

Influence of Additives on Enhanced *in Vitro* Shoot Multiplication of *Stevia rebaudiana* (Bert.)—An Important Anti Diabetic Medicinal Plant

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ABSTRACT

The present study was designed to develop an efficient protocol for micro propagation of *S. rebaudiana* from nodal explants and study the influence of additives on enhancement of shoot proliferation. A two-step protocol has been standardized in which, first step comprising growth hormones concentration is optimized and it was found that MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA turned out to be the best treatment for shoot induction. In the second step, the best treatment for shoot induction was fortified with different growth additives for further shoot proliferation. Among the different types of additives used, casein hydrolysate at 0.05% (w/v) was found to be most effective, resulted with maximum of 15.0 shoots. 90% regeneration frequency and shoot length of 6.0 cm were recorded per explant. Thus, the procedure described is a quick and reliable method which could be applied for efficient large scale propagation, genetic transformation assays and secondary metabolite production of *Stevia*.

KEYWORDS

Stevia rebaudiana; Nodal Explants; MS Medium; Growth Hormones; Additives; Shoot Proliferation

1. Introduction

Stevia rebaudiana (Bert.) is a herb from the asteraceae family indigenous to higher elevations of northern Paraguay near Brazilian borders [1], now being cultivated in many tropical and sub tropical countries. The leaves of *Stevia rebaudiana* mainly synthesize non-caloric thermo stable intense sweeteners, mainly steviolosides that find applications in food and pharmaceutical industries. Purified steviolosides imparts about 300 times sweetener than sucrose (0.4% solution) apart from being a calorie free biosweetener [2]. Another glycoside namely rebaudioside “A” which is about 400 times sweeter than sucrose is also present in *Stevia* leaves. *Stevia rebaudiana* leaves accumulate mixture of diterpene glycosides derived from the tetra cyclic diterpene steviol. These natural products

taste intensely sweet and have similar biosynthetic origins to those of gibberellic acid [3].

About 30 million Indians are presently suffering from diabetes and it is estimated that by 2025 India’s contribution to the diabetic global population would be a whooping 80 million [4]. Therefore, the wave of “sugar free” has become a welcome trend. *Stevia* shows calorie free high potency sweetener does not contain calcium cyclamate, saccharin, and aspartame and causes no side effects [5]. The sweet compounds pass through the digestive process without chemically breaking down making *Stevia* safe for diabetes and obese people [6]. *Stevia* leaf tea offers excellent relief for an upset stomach. Like cucumber, a wet *stevia* leaf bag provides a cooling effect to eyes and helps to reduce weight and blood sugar management [7]. The *Stevia* powder also helps in rejuvenating the pancreatic gland [8]. Stevioloside and rebaudioside

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induce insulin secretion [9] and the former acts as anti-tumour agent [10].

Propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in important features like sweetening levels and composition, further vegetative propagation is not very successful because of low yield of cuttings from mother plants [11]. Hence, *in vitro* propagation is the sound technique for mass propagation of this plant because the species is becoming rare in natural habitat condition due to habit destruction and over exploitation.

Minor quantities of complex organic nutrients like amino acids, peptides, fatty acids, carbohydrates, vitamins on high proliferation and regeneration of plants *in vitro*, are well documented in many medicinal crops [12-14].

Optimization of regeneration protocol supporting the action of growth additives as a supplement of growth regulators will be useful in the establishment of reliable regeneration protocols for various medicinal herbs of economic importance; however, no such attempts have been made in *Stevia*.

In this study, an attempt has been made to understand the role of different growth additives at various concentrations on enhancing the efficiency of shoot multiplication rate in *Stevia rebaudiana*.

