African Crop Science Journal, Vol. 23, No. 1, pp. 59 - 66
ISSN 1021-9730/2015 $4.00

BANANA JUICE AS AN ALTERNATIVE ENERGY SOURCE FOR BANANA *IN VITRO* GROWTH MEDIUM

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(Recieved 24 January, 2014; accepted 23 February, 2015)

ABSTRACT

Energy sources in tissue culture media are important for plants whose photosynthetic efficiency is insufficient under *in vitro* conditions. However, the cost of tissue culture grade energy sources is high, thus making tissue culture derived plantlets expensive. The cost of table sugar commonly used in commercial tissue culture laboratories and a substitute for tissue culture grade sucrose in Uganda, is also relatively high given the volumes used. The aim of this study was to evaluate the possibility of exploiting banana (*Musa* spp.) juice, as an energy source in place of table sugar or tissue culture grade sucrose. Banana juice was extracted from the locally available East African Highland Banana (EAHB) beer cultivars, Mbidde-Kabula, Pisang awak (Kayinja) and Km 5, and used at levels of 20, 30, 40 and 50 ml l^{-1}. The quality and amount of juice necessary to support *in vitro* growth of cooking EAHB cultivars Nakabululu, Nakitembe and Nakinyika was evaluated. The juice had varied composition of salts, sugars and organic acids; but with pH compared with table sugar solution. The highest number of shoots and shoot height was observed when bananas were cultured on media supplemented with 50 ml l^{-1} Kayinja juice. This response was greater than that observed with culture media supplemented with the control energy source of 30 g l^{-1} of table sugar. Results also showed that banana juice not only enhanced micropropagation but also improved *in vitro* plantlet vigour and reduced the cost of energy sources by 30%.

Key Words: Carbon source, East African Highland Banana, micropropagation, *Musa* spp.

RÉSUMÉ

Les sources d'énergies sont importantes dans les milieux de culture *in-vitro* des plantes dont l'efficacité photosynthétique est insuffisante dans les conditions de culture *in-vitro.* Cependant, le coût des sources d'énergies utilisées en culture *in-vitro* de tissus est élevé, ceci rend couteux les plantules produites par culture *in-vitro*. Le coût du sucre de table habituellement utilisé dans les laboratoires commerciaux de culture de tissus *in-vitro* ainsi que celui du substitut de sucrose utilisé en Ouganda reste relativement élevé, étant donné les volumes utilisés. L'objectif de cette étude était d'évaluer la possibilité d'exploiter le suc de bananier (*Musa* spp.), comme une source d'énergie en lieu et place du sucre de table ou du sucrose. Le suc de bananier a été extrait des variétés de bananier localement disponible ; bananier de terre ferme de l'Afrique de l'Est (EAHB), Mbidde-Kabula, Pisang awak (Kayinja) and Km 5, et utilisé à différentes concentrations telles que 20, 30, 40 and 50 ml l^{-1}. La qualité et la quantité de suc nécessaire pour assurer la croissance *in-vitro* des variétés EAHB, Nakabululu, Nakitembe and Nakinyika a été évalué. Le suc avait des concentrations variées en sels, sucres et acides organiques; mais avec un pH comparable à celui du sucre de table en solution. Le plus grand nombre de rejetons et les pousses les plus hautes ont été obtenues lorsque les tissus du bananier sont cultivés dans un milieu contenant 50 ml l^{-1} de suc de Kayinja. Cette réponse était plus élevée que celle obtenue avec culture sur un milieu témoin (30 g l^{-1} de sucre de

table). Les résultats indiquent que le suc de bananier au-delà de renforcer la micro propagation, améliore aussi la vigueur des plantules en verre et réduit de 30% le coût des sources d'énergies utilisées en culture de tissus in-vitro.

Mots Clés: Source de carbone, Bananier de terres fermes de l'Afrique de l'Est, micro propagation, *Musa* spp.

INTRODUCTION

Addition of a carbon source to a nutrient medium is crucial for proper *in vitro* growth and development of plant species. This is because the photosynthetic capacity of *in vitro* cultures is often under-developed and, thus is insufficient (George, 1993). Sucrose has been found to be an appropriate energy source in micropropagation; other alternatives energy sources include galactose, maltose, fructose and lactose (George, 1993).

