Effect of Various Basal Salts on *in vitro* growth of *Stevia rebaudiana* (Bert.)

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Abstract

Stevia rebaudiana (Bert.) commonly known as sweet leaf, sugar leaf or simply *Stevia* is originally a south American wild plant but it could be found growing in semiarid habitat. Leaves of this plant produce zero caloric deterpene glycosides (Stevioside and rebaudioside) a non-nutritive, high potency sweetener and is of therapeutic values such as anti hyperglycemic, anticancerous and antihypertensive agent. But seed germination is very poor so plant tissue culture is best option for multiplication of *Stevia rebaudiana*. Present study shows brief review of shoot multiplication of *Stevia rebaudiana* by using various basal salts that is Murashige and Skoog (1962) (MS), Schenk and Hildebrandt (S & H) medium and Woody plant medium (WPM) supplemented with different concentration of BAP at (0.0, 0.5 and 1.0mg/l) or concentration of NAA at (0.0, 0.05 and 0.10 mg/l) was investigated for in vitro shoot multiplication of *Stevia rebaudiana* (Bert.) using shoot tip explants. The best results obtain from WPM medium supplemented with BAP concentration of 1.0 mg/l for shoot multiplication.

Key Words: *Stevia rebaudiana* (Bert.), MS Medium, S & H Medium, WPM, Multiplication rate.

Introduction

Stevia rebaudiana (Bert) is an herbaceous perennial plant of the Asteraceae family, native to Paraguay (South America). It is a natural non caloric sweet testing plant used around the world for its intense sweet taste. Diterpene glycosides produced by *Stevia rebaudiana* (Bert) leaves are many times sweeter than sucrose. They can be utilized as a substitute to sucrose (Robinson 1930; Soejarto *et al*. 1982, 1983; Lyakhoukin *et al*. 1993; Matsui 1996; Megeji *et al*. 2005). *Stevia rebaudiana* (Bert), is non-toxic, non-calorie, non-plaque, non-fermentative, flavor enhancing, non-carcinogenic, non-addictive sweetness. Stevioside and rebaudioside induce insulin secretion and the former acts as anti-tumor agent. Propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in important features like sweetening levels and composition (Nakamura and Tamura, 1985). Vegetative propagation too is limited by the lower number of individuals that can be obtained simultaneously from a single plant (Sakaguchi and Kan, 1982). Due to the above-mentioned difficulties, tissue culture is the only alternative for rapid Multiplication of *Stevia rebaudiana* (Bert), plants.

Material and Method: -

Plant Collection: -

1cm length of shoot tip segments of *Stevia rebaudiana* (Bert) collected from the established in vitro shoot cultures at Tissue culture Labortory, Cadilla Pharmaceutical limited, Ahmedabad, were used for experiments.

Culture Medium: -

Separate stock solutions were prepared according to the composition of the medium and were stored at 7-8 °C temp. The pH of the media was adjusted to 5.8 ± 0.1 using 0.1 N NaOH or 0.1 N HCL. Various concentration of Cytokinin (BAP) in combinations with Auxin (NAA) and alone i.e. T1 (BAP 0.0 mg/l, NAA 0.0 mg/l), T2 (BAP 0.05 mg/l, NAA 0.0 mg/l), T3 (BAP 0.10 mg/l, NAA 0.0 mg/l), T4 (BAP 0.0 mg/l, NAA 0.05 mg/l), T5 (BAP 0.05 mg/l, NAA 0.05 mg/l), T6 (BAP 0.10 mg/l, NAA 0.05 mg/l), T7 (BAP 0.0 mg/l, NAA 0.10 mg/l), T8 (BAP 0.05 mg/l, NAA 0.10 mg/l), T9 (BAP 0.10 mg/l, NAA 0.10 mg/l)were incorporated in MS, S & H and WPM medium along with 3 % sucrose and 0.7 % agar and then observations were recorded.

Culture Condition: -

All the cultures were incubated under in growth room with 16 hr photoperiod of light having 65.33 µ molm-2. S-2 intensity (1500 flux). Temperature of growth room was maintained at 26 ± 2 °C with 55-60 % relative humidity.

