Nile Valley University Publications
Nile Journal for Agricultural Sciences (NJAS)
(ISSN: 61 –71)
Volume 11, NO. 1, 2026
nilevalley.edu.sd

Using a Low-cost Energy (carbon) Source to Produce Tissue Cultures Banana

Islam Fath Alrhman Ahmed Elmahi [1], Abdelazim Mohamed Ali[2], and Ibrahim Abdelmaksoud Ibrahim[3]

1Faculty of Agriculture, Nile Valley University, Atbara, Sudan

2 Kaffaa Project, Berber, Sudan

Corresponding author: azimali58@yahoo.com

Received May 2025 Accepted Dec. 2025 Published :20 April2026

Abstract

This study aimed to test commercial sugar, available in Sudan, as a carbon source instead of pure sucrose as a low-cost energy source for tissue culture-derived banana seedling production. Grand Nain banana offshoots were used as initial source of explants in a series of subculturing to produce healthy plantlets and their subsequent regeneration using two sugar types with low and high concentrations (15 and 30 g per liter). Results obtained indicated that sugar concentration rather than sugar type affected significantly and positively bud formation in the first two week after subculturing. However, in the last weeks low concentration of commercial sugar behave in a similar way to high concentration of both sugar types on its effect on bud regeneration. Regarding leaf appearance, high concentration of both sugars significantly produced more leaves in the first week only, and then after, leaf appearance was same in both type and concentration of sugars. Higher rooting was significantly recorded by low concentrations of both types of sugars in 2 later weeks of plantlets.

Keywords: *Banana, Tissue culture, Sugar concentration, Commercial sugar*

استخدام مصدر طاقة (كربون) منخفض التكلفة لإنتاج شتلات الموز بزراعة الانسجة

إسلام فتح الرحمن أحمد الماحي[1]، عبد العظيم محمد علي[2]، و إبراهيم عبد المقصود إبراهيم[3]

1 كلية الزراعة، جامعة وادي النيل، عطبرة، السودان

2 مشروع الكفاءة ، بربر، السودان

ممثل المؤلفين : azimali58@yahoo.com

المستخلص:

هدفت هذه الدراسة إلى اختبار السكر التجاري المتوفر في السودان كمصدر للكربون بدلاً من السكروز النقي، وذلك كمصدر طاقة منخفض التكلفة لإنتاج شتلات الموز بتقنية زراعة الأنسجة. استُخدمت شتلات موز جراند نائين كمصدر أولي للأجزاء النباتية في سلسلة من عمليات الاستزراع المتكررة لإنتاج شتلات صحية، ثم تجديدها باستخدام نوعين من السكر بتركيز منخفض وعالٍ (15 و30 غرامًا لكل لتر). أشارت النتائج إلى أن تركيز السكر، وليس نوعه، هو ما يؤثر بشكل ملحوظ وإيجابي على تكوين البراعم خلال الأسبوعين الأولين بعد إعادة زراعة الأنسجة. مع ذلك، في الأسابيع الأخيرة، أظهر التركيز المنخفض للسكر التجاري تأثيرًا مشابهًا للتركيز العالي لكلا نوعي السكر على تجديد البراعم. أما بالنسبة لظهور الأوراق، فقد أدى التركيز العالي لكلا نوعي السكر إلى زيادة ملحوظة في عدد الأوراق خلال الأسبوع الأول فقط، ثم أصبح مظهر الأوراق متماثلًا بعد ذلك بغض النظر عن نوع السكر أو تركيزه. وتم تسجيل تجذير أعلى بشكل ملحوظ عند استخدام التركيز المنخفض لكلا نوعي السكر خلال الأسبوعين الأخيرين من عمر المستنبتات.

الكلمات المفتاحية: *الموز، زراعة الأنسجة، تركيز السكر، السكر التجاري*

Introduction

Bananas are grown in many regions of the world, including tropical, subtropical, and even sometimes, cooler zones. The area planted with bananas in Sudan has increased to over 23,000 hectares, planted with various banana varieties, most of which are the Dwarf Cavendish variety, producing over 1,150,000 tons. The Ministry of Agriculture, along with some producers and research centers, have imported, tested, and cultivated some other so-called excellent varieties. These varieties have proven their ability to grow and produce well, which foretells well the potential to increase the export capacity of this crop (Osman et al., 2015).