2. Materials and Methods

2.1. Explant Preparation

Stevia rebaudiana plants were maintained in shade nets at Indian Institute of Horticultural Research, Bangalore, India. Surface sterilization of nodal explants was performed by washing the explants under running tap water. It was then washed with 1% (v/v) labolene solution and rinsed thoroughly with sterile water. The explants were disinfected by soaking in 0.2% (w/v) bavistin (Carbendazim 50% WP fungicide) for 5 min and washed five times with sterile distilled water. Surface decontamination was performed under aseptic conditions by immersing the explants in 0.1% (w/v) mercuric chloride for 2 min followed by rinsing 4 - 5 times with sterile distilled water to remove all the traces of mercuric chloride. Nodal explants were further trimmed at the cut ends and they were used as explants for *in vitro* regeneration.

2.2. Medium and Culture Conditions

Murashige and Skoog (MS) medium [15] used in this study was prepared by adding 3% (w/v) sucrose to MS basal salts and vitamins. Different concentrations of plant growth regulators (PGRs) were supplemented to the medium and the pH was adjusted to 5.7 before adding 0.7% (w/v) agar. MS medium devoid of growth regulators

served as control. Agar fortified medium (10 mL) was dispensed into culture tubes and plugged with non absorbent cotton plugs. Culture bottles containing 50 mL medium were tightly closed with polypropylene caps. The tubes and bottles containing the media were autoclaved at 121°C for 15 min. The cultures were incubated under 16 hrs photoperiod, irradiance of 40 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ and temperature of at $24 \pm 2^\circ$.

Murashige and Skoog (MS medium composition)

Major salts (macronutrients)

• Ammonium nitrate (NH_4NO_3)	1650 mg/l
• Calcium chloride ($\text{CaCl}_2\cdot 2\text{H}_2\text{O}$)	440 mg/l
• Magnesium sulphate ($\text{MgSO}_4\cdot 7\text{H}_2\text{O}$)	370 mg/l
• Potassium phosphate (KH_2PO_4)	170 mg/l
• Potassium nitrate (KNO_3)	1900 mg/l

Minor salts (micronutrients)

• Boric acid (H_3BO_3)	6.20 mg/l
• Cobalt chloride ($\text{CoCl}_2\cdot 6\text{H}_2\text{O}$)	0.025 mg/l
• Cupric sulphate ($\text{CuSO}_4\cdot 5\text{H}_2\text{O}$)	0.025 mg/l
• Ferrous sulphate ($\text{FeSO}_4\cdot 7\text{H}_2\text{O}$)	27.80 mg/l
• Manganese sulphate ($\text{MnSO}_4\cdot 4\text{H}_2\text{O}$)	22.30 mg/l
• Potassium iodide (KI)	0.83 mg/l
• Sodium molybdate ($\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$)	0.25 mg/l
• Zinc sulphate ($\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$)	8.60 mg/l
• $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$	37.20 mg/l

Vitamins and organics

• Myo-Inositol	100 mg/l
• Niacin	0.5 mg/l
• Pyridoxine·HCl	0.5 mg/l
• Thiamine·HCl	0.1 mg/l
• Glycine	2.0 mg/l

2.3. Bud Break and Shoot Multiplication

For axillary shoot initiation and multiplication, nodal explants were inoculated on MS medium supplemented with different hormones such as 6-benzyl amino purine (BAP) (0.5 - 3.0 mg/l) and Kinetin (Kin) (0.1 - 1.0 mg/l) and in combination with auxins such as NAA and IAA (0.1 mg/l). Combination of both the cytokinins (BAP - 1.0 - 2.0 mg/l and KIN - 0.50 mg/l) was also used for this study. Total number of shoots per explant and length of the shoots were recorded after 4 weeks of culture.

2.4. Fortification of Growth Additives to Culture Medium

To study the influence of various additives on shoot proliferation, explants were cultured on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA along with different growth additives such as casein hydrolysate (CH), malt extract (ME), yeast extract (YE) (0.025%, 0.050%, 0.075% & 0.1%, w/v) and coconut water (CW) (5%, 10%, 15%, 20%, v/v). Sub cul-

turing was done two times in the same medium after a 4 weeks interval. The number and length of the shoots per explant were recorded after 4 weeks of culture.

