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Integrated effect of different growth regulators, substrate media and stem portions on rooting of stevia cuttings

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Abstract
Stevia rebaudiana (Bert.) is an emerging natural, non-caloric sugar alternative and anti-diabetic plant in Pakistan. Its propagation through seed is very difficult due to bottlenecks in seed setting and seed germination. Even people did not know the exact time and method for its propagation. To contribute in filling the existing information and knowledge gaps on asexual propagation of Stevia (*Stevia rebaudiana* Bertoni), an experiment was conducted with objective to establish feasible propagating media combination for healthy production of Stevia cuttings. In the present study, different stem portions (apical, middle, lower), substrate media (soil, sand, silt, FYM, coirdust) and growth regulators (Control, IBA, *Trichoderma varidi* culture) were investigated for higher propagation productivity. The experiment was laid out in complete randomized design (CRD). All the treatments were placed under polythene sheet completely closed from all sides to maintain required humidity level i-e >90%. Recorded data on various seedling and root parameters was analyzed by applying one way ANOVA and the treatments' means were compared for significance by Least Significance Difference (LSD) test at 0.05% P. The study revealed that apical portion of stem grown in soil + sand + silt medium and treated with IBA 500 ppm was effective in induction of better rooting (95.84%) followed by apical portion of stem grown in soil + sand + silt media and treated with *Trichoderma varidi* culture (87.50%). Lowest values were observed when cuttings were not treated with any growth regulator. So, after this study, combination of apical portion of stem grown in soil + sand + silt medium and treated with IBA 500 ppm could be recommended to growers to propagate Stevia with high success on commercial scale.

Keywords: Stevia, *Trichoderma varidi*

Introduction

Sugar forms an indispensable ingredient in the food of the human beings. With increasing awareness of the common man about obesity, diabetes and heart diseases, people are becoming more and more conscious about the use of sugar in their daily meals. The leading source of sugar has been sugarcane and sugar beet but other than these, some artificial sugars like saccharine, aspartame, sucralose, neotame, and acesulfame potassium are also used in some parts of world. These compounds are all high intensity sweeteners, but they have high health risks like aspartame can cause cancer. Similarly patients of phenylketonuria cannot use aspartame in their diet due to the formation of phenylalanine during its metabolism (Butchko *et al*., 2001) [6]. Saccharin is considered to be associated with bladder cancer (Pearson, 2001) [27]. Cyclamate has a major metabolite, cyclohexylamine, which causes testicular atrophy and at high doses, it has unwanted cardiovascular effects (Bopp and Price, 2001) [5]. In such circumstances where chemical sweeteners and sugar are considered health hazards, the interest is now being concentrated on natural sweeteners (Pinhiero and Oliviera, 2005). *Stevia rebaudiana* (family: Asteraceae) is an emerging natural sweetner throughout the world (Sreedhar *et al.,* 2008) [34]. This species originated from Paraguay and Brazil, and currently considered as an alternate substitute of cane and beet sugar (Ahmad *et al*., 2011a, b) [2-3]. It is considered as an important plant due to its active compound present in the leaves known as steviol glycosides. The purified form of steviol glycoside is stevioside which is 150-300 times sweeter than commercially available sucrose and non-caloric in nature without any harmful effects (Dacome *et al.,* 2005; Hwang, 2006) [11, 20]. Other sweetening compound present is Rebaudioside-A which is 180-400 times sweeter than sugar. The sweetening effect of these compounds is purely by taste as natural steviosides cannot enter into the blood stream due to the absence of receptor for absorbance. They are undigested and no part of chemical is absorbed by the body, hence they are of no nutritional value. In its pure form, it is non-caloric and specially used for the treatment of diabetic patients' alongwith hypoglycemics (Thomas and Glade, 2010) [40] and it is expected that 57 million people would be affected by diabetes in the year of 2025. It has other good properties such as immunomodulatory, anti-bacterial, anti-inflammatory, anti-oxidant and can be used as a cardio tonic (Hsieh *et al*., 2003; Taylor, 2005; Madan *et al*., 2010) [19, 37, 25]. It is reported that stevia extracts have no side effects and can be used as an alternative to sugar and other synthetic sweeteners ((Ahmad *et al*., 2011a; Thiyagarajan and Venkatachalam, 2012) [2, 39]. Stevia is a new crop in Pakistan and was 1st introduced by National Agriculture Research Centre (NARC), Islamabad in 2003 to check the climate

