Biosci. Biotech. Res. Asia Vol. **3,** No.(**1**) 157-159 (2005)

CALLUS FORMATION AND PLANT INDUCTION FROM AXILLARY BUDS OF *Stevia rebaudiana* Bertoni (HONEY-LEAF)

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(Received May 02, 2005; Accepted June 21, 2005)

ABSTRACT

In vitro regeneration of stevia rebaudiana Betroni. (Honey-leaf) was initiated from axillary buds onto Ms Medium[9] , supplemented with different concentration of BAP and KN in higly aseptic conditions. Shoot differentiated best from axillary shoot base callus on MS medium containing BAP (1Mgl^{-1}) where maximum bud break (80%), number of leaves 5-6 and shoot length 2-3 cm. Were shown and in comparision with medium containing KN (1.5 mgl^{-1}) maximum bud break (60%), number of leaves 3-4 and shoot length 1-2 cm were observed. Regenerated shoots were sub cultured for roots formation.

Keywords: *Stevia rebaudiana,* BAP-6- benzylaminopurine, KN-6- furfurglaminopurine, MS Medium and axillary buds.

INTRUDOCTION

Stevia rebaudiana Bertoni. is a small perennial exotic herb of the compositae family. The nature occurrence of Honey - leaf is between 22-24°s and 53-56° in Paraguay and Brazil.

Stevia rebaudiana Bertoni. is an important medicinal plant of Diabetes Mellitus. It is a pant that produces a variety of high potency low-calorie sweeteners. Honey-leaf has no calcium cyclamate, saccharin, as partame and calories. It is safe for Diabetes, as it does not have the neurological or renal side effects of some of the artificial sweeteners. The sweeteners in leaf is due to the presence of an intensive- sweeteners agent called stevioside[1], whose sweetening power ranges from 100-300 times higher than sucrose, depending on the sucrose concentration of the reference solution[5], Hence, *Stevia rebaudiana* Bertoni. has been named as "Colorie free Bio- sweetener of High-Quality". Cultivation of Honey-leaf for cold and dry regions are not appropriate ? This is a short day plant with critical photoperiod between 13-14 hrs[15] sexual propagation of the species is possible and it is largely employed despite the low fertility index[3]. vegetative propagation through cuttings or eluviation of the nature plants is also employed[14], but this method is limited by the lower number of individuals that can be obtained simultaneously from a single plant.

MATERIAL AND METHODS

Micro propagation by axillary buds and nodal cultures are the commonly used cultures system for rapid clonal propagation and for the germplasm conservation of *Stevia rebaudiana* Bertoni.

Axillary buds were collected from Medicinal Garden of Sarojini Naidu Govt. Girls P.G. college, Bhopal. The explants were washed in a mils soap for 10-20 mint. and then in running tap water for ½ an hours. Surface sterilization was achieved by passing through 70% ethyl alcohol and 0.1% $HgCl_2$ for 6 mint, respectively. After 4-5 rinse in sterile double distilled water.

The single nodes were finally dissected and rinsed once again in distilled water. The explants were finally placed on sterile petriplates with a filler paper and then inoculated on MS medium[9], supplemented with sucrose and agar was used with various concentration of BAP (0.5, 1.0, 1.5, 2.0, 2.5 mgl^{-1}) and KN (0.5, 1.0, 1.5, 2.0, 2.5 mgl^{-1}). The pH of the medium was adjusted to 5.8 prior to the addition of the gelling agent.

The medium was autoclaved at 121°C and 108 KP_a for 20 min. All the cultures were incubated at 24±2°C and 16 hrs photoperiod from cool white fluorescent tube giving 1000 lux at culture level.

RESULTS AND DISCUSSION

Callus initiation

Callus induction and shoot regeneration from axillary buds were achieved after 7-8 days of culture. Similarly after 14-15 days of culture leaves and elongated shoots were shown. Callusing was best (90±1.5) on medium supplemented with BAP (1.0 mgl^{-1}) and in KN (1.5 mgl^{-1}) it was best (68±0.8) intitation[8, 10, 4].

- The experiments were repeated twice, each experiment consisting of 25 replicates.
- Value of mean ± SD callusing at the cut ends.
- SD = standard deviation.

Shoot regeneration

Shoot regeneration from stevia rebaudiana Bertoni explants were cultured onto MS medium[9] supplemented with various concentration of BAP and KN summarized in Table-1 shoot buds were initiated highest (80%) in Ms medium[9] , supplemented with BAP (1.0 mgl^{-1}) and with KN (1.5 mgl^{-1}) bud breaks were achieved (60%) maximum. Similarly in Wame PGR_s concentration results were shown highest in leaves development (5-6) and shoot elongation (2-3 cms) with BAP and number of leaves (3-4) and shoot elongation (1-2 cm) with KN Comparison with PGR_s faster multiplication were shown in MS medium[9] supplemented with BAP and also survival rates were high similar works have been done by Phukan *et. al.*, Shrivastav Joshi, Sthapak. This species is economic interest for its wide ranging pharmacological activity and one of the major constrains in utilizing natural populations is the existence of plant to plant chemovariability. It is hoped that a standard protocol to induce multiple shoots in culture may. Provide a more homogenous source of plants.

Table-1: *In vitro* responses from axillary buds of *Stevia rebaudiana* Bertoni.

Source of Explants	Number Culture	PGR^S (Mgl^{-1}) per plant	Precentage Callusing X±SD	Bud Break %	Number of leaves	Shoot length
BAP						
	25	0.5	75±0.9	70	4-5	1-2
	25	1.0	90±1.5	80	5-6	2-3
Axillary	25	1.5	76±0.8	70	4-5	1-2
buds	25	2.0	74±1.6	62	3-4	0-1
	25	2.5	72±1.0	60	3-4	0-1
KN						
	25	0.5	55±0.4	40	1-2	0-1
	25	1.0	65±0.7	56	2-3	0-1
	25	1.5	68±0.8	60	3-4	1-2
	25	2.0	54±1.2	40	1-2	0-1
	25	2.5	52±1.5	50	1-2	0-1

Stevia rebaudiana Bertoni.

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