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Sugarcane Juice as an Alternative Carbon Source for *in vitro* Culture of Plantains and Bananas

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ABSTRACT

The suitability of sugarcane juice for the *in vitro* culture of bananas and plantains was investigated in this study. Two concentrations of sugar cane juice 5, 10% and 30 g L⁻¹ laboratory sucrose were used. There was also a control medium without carbon source. Explants were prepared from field grown sword suckers. The preparation of the explants involved, sterilization with 70% ethanol, followed by 1% Sodium hypochlorite+1% tween 20. The explants were cultured at 26°C, under 16 h photoperiod, light intensity of 3000 lux and a relative humidity of 60%. Plants that were cultured on 5% sugarcane juice had higher average fresh weight values of 16.6 g per plant compared to those that were cultured on the 30 g L⁻¹ sucrose and 10% sugarcane juice which had fresh weight of 15.6 g per plant and 11.0 g per plant, respectively. Oniba had higher fresh weight on medium supplemented with 5% sugarcane juice compared to the two other cultivars. In terms of dry weight, 'Oniaba' again had the highest value of 7.2 g per plant which was obtained on medium supplemented with 5% sugarcane juice. Plants that were cultured on 5% sugarcane juice were better in terms of shoot height and number of shoots per plant than those cultured on 30 g L⁻¹ sucrose and the differences between the 5% sugarcane juice and the other treatments were statistically different. The various carbon sources and their concentrations also affected the water potential of the media. Medium supplemented with 10% sugarcane juice had the lowest water potential of -0.8 MPa compared to the control medium that had a water potential of -0.1 MPa. Medium supplemented with 5% sugarcane juice and 30 g L⁻¹ sucrose had in a medium water potential of -0.3 MPa. Five percent sugarcane juice was found to be a better substitute for laboratory grade sucrose for the *in vitro* propagation of *Musa* sp.

Key words: Sugarcane juice, sucrose, *Musa* sp., carbon source, *in vitro* culture

INTRODUCTION

Plant tissue culture is the aseptic growth of cells, tissues, organs and whole plants on artificial or definable media. The advantages of plant tissue culture are enormous and strategic in plant biotechnology (Okonko *et al.*, 2006).

Tissue culture techniques have become an attractive field of biotechnological research and their roles are particularly appreciated in the areas of large scale clonal propagation and crop improvement. The success of tissue culture among other things relies on shoot regeneration and its efficiency requires a better understanding of the influence of culture conditions on shoot regeneration and development. Plant cells and tissues *in vitro* culture do not have autotrophic

ability and so require exogenous carbon source for their energy needs. Sucrose has been the main source of carbon for such *in vitro* culture of plants (Ahmad *et al.*, 2007; Hilae and Te-Chato, 2005). The optimal concentration of sucrose in a medium should be enough to satisfy the energy needs for cell division and differentiation without having any negative osmotic effect on shoot formation (Javed and Ikram, 2008).

The *in vitro* culture of plants is a technique that has caught up in Ghana with crops like cassava, coconut, plantains/bananas, yam, potatoes, pineapple and many more now being multiplied *in vitro* as a way of producing large quantities of healthy planting materials. Although *in vitro* multiplication of plants started some 40 years ago (Bud, 1993) and is promising, extensive use of this technology is limited by the high cost of media components. Media chemicals account for less than 15% while the carbon sources such as laboratory grade sucrose contributes about 34% of the production cost (Demo *et al.*, 2008).

For most of the developing countries like Ghana to benefit from the direct use of tissue cultured material, the cost of commercial micro propagation has to be drastically reduced without compromising the quality of micro propagules (Demo *et al.*, 2008). This can be done through identifying cheaper alternatives to some of the expensive media components.

Sugarcane has been identified as a plant that has high amount of sucrose and other sugars (Walford, 1996) which could possibly be used as an alternative source of carbon. Prakash *et al.* (2002) and Alkhateeb (2006, 2008) have used date palm syrup as a carbon source for the *in vitro* culture of date palm.

