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Micropropagation of an Anti diabetic Plant - *Stevia rebaudiana* Bertoni, (Natural Sweetener) in Hadoti Region of South-East Rajasthan, India

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Abstract

This review highlights the recent development and achievements made for the micropropagation of Stevia rebaudiana Bertoni (an antidiabatic sweetener herb) in Hadoti region of south-east Rajasthan. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments was the highest in MS medium supplemented with 0.5 mg/l BAP+2.0 mg/l Kn. For rooting different concentration of IBA were used and highest rooting was recorded on MS medium with 1.0 mg/l IBA. The rooted Plantlets were hardened initially in culture room conditions and then transferred to misthouse.

Keywords: Anti diabetic, micropropagation, shoot multiplication sweetener.

Introduction

Stevia rebaudiana Bertoni, is a small, herbaceous, semi-bushy, perennial shrub of Compositae family originated from Paraguay. It is a natural sweetener plant known as "Sweet Weed", "Sweet Leaf", "Sweet Herbs" and"Honey Leaf", which is estimated to be 300 times sweeter than cane sugar[1]. It grows well at the temperature ranging between 15-30°C. It is one of 154 members of the genus Stevia, which produces stevioside, a diterpinoid glycoside isolated from plant leaves[2]. Stevioside of special intrestto diabetics, persons with hyperglycemia and the diet conscious. *Stevia* has various properties such as antibacterial, anticandidal, antifungal, antiviral, cardio tonic (tones, balances, strengthens the heart), diuretic, hypoglycemic, vasodilator. Dry leaves of this plant are 30 times sweeter than sugar with zero calories. The first report of commercial cultivation in Paraguay were in 1964 began a large effort aimed at establishment[3]. The herbal drug industry is considered to be a high growth industry of the late 90s and seeing the growing demand, it is all set to flourish in the next century in ancient Indian traditional Ayurvedic system of medicine. *Stevia* has various properties such as antibacterial, anticandidal, antifungal, antiviral, cardio tonic (tones, balances, strengthens the heart), diuretic, hypoglycemic, vasodilator.

The seeds of stevia show very less vigour and propagation and do not allow the production of homogenous population which leads to variability in sweetening level and composition[4-5]. Poor seed germination percentage is the limiting factor to large scale cultivation of this species. Vegetative propagation is also limited by the low number of individuals obtained from single plant. Hence, to overcome all these obstacles, micropropagation or *in vitro* culture technique can play a vital role for masspropagation and the production of genetically identical plants of *S. rebaudiana* and the present study was aimed at the findings of efficient protocol for *in vitro* mass propagation of *S. rebaudiana* in hadoti-Kota region. There has been few report of *in vitro* micropropagation from shoot tip and leaf.

Material and Methods

The branches (about 5-6 cm) of shoots of *Stevia rebaudiana* plants were collected from the Herbal Garden, Jhalra Paten. The branches with node explants were washed in running tap water and then washed again thoroughly by adding a few drops of tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing them five times with double distilled water inside the laminar air flow chamber. Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same experiments were repeated for shoot multiplication.

The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9+0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were sub cultured

on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations and combination of BAP and Kn. Among these, the maximum number of shoots (3.42±0.58) was developed on MS media fortified with 0.5 BAP±2.0 Kn. Maximum shoot length was observed as 7.54±0.31cm.of a medium supplemented with 0.5 BAP+0.5 Kn. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (0.5-2.5 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose. The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with soilrite composition (soil: sand: peat moss) and irrigated with 1/4 MS salt solution. These bottles were kept in controlled environmental conditions of culture room. After 3 wk of growth, the plantlets were transferred to misthouse for further growth.

Results and Discussion

The nodal explants, when inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 3.0 mg/l concentration evoked best response. Incorporation of NAA or IAA improved bud proliferation but the shoots remained stunted. Shoots after their initial proliferation on medium containing 3.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than Kn and the maximum number of shoot was obtained on its 3.0 mg/l concentration (table-1, figure-1 A, B, figure-2). When BAP was used in combination with Kn a variety of responses were observed (table-2, figure-1 C, Figure-3). But best response was observed on medium containing 0.5 mg/l BAP + 2.0 mg/l Kn (average number of shoots (3.42±0.39) and best shoot length was observed on medium containing 0.5 mg/l BAP+ 0.5 mg/l Kn (average shoot length 7.54±0.31 cm). The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins.

