

Documents

Zainuddin, Z.^{a b}, Urbi, Z.^c, Halim, N.M.A.^d

In vitro Regeneration and ISSR-Based Genetic Fidelity Evaluation of *Stevia rebaudiana*
(2025) *Malaysian Applied Biology*, 54 (1), pp. 76-86.

DOI: 10.55230/mabjournal.v54i1.3192

^a Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Pahang, Kuantan, 25200, Malaysia

^b Plant Productivity and Sustainable Resource Unit, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Pahang, Kuantan, 25200, Malaysia

^c Department of Industrial Biotechnology, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Pahang, Kuantan, 26300, Malaysia

^d Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Pahang, Kuantan, 25200, Malaysia

Abstract

Steviol glycoside, which is a natural sweetener extracted from *Stevia rebaudiana*, is globally recognized. For consumers, this compound is widely utilized by diabetic patients and demonstrates numerous therapeutic effects. However, the escalating demand for this natural sweetener and medicinal herb impacts the availability of stevia. Conventional propagation methods, such as seed and stem cutting, frequently result in low germination rates. To address these limitations, the present research explores the potential of in vitro clonal propagation to ensure a consistent supply of planting material. Therefore, the objective of this study was to develop an efficient protocol for tissue culture of *S. rebaudiana* accession MS007. The highest regeneration frequency (85.19% & 86.67% for shoot tips & nodes, respectively) and maximum shoot number (14.30 & 12.77 shoots/explant, respectively) were observed on Murashige and Skoog (MS) medium supplemented with 1.0 mg/L 6-benzylaminopurine (BAP). Half-strength MS medium supplemented with 0.5 mg/L indole-3-butyric acid (IBA) was the optimal medium for rooting, exhibiting the highest rooting percentage, root number, and root length. Subsequently, the plantlets were acclimatised in plastic cups containing peat moss, and it was observed that 86.67% of plantlets survived when transplanted to the field. The outcome of inter-simple sequence repeat (ISSR) analysis using 38 ISSR primers confirmed the genetic fidelity between the in vitro regenerated plants and the mother plant. This study successfully developed an in vitro propagation technique for stevia to produce true-to-type clonal plants. The obtained results can be used to mass-produce stevia accession MS007 to meet market demand. © 2025 Malaysian Society of Applied Biology.

Author Keywords

Direct organogenesis; genetic fidelity; in vitro; ISSR markers; stevia

References

- Abdel-Aaal, R.A., Abdel-Rahman, M.S., Al Baypumi, S., Ali, L.A.
Effect of stevia aqueous extract on the antidiabetic activity of saxagliptin in diabetic rats
(2021) *Journal of Ethnopharmacology*, 265, p. 113188.
- Abdul Rashid, R., Mohamad, A., Awang, M.R., Mutaat, H.H., Mohamad, S.A., Abu Hasan, A., Maskom, M.M., Mohd Nahar, S.K.
Isolation and optimization of inter-simple sequence repeat (ISSR) technique for *Pleurotus sajor caju* towards environmental study in support of the country's nuclear power programme
(2013) *Jurnal Sains Nuklear Malaysia*, 25 (1), pp. 1-8.
- Ahmad, N., Anis, M.
Rapid clonal multiplication of a woody tree, *Vitex negundo* L. through axillary shoots proliferation
(2007) *Agroforestry Systems*, 71, pp. 195-200.
- Ahmed, M.E.A.E.
In vitro propagation for conservation and genetic fidelity of the near threatened *Dimocarpus longan* plant
(2022) *Journal of Genetic Engineering and Biotechnology*, 20, p. 130.