2.5. Culture Conditions and Statistics

The cultures were incubated under 16 hrs photoperiod, irradiance of $40 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ and temperature of $24^\circ\text{C} \pm 2^\circ\text{C}$ with a relative humidity of 55% - 60%. Explants were sub cultured every 4 week, data were recorded after four weeks of multiple shoot induction, each treatment had five replicates of 10 explants each. All data are statistically analyzed by analysis of variance (ANOVA).

3. Results and Discussion

3.1. Influence of Plant Growth Regulators on Multiple Shoot Induction

Axillary shoot system is the best suited *in vitro* culture system for conservation purposes, since it eliminates the risk of somaclonal variation [16]. Bud break from nodal explant were observed *in vitro* one week after inoculation on MS basal medium supplemented with various plant growth regulators.

3.2. Influence of Cytokinins

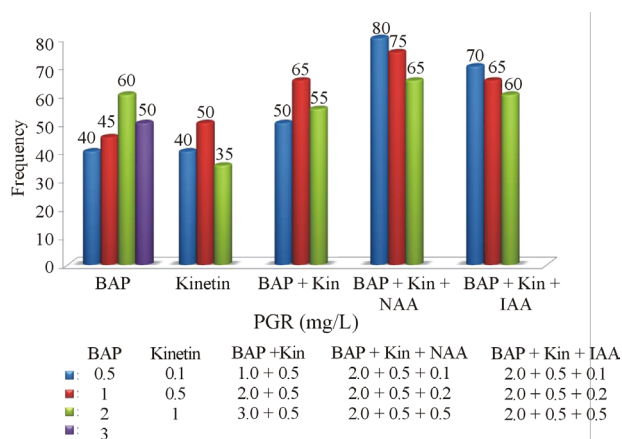
Morphogenetic responses of nodal explants to various cytokinins alone (BAP and Kin) and in combination with auxins (NAA and IAA) are summarized and presented in **Table 1**. Nodal explants that were cultured on MS medium devoid of PGR's (control) did not showed any regeneration response. However, the multiplication rate and shoot number were remarkably increased in cultures supplemented with PGR's. The percentage response (represented in **Graph 1**), average number of shoots per explant as well as the mean length of shoots varied with the type of growth regulator used as well as its concentration and the results have been presented (**Table 1**).

Among the two cytokinins tested, BAP was found to be more efficient than Kin with respect to shoot initiation and subsequent multiplication. Medium supplemented with BAP alone (1.0 - 3.0 mg/L) recorded 2.0 - 2.80 number of shoots with an average shoot length of 1.2 - 2.4 cm. Among various concentrations of BAP, 2.0 mg/L BAP was proved to be most effective with 60% regeneration and a maximum of 2.80 number of shoots with 2.40 cm shoot length per culture. Similar findings were reported earlier in nodal explants of *Orthosiphon stamineus* cultured on MS medium containing BAP [17]. In Kinetin alone (0.1 - 1.0 mg/l) supplemented medium, an average of 1.2 - 2.0 shoots with 2.6 - 5.6 cm shoot length was obtained. The superiority of BAP over Kinetin on induction of multiple shoots has been reported in several

Table 1. Effect of BAP alone or in combination with Kinetin & NAA/ IAA on multiple shoot induction from nodal bud explants of *S. rebaudiana* in MS medium. Observations were taken after 4 weeks of culture.

