REVIEW ARTICLE Agricultural Reviews, Volume 46 Issue 4: 681-685 (August 2025)

Advancements in Sugarcane Tissue Culture: A Review

Vijeta Gupta[1], Sudhir Sharma[1]

10.18805/ag.R-2723

ABSTRACT

Sugarcane (*Saccharum spp.*) is a vital crop with significant economic importance globally, primarily for its sugar and bioenergy production. Tissue culture techniques have emerged as valuable tools for sugarcane improvement, offering rapid multiplication, genetic transformation and germplasm conservation. This review provides a comprehensive overview of recent advancements in sugarcane tissue culture, focusing on protocol optimization, genetic transformation, somaclonal variation and their applications in sugarcane breeding programs. Various factors influencing the success of tissue culture protocols, such as explant selection, culture media composition, growth regulators and environmental conditions are discussed. Furthermore, the paper highlights the challenges and future prospects of sugarcane tissue culture, including the development of stress-tolerant cultivars, enhanced production of bioenergy and the integration of molecular techniques for precise trait manipulation.

Key words: Protocol optimization, Somaclonal variation, Sugarcane, Tissue culture.

Sugarcane (*Saccharum spp*.) is one of the most important crops worldwide, serving as a major source of sugar, ethanol and bioenergy. The conventional methods of sugarcane propagation such as stem cuttings and seed propagation are associated with several limitations, including the transmission of diseases and slow multiplication rates (Lal *et al*., 2015). Tissue culture techniques offer viable alternatives to conventional methods by enabling rapid multiplication, production of disease-free plants, genetic transformation for trait improvement (Shukla and Shukla, 2024), play an important role in the manipulation of plants for improved agronomic performance and also an integral part of molecular approaches to plant improvement (Bijalwan, 2021). Over the past few decades, significant progress has been made in optimizing tissue culture protocols and understanding the underlying cellular and molecular mechanisms governing sugarcane regeneration. This review aims to summarize the recent advancements in sugarcane tissue culture and their implications for sugarcane improvement.

Protocol optimization

Standardization of efficient protocol for the *in vitro* micropropagation is essential to get efficient *in vivo* developed replicates (Prasanna *et al*., 2022). Optimizing tissue culture protocols is essential for achieving high efficiency in sugarcane regeneration. Key factors influencing the success of tissue culture include explant selection, culture media composition, growth regulators and environmental conditions. Explant selection plays a crucial role in determining the regeneration capacity and genetic stability of the derived plants. Commonly used explants in sugarcane tissue culture include shoot apices, nodal segments and meristem tips. The choice of culture media and growth regulators significantly affects the frequency and quality of regeneration. Hormonal balance, particularly auxins and cytokinins, regulates the processes of shoot proliferation, rooting and callus formation.

[1]CCS Haryana Agricultural University, Regional Research Station, Uchani, Karnal-132 001, Haryana, India.

Corresponding Author: Vijeta Gupta, CCS Haryana Agricultural University, Regional Research Station, Uchani, Karnal-132 001, Haryana, India. Email: vijetagupta1994@gmail.com

How to cite this article: Gupta, V. and Sharma, S. (2025). Advancements in Sugarcane Tissue Culture: A Review. *Agricultural Reviews*. **46(4):** 681-685. doi: 10.18805/ag.R-2723.

Submitted: 08-05-2024 **Accepted:** 15-06-2024 **Online:** 21-08-2024

Fine-tuning the concentrations of growth regulators and optimizing the culture conditions are critical for enhancing the regeneration efficiency and minimizing somaclonal variation.

