CLONALPROPAGATION OF STEVIAREBAUDIANA –THEBIOS WEETNER

Tajo Abraham* and Smrithi P K

Department of P G Studies and Research in Botany, Sir SyedCollege.
*e-mail:tajo.abraham@gmail.com

Abstract:

Steviarebaudiana is a biosweetner with zero calorific value and also economically important medicinal plant for diabetic patients.Present investigation develops a protocol for accelerating the growth of stevia callus and also large scale production by using node, leaf and callus as explants. Optimal shoot initiation was done by BAP and IAA combinations. Callus was induced by BAP and NAA in MS medium.

Key words:*Stevia,* MS medium, callus, phytohormones.

INTRODUCTION

Every year world health day is celebrated on 7thApril to make the anniversary of the founding of WHO (World Health Organization) in 1948. The theme for 2016 is "beat diabetes".Diabetic mellitus(DM)commonly referred to as diabetes is, a group of metabolic disease in which there are high blood sugar levels over prolonged period (WHO, 2014).Indian express reports that there is a fourfold rise in the number of diabetics from 108 million in 1980 to 422 million in 2014 and half of them live in India, China,USA,Brazil and Indonesia. According to their studies China,India and USA are among the top three countries with high number of diabetic populations. While the numbers climbed from 20.4million in China.In 1980to 102.9 million in 2014, the rise has been equally dramatic in India from 11.9 million in 1980 to 64.5 million in India. Prevalence of diabetes has more thandoubled for men in India and China.It has also increased by 80% among women in India. The main reason for diabetics is the artificial sweeteners we consumed through various food products. In 2009, the American Heart Association released a scientific statement calling for reductions in added sugar intake to 100 to 150 kcal/day for most Americans as a means of reducing obesity and cardiovascular disease (CVD) risk (Johnson, et al., 2009).Sucralose and xylitol have been shown through scientific research to have adverse effects. While they are low caloric and are not metabolized much within the human body, do seem to have significant effects on the microflora within the gastrointestinal system. Specifically sucralose and xylitol have been proved through scientific experimentation to cause decrease in beneficial micro-organisms (sucralose) or diarrhea (xylitol). Aspartame, has been thought to cause brain damage because of one of its component molecules, phenylalanine. Aspartame's incorporation into the general public's diet raised the issue that the population would be exposed to consuming unnecessarily high doses of phenylalanine, resulting in possible brain damage even in individuals who

do not have homozygous phenylketonuria. While the average person may not be able to consume excessively high amounts of aspartame enough to cause brain damage, some individuals with a more sedentary, diet beverage-dependent lifestyle, or individuals who rely heavily on artificial sweeteners in any way may consume excess phenylalanine and experience neurological disorders (Lebedev *et al*., 2010).

Natural sweeteners like honey, sucrose, and the new emerging trend, *Steviaare* very safe to use and *Stevia* contains zero calories. *Stevia* leaves were used by indigenous peoples in Paraguay and Brazil since before recorded history (Lee, 1979 and Soejarto, 2002). In the 1887, M. S. Bertoni, a Botanist was the first European to document *Steviaand* later on in 1931, French chemists extracted stevioside, the main sweet component in the form of an extremely sweet, white crystalline compound. Afterwards *Steviawas* considered to utilize as a sweetener for food shortages experienced by Britain during World War II, conversely, interest faded when sugar again became available. Japan used *Steviain* replace of saccharin after it was banned in the 1970s and *Steviasweeteners* have been consumed in Japan in large amount than in any other country. *S.rebaudianabelongs* to Asteraceae, a small perennial herb, growing up to 65-80 cm tall, with sessile, oppositely arranged leaves. Different species of *Stevia* contain several potential sweetening compounds, with S. rebaudiana being the sweetest of all.*Stevia* has been successfully cultivated in recent years in many areas of Indian states: Rajasthan, Maharashtra, Kerela and Orissa. The increasing demands for natural sweeteners have driven the farmers in India toward large-scale *Stevia* cultivation. Diterpene glycosides are the group of natural sweeteners that have been extracted from *Stevia*. The leaves of wild *Stevia* plants contain 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside.

