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RESEARCH ARTICLE

EVALUATION OF SUGARCANE MOLASSES AS AN ALTERNATIVE CARBON SOURCE FOR *IN-VITRO* ROOTING OF SOUR BANANA (MYSORE AAB)

Piyarathna KKCTP[1], Vassanthini R[1*], Safeena MIS[2], Rohonadeera H[3]

[1]Department of Biosystems Technology, Faculty of Technology, Eastern University, Chenkalady, Sri Lanka

[2] Department of Biological Sciences, Faculty of Applied Sciences, South Eastern University of Sri Lanka

[3] Institute for Agro-Technology and Rural Science, University of Colombo, Hambanthota, Sri Lanka.

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ABSTRACT

Banana (*Musa* spp.) is one of the most important fruit crops globally, and *in-vitro* propagation plays a crucial role in large-scale multiplication, particularly for cultivars like sour bananas (Mysore AAB). In tissue culture, sucrose is the primary carbon source, typically used in the form of commercial white sugar. However, this study investigates the potential of sugarcane molasses as a cost-effective and sustainable alternative to commercial white sugar in Murashige and Skoog (MS) medium for *in-vitro* sour banana propagation. The experiments were conducted using MS medium supplemented with varying concentrations of sugarcane molasses (10 g/L, 20 g/L, 30 g/L, and 40 g/L) compared to the standard 30 g/L of commercial white sugar. Growth parameters such as root initiation, root number, root length, plant height, root fresh and dry weight, and total plant fresh and dry weight were evaluated over six weeks. The MS medium with sugar (T1) showed the best results, significantly outperforming all other treatments ($p < 0.05$), with the highest plant height (53.36 mm), root number (5.41), root length (7.68 cm), and plant dry weight (0.09 g). Sugarcane molasses in low concentration with MS media (T2, 10 g/ L) exhibited promising performance, it significantly lower than T1 with plant height (34.4 mm), root number (4.4), and shoot dry weight (0.05 g). However, higher concentrations of molasses resulted in media browning and reduced root growth, likely due to excessive impurities and phenolic compound accumulation. This study demonstrates that sugarcane molasses in low concentrations with further optimization could be a viable, cost-effective, and sustainable alternative carbon source for commercial micro propagation of banana. Therefore, further research is recommended to refine molasses treatment methods to minimize impurities and optimize plantlet development.

Keywords: Alternative carbon source, Banana (*Musa* spp), Cost-effectiveness, *In-vitro* propagation, Sugarcane molasses, Sucrose

INTRODUCTION

Bananas are monocotyledonous, herbaceous, perennial, and succulent plants, grouped as members of the genus *Musa* and the family Musaceae (Ozukum *et al.,* 2024). Bananas are a major source of tropical fruits for the worldwide market, a most important staple food, and a major export commodity for many tropical and subtropical countries.

Bananas are vegetatively propagated through suckers. However, conventional methods of breeding for improving edible bananas are difficult and time-consuming because the majority of them are triploid, almost sterile, and require parthenocarpy. Biotechnological methods and mutation breeding offer useful tools for improving bananas (Singh *et al*., 2011). New possibilities for *in-vitro* mutagenesis and selection among several banana cultivars have been made possible by several propagation experiments (Beshir *et al.,* 2012).

Global demand for bananas is steadily increasing, driven by their high nutritional value and significant economic importance (FAO, 2023). However, large-scale banana cultivation is often challenged by limitations in propagation methods (George *et al*., 2008). Traditional vegetative propagation through

Corresponding author: vassanthinir@esn.ac.lk

suckers is inefficient, prone to disease transmission, and unable to meet commercial demand. As a result, *in-vitro* propagation has become the preferred method for producing disease-free and high-quality planting material (Sirohi *et al.,* 2025). Despite its advantages, one of the major constraints of banana micro propagation is the high cost of tissue culture media, particularly the carbon source (George *et al*., 2008).

Sucrose has been reported to be the best carbon and energy source. *In-vitro* plant cells, tissues, and organ cultures are not fully autotrophic, requiring an external carbon source to maintain osmotic potential and supply energy for key developmental processes such as shoot proliferation, root induction, embryogenesis, and organogenesis, all of which are energy-intensive (Yaseen *et al.,* 2013).

