



## **Adaptability of Sugarcane (*Saccharum officinarum* L.) Meristem Culture to Fermented Plant-Based Media under In Vitro Conditions**

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### **ABSTRACT**

Sugarcane (*Saccharum officinarum* L.) is an economically important crop widely propagated vegetatively; however, this method often results in heterogeneous and disease-prone planting materials. Plant tissue culture offers an effective approach to producing uniform, disease-free seedlings, with the composition of the culture medium playing a critical role in successful morphogenesis. This study evaluated the adaptability of sugarcane meristematic tissues to fermented plant-based culture media under in vitro conditions. The experiment was conducted using a Completely Randomized Design with four treatments. The treatments were Murashige and Skoog (MS) medium as a control, N6 medium, and fermented plant-based solutions as macronutrient and plant growth hormone substitutes, respectively. Each treatment was replicated four times with 20 culture bottles per replicate. Results showed that MS medium produced the highest shoot initiation (3.00) and the earliest shoot emergence (7.00 days). Fermented plant-based macronutrient and plant growth hormone treatments showed delayed shoot initiation, with mean emergence at 19 and 29 days, respectively. The N6 medium did not support shoot initiation. In terms of contamination, fermented plant-based treatments showed comparable or lower contamination rates (1.00) than MS medium (2.00), while N6 recorded the highest contamination (3.00). The results of the study indicate that fermented plant-based solutions support shoot initiation and maintain low levels of contamination. However, the MS medium remains superior at promoting rapid and efficient shoot development. Further optimization of nutrient composition and the balance of plant growth hormones is recommended to enhance the performance of fermented plant-based media in plant tissue culture systems.

### **RESUMO**

A cana-de-açúcar (*Saccharum officinarum* L.) é uma cultura de grande importância econômica, amplamente propagada de forma vegetativa; entretanto, esse método frequentemente resulta em materiais de plantio heterogêneos e suscetíveis a doenças. A cultura de tecidos vegetais oferece uma abordagem eficaz para a produção de mudas uniformes e livres de patógenos, sendo que a composição do meio de cultura desempenha um papel fundamental no sucesso da morfogênese. Este estudo avaliou a adaptabilidade de tecidos meristemáticos de cana-de-açúcar a meios de cultura à base de plantas fermentadas sob condições *in vitro*. O experimento foi conduzido utilizando um Delineamento Inteiramente Casualizado, com quatro tratamentos. Os tratamentos consistiram em meio de Murashige and Skoog (MS) medium como controle, meio N6 e soluções fermentadas de origem vegetal como substitutos de macronutrientes e reguladores de crescimento vegetal, respectivamente. Cada tratamento foi replicado quatro vezes, com 20 frascos de cultura por repetição. Os resultados mostraram que o meio MS apresentou a maior iniciação de brotos (3) e a emergência mais precoce (7 dias). Os tratamentos com soluções fermentadas de macronutrientes e reguladores de crescimento vegetal apresentaram atraso na iniciação dos brotos, com emergência média aos 19 e 29 dias, respectivamente. O meio N6 não promoveu a iniciação de brotos. Quanto à contaminação, os tratamentos à base de plantas fermentadas apresentaram taxas comparáveis ou inferiores (1) em relação ao meio MS (2), enquanto o meio N6 registrou a maior taxa de contaminação (3). Os resultados do estudo indicam que as soluções vegetais fermentadas são capazes de sustentar a iniciação de brotos e manter baixos níveis de contaminação. No entanto, o meio MS ainda se mostra superior na promoção de um desenvolvimento rápido e eficiente dos brotos. Recomenda-se a otimização adicional da composição nutricional e do equilíbrio de reguladores de crescimento vegetal para melhorar o desempenho dos meios à base de plantas fermentadas em sistemas de cultura de tecidos vegetais.

