Short communication

Vegetative propagation of stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] through stem cuttings

G.R. Smitha[1]* and K. Umesha[2]

[1]Directorate of Medicinal and Aromatic Plants Sciences, Boriavi, Anand 387 310, Gujarat, India; [2]Division of Horticulture, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bengaluru 560 065, Karnataka, India

Received 6 December 2011; received in revised form 30 March 2012; accepted 30 March 2012.

Abstract

Stevia rebaudiana (Bertoni) Hemsl. is a non-caloric bio-sweetener wherein seed propagation is difficult. Propagation of stevia through stem cuttings was evaluated under two environmental conditions (natural shade and greenhouse with intermittent mist) and four combinations of sand, soil, FYM, vermicompost, and coir dust, with growth regulator (IBA), bio-inoculants (*Trichoderma viride*), and cow's urine treatments. Stem cuttings treated with *T. viride*, placed in soil, sand, and vermicompost rooting media (1:1:1) under greenhouse conditions recorded better shoot and root growth parameters, besides better field establishment (90%), over other treatment combinations.

Keywords: Bio-inoculants, Environmental conditions, Growth regulators, Rooting media

Stevia [(*Stevia rebaudiana* (Bertoni) Hemsl.], commonly known as "sweet weed", "sweet leaf", "sweet herb" and "honey leaf", is a perennial herb belonging to the family Asteraceae. Leaves of stevia contain around 10 sweetening glycosides, of which stevioside (3–10%), rebaudiside-A (13%), and rebaudiside-B, C, D are important (Yoshida, 1986). With a huge share of the Indian population being diabetic (Siegel et al., 2008), non-caloric, natural sweeteners safe to diabetics will receive greater focus in future. Although there is greater interest in this plant now as a natural alternative to artificial sweeteners like saccharin, aspartame, asulfam-K, progress towards large scale commercialization of stevia sweetners has been rather slow, largely due to difficulties in propagating the crop. Stevia is propagated either through seeds or cuttings. However, low and erratic seed germination (owing to its small seed size and related bottlenecks in nutrition) and slow establishment of seedlings warrant its propagation through vegetative means (Randi, 1980). Among different vegetative means, stem cutting is a cheaper and better alternative although tissue culture methods have been standardized. Some basic work on vegetative propagation of stevia regarding use of polythene cover, sand: perlite (1:3) rooting media, and use of growth regulators particularly IBA and NAA to enhance rooting of stevia cuttings (Rajashekara, 2004; Ingle and Venugopal, 2009) also have been carried out. However, information on naturally available, cost effective rooting media, bio-inoculants, and their interaction with the growing conditions for multiplication of stevia has been scarce. Hence, an effort was made to standardize the effects of rooting media, growth regulators, bio-incoulants, and growing con-

*Author for correspondences : Phone +912692271605/606 Ext. 222; Mobile+91-9974303326; Email <smithagingade@yahoo.com>.

ditions on propagation of stevia through stem cuttings.

An experiment was carried out at Sanjeevini Vatika, Bengaluru (12°58" N; 77°55" E; 930 m altitude) during 2009–'10. The trial was laid out in a factorial controlled randomized design (CRD) with two environmental conditions: natural shade of Singapore cherry (*Muntingia calubura* L.) and greenhouse with intermittent mist, four media: soil+sand (1:1), soil+sand+FYM (1:1:1), soil + sand + vermicompost (1:1:1) and soil+sand+coirdust (1:1:1), and six growth regulators and bio-inoculants (control, IBA 500 ppm, IBA 1000 ppm, IBA 2000 ppm, *Trichoderma viride*, and cow's urine). The media were prepared as per the treatment protocol and filled in the seed pan leaving a head space of 2 cm. *Trichoderma viride* was prepared by mixing 0.5 kg of culture in 0.5 L water to form a uniform slurry. Fresh cow urine was collected and diluted with water in 1:10 ratio. Semi-hardwood cuttings with three nodes were prepared using matured basal portions of current year stem. Basal portion of the cuttings (1 to 2 cm) was dipped in growth regulator solution for 10 seconds, as per the treatment protocol. The cuttings were planted in seed pans after treating with growth regulators or bio-inoculants with one basal node buried inside the medium. The cuttings were kept under the shade of Singapore cherry and in the medium cost polyhouse with intermittent misting (LDPE with 150 µM thickness, average temperature 30ºC, and Relative humidity 85%). Observations on shoot parameters were recorded from five labelled plants in each replication at regular intervals up to 60 days after planting. Rooting parameters such as rooting percentage, root length, and thickness were recorded by destructive sampling (uprooting five labelled cuttings in each replication at 60 days after planting). The data were statistically analyzed using ANOVA.

Among the two environmental conditions evaluated, early sprouting (20 days), maximum bud sprout (79%), maximum sprout length (23 cm), highest number of leaves per rooted cutting (27), highest dry weight of the shoot (3.04 g), highest rooting

Table 1. Influence of environmental conditions, rooting media and growth regulators on shoot parameters of stevia cuttings.