Banana seedlings can be produced using micro propagation. This technique produces a large number of high-quality, disease-free plants in a short period of time, regardless of the season and weather (Anonymous, 2004). Micro propagated banana plantlets are progressively becoming the suitable planting material due to its disease and nematode control, crop growth uniformity and also due to its rapid multiplication nature. However, growers have to face higher costs of seedlings compared to conventional suckers. Bananas, however, require a suitable culture medium that is

inexpensive. Conventionally it requires quantities of sucrose, growth regulators, gelrite or agar, and components of the Murashige and Skoog culture medium salts (Nasr et al., 2014). The main

obstacle to plant tissue culture propagated seedlings is its relatively high costs of materials and high level working skill demand compared to conventional cultivation methods. Therefore, low-cost alternatives are preferred to reduce production costs (Ssamula et al., 2015). The use of chemicals, a carbon source, a gelling agent, organic and inorganic nutritional supplements, and growth regulators in the culture medium makes this technique relatively expensive. Sucrose is usually used as a carbon source and agar as a gelling agent, and together they constitute the most expensive components of the culture medium (Rakshi et al., 2017).

Commercial sugar as carbon source and locally available salts, such as nutrients, can be used as an alternative source to reduce the cost of the medium (Gitonga et al., 2010). The carbon source, sucrose, which is very important for micro propagation of plants, contributes approximately to 34% of the production cost (Demo et al., 2008). Kaur et al., 2005, found that replacing sucrose with commercial sugar in the banana culture medium reduced the cost of tissue culture by 90%. Therefore, the use of low-cost materials for banana growth and development contributes to increasing banana production worldwide. This system can be adopted to reduce costs for farmers and establish low-cost tissue culture laboratories in their regions to increase banana production (Dhanalakshmi and Stephan, 2014). To reduce the cost of banana tissue culture, Stephan and Dhanalakshmi (2014) replaced the conventional medium with micronutrients and macronutrients, adding 30 g/L of commercial sugar and 8 g/L agar.

Laboratory grade sucrose, common grade sugar, cube sugar, rock sugar, candy sugar, glucose, jaggery and sugarcane juice were evaluated for in vitro propagation of banana cv. GrandNaine. Best response in terms of shoot multiplication and rooting were achieved with rock sugar and common grade sugar, respectively which could be compared well with that of analytical grade sucrose. The results showed the possibility of successful use of cheaper carbon sources for micro propagation of banana cv. GrandNaine (Prabhuling and Sathyanarayana, 2017).

Two banana cultivars (Monthan and Poovan) were grown and the cost of nutrients used in the medium was determined. Results indicated that by 61.4% cost reduction, a reasonable number of buds were produced on the low-cost medium compared to the conventional medium. The average production of the Mothan cultivar was 4.6 buds per plant, while the average production of the Poovan cultivar was 4.5 buds per plant on the low-cost medium. The production of the Poovan cultivar was 5.1 buds per plant compared to the Mothan cultivar, which had an average of 4.9 buds per plant on the conventional medium. We find that both cultivars produced the highest number of buds in Traditional medium compared to low-cost medium. In root production, the traditional medium produced a greater number of roots compared to the low-cost medium.

Dhanalakshmi and Stephan (2016) Stated that the overall comparative cost reduction analysis percentage was 73.20 % for medium used with conventional nutrient salts and table sugar. Ahamad (2024) Suggest replacement of highly expensive media components such as agar,

Sucrose, and water that cost more than 70-85 % of total production cost by cheaper alternatives. He suggest refined household sugar and other types instead of pure sucrose.

This study aimed to test commercial sugar, available in Sudan, as a carbon source instead of pure sucrose as a low-cost energy source for tissue-derived banana production.