Growth regulator (mg/l)	Avg. no. of shoots Mean \pm SE	Avg. shoot length (cm) Mean \pm SE
*Control	-	-
BAP		
1.0	2.25 \pm 0.75	1.80 \pm 0.72
2.0	2.80 \pm 0.83	2.40 \pm 0.19
3.0	2.00 \pm 0.50	1.20 \pm 0.95
Kinetin		
0.1	1.60 \pm 0.10	2.62 \pm 0.40
0.5	2.00 \pm 0.12	3.00 \pm 0.12
1.0	1.20 \pm 0.90	5.60 \pm 1.10
BAP + Kinetin		
1.0 + 0.5	3.40 \pm 0.28	3.05 \pm 0.12
2.0 + 0.5	5.20 \pm 0.20	4.20 \pm 0.21
3.0 + 0.5	3.00 \pm 0.50	2.60 \pm 0.22
BAP + Kinetin + NAA		
2.0 + 0.5 + 0.1	8.60 \pm 0.10	5.60 \pm 0.33
2.0 + 0.5 + 0.2	6.00 \pm 0.15	4.80 \pm 0.90
2.0 + 0.5 + 0.5	5.00 \pm 0.50	4.20 \pm 0.70
BAP + Kinetin + IAA		
2.0 + 0.5 + 0.1	5.00 \pm 0.12	4.40 \pm 0.60
2.0 + 0.5 + 0.2	4.00 \pm 0.15	3.80 \pm 0.90
2.0 + 0.5 + 0.5	3.25 \pm 0.75	2.50 \pm 0.30
SEM	0.36	0.35
CD@ 5%	1.02	0.99
CD@ 1%	1.36	1.32

*Control: MS medium devoid of growth regulators treated as control.



Graph 1. Frequency of regenerated shoots on MS medium supplemented with growth regulators at different concentrations.

medicinal species such as *Salvia branchyodon* [18], *Pogostemon heyneanus* [19] and *Penthorum chinense* [20]. However, the highest mean shoot length (5.6 cm) was recorded in Kinetin (1.0 mg/l) cultures. Earlier *In vitro* studies also reported the effect of kinetin on shoot length in *Celastrus paniculatus* [21]. Upon increasing the concentration of growth regulators, a reduction in the number of shoots per culture was observed. Similar trend was observed in *Solanum nigrum* using BAP at higher concentrations [22].

MS medium supplemented with a combination of (BAP 1.0 - 3.0 mg/l) and Kinetin (0.5 mg/l) in different concentrations was found to be effective in enhancing the shoot number and length per explant (Table 1). Maximum regeneration response of 65% was observed with BAP and Kin at 2.0 mg/l and 0.5 mg/l respectively, producing an average of 5.2 number of shoots and 4.2 cm shoot length per culture. The combined effect of BAP and Kinetin on efficient shoot induction has been well documented and proved in another asteraceae member, *Capsicum chinense* [23] and *Bambusa balcooa* [24].

3.3. Influence of Two Cytokinins and Auxin Combination

It is well established that proper ratio of auxin and cytokinins is necessary for morphogenesis leading the formation of complete plantlets [25]. The efficiency of the optimal concentration of BAP and Kinetin (1.0 - 3.0 mg/l) with auxins (0.1 mg/l) was also tested for multiple shoot induction. Highly efficient regeneration frequency of 60% - 80% was observed in all the combinations tested. Increase in frequency and number of shoot formation was observed when BA and Kin used in combination with auxins (both NAA and IAA at 0.1 mg/l). Similar response was also observed in the propagation of *Hemidismus* [26]. High frequency (80%) and maximum number (8.6) of multiple shoots with shoot length of (5.0 cm) were induced on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA. When BAP and Kinetin were combined and used at their optimal concentrations with NAA, healthy shoots were produced. MS medium supplemented with BA and kinetin in combination with NAA found to be the best in shoot proliferation compared with same combination using NAA. The maximum regeneration frequency (70%), shoot number (5.0) and shoot length (4.4 cm) were observed in the combination of 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l IAA.

In the present study BAP and Kinetin in combination with NAA was found to be an effective combination for shoot regeneration and multiplication. Similar regeneration response was reported in *Gymnema sylvastre* [27] using BAP, Kinetin and NAA at their optimum concentrations.

3.4. Influence of Growth Additives on Multiple Shoot Induction

Synergistic effect of complex organic extracts such as casein hydrolysate, coconut water, malt extract and yeast extract was studied after determining the optimum cytokinin and auxin levels for shoot sprouting to improve the shoot multiplication rate *in vitro*. The proliferation effect of *in vitro* shoot buds on growth additives induced medium with different concentrations of cytokinins in combination with auxins showed variation in the regeneration percentage (represented in Graphs 2 and 3), no of shoots and shoot length and the results have been presented (Table 2 and Figure 1).