Results and Discussion: -

In the present experiment, attempts were made to study the effect of various basal salts and phytohormones on various aspects of shoot multiplication in *Stevia rebaudiana* (Bert). Various concentration and combination of BAP and NAA were used in MS medium, S & H medium and WPM medium, and observed for the morphogenic responses of explants of *Stevia rebaudiana* (Bert).

After 30 days of inoculation, the highest number of shoot induction and highest length of shoot was observed in Treatment T5 (5.83) and Treatment T6 (2.52 cm) in MS basal media and The lowest average number of shoot induction and lowest length of shoot was found in Treatment T4 (0.48) and Treatment T4 (0.77) in MS basal media. In the S & H basal medium, the highest average no. of shoot induction was observed in Treatment T_9 (8.66) and the lowest average no. of shoot induction was found in Treatment T_7 (1.28). While in WPM basal medium the highest number of shoot induction and highest length of shoot was observed in Treatment T3 (15.8) and Treatment T8 (5.79 cm) and The lowest average number of shoot induction and lowest length of shoot was found in Treatment T4 (0.99) and Treatment T6 (3.08 cm). This result indicates that the high level of Cytokinin alone gives best number of shoots as compared to combination with Auxin and absence of Cytokinin in medium decreased the length of shoot. Same result shows that high level of Cytokinin proved best for the shoot induction and shoot length as compared to combination with Auxin in this plant which is supported by. [(11)] He reported that the highest Numbers of Shoots produced on medium supplemented with BAP alone were best then other media.

After 30 days of inoculation, the highest length of longest shoot was measured in Treatment T_6 (2.52 cm) and the lowest length of shoot was measured in Treatment T_4 (0.77) in MS basal media. In the S & H media the highest length of longest shoot was measured in Treatment T_4 (4.08 cm) and the lowest length of shoot was measured in Treatment T_9 (2.47 cm). While in WPM basal medium the highest length of longest shoot was measured in Treatment T_8 (5.79 cm) and the lowest length of shoot was measured in Treatment T_6 (3.08 cm). same results show that the high level of cytokinine proved best for the length of shoot reported by.

In MS basal media, the number of internodes was highest in Treatment T5 (2.28) the lowest number of internodes was obtained in Treatment T4 (0.60), In the S & H media, the highest no. of internodes was observed in Treatment T_4 (3.61) and the lowest no. of internodes was obtained in Treatment T_1 (2.77). while in WPM basal the highest number of internodes were observed in Treatment T7 (4.28) and the lowest number of internodes was obtained in Treatment T2 (2.49). This indicates that absence of Cytokinin in medium

decrease the number of internodes in MS medium while the absence of Cytokinine increases number of internodes in WPM medium. High level of Cytokinin and lower level of NAA combination is proved best for more growth of internodes as compared to Auxin alone. Similar results were reported by12.

Higher level of Cytokinin proved its ability to induce more shoots which was observed during the experiment. Lower level or absence of Cytokinin resulted into lower multiplication rate or inhibit the multiplication respectively. During experiment it was observed that the highest multiplication rate was observed in Treatment T6 (3.17) whereas the lowest multiplication rate was observed in Treatment T7 (0.32) with MS basal, In the S & H medium the highest multiplication rate was observed in Treatment T_5 (5.28), whereas the lowest multiplication rate was observed in Treatment T_1 (1.66) (Figure - 2) and the highest multiplication rate was observed in Treatment T3 (11.7), whereas the lowest multiplication rate was observed in Treatment T4 (2) with WPM basal medium. (Figure-3).

Though BAP at its high level induces more multiplication rate, it also induces the callus indicating imbalance of Auxin -Cytokinin ratio with basal medium[13] reported rapid and reproducible regeneration in vitro protocol with BA for Stevia rebaudiana (Bert) via nodal shoot multiplication. Similar observation was also reported by[13].

Table 1:- Different Combination of Phytohormones (BAP and NAA) used with MS, S & H and WPM basal medium.

Auxin (mg/l)	**Cytokinin (mg/l)**			
	BAP / **NAA**	**0.0 (mg/l)**	**0.05 (mg/l)**	**0.10 (mg/l)**
	0.0 (mg/l)	T_1	T_2	T_3
	0.05 (mg/l)	T_4	T_5	T_6
	0.10 (mg/l)	T_7	T_8	T_9

Table 2:- Data on Different Parameters under Study (Full MS basal Salts).