The plant has gained wide access to many countries, where in recent decades it is being cultivated domestically, used in its raw leaf form and is now commercially processed into sweetener. Seed germination of *Stevia* is often poor (Miyazaki and Wantenabe, 1974), and vegetative propagation is limited by lower number of individuals. Tissue culture is the only rapid process for the mass propagation of stevia and there have been many reports of in vitro growth of stevia (Miyagawa and Fujioka.,1986)in vitro micropropagation-from shoot tip and leaf(Akita , et al.,1994;Constantinovici, and Cachita.,1997;Ferreira and Handro., 1988;Kornilova, And Kalashnikova., 1996; Sivaram, and Mukundan, 2003; Uddin., et al 2006). Ahmed et al., (2007) regenerated multiple shoots from nodal segments and found highest regeneration rate in MS medium supplemented with 1.5 mg/L BA + 0.5 mg/L KIN. For rooting 97.66% rooting was recorded on MS medium with 0.1 mg/L IAA. Optimal shoot initiation by 0.3mg/l KIN by (Taware, et al., 2010).The present investigation was undertaken to find out suitable sources of explants and suitable concentration for callus and shoot induction in micro propagation of *S. rebaudiana.*

MATERIALS AND METHODS

Plant materials

Explants (node and leaf) are collected from the college garden, and cut into small pieces(about 1 cm long) and treated with detergent for 4-5 min with constant shaking and thoroughly washed with distilled water. Then the explants are surface sterilizedwith 0.1%mercuric chloride and it washed off with sterilized distilled water. Explants are then inoculated in Murashige and Skooge, medium with different combinations of growth promoters.

Preparation of growth medium

After adding macro, micro nutrients and organic supplements pH was adjusted to 5.6 then added agar(8g/l) for solidifying the medium. BAP, IBA, IAA, NAA and KIN were used for growth induction. The prepared medium poured into the culture tubes and autoclaved 121^{0}C temperature.

Inoculation of explants:

To avoid source of infection, the tissue must be thoroughly surface sterilized before planting it in the nutrient medium. Tissues with systemic fungal or bacterial infection are usually discarded in tissue culture studies. Leaf and node are used as the explant and callus sub culturing is also done.

1. Callus induction from leaf explants

Incubation

All cultures were grown in an air- conditioned culture room.The temperature of the culture room was maintained around 25°C. The photoperiod was maintained as 16 h light and 8 h darkness. Visual observation of culture was made every week and data were recorded after 2 weeks of inoculation