Treatments	Days taken for sprouting	Sprouting percentage	Number of sprouts/ rooted cutting	Length of longest sprout (cm)	Girth of sprout (mm)	Number of leaves per rooted cutting
Environmental conditions						
Natural shade	21.52^{b}	70.65^{b}	2.44^{a}	13.94^{b}	1.14^{a}	26.44^{b}
Greenhouse	20.35^{a}	79.18^{a}	2.17^{b}	23.06^{a}	1.05^{b}	32.64^{a}
Media						
Soil+sand (1:1)	20.64	73.88^{b}	2.27	18.79^{b}	0.96^{c}	30.42^{a}
Soil+sand +FYM (1:1:1)	21.47	71.35^{c}	2.37	19.90^{a}	1.12^{b}	33.27^{a}
Soil+sand+vermicompost (1:1:1)	20.72	73.20^{b}	2.39	20.12^{a}	1.24^{a}	31.76^{a}
Soil+sand+coirdust (1:1:1)	20.89	81.22^{a}	2.20	15.21^{c}	1.06^{b}	22.73^{b}
Growth regulators						
Control	21.79^{c}	64.04^{d}	2.31^{b}	14.93^{c}	0.92^{C}	23.46^{c}
IBA 500 ppm	21.88^{c}	76.81^{b}	2.54^{a}	19.98^{b}	1.09^{b}	33.63^{a}
IBA 1000 ppm	21.29^{b}	76.80^{b}	2.11^{c}	19.18^{b}	1.02^{C}	29.21^{b}
IBA 2000 ppm	20.25^{b}	70.17^{c}	2.07^{c}	16.29^{c}	1.16^{a}	25.19^{c}
Trichoderma viride	19.29^{a}	86.98^{a}	2.56^{a}	22.06^{a}	1.26^{a}	34.87^{a}
Cow's urine	21.08^{b}	74.70^{b}	2.25^{b}	18.59^{b}	1.12^{b}	30.92^{b}

Means with the same superscript do not differ significantly (5%).

(77%), maximum number of roots (14), longest root length (8.02 cm), maximum root girth (0.7 mm) and maximum root dry weight (0.37 g) were recorded for the greenhouse treatment (Tables 1 and 2; Fig.1). Quicker and better sprouting under greenhouse conditions might be attributed to the warmer temperature (30ºC) and higher relative humidity (85%) prevailing under it compared to that of natural shade, which accelerated sprouting of the dormant buds (Umesha et al., 2011).

As regards to growth media, soil + sand + vermicompost media produced longer and thicker sprouts, more roots and higher dry weights for shoot and root (Tables 1 and 2). Higher biomass production in vermicompost media may be due to increased nutrient uptake and enhanced availability of nutrients and growth promoting substances (Thankamani et al., 2005). Among the six growth regulators and bio-inoculants tried, *T. viride* was clearly superior. *Trichoderma* is also capable of increasing nutrient uptake by secreting enzymes that solubilise soil nutrients (Harman et al., 2004). Among the interaction effects, cuttings treated with *T. viride* planted in soil+sand+vermicompost media and kept in greenhouse gave maximum number of leaves (49.67) and maximum root length (12.29 cm). Similarly, the highest field establishment of the rooted cuttings (Table 2) was recorded in greenhouse conditions (90.79%) for cuttings dipped in *T. viride* (91.13%). Overall, cuttings treated with *Trichoderma viride* and planted in a media containing soil, sand, and vermicompost (1:1:1) kept in the greenhouse showed better shoot and root parameters

