Low cost options of carbon sources, gelling agents and supporting materials used for micropropagation of shoot tips of banana cultivar Grand Nain

Hala M. Abdallah[1], Mohamed A. Ali[1] and Adil Yousif Yagoub[2]

[1]Tissue Culture Unit, Agricultural Research Corporation, Wad Medani, Sudan.
[2]Agricultural Economics and Policy Research Center, Agricultural Research Corporation, Wad Medani, Sudan

ABSTRACT

The high cost of materials used for media preparation is chiefly constituted by gelling agents and sucrose. The objective of this study was to develop efficient micropropagation techniques for the newly released banana cultivar Grand Nain at a low cost by testing different alternatives of carbon sources (sucrose),gelling agents and supporting materials. Three experiments were conducted to test different carbon sources, gelling agents and support matrices. In the first experiment, different carbon sources were tested. These included the refined imported sugar special for tissue culture from Sigma Company, Kenana (refined local sugar), Alguneid (brown local sugar) and beet crystal imported sugar from the local market. Results showed that all sources of sucrose resulted in the highest number of explants with shoots, however, Alguneid sugar induced significantly higher number of shoots per explants. There was 99.4% reduction in the medium cost compared to the standard medium of Sigma sugar. The second experiment was conducted to test different gelling agents such as corn starch at 50.0g/l plus agar at 3.0 and 4.0 g/l, corn starch at 50.0g/l plus phytagel at 1.25 and 1.5 g/l, respectively. Phytagel at 2.5 g/l and agar at 9 g/l were used as control. Gelling agents did not differ in the percentage of explants with shoots. However, corn starch at 50.0g/l and phytagel at 1.5 g/l resulted in the highest number of shoots which reduced the medium cost by 30.7%. In the third experiment, different supporting materials were used as low cost options including stone matrices, glass beads, cotton fiber and filter paper. Rock stones were the best supporting material in liquid medium for propagation of banana cv. Grand Nain. It resulted in significantly higher percentage of explants with shoots, number of shoots per explant and plant height.

INTRODUCTION

Banana (*Musa* spp.*)* is an important fruit crop. Micropropagation of banana is highly efficient, allowing a large turnover of plants in a very short period of time within very little space (Arvanitoyannis *et al*., 2007). Although conventional plant tissue culture has been applied for decades, the high cost of tissue production is a drawback for laboratories with limited resources, especially in the developing countries (Savangikar, 2002).

Plant tissue culture, has three components, namely, nutrients/media chemicals (plant growth hormones, vitamins and mineral nutrients), plant inocula, equipment (culture containers, autoclave, laminar flow, instruments used for micropropagation, pH meter *etc*.) and the structures

(media preparation, inoculation, growth and hardening rooms). All these form points of interventions in cost reduction (Ganapathi *et al.*, 1995).

The cost of medium preparation (chemicals and energy) account for 30-35% of the cost of micropropagation of plants (Savangikar, 2002). On the other hand, the gelling agents such as agar contribute 70% of the total cost of the media (Savangikar *et al.*, 2002).

Use of support matrices, locally available macronutrients, micronutrients, sugar, equipment and facility reduced the cost of consumable material for banana tissue culturing by about 94% (Gitonga *et al*., 2010). Vora and Jasari (2011) reported that the cost of the components used in media preparation, is chiefly constituted by agar 49.61 %, sucrose 38.49%, BA 7.78 % and the rest of the components 4.12%. Kwame *et al*. (2012) substituted the conventional sources of Murashige and Skoog (1962) medium salts with two grams Easygro® vegetative fertilizer containing both macro and micronutrients, which reduced the cost of the nutrients used in media preparation by 96.9%.

Various plant derived gelling agents such as corn, potato, cassava flour were used as agar substitute or in combination with agar in growth media (Shalini and Deepa 2014). Sucrose is the most commonly used carbon source in the micropropagation of plants. Household sugar and other sugar sources can be used to reduce the cost of the medium (Agrawal *et al*., 2010).