The high cost and restricted availability of laboratory-grade sucrose remain significant constraints for plant tissue culture laboratories, particularly in developing countries. To overcome these limitations, commercially available table sugar has been employed as a cost-effective substitute, substantially reducing expenditure without adversely affecting micropropagation efficiency or plantlet quality. In addition, naturally derived supplements such as cane molasses, banana extract, and coconut water have been incorporated into culture media as economical alternatives, supplying carbohydrates, essential vitamins, and inorganic ions that support *in-vitro* plant growth (George & Manuel, 2013).

The most popular carbon source in plant tissue culture is commercial white sugar, or sucrose, because of its function in osmotic control and energy metabolism. Large-scale production is, however, severely constrained by the increasing cost of sucrose and the need for sustainable alternatives (Thorpe, 2007). An economical and plentiful byproduct of the sugar industry, sugarcane molasses has been proposed as an alternative carbon source (Singh *et al*., 2015). While molasses is successful in various plant tissue culture applications, its function in banana micropropagation, specifically *in-vitro* roots, is still unknown (Kumar *et al.,* 2021).

Sugarcane molasses is a viscous byproduct of sugar production, obtained after the crystallization of sucrose during sugar refining. It is composed of approximately 48–55% total sugars, including sucrose, glucose, and fructose, and also contains amino acids, vitamins, and essential minerals such as potassium and calcium (Xu *et al.,* 2015). However, there is a significant environmental concern when this waste is disposed of in the environment. Thus, innovative applications of sugarcane molasses should be explored. Understanding its effect on *in-vitro* rooting is essential for optimizing tissue culture protocols and reducing dependency on expensive commercial sugars.

The benefits of using sugarcane molasses as an alternative carbon source to commercial sucrose, both economically and environmentally, serve as the justification for this study. Molasses has the potential to significantly reduce production costs, opening up banana tissue culture to both commercial and small-scale growers. Reusing an industrial byproduct also promotes the circular economy, reduces waste, and aligns with sustainable agriculture practices (Abderrahmane *et al*., 2024). Therefore, this aims to evaluate the sugarcane molasses as an alternative carbon source for *in-vitro* rooting of sour banana (Mysore AAB).

MATERIALS AND METHODS

Study area

The study was conducted at the Plant Tissue Culture Research & Development Laboratory, Institute for Agro Technology and Rural Sciences, Weligatta, Hambanthota, University of Colombo

Experimental design

The experiment was conducted using a completely randomized design (CRD) with five treatments, each replicated 10 times.

Sample collection

Sugarcane molasses was collected for this study from the Lanka Sugar Company, Sevanagala.

Laboratory procedures

This study selected banana shoots with 2 leaves from the six sub-culturing cycles as explant materials. Explants were established on the Murashige and Skoog medium. The macronutrient solution was prepared as three separate stock solutions: Macronutrients I, II, and III. Each component was weighed using an analytical balance according to Sharma *et al*., 2016 mentioned and dissolved in a small volume of distilled water with a magnetic stirrer. The solutions were transferred into 1 L volumetric flasks and diluted to the final volume with distilled water. Micronutrient solution was prepared by dissolving boric acid, cobalt chloride, copper sulfate, manganese sulfate, potassium molybdate, sodium molybdate, and zinc sulfate (Sharma *et al*., 2016) in distilled water, and the final volume was adjusted to 1 L. The vitamin solution, consisting of glycine, thiamine-HCl, pyridoxine-HCl, and nicotinic acid (Sharma *et al*., 2016), was dissolved in 50 ml of distilled water and made up to 500 ml. The ferrous solution was prepared by dissolving 27.8 mg of ferrous sulfate and 33.6 mg of Na_2EDTA separately in 50 ml of distilled water and combining them in a 1 L volumetric flask. These stock solutions were used in media preparation as per the volumes as mentioned in (Saad, 2012). For the control treatment (T1), MS medium was prepared by adding 30 g sugar and 6 g agar to 800 ml of distilled water, followed by the addition of macronutrients, micronutrients, vitamins, and ferrous solutions, along with 1 ml each of BAP and IAA, and adjusted to 200 ml. The pH was set to 5.8, and the medium was autoclaved at 121 °C for 1 hour. Experimental treatments (T2, T3, T4, T5) were prepared using sugarcane molasses at 5 g, 10 g, 15 g, and 20 g per 500 ml of media, respectively. These were mixed with 12 g agar/ 100, stock solutions, 2 ml BAP, and 2 ml IAA in a 2 L total volume, divided, pH-adjusted to 5.8, sterilized, and stored. All cultures were incubated under 22 °C–25 °C temperature, 2500 lux light intensity, 16/8 h light/dark cycle, and 75% relative humidity in a sanitized growth room. At the end of the experiment, the data for each week for each treatment was calculated. Data were taken as the average of each medium root length, number of roots, plant height, fresh weight, and dry weight.