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## Introduction

Sugarcane (*Saccharum officinarum* L.) is a domesticated tropical, perennial grass known for its sweetness, with its stalks harvested for cash or used in industries (Hamza and Alebjo, 2017). Brazil is the world's top producer and exporter of sugarcane, which supplies 50% of the world's sugar, producing 654.8m tons of sugarcane, 41.25m tons of processed sugar, and 29.7 billion liters of ethanol annually (Cursi et al., 2022). While ASEAN countries such as Thailand, Indonesia, Vietnam, the Philippines, Cambodia, Myanmar, and Lao PDR account for 10% of global sugar production, producing over 17 million tons of sugar and consuming around 15 million tons annually. Thailand is the second largest sugar exporter after Brazil (Solomon et al., 2016).

Sugarcane varieties are highly heterogeneous and are generally propagated vegetatively by stem cutting (Khan et al., 2009). In tropical countries, nodal sections of sugarcane with 2 or 3 nodes are commonly used as a planting material (Hamza & Alebjo, 2017). In this regard, it is necessary to have good planting materials (cane points), as they contribute to higher yields and productivity in sugarcane production (Ibrahim et al., 2016). Availability of disease-free, genuine-to-type planting material is essential for achieving high yields in sugarcane cultivation (Singh & Singh, 2015).

Plant tissue culture emerged as a reliable technique for the rapid multiplication of uniform, disease-free planting materials. This method enables large-scale propagation through controlled in vitro conditions, supporting consistent plant development and genetic stability (Debergh & Read, 1991). A key component influencing the success of tissue culture is the composition of the culture medium. The effectiveness of the culture medium depends on the availability of nutrients and plant growth hormones needed for division, differentiation, and organogenesis.

The Murashige and Skoog (MS) medium is widely used as a standard formulation due to its balanced supply of macro- and micronutrients. There is also a wide compatibility of MS medium as a culture medium standard in various plant species. However, recent studies on the exploration and incorporation of organic-derived inputs, such as plant-based extracts and fermented waste materials, have been incorporated into the plant tissue culture systems. For instance, palm mill oil effluent (POME) and vinasse have been investigated as alternative nutrient sources and have shown potential for supporting in vitro plant growth (Nadirah et al., 2019; Da Silva et al., 2014). These studies suggest that organic-based media may influence development through the gradual release of nutrients and the presence of bioactive compounds.

Despite the advancements, there is still limited information on the effectiveness of fermented plant-based solutions as substitutes for specific components in plant tissue culture media, particularly for macronutrients and plant growth hormones in response to sugarcane

meristematic culture. Moreover, the effects of these fermented plant-based solutions on shoot initiation, time of shoot emergence, and contamination rate to sugarcane meristem culture are not yet understood under controlled in vitro conditions.

Therefore, this study was conducted to evaluate the adaptability of sugarcane meristematic tissues to fermented plant-based culture media by assessing their shoot initiation, time of shoot emergence, and contamination rate. The findings may contribute to a better understanding of the effects of organic-based inputs on morphogenesis and culture stability in sugarcane meristem tissue culture.

## Materials and Methods

The study used the Completely Randomized Design following Gomez and Gomez (1984). The four treatments were evaluated; the Murashige and Skoog (MS) as control (t<sub>1</sub>), the N6 medium (t<sub>2</sub>) based on Chu formulation as the culture medium for sugarcane tissue culture, the MS medium supplemented with fermented plant-based solution as macronutrient substitute (t<sub>3</sub>), and the MS medium supplemented with fermented plant-based solution as plant growth hormone substitute (t<sub>4</sub>). These treatments were replicated 4 times, with 20 culture bottles per replicate, for a total of 320 culture bottles per experimental run. The experiment was conducted three times to ensure data reliability and reproducibility.

The fermented plant-based solution was prepared using a combination of young kakawate (*Gliricidia sepium*) leaves, finely-chopped banana trunks, and fruit peels. The prepared materials were mixed with brown sugar at a 1:1 (w/w) ratio and fermented for 14 days under ambient temperature. After fermentation, the solution was filtered using a sterile muslin cloth, collected, and stored in a refrigerator before use. Representative samples were analyzed at the University of the Philippines, Los Baños, laboratory for nutrient composition. The fermented solutions contained approximately 950 mg/L nitrogen, 277 mg/L phosphorus, 2776 mg/L potassium, 390 mg/L calcium, 190.48 mg/L magnesium, 0.2 mg/L copper, 1.82 mg/L zinc, and 16.3 mg/L iron.