The main objective of this study was to test low cost alternatives of carbon sources, gelling agents and supporting material for micropropagation of the newly released banana cultivar Grand Nain.

MATERIALS AND METHODS

This research work was conducted at the Tissue Culture Unit, Agricultural Research Corporation, Wad Madani, Sudan in 2013-2014. Shoot tip explants of banana variety Grand Nain (GN) were propagated *in vitro* on Murashige and Skoog (1962) medium using a protocol released by Ali and Tokporo (2007). The gelling agents and carbon sources as low cost options, were tested in three experiments using the completely randomized design (CRD) with five replicates and four explants per replicate. Four sources of sucrose including Sigma (refined imported sugar), Kenana (refined local sugar from Kenana Sugar Company), brown local sugar from Alguneid Sugar Factory and the beet crystal imported sugar were tested. All sugar types were added to the media at a rate of 3%. The second experiment was established to test the morphogenesis of banana on media with partial substitution of gelling agents by corn starch (CS): agar (Ag) at the following ratios (50:0:3 and 50:0:4) and corn starch (CS): phytagel (Py) at the ratios of 50:0:1.25 and 50:0:1.5 compared with phytagel at 2.5g/l and agar at 9.0g/l as control. In the third experiment, different supportive materials in liquid media were tested as alternatives to agar including rock stones, glass beads, cotton fiber and filter paper. The pH of culture media was adjusted to 5.8± 0.1 before autoclaving at 121°C under pressure of 1.1 kg cm^{-2} for 15 minutes. All cultures were maintained in a growth chamber at 25º ± 2ºC under 16/8 h (light/dark) photoperiod.

Data on percentage of explants with shoots, number of shoots per explant and plant height were taken after 4 weeks of incubation. Roots were counted after 4 weeks of culture on the first experiment. Data were analyzed using the analysis of variance procedure. Duncan's Multiple Range Test (DMRT) was used for means separation.

RESULTS AND DISCUSSION

Table 1 shows that all banana explants cultured on MS medium with different sugar sources successfully regenerated shoots and the frequency of shoot regeneration was comparable on all types of sugar. There were very highly significant differences between the different sucrose sources with respect to the number of shoots per explant, plant height and number of roots per explant. Alguneid sugar resulted in the maximum number of shoots per explant while Kenana sugar resulted in the longest plantlets and maximum number of roots per explants. So, substituting refined sugar (Sigma) with local sucrose positively affected the number of shoots and roots produced by the explants and length of plantlets one month after inoculation.

Results concluded that locally produced sugars can be used effectively as carbon sources for micropropagation of banana cv. Grand Nain. This is in agreement with the results of Agrawal *et al*. (2010) who reported the use of market sugar instead of sucrose to reduce the cost of *in vitro* propagation of banana, Karpura chakkarakeli cultivar, with no significant effect on regeneration compared to sucrose. Dhanalakshmi and Stephan (2014) reported the use of table sugar as a low cost media option for the production of banana through plant tissue culture which led to 84.7% cost reduction. Kodym and Zapata-Arias (2001) successfully used table sugar for multiplication of banana, potato, orchids, chrysanthemum, shoot regeneration and rooting of lentil, peanut and chickpea. Results obtained in this study concluded that the unrefined sugar was better than high grade laboratory sugar for the multiplication of banana.

Table 1. Effect of different sources of sucrose on *in vitro* morphogenesis of banana cv. Gran Nain shoot tip explants cultured on MS medium after 4 weeks.

Sources of sucrose	Explants with shoots (%)	Number of shoots/explant	Plant height (cm)	Number of roots/explant
Sigma	100	4.1b	2.5b	2.7b
Kenana	100	4.0b	3.3a	3.3a
Alguneid	100	4.7a	2.7b	2.2c
Beet	100	4.1b	2.7b	2.7b
Sig. Level	NS	***	***	***
SE ±		0.08	0.08	0.10
CV%		5.6	7.6	8.6

*** and NS indicates significance at P< 0.001 and not significant, respectively.
Means within the same column followed by the same letters are not significantly different at p<0.05 level of probability using DMRT.

