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**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

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**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

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**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