Sugarcane grows abundantly in Ghana in seven out of the ten regions of the country. The canes are consumed raw for its juice and the bulk is used in the preparation of local gin. The price of the raw sugarcane is very cheap compared to ordinary sugar, therefore if sugarcane juice can be used as an alternative carbon source for the *in vitro* culture of plants, it will reduce the cost of commercial tissue culture. This study therefore seeks to investigate the possibility of using sugarcane juice as an alternative carbon source for the *in vitro* culture of plantains and bananas.

MATERIALS AND METHODS

Plant materials and explant preparation: The study was conducted at the tissue culture laboratory of the Plant Genetic Resource and Research Institute Bunso, Ghana from November 2009 to June 2010. Two plantain cultivars Oniaba, Apantu (*Musa paradisiaca*) and one Dwarf Cavendish banana cultivar (*Musa accuminata*) were used as the plant materials.

Plant materials were taken from sword suckers of the three cultivars of *Musa* sp. that had been grown in the open field at Bunso in the Eastern Region of Ghana, under good watering regime. The materials were taken early in the morning by using earth chisel to separate the sucker from the parent at the point of attachment. Before washing with running tap water, the roots and the top of the shoots were trimmed off.

The sheaths that form the pseudostem were carefully removed to reduce the size of the material to about 4 leaf sheaths. They were then sterilized with 70% ethanol for 3 min and washed three times in sterilized distilled water (Buah *et al.*, 2000).

More leaf sheaths were then removed aseptically in a clean bench until about two leaves covered the shoot meristem. This process was followed by sterilization with 1% Sodium hypochlorite solution containing a drop of polyoxyethylenesorbitanmonolaurate (Tween 20) for five minutes with occasional shaking and there after washed three times with sterilized distilled water. Prior to their inoculation on the medium, each shoot tip (about 1cm) was longitudinally divided into two halves and again sterilized with 1% Sodium hypochlorite (NaClO) as above for 1 min.

Preparation of sugarcane juice: Sugarcanes were obtained from a farmer's field early in the morning. They were cut into pieces and washed thoroughly under running tap for several minutes. The canes were then peeled and further cut into smaller pieces and washed again. They were then placed in a previously cleaned and dried sugarcane crusher to extract the juice. The phenol-sulphuric acid assay spectrophotometer was used to check the sucrose content of the juice (Masuko *et al.*, 2005).

Media composition: MS medium (Murashige and Skoog, 1962) supplemented with 4.5 mg L⁻¹ 6-Benzylaminopurine was used. Three different media were prepared according to their carbon source and each treatment was replicated 10 times for each of the three *Musa* sp. used. The culture bottles were arranged in a completely randomized fashion. As 10% sugarcane juice, 5% sugarcane juice, 30 g L⁻¹ sucrose and No carbon source added (control).

Thirty grams per liter sucrose was used because it had been the optimal sucrose concentration from previous work with *Musa* species (Buah *et al.*, 2000).

pH of the media used was adjusted at 5.8 and 0.9 g L⁻¹ gerlite was then added to the media before autoclaving for 15 min at 121°C.

The explants were inoculated into the media and kept under a temperature of 26°C, 16 h photoperiod with an intensity of 3000 Lux and a relative humidity of 60%. The initial sub culturing was done 4 weeks after placing the explants in the media and subsequently at two weeks interval. During subculturing, materials with multiple shoots were separately removed and placed into different vessels. In all, seven subcultures were done during which data were taken.

The isopiestic psychrometer (Tang *et al.*, 2002) was used in measuring the water potential of the various media before inoculating them with the explants. Prior to the measurements, the jointed parts of the psychrometer were made airtight by applying vaseline to prevent the escape of water vapor from the samples. The thermocouples were then cleaned with 90% ethanol and rinsed in sterilized distilled water before drying with an air stream. About 0.4 g of each of the media samples were placed one at a time in the chamber and covered with the cylinder containing the thermocouple. A drop of sucrose at a designated concentration was placed at the tip of the thermocouple before inserting it into the cylinder that covers the chamber.

Data were taken from the first subculture on Number of shoots, Fresh weight (g), Shoot height (cm), Shoot dry weight (g) and Media water potential (Mpa).