adoptability in different areas of the country (Tanvir, 2005) [36]. But still now suitable method of cultivation at field level has not been developed.
Stevia can be propagated by seed, tissue culture and by stem cuttings. Seed germination is notably very poor in stevia due to infertile seed, small size seed and self-incompatibility (Randi, 1980; Midmore and Rank, 2002) [30, 26]. This is an emerging species in Pakistan and most of the researchers are unaware regarding the exact time of seed sowing and cuttings. Even the most important thing in propagation involves tissue culture which is not feasible for the farmer of our country due to requirement of highly sophisticated technologies and equipments. So, this research was aimed to develop a protocol for propagation of Stevia (*Stevia rebaudiana*) which is compatible with the socio-economic conditions of Pakistan. As stevia doesn't produce fertile seed so asexual propagation through stem cuttings has given attention for this particular study. 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 indole butyric acid (IBA) and Naphthalene acetic acid (NAA) to enhance rooting of stevia cuttings (Rajashekara, 2004; Ingle and Venugopal, 2009) [29, 21] have been carried out. However, literature on easily available, cost effective substrate media (Soil, Sand, Silt, FYM, Coirdust), plant growth regulator and bio-inoculant (IBA and *Trichoderma varidi* culture) and their combination with the portion of stem cuttings for multiplication has been scarce. Therefore, this study was aimed to standardize the method for vegetative propagation of stevia by using different combination of substrate media, growth regulator, bio-incoulant and stem portions.

Material and Methods

Design and Layout of Experiment

Experiment was laid out in November 2016 at Research area, Rahim yar Khan of using complete randomized design (CRD). Forty eight (48) different treatment combinations were used to evaluate best rooting media (Table 2.1). Each treatment was replicated twice, with 12 cuttings per replication. The cuttings were placed under polythene sheet completely closed from all sides to attain required humidity level i-e >90%.

Source of Cuttings and their Preparation

The cuttings were procured from the Plant Physiology Research section, Ayub Agricultural Research Institute (AARI), Faisalabad. Three different portions of stem (i) Young portion (ii) Middle portion and (iii) Lower portion of one genotype (Bertoni) was used for propagation. The portion of the cutting with a length of 8 cm and 3-4 nodes without flower and branches were selected with uniform thickness and 2-3 leaves per cutting.

Use of Growth Media

The cuttings were grown in plastic cups filled with four different substrate media combinations (i) Soil + Sand + Coirdust (ii) Soil + Sand + FYM (iii) Soil + Sand + Silt (iv) Soil + Sand + Silt + FYM. Before sowing cuttings were treated with different growth regulators as follows (i) Control (ii) IBA 300 ppm (iii) IBA 500 ppm (iv) *Trichoderma varidi* culture.

Treatment and Sowing of Cuttings

After preparing required concentration of the growth regulator solution and bio-inoculant culture, the cuttings were dipped upto 4 cm deep in the solution for 5 seconds and kept for 2-3 minutes for the auxin to be absorbed into the cut shoot through the xylem. After treatment all cutting portions were planted in plastic cups filled with substrate media and placed under polythene sheet.

Preparation of Stock Solutions

For preparing the stock solution, IBA was dissolved in 0.1 N NaOH solution. The stock solution of 500 ppm was prepared by dissolving 500 mg IBA in half litre of distilled water. The makeup volume was maintained to 100 ml.

