجرام/لتر Kin و0.01 مليجرام/لتر NAA. و سجلت معاملة التوليفه 0.3 مليجرام/لترKin مع 0.03 مليجرام/لتر NAA افضل النتائج لمتوسط طول السيقان مقارنةً بالتوليفات الاخرى التي اختبرت وتم الحصول على اعلى متوسط لعدد الجذور عند التركيز 1.0مليجرام/لترKin و0.1 مليجرام/لترNAA و على اطول الجذور على وسط غذائي خالٍ من منظمات النمو. تمت اقلمة النبيتات المجذره ومن ثم تاسيسها في التربه مع تحقيق أعلى معدل للنجاح مع تلك المنتجه على الوسط الغذائي المحتوي على السكروز. كان نمو هذه النباتات قوياً و يبدو طبيعياً.