Data was analyzed with Genstat version 7.1 for analysis of variance and Excel 2007 for the plotting of graphs (Hilbe, 2007).

RESULTS

The various sources of carbon used in this experiment affected some physical properties (water potential) of the medium as well as the growth of the explants. Sugarcane juice was found to be a good alternative to the laboratory sucrose and was evidenced in almost all the parameters measured.

The explants were taken through seven cycles of sub culturing from the 4th to the 16th week after inoculation. Generally, explants of the three *Musa* sp. cultured on medium supplemented with 5% sugarcane juice had higher fresh weight values than all the other treatments (Fig. 1a-c).

On the average, all the three types cultivars of *Musa* sp. obtained a fresh weight of 16 g per plant at the end of sixteen weeks, on medium supplemented with 5% sugarcane juice. This was followed by plants cultured on medium supplemented with 30 g L⁻¹ sucrose which had fresh weight

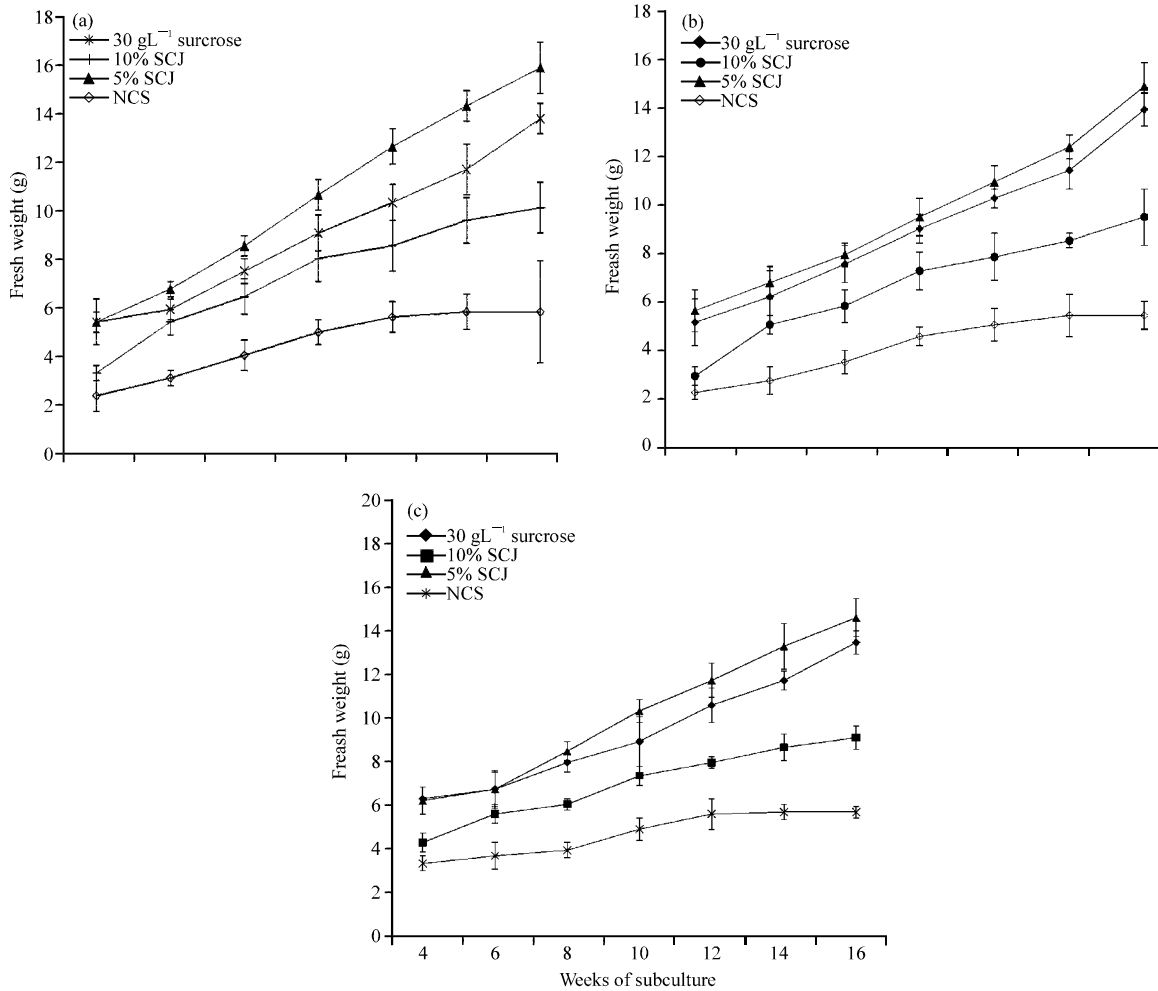


Fig. 1: Fresh weight g/plant of *Musa* sp. (a) Apantu, (b) Oniaba and (c) Dwarf cavendish cultured for 16 weeks on media supplemented with different carbon sources. SCJ: Sugarcane juice NCS: No carbon source

of 15 g per plant. These were better than those cultured on the 10% sugarcane juice which had average fresh weight of 8 g/plant. Plants that were cultured on the medium without a carbon source had the least fresh weight values of between 6.1 and 6.4 g per plant for all the *Musa* sp.

Oniaba had a slightly higher fresh weight 15.6 and 16.6 g on the 30 g L⁻¹ sucrose and the 5% sugarcane juice respectively (Fig. 1b) compared with Apantu and Dwarf cavendish (Fig. 1a, c).

Even though the shoot height of plants cultured on media supplemented with the different carbon sources increased throughout the experimental period, there were clear differences among the various types of carbon treatments (Fig. 2a-c).

Similar to the pattern found with the fresh weight, plants cultured on 5% sugarcane juice and 30 g L⁻¹ sucrose grew taller than those cultured on 10% sugarcane juice and the control medium. Plants cultured on 5% sugarcane juice had higher values of 17.9, 16.9 and 14.6 cm for Apantu, Oniaba and Dwarf Cavendish, respectively (Fig. 2a-c) compared with 17, 16.0 and 14.2 cm for

