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*Full Length Research Paper*

# Table sugar as an alternative low cost medium component for *in vitro* micro-propagation of potato (*Solanum tuberosum* L.)

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**Most developing countries are limited in maximizing tissue culture technology due to the overhead costs involved. In view of this, the aim of this research was to evaluate alternative cheap sources of carbon and energy in potato culture media in order to reduce the overall cost of micro-propagation. A randomized complete block design was used to compare laboratory grade sucrose with two types of local commercial table sugar, specifically white and brown sugar. Three selected Kenyan potato cultivars, Tigoni, Asante and Kenya Sifa were cultured on full strength Murashige and Skoog (MS) medium at 3% (w/v) in combination with the 3 different sugars. The variation in growth performance of the cultivars was then observed. Plantlet survival of 100% was recorded after four subculture generations on all sugars for all the cultivars. The mean number of nodes per plantlet was significantly higher in brown sugar for cultivars Kenya Sifa and Asante. Brown sugar enhanced significantly higher mean number of roots per plantlet after four subculture generations for all cultivars. There was no significant difference in percentage of plantlet survival after transplanting for cultivars Asante and Kenya Sifa but significantly lower for cultivar Tigoni on grade sucrose medium. Results also showed that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 34 to 51% when compared with the analytical grade sucrose.**

**Key words:** Table sugar, micro-propagation, single node cultures, *Solanum tuberosum* L.

## INTRODUCTION

The potato (*Solanum tuberosum* L) is the fourth most important food crop in the world, behind rice, wheat and corn (Manrique, 2000; CIP, 2002; Nyende et al., 2005). Potato as a vegetable is indispensable for its high quality proteins, substantial amounts of essential vitamins, minerals, trace elements, very low fat content and even medicinal properties (Khan, 1993). The major problem cited in all potato growing regions has been lack of high quality clean certified seeds, less than 5% of potato farmers in Kenya have access to certified seeds (Nyende et al., 2002). For that reason, farmers use previous harvest as seed tubers. This favours seasonal build up of the tissue borne pathogens, such pests lead to significant loss of yield and tuber quality via seed degeneration (Struik and Wiersema, 1999; Nyende et al., 2005).

To circumvent these problems, a number of rapid multiplication techniques have been developed including: *in vitro* tuberisation (Kaur et al., 2000), nodal cuttings from *in vitro* shoots (Sarkar et al., 1996; Djurdjina et al., 1997), multimeristems developed from axillary buds and layering of *in vitro* shoots (Sarkar et al., 1996; Chandra and Upadhya, 1998), synthetic seeds (Nyende et al., 2003; Nyende et al., 2005).

Although, in vitro multiplication of potato was achieved 40 years ago (Coleman et al., 2001), and is promising, extensive use is limited by the high costs of media com-

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ponents. Media chemicals, account for less than 15% while the carbon sources such as grade sucrose contribute about 34% of the production cost. Therefore, for most of developing countries to benefit from direct use of tissue cultured material, the cost of commercial micropropagation has to be drastically reduced without compromising on the quality of micropropagules (Kuria et al., 2008). These can be done through identifying cheap alternatives to expensive grade sucrose. The cost of local sugar (2006 super market prices in Kenya) was about Kshs 74/kg against Kshs 3680/kg for the imported grade sucrose. It is against this economic backdrop that potential of locally available table sugar as a carbon source was investigated.

## MATERIALS AND METHODS

The study was carried out in the plant tissue culture laboratory and in the glass house at the National potato Research Centre of Kenya Agricultural Research Institute (KARI) Tigoni between June 2005 and April 2006.

### Plant materials

Three popular Kenyan cultivars viz., Asante (CIP 381381.20), Tigoni (CIP 381381.13) and Kenya Sifa (720097) were provided as *in vitro* plantlets by Kenya Agricultural Research Institute Tigoni centre. The plants were certified as disease free and *in vitro* plantlets were initiated through meristem shoot tip culture. They were then routinely multiplied every four weeks using nodal cuttings on basal Murashige and Skoog medium (1962).

### Preparation of MS nutrient medium

Three different types of carbon sources namely analytical grade sucrose (Sigma Chemical Co. Germany), white sugar, and brown sugars (purchased in local Kenyan supermarket) were compared at 0.3% (w/v). The medium was prepared using full strength Murashige and Skoog (1962) (MS) basal salts amended with 0.25% (w/v) phytagel (Sigma Chemical Co. Germany) and 0.001 mg/l gibberellic acid. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 0.1 M HCL. Ten millilitre of culture medium was dispensed in 20 mm test tubes and covered using aluminium foil preceding autoclaving at 1.06 kg $cm^{-2}$ and 121$^{o}$C for 15 min.