Materials and Methods

Experimentation Site

This experiment was conducted in the tissue culture laboratory of Al Rajhi Company in Berber, 5 km from the city. Berber is located in the center of River Nile State, on the eastern bank of the Nile River, parallel to the course of the Nile. latitudes 17.40 and longitudes 32.20 north of Atbara.

Plant Source**:**

Grand Nain banana offshoots were brought from the University of Gezira farm, east of Wad Medani, to the tissue culture laboratories of the Kafaa Project in Berber.

Experimentation:

Sterilization and cultures initiation:

The plants were washed for cleaning under running water, and the corms were brought to the company's tissue culture laboratory. Prepared explants were cleaned and surface sterilized with sodium hypochlorite, followed by pre cultivation treatments and incubation as stated in the steps bellow. Some of regenerating buds were subsequently taken for further studies according to the suggested investigation treatments

In the first stage, Murashige and Skoog's (1962) medium salts were prepared as nutrient solutions in liquid medium with activated charcoal. The pH of the medium was adjusted to 5.8 using both potassium hydroxide and hydrochloric acid. Sucrose was used as a carbon source, and a growth regulator was added. In every 250 ml containers 30 ml of the medium was dispensed and covered with Teflon caps. All containers were autoclaved at 121°C and 15 psi for 30 minutes to sterilize the medium. In a dark room at 27°C the containers were then incubated for 4 days and examined for contamination before planting. Explants were then planted in the culture medium under a sterilization cabinet (hood). The cultures were then incubated at 27°C in complete darkness and transferred to a fresh medium every month three times. They were then transferred to the multiplication medium specified for each experimental treatment as stated bellow. The plantlets that had 4-5 shoots were selected and spliced into cuttings for further culturing. The sucker cuttings were put in low cost fresh prepared medium of the composition as in Table (1). Morphological changes were observed and recorded weekly. After the 4th week of culturing, final plantlets produced per each treatment was compared to others for detecting suitability of culture medium for further recommendation.

Four treatments were used in which two types of sugar (sucrose and commercial sugar) each in two concentrations (15 and 30 grams per liter), while MS salt strength was kept at 3.3 grams per liter and with 2 grams of gelrite and 5 mg benzyel adenine as indicated in Table (1).

Ten cultures were grown to be tested for growth parameters with four replicates, and the experiment was statistically analyzed according to a randomized complete block design (RCBD). Readings were taken at different periods of the experiment, as shown in the results tables (bud emergence, leaf emergence, and root emergence). At the end of the experiment (after 4 weeks), the number of shoots, leaves, and roots in the culture were calculated.

Table (1) Media component used for banana bud regeneration in comparison between two types of sugar

Treatment	Ms salt strength gram/liter	Sucrose concentration gram/liter	Commercial sugar concentration gram/liter	Benzyel adenine concentration mg/ liter	Added Gelrite gram/liter
1	3.3	15	0	5	2
2	3.3	30	0	5	2
3	3.3	0	15	5	2
4	3.3	0	30	5	2

Results and discussion

Effect of commercial sugar:

The effect of sugar type and concentration on the formation and growth of banana buds in plant tissue culture were shown in Figure (1) in the initial reading seven days after the cultures were grown, the high sugar concentration (30 g/L) resulted in a significant increase in the number of bud-forming culture containers. The percentage of bud-forming culture containers was 20%, compared to the low sugar concentration (15 g/L) containers, which represented 10% of the incubated cultures containers ($p<0.0060$). No significant difference was found between the two sugar types.

In the second reading, 14 days after culture, the high sugar concentration of both types resulted in a significant increase in the number of containers forming new buds (30%) compared to the low sugar concentration of both types (15 g/L) which was 20% with commercial sugar, and 10% of bud forming containers of 15 g/L sucrose ($p<0.0005$).

In the third reading, 21 days after culture, the highest percentage of regenerating containers forming buds was produced with both 30 g/L sucrose and 30 and 15 g/L commercial sugars, representing 40% of total number of tested containers. No increase was observed with sucrose 15 g/L compared to the previous week which was only 10% of total number of containers, and the differences were highly significant ($p<0.0001$).