Table 1

Concentration of IBA required	Amount of stock solution taken and made up to 100 ml using distilled water
300 ppm IBA	30 ml
500 ppm IBA	50 ml

Table 2: Treatment Details

Sr. No	Treatment	Sr. No	Treatment
T1	S1 + M1 + G1	T25	S2 + M3 + G1
T2	S1 + M1 + G2	T26	S2 + M3 + G2
T3	S1 + M1 + G3	T27	S2 + M3 + G3
T4	S1 + M1 + G4	T28	S2 + M3 + G4
T5	S1 + M2 + G1	T29	S2 + M4 + G1
T6	S1 + M2 + G2	T30	S2 + M4 + G2
T7	S1 + M2 + G3	T31	S2 + M4 + G3
T8	S1 + M2 + G4	T32	S2 + M4 + G4
T9	S1 + M3 + G1	T33	S3 + M1 + G1
T10	S1 + M3 + G2	T34	S3 + M1 + G2
T11	S1 + M3 + G3	T35	S3 + M1 + G3
T12	S1 + M3 + G4	T36	S3 + M1 + G4
T13	S1 + M4 + G1	T37	S3 + M2 + G1
T14	S1 + M4 + G2	T38	S3 + M2 + G2
T15	S1 + M4 + G3	T39	S3 + M2 + G3
T16	S1 + M4 + G4	T40	S3 + M2 + G4
T17	S2 + M1 + G1	T41	S3 + M3 + G1

T18	S2 + M1 + G2	T42	S3 + M3 + G2
T19	S2 + M1 + G3	T43	S3 + M3 + G3
T20	S2 + M1 + G4	T44	S3 + M3 + G4
T21	S2 + M2 + G1	T45	S3 + M4 + G1
T22	S2 + M2 + G2	T46	S3 + M4 + G2
T23	S2 + M2 + G3	T47	S3 + M4 + G3
T24	S2 + M2 + G4	T48	S3 + M4 + G4

S1= Apical portion/Upper portion **M1**= Soil + Sand + Coirdust **G1**= Control
S2= Middle portion **M2**= Soil + Sand + FYM **G2**=IBA (300ppm)
S3= Lower portion **M3=** Soil + Sand + Silt **G3**=IBA (500ppm)
M4=Soil + Sand + Silt + FYM **G4**=*T.varidi* culture

Preparation of *Trichoderma viride* culture

Trichoderma viride culture was prepared by mixing 0.25 kg of culture in 0.5 L water to form uniform slurry (Smitha and Umesha, 2012) [33].

Observations Recorded

Data of seedling and root traits were collected after 30 days interval. For this purpose, rooted cuttings were carefully taken out (three rooted cuttings from each replication and each treatment) and were directly dipped in water for some time to loosen the soil. The cuttings were pulled carefully without harming the roots. Observations in relation to seedling parameters such as sprouting percentage, no. of sprouts/rooted cuttings, length of seedling, no. of new leaves/rooted cuttings were taken. Other observations regarding root parameters taken were length of the longest root, no. of roots per cutting, fresh and dry weight of roots.

Statistical Analysis

For statistical analysis, the generated data were analyzed through one way analysis of variance (ANOVA) (Steel *et al*., 1997) [35]. The treatment means were compared for significance by Least Significant Difference (LSD) test at 0.05% P using Statistics 8.1 software.