3.5. Casein Hydrolysate (CH)

Casein hydrolysate can be a source of calcium, phosphate, several micro elements, vitamins and most importantly, a

Table 2. Effect of different growth additives on multiple shoot regeneration from nodal bud explants of *S. rebaudiana* in MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kinetin + 0.1 mg/l NAA. Observations were taken after 4 weeks of culture.

Growth additive	Concentration	Avg. no. of shoots Mean ± SE	Avg. shoot length Mean ± SE
*Control		8.60 ± 0.10	5.0 ± 0.33
Casein hydrolysate (W/V)	0.025	10.0 ± 0.50	5.4 ± 0.30
	0.050	15.0 ± 0.62	6.0 ± 0.80
	0.075	11.0 ± 0.65	5.2 ± 0.70
	0.1	9.0 ± 0.10	4.4 ± 0.01
Coconut water (V/V)	5	8.6 ± 0.10	5.8 ± 0.30
	10	9.4 ± 0.50	6.4 ± 0.84
	15	11.0 ± 0.40	8.0 ± 0.10
Malt extract (W/V)	20	8.0 ± 0.22	5.6 ± 0.80
	0.025	9.0 ± 0.55	5.0 ± 0.70
	0.050	12.0 ± 0.60	6.0 ± 0.40
	0.075	10.4 ± 0.15	4.8 ± 0.50
Yeast extract (W/V)	0.1	8.0 ± 0.60	4.0 ± 0.50
	0.025	8.2 ± 0.88	4.8 ± 0.90
	0.050	10.0 ± 0.50	5.8 ± 1.00
	0.075	9.0 ± 0.55	4.6 ± 0.86
	0.1	7.6 ± 0.07	3.8 ± 0.10
	SEM	0.33	0.35
	CD@ 5%	0.96	0.99
	CD@ 1%	1.28	1.32

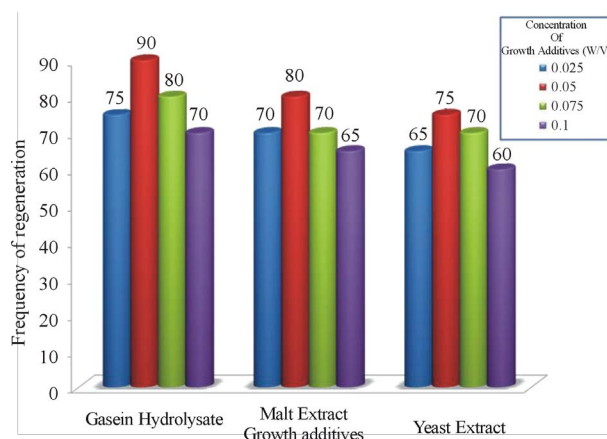
*Control: MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA and devoid of additives treated as control.



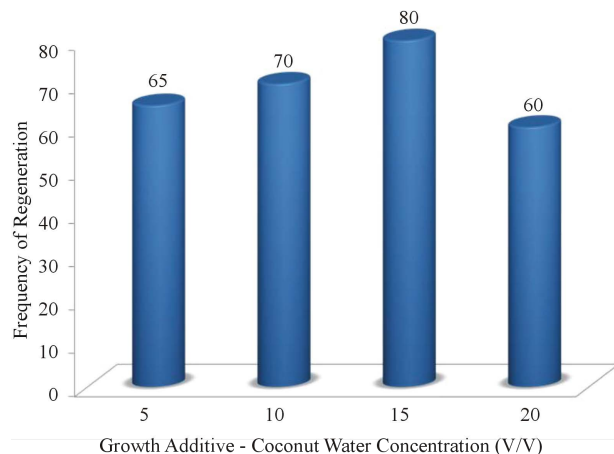
Figure 1. Shoot initiation from nodal bud explants cultured on (A) MS + BAP (2.0 mg/l). Shoot multiplication from nodal bud explants cultured on (B) MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) + 0.05% Malt extract. (C) MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) + 10% Coconut extract. (D) MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) + 0.05% Casein hydrolysate. Bar (A & C)—1 cm; Bar (B & D)—0.9 cm.