Ullah and Khan (2022) evaluated different auxins [Dichlorophenoxy Acetic acid (2,4-D), Nephthalene Acetic Acid (NAA)], cytokinin [Benzyl Amino Purine (BAP)] either alone or in combination with each other and carbon sources (sucrose, glucose and fructose) with varying concentrations (2, 4 and 6%) for their effect on the tissue culture of sugarcane variety-CP 77/400. For callus induction, auxin 2, 4-D was found to be most effective. High regeneration capacities were achieved using combination of BAP and NAA. Amongst the carbon sources, sucrose was most effective both for callus induction and regeneration with 50.33% callus induction. Maximum callus induction was shown by carbon sources at 4% concentration. Maximum regeneration (72.06%) was observed on shooting media SM-2 supplemented with BAP (2 mg L^{-1}), NAA (0.25 mg L^{-1}) and 6% sucrose. Roots were established on ½ MS having 1.5 mg L^{-1} NAA and regenerated plantlets successfully acclimatized to greenhouse conditions. Alberto da Silva *et al*. (2020) evaluated different methods of initiation, multiplication and rooting by tissue culture for several varieties of sugarcane (*Saccharum officinarum* L.) and sugarcane-related species such as *Erianthus spp*.,

Miscanthus spp. and *Sorghum spp*. × sugarcane hybrids. For all the micro propagated genotypes, liquid or semisolid Murashige and Skoog (MS) medium was used which was further supplemented with 0.1-0.2 mg L^{-1} BAP, 0.1 mg L^{-1} kinetin, 0-0.1 mg L^{-1} NAA and 0-0.2 µg L^{-1} giberellic acid. These cultures produced shoots between 4 and 8 weeks after initiation whereas shoot regeneration from leaf rolls or immature inflorescences was observed as early as 4 weeks after initiation. Higher combined multiplication rate was observed when these were grown under four or five LED lamps or under fluorescent lights in a growth chamber. Faster and better rooting was observed in all of the genotypes tested by addition of 2 mg L^{-1} NAA. By this, a protocol was standardized for different energy cane clones that were recommended for their biomass production and cell wall composition.

An efficient protocol for meristem culture of sugarcane was developed by Salokhe (2021). The potting mix for the survival of meristem cultured plantlets in a greenhouse was standardized. Multiple shoots were observed on Murashige and Skoog's (MS) medium supplemented with BAP 0.2 mg/l and Kinetin 0.1 mg/l while best rooting was observed on MS media supplemented with IAA 1 mg/l and NAA 1 mg/l. Soil mixture containing [Soil + Vermiculite + Sand in 4: 1: 1 proportion (by volume)] + Vermicompost @ 25% by volume of medium + NPK is best suited for hardening tissue cultured plants in a greenhouse. Setts obtained from tissue cultured plants planted at 15 cm spacing gave higher yield.

Iqbal *et al*. (2023) evaluated the TCL technique for somatic embryo production and plantlet regeneration of sugarcane plant. Explants from young leaf whorls (tTCLs) were cultivated in MS culture medium supplemented with 3% sucrose and different concentrations of 2, 4-dichlorophenoxyacetic acid (2,4-D) and incubated under dark condition at 25±2°C for callus formation. The explants cultured on 2.0 mg/l 2, 4-D produced good-quality callus as compared to other concentrations. The calli when further evaluated for regeneration, maximum number of shoots per callus mass (25±1.6) with highest shoot length of 6±0.5 cm were regenerated using MS medium supplemented with 2.0 mg/l BAP (Benzyl aminopurine) and 0.2 mg/l NAA (naphthalene acetic acid) whereas highest number of root emergence (28.2±0.8) and maximum root length (2.8±0.09 cm) were achieved on MS medium supplemented with 4.0 mg/l NAA. The multiplication of sugarcane (*Saccharum spp*.) variety LAICA 04-809 by direct organogenesis was evaluated by Orozco-Ortiz *et al*. (2023) (1) using a semisolid medium and (2) three temporary immersion systems with liquid medium (RITA®, BIT® and SETIS™). In addition, three planting densities (10, 30 and 50 mL of medium per explant) were studied for each system as initial inoculum. In all three temporary immersion systems, photomixotrophic metabolism was identified. In addition, at density of 30 mL medium/explant, the BIT® bioreactor generated the highest number of quality shoots (425.33±24.58). No hyperhydricity was observed in the explants. The results of this study suggest that the combination of the BIT® system with the 50 mL volume of medium/explant represents the best conditions for mass propagation of sugarcane shoots after four weeks of cultivation.