The MS medium was prepared using the standard stock solutions consisting of macronutrients (ammonium nitrate, potassium nitrate, calcium chloride, magnesium sulfate, and potassium phosphate), micronutrients, Fe-EDTA, vitamins, plant growth hormone, agar, and sugar. For each liter of culture medium, the following components were measured: 60 mL macronutrient stock solution, 1 mL micronutrient solution, 5 mL Fe-EDTA solution, 1 mL vitamins, 30 mL BAP solution at 3 ppm, 30 grams of sugar, and 5 grams of agar. On the other hand, N6 culture medium followed a similar preparation, except that the macronutrient formulation excluded ammonium nitrate, as reported by Manglar et al. (2022). For treatment 3, 60 mL of the fermented plant-based solution was added as a replacement for the macronutrient component. Treatment 4 used 30 mL of a fermented plant-based solution in

place of BAP. The media components were mixed thoroughly, and the pH was adjusted to 5.8. The media were heated to boiling for 15 minutes, dispensed into culture bottles of approximately 50 mL, and sterilized by autoclaving at 121 degrees Celsius and 15 psi for 30 minutes.

The meristematic explants were obtained from healthy sugarcane plants aged five months and younger (Salokhe, 2021). The collected explants were brought to the preparation room of the tissue culture laboratory, trimmed into smaller sections, and washed under running tap water for 10 minutes to remove surface contaminants. Surface sterilization was performed using a 5% v/v sodium hypochlorite solution for 30 minutes, followed by rinsing with distilled water 5 times to remove residual chlorine. The rinsed meristematic explants were brought to the culture room. Before inoculation, the laminar airflow cabinet was cleaned and disinfected using the 70% ethyl alcohol (Singh, 2003).

The sterilized explants were aseptically inoculated into prepared culture media. After inoculation, the culture bottles were placed on the culture shelves at 22 degrees Celsius and exposed to 8 hours of light from 40-watt fluorescent lamp. The culture bottles were observed for 1 month to assess the initiated shoots, time to shoot emergence, and contamination rate. The data were analyzed using the STAR statistical software. Mean comparisons were performed using the Honest Tukey Significant Difference (HTSD) test at 5% level of significance.

## Results and Discussions

Table 1 shows the effect of different culture media on shoot initiation, time to shoot emergence, and contamination rate of sugarcane meristem cultures. Shoot initiation, time to shoot emergence and contamination rate were counted manually. The culture bottles were monitored daily and recorded any observations found.

**Table 1**

Effects of different culture media on shoot initiation, time to shoot emergence, and contamination rate of sugarcane (*Saccharum officinarum* L.) meristem cultures

Treatment	Means of Initiated Shoots	Time to Shoot Emergence (days)	Means of Contamination (culture bottles)
MS Culture Media	3.00 <sup>a</sup>	7.00 <sup>a</sup>	2.00 <sup>b</sup>
N6 (Chu)	0.00 <sup>c</sup>	0.00 <sup>d</sup>	3.00 <sup>a</sup>
Fermented Plant-Based as Macronutrients	1.00 <sup>b</sup>	19.00 <sup>b</sup>	1.00 <sup>b</sup>
Fermented Plant-Based as PGH	1.00 <sup>b</sup>	29.00 <sup>c</sup>	1.00 <sup>b</sup>
CV (%)	46.95	7.10	28.17

Means of the same letter did not differ significantly

### **Shoot Initiation Success Rate**

Table 1 showed that MS culture media produced 3.00 shoots, which were significantly higher than those of the other treatments. However, the table showed that fermented plant-based macronutrients and plant growth hormone produced 1.00 shoot, respectively. The two fermented plant-based solutions are statistically comparable. On the other hand, the N6 culture medium preparation did not show an initiated shoot.