In the fourth reading after 28 days of planting the cultures, commercial sugar at a concentration of 15 g/L showed the highest percentage of containers forming new buds (80% of total number of containers), followed by sucrose at a concentration of 30 g/L (50% of total number of containers), while there was no increase in the percentage of cultures forming buds at a concentration of

commercial sugar of 30 g (40% of total number of containers) and sucrose of 15 g/L (10% of total number of containers), and the differences were highly significant ($p < 0.0001$).

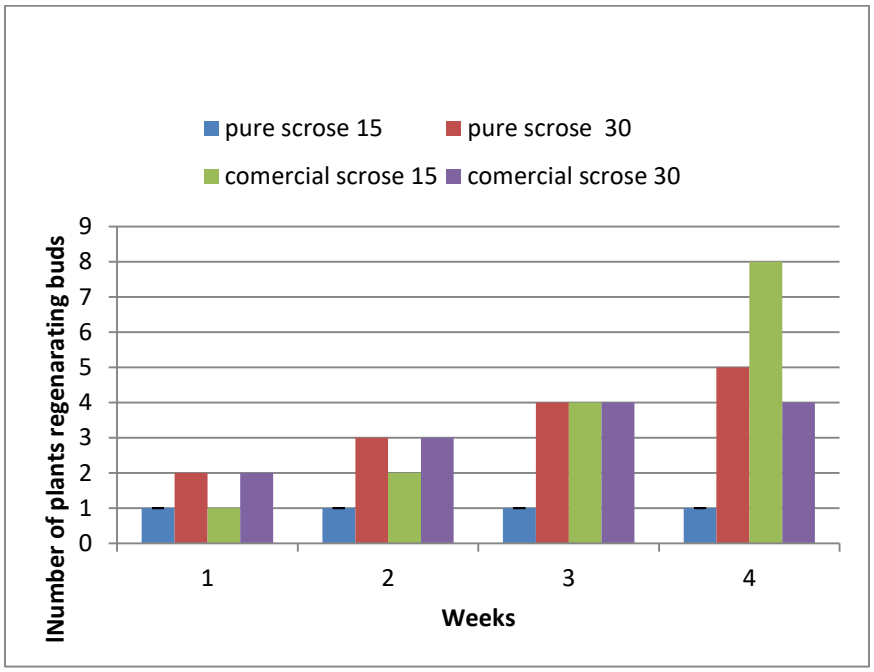

Figure (1) Effect of sugar type and concentration on the regenerated cultures

Figure (2) shows a significant difference in leaf growth regarding sugar type and concentrations. In the first reading, seven days after the culturing, as a result of the different treatments. The highest percentage of leaf formation resulted from the high sugar concentration of 30 g/L (100% leaf-forming cultures), compared to the low concentration of both sugar types (15 g/L), where the percentage of leaf-forming culture containers was 70% ($p<0.0006$).

In the second reading, 14 days after the culturing, the highest leaf growth resulted from high sucrose concentration (30 g/L) and commercial sugar of 30 and 15 g/L concentrations, where the percentage of leaf-forming culture containers was 100%, compared to the low sucrose concentration (15 g/L), the percentage of leaf-forming culture containers was 80% of the total containers number. The differences were highly significant ($p<0.0001$).

In the third reading, 21 days after culturing, the highest percentage of leaf-forming containers resulted from the high sugar concentration of the two types and from low concentration of commercial sugar (15 g/L). The percentage of leaf-forming containers was 100%, and the lowest percentage was from the low sucrose concentration (15 g/L), despite the increase in the percentage of leaf-forming containers to 90% ($p<0.0877$).

In the fourth reading, 28 days after culturing, all sugar concentrations of both types resulted in complete leaf development, and there was no difference between treatments ($p<0.436$).