Data analysis

Data analysis was performed using Minitab software (version 18) to compare the mean values of all treatments via analysis of variance (one-way ANOVA), and mean Tukey separation was conducted at a 5% significance level.

RESULTS AND DISCUSSION

The effect of sugarcane molasses as an alternative carbon source for commercial white sugar on *in-vitro* rooting of sour banana (Mysore AAB) was evaluated. The results showed significant differences among the four different concentrations of sugarcane molasses with commercial white sugar in the standard MS medium. The results revealed significant differences ($p < 0.05$) in plant growth across treatments, indicating that sugar concentration markedly influences *in-vitro* development (Table 1).

Table 1. Plant height (mm) over the six weeks

Weeks	T1	T2	T3	T4	T5	*P* value
1st Week	21.84±0.5^{a}	22.31±0.3^{ab}	21.32±0.1^{b}	19.06±0.2^{c}	17.35±0.2^{d}	0.00
2nd Week	27.55±0.6^{a}	24.18±0.8^{b}	23.03±0.2^{b}	19.86±0.3^{c}	18.84±0.4^{c}	0.00
3rd Week	36.90±1.4^{a}	26.51±0.7^{b}	25.12±0.3^{b}	20.70±0.4^{b}	18.27±0.5^{a}	0.00
4th Week	43.83±1.7^{a}	29.17±0.7^{b}	27.40±0.6^{b}	21.73±0.5^{c}	19.02±0.2^{c}	0.00
5th Week	49.12±1.7^{a}	31.60±0.5^{b}	29.41±1.0^{b}	22.29±0.5^{c}	19.28±0.4^{c}	0.00
6th Week	53.36±1.5^{a}	34.41±0.7^{b}	31.83±1.3^{b}	24.18±0.4^{c}	20.06±0.4^{c}	0.00

*Values represent the mean ± standard error of ten replicates. Mean values in a row having dissimilar letters indicate significant differences at a 5% level of significance according to Tukey's HSD Test. T1: 30 g/l sugar, T2: 10 g/l, sugarcane molasses, T3: 20 g/l sugarcane molasses, T4: 30 g/l sugarcane molasses T5: 40 g/l sugarcane molasses.

Among the treatments, T1 (30 g/L sugar) consistently produced the highest plant height (53.36 mm), suggesting optimal carbohydrate availability for growth. T2 (10 g/L sugarcane molasses) also supported growth, though significantly lower than T1. Moderate growth was observed in T3 (20 g/L sugar), while T4 (30 g/L sugarcane molasses) and T5 (40 g/L sugarcane molasses) exhibited reduced growth, with T5 showing the poorest performance (17.35 mm) at the 1st week (Table 1). These findings suggest that excessive concentrations of sugarcane molasses may inhibit growth, possibly due to the presence of complex sugars, organic acids, and phenolic compounds. Phenolics are known to induce oxidative stress, impairing cell division and elongation *in-vitro* (Shafiqa-Atikah *et al*., 2020). The decline in growth from the third week onward further supports the inhibitory effect of high sugarcane molasses concentrations. Thus, moderate sugar concentrations appear optimal for promoting plant growth *in-vitro*.

T1 (30 g/L sugar), with the highest number of roots across all six weeks, was the most effective in promoting growth, as evidenced by its consistently higher values (5.41) (Table 2). T2 (10 g/L Sugarcane molasses) also supported substantial growth, but was slightly lower than T1 in week 3, suggesting a small but significant difference in root number (4.63) between the two treatments. T3 (20 g/L Sugarcane molasses) showed moderate growth with values significantly lower than both T1 and T2 in the early weeks but still more favorable compared to T4 (30 g/L Sugarcane molasses) and T5 (40 g/L Sugarcane molasses), indicating a somewhat effective but not optimal sugar concentration. In contrast, T4 and T5 exhibited the lowest root number. Overall, T4 performed the lowest, showing significantly slower growth than the other treatments, particularly in the earlier weeks.