Maruprolu *et al*. (2022) optimized *in vitro* callus induction protocol and whole plant regeneration for developing red rot resistant somaclones in commercial sugarcane variety COC 671 using immature leaf sheaths as explants. In the whole experiment, the best medium for callus induction was MS medium supplemented with 2, 4-D (2.0 mg/l) recorded callus induction frequency of 100 per cent in eight days. The compact and friable embryogenic calli were regenerated in MS medium supplemented with KIN (1.0 mg/l) producing 21 shoots in 10 days with a shoot length of 8.69 cm. The well grown shoots were rooted in MS medium supplemented with NAA at 3.0 mg/L with 9 roots in 9 days which had an average root length of 3.63 cm. The regenerated plantlets were successfully acclimated under field conditions. This protocol would further be used for developing red rot resistant somaclonal variants in sugarcane.

Baday (2020) showed 1.5 mg/L 2,4-D regulator was more potent in the induction of the callus and callus subsequent growth. The effected interactions of auxin and cytokinin regulators were not significant with respect to the formation of callus. The best regeneration response was achieved by using growth regulators. In Co8371, best induction of the shoot response was found on MS medium with concentration of 1.0 mg/L BAP compared to Co85004. MS medium using of 0.5 mg/L BAP and 0.25 mg/L Kinetin regulator exhibited best organogenesis. Aisyah *et al*. (2022) determined the appropriate pH and concentration of sucrose in the liquid culture of embryogenic cells enabling to produce good quality virus and disease free seedlings and can generate a large quantity in a short time. The results showed that pH 6.5+3% sucrose (A3B1) was the best combination treatment of pH and sucrose for sugarcane callus proliferation in liquid culture media that produced the highest number of callus with the average 29 callus on the first week, 833 callus on the second week and the third week 433 callus and the color did not easily turn to brown.

Genetic transformation

Genetic transformation holds immense potential for introducing desirable traits, such as disease resistance, abiotic stress tolerance and improved biomass yield into sugarcane cultivars. Agrobacterium-mediated transformation and particle bombardment are the two primary methods used for introducing foreign genes into sugarcane cells. Various factors, including vector design, selection markers, tissue culture conditions and regeneration protocols influence the success of genetic transformation. Despite significant progress in sugarcane transformation, several challenges remain including low transformation efficiency,

genotype dependency and transgene silencing. Future efforts should focus on developing efficient transformation protocols, enhancing transgene expression and improving the selection and regeneration of transformed plants.

Genetic transformation has the potential to improve economically important properties in sugarcane as well as diversify sugarcane beyond traditional applications, such as sucrose production. Traits such as herbicide, disease and insect resistance, improved tolerance to cold, salt and drought and accumulation of sugar and biomass have been some of the areas of interest as far as the application of transgenic sugarcane is concerned. Since the early 1990's, different genetic transformation systems have been successfully developed in sugarcane, including electroporation, *Agrobacterium tumefaciens* and biobalistics (Budeguer *et al*., 2021). However, genetic transformation of sugarcane is a very laborious process, which relies heavily on intensive and sophisticated tissue culture and plant generation procedures that must be optimized for each new genotype to be transformed. Therefore, it remains a great technical challenge to develop an efficient transformation protocol for any sugarcane variety that has not been previously transformed. Tripathy and Ithape 2020 reported efficient *in vitro* culture protocol for genetic transformation in a popular sugarcane cv. Sabita (CoOR 03151) of Odisha. 3.0 mg/l 2, 4-D resulted in highest callus induction frequency (87.8%) with white, friable and nodular embryogenic calli suitable for plantlet regeneration. 2 mg/l BAP resulted in moderately higher number of shoots and higher percentage of plant survival. A combination of Paclobutrazol (0.05 mg/l) with BAP (2 mg/l) and Thidiazuron (0.05 mg/l) revealed profuse multiple shoots and higher percentage of survival during follow-up plant establishment. Among various hormone recipes, MS medium with 3.0 mg/l NAA resulted in excellent rhizogenesis response (88.0%) with more or less normal rooting within 2 weeks. The above efficient and highly reproducible *in vitro* culture system can be amenable for genetic transformation in this crop.