- Ajami, M., Seyfi, M., Hosseini, F.A.P., Naseri, P., Velayati, A., Mahmoudnia, F., Zahedirad, M., Hajifaraji, M.
Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: A randomized controlled trial
(2020) *Avicenna Journal of Phytomedicine*, 10 (2), pp. 118-127.
- Ali, H., Musa, I.F., Abu Bakar, N.A., Karsani, S.A., Yaacob, J.S.
In vitro regeneration and ISSR-based genetic fidelity analysis of *Ortosiphon stamineus* Benth
(2019) *Agronomy*, 9 (12), p. 778.
- Altaher, A.G.A., Yusof, Z.N.B., Mahmood, M., Shaharuddin, N.A.
High-frequency induction of multiple shoots and plant regeneration from cotyledonary node explants of Tongkat Ali (*Eurycoma longifolia*)
(2020) *Applied Ecology and Environmental Research*, 18 (5), pp. 6321-6333.
- Anbazhagan, M., Kalpana, M., Rajendran, R., Natarajan, V., Dhanave, D.
In vitro production of *Stevia rebaudiana* Berton
(2010) *Emirates Journal of Food and Agriculture*, 22 (3), pp. 216-222.
- Asande., L.K., Ombori, O., Nyaboga, E.N., Oduor, R.O.
Efficient shoot organogenesis using leaf disc and nodal explants of passion fruit (*Passiflora edulis* Sims) and genetic fidelity assessment using sequence-related amplified polymorphism (SRAP) markers
(2020) *International Journal of Agronomy*, 2020, p. 3205710.
- Bhojwani, S.S., Dantu, P.K.
(2013) *Plant Tissue Culture: An Introductory Text*, Springer, New Delhi
- Borsai, O., Hârta, M., Szabo, K., Kelemen, C.D., Andrean, F.A., Codrea, M.M., Clapa, D.
Evaluation of genetic fidelity of in vitro-propagated blackberry plants using RAPD and SRAP molecular markers
(2020) *Horticultural Science (Prague)*, 47, pp. 21-27.
- Castañeda-Saucedo, M.C., Tapia-Campos, E., del Pilar Ramírez-Anaya, J., Beltrán, J.
Growth and development of *Stevia* cuttings during propagation with hormones in different months of the year
(2020) *Plants*, 9 (3), p. 294.
- Ceole, L.F., Companhoni, M.V.P., Lopes, S.M.S., de Oliveira, A.J.B., Gonçalves, R.A.C., Filho, B.P.D., Nakamura, C.V., Ueda-Nakamura, T.
Anti-herpes activity of polysaccharide fractions from *Stevia rebaudiana* leaves
(2020) *Natural Product Research*, 34 (11), pp. 1558-1562.
- Chirumamilla, P., Gopu, C., Jogam, P., Taduri, S.
Highly efficient rapid micropropagation of genetic fidelity of regenerants by ISSR and SCoT markers of *Solanum khasianum* Clarke
(2021) *Plant Cell, Tissue and Organ Culture*, 144, pp. 397-407.
- Deonas, A.N., Zorzenon, M.R.T., de Oliveira, J.P., da Silva, D.V., Cabeça, C.L.S., Mori, M.A., da Costa, S.C., de França, E.J.G.
Stevia Rebaudiana* fraction presents broad-spectrum antibacterial action and antibiofilm action for *Staphylococcus aureus
(2022) *Brazilian Journal of Development*, 8 (7), pp. 49529-49541.
- Dönmez, D., Erol, H., Biçen, B., Şimşek, Ö., Kaçar Aka, Y.
The effects of different strength of MS media on in vitro propagation and rooting of *Spathiphyllum*

(2022) *Anadolu Journal of Agricultural Sciences*, 37 (3), pp. 583-592.
M

- Doyle, J.J., Doyle, J.L.
A rapid DNA isolation procedure for small quantities of fresh leaf tissue
(1987) *Phytochemical Bulletin*, 19, pp. 11-15.
- Ferris, E.
The benefits and applications of plant tissue culture: A revolutionary method of plant propagation
(2023) *Journal of Plant Biotechnology and Microbiology*, 6 (1), p. 133.
- Ghose, A.K., Abdullah, S.N.A., Md Hatta, M.A., Megat Wahab, P.E.
In vitro regeneration of Stevia (Stevia rebaudiana Bertoni) and evaluation of the impacts of growth media nutrients on the biosynthesis of steviol glycosides (SGs)
(2022) *Agronomy*, 12, p. 1957.
- Hadia, A.H., Badawy, O.M., Afaf, M. H.
Genetic relationships among some Stevia (Stevia rebaudiana Bertoni) accessions based on ISSR analysis
(2008) *Research Journal of Cell and Molecular Biology*, 2 (1), pp. 1-5.
- Hassanen, S.A., Khalil, R.
Biotechnological studies for improving of stevia (Stevia rebaudiana Bertoni) in vitro plantlets
(2013) *Middle-East Journal of Scientific Research*, 14 (1), pp. 93-106.
-
- Jadid, N., Anggaraeni, S., Nur Ramadani, M.R., Arieny, M., Mas'ud, F.
In vitro propagation of Indonesian stevia (Stevia rebaudiana) genotype using axenic nodal segments
(2024) *BMC Research Notes*, 17, p. 45.
- Joshi, P.R., Pandey, S., Maharjan, L., Pant, B.
Micropropagation and assessment of genetic stability of Dendrobium transparens Wall. Ex Lindl. using RAPD and ISSR markers
(2023) *Frontiers in Conservation Science*, 3, p. 1083933.
- Kim, N.C., Kinghorn, A.D.
Highly sweet compounds of plant origin
(2002) *Archives of Pharmacological Research*, 25 (6), pp. 725-746.
- Ludwig-Müller, J.
Indole-3-butyric acid in plant growth and development
(2000) *Plant Growth Regulation*, 32 (2-3), pp. 219-230.
- Madan, S., Ahmad, S., Singh, G.N., Kohli, K., Kumar, Y., Singh, R., Garg, M.
Stevia rebaudiana (Bert.) Bertoni-A review
(2010) *Indian Journal of Natural Products and Resources*, 1, pp. 267-286.
- Mahardhini, A.D., Anwar, S., Karno
BAP and Kinetin application for in vitro bud induction of vanilla (Vanilla planifolia Andrews.) from stem node explants in the initiation stage
(2022) *IOP Conference Series: Earth and Environmental Science*, 1246, p. 012030.
- Majumder, S., Rahman, M.M.
Micropropagation of Stevia rebaudiana Bertoni. through direct and indirect organogenesis
(2016) *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 3 (3), pp. 47-56.