### Inoculation and inoculation

Inoculation was carried out in a sterile laminar airflow hood chamber. Surface sterilisation was achieved through spraying 70% (v/v) ethanol. Nodal cuttings were dissected from four weeks-old *in vitro* plantlets using sterile blade and forceps. Single nodal cuttings were inoculated into a test tube containing 10 ml MS medium amended with the different sugars. These were incubated at 22 ± 2$^{o}$C under a 16-h photoperiod with a photosynthetic photon flux density of 40 µmol $m^{-2}$ $s^{-1}$ provided by overhead cool fluorescent lamps (Philips, India 30 Watts) for 35 days.

### Transplanting

Four weeks old *in vitro* plantlets were transplanted in 15 cm diameter pots half filled with well-watered mixture of steam sterilised soil substrate (forest soil, humus, ballast and sand in the ratio of 4:1:1:1) amended with five grams per pot of Di-ammonium phosphate fertilizer (DAP). The transplants (seedlings) were then kept in glasshouse for observation. The plants were irrigated every alternative day with tap water for two weeks and twice a week thereafter.

### Data collection

Collection of data on *in vitro* plantlet performance commenced two weeks after inoculation and on weekly interval for five weeks during incubation. While in the glass house the following growth characteristics were determined: transplant survival, number of nodes, plantlet vigour, and percentage plants survived at harvest. Further, a cost/benefit analysis of the different carbon sources was done.

### Experimental design and data analysis

A two-factor experiment in a randomized complete block design was conducted with three replicates per treatment and seven test tubes per experimental unit. The *in vitro* plantlets were sub-cultured four times (each lasting 35 days) in the same treatment. Data were subjected to analysis of variance (ANOVA) to test the significance of the differences between treatments using SAS statistical software package (Release 8.2, SAS Institute, Inc., Cary, NC, USA). Treatment means were separated using the Least Significant Difference test at $p < 0.05$.

## RESULTS

### Plantlet survival

The survival rates at 14 days after culturing (DAC) was 100% in media supplemented with white sugar, brown sugar and sucrose, respectively (Table 1). This was observed in all the three cultivars throughout the four generations of subculture.

### Number of nodes

For the cultivar Tigoni, the carbon sources were not significantly different ($P < 0.05$) with respect to the number of nodes per plantlet during the four generations of culture. For cultivar Asante, analytical grade sucrose gave significantly higher number of nodes in the third and fourth generations. Kenya Sifa to the contrary gave significantly more nodes with brown sugar as a carbon source in the same generations (Table 2).

### Number of roots

During the first generation of culture, the external sources of carbon did not differ significantly ($p < 0.05$) in the mean number of roots for all the cultivars (Table 3). However, during the second generation of culture for the cultivar Asante, a significant difference ($p < 0.05$) was observed,

**Table 1.** Survival of *in vitro* plantlets cultured in media with different carbon sources during the four generation of culture.

| Carbon source | Survival (%) | | | |
|---|---|---|---|---|
| | Generation 1 | Generation 2 | Generation 3 | Generation 4 |
| White sugar | 100 | 100 | 100 | 100 |
| Grade sucrose | 100 | 100 | 100 | 100 |
| Brown sugar | 100 | 100 | 100 | 100 |
| **Mean** | **100** | **100** | **100** | **100** |
| LSD (0.05) | 0 | 0 | 0 | 0 |

†Comparison of means by least significant difference (LSD) at P < 0.05 within the columns. ‡ Generation = Interval between subcultures (35 days).

**Table 2.** Mean number of nodes per plantlet during the four generations of culture for the cultivars Tigoni Asante and Kenya Sifa derived from three different carbon sources.

| Cultivar/ carbon source | Number of nodes per plantlet | | | |
|---|---|---|---|---|
| | Generation 1 | Generation 2 | Generation 3 | Generation 4 |
| **Tigoni** | | | | |
| White sugar | 8.9a | 9.2a | 8.9a | 9.6a |
| Grade sucrose | 10.1a | 9.3a | 9.6a | 9.6a |
| Brown sugar | 9.8a | 8.8a | 9.0a | 9.7a |
| **Mean** | **9.6** | **9.0** | **9.2** | **9.6** |
| **Asante** | | | | |
| White sugar | 8.9a | 8.8a | 7.9b | 7.8b |
| Grade sucrose | 8.8a | 8.2a | 9.4a | 9.1a |
| Brown sugar | 9.9a | 8.7a | 7.9b | 8.5b |
| **Mean** | **9.2** | **8.6** | **8.4** | **8.5** |
| **Kenya Sifa** | | | | |
| White sugar | 8.1a | 7.0a | 7.0b | 5.7b |
| Grade sucrose | 8.8a | 7.5a | 7.7b | 5.9b |
| Brown sugar | 8.7a | 7.7a | 9.5a | 9.3a |
| **Mean** | **8.5** | **7.4** | **8.1** | **7.0** |
| LSD (0.05) | 1.5 | 0.9 | 0.9 | 0.8 |
| CV% | 9.8 | 6.4 | 6.0 | 5.7 |