Results and Discussion

Data regarding different seedling and root traits (sprouting percentage, number of sprouts, length of seedling, number of new leaves per rooted cuttings, number of roots, length of the longest root, fresh weight of roots and dry weight of roots) are furnished in table 2 and 3 respectively. Different cutting portions showed variation in sprouting percentage under different treatment combinations. Maximum sprouting (95.84%) was observed when Apical portion of stem (S1) grown in Soil + Sand + Silt medium (M3) and treated with IBA solution 500 ppm (G3) followed by (87.50%) three treatment combinations i-e (i) Apical portion (S1) Soil + Sand + Coirdust medium (M1) IBA 500 ppm (G3) (ii) Apical portion (S1) Soil + Sand + Silt (M3) *T.varidi* culture (G4) (iii) Middle portion (S2) Soil + Sand + Silt medium (M3) IBA 500 ppm (G3). Smitha and Umesha (2012) [33] recently reported sprouting (76.8%) with the application of IBA 500 ppm. However, 54.17% sprouting was recorded in control when lower portion of stem (S3) sown in Soil + Sand + FYM medium and treated with no growth regulator (G1). With regard to seedling length, significant differences were observed among different treatments. The maximum length (18.50 cm) of the seedling was recorded in apical portion of stem (S1) Soil + Sand + Silt medium (M3) IBA 500 ppm (G3) followed by (17.30 cm) apical portion of stem (S1) Soil + Sand + Coirdust (M1) IBA 500 ppm (G3) and apical portion of stem (S1) Soil + Sand + Silt (M3) *T.varidi* culture (G4). Lower value (14.47 cm) was observed in treatment combination of Lower portion of stem (S3) Soil + Sand + Silt + FYM (M4) Control (G1) with no growth regulator treatment. The probable reason for increase in length of seedling may be the better utilization of carbohydrates, nitrogen and other nutrients which has been aided by growth regulators (Chandramouli, 2001). The increase in the length of the seedling with the increase in concentration of IBA was also noted by Shivanna *et al.* (2006) in Jeevanthi (*Leptadenia reticulata*). Maximum number of sprouts (3.34) was recorded in the treatment having combination Apical portion of stem (S1) grow in Soil + Sand + Silt medium (M3) and treated with IBA 500 ppm (G3) followed by (2.84) Apical portion of stem (S1) Soil + Sand + Coirdust medium (M1) and IBA 500 ppm (G3). Smitha and Umesha (2012) [33] recently reported sprouting (2.54) with the application of IBA 500 ppm. Lowest number of sprouts (0.67) were recorded when cuttings were not treated with any growth regulator in following combination (i) Lower portion (S3) Soil + Sand + FYM (M2) Control (G1) (ii) Lower portion (S3) Soil + Sand + Silt (M3) Control (G1) (iii) Lower portion (S3) Soil + Sand + Silt + FYM (M4) Control (G1).

Different concentrations of IBA caused significant difference over control with regard to number of new leaves developed per cutting. Maximum number of new leaves (18.17) was noted in the cuttings grown in combination of apical portion of stem (S1) Soil + Sand + Silt medium (M3) IBA 500 ppm (G3) followed by (16.17) apical portion of stem (S1) Soil + Sand + Silt (M3) *T.varidi* culture (G4). On the other hand the lowest number of new leaves (10.51) was recorded in treatment of apical portion (S1) Soil + Sand + Coirdust (M1) Control (G1). The results reported by Chalapathi *et al.* (1999) [8], Chalapathi *et al.* (2001) [9] and Kassahun *et al.* (2013) [22] in Stevia followed the similar trend. Similarly, Ingle and Venugopal (2009) [21] obtained the highest number of leaves (37.86) with the application of 500 ppm IBA solution. The possible reason for such increase may be due to the activation of shoot growth which probably increased the number of nodes that lead to development of more number of leaves.

Table 3: Integrated Effect of Stem Cuttings, Substrate Media and Growth Regulators on Seedling Traits of Stevia Cuttings.

Treatment	SP. %	No. Sp.	L Seedling	NNL
S1 + M3 + G3	95.84 A	3.34 A	18.50 A	18.17 A
S1 + M1 + G3	87.50 AB	2.84 B	17.30 B	12.83 GHIJK
S1 + M3 + G4	87.50 AB	1.67 G	17.30 B	16.17 B

