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EFFECT OF COPPER SULPHATE ON *IN VITRO* SHOOT REGENERATION FROM SHOOT TIP EXPLANT OF *STEVIA REBAUDIANA* BERT.

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Abstract

The present investigation was carried out to produce an efficient and reproducible protocol for direct organogenesis and to find out the effect of copper sulphate on *in vitro* shoot regeneration from shoot tip explant of *Stevia rebaudiana*. Maximum number of multiple shoots 12.0±0.27 regenerated from the shoot tip explants cultured on MS medium fortified with BAP (1.5mg/l) + Kinetin (1mg/l). The highest culture response 18.4±0.17 shoots per explant was observed in the treatment with MS medium supplemented with copper sulphate (0.2 mg/l) in combination with BAP (1.5 mg/l) and Kinetin (1.0 mg/l).

Key words : *Stevia rebaudiana*, shoot tip, copper sulphate, BAP, multiple shoots.

Introduction

Stevia rebaudiana Bert, belongs to the family Asteraceae is a perennial shrub which grows up to 1m. The leaves of *Stevia* are the source of the diterpene glycosides, *viz*. stevioside and rebaudioside, which are estimated about 100–300 times sweeter than sucrose (Ishima and Katayama, 1976). The leaf powder can be incorporated in tooth paste which is having the capacity to reduce tooth cavity and decay. It also possesses antiwrinkle properties in it. It is frequently used in preparation of many food products. The germination capacity of seeds is found to be very poor (Nakamura and Tamura, 1985). Plant breeding by seeds does not allow the production of homogeneous populations, resulting variation in important features like sweetening levels and composition. By means of vegetative propagation limited number of propagules can be obtained simultaneously from a single plant (Sakaguchi and Kan, 1982). Due to the above-mentioned difficulties, *in vitro* culture technique is the only alternative for rapid mass propagation of Stevia plants. Copper is one of the important microelement which is essential for normal growth and development of plants. In plants copper plays a very important biochemical and physiological functions. It takes part in the processes of photosynthesis, respiration, conversion of nitrogen compounds and transport of carbohydrates and also regulating the process of DNA replication. (Podlesna and Wojcieska-Wyskupajtys, 1996). In the present study, the effect of copper sulphate on *in vitro* regeneration of *S. rebaudiana* was analysed.

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Materials and Methods

Plant material

Stevia rebaudiana plants were collected from Horticultural institute, Yercaud, Tamilnadu, India and maintained in green house.

Preparation of shoot tip explant

Shoot tips were collected from 30 days old plants and thoroughly washed with 0.1 percent teepol followed by washing with sterilized distilled water thrice. The explants were then surface sterilized with 0.1% mercuric chloride for 5minutes, followed by washing with sterile distilled water. The outer leaves in each explant were removed and inner most shoot tip, measuring 0.5-1.0 cm in length, having 1 or 2 leaf primordial were excised.

Fig. 1 : Multiple shoot induction from Shoot tip explants of *Stevia rebaudiana* on MS medium fortified with BAP (1.5mg/l) + Kinetin (1mg/l).

Fig. 2 : Multiple shoot induction from Shoot tip explants of *Stevia rebaudiana* on MS medium fortified with copper sulphate (0.2 mg/l) in combination with BAP (1.5 mg/l) and Kinetin (1.0 mg/l).

Table 1 : Effect of BAP with Kinetin (1.0 mg/l) on multiple shoot induction from Shoot tip explants of *Stevia rebaudiana* on MS medium.

BAP (mg/l)	Explants Responded (%)	Number of shoots produced	Basal callus
0.5	59.0±0.22^{e}	7.2±0.28^{e}	-
1.0	68.2±0.31^{b}	9.3±0.21^{d}	-
1.5	72.1±0.34^{a}	12.0±0.27^{a}	-
2.0	65.2±0.38^{c}	11.2±0.17^{b}	+
2.5	60.2±0.19^{d}	10.0±0.19^{c}	+

Triplicates were maintained for each experiment (3X) with 20 explants per treatment. Values represent the means ± the standard error. Mean value within the column followed by the same letter in superscript are not significantly different at $P < 0.05$

Shoot regeneration

Explants were cultured in sterilized MS media containing 3% sucrose, 0.8% agar along with growth regulators with different concentration of BAP and kinetin (0.5 - 2.5 mg/l). The shoot tip explants were inoculated aseptically and incubated in the culture room, maintaining a temperature of 25±2^{0}C and a humidity of 75 percent. The light cycle of 16 hours and 8 hours dark was maintained with 2000-3000 lux intensity. Explants were subjected to two sub cultures at an interval of ten days each in the MS medium supplemented with the same concentration of growth regulators. To find out the effect of copper sulphate, Murashige and Skoog medium was prepared with different concentration of copper sulphate ranging from 0.1-0.5 mg/l along with BAP (1.5 mg/l).

Results and Discussion

Various growth regulators at different combinations and concentrations were examined for regeneration of multiple shoots from shoot tip explants of *Stevia rebaudiana*. The maximum shoots (12.0±0.27) were observed in the combination of BAP (1.5 mg/l) and kinetin (1mg/l) (table 1, fig. 1). This observation was similar to that of Ferreira and Handro (1988). Though, shoot regeneration from the shoot tips was found to be effectively enhanced by the addition of copper sulphate, the highest culture response (18.4±0.17) was observed in the treatment in which MS medium supplemented with copper sulphate (0.2 mg/l) in combination with BAP (1.5 mg/l) and Kinetin (1.0 mg/l) (table 2, fig. 2). The culture response increased with the increasing concentration of copper sulphate, but a reduction in culture response was observed at the concentration above 0.3 mg/l. It is interesting to note that there was no response when the explants were cultured in the MS medium without any

Table 2 : Effect of Copper sulphate with BAP (1.5mg/l) on multiple shoot induction from Shoot tip explants of *Stevia rebaudiana* on MS medium.

Copper sulphate(mg/l)	Explants Responded (%)	Number of shoots produced
0.1	68.2±0.15[b]	10.5±0.14[b]
0.2	75.2±0.18[a]	18.4±0.17[a]
0.3	60.0±0.19[c]	8.7±0.19[c]
0.4	42.2d±0.20[e]	6.6±0.11[e]
0.5	43.2±0.23[d]	7.4±0.15[d]

Triplicates were maintained for each experiment (3X) with 20 explants per treatment. Values represent the means ± the standard error. Mean value within the column followed by the same letter in superscript are not significantly different at $P < 0.05$.

supplementation, suggested that it is essential to add growth regulators exogenously to obtain desirable results. The metals like cobalt, iron, manganese, copper and zinc are essential for plant life but are required in a very small or trace amounts and become toxic at higher concentrations (Hussein *et al*., 2010). In the present study, it was concluded that copper sulphate in MS Medium significantly enhanced direct shoot bud induction and proliferation from cultured explants.

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