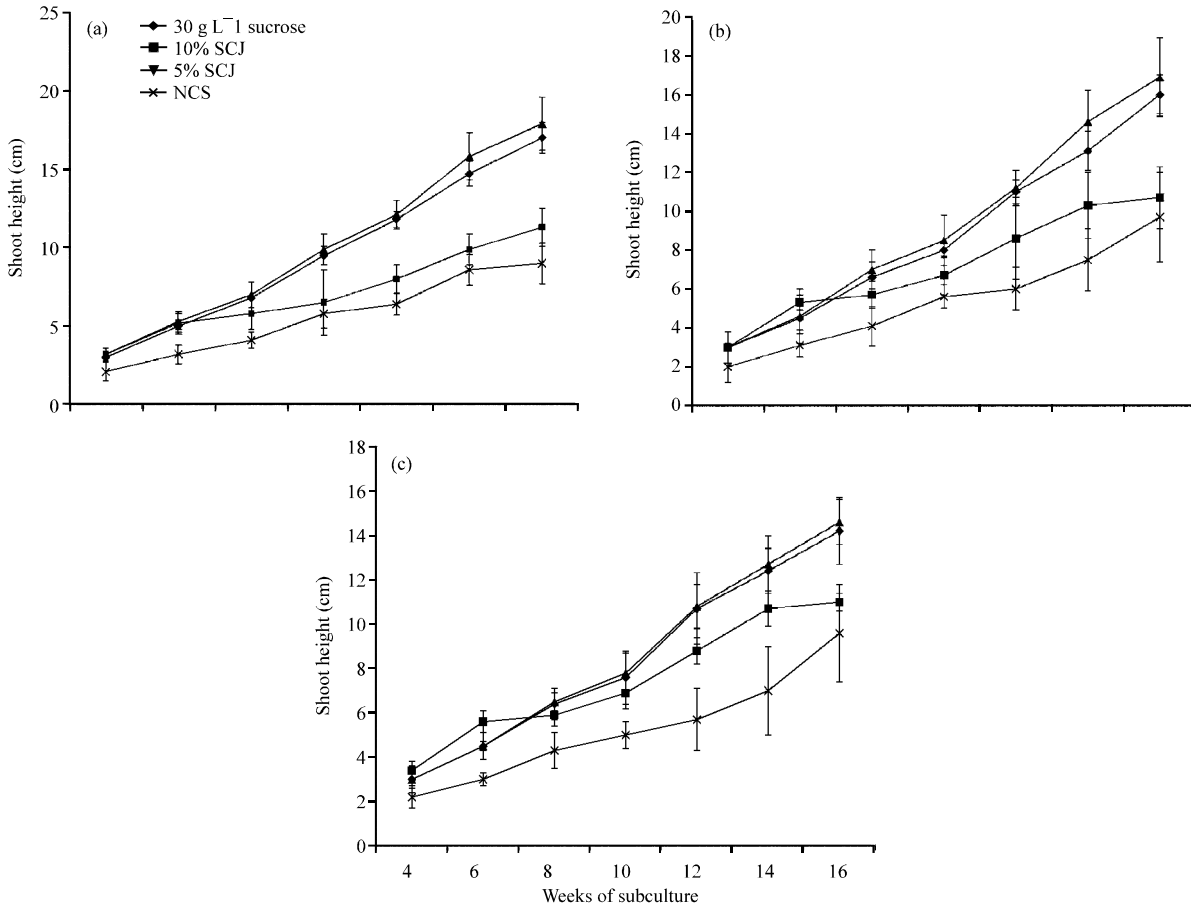


Fig. 2: Shoot height (cm) of *Musa* sp. (a) Apantu, (b) Oniaba and (c) Dwarf cavendish cultured for 16 weeks on media with different carbon sources. SCJ: Sugarcane juice NCS: No carbon source

Apantu, Oniaba and Dwarf Cavendish that were cultured on 30 g L⁻¹ sucrose. Among the three *Musa* sp., Apantu and Oniaba grew slightly taller than the dwarf Cavendish on media supplemented with 5% sugarcane juice and 30 g L⁻¹ sucrose.

Media supplemented with 10% sugarcane juice and the control gave the least shoot height among the various sugar concentrations. However, plants on 10% sugarcane juice had higher values of about 11 cm compared to plants on the control medium which had values of about 9.5 cm. It was observed that plants cultured on 10% sugarcane juice grew taller initially than the other treatments and this however declined after the second sub culture.

All the three *Musa* sp. produced more shoots on medium supplemented with 5% sugarcane juice than those on the other media. The highest number of shoots per explant was recorded with Oniaba which produced 20.72 shoot per explant, followed by Dwarf Cavendish and Apantu with 20.10 and 15.53 shoot per explant respectively as shown in Table 1. The number of shoots produced by Oniaba (20.72) and Dwarf Cavendish (20.10) on medium supplemented with 5% sugarcane juice were statistically different from those produced by Apantu (Table 1). Also, the number of shoots produced by the three *Musa* sp. on 5% sugarcane juice were different statistically, from the other media treatments.

Table 1: Effect of carbon source on mean number of shoots for three cultivars of *Musa* sp. cultured for 16 weeks

Carbon source	Mean number of shoots		
	Apantu	Dwarf cavendish	Oniaba
30 g Sucrose L ⁻¹	13.99	14.92	17.60
10% sugarcane	9.03	10.86	12.42
5% sugarcane juice	15.53	20.10	20.72
No carbon source	7.30	9.51	10.52
SED	0.348	0.579	0.466
LSD _{0.05}	0.706	1.175	0.945

Table 2: Effect of carbon source on mean dry weight for three cultivars of *Musa* sp. cultured for 16 weeks

Carbon source	Mean dry weight (g plant ⁻¹)		
	Apantu	Dwarf cavendish	Oniaba
30 g Sucrose L ⁻¹	4.10	4.00	5.82
10% sugarcane	3.10	3.23	3.52
5% sugarcane juice	6.07	6.72	7.25
No carbon source	2.32	2.63	2.80
SED	0.2564	0.1798	0.2451
LSD _{0.05}	0.5201	0.3646	0.4972

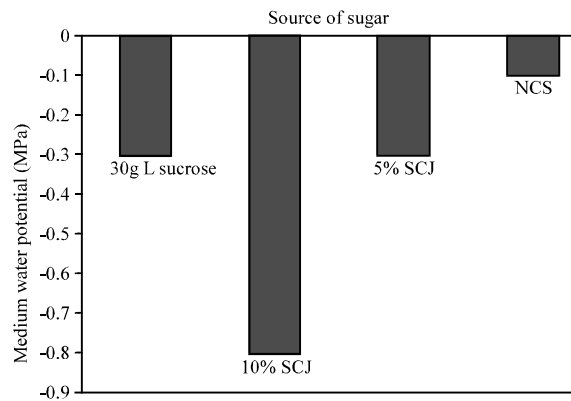


Fig. 3: Water potential of media supplemented with different carbon sources

The dry weight of plants cultured on 5% sugarcane juice was higher than those obtained from the three other media. Oniaba had the highest dry weight of 7.25 g per plant compared with 6.72 per plant and 6.07 g per plant for Cavendish and Apantu, respectively (Table 2). The dry weight values for plant cultured on 5% sugarcane juice were statistically different from the other treatments (Table 2). Five percent sugarcane juice was followed in terms of shoot dry weight by the 30 g L⁻¹ sucrose which had dry weight values of 4.10, 4.00 and 5.82 g per plant for Apantu, Dwarf Cavendis and Oniaba, respectively and these were also statistically higher than the values obtained for 10% sugarcane juice and the control treatments.

The water potential of the culture medium is very important for the growth of plants *in vitro*. The various carbon sources and their concentrations in this study, affected the water potential of the media (Fig. 3). Medium supplemented with 10% sugarcane juice had the lowest water potential

of -0.8 MPa. The highest water potential of -0.1 MPa was obtained from the control medium whilst the 5% sugarcane juice and 30 g L⁻¹ sucrose all had water potential values of -0.3 MPa.

DISCUSSION

The type of sugar and their concentrations in a culture medium affect the growth of plants *in vitro*. The plant materials that were cultured on the different carbon sources and their concentrations exhibited differences in their growth. This observation has also been made by Sul and Korban (1998) who reported that the type of carbon source affects the *in vitro* growth of plants and this depends on the plant and the part of the plant that is used as the explant.

Generally, medium supplemented with 5% sugarcane juice and 30 g L⁻¹ sucrose produced plants with higher fresh weight compared to the 10% sugarcane juice and the medium without a carbon source. The superiority of sugarcane juice as reported in our work is also corroborated by Sul and Korban (1998) who found sugarcane juice superior to laboratory grade sucrose. Sugarcane juice has other reducing sugars apart from sucrose, known to speed up cell division thus leading to an increase in the volume and weight of tissues. Moreover, the sugars in sugarcane juice are better translocated and assimilated than pure sucrose. The juice also contains other elements like iron, phosphorus, potassium and sodium compared to the scant traces in laboratory sucrose. Such additional nutrients may further boost growth of *in vitro* plantlets (Demo *et al.*, 2008).

Kodym and Zapata-Anas (2001) also confirmed this by comparing table sugar, sugar beet and sugarcane juice as carbon substitutes for the *in vitro* culture of Grand Naine banana and found sugarcane juice and table sugar to be superior.

On the contrary however, other researchers have found laboratory grade sucrose as the best carbon source for the *in vitro* culture of plants (Bahmani *et al.*, 2009; Esan *et al.*, 2009). Similarly, Rahman *et al.* (2004) have also found 30 g L⁻¹ laboratory grade sucrose as the best carbon source for the *in vitro* shoot growth and development. The difference between our work and theirs however, are the plant materials. Whilst *Musa* sp. were used in this study, *Arariae lata* was used in the case of Rahman *et al.* (2004). Their cultures were also placed under different culture conditions and this could explain the differences in our findings with sucrose and theirs. Gawad *et al.* (2010) and Shatnawi *et al.* (2006) have also reported the superiority of fructose over laboratory grade sucrose for the *in vitro* growth of banana plants.

It is believed that the addition of sugar to the culture medium promotes plant growth *in vitro* and compensate for the low or negative net photosynthetic rate as a result of poor photosynthetic ability, thus increasing the survival rates of tissue sections cultured *in vitro* (Demo *et al.*, 2008; Kubota, 2002).

The differences in the suitability of the various carbon sources as reported by the various researchers is indicative of the fact that one carbon source may not be suitable for the *in vitro* culture of all plants but rather the type of plant and the culture conditions may play a role.

The pattern of fresh and dry weights of plants cultured on the various sources of carbon were similar since they all decreased with increasing concentration of sugarcane juice from 5% to 10%.

Similar observation have been made by Asemota *et al.* (2007) and Bahmani *et al.* (2009) who reported a decrease in fresh weight of date palm on high concentrations of sucrose and date palm syrup. The detrimental effects of high levels of sugars in culture medium on the development of *in vitro* plants have also been confirmed by Hilae and Te-Chato (2005) and Yildzil *et al.* (2007). It is possible that the accumulation of phenolic compounds in the medium had inhibitory osmotic effect which adversely affected shoot growth and development as a result of stress (Karim *et al.* 2007).

On the other hand however, Baksha *et al.* (2003) and Shatnawi *et al.* (2006) have obtained better shoot formation and higher fresh weights in *Lilium* species that were cultured on higher concentration of sucrose.

Plants which were cultured on our control medium also had low fresh and dry weight values which could be attributed to the unavailability of sufficient energy to carry out the metabolic processes in the no sugar culture environment. In other cases, shoot have failed to form on medium that has no added sugar as was observed by Yildzil *et al.* (2007).

The water potential of a medium is very important to the growth and development of plants *in vitro* since it determines the movement of water and mineral elements into the plant material and also maintains a better turgor for the plant cells. One of the factors that affects the water potential of media is the type and concentration of sugar used (Buah *et al.*, 2000). Water potential of the medium without sugar was the highest -0.1 MPa whilst the medium with the 10% sugarcane juice had the least water potential of, -0.8 MPa. This difference could only be attributed to the concentrations and types of carbon source since they were the only variables. In previous work by Buah *et al.* (2000), medium supplemented with 30 g L⁻¹ sucrose had a water potential of -.03 MPa and the value obtained in this work confirms this. It is possible that the differences in the growth of the *Musa* sp. on the various media could be due to the effect these sugar sources and concentrations had on the media water potentials.

Daozhi *et al.* (2005) and Tang *et al.* (2002) have reported a decrease in leaf conductance in various plants due to decreased leaf water potential which consequently led to a decrease in the growth of the plant *in vitro*. Similarly, Tang *et al.* (2002) have reported a decrease in the fresh and dry weight of rose when leaf water potential decreased from -0.5 to -0.8 MPa. This was attributed to decreased photosynthetic activity which may be the result of decreased leaf conductance. This phenomenon may explain the better growth of plants cultured on 5% sugarcane juice and 30 g L⁻¹ sucrose all of which had a medium water potential of -0.3 MPa.

Even though cost analysis was not done, it is believed that the replacement of laboratory grade sucrose with sugarcane juice could have a positive effect in reducing the cost of *in vitro* rapid multiplication of plantains and bananas. Preeti *et al.* (2009) successfully reduced the cost of banana tissue culture by 90% by replacing the tissue culture grade sucrose with table sugar.

CONCLUSION

It has been demonstrated that sugarcane juice as a carbon source for the *in vitro* culture of plantains and bananas, is comparable or better than laboratory grade sucrose in terms of the growth of the plantlets *in vitro*. Plants which were cultured on 5% sugarcane juice gave higher fresh and dry weights as well as plant heights compared to those cultured on 30 g L⁻¹ laboratory grade sucrose. Therefore the use of sugarcane juice in place of laboratory grade sucrose could reduce the cost of *in vitro* multiplication of *Musa* sp. without compromising on the quality of growth. Comparing the two concentrations of sugarcane juice used (5 and 10%), the 5% was optimal for the growth of *Musa* sp. *in vitro*.

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