S2 + M3 + G3	87.50 AB	2.00 EF	16.87 BCDE	15.5 BC
S1 + M4 + G3	83.33 BC	2.00 EF	16.64 BCDEF	14.83 BCDE
S1 + M1 + G4	79.17 BCD	2.33 CD	16.64 BCDEF	12.17 IJK
S1 + M2 + G3	79.17 BCD	2.50 C	16.67 BCDEF	13.17 FGHIJ
S1 + M2 + G4	79.17 BCD	2.17 DE	16.27 CDEFGHIJK	12.50 HIJK
S1 + M3 + G2	79.17 BCD	1.67 G	16.14 DEFGHIJKL	15.17 BCD
S1 + M4 + G2	79.17 BCD	1.67 G	16.00	13.83 DEFGH
S1 + M4 + G4	79.17 BCD	1.67 G	16.17 DEFGHIJK	14.17 CDEFG
S1 + M3 + G1	79.17 BCD	1.84 FG	15.64	15.17 BCD
S1 + M4 + G1	79.17 BCD	1.67 G	15.47 IJKLMNO	13.83 DEFGH
S2 + M1 + G3	79.17 BCD	1.67 G	17.07 BCD	13.17 FGHIJ
S3 + M1 + G3	79.17 BCD	1.33 H	16.44 BCDEFGH	14.50 CDEF
S2 + M4 + G3	75.00 CDE	1.67 G	17.20 BC	15.17 BCD
S3 + M3 + G3	75.00 CDE	1.33 H	16.50 BCDEFG	14.50 CDEF
S1 + M1 + G2	70.84 DEF	1.67 G	16.40 BCDEFGHI	11.51 KL
S1 + M2 + G2	70.84 DEF	1.84 FG	15.97	11.83 JKL
S1 + M1 + G1	70.84 DEF	1.33 H	15.64	10.51 L
S1 + M2 + G1	70.84 DEF	1.67 G	15.40 KLMNO	11.83 JKL
S2 + M1 + G4	70.84 DEF	1.33 H	16.27 CDEFGHIJK	12.50 HIJK
S2 + M2 + G3	70.84 DEF	1.33 H	16.24 DEFGHIJK	13.83 DEFGH
S2 + M3 + G2	70.84 DEF	1.33 H	16.27 CDEFGHIJK	14.17 CDEFG
S2 + M3 + G4	70.84 DEF	1.00 I	16.47 BCDEFGH	15.17 BCD
S2 + M4 + G2	70.84 DEF	1.00 I	16.20 DEFGHIJK	14.83 BCDE
S2 + M3 + G1	70.84 DEF	1.00 I	15.74	13.83 DEFGH
S2 + M4 + G1	70.84 DEF	1.00 I	16.00	14.17 CDEFG
S3 + M1 + G4	70.84 DEF	1.00 I	16.04	13.51 EFGHI
S3 + M2 + G3	70.84 DEF	1.33 H	15.90	13.51 EFGHI
S3 + M3 + G2	70.84 DEF	1.00 I	15.17 MNO	13.17 FGHIJ
S3 + M3 + G1	70.84 DEF	0.67 J	15.07 NO	13.17 FGHIJ
S3 + M4 + G3	66.67 EF	1.00 I	16.30 CDEFGHIJK	13.51 EFGHI
S3 + M2 + G4	66.67 EF	1.00 I	15.44 JKLMNO	13.17 FGHIJ
S3 + M3 + G4	66.67 EF	1.00 I	16.07	13.51 EFGHI
S2 + M1 + G2	62.50 FG	1.33 H	16.37 BCDEFGHIJ	12.17 IJK
S2 + M2 + G2	62.50 FG	1.00 I	16.00	12.83 GHIJK
S2 + M2 + G4	62.50 FG	1.33 H	16.14 DEFGHIJKL	14.17 CDEFG
S2 + M4 + G4	62.50 FG	1.00 I	16.47 BCDEFGH	14.83 BCDE
S2 + M1 + G1	62.50 FG	1.33 H	15.64	11.51 KL
S2 + M2 + G1	62.50 FG	1.00 I	15.54 HIJKLMNO	12.17 IJK
S3 + M1 + G2	62.50 FG	1.00 I	15.17 MNO	13.17 FGHIJ
S3 + M4 + G2	62.50 FG	1.00 I	14.84 O	13.17 FGHIJ
S3 + M4 + G4	62.50 FG	1.00 I	16.14 DEFGHIJKL	13.17 FGHIJ
S3 + M1 + G1	62.50 FG	1.00 I	14.90 O	12.83 GHIJK
S3 + M4 + G1	62.50 FG	0.67 J	15.17 MNO	13.17 FGHIJ
S3 + M2 + G2	54.17 G	1.00 I	15.20 LMNOP	13.17 FGHIJ
S3 + M2 + G1	54.17 G	0.67 J	14.80 O	