RESULTS AND DISCUSSIONS

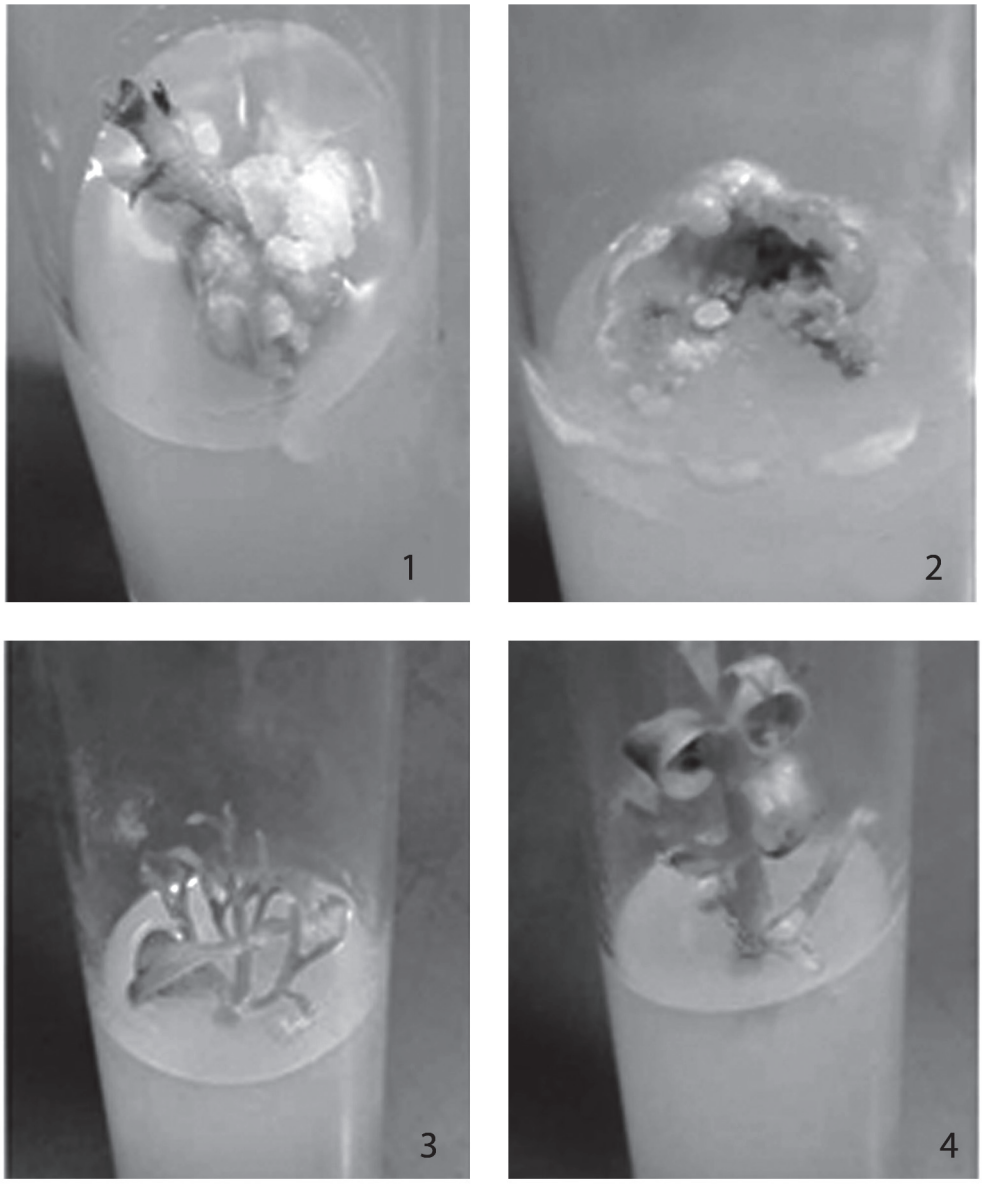
The result of our studies and earlier reports support invitro method of Steviafor large scale production.BAP and NAA combinations promotes the callus proliferation by using callus as explant. Node used as explant for BAP and IAA combinations with varying concentrations which induces shoot initiation and leads to faster growth (Table 1). Leaf segment initiated dark and hard callus and callus induces fleshy and green ones.Furthermore, survival rate of regenerated plants were 40-60% during hardening and shifting to green house, Callus sub cultured on medium with reduced concentration of BAP and NAA (1. mg/l) became embryogenic.1mg/L IBA and KIN 3mg/L and also BAP (3mg/l) and IAA (1mg/l) initiates callus formation which is green, soft and fleshy callus. Studies revealed that leaf explants was much more effective for callus formation compared to nodal and shoot tip explants on MS medium. Varying concentrations of different growth regulators BAP, KIN, IBA, NAA and IAA were applied through growth medium to assess their effects on explants development while NAA and IBA were applied to assess their role in root formation.(fig 1).

Common Sweeteners Approved for Use in India by FSSAI (Food safety and standard authority of India) are, Aspartame, Acesulphame K2, Saccharin and Sucralose. Stevia is a healthy way to reduce blood sugar level. In vitro propagation can become an important alternative to conventional propagation and breeding procedures for wide range of plant species and an improved analytical method HPLC also helps to analyze stevioside extracted from callus of Stevia rebaudiana. Stevioside is the predominant sweetener, from dried leaves with sweetening power 300 times that of sucrose and is known to be non-caloric.