T1 (30 g/L sugar), with the highest number of roots across all six weeks, was the most effective in promoting growth, as evidenced by its consistently higher values (5.41) (Table 2). T2 (10 g/L Sugarcane molasses) also supported substantial growth, but was slightly lower than T1 in week 3, suggesting a small but significant difference in root number (4.63) between the two treatments. T3 (20 g/L Sugarcane molasses) showed moderate growth with values significantly lower than both T1 and T2 in the early weeks but still more favorable compared to T4 (30 g/L Sugarcane molasses) and T5 (40 g/L Sugarcane molasses), indicating a somewhat effective but not optimal sugar concentration. In contrast, T4 and T5 exhibited the lowest root number. Overall, T4 performed the lowest, showing significantly slower growth than the other treatments, particularly in the earlier weeks.

Table 2: Growth of root numbers over time

Weeks	T1	T2	T3	T4	T5	*P* Value
1st Week	1.74±0.25^{a}	1.69±0.11^{a}	1.47±0.14^{b}	0.40±0.05^{b}	0.53±0.10^{b}	0.00
2nd Week	2.79±0.29^{a}	2.88±0.07^{a}	2.30 ±0.16^{a}	0.44±0.07^{b}	0.62±0.11^{b}	0.00
3rd Week	3.39±0.29^{a}	3.19±0.18^{b}	2.96 ±0.20^{b}	0.46±0.08^{b}	0.82±0.15^{b}	0.00
4th Week	3.88±0.24^{a}	3.66±0.22^{a}	3.51±0.21^{a}	0.59±0.20^{b}	1.05±0.09^{b}	0.00
5th Week	4.43±0.24^{a}	4.01±0.23^{a}	3.96±0.22^{a}	1.15±0.18^{b}	1.08±0.12^{b}	0.00
6th Week	5.41±0.35^{a}	4.63±0.20^{a}	4.50±0.21^{ab}	1.59±0.08^{c}	1.25±0.09^{c}	0.00

*Values represent the mean ± standard error of ten replicates. Mean values in a row having dissimilar letters indicate significant differences at a 5% level of significance according to Tukey's HSD Test. T1: 30 g/l sugar, T2: 10 g/l, sugarcane molasses, T3: 20 g/l sugarcane molasses, T4: 30 g/l sugarcane molasses T5: 40 g/l sugarcane molasses.

This suggests that the conditions in these treatments were not conducive to optimal growth, likely due to low sugar concentrations or other limiting factors. The reduction in growth at this concentration could be due to the presence of inhibitory compounds in molasses such as phenolics and excess minerals, which might have a toxic effect on plant cells (Yaseen *et al*., 2013). However, T5 roots grow lower than all other treatments. Higher concentrations of sugarcane molasses can't be used as a carbon source, as they

provide unfavorable conditions in the media for root growth and development.

Significant differences in root length performance among the five treatments over six weeks, with T1 (30 g/L sugar) showing the most effective and consistent root growth (7.68 cm) (Table 3). This suggests that T1 contains an optimal mix of nutrients or growth regulators suitable for promoting rapid root development. T2 (10 g/L Sugarcane molasses), though less effective than T1, still supported moderate growth (1.66 cm), indicating its potential for use in controlled growth environments. In contrast, treatments T3 (20g/l Sugarcane molasses), T4 (30 g/L Sugarcane molasses), and T5 (40 g/L Sugarcane molasses) exhibited minimal root elongation, with T3 being the least effective (0.18 cm). These results indicate that higher concentrations of sugarcane molasses negatively affect root development, possibly due to the presence of inhibitory compounds. As such, molasses may require further purification to be used effectively in tissue culture media (George *et al*., 2008). Alternatively, combining sucrose with low concentrations of molasses (<10 g/L) could offer a more cost-effective yet efficient approach for *in-vitro* rooting.

Table 3. Growth of root length over six weeks (cm)

Weeks	T1	T2	T3	T4	T5	*p* value
1st Week	0.44±0.04^{a}	0.12±0.01^{b}	0.10±0.00^{b}	0.11±0.01^{b}	0.10±0.00^{b}	0.00
2nd Week	2.38±0.40^{a}	0.80±0.08^{b}	0.13±0.01^{b}	0.15±0.01^{b}	0.17±0.01^{b}	0.00
3rd Week	3.92±0.55^{a}	1.05±0.14^{a}	0.15±0.01^{a}	0.21±0.01^{b}	0.19±0.09^{b}	0.00
4th Week	5.10±1.51^{a}	1.30±0.18^{b}	0.16±0.01^{c}	0.32±0.02^{c}	0.22±0.02^{c}	0.00
5th Week	6.30±0.45^{a}	1.43±0.23^{b}	0.17±0.01^{c}	0.35±0.02^{c}	0.26±0.032^{c}	0.00
6th Week	7.68±0.49^{a}	1.66±0.24^{b}	0.18±0.01^{c}	0.40±0.02^{c}	0.30±0.02^{c}	0.00

*Values represent the mean ± standard error of ten replicates. Mean values in a row having dissimilar letters indicate significant differences at a 5% level of significance according to Tukey's HSD Test. T1: 30 g/l sugar, T2: 10 g/l, sugarcane molasses, T3: 20 g/l sugarcane molasses, T4: 30 g/l sugarcane molasses T5: 40 g/l sugarcane molasses.