- Mattick, C. S.
Cellular agriculture: The coming revolution in food production
(2018) *Bulletin of the Atomic Scientists*, 74 (1), pp. 32-35.
- Myint, K.Z., Zhou, Z., Shi, Q., Chen, J., Dong, X., Xia, Y.
Stevia polyphenols, their antimicrobial and anti-inflammatory properties, and inhibitory effect on digestive enzymes
(2023) *Molecules*, 28, p. 7572.
- Nower, A.A.
In vitro propagation and synthetic seeds production: an efficient method for *Stevia rebaudiana* Bertoni
(2014) *Sugar Technology*, 16 (1), pp. 100-108.
- Othman, H.S., Osman, M., Zainuddin, Z.
Genetic variabilities of *Stevia rebaudiana* Bertoni cultivated in Malaysia as revealed by morphological, chemical and molecular characteristics
(2018) *AGRIVITA Journal of Agricultural Science*, 40 (2), pp. 267-283.
- Papaefthimiou, M., Kontou, P.I., Bagos, P.G., Braliou, G.G.
Antioxidant activity of leaf extracts from *Stevia rebaudiana* Bertoni exerts attenuating effect on diseased experimental rats: A Systematic review and meta-analysis
(2023) *Nutrients*, 15, p. 3325.
- Patel, R.M., Shah, R.R.
Regeneration of *Stevia* plant through callus culture
(2009) *Indian Journal of Pharmaceutical Sciences*, pp. 46-50.
- Pradhan, S., Paudel, Y.P., Qin, W., Pant, B.
Genetic fidelity assessment of wild and tissue culture regenerants of threatened orchid, *Cymbidium aloifolium* using molecular markers
(2023) *Plant Gene*, 34, p. 100418.
- Qi, F., Zhang, F.
Cell cycle regulation in the plant response to stress
(2020) *Frontiers in Plant Science*, 10, p. 1765.
- Razali, A., Samsulrizal, N.H., Zainuddin, Z.
Identification of genes involved in flowering in *Stevia rebaudiana* using expressed sequence tags (ESTs)
(2020) *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 28 (2), pp. 105-112.
- Rolnik, A., Olas, B.
The plants of the Asteraceae family as agents in the protection of human health
(2021) *International Journal of Molecular Sciences*, 22, p. 3009.
- Royani, J.I., Chotimah, S., Utami, R.N., Fatma, W.S., Susiyanti, Fatmawaty, A.P.
Effect of benzilaminopurine and kinetin for shoot multiplication of *Indigofera* (*Indigofera zollingeriana* Miq.) by in vitro culture
(2021) *IOP Conf. Series: Earth and Environmental Science*, 637, p. 012053.
- Saad, A., Khan, F.A., Hayee, A., Nazir, M.S.
A review on potential toxicity of artificial sweeteners vs safety of *Stevia*: A natural bio-sweetner
(2014) *Journal of Biology, Agriculture and Healthcare*, 4 (15), pp. 137-147.
- Saadh, M.J., Thiagarajan, S., Cotrina-Aliaga, J.C., Suleiman, M.I., Fahdil, A.A., Ahjel, S., Soleimanian, A., Harismah, K.

