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In Vitro Propagation of Stevia (*Stevia rebaudiana* Bert.)- An Elite Sweetening Herb

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ABSTRACT

The experiment was conducted at Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. Shoot tip, nodal segment and leaf base with petiole of Stevia were cultured on MS media supplemented with varying concentrations of BA for shoot production. Nodal segment initiated shoots earlier than shoot tip explant. Higher number of shoot per culture and micro-cuttings were obtained from shoot tip explant than nodal segment. Leaf base with petiole failed to produce shoot on any of the media used. The highest number of shoots (3.2) and micro-cuttings (5.8) were found in the MS medium with 3.0 mgl^{-1} BA and 2.0 mgl^{-1} BA respectively. Interaction effects of explants and shooting media were significant for days of shoot initiation, number of shoots per culture and number of micro-cuttings per culture. Among four media the highest number of root per shoot was obtained in MS medium supplemented with 1.0 mgl^{-1} NAA followed by 1.5 and 0.5 mgl^{-1} NAA respectively. No roots were produced in the medium without auxin (NAA).

Key words : Stevia, *in vitro*, propagation, BA, NAA.

INTRODUCTION

Stevia plant belongs to the family Compositae. Centuries ago, natives of Paraguay used the leaves of this small, herbaceous, semi-bushy, perennial shrub to sweeten their bitter drinks. The property of the species that called attention to the plant was the intense sweet taste of the leaves in aqueous extracts. From the leaves of stevia, stevioside, sweet crystalline diterpene glycosides are extracted. The dry leaves of this plant are 30 times sweeter than sugar, with zero calories. Where as pure extract stevioside is non-caloric and 300 times sweeter than sugar (Bhosle, 2004). It is used as a table top sweetener, in soft drinks, baked goods, pickles, fruit juices, tobacco products, confectionery uses, jams and jellies, candies, yogurts, pastries, chewing gum, sherbets, etc. Stevioside is of special interest to diabetics, persons with hyperglycemia and the diet conscious. Additions of Stevia powder or liquid a pinch or drops at a time to tea, coffee,

Progress towards large-scale commercial cultivation has been slow mainly due to difficulties in multiplication and producing the crop. The plants are herbaceous perennial, leaves alternate, flowers in indeterminate heads, small, white with pale purple throat. Small seeds with little endosperm disperesed in the wind via a hairy pappaus. Seed germination of Stevia is often poor (Miyazaki and Wantenabe, 1974). Therefore, there are

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basically two options for multiplication; the first is the tissue culture and second the stem cutting. The tissue culture is proved to be the best option. Therefore, the present investigation was undertaken for in vitro multiplication of this plant. dairy products, or juices sweeten to taste. Some people detect a slight licorice aftertaste, depending on potency. Due to sweetness, this plant is also called honey plant.

MATERIALS AND METHODS

The experiment was conducted at Biotechnology laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. The explants, shoot tips, leaf base with petiole and nodal segments (Plate 1) were collected from 4-6 month old yard grown plants at Biotechnology Laboratory, BSRI. The explants were cut into small pieces (about 1cm long) and then were treated with 1% savlon for 5-6 min with constant shaking and washed thoroughly with distilled water. Then the explants were taken under laminar airflow cabinet and surface sterilization was done with a 0.1% mercuric chloride solution containing two to three drops of tween-80 for five min under aseptic condition and then washed four times with sterilized distilled water. After steriliztion explants were inoculated aseptically for *in vitro* propagation on MS (Murashige and Skooge, 1962) medium with different concentrations and combinations of growth regulators (Plate 2). For shoot induction explants were inoculated on media MS supplemented BA (1.0, 2.0 and 3.0 mgl^{-1}). Shoots were rooted on the media MS supplemented with NAA (0.5, 1.0 and 1.5 mgl^{-1}). Visual observation of culture was made every week. Data were recorded after 2 weeks of culture and subjected to statistical analysis using computer software MSTAT-C. Means were compared using Dancan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The explants, shoot tip, leaf base with petiole and nodal segment of Stevia were cultured on four different media for differentiation and proliferation of shoots. Four types of shooting media such as MS+ BA (0mgl^{-1}), MS+ BA (1mgl^{-1}), MS+ BA (2mgl^{-1}), and MS+ BA (3mgl^{-1}) were used.