Due to high cost of analytical grade carbon sources, table sugar has been widely used as a substitute (Demo *et al*., 2008). However, like other alternative carbon sources, the physiochemical characteristics of table sugar depend on genotypes and growth environments. The cost of energy sources increases the cost of growth media and subsequently leads to increased prices of tissue culture-derived banana plantlets. This justifies the need for alternatives like banana (*Musa* spp.) juice, which can be obtained locally in Sub-Saharan countries. Bananas grow abundantly in countries such as Uganda, and some varieties are specialised for juice extraction (Gensi *et al*., 1994).

Although banana juice has been found to contain high amounts of sugars (Kyamuhangire, 2002), its energy or carbon source potency in plant micropropagation remains unexploited. Some varieties have been reported to yield over 10 litres of juice per bunch (Gensi *et al*., 1994); an amount that can make up to 200 litres of media. The aim of this study was to determine the amount and potency of banana juice for *in vitro* propagation of bananas.

MATERIALS AND METHODS

Source of explants. Sword suckers of cooking East African Highland Bananas (EAHB)-AAA, cultivars Nakabululu, Nakitembe and Nakinyika were obtained from Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) banana mother garden. These were surface sterilised according to Talengera *et al*. (1994). The explants were initiated on Murashige and Skoog (MS) media (Murashige and Skoog, 1962) supplemented with locally extracted banana juice, in different volumes.

Banana juice extraction and quality analysis. Mature fruits of beer banana cultivars Mbidde-Kabula, Pisang awak (Kayinja) and Yagambi-Km 5 (Km 5) were obtained from the banana germplasm at National Agricultural Research Laboratories (NARL) at Kawanda in Uganda. The banana juice was extracted according to Kyamuhangire (2002). The extracted juice was weighed and its pH and Brix determined using a pH meter and refractometer, respectively. The juice was then packaged in one liter pre-sterilised plastic bottles according to Mannheim *et al*. (2006).

Proximate analysis of the extracted juices was done using the modified method of Ronald and Ronald (1991). The juices were analysed for potassium, sodium, phosphorus, titratable acidity, glucose, sucrose, maltose, fructose, ash, ascorbic acid and viscosity. Similar analyses were done on a control table sugar solution, with a concentration of 30 g L^{-1}, that is routinely used in banana tissue culture at Makerere University Plant Tissue Culture Laboratory (Sadik *et al*., 2012). The control sugar solution was prepared by dissolving 30 g of table sugar in 1000 ml distilled sterile water.

Media preparation. Culture medium was prepared basing on Murashige and Skoog's (1962) (MS) medium, with modifications (Talengera *et al*., 1994). The pH of the medium was adjusted to 5.8, using 0.1M HCl and 0.1M NaOH solutions, just before autoclaving. Twenty millilitre aliquots were dispensed into culture tubes and later sterilised by autoclaving at 121 ^{o}C, 1.05 kg cm^{-2} (103.4 KPa), for 15 min.

Inoculation, incubation and sub-culturing. Sterile explants were inoculated into culture vessels containing tissue culture media under an air laminar flow hood (Talengera *et al*., 1994). Ten glass culture bottles per treatment, per explant, were inoculated and the cultures maintained under 14 hr photoperiod, light intensity of 30-40 ìmole M^{-2} S^{-1}, supplied by white fluorescent tubes, and at temperature of 25-27 °C. Monthly sub-cultures were carried out by separating the shoot clusters into individual shoots, which were then trimmed down to shoot tips and re-cultured under the same conditions. This process was repeated up to the second sub-culture level, on table sugar (30 g l^{-1}) supplemented media, after which they were transferred onto media supplemented with banana juice at 10, 20, 30, 40 and 50 ml l^{-1} for each banana type. A medium containing table sugar at 30 g l^{-1} was used as the control.

Experimental design and data analysis. The experiment was laid out in a completely randomised design, in which ten shoot tips of banana cultivars Nakabululu, Nakitembe and Nakinyika were used for each media type. Shoot clusters from the various MS media types, supplemented with banana juice, were monitored for the number of shoots and shoot height. This procedure was repeated three times and data on number of shoots and shoot height was subjected to analysis of variance (ANOVA) using GenStat, 12th edition (VSN international).