Shoot regeneration was induced on all explants of banana cultured on MS medium with different gelling agents. Highly significant differences were obtained between the different treatments on the number of shoots per explant (Table 2). Shoot proliferation was better on MS medium solidified with 50.0g/l corn starch plus 1.5g/l phytagel. This medium resulted in the maximum number of shoots per explant and it was comparable with MS medium solidified with 2.5g/l phytagel. Concerning plant height, significant differences were obtained between the different gelling agents used in this study. The longest plantlets were found on MS medium solidified with 2.5g/l phytagel. These results were similar to those induced on MS medium solidified with 50.0g/l corn starch plus 3.0g/l agar or 50.0g/l corn starch plus 1.5g/l phytagel (Table 2).

Results obtained in this study were in line with those reported by Kodym and Zapata-Arias (2001) who successfully replaced gelrite in the medium with locally available starch/gelrite mixture for micropropagation of *Musa* Grand Nain by shoot tip culture. Starch of corn or potato was able to partially substitute gelrite and agar. Agrawal *et al*. (2010) reported significantly higher survival rate (100%) of cultures of banana cv. Karpura Chakkarakeli on isabgol-media, than that on agar-media (79–83%) and on phytagel-media (51–57%). Similar to the results of this study, Norhayati *et al*. (2011) used various commercial starch, namely, cassava flour, rice flour,

Table 2. Effect of different gelling agents on *in vitro* morphogenesis of banana cv. Gran Nain shoot tip explants cultured on MS medium after 4 weeks of incubation.

Gelling Agents (g/l)			Explants with shoots (%)	Number of shoots/explant	Plant height (cm)
Corn starch	Phytagel	Agar			
50	0.0	3.0	100	3.0bc	2.5ab
50	0.0	4.0	100	2.7c	2.2b
50	1.25	0.0	100	3.2b	2.2b
50	1.5	0.0	100	4.0a	2.5ab
0.0	2.5	0.0	100	3.7a	2.7a
0.0	0.0	9.0	100	3.0bc	2.3b

Sig. Level	NS	***	*
SE±		0.10	0.06
CV%		10.2	12.8

NS, * and *** indicate not significant, significance P< 0.05 and 0.001, respectively.
Means within the same column followed by the same letters are not significantly different at p<0.05 level of probability using DMRT.

corn flour and potato starch as alternative gelling agents in *in vitro* micropropagation of *Celosia* sp. The best concentration for shoot regeneration was corn starch (40g/l), rice flour (40g/l), potato flour (60g/l) and blend of cassava flour and agar (40g/l and2g/l). Ayelign *et al*. (2012) used *Enset ventricosum* flour, 'Bulla' at 80g/l, as agar substitute. It showed no significant differences in number of shoots, number of roots, shoot height, number of leaves and fresh weight of *Ananas comosus* var. Smooth Cayenna plantlets besides good gelling ability than agar, with 72.5% reduction of agar cost per litre of media. In contrast to the results of this study Abdul *et al*. (2012) investigated eleven putative gelling agents of different concentrations and combinations as agar substitutes. These included arrowroot (*Maranta arundinaceae*), coconut powder (*Cocos nucifera*), corn flour (*Zea mays* var. amylacea), gelrite (a water-soluble polysaccharide produced by *Sphingomonas elodea*, glue (*Cyanoacrylates*), katira gum (*Cochlospermum religiosum*), guar gum (*Cyamopsis tetragonolobus* L.), isubgol husk (*Plantago ovata*), pectin and rice (*Oryza sativa* L.) powder. Among these, 2.8% guar gum was found a promising alternative for agar.

Shalini and Deepa (2014) reported that a low cost gelling agent can be developed from naturally available plant sources provided that its properties such as clarity, osmotic properties and gelling properties were unaffected by altered pH to substitute tissue culture grade agar in culture medium. They added that the high amount of impurities of a low cost gelling agent may induce adverse toxic effects to the micropropagules produced.