†Comparison of means by least significant difference (LSD) at P < 0.05 within the columns. ‡ Generation = Interval between subcultures (35 days).

with the medium supplemented with analytical sucrose having fewer roots compared to table sugars (Table 3). During the third and fourth generations of culture for the cultivar Kenya Sifa, significantly more nodes were recorded in plantlets cultured in media amended with brown sugar (Table 3).

## Plantlet vigour

Table sugar gave similar growth vigour as analytical sucrose for cultivars Asante and Tigoni. For the cultivar Kenya Sifa, brown sugar resulted to significantly more vigorous plantlets compared to analytical sucrose during the third and fourth generations of culture (Table 4).

## Transplant survival

Variation was observed on the *in vitro* plantlets of the cultivar Tigoni, with those from brown sugar having better survival in the glass house (Table 5). No significant differences ($p < 0.05$) were observed on the survival of transplants of cvs Asante and Kenya Sifa derived from the three different carbon sources (Table 5).

Figure 1 shows *in vitro* plantlets well established in glass house 62 days after transplanting. Visually, the seedlings grew normally to become identical to their origi-

**Table 3.** Mean number of roots of potato plantlets derived from nodal explants cultured in media with different carbon sources during the four generations for the cultivars Tigoni, Asante and Kenya Sifa.

| Cultivar/ carbon source | Mean number of roots per plantlet | | | |
|---|---|---|---|---|
| | Generation 1 | Generation 2 | Generation 3 | Generation 4 |
| **Tigoni** | | | | |
| White sugar | 6.3a | 7.5a | 8.0a | 7.8b |
| Grade sucrose | 7.2a | 7.5a | 7.4a | 7.9b |
| Brown sugar | 7.5a | 7.2a | 7.5a | 8.8a |
| **Mean** | **7.0** | **7.4** | **7.7** | **8.2** |
| **Asante** | | | | |
| White sugar | 6.7a | 8.0a | 6.8a | 6.5b |
| Grade sucrose | 6.9a | 7.1b | 7.0a | 7.5a |
| Brown sugar | 7.5a | 7.5ab | 6.9a | 7.5a |
| **Mean** | **7.0** | **6.2** | **6.9** | **7.2** |
| **Kenya Sifa** | | | | |
| White sugar | 6.7a | 5.7a | 6.2b | 5.9b |
| Grade sucrose | 6.5a | 5.9a | 6.4b | 5.7b |
| Brown sugar | 6.0a | 6.2a | 8.0a | 7.9a |
| **Mean** | **6.4** | **5.8** | **6.9** | **6.5** |
| LSD (0.05) | 1.3 | 0.8 | 0.9 | 0.8 |
| CV% | 11.0 | 7.5 | 7.4 | 6.5 |

†Comparison of means by least significant difference (LSD) at P < 0.05 within the columns. ‡ Generation = Interval between subcultures (35 days).

**Table 4.** Mean plantlet vigor of the potato cultivars Tigoni Asante and Kenya Sifa during the four generations of culture as influenced by the carbon source.

| Cultivar/ carbon source | Mean plantlet vigour | | | |
|---|---|---|---|---|
| | Generation 1 | Generation 2 | Generation 3 | Generation 4 |
| **Tigoni** | | | | |
| White sugar | 5.0a | 4.7a | 5.0a | 5.0a |
| Grade sucrose | 5.0a | 4.7a | 5.0a | 5.0a |
| Brown sugar | 5.0a | 5.0a | 5.0a | 5.0a |
| **Mean** | **5.0** | **4.8** | **5.0** | **5.0** |
| **Asante** | | | | |
| White sugar | 5.0a | 5.0a | 5.0a | 5.0a |
| Grade sucrose | 5.0a | 5.0a | 5.0a | 5.0a |
| Brown sugar | 5.0a | 5.0a | 5.0a | 5.0a |
| **Mean** | **5.0** | **5.0** | **5.0** | **5.0** |
| **Kenya Sifa** | | | | |
| White sugar | 4.3a | 4.7a | 4.7ab | 4.7ab |
| Grade sucrose | 4.3a | 4.3a | 4.0b | 4.3b |
| Brown sugar | 4.7a | 5.0a | 5.0a | 5.0a |
| **Mean** | **4.4** | **4.7** | **4.6** | **4.7** |
| LSD (0.05) | 0.7 | 0.7 | 0.3 | 0.5 |
| CV% | 8.3 | 8.3 | 3.9 | 5.9 |

†Comparison of means by least significant difference (LSD) at P < 0.05 within the columns. ‡ Generation = Interval between subcultures (35 days).