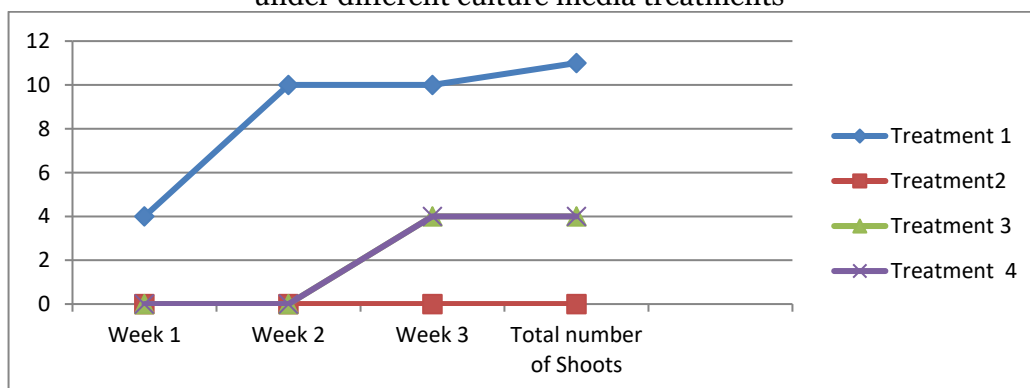
The higher shoot initiation rate in MS culture medium is due to complete nutrient supplementation. This medium is also used in the laboratory, thereby standardizing the preparations. On the other hand, the effect of the fermented plant-based culture medium on sugarcane shoot initiation is attributed to its nutrient composition. The macronutrients present in the analysis are enough to initiate a shoot. However, when it served as a substitute for plant growth hormone, the culture medium did not produce more shoots as expected. The fermented plant-based solutions could not be used as a substitute for plant growth hormone. Treatment 2 (N6) was considered non-significant, yielding a mean of 0.00, because all explants became rotten and/or discolored from green to entirely black, showing no possible signs of shoot initiation.

To further explain what happened during shoot initiation, Figure 1 presents the weekly initiation response of sugarcane meristem cultures to different culture media treatments. The culture media shows the greatest number of initiated explants having four (4) culture vessels in the first week, with an additional six (6) explants in week 2, and was maintained up to week 4 with a total of 10 explants/culture vessels that constitutes to 62.5% success rate of the initiated culture.

There are a total of 11 shoots formed out of those ten (10) initiated explants. However, the number of initiated shoots was not recorded or statistically analyzed, since the goal of this study is to determine the sugarcane’s meristematic tissue response to fermented plant-based macronutrients and plant growth hormone. While fermented plant-based macronutrients and plant growth hormone both exhibited zero (0) initiated explants in the first and second weeks, explants were initiated in weeks 3 and 4, respectively, with corresponding 25% success rates. There are a total of four shoots formed. For the N6 culture medium, no cultures were initiated, as all samples turned black and showed no signs of possible shoot initiation during the study.

**Figure 1.**

Weekly shoot initiation response of sugarcane (*Saccharum officinarum* L.) meristem cultures under different culture media treatments



The superior shoot initiation observed in the MS culture medium (t1) is attributed to its complete and accurate composition of macronutrients, micronutrients, vitamins, and plant

growth hormones. The presence of synthetic phytohormones such as BAP, along with other growth hormones, promotes cell division and organogenesis. The MS medium supplemented with BAP, Kn, and IBA produced multiple shoots from the cultured sugarcane shoot tips, as the findings of Baksha et al. (2002).

In addition, a study emphasized that the interaction and concentration of auxins and cytokinins influence morphogenesis and shoot regeneration (Yutaka et al. 1998). The effectiveness of MS medium is further supported by Kurimella (2016), who identified it as the preferred and suitable culture medium of a wide range of plant species due to its balanced nutrient formulation.

In contrast, the lower shoot initiation observed in treatments with fermented plant-based solutions (t3 and t4) suggests a delayed or reduced response compared to the synthetic medium. Although these treatments supported shoot formation at later stages, their performance may be influenced by the variability in nutrient mineralization and availability, as well as the absence of defined concentrations of plant growth hormones. Unlike the MS medium, where hormone levels are standardized, fermented solutions may contain inconsistent levels of bioactive compounds, resulting in slower or less uniform shoot induction.