mixture of up to 18 amino acids [28]. Casein hydrolysate supplementation to the culture medium successfully induced shoot initiation within 4 - 5 days and subsequent multiplication from nodal bud explants. These results are in agreement with the previous report, where CH without growth regulators in the nutrient medium reduces the time period of shoot initiation up to 3 - 4 days as well as new shoot proliferation from nodal buds and also increased the percentage of explant response [29].

High frequency (90%) and maximum number (15) of shoots was obtained on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA when 0.05% CH was added. Similar results have also been found with CH in *Anogeissus pendula*, *A. latifolia* *in vitro* studies [30]. Induction of healthy shoot formation has



Graph 2. Frequency of regenerated shoots on MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) supplemented with growth additives at different concentrations.



Graph 3. Frequency of regenerated shoots on MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) supplemented with growth additive-Coconut Water at different concentrations.

been reported in *Crataeva nurvala* [31] using CH as medium supplement. Higher concentrations of casein hydrolysate (0.075% & 0.1%) resulted with no further increase in the number of shoots. The higher concentrations of CH (0.1%) in the growth medium did not support shoot growth as a consequence of which shoots remained compact and stunted. At higher doses (0.075% & 0.1% CH) basal callus was formed at the cut ends, thereby which further diminishes the absorbance of nutrients resulted in stunted growth of the shoots (Figure 2). This is possibly due to maladjustment of *in vitro* cultured cells to the excessive organic nitrogen in the medium. Similar research findings have been reported in *Asclepias* regeneration studies, where CH in the growth medium produces appreciable amount of callus at the cut ends and readily turned brown and retarded the vigorous growth of shoots [32].

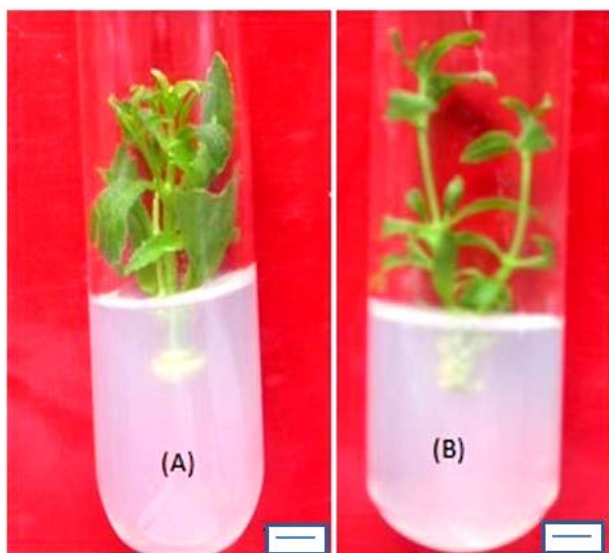


Figure 2. Shoot showing basal callus growth at cut portions from nodal explants cultured on (A) MS + BAP (2.0 mg/l) + NAA (0.1 mg/l) + 0.75% Casein hydrolysate. (B) and (C) MS + BAP (2.0 mg/l) + Kin (0.5 mg/l) + NAA (0.1 mg/l) + 0.75% Yeast extract. Bar (A)—1 cm; Bar (B)—0.9 cm.