Among explants used shoot tip and nodal segment were able to produce multiple shoots. Results of shoot production and multiplication are presented in the Table 1. Nodal segments initiated shoots earlier than shoot tip explant. Higher number of shoot per culture was obtained from shoot tip explant than nodal segments. Shoots produced per culture were segmented to micro-cuttings and were cultured on the same shooting medium for rapid multiplication. Significantly higher number (5.3) of micro-cuttings was obtained from shoot tip culture. The multiplication rate was higher in shoot tip explant than nodal segments. Leaf base with petiole failed to produce shoot on any of the media used.

Four media were used for shoot production. Effects of shooting media on shoot production and multiplication are shown in the Table 2. Early response for shoot initiation was recorded from the medium where MS medium was supplemented with higher amount (3.0 mgl^{-1}) of BA. Days of shoot initiation was inversely related with amount of BA used. Number of shoots produced per culture varied significantly due to media composition. The highest number of shoot (3.2) was found in the MS medium with 3.0 mgl^{-1} BA and was statistically identical to 2.0 mgl^{-1} BA used. The lowest and similar number of shoots (1.8) per culture was found in the media where no BA was used and BA 1.0 mgl^{-1}

respectively. In case of number of micro cutting, media showed significant effect. The highest number of micro-cutting (5.8) was obtained from the shooting medium where 2.0 mgl^{-1} BA was used which was statistically same as where 3.0 mgl^{-1} BA was used followed by MS medium supplemented with 1.0 mgl^{-1} BA. Higher number of micro cutting per culture was obtained in the medium supplemented with 2.0 mgl-1 BA than 3.0 mgl^{-1} BA possibly due to higher shoot length (Plate 3). Multiplication rates showed the same trend as number of micro-cutting per culture. The hormone free medium produced shoots identical to 2.0 mgl^{-1} BA used. Salas (2001) also reported that micro-cutting with apical and axial bud produced shoots successfully on hormone free medium. Micro-cutting production from in vitro grown shoots was significantly influenced by media used. Although higher but statistically similar number of shoots per culture was obtained from 3.0 mgl^{-1} BA compared to 2.0 mgl^{-1} BA. The highest number of micro-cutting (5.8) was obtained from the shooting medium where 2.0 mgl^{-1} BA was used. This was possibly due to higher length of shoots produced in BA 2.0 mgl^{-1} compared to BA 3.0 mgl^{-1}.

Interaction effects of explants and shooting media were significant for days of shoot initiation, number of shoots per culture and number of micro-cutting per culture. Results are presented in the Table 3. The lowest days required for shoot initiation was recorded in nodal segment cultured on MS medium where no hormone was used. Shoot tip explant cultured on MS medium supplemented with 3.0 mgl^{-1} BA showed the statistically similar result with nodal segment cultured on MS medium without supplementation of BA. The highest number of shoot per culture was produced from nodal segment cultured on MS medium supplemented with 3.0 mgl^{-1} BA followed by 2.0 mgl^{-1} BA used for both the explants. The lowest number of shoot per culture was observed in both explant cultured on media without BA. The highest number of micro-cutting per culture was obtained from shoot tip explant cultured on MS medium supplemented with 3.0 mgl^{-1} BA which was statistically similar with medium where 2.0 mgl^{-1} BA was used followed by nodal segment cultured on MS medium supplemented with 2.0 and 3.0 mgl^{-1} BA respectively. Higher multiplication rate was recorded from shoot tip culture than nodal segment. Results indicate that shoot tip and nodal segment initiated growth of shoots on hormone free medium within shorter period of time than hormone supplemented medium. This was perhaps due to apical dominance of shoot tip and axial bud of nodal segments.

For root production in multiplied shoots, rooting media were used. The rooting media showed remarkable influence on shoot growth in consequence with root production. Results of root production and influence on shoot length are shown in the Table 4. Numbers of root production were significantly different for different rooting media. The highest number of root per shoot was recorded in MS medium supplemented with 1.0 mgl^{-1} NAA (Plate 5), followed by 1.5 and 0.5 mgl^{-1} NAA used. No roots were produced on the medium where no hormone (NAA) was used. Shoot length during root production was the highest on the medium where the highest number of roots was recorded perhaps due to higher amount of nutrient absorbed by higher number of roots. Anis et al. (2003) reported that 80% rooting was obtained from shoots culture on the MS supplemented with NAA (1.0 mgl^{-1}) in mulberry. No root produced in the medium without NAA. Therefore, NAA is indispensable for root production of in vitro culture of Stevia. Shoot length during root production was the highest on the medium where the highest number of roots was recorded perhaps due to higher amount of nutrient absorbed by higher number of roots.