Despite this limitation, the observed shoot initiation in t3 and t4 supports previous findings that organic and fermented plant-based inputs can serve as alternative nutrient sources in plant tissue culture. A study by Nadirah et al. (2019) reported that fermented palm oil mill effluent (POME) promoted the *in vitro* regeneration of *Musa acuminata* by enhancing nutrient availability. In addition, a similar study demonstrated that natural additives such as coconut water and fruit extracts promote shoot regeneration in *Celosia* sp. (Daud, 2011). Furthermore, Yusuf (2012) found that fermented fruit waste improved *in vitro* seed germination in pitaya, indicating the potential of organic substrates in supporting plant growth.

Moreover, Nadirah et al. (2019) explained that the nutrient composition of fermented solutions can be physiologically comparable to standard tissue culture media, depending on plant requirements. This supports the present findings, in which fermented plant-based solutions initiate shoots, albeit at a lower rate and slower pace than in MS medium. The delayed response in fermented plant-based treatments may be due to the gradual release and transformation of nutrients during fermentation, which differs from the immediate availability of nutrients in synthetic media.

In general, the results show that fermented plant-based solutions can be considered as a supplementary component for MS culture medium. Thus, there is still a need to further optimize the nutrient concentrations and hormonal balance of the fermented plant-based

culture medium to improve its effectiveness and achieve results comparable to those of standard synthetic media.

### **Time to Shoot Emergence**

Table 1 shows that the MS culture medium (t1) significantly promoted earlier shoot emergence, with an average of 7 days after inoculation, compared to all other treatments. This result confirms the efficiency of MS medium in supporting morphogenesis, due to its complete and balanced nutrient and plant growth regulator composition. The presence of macro- and micronutrients and cytokinins facilitates faster cell division and shoot differentiation. The concentration and interaction of auxins and cytokinins play a critical role in shoot regeneration (Baksha et al. 2002). This further proved that sugarcane explants cultured in MS medium produced shoots within 10 days, demonstrating the effectiveness in promoting early shoot development.

In contrast, the fermented plant-based macronutrient treatment (t3) initiated shoot days. Later, this showed a delayed response in shoot regeneration compared to the synthetic medium. The delay is attributed to the gradual mineralization and nutrient release from the fermented plant-based solution. Thus, the nutrients are unavailable to the explants. Unlike MS medium, where nutrients are present in readily absorbable inorganic forms, the fermented plant-based inputs require biochemical transformation before becoming accessible for plant uptake. This finding aligns with the concept that nutrient availability from organic sources is slower and dependent on decomposition processes (Nadirah et al., 2019).

Furthermore, the fermented plant-based solution used as a substitute for plant growth hormone (t4) had the longest time to shoot emergence at 29 days, which was significantly different from all other treatments. The result suggests a lack of specific balance and concentration of the plant growth hormone required for efficient shoot induction in the fermented plant-based solution. Plant growth hormones, such as cytokinins and auxins, are essential for initiating shoot organogenesis. The insufficient concentrations of these growth hormones significantly delay morphogenic responses (George et al., 2008). This supports the observation that replacing synthetic plant growth hormones with fermented plant-based solutions reduces shoot initiation efficiency.

The N6 medium (t2) did not produce any shoots during the observation period, indicating its unsuitability for sugarcane meristem culture under the given conditions. This may be due to differences in nutrient composition, particularly the absence of ammonium nitrate, which is a critical nitrogen source for rapid cell division and growth in many plant tissue culture systems.

Overall, the results demonstrate that the MS culture medium remains superior in promoting early shoot emergence. On the other hand, fermented plant-based solutions delay

shoot initiation by several days. The delay in shoot emergence highlights the need to further optimize nutrient composition and supplementation of plant growth hormones when using organic-based alternatives in plant tissue culture systems.