Khan *et al.* (2021) optimized callus induction and transformation of the sugarcane variety CP-77-400 with the promoter region of the OsC_3H_{52} gene to analyze its regulatory function under drought and salt stress. Calli were induced using different callus induction media (CIM). The promoter region of the OsC_3H_{52} gene was cloned into two different expression vectors *i.e.* pBI221 and pGreenII0129, which were subsequently transformed to sugarcane calli through agrobacterium and biolistic transformation methods. Among various callus induction media, 5 mg/l 2,4-D + 10% coconut water showed high callus induction (93%). Biolistic transformation using recombinant pGreenII0129 at 8.5 µg/µl concentration gave 28% transformation while the agrobacterium mediated transformation using recombinant pBI221 plasmid gave 13% transformation efficiency. Acetosyringone used in agrobacterium mediated transformation showed 13% of transformation efficiency.

Somaclonal variation

Somaclonal variation refers to the genetic and phenotypic changes observed in plants regenerated through tissue culture. Although tissue culture offers a rapid means of clonal propagation, it is also associated with the accumulation of somatic variations (Kumari and Verma, 2001). Somaclonal variation can lead to phenotypic abnormalities, such as altered growth patterns, morphology and stress responses, which may affect the agronomic performance and stability of regenerated plants. Understanding the mechanisms underlying somaclonal variation is crucial for minimizing its adverse effects and ensuring the genetic stability of regenerated sugarcane plants. Molecular markers, cytogenetic analysis and genomic sequencing techniques can be employed to characterize somaclonal variants and assess their potential impact on sugarcane breeding programs.

Abo-Elwafa *et al*. (2021) found significant genetic differences among the somaclones and also their donor. The highest values of GCV and PCV were estimated for cane yield (18.11 and 18.53%) and sugar yield (17.65 and 17.76%) over two ratoon crops, respectively. The heritability in agronomic traits ranged from 50.39 (stalk diameter) to 98.46% (cane yield) and in technological traits varied from 73.02 (purity) to 98.78% (sugar yield) over the both ratoon crops. Somaclones no. 7 and 8 surpassed the donor in stalk height, stalk weight, stalk number/fed and cane yield, respectively while the somaclone no. 4 surpassed the donor in sugar yield, brix, sucrose%, purity%, pol% and sugar recovery%. These results exhibited genetic variability among the obtained somaclones (somaclonal variation), which could be used to invent new superior somaclones and overcome the accomplishments of traditional cane breeding.

Rahman *et al*. (2022) evaluated the feasibility of incorporating five exotic non-flowering sugarcane genotypes into a traditional breeding programme through somaclonal variations. Explants from the leaf sheath of donor plant were grown on modified MS media to generate genetic diversity. Within six to twelve days of cultivation, callus formation occurred. For shoot regeneration, MS medium supplemented with BA + NAA was utilized, while NAA was used for roots from micro shoots. 834 somaclones were acclimatised and planted in the field for evaluation, where 520 somaclones survived in the G_0 generation. On the basis of three years of field study under three generations, three somaclones were chosen for their profuse flowering and better agronomic traits which were found to be significantly different from the mother plants.

