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(RESEARCH ARTICLE)

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In vitro micropropagation and mass multiplication of *Stevia rebaudiana* Bertoni

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Abstract

Stevia rebaudiana Bertoni, an ancient perennial herb produces steviol glycosides including stevioside and rebaudioside-A that are valuable as low calorie sweeteners, about 300-400 times sweeter than saccharose. Conventional propagation methods are not produce adequate planting material. Micro propagation is an imperative technology that could be extensively exploited to meet the growing demands of elite planting material for commercially cultivated. The prime objective of this study was to established standard protocol for *in vitro* regeneration and mass multiplication in *Stevia*. In present investigation, different concentrations of Auxin and Cytokinin were tried for optimization of shoot initiation, shoot multiplication, root initiation and callus induction of *Stevia* from different explant. BAP and IBA (1.0+0.5) mg/l, IBA (2.0) mg/l shows promising results of shoot multiplication and root initiation respectively.

Keywords: *Stevia rebaudiana*; *In vitro*; Micropropagation; MS medium; Low calorie sweetener

1. Introduction

The International Diabetes Federation (2021) reports that 10.5% of the adult population (20-79 years) has diabetes, with almost half unaware that they are living with the condition [1]. In other words every single individual in ten worldwide currently suffer from diabetes. The increasing number of people living with diabetes is an alarming condition regarding to the health and wellbeing of individuals in India. To solve this issue one of the significant substitutes for table sugar is *Stevia*. *Stevia rebaudiana* Bertoni, an ancient perennial herb [2] produces steviol glycosides including stevioside and rebaudioside-A that are valuable as low calorie sweeteners, about 300-400 times sweeter than saccharose [3]. It shows heterozygous and self-incompatibility natures which lead to the lack of fertilization and due to this plant are not planted extensively [4]. For mass multiplication and production, micropropagation is a suitable alternative method and hence used.

2. Material and methods

2.1. Preparation of explant and sterilization

The explant like leaf, stem and node were collected from Wagh Nursery, Manjari Farm, Pune where they maintained stock plants under observation. All these explant were washed with running tap water for 5 minutes, followed by 70% ethanol for 2 minute and finally with distilled water for 5 minutes. Surface sterilization of explant was carried out by washing with sterile distilled water for 5 minutes followed by various concentration of mercuric chloride ($HgCl_2$), leaf explant sterilized with 0.2% whereas stem node with 0.2% of $HgCl_2$. Two more rinses in laminar airflow with sterilized

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double distill water came after it. All of these explants were cut into tiny pieces and inoculated on the appropriate medium.

2.2. Culture medium

All experiments of present study were tried on MS media (Murashige and Skoog, 1962) supplemented with varying concentrations of growth regulators. Culture medium was enriched with 40 gm sucrose and 2.5 to 3 gm clerigar for solidification, and the pH was set to 5.6-5.8. The media were steam sterilized in an autoclave at 15 psi and 121 °C.

2.3. Culture condition

After the inoculation, culture bottles were shifted to a culture room with a temperature of 25±2°C and a 16-hour photoperiod provided by cool white fluorescent cool tubes.

3. Results and discussion

Standard protocol for surface sterilization of explant was analyzed by trial and error method. Surface sterilization of leaf and stem node explant were tried with 0.1-0.5% of $HgCl_2$ for 1 to 5 minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.2% of $HgCl_2$ for leaf and stem node explant during the present study. BAP 1.0 mg/L showed most promising results of initial shoot forming and hence used for further experiments. Several concentrations ranges from 0.5-3.0 mg/L of different auxins mainly IAA, IBA, NAA and 2,4-D along with BAP 1.0 mg/L were tried for the shoot multiplication. BAP and IBA (1.0 + 0.5) mg/l showed promising results of shoot multiplication in *Stevia* respectively (Table 1). After successful shoot multiplication root regeneration was achieved from both explants from different auxins having concentrations ranges from 0.5-3.0 mg/L. Lower concentration of auxin except IBA 2.0 mg/L was found effective to induced root regeneration however higher concentration revealed poor result of root regeneration (Table 2). Similar kinds of result were reported by Singh *et al*., [5].