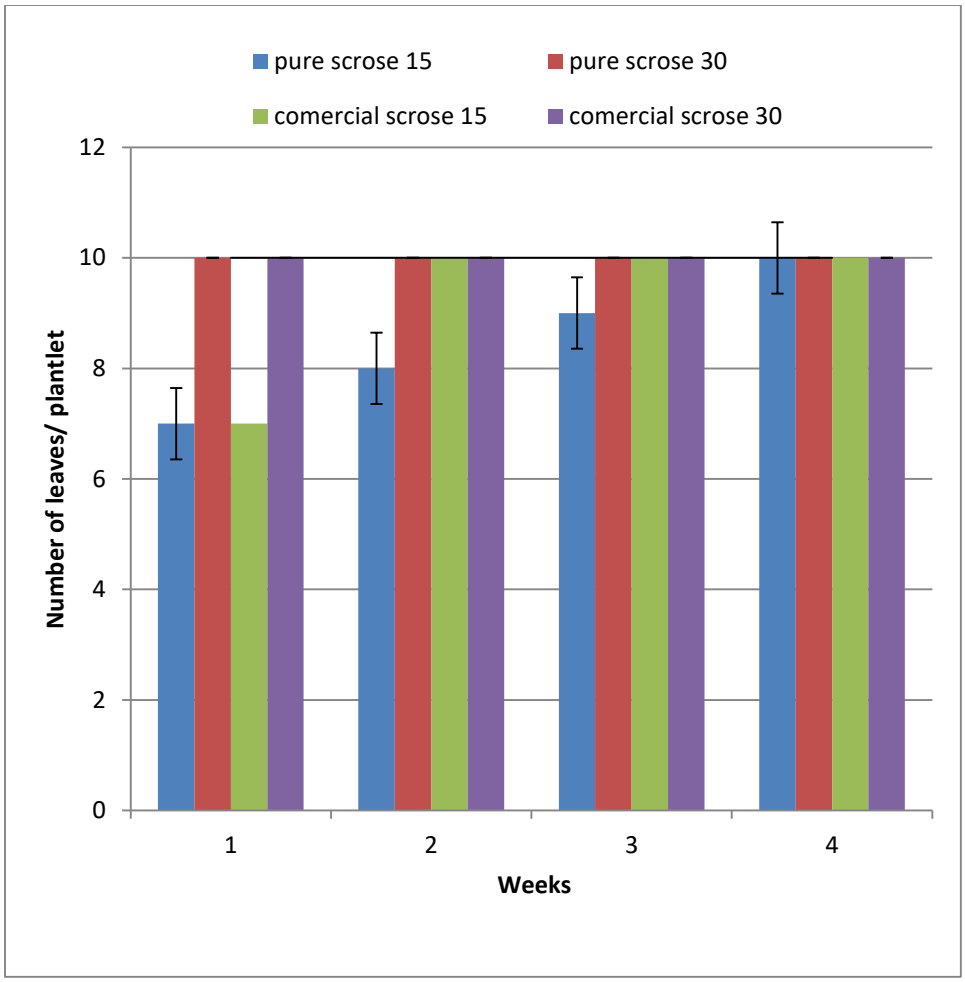

Figure (2) Effect of sugar type and concentration on leaf emergence on banana shoots in tissue culture

The effect of sugar type and concentration on plantlets rooting in banana tissue culture treatments was shown in figure (3). Results showed a significant effect of sugar type and concentration on the percentage of rooting containers. In the initial reading, seven days after the cultures were made, the effect of sugar concentration and type showed significant differences in the number of rooting containers. The highest percentage of rooting containers (20%) was registered by the highest sugar concentration of 30 (g/L) of both sugar types and the commercial sugar concentration of 15 g/L, the percentage of culture containers with roots was 10% ($p<0.015$).

In the second reading, 14 days after culture, the type and concentration of sugar produced significant differences in the percentage of rooting culture containers. The highest percentage of rooting culture containers (50%) was recorded by concentration of 15 g/L of both sugar types, followed by sucrose at 30 g/L, with a 40% rooting culture container percentage. The least rooting

occurred at a commercial sugar concentration of 30 g/L, with a 30% rooting of the total number of culture container ($p<0.0291$).

In the third reading, 21 days after culture, an increase was observed in the percentage of containers of rooting culture, reaching 50% for the low sugar concentration compared to 40% for the high sugar concentration. The differences were significant ($p<0.0460$).