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

S1= Apical portion/Upper portion **M1=** Soil + Sand + Coirdust **G1=** Control
S2= Middle portion **M2=** Soil + Sand + FYM **G2=** IBA (300 ppm)
S3= Lower portion **M3=** Soil + Sand + Silt **G3=** IBA (500 ppm
M4= Soil + Sand + Silt + FYM **G4=** *T. varidi* culture
SP. %= Sprouting Percentage **N SP.** = No. of Sprouts/Cutting **L SP. =** Length of Sprout
L Seeedling = Length of Seedling **NNL =** No. of new Leaves/Rooted Cutting

It was found that number of roots per cutting was found to be increasing with the application of IBA. The number of roots was found to be more (11.34) in the cuttings treated with IBA 500 ppm (G3) in combination of apical portion of stem (S1) and Soil + Sand + Silt medium (M3) followed by (10.34) apical portion (S1) Soil + Sand + Coirdust (M1) and IBA 500 ppm (G3). The reason may be attributed to enhanced tissue sensitivity and increased rooting via increased internal free IBA resulting in increased number of roots. These results are in close agreement with Carvalho *et al.* (1995) [7], Chalapathi *et al.* (2001) [9], Debnath (2008) [12] and Abdullateef and Osman (2012a) [1]. Another possible reason may be due to translocation of carbohydrates from the leaves which plays important role in root development (Carvalho *et al.*, 1995) [7]. Similarly, Ingle and Venugopal (2009) [21] documented that IBA 500 ppm influenced maximum number of roots in stem cutting of Stevia. Increased number of roots due to auxin application is a common feature in many herbaceous perennial crops (Haissing and Davis, 1984; Hartmann *et al.*, 2002) [14, 17]. The lowest numbers of roots (5.67) were recorded in Lower portion (S3) Soil + Sand + Coirdust (M1) Control (G1) treatment combination.

The length of the root differed significantly among different growth regulator treatments and it was found to be superior over control. The length of root was found to be higher

(11.55 cm) in the cutting treated with IBA 500 ppm (G3) in combination having apical portion of stem (S1) and Soil + Sand + FYM medium (M2) followed by (3.26 cm) apical portion (S1) Soil + Sand + Silt (M3) IBA 500 ppm (G3). Minimum length of root was observed (2.06 cm) in control (G1) alongwith Lower portion (S3) Soil + Sand + Coirdust medium (M1) treatment combination. The increase in length of the roots might be due to an early initiation of roots at higher concentrations of IBA and more utilization of the food materials due to early formation of the roots. Similar trend has been reported by Chalapathi *et al.* (2001) [9] and Debnath (2008) [12] in Stevia. It is also attributed to the action of auxin activity which might have caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings and resulted in accelerated cell elongation and cell division in suitable environment (Singh *et al.*, 2003) [32]. The fresh weight of roots was found to be significantly higher (0.68 g) in the cuttings having apical portion (S1) Soil + Sand + Silt (M3) IBA 500 ppm (G3) followed by (0.48 g) in the cuttings having Lower portion (S3) Soil + Sand + Silt + FYM (M4) Control (G1). The lowest fresh weight (0.06 g) was recorded in the treatment combination of Lower portion (S3) Soil + Sand + Coirdust (M1) Control (G1).

