RESEARCH ARTICLE | *International Journal of Plant Sciences,* (January to June, 2010) Vol. 5 Issue 1 : 284-289

Somatic embryogenesis in *Stevia rebaudiana* Bertoni using different concentration of growth hormones

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Accepted : November, 2009

SUMMARY

In vitro propagation of *Stevia rebaudiana* through somatic embryogenesis was successfully achieved from axillary buds using nodal and leaf as explants in basal medium (MS) with vitamins, sucrose (30g/l), agar (0.9% w/v) and supplemented with 2, 4- D (2.0 mg/l) + BAP (0.2mg/l) + TDZ (0.2mg/l). These conditions yielded friable callus cultures. Callus sub cultured on medium with reduced concentration of 2, 4- D (1.0 mg/l) became embryogenic. Organogenesis of embryonic callus was then achieved by eliminating the agar and modulating the mediums with hormones BAP (1.0 mg/l) + IBA (0.5mg/l) and increasing concentration of sucrose concentration up to 40g/l for 1 week followed by transfer of mature embryonic callus to ½ strength MS medium containing IBA (1.5 mg/l) were 83% of embryos developed into micro shoots. Through sequential hardening process, well rooted plantlets with survival rate (95%) were established in the field.

Key words : *In vitro,* Somatic embryogenesis, *Stevia rebaudiana*, Growth hormones, Organogenesis

Stevia, one of the 950 genera of the *Asteraceae* family is a genus of more than 200 species family, it grows up to 1 meter with an extensive root system and brittle stem producing small, elliptic leaves. Members of *Stevia* comprise mostly of herbs but also shrubs and trees. Originally it is said to be native to subtropical South America (Paraguay and Brazil) (Soejarto *et al.*, 1982). The Guarani Indians of Paraguay were the first to exploit this sweetener for mate tea. It has been cultivated domestically in continental China, Taiwan, Thailand, Korea, Brazil and Malaysia (Brandle and Rosa, 1992, Fors, 1995). Pure extract stevioside is non–caloric and 300 times sweeter than sugar with a delicious and refreshing taste (Bhosle, 2004). The other attributes of this natural, high intensity sweetener include non-fermentable, non-discoloring, maintain heat stability at 100° C and features a lengthy shelf life. The leaves are the source of the diterpene glucoside vis. stevioside, rebaudioside A and C, and along with it also contain rebaudioside A and C, dulcoside. Various studies have found the leafs to contain protein, fibers, carbohydrates, iron, phosphorous, calcium, potassium, sodium, magnesium, zinc, rutin (flavonoid), true vitamin A, vitamin C and an oil which contain 53 other contitutents. In addition to its sweetening property it has therapeutic values such as antihyperglycemic, anticanerous (Jeppensen *et al.*, 2002, 2003), antihypersensitive agent (Chan *et al.*, 1998) contraceptive (Melis, 1999) and prevention of dental caries (Fujita H *et al.*, 1979). *Stevia* can also inhibit bacterial and fungal growth (Cerda-Gercia-Rojas and Pereda Miranda, 2002).

The main problem in cultivation of these plants is that they are heterozygous and self- incompatibility leads to low germination percentage and with that vegetative propagation too is limited by the lower number of individuals that can be obtained simultaneously from a single plant (Sakaguchi and Kan, 1982). To overcome all these, multiplication and improvement of this medicinal plant though tissue culture may be an alternative for rapid mass propagation of *Stevia*. Somatic embryogenesis and organogenesis have been the common pathways for clonal propagation of superior medicinal plant species. Somatic embryogenesis enables large numbers of plantlets to be produced within a short span of time.

MATERIALS AND METHODS

The methods of plant tissue culture were the standard method as described in plant cell, tissue and organ culture fundamental methods (Gamborg and Phillips, 2004).

Plant material and sterilization:

The leaves were excised from both mature field grown of *Stevia rebaudiana* plant collected from "Swariya Musli" farm, and controlled cultured *in vitro* plants. The leaf disc (15mm diameter) were cut into small pieces and were washed thoroughly under running tap water, then soaked in labolene (a commercial neutral detergent Quligens, India) (5% v/v) for 5 minutes. After

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