ISSN 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

In -Vitro propagation of *Stevia rebaudiana* Bertoni: Review

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Abstract

Stevia rebaudiana (SB) Bertoni commonly Known as sweet leaf, sugar leaf or simply stevia is originally a south American wild plant but it could be found growing in semi arid habitat. It is small herb, oppositely arranged, lanceolate to oblanceolate leaves, serrated above the middle. 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. Seed germination is very poor so plant tissue culture is best option for multiplication of *Stevia rebaudiana* present study show a brief review of micropropagation of *stevia rebaudiana* by using liquid media that is Murashigue and Skoog medium and shoot proliferation of *Stevia rebaudiana*.

Keywords: *Stevia rebaudiana* Bertoni (SB)., Murashigue and Skoog (MS)., 2,4-D., IBA., NAA., BAP.

Introduction

Stevia rebaudiana Bertoni belongs to the family compositae is one of the most valuable tropical medicinal plants. It is originally a South American wild plant[1], but it could be found growing in semi-arid habitat ranging from grassland to scrub forest to mount terrain. it is commercially grown in many parts of Brazil, Paraguay, Uruguay, Central America, Israel, Thailand and China but its cultivation has now became popular world wide[2].*Stevia rebaudiana* is a natural, non-caloric, sweet tasting plant used around the world for its intense sweet taste. The sweet herb of Paraguay, stevia produces sweeteners in its leaves are natural plants products[3]. Leaves of this plant produces of zero calorie deterpene glycosides (Stevioside and rebaudioside)[4], a non-nutritive, high potency sweetener and substitute to sucrose being sweet ever than sucrose. It is small herb perennial growing up to 65 cm tall, with sessile, oppositely arranged, lanceolate to oblanceolate leaves, serrated above the middle[5]. In addition to its sweetening property it has therapeutic values such as anti-hyperglycemic, anticancerous[6-7], antihypersensitive agent[8], contraceptive properties[9] and prevention of dental caries[10] (Fujita et al., 1979). Stevia can also inhibit bacterial and fungal growth[11] . Propagation by seeds does not allow the production of homogenous plant population resulting in great variability in important features like sweetening levels and compositions[12-13]. Vegetative propagation too is limited by lowering number of individuals that can be obtained from single plant[14].

Due to the above mentioned difficulties tissue culture is the only alternative for rapid mass propagation of stevia plants. Synthetic growth regulators enhance and accelerate the production of in-vitro plants with good agronomical traits. The success of in-vitro culture depends on the growth condition of the source material medium composition and culture conditions and on the genotypes of donor plants[15]. Stevia has the ability to inhibit the growth of certain bacteria helps to explain its traditional use in treating wounds, sores and gum disease. The biological activity for compound stevia has been studied[16]. They have studied bactericidal activity of a fermented hot-water extract from *Stevia rebaudiana* Bertoni towards Enteromorrhagic Escherichia coli and other food born pathogenic bacteria. The present investigation was undertaken to find out the different approaches for in-vitro regeneration suitable sources of explants and suitable concentration of media and hormones for callus induction in micropropagation of *Stevia rebaudiana*.

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ISSN 0976-7126

Different strategies adopted

Stevia rebaudiana was identified and characterized and in - vitro work was initiated shoot tips and nodal explants were used as a explants for shoot multiplication. The explants were cut into small pieces (about 1.5c.m. long) and then washed or rinsed in a running tap water for 20 minutes after three washes with double distilled water the explants were taken under laminar air flow cabinet and surface sterilized with 0.1% mercuric chloride solution for 5 minutes under aseptic condition followed washing 7-8 times with sterilized distilled water. Then inoculation of stevia rebaudiana explants was done in Murahsige and Skoog medium[17-18].

Preparation of stock solution

After mixing all salts and organic components the pH was adjusted to 5.7 by adding 1 m NaOH. Then 5.6g. agar was mixed to the media (by using microwave oven) for making it semi solid. Total amount of media was then sub divided into four clinical flasks each containing 250 ml. Then the hormone 2, 4-D was added at 2,3,4 and 5mg/L. Each 250 ml media was then poured into 18 test tubes. Thus a total of 72 test tubes were filled with media. In the same way other 36 test tubes were prepared and finally 108 test tubes were used in the experiment. The test tubewere autoclaved 121^0C temperature and 15 psi for 20 minutes. 6-benzyladenopurine (BAP) or Kinetin (Kn) at varying concentrations (0, 0.5, 1.0 and1.5 mg) were prepared[17].

Callus induction medium

MS basal medium supplemented with 2,4-D at varying concentrations 2-5 mg/l were prepared for callus induction. After mixing all stock solutions and 3% sugar, pH of the medium was adjusted to 5.5-5.8. The agar (0.6%) was dissolved and media were dispersed in the test tubes and capped with cotton plugs. Test tubes containing media were autoclaved at 121^0C at 15 psi for 20 minutes[17].

Shoot prolferation medium

MS media in two strengths i.e. full MS and 1/2 MS supplemented with either BAP or kn at varying concentrations (0,1.0, 2.0 and 3.0 mg/L) were prepared for shoot proliferation. After mixing all stock solutions 3% or 1.5 % sugar was dissolved and the medium was dispersed in the test tubes and capped with cotton plugs and autoclaved.

