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Caulogenesis and Chlorophyll Content in *STEVIA REBAUDIANA* (BERT) Bertoni by Using Glutamine in the Culture Medium

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Abstract: ***Incorporation of Glutamine in MS medium significantly enhanced direct shoot bud induction from nodal explants from juvenile plants of S. rebaudiana. Shoot bud induction medium was supplemented with BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}) When the concentration of Glutamine in the induction medium 100mgL^{-1}. There was significant increase in percentage response along with healthy, well developed with dark green broader leaves. Here was remarkable increase in total biomass and chlorophyll content at 100mgl^{-1} Level in the medium. During the proliferation stage, also presence of Glutamine in the medium favored increase in shoots bud number per explants.***

Keywords: *S. rebaudiana*, MS medium, Glutamine, chlorophyll

1. Introduction

Stevia rebaudiana (Bert) Bertoni is a perennial herb, belonging to family Asteraceae. The herb is nutrient rich, contains substantial amounts of protein, calcium, phosphorous, sodium, Magnesium, zinc, Vitamin A, Vitamin C and other nutrients, yet has no caloric value. Hence, Stevia has been named as calorie free "Biosweetener of high quality". The leaves of Stevia are source of diterpenoid glycosides, such as stevioside and rebaudioside, which are estimated 100-300 times sweeter than sucrose [1]. Besides, Stevioside acts as anti-tumour agent, Stevia possess anti-fungal, antibacterial property also in addition to its other versatile uses. It is widely used as non-caloric natural sweetener for diabetic and diet conscious people. Its medicinal and commercial value led to the urgent demand for large- scale production of Stevia from elite germplasm. The seeds of Stevia show very low germination percentage [2]. *In-vitro* culture can provide genetically uniform plants in large numbers. There are few reports of micropropagation from leaf and nodal cultures.

2. Materials and Methods

2.1 Collection of Plant material

S. rebaudiana plantlets were supplied by Grow more biotech ltd. Hosur. Tamilnadu.The plantlets were grown and maintained in greenhouse. Nodes of size 1cm from field-grown plants were used as explants.

2.2 Explant Sterilization

The nodes were washed in tap water and gently washed with 1% Mercuric Chloride for 1min and then rinsed with 3 changes of sterile double distilled water. MS medium [3] was prepared with 30g sucrose and BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}) and solidified with 8g agar (Qualigens, Bacteriological Grade).The $_{p}$H of the medium was adjusted to 5.6 before autoclaving at 121° c and for 20 min.

2.3 Culture Establishment

Nodal explants were cut from stem and placed in sterile medium with supplemented BAP(2.5mgl^{-1})) and IAA(2.5mgl^{-1}).All the cultures were incubated in growth chamber at a temperature of 26° C,16h photoperiod and light intensity of 25µmol/(m^{-2} S^{-1}) provided by white fluorescent tubes. After 2 weeks, multiple shoots developed from nodal explants. Multiple shoots were subculted every 4weeks. The materials were cut into required amount and transferred to fresh medium with the help of sterile forceps inside the inoculation chamber. After subculture, they were transferred to the culture room. After 2-4 weeks, there was significant increase in shoot number. The nodal explants from these regenerated shoots were used as explants for studying the effect of different concentration of glutamine in MS medium on regeneration in Stevia nodal explants culture. The explants were placed on MS medium supplemented with, BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}). This was considered as control induction medium containing a usual BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}). Different levels of Glutamine (50, 100, 150, and 200) were added in MS medium with BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}). At the same levels as the control induction medium. The elongated shoots were sub cultured in proliferation medium supplemented with BAP (2.5mgl^{-}) and IAA (2.5mgl^{-1}).along with different levels of Glutamine (50, 100, 150, 200 µM). The cultures were kept under controlled conditions in culture chamber and observed regularly for 4 weeks.

2.4 Chlorophyll Estimation

The chlorophyll content was determined after extraction of the pigment with 80% acetone [4]. Fresh mass of leaves (1g) was ground in small volumes of acetone solution and then the extract obtained was diluted to a final volume of 4cm^{3}.