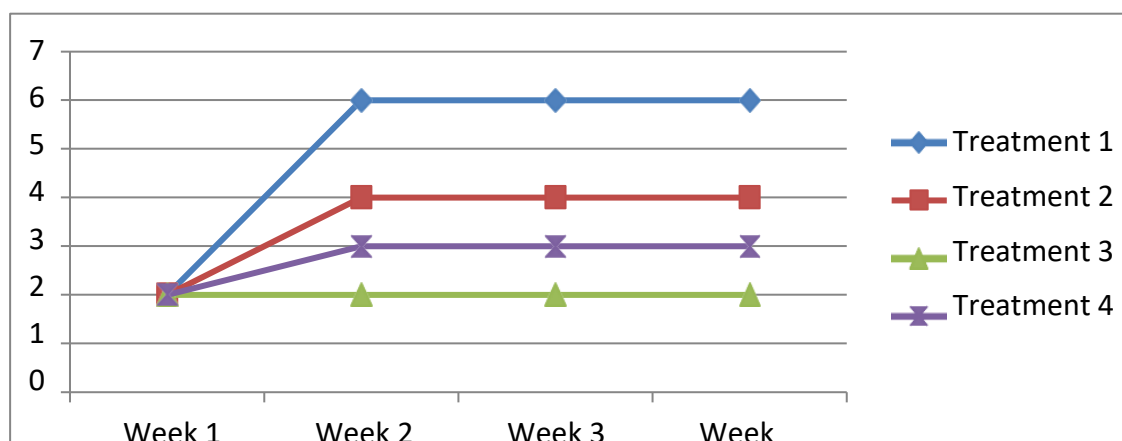
### **Contamination Rate**

Table 1 shows that MS medium (t1) has 2 bottles contaminated, and the fermented plant-based solutions (t3 and t4) are statistically comparable in contamination levels, with average contamination levels of 2 and 1 bottles, respectively. In contrast, the N6 medium (t2) showed the highest contamination across 3 culture bottles, indicating greater susceptibility to microbial growth under the conditions of this study. These results suggest that while MS medium remains the standard for plant tissue culture, the fermented plant-based solutions demonstrated comparable, and in some cases lower, contamination levels despite being derived from organic materials.

The temporal trend in Figure 2 further supports these findings. Fermented plant-based macronutrient (t3) maintained the lowest contamination level of 12.5% throughout the four-week observation period. Fermented plant-based growth hormone (t4) showed a slight increase from week 1 to week 2, then stabilized, with a total contamination rate of 18.75%. In contrast, MS medium (t1) exhibited a 37.5% increase in contamination from week 2 onward, while N6 medium (t2) consistently showed higher contamination across observation periods. This pattern indicates that organic-based treatments did not increase contamination risk and may even contribute to a more stable culture environment.

**Figure 2.**

Weekly observations of the contaminated culture bottles as effect of the MS medium and fermented plant-based solutions



Contamination in plant tissue culture remains a major challenge. The culture medium provides nutrients, sugars, agar, and moisture. However, these ideal environments entice microbial proliferation. The explants cultured in vitro are physiologically stressed and more

susceptible to infection, even by non-pathogenic microorganisms (Habiba et al., 2002). Additionally, the MS culture medium promotes microbial growth due to the variations in preparation or sterilization (Wojyania et al., 2005). Despite the application of sterilization protocols, complete elimination of contaminants is difficult due to endogenous microorganisms and limitations in detection (Leifert & Cassells, 2001).

The relatively lower contamination observed in fermented plant-based treatments may be attributed to the fermentation process that produces bioactive compounds with antimicrobial properties. These bioactive compounds might suppress the microbial growth in the culture (Nadirah et al., 2019). In addition, the nutrient composition of these organic solutions is less available for microbial growth and contamination. The synthetic medium contains nutrients, which are highly soluble and readily accessible. This could result in reduced microbial proliferation while still supporting plant growth at a slower rate.

On the other hand, the higher contamination values observed in N6 medium might be attributed to the nutrient composition and imbalance in carbon and nitrogen sources. The imbalance of nutrient composition affects plant tissue viability and microbial susceptibility. Aside from that, modifications to nutrient components of the medium, such as ammonium nitrate, may hinder explant growth, increasing vulnerability to contamination. Furthermore, the type and concentration of carbon sources in the culture medium play a crucial role in morphogenesis and microbial interactions, as reported by Yaseen et al. (2013).

The overall results indicate that the fermented plant-based media are not only viable for shoot initiation but also demonstrate comparable or lower contamination levels than conventional MS medium.