Significantly higher dry weight (0.26 g) of the roots per cutting was recorded in
the cuttings treated with IBA 500 ppm (G3) in combination of apical portion of stem (S1) and Soil + Sand + Silt medium (M3) followed by (0.23 g) the treatment combination of apical portion (S1) Soil + Sand + Coirdust (M1) IBA 500 ppm (G3) and apical portion (S1) Soil + Sand + FYM (M2) IBA 500 ppm (G3). On the other hand, the lowest dry weight (0.05 g) was found in three treatment combinations as follows. (i) Lower portion (S3) Soil + Sand + Silt + FYM (M4) Control (G1) (ii) Lower portion (S3) Soil + Sand + Coirdust (M1) Control (G1) (iii) Lower portion (S3) Soil + Sand + FYM (M2) Control (G1). Likewise, Ingle and Venugopal (2009) [21] and Smitha and Umesha (2012) [33] reported maximum of 0.29 g and 0.27 g of dry weight respectively after treatment with IBA 500 ppm. The higher dry weight of the roots may be attributed to increased number of roots and length of longest root. Similar effect has also been observed by Farooqi *et al.* (1994) [13] in *Rosa damascena.*

As regards to substrate media, Soil + Sand + Silt media produced longer and thicker sprouts, maximum newly emerged leaves, more roots and higher dry weight of roots (Table 2 and 3). Higher biomass production in Soil + Sand + Silt media may be due to increased nutrient uptake and enhanced availability of nutrients and growth promoting substances (Thankamani *et al*., 2005) [38]. Our findings are in accordance with the results reported by Hasan *et al*. (2009) [18], Koppad and Gouda (2010) [23], Abdullateef and Osman (2012a) [1], Smitha and Umesha (2012) [33] and Kumar (2013) [24]. Among the different stem portions used for propagation of Stevia it was noted that apical portion of stem produced the best results regarding root and seedling parameters. Beemnet and Solomon (2012) [4] and Kassahun *et al.* (2013) [22] also suggest apical portion of stem for stevia propagation through stem cuttings. The reason may correlate with the structure of the stem or difference in chemical composition of the plant along the stem as reported by Hartman *et al*. (1997) [16] and Hansen (1986) [15].

Table 3: Integrated Effect of Stem Cuttings, Substrate Media and Growth Regulators on Rooting Traits of Stevia Cuttings