Auxins in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 1.0mg/l IBA, where roots measuring 1.68±0.32 cm (average) were formed (table-3, figure-1 D, figure- 4). *In vitro* rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 week, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly (figure-E).

Table-1
Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana*

Hormone Concentration (mg/ l)	Hormone Concentration (mg/ l)	Response (%)	Number of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)
BAP	**Kn**			
1.0	**-**	70	2.28±0.71	6.56±0.84
2.0	**-**	65	2.71±0.56	7.62±0.53
3.0	**-**	80	3.42±0.58	6.51±0.76
4.0	**-**	55	3.28±0.36	5.08±0.51
5.0	**-**	40	2.85±0.51	3.31±0.33
-	1.0	75	2.42±0.39	6.30±0.26
-	2.0	60	2.28±0.36	6.47±0.29
-	3.0	55	1.85±0.27	6.15±0.24
-	4.0	40	1.57±0.40	5.70±0.41
-	5.0	30	1.28±0.36	4.92±0.51

Medium: MS+ additives; mean± SD, n= 7 replicates, means having the same letter in each column, do not different significantly at P< 0.05 (Tukey's test)

Table-2
Interactive effect of cytokine (BAP+ Kn) on shoot multiplication by sub culture of shoot clumps of *Stevia rebaudiana*

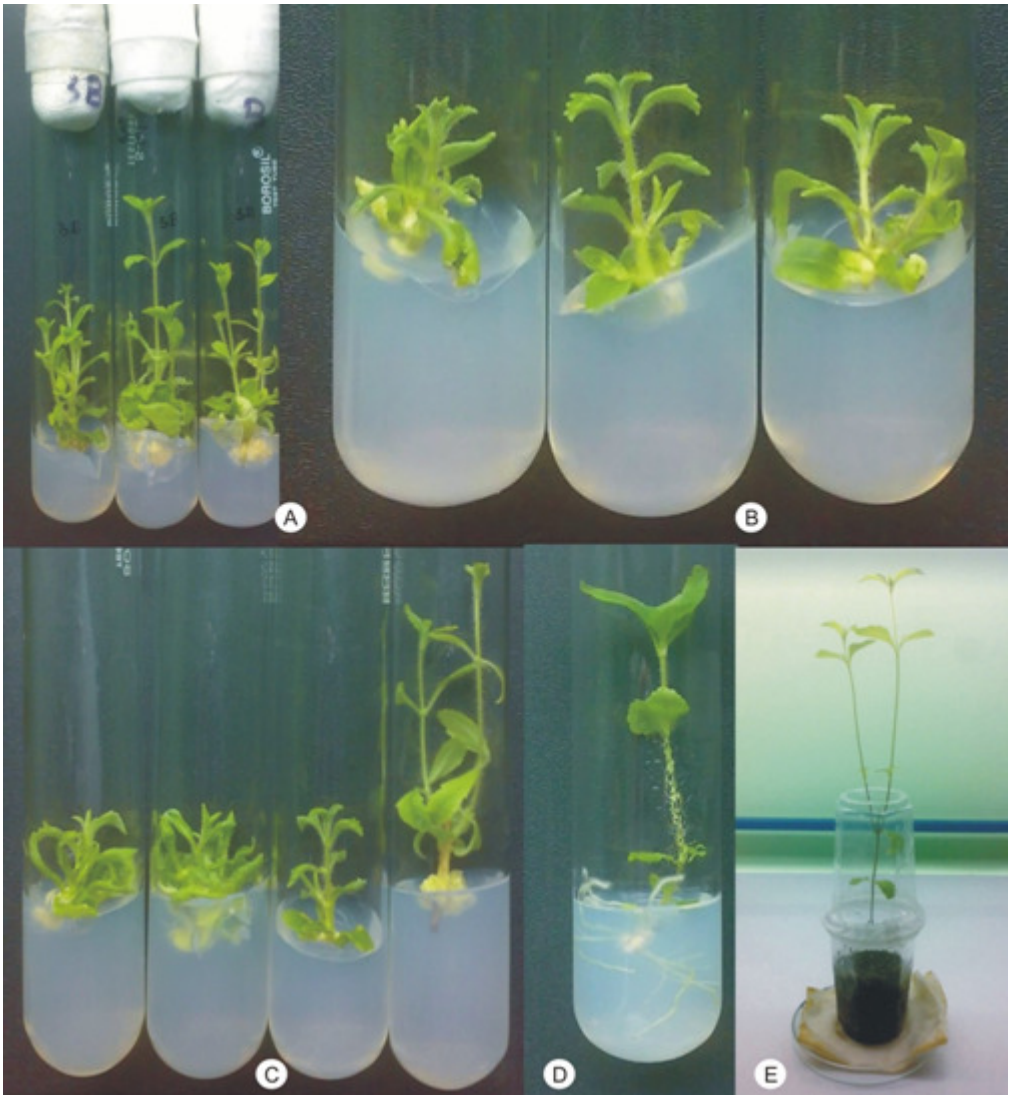
Hormone Concentration (mg/ l)	Number of Shoot/explant	Shoot length (in cm)	Shooting Response (%)
0.5 BAP + 0.5 Kn	1.71±0.38	7.54±0.31	70
0.5 BAP + 1.0 Kn	2.14±0.51	6.70±0.39	80
0.5 BAP + 2.0 Kn	3.42±0.39	5.70±0.41	90
0.5 BAP + 3.0 Kn	2.71±0.36	4.71±0.29	85
0.5 BAP + 4.0 Kn	2.57±0.40	3.70±0.28	82