## **Conclusion**

This study demonstrated the potential of fermented plant-based solutions as alternative sources in the *in vitro* micropropagation of sugarcane. The results showed that modified MS media supplemented with fermented plant-based inputs supported shoot initiation and organogenesis, indicating the adaptability of sugarcane meristematic tissues to organic-based culture conditions. Notably, the fermented plant-based treatments exhibited contamination rates comparable to or lower than those of the conventional MS medium. However, despite the adaptability, the standard MS medium remained superior in terms of rapid higher shoot initiation and time of shoot emergence. Thus, to further optimize the fermented plant-based solutions, studies on concentration levels, standardization of nutrient composition, and cost-benefit analysis must be conducted to address the limitations of this study.

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## REFERENCES

- Ali, A., Naz, S. H. A. G. U. F. T. A., Siddiqui, F. A., & Iqbal, J. (2008). An efficient protocol for large scale production of sugarcane through micro propagation. *Pakistan Journal of Botany*, 40(1), 139.
- Baksha, R., Alam, R., Karim, M.Z., Paul, S.K., Hossain, M.A., Miah, M.A.S., and Rahman, A.B.M.M. (2002). In vitro Shoot Tip Culture of Sugar-cane (*Saccharum officinarum*) Variety Isd 28. *Biotechnology*, 1: 67-72.
- Chimdessa, E. (2020). Composition and preparation of plant tissue culture medium. *J. Tissue Cult. Bioeng*, 3, 281-286.
- Cursi, D. E., Hoffmann, H. P., Barbosa, G. V. S., Bressiani, J. A., Gazaffi, R., Chapola, R. G., ... & Carneiro, M. S. (2022). History and current status of sugarcane breeding, germplasm development and molecular genetics in Brazil. *Sugar Tech*, 24(1), 112-133.
- Da Silva, A. L. L., Costa, J. D. L., Gollo, A. L., Dos Santos, J. D., Forneck, H. R., Biasi, L. A., ... & Soccol, C. R. (2014). Development of a vinasse culture medium for plant tissue culture. *Pak J Bot*, 46(6), 2195-2202.
- Dash, M., Chiellini, F., Ottenbrite, R. M., & Chiellini, E. (2011). Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Progress in polymer science*, 36(8), 981-1014.
- Daud, N., Taha, R. M., Noor, N. N. M., & Alimon, H. (2011). Provision of low-cost media options for in vitro culture of *Celosia* sp. *African Journal of Biotechnology*, 10(80), 18349- 18355.
- Debergh, PC and Read, PE. (1991). Micropropagation. In *Micropropagation*. (pp.1-13). Springer, Dordrecht.
- Duda-Chodak A, and Tarko T. (2007). Antioxidant Properties of Different Fruit Seeds and Peels. *Acta Scientiarum Polonorum Technologia Alimentaria*. (pp. 29-36).
- George, E. F., Hall, M. A., & De Klerk, G. J. (2008). *Plant propagation by tissue culture* (3rd ed.). Springer.
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*.

John Wiley & Sons.