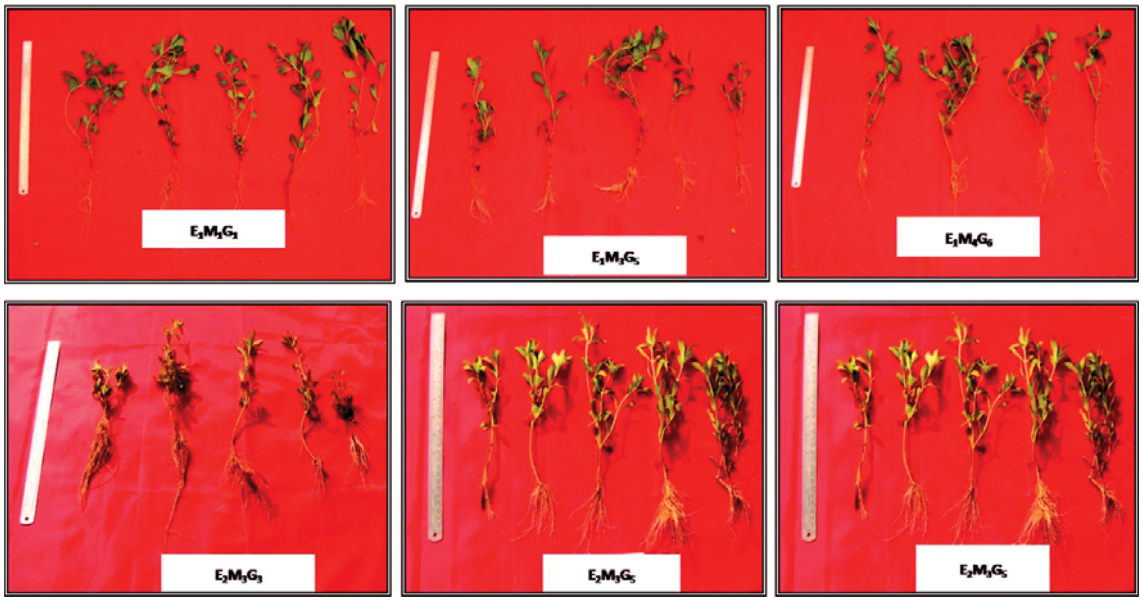

Figure 1. Rooting in stevia cuttings as influenced by different environmental conditions, rooting media and growth regulators.

Legends:

Environment (E)
E_1- Natural shade
E_2- Greenhouse with intermittent mist

Media (M)
M_1-Soil+Sand (1:1)
M_2- Soil+Sand+FYM (1:1:1)
M_3- Soil+Sand+Vermicompost (1:1:1)
M_4- Soil+Sand+Coir pith (1:1:1)

Growth Regulators (G)
G_1- Control
G_2- IBA 500 ppm
G_3-IBA 1000 ppm
G_4-IBA 2000 ppm
G_5-*Trichoderma viride*
G_6-Cow's urine (1:10)

Table 2. Effect of environmental conditions, rooting media and growth regulators on root parameters of stevia cuttings.

Treatments	Rooting percent	Number of roots per rooted cutting	Length of longest root (cm)	Girth of the roots (mm)	Dry weight of roots (g/plant)	Field survival percentage
Environment						
Natural shade	67.09[b]	11.14[b]	6.68[b]	0.56[b]	0.18[b]	89.41[b]
Greenhouse	76.93[a]	13.55[a]	8.02[a]	0.75[a]	0.37[a]	90.79[a]
Media						
Soil+sand (1:1)	70.33[b]	11.31[b]	7.53	0.64	0.20[d]	89.30
Soil+sand +FYM (1:1:1)	67.00[c]	12.84[a]	7.37	0.67	0.24[c]	90.50
Soil+sand+vermicompost (1:1:1)	70.83[b]	13.38[a]	7.30	0.65	0.32[b]	90.39
Soil+sand+coirdust (1:1:1)	79.73[a]	11.86[b]	7.20	0.66	0.35[a]	90.22
Growth regulators						
Control	59.92	7.39[d]	6.63[c]	0.60[d]	0.13[e]	88.83[c]
IBA 500 ppm	74.26[b]	12.18[b]	7.58[b]	0.66[b]	0.27[c]	89.82[c]
IBA 1000 ppm	75.63[b]	13.29[b]	7.49[b]	0.66[b]	0.33[b]	90.00[b]
IBA 2000 ppm	66.67	15.03[a]	7.23[b]	0.63[c]	0.37[a]	90.19[b]
Trichoderma viride	84.61[a]	16.39[a]	8.25[a]	0.76[a]	0.38[a]	91.13[a]
Cow's urine	70.68[b]	9.80[c]	6.92[c]	0.63[c]	0.17[d]	90.65[a]

Means with the same superscript do not differ significantly (5%).

and is appropriate for commercial propagation of stevia.

References

Harman, G.V., Homell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma sps*. Opportunistic, avirulenmt plant symbionts. Nature Rev. Microbiol., 2: 43–56.

Ingle, M. R. and Venugopal, C. K. 2009. Effect of different growth regulators on rooting of stevia (*Stevia rebaudiana* Bertoni) cuttings. Karnataka J. Agric. Sci., 22(2): 460–461.

Rajashekara, 2004. Standardization of vegetative propagation of stevia (*Stevia rebaudiana* Bertoni) through stem cuttings. M. Sc. (Hort.) thesis, University of Agricultural Sciences, Bengaluru, India, pp. 28–36.

Randi, A.M. 1980. Germinacho de Stevia rebaudiana Bert., M. Sc. Thesis, Univeridade Estadual de Campinas, Campinas.

Siegel, K., Narayan, K.M., and Kinra, S. 2008. Finding a policy solution to India's diabetes epidemic. Health Aff. (Millwood), 27: 1077–1090.

Thankamani, C.K., Sreekala, K., and Anandaraj, M. 2005. Effect of *Pseudomonas fluorescens* (IISR-6) and *Trichoderma harzianum* (P-26) on growth of black pepper (*Piper nigrum* L.) in the nursery. J. Spices Arom. Crops, 14(2): 112–116.

Umesha, K., Murthy, G., and Smitha, G.R. 2011. Environmental conditions and type of cuttings on rooting and growth of vanilla (*Vanilla planifolia* Andrews). J. Trop. Agric., 49 (1–2): 121–123.

Yoshida, S. 1986. Studies on the production of sweet substances in *Stevia rebaudiana*: I. Simple determination of sweet glucosides in *Stevia* plant by thin layer chromato-scanner and their accumulation patterns with plant growth. Jap. J. Crop Sci., 55(2): 189–195.