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IN VITRO CLONING AND STEM CUTTING OF STEVIA (*STEVIA REBAUDIANA* BERTONI.) FOR MASS PROPAGATION IN CHITTAGONG, BANGLADESH

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ABSTRACT

Stevia (Stevia rebaudiana Bertoni.) is non-caloric sweetening plant. It was cloned using shoot tip in vitro MS medium supplemented with 6-Benzyl Amino Purine (BAP) and Kinetin (Kn). Excised shoots were rooted in half strength Murashige and Skoog (MS) medium supplemented with Indole-3 Butyric Acid (IBA). The rooted plantlets were transferred to soil and coconut husk filled bags and beds. Stem cutting method in sand medium with water misting propagation bed without plant growth regulator was effective for mass propagation. The plantlets derived from in vitro and stem-cutting grew luxuriantly in cultivating field during long day months (March to September) and produced gregarious flowering during short day months(October to February). Similar type of flowering behavior was observed between these two types of propagules of Stevia.

Keywords: *In vitro*, Stevia, Cloning, Stem-cutting, Sand-medium, Mass propagation, Flowering, Long day, Short day.

Contribution/ Originality

The studies are used *in vitro* propagation methods and stem cutting in mist propagation bed as simple as vegetative propagation. Here investigated rooting, vegetative growth and flowering behavior. This paper contributes an easy propagation method of stem cuttings which is applicable for the farmers.

1. INTRODUCTION

Stevia is a genus having 150 species of herbs and shrubs. It belongs to family Asteraceae. It is native to tropical and subtropical South America (en.wikipedia.org/wiki/stevia, 2006). Stevia (*Stevia rebaudiana* Bertoni.) plant, claiming the sweetening power is so superior to sugar. The chemist isolated *glycosides* - the chemical components that give Stevia its sweet taste and called them steviosides and rebaudiosides. The pure form of each of these compounds is up to three

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hundred times sweeter than sugar. The sweetener can be found in the leaves of the Stevia plant; the liquid extracted from those leaves, and purified forms of the mentioned steviosides (http.//www.stevia.net/, 2004). Japan has used all forms of this Stevia commercially in manufacturing food or soft drinks, and as a table sweetener since 1977. Today the demand and use of Stevia has increased worldwide (http.www.stevia.net/cspi.htm, 2000).

Stevia plants is propagated by stem cutting using plant growth regulators Indole Acetic Acid (IAA), IBA and Naphtalene Acetic Acid (NAA) (www.cabi.org, 2006). *In vitro* study on Stevia is a common interest among scientists of different countries (Ferreira and Handro, 1988; Akita *et al*., 1994; Kornilova and Kalashnikova, 1996; Uddin *et al*., 2006). In Bangladesh, Stevia plants are propagated through micropropagation and stem cutting. Bangladesh Rural Advancement Committee (BRAC), an NGO is cultivating Stevia at large scale, and marketing propagules and its leaf powder (Anonymous, 2008). However, it is difficult task for the farmer to cultivate Stevia through micropropagation or stem cutting using plant growth hormone. Considering the fact study was taken to find out easy way of cloning and cultivation. The paper deals with some basic information on mass propagation through *in vitro* and stem cutting and also flowering of Stevia.

2. MATERIALS AND METHODS

Stevia plant was collected from Bangladesh Sugar Cane Research Institute (BSRI), Ishwardi in 2006 and experiment conducted in the nursery and tissue culture laboratory of Silviculture Genetics Division, Bangladesh Forest Research Institute, Chittagong, Bangladesh. Young shoot tips were collected as explants, sterilized with mercuric chloride (0.1%w/v) and washed with sterilized distilled water. Sterilized shoot tips were inoculated in autoclaved MS basal medium supplemented with BAP (1.0mg/l). BAP and Kn were supplemented in MS medium for induction of multiple shoots. Growth room temperature was maintained at 27± 2^0 C with 16 hours light and 8 hours dark period. Half strength MS medium supplemented with IBA was used for root induction. Rooted plantlets were thoroughly washed under tap water and they were kept in culture tubes in water for a week in the growth room. Then the plantlets were transferred to water misting beds of soil and coconut husk, and in polybag (filled with compost of coconut husk) in the nursery maintaining high humidity (RH% 70-90). Stem cuttings were raised from original stock plant and tissue culture (T.C) derived plants of Stevia in the sand-media of water misting propagation bed. This was done repeatedly done in the long (March-September) and short (October-February) day period months without plant growth regulator. Both *in vitro* and stem cutting propagules were planted in well prepared field and observed growth patterns.Data were recorded in February 2008 on height, number of branch(s), leaves, flowers, root(s) number and length of three month aged cuttings from original stock plant and tissue culture derived plants.