Medium: MS+ additives; mean± SD, n= 7 replicates, means having the same letter in each column, do not different significantly at P< 0.05 (Tukey's test)

Table-3
Effect of auxin (IBA) on root induction from isolated shoot of *Stevia rebaudiana*

Hormone Concentration (mg/ l)	Number of roots/explants	Root length (in cm)	Rooting Response (%)
0.5 IBA	2.80±0.73	0.43±0.33	90
1.0 IBA	3.60±0.51	1.68±0.32	85
1.5 IBA	1.40±0.52	1.06±0.08	80
2.0 IBA	1.38±0.37	0.92±0.10	78
2.5 IBA	1.08±0.19	0.51±0.05	73

Medium: MS+ additives; mean± SD, n= 7 replicates , means having the same letter in each column do not different significantly at P< 0.05 (Tukey's test)



A. Shoot multiplication on MS medium supplemented with 3.0 mg/l BAP, B. Shoot multiplication on MS medium supplemented with 1.0 mg/l Kn, C. Shoot multiplication on MS medium supplemented with 0.5 mg/l BAP+0.5 mg/l Kn, D. In vitro root induction on ¼ of MS medium supplemented with 0.5 mg/l IBA, E. 5 week's old hardened plant growing on soilrite moistened with basal medium.

Figure-1
(A-E) Micropropagation of *Stevia rebaudiana* from nodal shoot explants

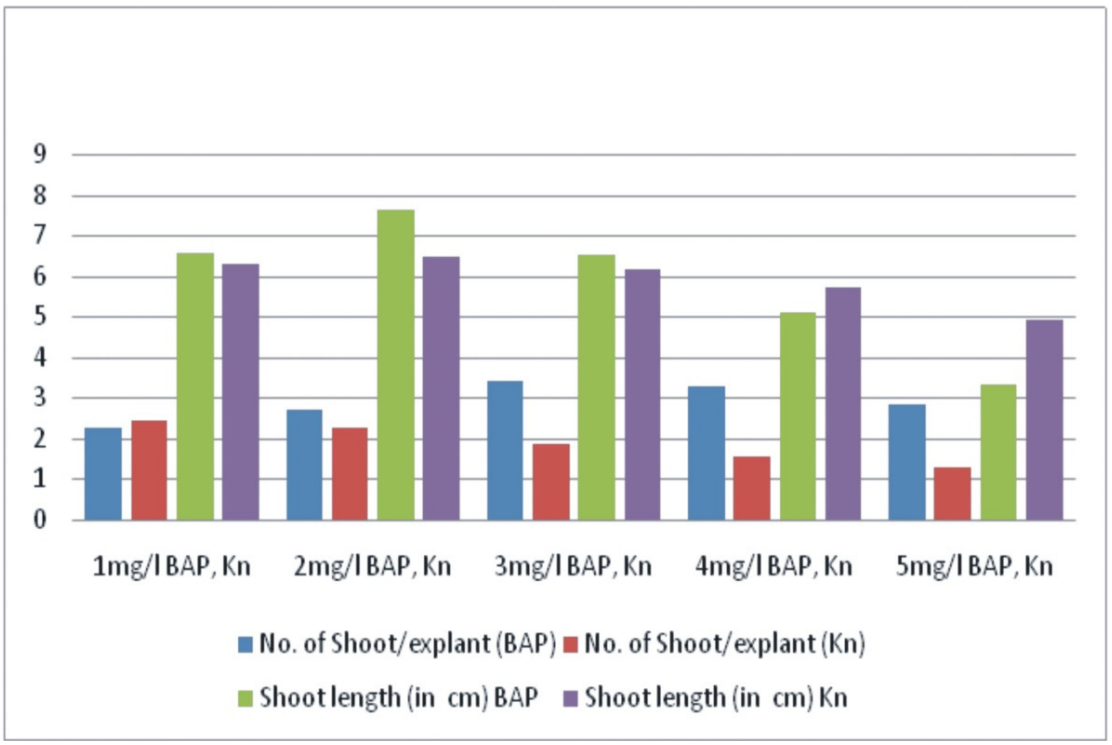


Figure-2
Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Stevia rebaudiana*

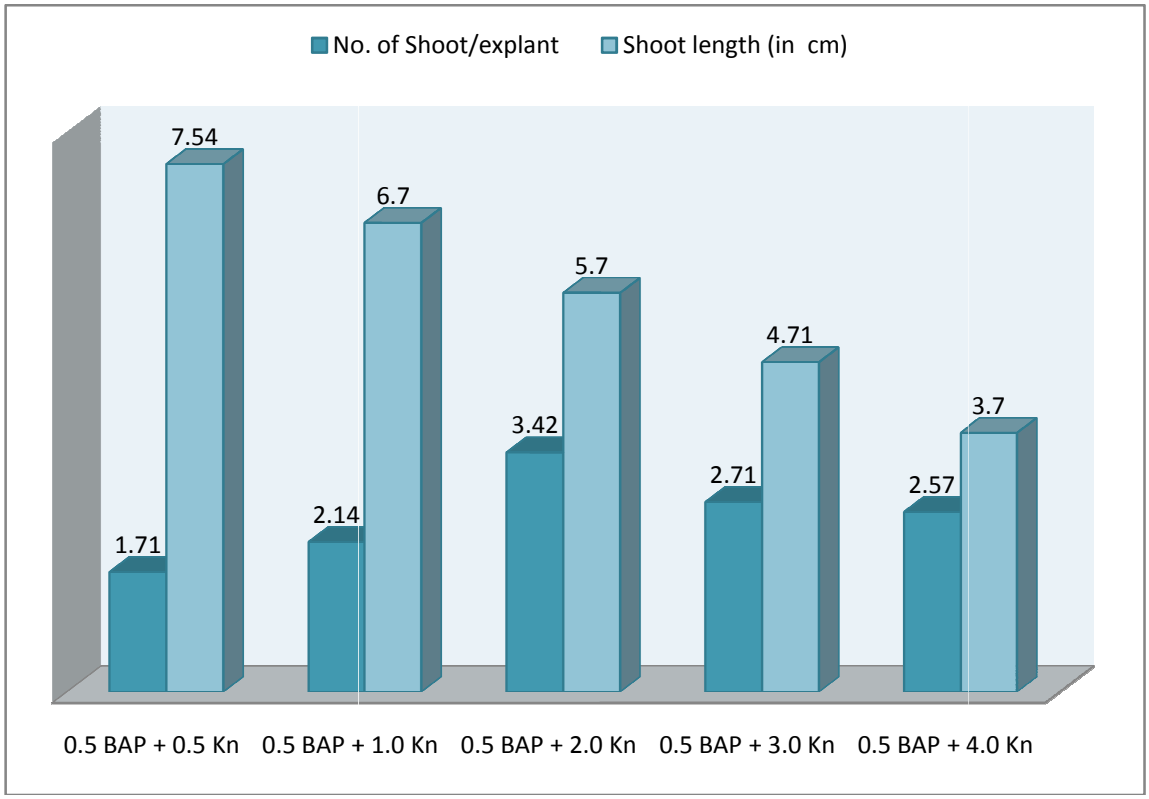


Figure-3
Interactive effect of cytokine (BAP + Kn) on shoot multiplication by subculture of shoot clumps of *Stevia rebaudiana*

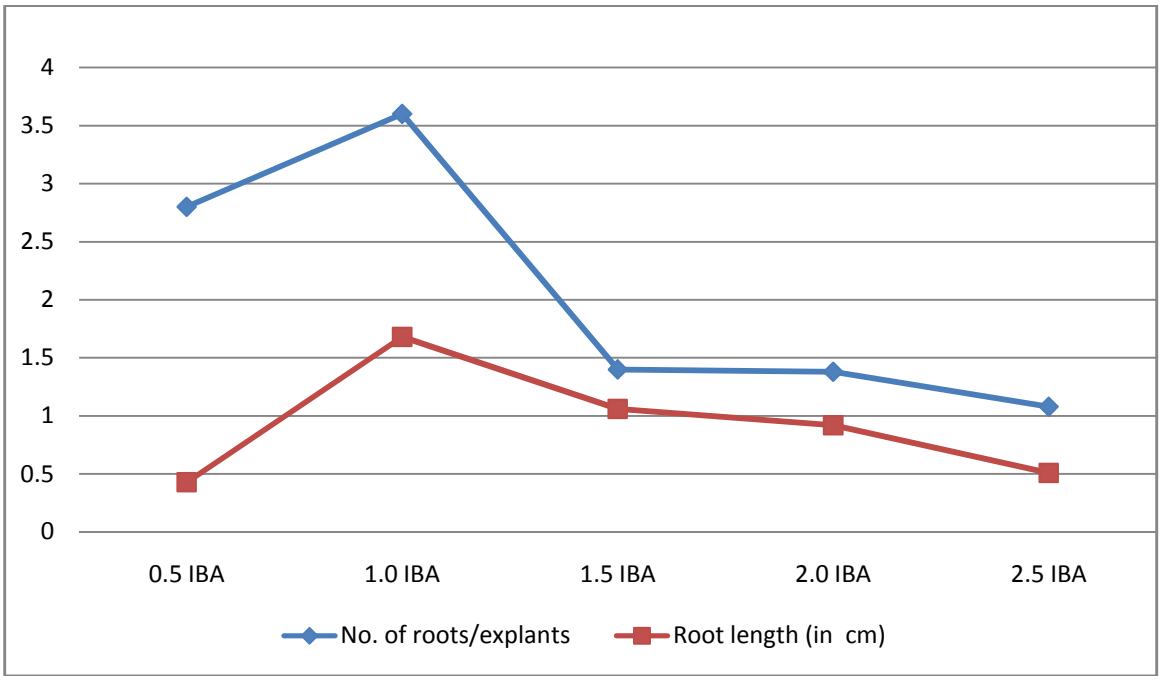


Figure-4
Effect of auxin (IBA) on root induction from isolated shoots of *Stevia rebaudiana*

Conclusion

The seedling derived explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Stevia rebaudiana* MS medium containing 3.0 mg/l BAP was the best for culture initiation. We have found that *Stevia rebaudiana* culture grew better on MS medium in comparison to other media. In *Stevia rebaudiana* 2.0 mg/l BAP was most suitable for shoot multiplication. We also observed improvement in shoot multiplication by different concentrations of Kn. (0.5-4.0 mg/l) in medium along with BAP (0.5 mg/l). Best shooting response was observed on media containing 0.5 mg/l BAP+ 2.0 mg/l Kn (average number of shoots 3.42±0.39) and 0.5 mg/l BAP+0.5 mg/l Kn (average shoot length 7.54±0.31 cm).

IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Stevia rebaudiana*, 1.0mg/l IBA proved to be the best for in vitro rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to mist house where they exhibited and hence, growth and 90% survival.

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