

In vitro regeneration through callus culture of medicinally important plant *Stevia rebaudiana* (Bert.) Bertoni

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SUMMARY

Stevia is versatile herb with incredible sweetness that can be safely used in herbal medicines; tonics for diabetic patients and also in the daily usage products. *Stevia rebaudiana* (Bert.) Bertoni is a small perennial herb of the family Asteraceae and it's a single sweetener has antidiabetic properties. Its sweetening power ranges from 100-300 times more than sucrose. The sweet compounds found in *Stevia* leaves are diterpene glycosides (Steviol glycosides), which are synthesized in the initial stages. Tissue Culture Technique has been adopted as an alternative method for rapid and large scale multiplication of *Stevia rebaudiana* for attaining market demand. Multiple shoots were obtained from callus obtained through leaves of *Stevia* when cultured on Murashige and Skoog's (1962) (MS) medium supplemented with 1.0-4.0 mg/l BAP and 0.5-1.0 mg/l NAA. These regenerated shoots when cultured on MS medium Supplemented with 2.0 mg/l BAP gave maximum shoot multiplication rate (10-12 folds). Rooting of these *in vitro* regenerated shoots were obtained on MS medium supplemented with 0.5-2.0 mg/l IBA.

Key words : MS medium, Tissue culture, Shoot multiplication, Rooting

The herb *Stevia rebaudiana* (Bert.) Bertoni (family: Asteraceae), commonly known as sweet leaf, sweet herb or honey herb is a native of highlands of Paraguay, where it has been used by aboriginals as a sweetener for centuries. It has been recently introduced in India and its name has been coined as "Madhu-patra". Due to its huge applications in food, drug and pharmaceutical industries, it is now commercial cultivated in many countries of the world viz., Brazil, Paraguay, Uruguay, Central America, Israel, Thailand, China and Japan. *Stevia* is a natural alternative to artificial sweeteners (Chalapathi *et al.*, 1999). Dry *Stevia* leaf is up to 100-300 times sweeter than sucrose due to the presence of two compound stevioside and rebaudioside A (Kinghorn and Soejarto, 1984; Liu and Liu, 1995; Striedner *et al.*, 1991; Sekihasti *et al.*, 2002; Brandle *et al.*, 1998; Nepovim and Vanek, 1998; Debnath *et al.*, 2008; Brandle and Rosa, 1992; Brandle, 1999). *Stevia* has been used for cavities, depression, diabetes, fatigue, heart support, hypertension, hyperglycemic, infections, obesity, sweet cravings, tonic, urinary insufficiencies and as a sweetener. In the US, *Stevia* is mostly employed as a sugar substitute. It is recommended for diabetes and has been used by humans with no side effects (Megeji *et al.*, 2005). There is growing

international market for *Stevia*. There are offers to buy container loads of *Stevia* leaves at a price of two-five dollars per kilo.

Stevia leaves can be used because of its anti-fungal and anti-bacterial property. Mild *Stevia* leaf tea offers excellent relief for an upset stomach. A wet *Stevia* leaf bag provides a cooling effect on eyes (similar to using cucumber). The leaves effectively tighten the skin and are good for wrinkles. *Stevia* has proved to give exceptional benefits when used regularly in skin care. It also has a healing effect on blemishes, wounds, cuts and scratches. *Stevia* is helpful in weight and blood pressure management. It has also been reported that *stevia* lowers incident of colds and flu. Medicinal uses of *stevia* include regulation of blood sugar, preventing hypertension, treatment of skin disorders and prevention of tooth decay (Planas and Kuc, 1968; Chen *et al.*, 2005).

Vegetative propagation of this plant through stem cuttings is also limited by the low rate of multiplication and the production of plants from seeds that otherwise have very low chances of germinating and growing. Propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in sweetening levels and composition (Sakaguchi and Kan, 1982). Tissue Culture Technique has been adopted as an alternative method for rapid and large scale multiplication of *Stevia rebaudiana* for attaining market demand (Nakamura and Tamura, 1985).

Tamura *et al.* (1984) established clonal propagation of *Stevia rebaudiana* by culturing stem tips with an increasing demand of stevioside in food industry. Ferreira

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