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Table 1. Effects of explants on shoot production and multiplication of Stevia.

Explant	Days of shoot initiation	Shoot numbers per culture	Number of micro-cutting per culture	Multiplication ratio
Shoot-tip	22.0a*	2.7	5.3a	1 : 2.0
Nodal segment	18.8b	2.3	3.6b	1 : 1.6

*Figures within column followed by different letters are significantly different by DMRT at p = 0.05.

Table 2. Effects of shooting media on shoot production and multiplication of Stevia.

Shooting media	Days of shoot initiation	Shoot number per culture	Number of micro-cutting per culture	Multiplication ratio
MS+BA(0mgl^{-1})	20.9ab*	1.8b	3.2b	1 : 1.7
MS+BA(1mg l^{-1})	22.1a	1.8b	3.0b	1 : 1.6
MS+BA(2mg l^{-1})	19.8bc	3.0a	5.8a	1 : 1.9
MS+BA(3mg l^{-1})	18.8c	3.2a	5.7a	1 : 1.8

*Figures within column followed by different letters are significantly different by DMRT at p = 0.05.

Table 3. Interaction effects of explants and shooting media on shoot production and multiplication of Stevia.

Explant x Media	Days of shoot initiation	Shoot number per culture	Number of micro-cutting per culture	Multipli cation ratio
Shoot-tip x MS+BA(0mg l^{-1})	25.6a*	2.3ab	4.3b	1 : 1.8
x MS+BA(1mg l^{-1})	24.0a	2.3ab	4.7ab	1 : 2.0
x MS+BA(2mg l^{-1})	20.6b	3.0a	6.0a	1 : 2.0
x MS+BA(3mg l^{-1})	17.8cd	3.0a	6.0a	1 : 2.0
Node x MS+BA(0mg l^{-1})	16.2d	1.3b	2.0c	1 : 1.5
x MS+BA(1mg l^{-1})	20.2b	1.3b	1.3c	1 : 1.0
x MS+BA(2mg l^{-1})	19.0bc	3.0a	5.7ab	1 : 1.9
x MS+BA(3mg l^{-1})	19.8b	3.3a	5.3ab	1 : 1.6

*Figures within column followed by different letters are significantly different by DMRT at p = 0.05.

Table 4. Effects of rooting media on root production and shoot growth of Stevia.

Rooting media	Number of roots per shoot	Shoot length in rooting media (cm)
MS+NAA (0.0mg l^{-1})	0.0b*	2.9d
MS+NAA (0.5mg l^{-1})	5.9a	4.0b
MS+NAA (1.0mg l^{-1})	7.3a	5.1a
MS+NAA (1.5mg l^{-1})	7.0a	3.2bc

*Figures within column followed by different letters are significantly different by DMRT at p = 0.05.

Plate 1. Explants (from left- shoot tip, leaf base with petiole and nodal segment)were used in the investigation.