Table 4 shows significant differences ($p < 0.05$) in root and total plant weights among the treatments. T1(30 g/L sugar) gave the best results with the highest root fresh weight (0.696 g), root dry weight (0.037 g), total fresh weight (1.700 g), and total dry weight (0.095 g). This means T1 supported the best plant growth. T2 (10 g/L Sugarcane molasses) and T3 (20 g/L Sugarcane molasses) showed moderate growth. T2 had 0.162 g root fresh weight and 0.531 g total fresh weight, while T3 had slightly lower values. T4 (30 g/L Sugarcane molasses) and T5 (40 g/L Sugarcane molasses) had the lowest growth, with T4 showing the smallest root fresh weight (0.072g) and T5 the lowest total fresh weight (0.145 g). These results show that sugar is important for plant growth in tissue culture. While sucrose is commonly used, sugarcane juice or molasses can be cheaper options. However, high concentrations of molasses may reduce growth. Using purified molasses or mixing small amounts with sucrose (less than 10 g/L) could be a better choice for good growth and lower costs.

Table 4. Banana plant's fresh and dry weight over time

Treatments	Root fresh weight (g)	Root dry weight (g)	Total fresh weight (g)	Total dry weight (g)
T1	0.696±0.039^{a}	0.037±0.001^{a}	1.700±0.117^{a}	0.095±0.055^{a}
T2	0.162±0.013^{b}	0.007±0.000^{b}	0.531±0.029^{b}	0.027±0.001^{b}
T3	0.136±0.011^{bc}	0.006±0.000^{b}	0.446±0.053^{bc}	0.021±0.003^{b}
T4	0.072±0.006^{bc}	0.005±0.000^{b}	0.241±0.013^{cd}	0.017±0.001^{b}
T5	0.091±0.007^{c}	0.007±0.000^{b}	0.145±0.008^{d}	0.025±0.001^{b}

*Values represent the mean ± standard error of ten replicates. Mean values in a row having dissimilar letters indicate significant differences at a 5% level of significance according to Tukey's HSD Test. T1: 30 g/l sugar, T2: 10 g/l, sugarcane molasses, T3: 20 g/l sugarcane molasses, T4: 30 g/l sugarcane molasses T5: 40 g/l sugarcane molasses. *p* value= 0.00

CONCLUSIONS

This study investigated the use of sugarcane molasses as an alternative carbon source for *in-vitro* rooting of sour banana (Mysore AAB). The results showed that the treatment with 30 g/L of sugar (T1) performed the best across all measured parameters, including plant height, root number, root length, and both fresh and dry weight. Treatments with lower concentrations of molasses (T2 and T3) supported some level of growth but were less effective than commercial white sugar, possibly due to insufficient energy supply. Higher concentrations of molasses (T4 and T5) significantly reduced growth, likely due to osmotic stress and the presence of impurities in the molasses. While molasses does contain sugars beneficial for plant growth, other components like minerals and organic acids may interfere with *in-vitro* development. Overall, sugarcane molasses show potential as a carbon source, but it may require further processing or optimization before it can effectively replace refined sugar in banana tissue culture. Sugarcane molasses at 10 g/L can be considered for further optimization in banana tissue culture protocols. Based on the above results, the following recommendations can be made to improve this research in the future. Future research can be conducted mixing sugarcane molasses with sugar may help balance nutrient composition and improve the growth performance of *in-vitro* plants. Further studies should test different dilution levels of molasses to identify an optimal concentration that minimizes osmotic stress while providing adequate carbon for growth. Future research can recommend purification for the sugarcane molasses using methods such as filtration, decolorization, or enzyme treatment, which could remove impurities that negatively impact *in-vitro* rooting. Also, a comparative analysis of filtered vs. unfiltered molasses should be conducted to assess whether purification improves its effectiveness.

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AUTHOR CONTRIBUTION

All the authors equally contributed to this study.

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