Applications in breeding programs

Sugarcane tissue culture has diverse applications in breeding programs, including rapid multiplication of elite clones, germplasm conservation, genetic transformation and trait introgression. Tissue culture enables the efficient propagation of disease-free planting material, there by

reducing the risk of pathogen transmission and enhancing crop productivity. Genetic transformation offers opportunities for introducing novel traits, such as resistance to biotic and abiotic stresses, improved sucrose content and enhanced biomass yield. Furthermore, tissue culture facilitates the preservation and utilization of genetic resources through cryopreservation and *in vitro* conservation techniques. Integrating tissue culture with conventional breeding approaches can accelerate the development of improved sugarcane cultivars with enhanced agronomic traits and resilience to environmental challenges.

Jamil *et al*. (2022) produced huge amounts of authentically grown, disease-free plant material quickly using plant tissue culture. Plant tissue culture can also be used to quickly reproduce recently released varieties with crucial agronomic characteristics. For this purpose, sugarcane callus culture was collected from the inner soft leaf sheath to increase genetic diversity. Ten different concentrations *viz*., 1.5, 1.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mgL^{-1} of 2, 4-dichlorophenoxy acetic acid in MS medium were used for callus development, along with 0 mgL^{-1} control was used. Different combinations and concentrations of BAP+ Kin were used for regeneration of shoots and auxin. IBA with 6 different concentrations (0. 0.3, 0.5, 0.7, 1.5 and 1.8 mgL^{-1}) were used for rooting of the shoots.

Garcia Merino *et al*. (2023) developed plants through plant tissue culture (PTC) in a relatively short time and limited space. They evaluated the bio-stimulant effect of sodium silicate (Na_2SiO_3) on *in vitro* propagation of sugarcane (CP 72-2086 and Mex 69-290). Plant tips were established for each variety. The shoots obtained were then cultivated in an MS medium supplemented with different concentrations of Na_2SiO_3 (0, 2.5, 5.0 and 10.0 mg L^{-1}). Morphometric analysis, quantification of photosynthetic pigments and stomatal index measurements were performed after 30 days. The CP 72-2086 variety had a 0.5 number of shoots, 6.36 leaves and 5.8 roots more compared to the control. In contrast, the Mex 69-290 variety had more sizable proportion of shoot length (1.42 times), root length (2.20 times) and dry weight (1.33 times) compared to the control. While the addition of Na_2SiO_3 in both varieties had a bio-stimulant effect on the content of photosynthetic pigments thus Na_2SiO_3 can be used as a bio-stimulant agent during the commercial micropropagation of sugarcane.

To understand the physio-biochemical response of sugarcane plants to salt (NaCl) stress, Singh *et al.* (2023) analyzed antioxidant enzymatic activity in callus cultures of tolerant (BO 91) and susceptible (Co 0239) cultivars under *in vitro* conditions. Shoot tip explants were excised from the tolerant and susceptible plants and exposed to media without (2.2 ECw; control) and with (4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 ECw) levels of salt for 90, 120 and 150 days. Significant effect of different salt concentrations was evident on catalase (CAT), peroxidase (POX), nitrate reductase (NR) and indole-acetic acid (IAA) oxidase activities. Under salinity stress conditions overall increasing trends of CAT and POX activity was found to be higher in callus cultures derived from tolerant cultivar compared to susceptible cultivar, indicates that antioxidant enzymes enhance cell protection against oxidative stress.

Prasetyo *et al*. (2024) provided large quantities of seedlings through scaling-up *in vitro* propagation method using bioreactors, such as Temporary Immersion Bioreactor (TIB). The sugarcane varieties used for the study were the rainfed sugarcane varieties (PSKA 942, PS 094) and the irrigated sugarcane varieties (PS 091, PS 881). The *in vitro* propagation stages were explants preparation and sterilization, callus initiation, callus proliferation and regeneration in TIB, shoot maturation and acclimatization. Results showed that all sugarcane varieties have been successfully induced to form callus ranged 11.6- 33.5%, proliferate and regenerate in TIB. However, in general, the rainfed sugarcane varieties showed better response, growth and regeneration rate than the irrigated sugarcane varieties.