Cost analysis. Computation of the cost benefit of using banana juice was done based on substitutions of table sugar, which is currently used in commercial tissue culture laboratories in Uganda, with banana juice that was locally extracted from beer bananas. Other components of the media were not altered. The cost of table sugar used in the analysis was the price in Uganda of Uganda shillings (UGX) 2,500 (US$ 1 = UGX 2,650 in 2013. The cost of 1 litre of banana juice concentrate was estimated at UGX 1,000, as per the prevailing market prices and was optimised to prepare 20 litres of media at 50 ml L^{-1} as described above. Therefore, it would cost UGX 50 of banana juice to prepare 1 litre of media. The control of table sugar at 30 g l^{-1} (30% of UGX 2,500) would cost UGX 75. Thus, the cost reduction of using banana juice in place of table sugar would be UGX 25 (30%).

RESULTS AND DISCUSSION

Banana juice yield and quality. Beer EAHB cultivars, Mbidde and Kayinja, yielded more banana juice per kilogramme of pulp; the least amount of juice was extracted from Km 5. The cultivars displayed differences in concentration of salts, sugars and organics acids (Table 1). The pH of table sugar solution was more acidic than that of banana juices. There was higher salt concentration in juice extracted from Mbidde than from other cultivars. Table sugar solution had the lowest salt concentration (Table 1). Juice extracted from 'Km 5' had higher sugar concentrations than other juice samples (Table 1), with table sugar solution having the lowest concentration.

Juice extracted from Mbidde had the highest concentration of titratable acids (Table 1); table sugar solution had the lowest concentration (Table 1). Juice from Kayinja had the highest total sugars (Brix), followed by Mbidde juice and the table sugar solution. Juice extracted from Km 5 was more viscous than other juices; while table sugar solution had the lowest viscosity.

Cultivar Kayinja yielded significantly more ascorbic acid, a component that plays a significant role in suppressing oxidation and browning of banana in vitro. Ascorbic acid has been used in banana tissue culture to control browning (Ko *et al*., 2009). In severe cases, plantlets *in vitro* can die due to browning. Therefore, use of banana juice in *in vitro* culture is likely to impart benefits of suppressing browning and its detrimental effects. The high brix levels and titratable acids (including Ascorbic acid, as well as high juice yield per pulp could be regarded as good attributes for cv Kayinja as a source of juice for banana micropropagation.

Shoot proliferation and vigour. The number of shoots significantly ($P<0.001$) differed on MS media supplemented with various volumes of banana juice (Table 2). The number of shoots of *in vitro* cultures increased with volume of banana

TABLE 1. Chemical characteristics of juice extracted from three banana cultivars in comparison to table sugar

Chemical component	Source of banana juice			Table sugar
	Cultivar Mbidde	Cultivar Kayinja	Cultivar Km 5	
Juice yield (l kg^{-1} pulp)	0.423	0.433	0.386	
pH	4.61	4.36	4.97	3.95
Salts				
Potassium (g l^{-1})	99.30	50.52	45.29	17.42
Sodium (g l^{-1})	22.11	22.11	17.69	17.42
Phosphorus (g l^{-1})	72.07	61.31	61.77	61.31
Ash (g l^{-1})	2.15	0.86	3.37	0.036
Sugars				
Glucose (g l^{-1})	15.60	22.42	47.22	8.29
Sucrose (g l^{-1})	11.16	24.65	86.79	2.63
Fructose (g l^{-1})	7.92	17.49	61.58	1.87
Maltose (g l^{-1})	5.40	11.93	42.00	1.29
Acids				
Titratable acid Malic acid (g l^{-1})	0.87	0.68	0.44	0.12
Ascorbic acid (g l^{-1})	0.33	1.49	0.60	0.33
Others				
Brix (%)	20.0	27.0	17.0	4.0
Viscosity (cp)	2.79	2.76	6.11	0.98

TABLE 2. Number of shoots and shoot height of *in vitro* bananas cultured on MS media supplemented with varying amount of banana juice of different beer cultivars