- Habiba, U., Reza, S., Saha, M. L., Khan, M. R., & Hadiuzzaman, S. (2002). Endogenous bacterial contamination during in vitro culture of table banana: Identification and prevention. *Plant Tissue Cult*, 12(2), 117-124.
- Hamza, T. A., & Alebjo, A. L. (2017). Sugarcane (*Saccharum Officinarum* L) Tissue Culture In Ethiopia: Opportunities For Ethiopia's Sugar Industries. *International Journal Of Scientific & Technology Research* Volume 6, Issue 08. [www.ijstr.org](http://www.ijstr.org),
- Ibrahim, M., Tolera, B., Biruma, M., Bedada, A., Shimelis, D., Aman, J., ... & Negi, T. (2016). Evaluation of tissue culture raised sugarcane planting materials against their donor conventional seed sources as initial source of seed cane at Tendaho Sugar Development Project, North-Eastern Ethiopia. *Journal of Horticulture*, 1-4.
- Idowu, P. E., Ibitoye, D. O., & Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, 8(16).
- Jaafar, F., Awal, A., Abdullah, S., Ismail, F., Sidik, N. J., & Yusuf, N. A. (2015). Bio-potential of fermented fruit waste solutions on in vitro seed germination and regeneration of *Lycium barbarum* and *Aquilaria malaccensis* Lamk. *Malaysian Applied Biology*, 44(3), 127-133.
- Khan, I. A., Dahot, M. U., Seema, N., Yasmeen, S., Bibi, S., Raza, G., and Naqvi, M. H. (2009). Direct regeneration of sugarcane plantlets: a tool to unravel genetic heterogeneity. *Pak. J. Bot*, 41(2), 797-814.
- Khairul Anuar NA, Mohd Shaain UH, Awal A, and Yusuf NA. (2016). Selected Fruits Peel as an Alternative Low Cost Medium Component for In Vitro Micro propagation of *Musa acuminata* cv. Berangan (AAA). Paper presented at regional Conference on Science, Technology and Social Sciences. Copthorne Hotel, Cameron Highlands, Brinchang, Malaysia.
- Kodym A and Zapata-Arias FJ. (2001). Low Cost Alternatives for the Micropropagation of Banana. In *Plant Cell, Tissue and Organ Culture*. 2001 Jul 1:66(1) (pp. 67-71)
- Kurimella, Ravindra. (2016). Re: Why MS media is most frequently used for plant tissue culture?. Retrieved from: <https://www.researchgate.net/post/Why-MS-media-is-most-frequently-used-for-plant-tissue-culture/579a103eeae3915e06e3f38/citation/download>.
- Leifert, C., & Cassells, A. C. (2001). Microbial Hazards in Plant Tissue and Cell Cultures. In *In Vitro Cellular & Developmental Biology. Plant*, Vol. 37, No. 2, pp.133-138.
- Mangla, Y., Khanduri, P., & Gupta, C. K. (2022). *Reproductive Biology of Angiosperms: Concepts and Laboratory Methods*. Cambridge University Press.

- Murashige, T. (1974). Plant propagation through tissue cultures. *Annual review of plant physiology*, 25(1), 135-166.
- Nadirah, M. N. A., Awal, A., Azma, Y. N., & Sulaiman, A. (2019, September). POME: An alternative nutrient source for bio-organic plant tissue culture media. In *IOP Conference Series: Earth and Environmental Science* (Vol. 327, No. 1, p. 012029). IOP Publishing.
- Salokhe, S. (2021). Development of an efficient protocol for production of healthy sugarcane seed cane through Meristem culture. *Journal of Agriculture and Food Research*, 4, 100126.
- Savangikar VA. (2004). Role of Low Cost in Tissue Culture. In *Low Cost Options for Tissue Culture Technology in Developing Countries. Proceedings of a Technical Meeting Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.*(pp. 11-15).
- Singh, J., & Singh, R. (2015). Sugarcane seed production: Indian scenario. *J. Biotechnology. Crop Sci*, 4, 43-55.
- Singh, R. (2003). Tissue culture studies of sugarcane. Thapar Institute of Engineering and Technology, Patiala, India.
- Solomon, S., Swapna, M., Xuan, V. T., & Mon, Y. Y. (2016). Development of sugar industry in ASEAN countries. *Sugar Tech*, 18(6), 559-575.
- Wojtania A, Pulawska J, Gabryszewska E (2005) Identification and elimination of bacterial contaminants from Pelargonium tissue cultures. *J Fruit Ornam Plant Res* 13:101–108
- Yaseen, M., Ahmad, T., Sablok, G., Standardi, A., & Hafiz, I. A. (2013). Role of carbon sources for in vitro plant growth and development. *Molecular biology reports*, 40, 2837-2849.
- Yutaka, T., Y. Tomohiro, M. Toshikazu and O. Takeshi. (1998). Plant regeneration via shoot organogenesis from cotyledons in two wild Cucumis species, *C. figarei* and *C. metuliferus*. *Jap. Agric. Res. Q.*, 32: 281-286.
- Yusuf NA. (2012) Bio-organic Tissue Culture Media for Pitaya's (*Hylocereus polyrhizus*) Shoot Growth (Master-degree thesis). Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia.