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Micropropagation of *Stevia rebaudiana* Bertoni. in different kind of basal medium

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ABSTRACT

Experiments were conducted for the standardization of in vitro culture technique for the mass propagation of Stevia rebaudiana Bertoni., a medicinally important, sweet tasted, an anti-diabetic herb with zero-calorie value. Mostly all micropropagation methods involve the proliferation of callusing via MS media. While such media have been moderately too highly successful in terms of multiplication yields, it has become increasingly important to improve productivity and reduce the time taken to multiply commercially important material.

In the present study, Shoot tip, nodal segment were used as explants and they were cultured on different mediums such as Eriksson (ER) medium, White's medium, Gamborg (B5) medium, Nitsch medium supplemented with different concentrations of BAP. Among all these mediums best results were obtained from Eriksson medium supplemented with 3.0 mg/l BAP. Satisfactory results were obtained in White's medium containing 1.0 mg/l BAP, In Gamborg (B5) medium good mass multiplication was found in 3.0mg/l BAP where as in Nitsch medium better growth was observed in 2.0 mg/l BAP.

***Key words**: Anti diabetic, micropropagation, shoot multiplication sweetener.*

INTRODUCTION

Stevia rebaudiana Bertoni. is a perennial herb belonging to Asteraceae family. It is indigenous of South America especially of northern Paraguay[16,11]. The plant has gained wide access to Pacific Rim countries, where in recent decades it is being cultivated domestically, used in its raw leaf form and is now commercially processed into sweetener. After first report of commercial cultivation in Paraguay in 1964[11], it has been introduced as a crop in a number of countries

including Brazil, Korea, United States, Mexico, Indonesia, Canada and Tanzania[1,5]. Stevia is a natural non-calorie sweetener so; it works as an alternative source to traditional sugars[3]. It is commonly known as honey leaf, sweet weed and sweet herb of Paraguay. Now a day, *Stevia rebaudiana* has obtained a great attention due to its high range of sugar content with zero calories.In addition to its sweetening property it has various therapeutic values such as anticancerous, antihyperglycemic[9], and antihypersensitive agent[4,10], contraceptive properties[13], prevention of dental caries[6] and inhibiting fat accumulation and lowering blood pressure in human being[4,17]. There are more than 180 species of the *Stevia rebaudiana* that gives the sweet essence[17]. The leaves of Stevia contains diterpene glycosides, such as steviolbioside, rubsoside, rebaudioside A, B, C, D, E and F, dulcoside and stevioside[18]. The biggest part of the sweet glycosides consists of the stevioside molecule[11]. The sweetener, stevioside[15] extracted from the plants is 300 times sweeter than sugar. The fresh leaves have a nice liquorice taste. It is recommended for diabetes and has been extensively tested on animals and has been used by humans with no side effects[14].

For anyone who suffers from diabetes, high blood pressure, chronic yeast infections, obesity and hypoglycemia, Stevia is the ideal sweetener.

Stevia is cultivated commercially by seeds. The poor seed germination is one of the factors limiting large scale cultivation[7,12]. Vegetative propagation too, is limited by lowering number of individuals that can be obtained from single plant[19]. Due to the above mentioned difficulties; tissue culture is the only alternative for rapid mass propagation of *Stevia* plants. Micropropagation of stevia through shoot tip or axillary bud culture allows recovery of genetically stable and true to type progeny[8]. In *in-vitro* propagation phytohormones play an important role. They enhance the production of plant in vitro with good agronomical characters.

The present investigation was aimed to find efficient protocol for rapid *in vitro* clonal propagation of *S. rebaudiana* by using variety of Media's such as Eriksson (ER) medium, White's medium, Gamborg (B5) medium, Nitsch medium. This investigation was also an attempt to compare *in-vitro* growth of *Stevia* in the given media's.

MATERIAL AND METHODS

The branches (about 5-6 cm) of shoots of *Stevia rebaudiana* Bertoni. 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 pH of the media was adjusted to 5.2-6.2 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 of BAP. Among these, the maximum number of shoots was developed on MS media fortified with 3.0 BAP in Eriksson (ER) medium.

RESULTS AND DISCUSSION

Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.

For the comparative study of shoot elongation Eriksson (ER) medium was used. When nodal shoot tips were incorporated in ER medium fortified with various concentrations of BAP then maximum shoots 4.11±0.21 with highest shoot length 6.54±0.32 cm were observed in 3 mg/l BAP and minimum number of shoots 2.15±0.29 with lower shoot length 4.38±0.33 cm were observed in 5.0 mg/l. (Table 1 and Fig. 1, 5-A)

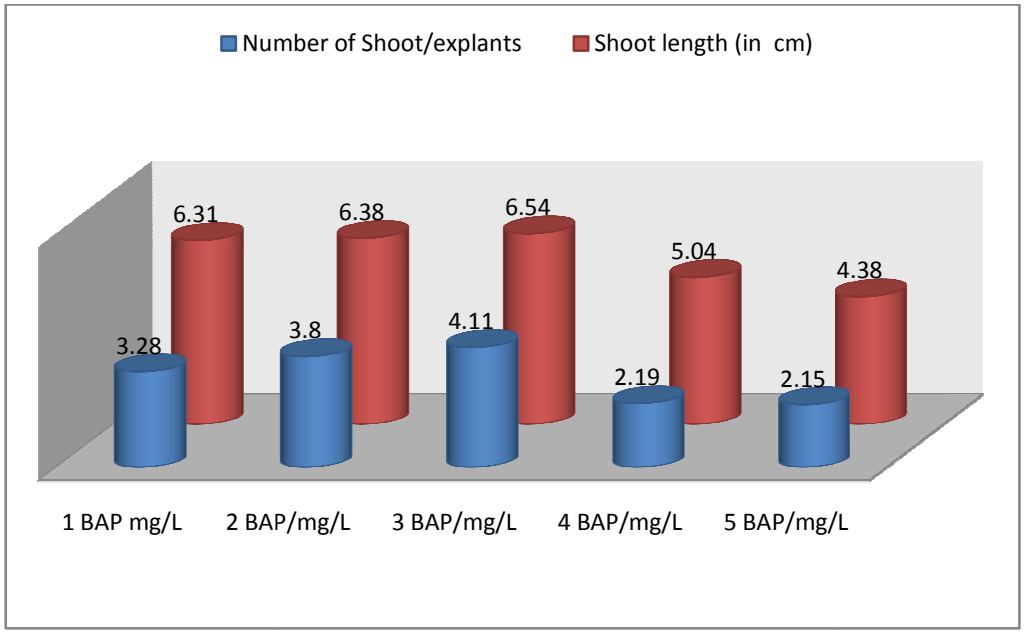
Table 1: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni. (after 45 days)

Hormone Concentration (mg/ L) BAP	Response (%)	Number of Shoot/explants (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	80	3.28±0.33	6.31±0.23
2.0	85	3.80±0.32	6.38±0.31
3.0	90	4.11±0.21	6.54±0.32
4.0	75	2.19±0.34	5.04±0.26
5.0	70	2.15±0.29	4.38±0.33

Medium: MS+ additives; mean± SE, n= 7 replicates

Means having the same letter in each Column dose not differentiate significantly at P< 0.05 (Tukey's test)

Fig. 1: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.



Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.

Similarly when Gamborg (B5) medium was used for the shoot multiplication then highest number of shoots 3.18±0.29 with shoots length 4.34±0.36 was observed onto Medium supplemented with 30.mg/l BAP where as minimum number of shoots 1.19±0.30 (shoot length 2.28±0.39 cm) were obtained in 5.0 mg/l BAP. (Table 2 and Fig. 2, 5-B)

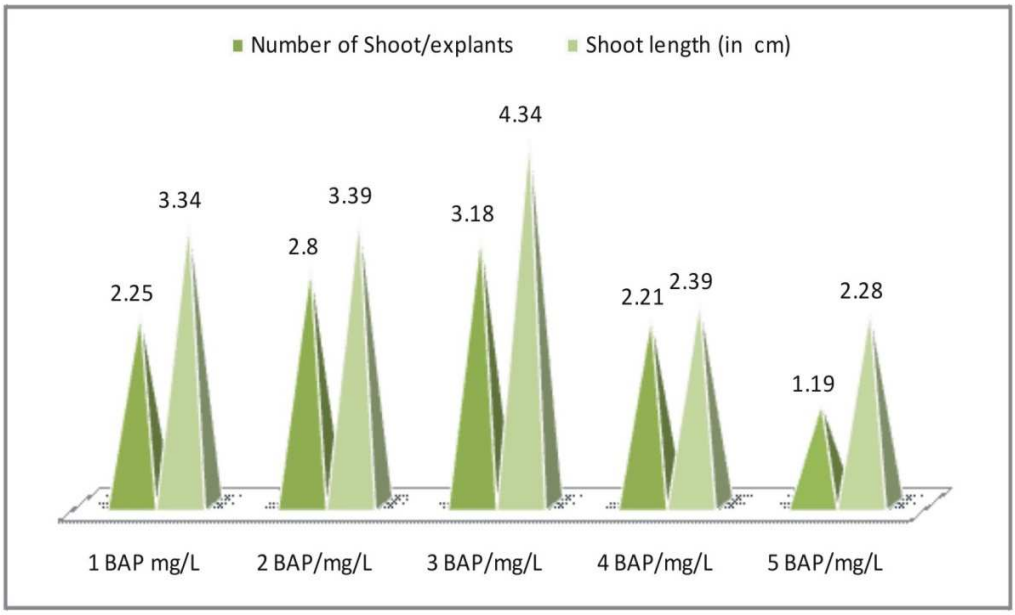
Table 2: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni. (after 45 days)

Hormone Concentration (mg/ L) BAP	Response (%)	Number of Shoot/explants (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	60	2.25±0.23	3.34±0.28
2.0	65	2.80±0.36	3.39±0.33
3.0	70	3.18±0.29	4.34±0.36
4.0	55	2.21±0.24	2.39±0.36
5.0	40	1.19±0.30	2.28±0.39

Medium: MS+ additives; mean± SE, n= 7 replicates

Means having the same letter in each Column dose not differentiate significantly at P< 0.05 (Tukey's test)

Fig. 2: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.



Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.

White's medium was also used with BAP for the study of its effect on shoot multiplication of *stevia rebaudiana.* After the inoculation of nodal segments on white's medium supplemented with BAP Maximum result were obtained onto 1.0 mg/l BAP (Number of shoots 3.21±0.33 and shoot length 3.24±0.23) and minimum result were obtained onto 5.0 mg/l (Number of shoots 2.15±0.31and shoot length 2.23±0.32). (Table 3 and Fig. 3, 5- c)

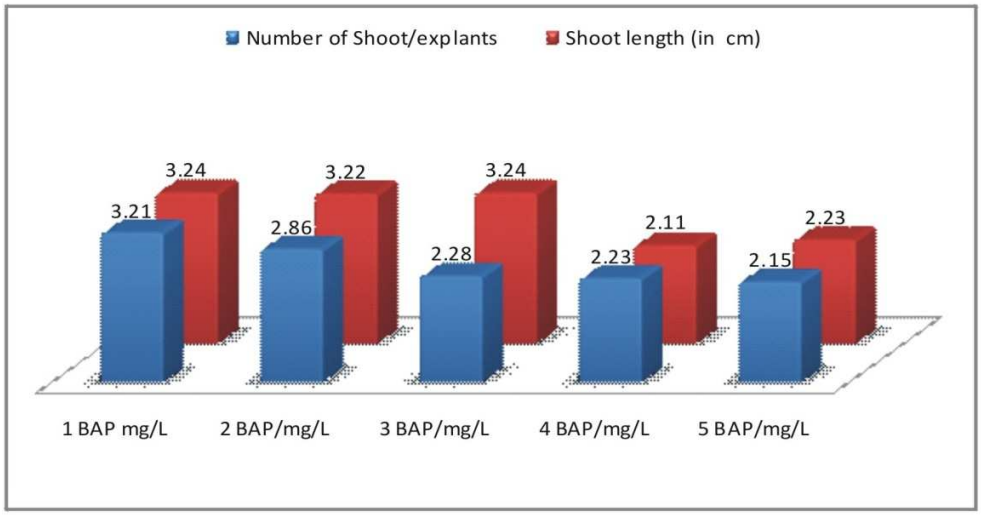
Table 3: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni. (after 45 days)

Hormone Concentration (mg/ L) BAP	Response (%)	Number of Shoot/explants (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	60	3.21±0.33	3.24±0.23
2.0	55	2.86±0.32	3.22±0.36
3.0	50	2.28±0.30	3.24±0.33
4.0	40	2.23±0.27	2.11±0.27
5.0	35	2.15±0.31	2.23±0.32

Medium: MS+ additives; mean± SE, n= 7 replicates

Means having the same letter in each Column dose not differentiate significantly at P< 0.05 (Tukey's test)

Fig. 3: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.



Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.

Nitsch medium was also used for the comparative study. In this greatest number of shoots 2.92±0.34 with 3.82±0.34 shoot length was observed in 2.0 mg/l BAP where as least amount of shoot 2.45±0.38 (shoot length 2.73±0.39) was obtained in 5.0 mg/l. (Table 4 and Fig. 4, 5-D)

Table 4: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni. (after 45 days)

Hormone Concentration (mg/ L) BAP	Response (%)	Number of Shoot/explants (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	50	2.74±0.23	3.54±0.23
2.0	55	2.92±0.34	3.82±0.34
3.0	53	2.88±0.37	3.74±0.33
4.0	45	2.53±0.25	2.81±0.37
5.0	42	2.45±0.38	2.73±0.39

Medium: MS+ additives; mean± SE, n= 7 replicates

Means having the same letter in each Column dose not differentiate significantly at P< 0.05 (Tukey's test)

Fig. 4: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Stevia rebaudiana* Bertoni.

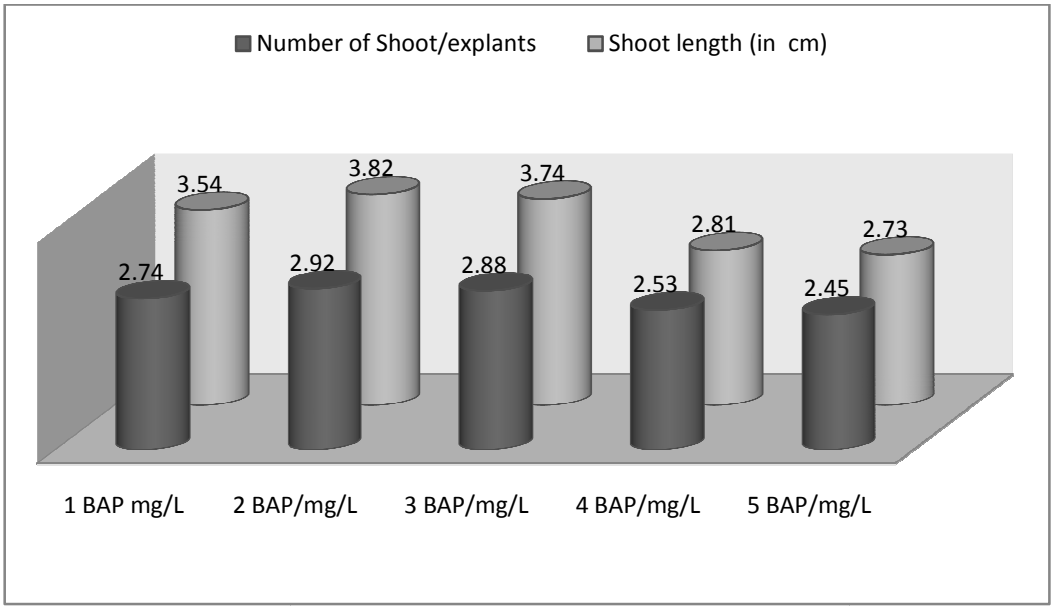


Fig. 5: (A-D) Micropropagation of *Stevia rebaudiana* Bertoni. from nodal shoot explants

A. Shoot multiplication on Eriksson (ER) medium supplemented with 3.0 mg/l BAP, B. Shoot multiplication on Gamborg (B5) medium supplemented with 3.0 mg/l BAP, C. Shoot multiplication on White's medium supplemented with 1.0 mg/l BAP, D. Shoot multiplication on Nitsch medium supplemented with 2.0 mg/l BAP

CONCLUSION

Nodal segments of plant were used for the fast propagation of plant *in-vitro*. It has found that *Stevia rebaudiana* culture grew better on Eriksson (ER) medium in comparison to other media. In Eriksson (ER) medium supplemented with 3.0 mg/l BAP proved best for shoot multiplication (maximum no. of shoots 4.11±0.21). When Stevia was cultivated on Gamborg (B5) medium then good growth was also obtained in 3.0 mg/l BAP (highest number of shoots 3.18±0.29) and when Stevia's nodal segments was sub-cultured on White's medium then satisfactory growth was found on 1.0 mg/l BAP (Number of shoots

3.21±0.33). Where as on Nitsch medium good growth was observed on medium supplemented with 2.0 mg/l BAP (greatest number of shoots 2.92±0.34).

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