Challenges and future prospects

Despite the significant advancements in sugarcane tissue culture, several challenges persist, hindering its widespread adoption and impact on sugarcane improvement. These challenges include genotype dependency, genotype recalcitrance to regeneration, somaclonal variation, low transformation efficiency and regulatory constraints associated with transgenic crops. Overcoming these challenges requires interdisciplinary approaches combining plant physiology, molecular biology genomics and biotechnology. Future research directions should focus on developing robust tissue culture protocols applicable to diverse sugarcane genotypes, enhancing the efficiency and precision of genetic transformation, addressing concerns related to transgene stability and biosafety and leveraging emerging technologies such as CRISPR/Cas9 for targeted genome editing. Furthermore, efforts should be directed towards enhancing the resilience of sugarcane cultivars to biotic and abiotic stresses, improving the sustainability and competitiveness of sugarcane-based bioenergy production and promoting collaborative initiatives between academia, industry and regulatory agencies to facilitate the translation of research findings into practical applications.

CONCLUSION

Sugarcane tissue culture represents a powerful tool for sugarcane improvement, offering opportunities for rapid multiplication, genetic transformation and germplasm conservation. Recent advancements in tissue culture protocols, genetic transformation techniques and understanding of somaclonal variation have enhanced our capacity to manipulate sugarcane genomes and develop improved cultivars with enhanced agronomic traits and resilience to environmental stresses. Despite the

remaining challenges, the continued integration of tissue culture with conventional breeding approaches and emerging biotechnologies holds promise for addressing the evolving needs of the sugarcane industry and ensuring food security, sustainability and economic prosperity in sugarcane-producing regions worldwide.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Abo-Elwafa, A., Bakheit, B.R., El-Taib, A.M. and Noby, N.Y. (2021). Evaluation of some new somaclones of sugarcane for yield and quality. *SVU-International Journal of Agricultural Sciences*. **3(1):** 129-139.

Aisyah, N.N., Widuri, L.I., Alfina, F.N. and Dewanti, P. (2022). The effect of pH and sucrose on the embryogenic cells growth of sugar cane (*Saccharum officinarum*) in liquid culture. *Tropical and Subtropical Agroecosystems*. **25(1)**. doi: 10.5 6369/tsaes.3897.

Baday, S.J. (2020). Sugarcane of Rapid Multiplication by Callogenesis. *In Journal of Physics: Conference Series*. **1660(1):** 0120 05. doi: 10.1088/1742-6596/1660/1/012005.

Bijalwan, P. (2021). Plant tissue culture-A new tool for vegetable improvement (Indian scenario): A review. *Agricultural Reviews*. **42(2):** 235-239. doi: 10.18805/ag.R-1979.

Budeguer, F., Enrique, R., Perera, M.F., Racedo, J., Castagnaro, A.P., Noguera, A.S. and Welin, B. (2021). Genetic transformation of sugarcane, current status and future prospects. *Frontiers in Plant Science*. **12:** 768609. doi: 10.3389/ fpls.2021.768609.

da Silva, J.A., Solis-Gracia, N., Jifon, J., Souza, S.C. and Mandadi, K.K. (2020). Use of bioreactors for large-scale multiplication of sugarcane (*Saccharum spp.*), energy cane (*Saccharum spp.*) and related species. *In vitro Cellular and Developmental Biology-Plant*. **56(3):** 366-376.

García-Merino, G.F., Ramírez-Mosqueda, M.A., Aguilar-Rivera, N., Hidalgo-Contreras, J.V., Burchi, G. and Leyva-Ovalle, O.R. (2023). Increase of the morpho-physiological responses of sugarcane plants *in vitro* (*Saccharum spp*. hybrids) using sodium silicate. *South African Journal of Botany*. **162:** 193-200.

Iqbal, A., Khan, R.S., Khan, M.A., Gul, K., Aizaz, M., Usman, M. and Arif, M. (2023). Efficient regeneration in sugarcane using thin cell layer (TCL) culture system. *Sugar Tech*. **25(1):** 168-176.