Amount of juice added (ml l^{-1})	Mean number of shoots			Mean shoot height (cm) cultivar		
	Cultivar Mbidde	Cultivar Kayinja	Cultivar Km 5	Cultivar Mbidde	Cultivar Kayinja	Cultivar Km 5
10	0.00c	0.58d	0.00c	0.00c	0.33d	0.00e
20	0.00c	2.25c	1.92b	0.00c	2.17c	1.21d
30	0.17c	2.67c	1.83b	0.08c	3.00b	1.75c
40	0.67b	3.33b	2.25b	0.25bc	3.25ab	2.58b
50	3.25a	3.50a	3.42a	3.42a	4.42a	3.46a

*Mean values in columns followed by the same letter are not significantly different at P<0.001 according to Tukey's least-significance difference range test

juice supplemented to MS medium. The highest number (3.5) was obtained on 50 ml l^{-1} Kayinja juice; while the lowest proliferation was on 10 -20 ml l^{-1} Mbidde juice and 10 ml l^{-1} Km 5 juice (Table 2). The number of shoots of each *in vitro* banana cultivar (Nakabululu, Nakitembe and Nakinyika) cultured on MS media supplemented with each of the various types and amounts of banana juice did not differ significantly (P>0.001).

Shoot height of bananas cultured on MS media supplemented with various volumes of banana juice from Mbidde, Kayinja and Km 5 significantly (P<0.001) differed (Table 2). The highest shoot height was observed when

'Kayinja' juice at 50 ml l^{-1} was used as carbon source. Cultures on MS media supplemented with 10, 20 ml l^{-1} Mbidde juice and 10 ml l^{-1} Km 5 juice failed to grow (Table 2). Although this was the case, a considerable height in this culture was obtained on media supplemented with 50 ml l^{-1} Mbidde juice or 50 ml l^{-1} 'Km 5' juice (Table 2). The different banana cultivars tested for multiplication responded similarly at different levels and types of energy sources. It was, therefore, apparent that banana juice from cultivar Kayinja was the best in terms of promoting shoot multiplication and shoot vigour.

It was apparent from our results that 50 ml l^{-1} of banana juice gave the best results of all the levels in terms of shoot number and height. Best results were observed under Kayinja, followed by Km 5. The highest mean shoot height was observed when bananas were cultured on medium supplemented with 50 ml l^{-1} Kayinja juice. This growth was greater than when bananas were cultured on medium supplemented with table sugar. The mean shoot height of bananas cultures on MS media supplemented with table sugar was comparable to than 50 ml l^{-1} Mbidde and Km 5. On the other hand, the mean number of shoots of bananas cultured on the different juices did not significantly ($P>0.001$) differ from the control medium (Table 3).

Mbidde juice at 50 ml, Kayinja juice at 20, 30, 40 and 50 ml and Km 5 at 40 and 50 ml were suitable alternatives to 30 g l^{-1} of table sugar as energy sources to *in vitro* banana media. As a plant extract, banana juice used in this study relates to many undefined supplements, which were employed in early tissue culture media (George *et al*., 2008). The first successful cultures of plant tissue involved the use of yeast extract (White, 1934; Robbins, 1992). Other additions reported include fibrin digest, malt, extract, pulps and juices from various fruits (Steward and Shantz, 1959; Ranga Swamy, 1963; Guha and Maheshwari, 1964). Despite the importance of sucrose, fructose, potassium and phosphorus showed in this study, other components could have come into play, contributing to positive response of EAHB cultivars like Nakabululu, Nakitembe and Nakinyika during *in vitro* banana growth. These could have largely been organic acids. Although the study revealed the presence of malic and ascorbic acid, various organic acids have been reported in banana juice. In fact Kayinja banana juice contained particularly higher ascorbic acid (Table 1) than all other banana juice sources and table sugar solution.

Organic acids were reported by George *et al*. (2008) to act as chelating agents, improving the availability of some micronutrients in the *in vitro* media. According to White *et al*. (1981), divalent organic acids such as citric, maleic, malic and malonic were found in the xylem sap of plants, where together with amino acids could complex with metal ions and assist their transport. These acids could also be secreted from cultured cells and tissues into the growth medium and would contribute to the conditioning effect. Ohira *et al*. (1973) discovered that malic and citric acids, released into the medium by rice cells, were able to make unchelated ferric iron available, thereby correcting an iron deficiency. This fact could have been supplemented by the various concentrations of banana juice from different sources as energy source and, thus enhancing nutrient availability for *in vitro* banana growth. In addition to the chelating role, organic acids have a nutritional role. This was also reported by George *et al*.