**Table 5.** Acclimatization (%) of *in vitro* plantlets of the potato cultivars Asante, Kenya Sifa and Tigoni in glasshouse 21 days after transplanting from different carbon sources.

| Carbon source | Cultivar | | |
|---|---|---|---|
| | **Asante** | **Kenya Sifa** | **Tigoni** |
| Brown sugar | 100.0a | 72.2a | 100.0a |
| Sucrose | 94.4a | 66.7a | 66.7b |
| White sugar | 94.4a | 66.7a | 88.9ab |
| **Mean** | **96.3** | **68.5** | **85.2** |
| LSD (0.05) | 19.7 | 13.9 | 27.8 |
| CV % | 8.2 | 8.1 | 13.0 |

†Means followed by the same letter within the column are not significantly different at P<0.05 using Least Significant difference.

**Figure 1.** Established *in vitro* plantlets in glasshouse 62 DAP.

nal mother stocks morphologically.

## Cost analysis

The cost of analytical grade sucrose and the table sugars used in the analysis were the current price in the Kenyan local market. The cost of one-litre MS medium using analytical grade sucrose worked out to be Kshs 312.76 and Kshs 204.58 with inclusion of 0.8% agar and 0.25% gelrite, respectively as a gelling agent (Table 6). When using table sugar as a carbon source, a cost reduction of 34 and 51% was achieved in medium gelled with agar and gelrite, respectively (Table 6).

## DISCUSSION

Sucrose grade 1 as well as the white and brown sugar enhanced high survival rates (100%) of the plantlets *in vitro* during the four generations of sub-cultures (Table 1). The source of external fixed carbon (sugar) and the success of aseptic procedures during tissue process are paramount for the survival of plantlets *in vitro*. According to Kubota et al. (2001), supply of sugar to the culture medium promote 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*. Therefore, potato plantlets require an initial source of carbon and hence energy from the medium until they are capable of using $CO_2$ as their main carbon source for efficient metabolism.

Each node cultured in MS medium developed into a plantlet and at 35 days of each culture passage the plantlets had well-developed aerial parts and good root system and occupied the full length of the test tubes. The regeneration index of potato plants observed in this work, by utilizing single node cuttings was 9 nodes per plantlet at 35 DAC (Table 2). The results are comparable with earlier report by Pierik (1997) 6-7 nodes and Rani and Singh (1999) 6 to 9 nodes per plantlet after four weeks in culture. In addition to high multiplication rates nodal explants became indistinguishable morphologically from the stock culture used as the source of explant, showing similar visual performance in terms of proliferation and rooting capacity. This is an indication that plantlets derived from nodal segments have maximum genetic uniformity with the stock plants used as a source of explant. During the routine subculture of the nodal cultures the shoot growth occurred rapidly in all the carbon sources; however, nodal production in cultivar Kenya sifa was lower compared to Tigoni and Asante. This indicates that some potato cultivars may be less responsive or produce smaller shoots *in vitro* when single node cuttings are used. Using the average number of new nodes produced per nodal cutting after five weeks, the number of shoots generated from the original nodal cutting after 1 year can be calculated as $Y^x$, where Y= node number produced per nodal cutting after the 5-week culture cycle and X= 10.4, the number of culture cycles per year. From this study approximately 100 million plantlets may be produced yearly using single node culture. This yearly production is quite large and provides opportunity to illustrate the power of single node

**Table 6.** Comparative cost of three carbon sources used for potato in vitro micro-propagation.

| Type of sugar | Cost/Kg (Kshs) | Conc. /L (% w/v) | Cost/L (Kshs) | | % Cost reductions | |
|---|---|---|---|---|---|---|
| | | | Agar | Gelrite | Agar | Gelrite |
| Lab sucrose | 3,679.50 | 3 | 313 | 205 | | |
| Table sugar | 73.59 | 3 | 205 | 99 | 34.6% | 51.4% |

micro-propagation technique.