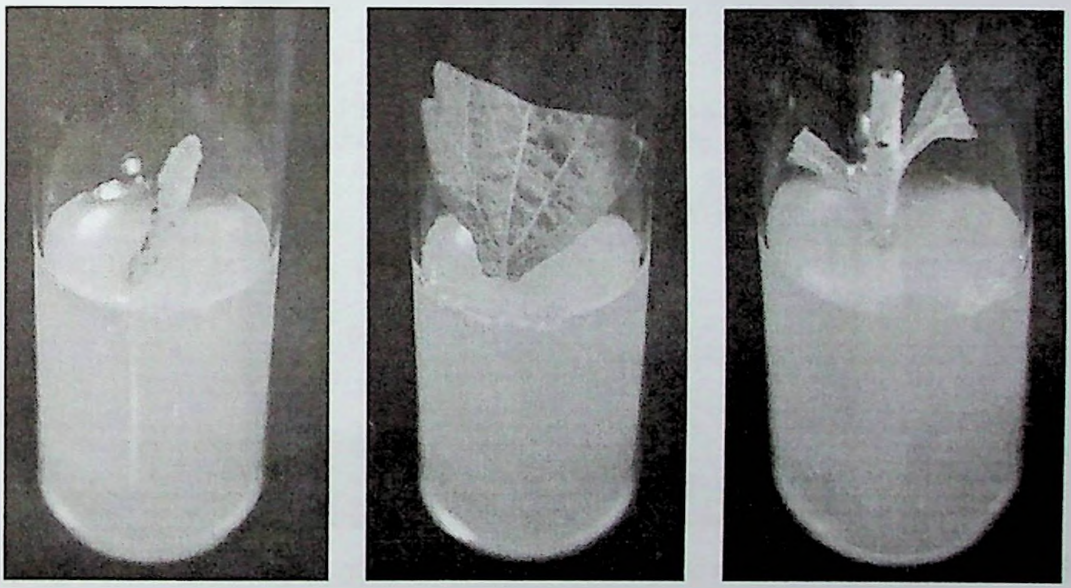

Plate 2. Explants (from left- shoot tip, leaf base with petiole and nodal segment) were inoculated on media in test tube.

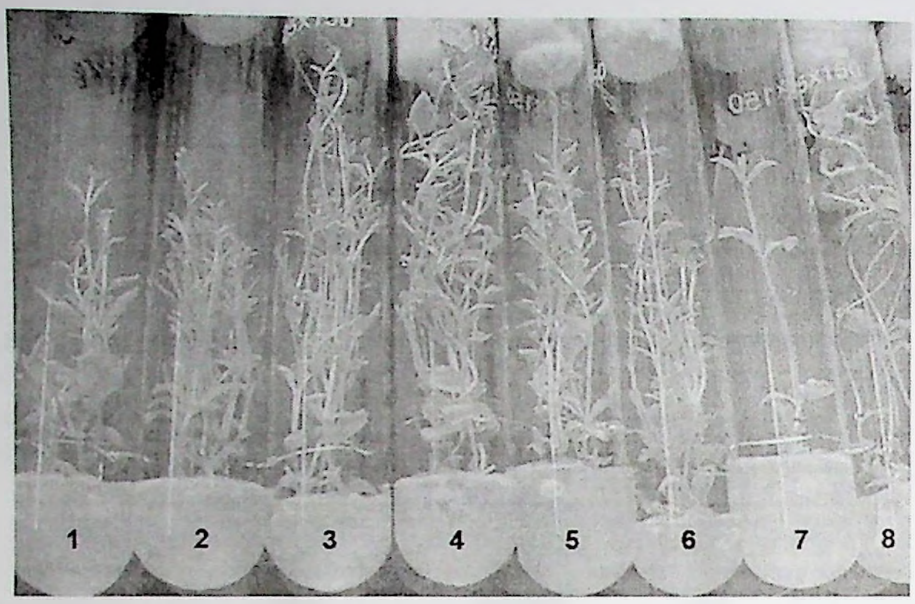

Plate 3. Multiple shoot production in explants (from left-tube 1 and 2, shoot production from MS medium without growth hormone; tube 3 and 4, shoot production from MS + BA 1mg l^{-1}; tube 5 and 6, shoot production from MS + BA 2mg l^{-1}; tube 7 and 8, shoot production from MS + BA 3mg l^{-1}).

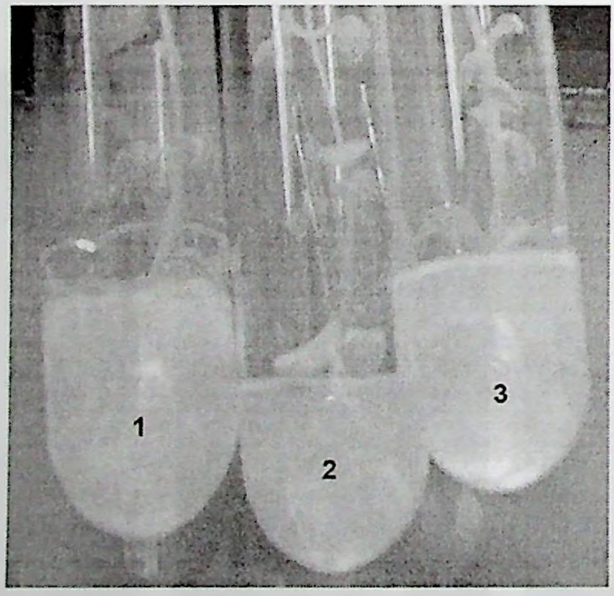

Plate 4. Roots production on shoot (tube 1, root production from MS+NAA 0.5 mg l^{-1}; tube 2, root production from MS+NAA 1.0 mg l^{-1}; tube 3, root production from MS+NAA 1.5 mg l^{-1}).

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