In the fourth reading, 28 days after culturing, the results appeared as in the third week, without an increase in the percentage of rooting containers.

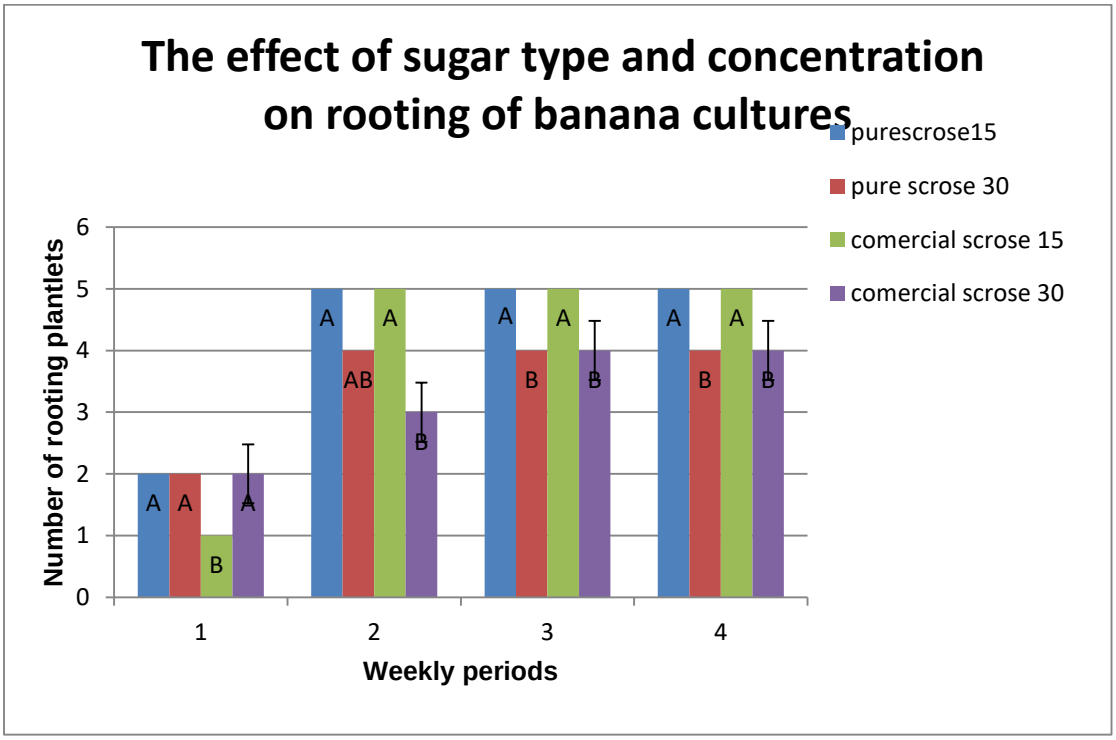

Figure (3) Effect of sugar type and concentration on banana rooting in tissue culture

Table (2) shows the performance of banana plantlets as affected by different type of sugars and their concentration at the end of the fourth week. Regarding bud formation, which demonstrates the doubling of banana plantlets during the multiplication phase, results indicate no significant differences in banana bud formation, whether pure sucrose or commercial sugar was used. The 15 g/L concentration achieved the best results in both sugar types. The average number of buds ranging between 3-4, with no significant differences between the two sugar types and concentrations. These results are similar to those obtained by Stephan and Dhanalakshmi (2014) for the number of regenerating buds when he used commercial sugar.

Regarding the number of leaves, there were no significant differences between treatments. Regarding the number of roots, the results indicate significant differences between the 30 g /l commercial sugar concentration and the remaining treatments.