There is a general consensus that the number of subcultures for explants should be limited to avoid somaclonal variation. Ahloowalia (2000) reported that in potato twenty subcultures may be done without expression of genetic variation.

Roots have an essential role and function in plant life and development, supplying water and nutrients to the plant from the environment (Schiefelbein et al. 1997). From the study, root system of the nodal cutting was fibrous arising from initials along the proximal end of the stem. Regeneration of roots from nodal explants occurred easily without inclusion of a rooting hormone in the medium. These results are in agreement with Vinterhalter et al. (1997) that potato is an easy to root species and nodal explants do not require exogenous hormone for rooting. Although Bensalim et al. (1998) reported that increasing day/night temperature dramatically enhanced root growth and root branching. Through four culture passages, root initiation was consistent in sucrose grade 1, white sugar and brown sugar respectively; roots were observed after seven days in culture in all the explants (Table 3). The high number of roots per explants allowed easier absorption of nutrients from the medium, which led to better plantlet growth.

The quality and performance of plantlets grown on sucrose grade 1 was comparable to those grown on white and brown sugar, respectively. All the sources of carbon fostered vigorous plantlet growth. However, at initial stages for every generation of culture, low vigour was observed but at 35 DAC all plantlets were very vigorous. This may be explained by physiological stress after excision of explants and undeveloped root system and photosynthetic pathways at initial stages. After four subcultures, plantlet vigour was not affected; even though Hu and Wang (1983) proposed that the vigour and multiplication capacity of certain species decrease rapidly after several subcultures and the morphogenetic potential may eventually be fully lost.

The locally available sugar at concentration of 0.3% (w/v) enhanced proliferation of plantlets similar or better than laboratory grade sucrose. This maybe mainly due to the fact that different carbon sources available in the culture medium were easily translocated and assimilated by the explants enhancing cell division thus leading to eventual growth. Loescher, (1987), Khuri and Moorby, (1995) reported that a number of carbohydrates besides sucrose are translocated in plants. It is then not surprising that white sugar and brown sugar respectively were effectively assimilated by potato cells to provide energy and carbon skeletons for metabolic processes conducted by the cells. From manufacturers analysis brown sugar has slightly more calories than white sugar, and contains 187 milligrams of calcium, 56 of phosphorous, 4.8 of iron, 757 of potassium and 97 of sodium, compared to only scant traces of those nutrients found in granulated white sugar. Therefore, such additional nutrients may further boost growth of *in vitro* plantlets. Gamborg (2002), Kodym and Zapata (2001) reported superior performances of *in vitro* plantlets of banana, chrysanthemum, peanut, and chickpea in medium supplemented with carbohydrates such as glucose, maltose, and table sugar.

Acclimatisation and high percentage survival is influenced by ability of plantlets to withstand transplanting stress and propensity to rapidly convert from heterotrophic or photomixotrophic to autotrophic growth (Ziv, 1986). The high percentage of acclimatisation observed in this study (Table 5; Figure 1) may be attributed to plantlets with functional root system, which continue to grow during *ex vitro* acclimatisation. Also the plantlets produced through micro-propagation technique were of high quality and vigorous with well developed leaves. They were therefore able to adjust to the *ex vitro* conditions. Potato plantlets require at least one root to acclimatise and establish *ex vitro* (Demo, unpublished).

Sucrose has been reported to be the best carbon and energy source (George, 1993). In spite of the widespread use, the cost of refined sucrose is far too high to justify the use at commercial scale (Table 6). Use of table sugar reduced the cost of the medium between 34 and 51% using 0.8% Agar and 0.25% gelrite, respectively as gelling agent. Zapata (2001) successfully reduced the cost of banana tissue culture by 90% by replacing the tissue culture sucrose grade with a commercial sugar. Table sugar can be processed locally from sugarcane. It can therefore find wide acceptability in developing countries needing to import analytical grade sucrose.

## Conclusions

Results achieved in this study demonstrate that micro-propagation is promising in the initial stage in potato seed systems. The high survival rates of 100% and the achievement of vigorous plantlets were indications that potato responds well to tissue culture and that it does not

loose its regeneration capacity after several subcultures.

Sucrose is of prime importance for cell growth; however significant cost incurred by importing analytical sucrose presents economic obstacle in full exploitation of tissue culture for certified potato seed production. The cost of tissue culture can be brought down by 34 to 51% utilizing locally available table sugar without compromising the quality of tissue cultured plants.

Potato plantlets can be utilized after four weeks in culture for minitubers production in glass house owing to high survival rates after transplanting. It is recommended that table sugar be considered as low-cost substitute for potato micro-propagation.

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