Treatment No.	**Growth hormone (mg/l)**		**Characters**			
	BAP	**NAA**	**Avg. No. of Shoots**	**Length of shoot (cm)**	**No. of Internodes**	**MR***
1	-	-	1.06	1.28	1.50	0.83
2	0.5	-	4.38	1.43	1.89	2.11
3	1.0	-	3.83	1.63	1.71	1.89
4	-	0.05	0.48	0.77	0.60	0.44
5	0.5	0.05	5.83	1.83	2.28	2.89
6	1.0	0.05	5.55	2.52	2.22	3.17
7	-	0.10	0.65	1.05	1.11	0.32
8	0.5	0.10	4.44	1.30	1.43	2.22
9	1.0	0.10	2.72	0.92	0.88	1.50

Table 3:- Data on different parameters under study (S & H basal salts).

Treatment No.	Growth hormone (mg/l)		Characters			
	BAP	NAA	Avg. No. of Shoots	Length of shoot (cm)	No. of Internodes	MR*
1	-	-	1.51	3.54	3.50	1.66
2	0.5	-	6.78	2.77	3.12	4.11
3	1.0	-	7	2.48	3.23	4.60
4	-	0.05	1.62	4.08	3.61	1.83
5	0.5	0.05	6.78	3.63	3.33	5.28
6	1.0	0.05	7.5	3.22	3.05	5.22
7	-	0.10	1.28	3.66	3.17	1.72
8	0.5	0.10	6.4	2.55	2.90	4.11
9	1.0	0.10	8.66	2.47	2.77	4.72

Table 4:- Data on Different Parameters under Study (WPM Basal Salts).

Treatment No.	Growth hormone (mg/l)		Characters			
	BAP	NAA	Avg. No. of Shoots	Length of shoot (cm)	No. of Internodes	MR*
1	-	-	1.55	3.44	2.83	2.04
2	0.5	-	8.11	3.37	2.49	6.66
3	1.0	-	15.8	3.67	2.77	11.7
4	-	0.05	0.99	3.73	3.17	2
5	0.5	0.05	10.5	4.41	3.34	10.2
6	1.0	0.05	6.40	3.03	3.22	6
7	-	0.10	1.55	4.16	4.28	2.45
8	0.5	0.10	11.1	5.79	3.99	10.6
9	1.0	0.10	13.3	5.33	3.95	10.6

*Multiplication rate

Figure 1: - *In Vitro* Growth in full MS Media Supplemented with Cytokinin and Auxin.

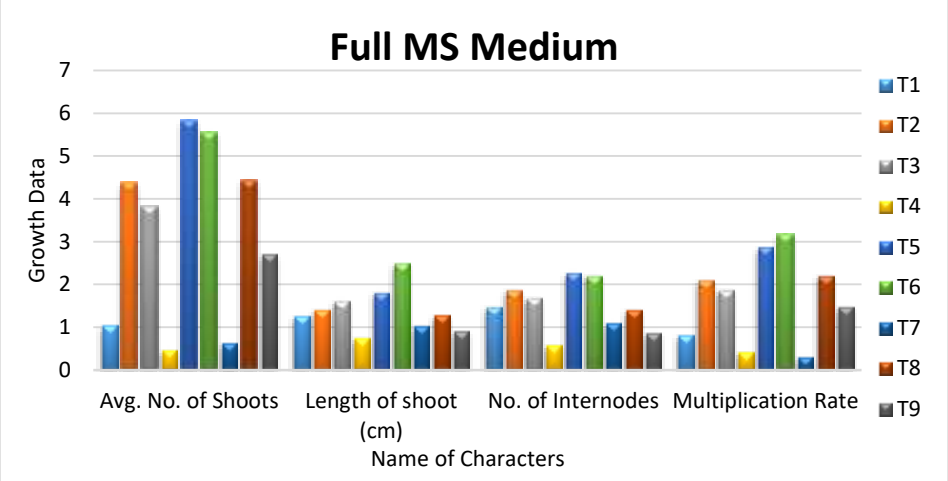


Figure 2: - *In Vitro* Growth in S & H Media Supplemented with Cytokinin and Auxin.

