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Effect of Sucrose Concentration on *In Vitro* Shoot Regeneration in *Stevia rebaudiana* Bertoni

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Abstract: ***Sucrose serves as the primary carbon source in plant tissue culture, influencing cellular growth and development. The present study investigates the effect of varying sucrose concentrations on shoot regeneration in Stevia rebaudiana using nodal explants. Experiments were conducted on Murashige and Skoog (MS) medium supplemented with different sucrose levels. The highest shoot proliferation (19.6±0.452) and maximum shoot length (11.66±0.172 cm) were observed at 40 g/L sucrose concentration. The study highlights the significance of optimizing sucrose levels for enhanced in vitro propagation of Stevia rebaudiana.***

Keywords: *Stevia rebaudiana*, sucrose concentration, micropropagation, plant tissue culture, *in vitro* regeneration

1. Introduction

Stevia rebaudiana Bertoni is a herbaceous perennial plant of the Asteraceae family, native to Paraguay [1]. *Stevia rebaudiana* is referred to as the sweet leaf or sugar leaf. *Stevia* is commonly known by different regional names in India, as cited in previous research [2]. It contains steviol glycosides, such as stevioside and rebaudioside-A, which serve as valuable low-calorie sweeteners and are among the significant alternatives to table sugar [3]. *Stevia* is utilised in several forms, including fresh and dried leaves, leaf powder, extracts, and liquid concentrates. The powdered leaves exhibit hypoglycemic properties and efficacy in lowering body weight. It is recommended for individuals with diabetes and those mindful of their diet [4]. *Stevia*'s sweet taste and high protein content make it a valuable raw resource in the food industry. It has several biochemical components, which show significant importance in the pharmaceutical and food industries [5]. *Stevia* germination and establishment from seed are generally poor or ineffective [6]. *Stevia* is often propagated through stem cuttings, which may establish roots quickly but need high labour inputs. To overcome this problem for mass multiplication and production, micropropagation is a suitable alternative method and hence is used. This study aims to determine the optimal sucrose concentration for *in vitro* shoot regeneration of *Stevia rebaudiana* to enhance its large-scale propagation. This research is significant as it provides insights into optimizing sucrose levels in plant tissue culture, facilitating mass propagation of *Stevia rebaudiana*, a plant valued for its natural sweeteners and medicinal benefits.

2. Material and Methods

2.1 Explant Preparation and Sterilization

The standard protocol of MS medium for micropropagation of plants was followed, which is earlier used by Misal and Chavan, 2024 [3]. The nodal segments used as an explant were collected from Khokarmoha, Beed (MS) where they maintained stock plants under observation. All these explants 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 0.2% of mercuric chloride ($HgCl_2$). Two more rinses in laminar airflow with sterilized double distill water came after it. All explants were cut into small sections and inoculated onto the appropriate medium.

2.2 Preparation of culture medium

All experiments of present study were tried on MS media (Murashige and Skoog, 1962) supplemented with varying concentrations of auxin and cytokinin. Culture medium was enriched with 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. To check the impact of different sucrose concentrations on the growth of nodal explants 20, 30, 40, and 50 g/L sucrose were tried.

2.3 Culture conditions

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. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.2% of $HgCl_2$. Several concentration ranges from 0.5 to 3.0 mg/L of different cytokinins, mainly BAP and KIN, were tested for the shoot initiation. BAP 1.0 mg/L showed most promising results of initial shoot forming and hence used for further experiments. Authors tested the effects of various sucrose concentrations after standardizing the PTC protocol.

20, 30, 40, and 50 g/L sucrose were tried along with standard MS medium. It was found that an increase in the concentration of sucrose shows significant effects on a plant's growth and development. 40 g/L sucrose showed most promising results with dense, thick, healthy and leafy shoots (Table 1 and figure 1).

Table 1: Impact of sucrose concentrations on *Stevia.*

Medium Used	Sucrose Concentration (g/L)	Average number of shoots/explant	Average Shoot length (cm)	Strength of shoots
MS + BAP (1.0 mg/L)	20	4.9±0.458	4.85± 0.073	+
	30	12.8±0.553	7.07± 0.073	++
	40	19.6± 0.452	11.66± 0.172	+++
	50	10.1± 0.348	6.89± 0.134	++

(Where, + indicates weaker shoots, ++ indicates moderate shoots, +++ indicates healthy and leafy shoots)

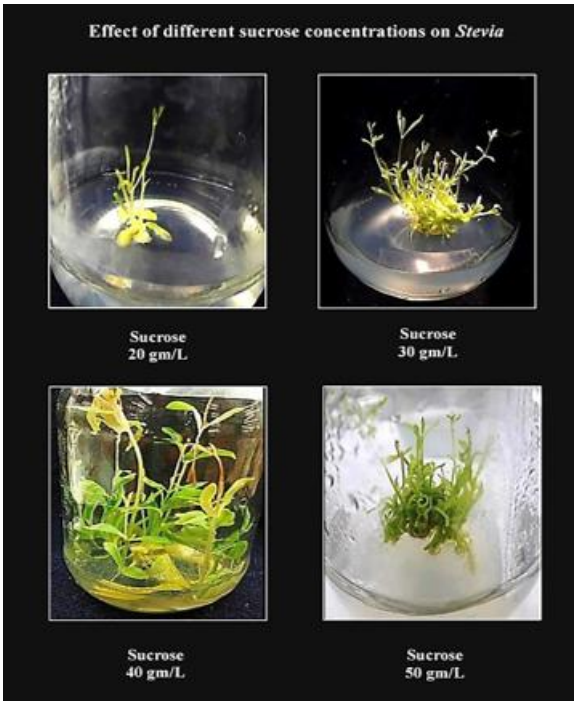

Figure 1: Impact of sucrose concentrations on the growth and development of *Stevia.*

4. Conclusion

This study demonstrates that sucrose concentration plays a crucial role in the in vitro propagation of *Stevia rebaudiana*. The results indicate that 40 g/L sucrose provides optimal conditions for shoot regeneration, yielding the highest shoot number and length. These findings contribute to the advancement of micropropagation techniques for *Stevia*, facilitating large-scale cultivation for medicinal and commercial use.

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