3. RESULTS AND DISCUSSION

Young shoot tips of stevia were cultured in MS medium supplemented with BAP and culture was established within two weeks. Multiple shoots (Fig.1) induction was observed in MS with BAP and Kn (Table- 1). Root induction was in half strength MS with low concentration of IBA (0.1 - 0.5mg/l) maintaining dark and low light intensity (Fig.2). Leafy stem-cuttings were easy from original stock plant and tissue culture derived plant of Stevia in the water misting sand-media propagation bed. Rooted plantlets (Fig.2, 3a&b) were easily established in soil beds (Fig.6 & 8) and also in soil and coconut husk filled polybags (Fig.4,5&7). The potting mixtures that allow adequate drainage and aeration is recommended for acclimatization of *in vitro* regenerated plants (Dustan and Turner, 1984) and excellent successes were obtained with soil-free potting mixture like vermiculite, partite, peat plug or small foam blocks (Mc Crown, 1986).

One node stem, two nodes stem, three nodes stem and about six centimeters tip cuttings were tried and success of rooting on stem was satisfactory (>70%) in sand-medium under water misting bed. No root inducing hormone was used.

The Stevia plants were found to grow luxuriantly in the experimental fields after planting in March to September and had better vegetative growth in the long photoperiodic days (Fig.6) were recorded. It was been observed that heavy rainfalls causes damage (Fig.7). In the short photoperiodic days, the plants grew slowly and flowering shoots emerged. The gregarious flowering (Fig.5&8) continued throughout the short day months and every new shoot came out with flower buds. At this stage very few and smaller leaves were grown in the plants. Stem cutting was also successful in short days and flowering buds are grown on new branches. The tissue culture plants and both stem cuttings of tissue plants and stalk plants those planted in soil beds were showed similar flowering behaviur (Table. 2,3&4). We could not observe seed germination but seed germination of Stevia were reported by Jeffrey and Ching (1999). It is necessary to conduct field management research for setting seeds in the flowers of flowering plants.

4. CONCLUSION

In vtro cultures of Stevia may be conserved in growth room during short day months. Leafy stem cuttings of Stevia are low cost and easy for propagation and cultivation.

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REFERENCES

Akita, M., T. Shigeoka, Y. Koizumi and M. Kawamura, 1994. Mass propagation of shoots of stevia

rebaudiana using large scale bioreactor. Plan Cell Rep. Japan, 13(3-4): 180-183.

Anonymous, 2008. Present status of BRAC plant biotechnology laboratory. Souvenir, Plant Tissue Culture & Biotechnology Conference 2008 11-13 April 2008, Dhaka, Bangladesh, pp: 13-15.

Dustan, D. and K. Turner, 1984. The acclimatization of micropropagated plants. In: Cell culture and somatic cell genetics of plants .Laboratory Procedures and Their Applications, Vasil IK (Eds). Orianddo: Academic Press, 1: 123-129.

en.wikipedia.org/wiki/stevia, 2006.

Ferreira, C. and W. Handro, 1988. Micropropagation of stevia rebaudiana through leaf explants from adult plants. Planta Medica, 54(2): 157-160.

http.//www.stevia.net/, 2004.

http.www.stevia.net/cspi.htm, 2000.