Table 1 Impact of cytokinins and auxin on shoot multiplication after 21 days

Concentration of plant growth regulators (PGRs) (mg/l)		**Explant used**			
		Leaf		**Nodal segment**	
		No. of shoots/ explant	**Shoot induction (%)**	**No. of shoots/ explant**	**Shoot induction (%)**
BAP + IAA	1.0 + 0.5	2.3±0.335	50	2.0±0.258	55
	1.0 + 1.0	2.5±0.372	80	2.4±0.305	80
	1.0 + 1.5	2.4±0.339	60	2.3±0.213	75
	1.0 + 2.0	2.3±0.366	55	2.2±0.326	60
	1.0 + 2.5	2.1±0.276	55	2.1±0.276	60
	1.0 + 3.0	2.1±0.276	50	1.8±0.200	50
BAP + IBA	1.0 + 0.5	6.1±0.348	100	5.5±0.477	100
	1.0 + 1.0	5.1±0.276	90	5.0±0.258	95
	1.0 + 1.5	5.0±0.258	90	4.7±0.260	90
	1.0 + 2.0	4.6±0.266	80	4.8±0.249	80
	1.0 + 2.5	4.5±0.307	70	4.3±0.260	75
	1.0 + 3.0	4.1±0.276	75	4.4±0.266	70
BAP + NAA	1.0 + 0.5	1.9±0.233	60	2.4±0.305	55
	1.0 + 1.0	3.2±0.249	70	2.9±0.233	70
	1.0 + 1.5	2.8±0.249	65	2.9±0.233	70

	1.0 + 2.0	2.8±0.200	60	2.7±0.152	55
	1.0 + 2.5	2.7±0.260	60	2.6±0.221	55
	1.0 + 3.0	1.9±0.233	55	2.3±0.213	50
BAP + 2,4-D	1.0 + 0.5	3.2±0.290	70	3.0±0.210	70
	1.0 + 1.0	3.2±0.249	85	3.2±0.249	85
	1.0 + 1.5	2.9±0.276	75	3.0±0.210	70
	1.0 + 2.0	2.8±0.249	70	2.9±0.233	70
	1.0 + 2.5	2.7±0.335	60	2.6±0.221	70
	1.0 + 3.0	2.6±0.305	60	2.5±0.268	60

Table 2 Impact of different concentrations of auxins on root initiation

MS + Hormone	**Concentration (mg/L)**	**Avg. no. of roots per explant**	**Root length (cm)**	**Observations**
IAA	0.5	12.6	0.61±0.033	Small, thin
	1.0	16.2	0.68±0.027	Normal, thick
	1.5	13.2	0.62±0.023	Thin
	2.0	13.3	0.56±0.020	Thin
	2.5	11.1	0.46±0.028	Thin
	3.0	-	-	-
IBA	0.5	14.7	0.48±0.023	Thin
	1.0	15.4	0.53±0.020	Thin
	1.5	16.3	0.67±0.028	Normal, thick
	2.0	18	0.88±0.034	Dense and thick
	2.5	15.9	0.59±0.022	Thin
	3.0	14.9	0.41±0.022	Small, thin
NAA	0.5	15.4	0.58±0.023	Normal, thick
	1.0	13.7	0.49±0.022	Thin
	1.5	13.6	0.47±0.024	Thin
	2.0	13.5	0.38±0.023	Small, thin
	2.5	12.1	0.29±0.022	Small, thin
	3.0	-	-	-
2,4-D	0.5	13.7	0.45±0.015	Normal, thick
	1.0	17.8	0.72±0.023	Dense and thick
	1.5	15.8	0.59±0.026	Normal, thick
	2.0	15.7	0.48±0.018	Thin
	2.5	13.7	0.42±0.018	Thin
	3.0	12.8	0.38±0.023	Small, thin

