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Effect of media amendments on invivo root and shoot organogenesis of stevia (*Stevia rebaudiana* L.)

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Abstract

Stevia, also known as sugar leaf or sweet weed, a highly valuable and medicinal plant domesticated recently in many countries of the world. Propagation of stevia usually by stem cuttings root easily, but require high labor inputs. Poor seed germination is one of the factors limiting large-scale cultivation. Therefore, an experimental study was carried out with objective to evaluate performance of nodal cutting of stevia under different media amendments (T_0 = Garden Soil, T_1 = IBA + Soil, T_2 = FYM + soil (1:1), T_3 = Leaf mold + soil (1:1). Randomized Complete Block Design (RCBD) with factorial arrangements having 4 treatments replicated 4 times were used during experimentation. Data analysis showed that highest value of survival percentage (91.75 %), plant height (36.18cm), number of branches (3.00), number of leaves (42.50), stem diameter (5.38mm), number of inflorescence (9.50), root length (10.00cm), fresh root weight (5.23g) and dry root weight (0.96g) were recorded in treatment T_1 (IBA+Soil). Whereas maximum number of days to spike emergence were taken by T_0 (47.00). It was concluded from the research findings that indole butyric acid (IBA) of 150ppm could be used as an alternative for enhancing the growth attributes of stevia under the agro-climatic conditions of National Agricultural Research Centre Islamabad.

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Introduction

Stevia *rebaudiana* L. belongs to family Asteraceae is a non-caloric natural-source alternative to artificially produced sugar substitutes. These compounds easily pass through the digestive process without chemically breaking down, making stevia safe for those who need to control their blood sugar level (Strauss 1995). Shock (1982) reported that stevia contains eight glucoside compounds, each featuring a three-carbon-ring central structure. Stevioside is the most abundant glucoside produced. An extract of one or more of these compounds may be up to 300 times sweeter than sugar (Duke 1993). *Stevia rebaudiana* (Bert) Bertoni is generally a perennial herb which is also called "sweet weed, sweet leaf, sweet herb, and honey leaf". It is frost susceptible hence its growth stops during winter season (Megeji *et al*., 2005).

Stevia plant develops up to the height of 60-70 cm approximately. It has a wide root system along with delicate stems consist of small elongated leaves. It grows well at altitudes of 200-500 meters above sea level, where mean temperature is 23°C (73.4°F) and annual rainfall ranges from 1500-1800 mm (Savita *et al*., 2004). Discovering in the South American wild area, it could be found growing in semi-arid habitation ranges from savannah to bush forest and highland environment. Where it is grown and cultivated in fertile soil certainly. As stevia plant get matured it grows up from 65 to 180cm. Being a short day plant it starts flowering in January-March in the southern high altitudes. Its flowers are normally white in color consisting a light purple throat. These are mini in size where its arrangement is like small corymbs (Goettemoeller and Ching, 1999; Singh and Rao, 2005). The most suitable soil is sandy soil, needing a sunny position not much colder. The appropriate accepted climate required is semi- humid subtropical where temperature excesses from 21 to 43°C and average should be 24°C (Huxley, 1992).

The introduction of stevia sugar obtained from stevia plant leaves has brought a change in world of sweetness in near past. That consists compounds which are 250 to 300 times sweeter than common table sugar. (Singh and Rao, 2005). *Stevia rebaudiana* has the highest sweetness levels among all the varieties (Carakostas *et al.,* 2008). This plant is presently under study as an alternate field crop for the southern United States. Field corn (maize) and Sugar cane has currently covered a huge space of the sweetener market. It is usually grown for the purpose to obtain stevioside (a sweet compound) which is about 300 times sweeter than common sugar (Kumar and Singh, 2012). Stevioside compounds are stable to heat and pH, and hence cannot be fermented. *Stevia rebaudiana* (Bertoni) among one of the 154 members of the genus *Stevia* Cav., the only one member of the genus that has sweet Ste viol glycosides (Brandle *et al*., 1998). The dry product obtained from the stevia leaves contains different compounds like amino acids, oligosaccharides, flavonoids, alkaloids, xanthophyll, chlorophylls, free sugars, lipids and trace elements (Goettemoeller and Ching, 1999).

Propagation of stevia is usually by stem cuttings which root easily, but require high labor inputs. Poor seed germination is one of the factors limiting large-scale cultivation. Shock (1982); Duke (1993); Carneiro (1997) all mention poor production of viable seeds. Seeds of *Stevia rebaudiana* L. are very small in size and the germination percentage is much low i.e. <10% (Sivaram and Mukundan, 2003). Propagation is a special concern for northern growers who must grow stevia as an annual. Apart from these limitations many research studies also showed that seed germination is not an appropriate method for vigorous biomass and for the production of sweet glycosides content. Homogeneous populations cannot be obtained through seed propagation, because it results a great variability in its significant features like sugar levels and its structure (Tamura *et al.,* 1984). Germination success of Stevia plant, normally grown from seed is very low; sometimes it germinates only up to 10% (Sakaguchi and Kan, 1982).