Table 3 shows highly significant differences between the different support materials in the percentage of shoot formation, number of shoots per explants and plant height. Rock stones as a support material were the most suitable alternative to the expensive gelling agents. It resulted in the highest percentage of explants with shoots, number of shoots per explant and longest plantlets. Explants cultured on liquid medium with filter paper developed the lowest percentage of shoots while cotton fiber resulted in the lowest number of shoots per explant and the shortest plantlets (Table 3).

Table 3. Effects of different support materials in liquid MS medium on in vitro morphogenesis of banana cv. Gran Nain shoot tip explants after 4 weeks of incubation.

Support material	Explants with shoots (%)	Number of shoots/explant	Plant height (cm)
Rock stones	100.0a	4.0a	2.7a
Glass beads	87.5c	3.1b	2.0b
Cotton fiber	93.8b	2.6c	1.4c
Filter paper	81.3d	2.7bc	2.2b
Sig. Level	***	***	***
SE ±	1.88	0.16	0.13
CV%	2.5	10.2	14.1

*** indicates significance at P< 0.001.

Means within the same column followed by the same letters are not significantly different at p<0.05 level of probability using DMRT.

Results show the possibility of using low cost options of physical matrices as supporting materials in liquid media for micropropagation of banana cv. Grand Nain. This finding was in agreement with Goel *et al*. (2007) who reported that support matrices have been used successfully as a low cost alternative to gelling agents. In contrast to the results of this study, Sharifi *et al*. (2010) used liquid medium with cotton substratum and different combinations of starch, semolina, potato powder and agar in a two step micropropagation (shoot induction and proliferation) of African violet (*Saintpaulia ionantha*). They found that the best shoot proliferation took place in liquid medium with cotton substratum.

Economic analysis

In this study, the purpose of using different sources of sugar, testing the alternative gelling agents to phytagel and agar and different supporting material in liquid media was to reduce the overall cost of micropropagating banana. The results showed that the productivity per unit of variable cost (plant/SDG) was better on the alternative sources of sucrose compared with Sigma sucrose. So, substitution of Sigma sucrose used in micropropagation of banana cv. Grand Nain with table sugar reduced the cost by 99.3%- 99.4% (Table 4). According to Goel *et al.* (2007), using of sugar in glass beads supported liquid medium caused up to 94% reduction in the cost of the medium used for culturing of *Rauwoflora seperpentina*. Gitonga *et al*. (2010) reported that the substitution of sucrose used in conventional tissue culture of banana with table sugar reduced the cost by 97.7%.

Table 5 shows that the higher productivity per unit of variable cost (plant/SDG) was observed on medium with 50.0g/l corn starch plus 1.5g/l phytagel. The partial replacement of phytagel and agar with 50.0g/l corn starch reduced the cost by 30.7%-40.7% and 54.7%- 65.8%, respectively. Substitution of gelling agents such as agar and gerlite with support matrices such as glass beads, cotton wool and vermiculite reduced costs by 94.2% (Gitonga *et al*., 2010).

In conclusion, local household sugar can be used as carbon source, corn starch as partial substitute for the gelling agents (agar or phytagel) and rock stones can be used as support materials in liquid media for micropropagation of the banana cv. Grand Nain.

Table 4. Productivity per unit of variable cost and percentage of cost reduction for the types of sugars compared with Sigma sucrose.

Sources of sucrose	Number of shoots/explants	Variable cost/l media	Productivity per litre of media (plant)	Productivity per unit of variable cost (plant/SDG)	Cost reduction (%)
Sigma	4.1	27.45	328	11.9	
Kenana	4.0	0.18	320	1777.8	99.3
Alguneid	4.7	0.159	376	2364.8	99.4
Beet	4.1	0.159	328	2062.9	99.4
Sig. Level	***				

*** indicates significance at P< 0.001.

Table 5. Productivity per unit of variable cost and percentage of cost reduction for the gelling agents.