TABLE 3. Response of *in vitro* bananas cultured on MS media supplemented with 50 ml l^{-1} of banana juice and table sugar as carbon sources

Growth attribute	Treatment (banana juice from different Cvs at 50 ml l^{-1})			Table sugar (30 g l^{-1})
	Cultivar Mbidde	Cultivar Kayinja	Cultivar Km 5	
Shoot height	3.44b	4.56a	3.22b	3.22b
Number of shoots	3.22a	3.56a	3.56a	3.33a

*Mean values in columns followed by the same letter are not significantly different at $P<0.001$ according to Tukey's least-significance difference range test

(2008) that adding Krebs' cycle organic acids to the medium enhances the metabolism of ammonium and, hence, protein manufacture in *in vitro* plants. Gamborg and Shyluk (1970) also found that some organic acids could promote ammonium utilisation, and that incorporation of small quantities of sodium pyruvate, citric, malic and fumaric acids into the medium, was one factor which enabled Kao and Michayluk (1975) to culture *Vicia hajastana* cells at low density. Chu *et al.* (1975) successfully cultured triticale anther callus on N6 medium supplemented with 35 mg l^{-1} of a mixture of sodium pyruvate, malic acid, fumaric acid and citric acid. This implied that banana juice had to be within specific concentrations for the nutrients to be adequately availed to the *in vitro* propagules to regenerate and for sucrose to be metabolised subsequently for energy and carbon source for *in vitro* development of 'Nakabululu', 'Nakitembe' and 'Nakinyika' banana varieties.

Cost benefit of using banana juice. It was interesting to note such a big reduction of 30% in the cost of the energy sources when table sugar was replaced by banana juice. At 50 ml l^{-1} of banana juice and 30 g l^{-1} of table sugar, more sugars were observed in the banana juices (Table 1). In plant tissue media, sucrose is the most frequently used carbon source at a concentration of 2-5% (Trigiano and Gray, 2010). Other carbohydrates used include lactose, galactose, maltose and starch and they were reported to be less effective than either sucrose or glucose (Kinnersley and Henderson, 1988). It was demonstrated that autoclaved sucrose was better for growth than filter sterilised sucrose. Autoclaving seems to hydrolyse sucrose into more efficiently utilisable sugars such as fructose (Vinterhalter and Vinterhalter, 1997). It was found that supplements of sugar cane molasses, banana extract and coconut water to basal media can be a good alternative for reducing medium costs. These substrates in addition to sugars are sources of vitamins and inorganic ions required growth (Dhamankar, 1992). These observations could explain the observed vigorous growth when the media was supplemented with banana juice.

CONCLUSION

Banana juice can be used as an energy source in *in vitro* media. Banana juice supports growth of shoots, with differences between cultivars from which the juice is extracted. However, environmental influence and extraction techniques are likely to influence the quality of banana juice even from the same cultivar. Although there are significant differences in the chemical composition of juice from different cultivars, it is difficult to establish a correlation between their concentration and *in vitro* growth of banana. Therefore, the major differences observed in Kayinja juice with respect to high brix and ascorbic acid levels, could have contributed to better *in vitro* growth. Besides provision of energy, banana juice could have contributed other conditioning elements that enhance multiplication and growth vigour. There is a need to further understand the specific conditioning elements besides ascorbic acid that we suspect to have positively contributed to enhanced growth. Other organic acids present in banana juice need to be established; and where possible, their individual contribution to plant growth when supplemented to *in vitro* media. At the current price of locally extracted banana juice, it is possible to realise a cost benefit of 30% in terms of energy sources. With improved and large scale banana juice extraction methods, it is possible to greatly lower the cost of banana plantlets through lowering the cost of media.

ACKNOWLEDGEMENT

The study was funded by the World Bank and the Government of Uganda through the Millennium Science Initiative (MSI) Program of the Uganda National Council for Science and Technology (UNCST), and the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) low cost tissue culture project (ASARECA/AB/2009/05).

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