Jeffrey, G. and A. Ching, 1999. Seed germination in stevia rebaudiana. In. J. Janick (Eds). Alexandria, VA: ASHS Press. pp: 510-511.

Kornilova, O. and E. Kalashnikova, 1996. Clonal micro-propagation of stevia (Stevia Rebaudiana Bertoni). Russia: Izvestiya Timiryzevskoi-Sel Skokhozyaistvennoi Adademi, 1: 99-104.

Mc Crown, D., 1986. Plug systems of micropropagules. In: Tissue culture as a plant production system for horticultural crops. (Eds). Zimmerman, H.R. Griesbach,R.J. Hammerschlag,F.A. and Lawson, R.H. Dordrecht: Martinus Nijhoff. pp: 53-60.

Uddin, M., M. Chowdhury, M. Khan, M. Uddin, R. Ahmed and M. Baten, 2006. In vitro propagation of stevia rebaudiana Bert in Bangladesh. Afr.J.Biotechnol, 5(13): 1238-1240.

www.cabi.org, 2006.

Table-1. Response of shoot tip *in vitro* culture with different growth regulators

Growth regulator(s)	Concentration mg/l	Observations(age- 4 weeks)	
		Agar gel (7g/l)	Liquid (paper bridge)
BAP	0.5	2-3 shoots, thick leaves	Elongated 2- 3 shoots, green normal leaves
BAP	1.0	Profuse shoots in cluster, thick leaves	Elongated 3-4 shoots, green normal leaves
Kn	0.5	1-2 shoots, thick leaves	1-2 shoots, with green normal leaves
BAP + Kn	0.5+0.5	2-3 shoots, thick leaves	Elongated 3-4 shoots with hard callus at base, green normal leaves
BAP + Kn	1.0+0.5	Profuse shoots in cluster, thick leaves	Elongated multiple shoots with hard callus at base, green normal leaves
IBA	1.0	2-3 thin roots	>3 thick roots, elongated shoot

Table-2. Three months old tissue culture origin plantlets grown in polybags, February 2008

Plant height (cm)	Branch(es)				
	Without flower		With flower(s)		
	Branch number(s)	Leaf numbers	Branch numbers	Leaf numbers	Flower numbers
13.0	1	6	2	51	25
22.0	1	15	2	64	25
15.0	2	17	2	47	42
14.0	4	35	5	46	49
15.0	6	53	4	49	34
15.8	2.8	25.2	3	51.4	35

Table-3. Three months old stem cuttings (original stock plant) grown in soil, February 2008

Plant height (cm)	Branch (es)					Roots	
	Without flower		With flowers			Number (s)	Length
	Branch. number(s)	Leaf number(s).	Branch number(s).	Leaf number(s)	Flower number(s)		
23.0	0	0	11	64	45	14	15.0
15.0	4	35	4	22	22	8	17.0
25.0	1	5	5	16	51	14	8.0
20.0	1	20	2	12	4	9	15.0
15.5	1	32	1	28	15	11	9.0
19.6	1.4	18.4	4.6	28.4	27.4	11.2	12.8

Table-4. Three months old stem cuttings of tissue culture origin plantlets grown in soil, February 2008

Plant height (cm)	Branch(es)					Roots	
	Without flower		With flowers			Numbers	Length (cm)
	Branch number(s)	Leaf number(s)	Branch number(s).	Leaf number(s).	Flower number(s)		
14.5	7	47	2	19	11	21	15.0
19.0	4	29	2	48	12	5	27.0
26.0	3	16	6	11	59	9	20.0
13.0	2	14	7	46	21	6	10.0
21.0	4	34	2	49	73	14	15.5
18.6	4	28	3.8	34.6	35.2	6.8	17.4

Fig-1. *In vitro* multiple shoots

Fig-2. *In vitro* rooted plantlet

Fig-3a. One node cutting

Fig-3b. Tip cutting

Fig-4. Poly bag plantlet

Fig-5. Gregarious flowering

Fig-6. Normal flowering

Fig-7. Abnormal shoots

Fig-8. Gregarious flowering