Table 1: Production of callus in growth promoters

NO	Medium used Mg/ l					Explant	Response	Nature of callus
	BAP	IAA	IBA	KIN	NAA			
1	3	-	-	-	1	Callus	Callus growth	Green fleshy and soft
2	4	-	-	-	2	Callus	Callus growth	„
3	2	-	-	-	2	Callus	Callus growth	„
4	2	2	-	-	-	Callus	Callus growth	„
5	-	-	1	3	-	Leaf	Callus	Dark green and hard callus
6	3	1				Leaf and node	Callus	Soft and fleshy Marginal response
7	4	1				Node	Shoot initiation	„
8.	3	1				Node	Shoot initiation	„

Fig 1-5: In vitro propagation of Stevia rebaudiana



BAP (3mg/l) and NAA(1.0 mg/l)

2. Callus induction from IBA(1.0 mg/l) and KIN (3 mg/l)
3. Shoot induction from node explants from BAP (4 mg/l) and IAA (1mg/l)
4. Shoot induction from node as explants BAP (3 mg/l) and IAA (1mg/l)

ACKNOWLEDGEMENT

The authors are grateful to KSCSTE for providing financial assistance.

REFERENCES

Johnson RK, Appel LJ, Brands M. (2009). Dietary sugars intake and cardiovascular health: a scien tific statement from the American Heart Association. Circulation.120 (11):1011–20.

IvanLebedev, Jayyoung Park and Ross Yaylaian.,(2010)Popular Sweeteners and Their Health Ef fects , Interactive Qualifying Project Report ,11–20.

Lee, C.K. (1979): Carbohydrate sweeteners: structural requirements for taste. In:Bourne, G.H. (Ed.), Some Special Aspects of Nutrition, Karger AG, Basel, Switzerland, World Review of Nutrition and Dietetics. 33: 142–197.

Soejarto, D.D. (2002): Ethnobiology of Stevia and *Steviarebaudiana.* In: Kinghorn, A.D.(Ed.), Stevia the genus Stevia (Medicinal and Aromatic Plants – Industrial Profiles). Taylor & Francis/CRC Press, New York/London, UK. 40–67.

Miyazaki Y, Wantenabe H (1974). Studies on the cultivation of stevia; on the propagation of plant (Eng. Abstr.). Jap. J. Trop. Agric. 17: 154- 157

Miyagawa, H. and N. Fujioka, (1986). Studies on the tissue culture of *Steviarebaudiana* and its components: II. Induction of shoot primordia. Planta Medica, 4: 321-323.

Akita, M., T. Shigeoka, Y. Koizumi and M. Kawamura, (1994). Mass propagation of Shoots of *Steviarebaudiana* using large scale bioreactor. Plant. Cell Rep., 13: 3-4, 180-183.

Constantinovici, D. and C.D. Cachita, (1997). Aspects of in vitro multiplication in *Steviarebaudi ana* Bert. Cercetari-Agronomic-in Moldova, 30: 80-86.

Ferreira, C.M. and W. Handro, (1988).Micropropagationof *Steviarebaudiana* through leaf ex plants from adult plants. Planta Medica, 54 (2): 157-160.

Kornilova, O.V. And E.A. Kalashnikova, (1996). Clonal micro-propagation of stevia (*Steviarebau diana*Bertoni). IzvestiyaTimiryzevskoi-SelSkokhozyaistvennoiAdademi. Russia, 1: 99-104.

Sivaram, L. and U. Mukundan, (2003). In vitro culture studies on *Steviarebaudiana*. In vitro Cell. Dev. Biol. Plant, 39: 520-523.

Uddin M.S., M.S.H. Chowdhury, M.M.M.H. Khan, M.B. Uddin, R. Ahmed and M.A. and Baten (2006). In vitro propagation of *Steviarebaudiana* Bert in Bangladesh. Afr. J. Biotechnol. 5(13): 1238-1240.

M.B. Ahmed, M. Salahin, R. Karim, M.A. Razvy, M.M. Hannan, R. Sultana, M. Hossain and R. Islam. (2007).An Efficient Method for in vitro Clonal Propagation of a Newly Introduced Sweetener Plant (*Steviarebaudiana* Bertoni.) in Bangladesh. American-Eurasian Journal of Scientific Re search 2 (2): 121-125.

Taware A. S, D S Mukadam, A M Chavan, S D Taware. (2010) comparative studies on invitro and invivo grown plants and callus of *Steviarebaudiana*(bertoni). IJIB. 9(1) 10-15. ■