Absorbance at 662nm (chl.a) and 645nm (chl b) was measured with spectrophotometer (Shimadzu)

3. Result

3.1. Effect of Bap and IAA on multiple shoot induction and proliferation

During induction of BAP and IAA at higher concentration was found to be optimum for regenerating maximum numbers, i.e. Maximum growth response in terms of average number of shoots/ explants (15.5), average height of shoots (2.7cm) and percentage frequency of shoot forming explants (20.5%), total chlorophyll content 21(mg/g fresh weight). was obtained on culture grown on media BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}) (Fig1).

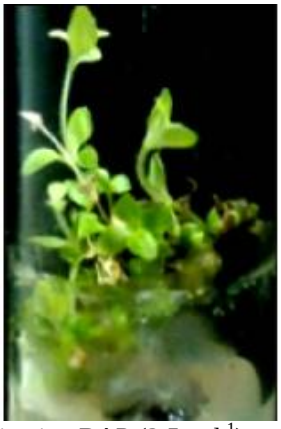

Figure 1: Combination BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1})

3.2. Effect of Bap and IAA with Glutamine on multiple shoot induction and proliferation.

Nodal explants grown on medium supplemented with BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}) with different concentration of Glutamine ((50, 100, 150, 200 mgl^{-1}). Maximum growth response in terms of average number of shoots/ explants (65.5),average height of shoots (4.5cm) and percentage frequency of shoot forming explants (80.5%),total chlorophyll content (29mg/g fresh weight) was obtained on culture grown on media BAP (2.5mgl^{-1}) and IAA (2.5mgl^{-1}) with Glutamine (100mgl^{-1}) (Fig2). (Table 1)

Figure 2: Combination BAP (2.5mgl^{-1}) + IAA (2.5mgl^{-1}) + Glutamine (100mgl^{-1}).

Table 1: Effect of Glutamine on growth of nodal explants

Concentration (mg/L)	Fresh weight(mg)	Dry weight(mg)	% frequency of shoot forming explants	Number of shoots/explants	Total chlorophyll (mg/g fresh weight)
50	826.7 ± 72.72	80.1 ± 7.04	58 ± 5.10	48 ±4.22	20 ± 1.76
100	2145 ± 188.72	209.8 ± 18.46	80.5 ± 7.08	75 ± 6.6	29 ± 2.54
150	1546 ± 136.02	148 ± 13.02	78 ± 6.86	61 ±5.32	27 ± 2.38
200	1010 ± 88.86	99.1 ± 8.72	68 ± 5.98	60 ± 5.28	23 ± 2.02

4. Discussion

L-Glutamine is the major amino acid used for proline synthesis via a constant pool of glutamate, is an unstable essential amino acid. In higher plants, Glutamine synthetase is a key enzyme involved in the assimilation of inorganic nitrogen into organic forms Mineral nutrients are the basic components of tissue culture media. Glutamine is one of the most abundant free amino acids in plants. In addition to protein and nucleotide biosynthesis, glutamine is a major donor for synthesis of amino acids and other nitrogen-containing compounds in plants. It acts as a fuel or precursor for protein synthesis. Glutamine can effectively support the growth of ***Stevia rebaudiana***. How rapidly a tissue grows and the extent the qualities of morphogenetic response are strongly influenced by the type and concentration of nutrients supplied [5]. Glutamine may enter the cell through amino acid transporters and can serve as a critical nitrogen source for plant growth and development. Stevia could effectively Use glutamine as a nitrogen source at a concentration significantly lower than that of ammonia nitrate. Based on parameters such as, shoot length and chlorophyll content. These results suggest that glutamine is a potential nitrogen source for plants in *in-vitro* culture. Glutamine has received attention for their stimulatory effect on plant growth and development of *Phaseolus vulgaris*[6]. Glutamine has been shown to induce a positive effect on the synthesis of secondary metabolites in Barley plants. The genotype, combination of growth regulator employed and supplemented of proline and glutamine showed significant effect on callus induction and subsequent plant regeneration [7]

5. Conclusions

In conclusion, optimization of the amount of amino acid in MS medium has a positive effect on *in-vitro* morphogenesis, chlorophyll, and total biomass in *Stevia rebaudiana*. (Fig.2*)*. this increase in chlorophyll content will directly affect Steviol glycosides production in Chloroplast of Stevia.

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Author Profile

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