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A simple and cost effective micropropagation system for stevia clonal plant production

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Stevia rebaudiana, commonly known as sweet leaf or sugar leaf, is a herbaceous perennial belonging to the family *Asteraceae*. Due to the presence of a sweet tasting glycoside, it is commonly used by diabetic patients as a low calorie, sweetener and as an antioxidant in treatment of cancer and heart diseases. Propagation of this plant species is through seeds or vegetative cuttings. Micropropagation of stevia has been applied to overcome poor seed germination rate, variability in sweetness and to meet the requirement of large number of healthy plantlets for the uniform growth and establishment of commercial plantations. The main objective of this study was to develop a simple and cost effective, clonal plant production in stevia. The literature review on micropropagation of stevia showed the utilization of growth hormones in the culture media. We have developed a simple protocol using growth hormone-free MS culture media for the regeneration and multiplication for stevia. The stem nodal segments and shoot tip explants of selected stevia plant were isolated and surface sterilized using commercial Clorox containing 1.25% sodium hypochlorite. The sterile shoot tip and stem nodal segment explants were inoculated into the MS culture medium without any plant growth hormones. All the cultures were maintained for a week time under total dark at 25 ± 1 °C. After a week, all the cultures were transferred to the growth room under 16 hours light and 8 hours dark at 25 ± 1 °C culture condition. All the cultures were sub-cultured in the same culture media once in 20 days. The shoot tip explant elongated into a single plantlet with roots and the nodal segments produced multiple shoots within 30 days. The micro-shoot multiplication of stem nodal segment was at the rate of 20/segment/30 day duration. Within 6 months duration, about 75,000 plantlets were produced starting from a single nodal segment. All the plantlets produced adventitious roots in the same culture media after 10 days duration and acclimatized using photoautotrophic culture system for 100% survival during the greenhouse hardening. The plants produced by our system showed uniform growth and high yield in the protected environment as well as in the open field. This micropropagation system is highly cost effective and can be used in commercial stevia plantations for more economic returns.

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