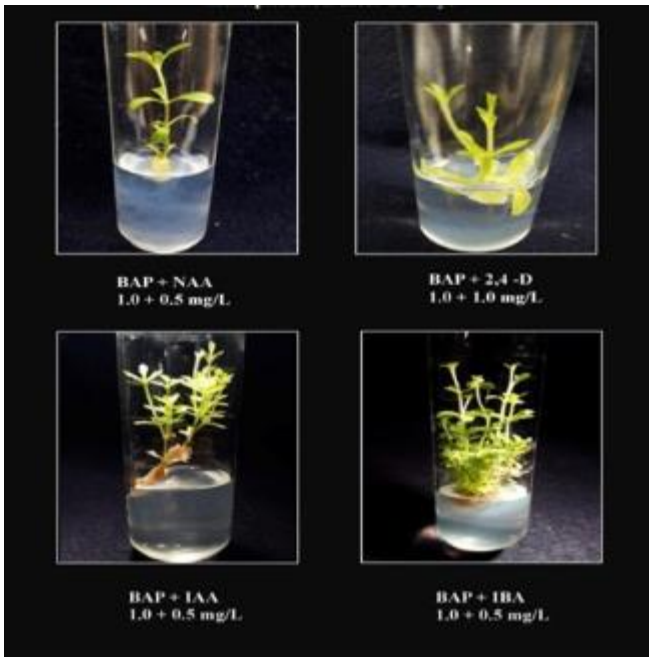

Figure 1 Impact of cytokinins and auxin on shoot multiplication after 21 days

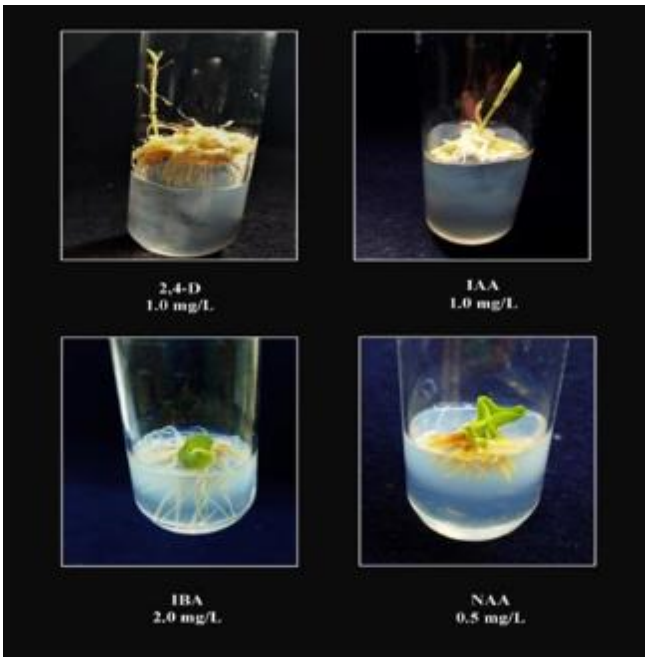

Figure 2 Impact of different concentrations of auxins on root initiation

4. Conclusion

Present research work was carried out to standardize the protocol for *in vitro* regeneration of *Stevia rebaudiana* Bertoni. Maximum shoot induction and multiplication was recorded 100% with 6.1±0.348 and 5.5±0.477 No. of shoots/ explant in leaf and nodal segment as explant at 1.0 mg/L of BAP in combination with 0.5 mg/L of IBA respectively. Thick and dense roots were obtained in IBA 2.0 mg/L with highest root length 0.88±0.034 cm. If these plants propagated through modern techniques like tissue culture, raw Material could be utilized as natural sweetener. Present piece of work is useful for developing micropropagation and mass multiplication of *Stevia* as well.

Compliance with ethical standards

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Disclosure of conflict of interest

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