Jamil, M.W., Ali, S.A., Sabir, W., Nasir, M., Irum, A., Khan, M.E., Naveed, M., Ullah, R., Nawaz, M.S., Saleem, S. and Khan, N.H. (2022). Optimization of protocol for callus formation and shoot development in sugarcane (*Saccharum officinarum* L.) cultivar CPF-248. *Journal of Global Innovations in Agricultural Sciences.* **10:** 229-235.

Khan, M.S., Ahmad, W., Ahmad, M.Z., Ullah, M. and Jan, A. (2021). Transformation of the osc 3 h 52 gene promoter in tissue culture of sugarcane. *JAPS: Journal of Animal and Plant Sciences*. **31(1)**. doi: 10.36899/JAPS.2021.1.0203.

Kumari, R. and Verma, D.K. (2001). Development of micro propagation protocol for sugarcane (*Saccharum officinaruml*)- A review. *Agricultural Reviews*. **22(2):** 87-94.

Lal, M., Tiwari, A.K., Gupta, G.N. and Kavita. (2015). Commercial scale micropropagation of sugarcane: Constraints and remedies. *Sugar Tech*. **17(4):** 339-347.

Maruprolu, S., Geetha, S., Gnanam, R., Viswanathan, R., Binodh, A.K. and Sudagar, R. (2022). Optimization of protocol for callus induction and whole plant regeneration for developing somaclonal variants in sugarcane cv. COC 671. *Electronic Journal of Plant Breeding*. **13(2):** 388-398.

Orozco-Ortiz, C., Sánchez, L., Araya-Mattey, J., Vargas-Solórzano, I. and Araya-Valverde, E. (2023). BIT® bioreactor increases *in vitro* multiplication of quality shoots in sugarcane (*Saccharum spp*. variety LAICA 04-809). *Plant Cell, Tissue and Organ Culture (PCTOC)*. **152(1):** 115-128.

Prasanna, S.U., Kumar, S.R., Nayaka, S.S., Ajith, S. and Krishna, V. (2022). Supplementation of urea as the nitrate source of ms medium and high-frequency regeneration of plantlets of *Musa paradisiaca* cv. malnad rasbale. *Indian Journal of Agricultural Research*. **56(6):** 705-711. doi: 10.18805/ IJARe.A-5970.

Prasetyo, M.E.R.B., Saptari, R.T., Sinta, M.M., Riyadi, I., Lindawati, S. and Sumaryono, S. (2024). *In vitro* propagation of some elite varieties of Indonesian sugarcane (*Saccharum officinarum* L.) using temporary immersion bioreactor. *In AIP Conference Proceedings*. **3080(1)**. https://doi.o rg/10.1063/5.0199110.

Rahman, A.M.S., Alam, R., Afrin, H.M. and Ahmed, M.M. (2022). Somaclonal variation-A gateway for varietal improvement of non-flowering sugarcane germplasms. *Bangladesh Journal*. **42:** 45-49.

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. https://doi.org/10.1016/j.jafr.2021.100126.

Shukla, S. and Shukla, S.K. (2024). Micropropagation for Crop Improvement and It's Commercialization Potential. In The Potential of Microbes for a Circular Economy. *Academic Press*. pp. 271-287.

Singh, R.B., Rao, V.P. and Sengar, R.S. (2023). Study of salinity induced oxidative stress and antioxidant responses in callus cultures of sugarcane. *Ecological Genetics and Genomics*. **26:** 100164. https://doi.org/10.1016/j.egg.2023. 100164.

Tripathy, S.K. and Ithape, D.M. (2020). High-throughput *in vitro* culture system targeting genetic transformation in sugarcane. *Journal of Crop Science and Biotechnology*. **23(4):** 325-335.

Ullah, M. and Khan, M.S. (2022). Effects of carbon sources and growth regulators on the tissue culture of sugarcane. *Sarhad Journal of Agriculture*. **38(1):** 312-321.