Gelling agents (g/l)			Number of shoots per explant	Variable cost	Productivity per litre of media (plant)	Productivity per unit of variable cost (plant/SDG)
Corn starch	Phytage	Agar				
50	0.0	3.0	3.0	23.21	240	10.3
50	0.0	4.0	2.7	30.75	216	7.0
50	1.25	0.0	3.2	3.84	256	66.6
50	1.5	0.0	4.0	4.49	320	71.3
0.0	2.5	0.0	3.7	6.48	296	45.7
0.0	0.0	9.0	3.0	67.84	240	3.5
Sig .Level			***			

*** indicates significance at P< 0.001

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الخيارات الاقتصادية للسكر والمواد الجلاتينية والمواد الداعمة للزراعات النسيجية المستخدمة للإكثار الدقيق للقمم النامية للموز الصنف قراند نين

هاله محمد عبدالله[1], محمد احمد علي[1] وعادل يوسف يعقوب[2].
[1]وحدة زراعة الأنسجة, هيئة البحوث الزراعية, واد مدني, السودان.
[2]مركز البحوث الاقتصادية والسياسات الزراعية, هيئة البحوث الزراعية, واد مدني, السودان.

الخلاصة

ارتفاع كلفة الزراعة النسيجية النباتية هو السبب في عدم تطبيقها علي نطاق واسع. إن تكلفة المواد الجيلاتينية (الآجار والفايتاجل) والسكر تمثل أكبر تكلفة بين العناصر المكونة للوسط الغذائي. الهدف من هذه الدراسة هو تطوير خيارات اقتصادية لتقنية زراعة الأنسجة للإكثار الدقيق للموز الصنف قراند نين المجاز حديثاً وذلك باختبار بدائل للسكر والمواد الجلاتينية وايجاد مواد داعمة للزراعات النسيجية في الأوساط الغذائية السائلة. أجريت الاختبارات في هذه الدراسة بإستخدام النظام العشوائي الكامل في خمس مكررات وأربع قمم نامية في كل مكرر. تم تنفيذ ثلاث تجارب بهدف خفض تكلفة الاكثار الدقيق للموز الصنف قراندنين. فى التجربة الأولى تم اختبار أربعة مصادر الكربون وهى: سيقما (سكر نقى مستورد خاص بزراعة الأنسجة من شركة سيقما), كنانة (سكر نقى منتج محلياً من شركة سكر كنانة), الجنيد (سكر بنى منتج محلياً من مصنع الجنيد) والبنجر(سكر بلورى مستورد). لا توجد فروقات معنوية بين أنواع السكر التي تم اختبارها في النسبة المئوية للقمم النامية للموز التي تم تحفيز نموات جديدة منها وعدد النموات الجديدة (السيقان)، لكن عدد السيقان النامية من كل قمة نامية علي سكر الجنيد أعلي معنويا من أنواع السكر الأخرى. باستخدام هذه البدائل تم تخفيض 99.4% من تكلفة السكر في الوسط الغذائي. أجريت التجربة الثانية لاختبار مواد جلاتينية مختلفة وهى: 50.0 جرام/لتر من نشا الذرة الشامية مع 3.0 جرام/لتر و4.0 جرام/لتر اجار,50.0 جرام/لتر من النشأ مع 1.25 جرام/لتر و1.5 جرام/لتر فايتاجل, 2.5 جرام/لتر فايتاجل و9 جرام/لتر اجار كشواهد. أوضحت النتائج أن الاحلال الجزئي للمواد الجلاتينية (الفايتاجل والاجار) بمقدار 50.0 جرام/لتر من نشا الذرة الشامية يمكن استخدامه للإكثار الدقيق للموز صنف قراندنين، حيث لا توجد فروقات معنوية بين كل المعاملات في النسبة المئوية لأجزاء الموز التي تم تحفيز نموات جديدة منها وعدد السيقان مع تخفيض30.7-40.7 %و54.7-65.8 % في كلفة الآجار والفايتاجل علي التوالي. فى التجربة الثالثة تمت زراعة القمم النامية فى وسط غذائي سائل باستخدام دعامات مختلفة وهى: مصفوفات من الحصى, البلى, ألياف قطنية و أوراق الترشيح. أثبتت النتائج أن مصفوفات الحصى هى أفضل الدعامات المختبرة فى الوسط الغذائي السائل.