3.6. Coconut Water (CW)

Coconut water is the colorless liquid endosperm of green coconuts (*Cocos nucifera*). However, coconut milk is the extract of white, solid endosperm of matured coconut after grinding and squeezing. Both of them are used in tissue culture media, but the coconut water is the more complex combination of compounds [33]. CW contains growth hormones and is liberally made use in tissue culture [12]. When added to the culture medium containing auxin, the liquid CW can induce plant cells to divide and grow rapidly this is clearly evident in the present research addition of CW into the media enhanced shoot growth and maximum shoot length (8.0 cm) was obtained on 15% CW supplemented medium. Similar research findings were obtained earlier with addition of CW enhanced the shoot growth and development of medicinal plants propagated *in vitro* [34]. Among the different concentrations tried, high frequency (80%) and maximum number (10.0) of shoots was obtained on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA when 15% CW was added. Whereas at higher concentrations (20%) callus formation at the cut ends was noted, there by which diminishes the shoot multiplication rate further.

3.7. Malt Extract (ME)

Malt extract, mainly a source of carbohydrates, was shown to initiate embryogenesis in nucellar explants [35]. Some plant hormones, such as auxins and gibberellins

have been identified in malt extract [36]. In malt extract fortified medium shoot initiation and multiplication was successfully induced from axillary bud explants. Among the different concentrations of malt extract used, at 0.05% ME supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA maximum number (12) of shoots with maximum shoot length (6.0 cm) was obtained. Similar trend was reported earlier, where ME was found to be necessary for improving the quality and quantity of shoot proliferation from nodal bud explants in *Gymnema sylvestris* [27]. In the present study shoot bud initiation and multiplication were higher at lower concentrations of ME (*i.e.* 0.025%, 0.05%), whereas at higher doses (*i.e.* 0.075%, 0.1% ME), the shoot number and shoot length was slightly decreased due to callus formation at the cut ended portions.

3.8. Yeast Extract (YE)

Addition of yeast extract to culture medium enhances the shoot proliferation rate *in vitro*. Among the different concentrations of yeast extract used, at 0.05% YE supplemented with 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA maximum number (10) of shoots with shoot length of (5.8 cm) was obtained. The promotive effect of YE on shoot proliferation was reported earlier in *Lavandula latifolia in vitro* studies [37]. In the present study similar to other growth additives such as CH, CW and ME, YE also favours the shoot multiplication, whereas at higher concentrations shoot multiplication rate was decreased due to basal callus formation at the cut ends of nodal explants.

Application of growth additives is adapted to the cultural needs [38] *i.e.* objectives of the experimental studies like micro propagation, regeneration, cytodifferentiation, androgenesis, biosynthesis of secondary metabolites and biotransformation of cells as well as the particular plant species taken. In the present study growth additives such as CH and ME have promontory effect on *in vitro* shoot multiplication rate compared to other growth additives. Where as CW has pronounced effect on axillary shoot length besides the number of shoots produced. YE have less beneficial effect on shoot proliferation and shoot length *in vitro*. Over all Casein hydrolysate has turned out to be the best growth additive for regeneration of *Stevia rebaudiana*. Hence, optimum medium composition for high frequency shoot multiplication where outlined.

4. Conclusion

The outlined procedure offers a potential system for improvement, conservation and mass propagation of *S. rebaudiana* from nodal bud explants. A two-step procedure

has been standardized for maximum shoot proliferation, where in the first step growth hormones concentration has been standardized and it was found that 2.0 mg/l BAP + 0.5 mg/l Kin + 0.1 mg/l NAA, proven as the best treatment. In the second step, the best hormone treatment was fortified with growth additives for further shoot proliferation, among which 0.05% CH was found to be the best growth additive concentration for enhancing shoot multiplication. The efficient regeneration protocol developed in the presented study for enhanced shoot multiplication seems to be beneficial for producing *Stevia* cultures on large scale to meet the industrial needs. Apart from this the present study appears highly useful for future line of work involving metabolic engineering of *Stevia* genes leading to genetic improvement of this highly commercial medicinal herb.

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Abbreviations

BAP—6 Benzyl Amino Purine;
Kin—Ki-netin;
NAA—Naphthalene Acetic Acid;
IAA—Indole Acetic Acid;
CH—Casein Hydrolysate;
CW—Coconut Water;
YE—Yeast Extract;
ME—Malt Extract.