Treatments	NR	LLR	FWR	DWR
S1 + M3 + G3	11.34 A	3.26 A	0.68 A	0.26 A
S1 + M1 + G3	10.34 B	3.19 A	0.43 C	0.23 B
S1 + M3 + G4	9.00 CD	2.73 DEF	0.26 H	0.18 FG
S2 + M3 + G3	7.67 FGH	2.63 FGH	0.26 HI	0.19 EF
S1 + M4 + G3	9.00 CD	2.96 B	0.33 G	0.21 C
S1 + M1 + G4	9.34 C	2.86 BC	0.36 E	0.13 OP
S1 + M2 + G3	8.67 CDE	3.29 A	0.38 D	0.23 B
S1 + M2 + G4	7.67 FGH	2.83 CD	0.35 F	0.16 HI
S1 + M3 + G2	8.34 DEF	2.56 GHI	0.25 IJ	0.17 GH
S1 + M4 + G2	8.00 EFG	2.66 FG	0.19 OP	0.15 JKL
S1 + M4 + G4	9.34 C	2.69 EF	0.21 M	0.19 E
S1 + M3 + G1	7.34 GHI	2.43 JKLM	0.09 T	0.06 ST
S1 + M4 + G1	8.00 EFG	2.43 JKLM	0.08 U	0.06 RS
S2 + M1 + G3	8.34 DEF	2.69 EF	0.24 JK	0.20 CD
S3 + M1 + G3	7.34 GHI	2.43 JKLM	0.23 K	0.16 IJK
S2 + M4 + G3	7.67 FGH	2.49 IJK	0.25 IJ	0.19 DE
S3 + M3 + G3	7.67 FGH	2.43 JKLM	0.22 LM	0.17 GH
S1 + M1 + G2	9.00 CD	2.53 HIJ	0.23 KL	0.14 LMN
S1 + M2 + G2	8.00 EFG	2.79 CDE	0.21 M	0.12 P
S1 + M1 + G1	8.34 DEF	2.83 CD	0.08 TU	0.07 RS
S1 + M2 + G1	8.34 DEF	2.63 FGH	0.08 U	0.06 ST
S2 + M1 + G4	7.34 GHI	2.43 JKLM	0.19 OP	0.18 FG
S2 + M2 + G3	8.34 DEF	2.66 FG	0.26 H	0.19 E
S2 + M3 + G2	7.34 GHI	2.46 IJKL	0.18 OPQ	0.13 OP
S2 + M3 + G4	7.34 GHI	2.46 IJKL	0.20 N	0.18 EFG
S2 + M4 + G2	6.67 IJK	2.36 LMNO	0.19 OP	0.13 OP
S2 + M3 + G1	7.34 GHI	2.39 KLMN	0.07 UV	0.07 RS
S2 + M4 + G1	6.34 JKL	2.23 P	0.07 U	0.07 R
S3 + M1 + G4	6.67 IJK	2.29 NOPQ	0.18 PQ	0.13 NOP
S3 + M2 + G3	7.00 HIJ	2.33 MNOP	0.25 HI	0.16 IJK
S3 + M3 + G2	7.00 HIJ	2.33 MNOP	0.15 S	0.12 P

S3 + M3 + G1	6.34 JKL	2.23 P	0.07 UV	0.06 ST
S3 + M4 + G3	7.34 GHI	2.36 LMNO	0.24 JK	0.16 IJ
S3 + M2 + G4	6.67 IJK	2.26 OPQ	0.20 N	0.13 OP
S3 + M3 + G4	7.00 HIJ	2.29 NOPQ	0.19 OP	0.15 KLM
S2 + M1 + G2	7.00 HIJ	2.53 HIJ	0.18 PQ	0.15 KLM
S2 + M2 + G2	7.34 GHI	2.36 LMNO	0.19 OP	0.13 NOP
S2 + M2 + G4	7.34 GHI	2.53 HIJ	0.19 NO	0.15 IJKL
S2 + M4 + G4	7.34 GHI	2.39 KLMN	0.19 OP	0.16 IJ
S2 + M1 + G1	7.67 FGH	2.33 MNOP	0.07 UV	0.07 R
S2 + M2 + G1	6.67 IJK	2.26 OPQ	0.07 U	0.07 RS
S3 + M1 + G2	6.00 KL	2.19 Q	0.16 R	0.11 Q
S3 + M4 + G2	6.34 JKL	2.26 OPQ	0.18 OPQ	0.13 OP
S3 + M4 + G4	7.00 HIJ	2.29 NOPQ	0.20 N	0.14 MNO
S3 + M1 + G1	7.34 GHI	2.06 R	0.06 V	0.05 T
S3 + M4 + G1	6.34 JKL	2.19 Q	0.48 B	0.05 T
S3 + M2 + G2	6.34 JKL	2.19 Q	0.17 Q	0.12 P
S3 + M2 + G1	6.00 KL	2.19 Q	0.07 UV	0.05 T

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

S1= Apical portion/Upper portion
S2= Middle portion
S3= Lower portion

M1= Soil + Sand + Coirdust
M2= Soil + Sand + FYM
M3= Soil + Sand + Silt
M4= Soil + Sand + Silt + FYM

G1= Control
G2= IBA (300 ppm)
G3= IBA (500 ppm)
G4= *T. varidi* culture

NR = No. of Roots/Rooted Cuttings
LLR = Length of Longest Root
FRW = Fresh Weight of Roots
DRW = Dry Weight of Roots

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