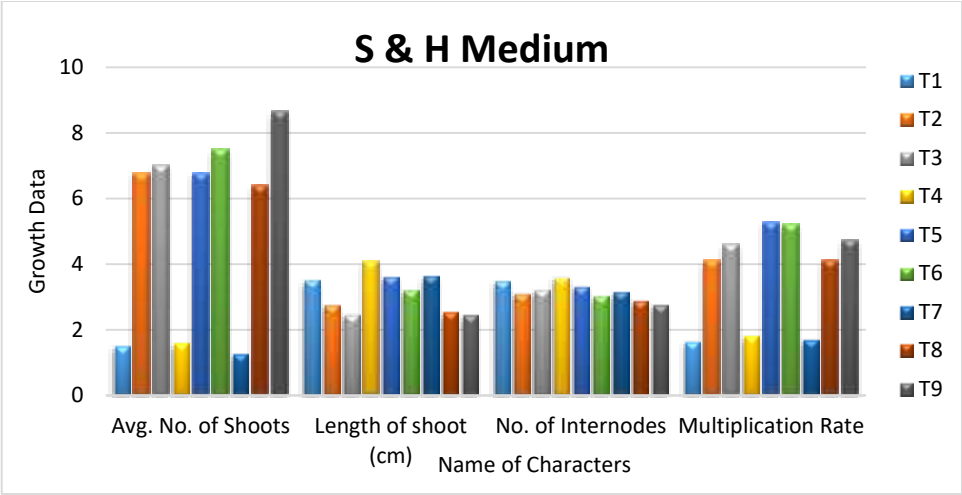
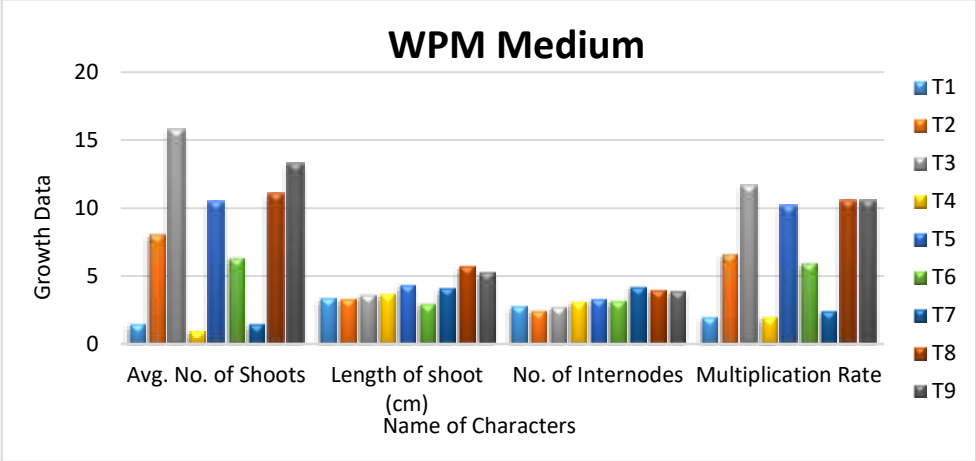


Figure 3: - *In Vitro* Growth in WPM Media Supplemented with Cytokinin and Auxin.



Conclusion:

In present experiment, single shoot of *stevia rebaudiana* (Bert) was used as an explants for different experiment based on the purpose of study, different level of phytohormones, basal salts and their combination were made to study their response on explants growth and development. Main findings of study for the characters viz, average no. of shoot, average length of shoot, average no. of internodes, multiplication rate, root induction, and callus growth are concluded as follows.

For no. of shoots, WPM is the best which indicated the more requirement of NH_4^+ for shoot development of *Stevia rebaudiana* (Bert). By exhibiting the normal behaviour of cytokinin i.e. BA at 1.o mg/l, WPM basal gave the highest multiplying rate among all treatment under study. It also supports the requirement of NO_3^- over NH_4^+. The normal effect of cytokinin and auxin was not seen in S & H also. The multiplication rate was also lower then WPM salts. It was may be due to lower NH_4^+ and higher NO_3^-.

For the study it can be concluded that *Stevia rebaudiana* (Bert) requires acidic phase of medium. High ratio of $NH4^+$/ NO_3^- and presence of higher Ca^{+2} and SO_4^{-2} were also beneficial for the higher multiplication. *Stevia rebaudiana* (Bert) also prefers the low salt combination for the best results.

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