- Effects of Stevia on hypertension of metabolic syndrome: A systematic review**
(2023) *Journal of Medicinal and Chemical Sciences*, 6, pp. 2407-2418.
- Samsulrizal, N.H., Zainuddin, Z., Noh, A.L., Sundram, T.C.
A review of approaches in steviol glycosides synthesis
(2019) *International Journal of Life Sciences and Biotechnology*, 2 (3), pp. 145-157.
 - Saraiva, A., Carracosa, C., Raheem, D., Ramos, F., Raposo, A.
Natural sweeteners: The relevance of food naturalness for consumers, food security aspects, sustainability and health impacts
(2020) *International Journal of Environmental Research and Public Health*, 17 (17), p. 6285.
 - Seo, E.H., Kim, H., Kwon, O.
Association between total sugar intake and metabolic syndrome in middle-aged Korean men and women
(2019) *Nutrients*, 11 (9), p. 2042.
 - Simlat, M., Szewczyk, A., Ptak, A.
Melatonin promotes seed germination under salinity and enhances the biosynthesis of steviol glycosides in *Stevia rebaudiana* Bertoni leaves
(2020) *PLoS One*, 15 (3), p. e0230755.
 - Sugla, T., Purkayastha, J., Singh, S.K., Solleti, S.K., Sahoo, L.
Micropropagation of *Pongamia pinnata* through enhanced axillary branching
(2007) *Vitro Cellular and Developmental Biology-Plant*, 43 (5), pp. 409-414.
 - Tegen, H., Mohammed, W.
The role of plant tissue culture to supply disease free planting materials of major horticultural crops in Ethiopia
(2016) *Journal of Biology, Agriculture and Healthcare*, 6 (1), pp. 122-129.
 - Thakur, S., Shruti, Hashmi, S., Mishra, S., Ekka, S.K., Kushwaha, A., Kujur, R.
A review on plant tissue culture
(2024) *Asian Journal of Biology*, 20 (2), pp. 14-18.
 - Thiyagarajan, M., Perumal, V.
Large scale in vitro propagation of *Stevia rebaudiana* (Bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb
(2012) *Industrial Crops and Products*, 37 (1), pp. 111-117.
 - Ting, W.T., Liew, F.C., Lim, W.Y., Subramaniam, S., Chew, B.L.
Shoot induction from axillary shoot tip explants of fig (*Ficus carica*) cv. Japanese BTM6
(2018) *Tropical Life Sciences Research*, 29 (2), pp. 165-174.
 - Uddin, M.S., Chowdhury, M.S.H., Khan, M.M.M.H., Uddin, M.B., Ahmed, R., Baten, M.
In vitro propagation of *Stevia rebaudiana* Bert in Bangladesh
(2006) *African Journal of Biotechnology*, 5 (13), pp. 1238-1240.
 - van Staden, J., Zazimalova, E., George, E.F.
Plant growth regulators II: cytokinins, their analogues and antagonists
(2008) *Plant Propagation by Tissue Culture*,
E. F. George, M.A. Hall and G.J. De Klerk (Eds). (3rd Edition). Springer, Dordrecht, Netherlands
 - Verma, N.K., Panda, P.
A study on *Stevia rebaudiana*: A review
(2018) *International Journal of Chemical Science*, 2 (2), pp. 1-6.

- Verma, P., Mathur, A.K., Jain, S.P., Mathur, A.
In vitro conservation of twenty-three overexploited medicinal plants belonging to the Indian sub continent
(2012) *The Scientific World Journal*, 2012, p. 929650.
- Watanabe, T., Fujikawa, K., Urai, S., Iwaki, K., Hirai, T., Miyagawa, K., Uratani, H., Shimamoto, K.
Identification, chemical synthesis, and sweetness evaluation of rhamnose or xylose containing steviol glycosides of Stevia (Stevia rebaudiana) leaves
(2023) *Journal of Agricultural and Food Chemistry*, 71 (29), pp. 11158-11169.
- Wijerathna-Yapa, A., Ramtekey, V., Ranawaka, B., Basnet, B.R.
Applications of in vitro tissue culture technologies in breeding and genetic improvement of wheat
(2022) *Plants*, 11, p. 2273.
- Yuan, X., Dai, Z.H., Wang, X.D., Zhao, B.
Assessment of genetic stability in tissue-cultured products and seedlings of Saussurea involucreta by RAPD and ISSR markers
(2009) *Biotechnology Letters*, 31 (8), pp. 1279-1287.

Correspondence Address

Zainuddin Z.; Department of Plant Science, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Pahang, Malaysia; email: zzarina@iiium.edu.my

Publisher: Malaysian Society of Applied Biology

ISSN: 01268643

Language of Original Document: English

Abbreviated Source Title: Malays. Appl. Biol.

2-s2.0-105001867512

Document Type: Article

Publication Stage: Final

Source: Scopus

ELSEVIER

Copyright © 2025 Elsevier B.V. All rights reserved. Scopus® is a registered trademark of Elsevier B.V.

 **RELX Group™**