Root indution medium

Full and half MS media supplemented with either 1- naphthalene acetic acid (NAA) or indole 3- butyric acid (IBA) at varying concentrations (0, 0.5, 1.0 and 1.5 mg/l) were prepared for rooting. Sugar was added.

Inoculation technique

General aseptic techniques concerning in vitro culture of the explants were followed in the present experiment. After surface sterilization the explants were cut into very small pieces (about 1 cm) and inoculated onto shoot induction and proliferation media. When the shoot was fully growth (for 1 month) the shoots were taken out from the test tube a laminar flow hood and were cut (about) 1c.m. Then the micro cuttings were sub cultured on root induction medium. All inoculations and aseptic manipulations were carried out in a laminar air flow cabinet. Before use the working surface of the cabinet was cleaned by swabbing with 90% ethyl alcohol and UV light (for 20 minutes) to reduce the chances of contamination. The instruments like scalpels, forceps, needles etc were sterilized by an alcoholic dip followed by flaming inside the laminar air flow cabinet. Other requirements like petridish, bottles, conical flasks, cotton, distilled water etc were sterilized by steam sterilization method. Before the onset of inoculations hands were washed thoroughly by soap and then swabbing with 70% ethyl alcohol. Cutting and transfer of the explants were carried out taking all possible care to ensure contaminations free inoculations.

Culture enviornment

All cultures were grown in air conditioned culture room illuminated by 40 W white fluorescent tubes with an intensity varied from 2000-3000 lux. The photo period was maintained as 16 hours light and 8 hours dark. The temperature of the culture room was maintained at 25 ± 1^0 C.

Data collection

Visual observation of culture was made every week and data were recorded after 2 week of inoculation.

Transplantation

Rooted plantlets were transplanted to puts filled with sterilized mixture of sand, vermicompost and soil (1:1:1) and growth for 5 weeks in greenhouse conditions to determine the percentage of plants that survived.[19]

ISSN 0976-7126

Summary and Conclusion

Among the explants used for the culture inter nodal segments were found to be able to produce profuse callus[17]. The results of callus production and multiplication are presented below in table 1:

Table 1: Production on *Stevia rebaudiana* Callus in different concentrations of hormones

2,4-D	Explant	Test Tube								
		1	2	3	4	5	6	7	8	9
2Mg/l	Leaf Internode Node	+ Failed Failed	+ + +	+++ ++ +	+ ++ ++	++ + +	+ + +	+ ++ +	+ ++ +	++ ++ +
3 mg/L	Leaf Internode Node	+ +++ Failed	+ +++ +	+ +++ +	++ +++ ++	+ +++ ++	++ +++ +	++ +++ ++	+ +++ +	+ +++ +
4mg/l	Leaf Internode Node	+ + +	+ ++ +	+ + +	++ + +	+ ++ ++	+ ++ +	+ + +	+ + +	+ ++ +
5mg/l	Leaf Internode Node	+ + +	++ + +	++ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

Inter nodal segments initiated callus earlier than leaf and nodal explants. The highest amount of callus was found in the MS medium with 3.0 mg /L 2,4-D. Root induction in microcuttings cutured on full MS and 1/2 MS media was significantly different for days of root imitation number of roots per plant and lengths of root is presented in the table to below:

Table 2: Effects of MS strengths on days of root initiation, number of roots per plant and length of roots

	Days of root initiation	No. of root plantlet	Lengths of root
MS	7.57b	8.61a	3.11a
1/2 MS	10.56b	0.06b	2.19b

Rooting was observed significantly earlier in full MS than in 1/2 ms (7.57 and 10.56 days respectively) and the higher number of roots per plantlet was observed in full MS than roots per plantlet in 1/2 MS (8-16 and 6.06 respectively).[18]

Shoot induction and proliferation

Both shoot tips and nodal segment explants produced multiple shoots. Initiation of shoots in shoot tip explants was earlier and significantly different from that in nodal segment 3-23 and 5-26 days respectively.

Table 3: Effect of Different explants an days of shoot initiation, number of shoots per culture, length of shoots per culture, length of shoots and number of micro cuttings per culture

Explants	Days of shoot	No. of shoots per culture	length of shoots (cm)	No. of micro cuttings per culture
Shoot tip	3.23b	1.98a	6.38	6.08a
Nodal Segment	5.26a	1.76 b	5.83	4.31b

Higher number of shoots per culture was obtained using shoot tips produced higher number of micro cuttings per culture than the nodal segments (6.38 and 5.83 respectively).[18]

Table 4: Effects of strengths of MS media on days of shoot initiation, number of shoots, length of shoots and number of micro cutting per culture

MS strength	Days of shoot initiation	No. of shoot per culture	Length of Shoots (cm.)	No. of micro cutting per culture.
MS	4.26	1.96a	6.64a	5.42
1/2 MS	4.23	1.78b	5.56b	4.96

ISSN 0976-7126

Stevia rebaudiana Bertoni is a natural alternative to artificial sweetener belongs to asteracae family and contains over hundred photochemical including well characterized stevioside. It is well known for its application in treatment of many diseases like diabetes, candidacies, high blood pressure and weight loss in various Indian traditional system of medicine. Because of instability of plantlets produced from stem cutting, micropropagation is the most reliable method of stevia propagation[20].

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