The average plant height did not differ significantly between sugar treatments, whether commercial sugar or sucrose used. It is well known that the carbon source is important for the growth of plant parts in tissue cultures, whether buds or other parts, due to the inability of cultures to provide the plant with energy through photosynthesis in its early stages. The above results demonstrate that the banana tissue culture medium is not affected by the type of sugar added to banana plant propagation. Therefore, the cost of seedlings and foreign currency can be reduced by using commercial sugar instead of pure sucrose, either at a concentration of 15 or 30 grams of sugar per liter of the nutrient medium containing essential macro and microelements, vitamins, amino acids, and plant growth regulators. Energy sources in tissue culture media are important for plants whose photosynthetic efficiency is not sufficient under laboratory conditions. The energy source in tissue cultures is high, making plant tissue culture expensive (Ssamula et al., 2015). In this experiment, sugar was used as a less expensive carbon source in tissue culture, which is expensive to produce plantlets at a lower cost due to the high cost of pure sucrose, its scarcity under Sudanese conditions, and the time it takes to import. Locally produced commercial sugar is about 5% of the price of pure sucrose at the time of experimentation.

Table (2) Effect of sugar type and concentration on growth parameters 4 weeks after banana explant culturing

Type and concentration of sugar in grams	Culture reading at the end of the 4th week			
	Number of shoots	Number of leaves	Number of roots	Average plant height in cm
Pure sucrose 15	3.55 a	5.11 a	1.11 b	2.03 a
Pure sucrose 30	3.22 a	5.11 a	1.33 ab	1.92 a
Commercial sugar 15	3.44 a	5.11 a	0.22 b	1.87 a
Commercial sugar 30	3.11 a	5.11 a	2.0 a	1.76 a
CV%	17.47	0.00	72.72	19.87
LSD	1.16	0.00	1.6902	0.76

Conclusion

This study indicated that commercial sugar can be used as low cost and safe media component for banana micro propagation. This can help in decreasing seedling cost of disease free material in banana production

References

Ahamad, S. (2024). Optimization of low-cost media for plant tissue culture: A step towards the formulation of cconomical culture medium Journal of Plant and Biolgical Crop Res.; 7(1): 1097

Anonymous. (2004). Low cost options for tissue culture technology in developing countries FAO/IAEA, Vienna , Austria .

Demo, P.; Kuria, P.; Nyende., A.B. and Kahangi, E.M. (2008). Table sugar as an alternative low cost medium component for *in vitro* micropropagation of potato (Solanum tuberosum L.). African Journal of Biotech. 7(15) :2578-2584.

Dhanalakshmi, s. and Stephan, R. (2014). Low cost media options for the production of Banana (*Musa paradisiaca L.*) through plant tissue culture. Journal of Acadimia and Industrial Research, 2(9): 509-512.

Dhanalakshmi,S.; Stephan, R. (2016).Low cost micro propagation package for Banana (Musa paradisiaca L.). International Journal of Advanced Research in Biological Sciences; 3(5): 240-253

Gitonga, N. M.; Ombori, O.; Murithi, K.S.D. and Ngugi M. (2010). Low technology tissue culture materials for initiation and multiplication of banana plants. African Crop Sci. 18 (4): 243-251.

Jain, N. and Babbar, S.H. (2002). Gum katira - a cheap gelling agent for plant tissue culture

Kaur, R., Gautam, H. and Sharma, D.R. (2005). A low cost strategy for micropropagation of strawberry (*Fragari ananassa*) cv. Chandler. Proc. of the VII Int. Symp. On temperate zone fruits in the tropics and subtropics.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Osman, A.A.; Daffalla, M.E. and Habeeb, B.I. (2015). Evaluating markets of banana for Sudan. International Journal of Innovative Science, Engineering & Technology, Vol. 2 (5).

Prabhuling, G.; Sathyanarayana, B.N. (2017).Cheaper carbon sources for micro propagation of banana cv. Grand Naine .International Journal of agric. Sci. 13 (1): 124-131

Rakshi, M.; Venkata, K.; Subbaiah, G.; Prabhuling, G.; Swamy, G.S.K . and Jholgiker, P. (2017). A review on Low cost micro propagation techniques in banana. Journal of Pharmacognosy and Phytochemistry, 2017; SP1: 357-359.

Ssamula, A.; Arinaitwe, G. and Mukasa, S.B. (2015). Banana juice as an alternative energy source for banana in vitro growth medium. African Crop Science Journal, 23, (1): 59-66.