Due to its commercial value, high demand and medicinal both in local and international market it gives higher profits and employment opportunities to farm families. Also, this crop is adapted over a wide-ranging climatic conditions. (Qui *et al.,*

2000).Environmental conditions normally provided in the nursery i.e. light, temperature and humidity play a key role in growth success, rooting and development of cuttings propagated (Hofferman, 1979). Best propagation methods (Sakaguchi and Kan, 1982) for stevia plants which are proven to be successful are cuttings or tissue culture technique. Due to the above-mentioned difficulties, cutting may be a suitable method for stevia propagation and conservation. Therefore an experiment was conducted to check the effect of different media amendment on rooting and other growth attributes of stevia in invivo condition.

Materials and methods

Site selection

The research was laid out at Directorate of Floriculture, National Agricultural Research Center Islamabad located at latitude of 33.6701° N and longitude of 73.1261° E (Basit *et al.*, 2018).

Soil characteristics of experimental site

Soil samples from the upper and lower surface were taken randomly before the cuttings transplantation and analyzed at Department of Soil and Environmental Sciences, The University of Agriculture Peshawar. The studied physio-chemical properties of soil samples are presented in the given table.

Plant materials and treatments

Randomized Complete Block Design (RCBD) with factorial arrangements was used during experimentation and each treatment were replicated four times. Experimental treatments comprised of following combinations of different growing media i.e. To = Soil, T_1 = IBA + soil, T_2 = FYM + soil (1:1), T_3 = Leaf mold + soil (1:1).

Preparation of IBA Solution

For 150ppm IBA solution, 150mg of IBA powder was mixed in 1 liter distilled water and uniformly dissolved on magnetic stirrer. The desired amount was taken and put the stevia cuttings in IBA solution for 10 minutes. The cuttings were then shifted to polythene bags filled with field soil and irrigated every day for 3-4 weeks. One year old stevia plants were used for propagation through shoot tip cuttings. The cuttings of selected plants were fresh, healthy, and disease free. Half shaded area, water source, sprinkler, garden tools, scissor/cutting tool, ruler/meter stick, polyethylene bags were used in materials etc. Whereas, garden soil, silt, FYM, leaf mold and IBA were used as media.

Growth parameters studied

Different parameters studied during this research study were: Survival percentage, number of branches plant^{-1} and number of leaves plant^{-1} were calculated. Plant height (cm) was calculated through measuring tapes from ground level to the top of the plant, stem diameter (mm) was measured through Vernier caliper and root length (cm) was measured through measuring tapes (Gilani *et al.*, 2018). Fresh root weight (gm.) was measured by using digital balance (Ohaus) and placed in oven at 70 °C for 12 hours and the dry root weight (mg) was then measured with the same scale.

Data analysis

Data analysis was performed using Statistix 8.1 software package. Where comparisons were made using one-way ANOVA (Analysis of Variance) and the means difference were compared at 5% level of significance (Basit *et al.*, 2018).

Results

Survival percentage

Fig 1 revealed that the media amendments significantly affected survival percentage of stevia. Research findings showed that the stevia cuttings treated with IBA (T_1) had highest survival percentage (91.75%) followed by T_2 (FYM) and T_3 (leaf mold), which are 63.50% and 54.25% respectively. Whereas lowest survival percentage (15.25%) was observed in T_0 (control).

Plant height (cm)

The data was statistically analyzed for the effect of IBA on plant height of stevia, which showed that the

effect of media amendments had a significant influence on plant height (Fig 2). The results showed highly significant difference of the treatments where IBA was applied compared to the treatments where IBA was not applied. Maximum plant height was observed in treatment T_1 (36.18cm) followed by treatment T_2 (23.18cm) and T_3 (18.90). While minimum was noted in T_0; Control (12.95cm).

Table 1. Soil characteristics of analysed samples.

Soil variables	pH	% O.M	% N	P (mg kg^{-1})	K^+(mg kg^{-1})
Values	6.6	0. 81	14	6.1	191

Number of branches plant^{-1}

It was obvious from Fig 2 that highest number of branches were recorded in treatment T_1 (3.00) followed by T_2 (1.25) and T_3 (1.00). Whereas minimum (0.75) were observed in treatment T_0; Control.

Number of leaves plant^{-1}

The mean data regarding number of leaves plant^{-1} are presented in Fig 2.The mean data indicated that highest number of leaves (42.5) were recorded in cuttings treated with IBA (T_1) followed by T_2 (30.75) and T_3 (28.75). While minimum was recorded in treatment T_0 (17.25).

Stem diameter

The statistical analysis of data revealed that thicker stem diameter was contributed by treatment T_1 (5.38mm), followed by T_2 (3.25mm) and T_3 (2.55mm). While thinner stem diameter was recorded in treatment T_0 (2.00mm) (Fig 2).

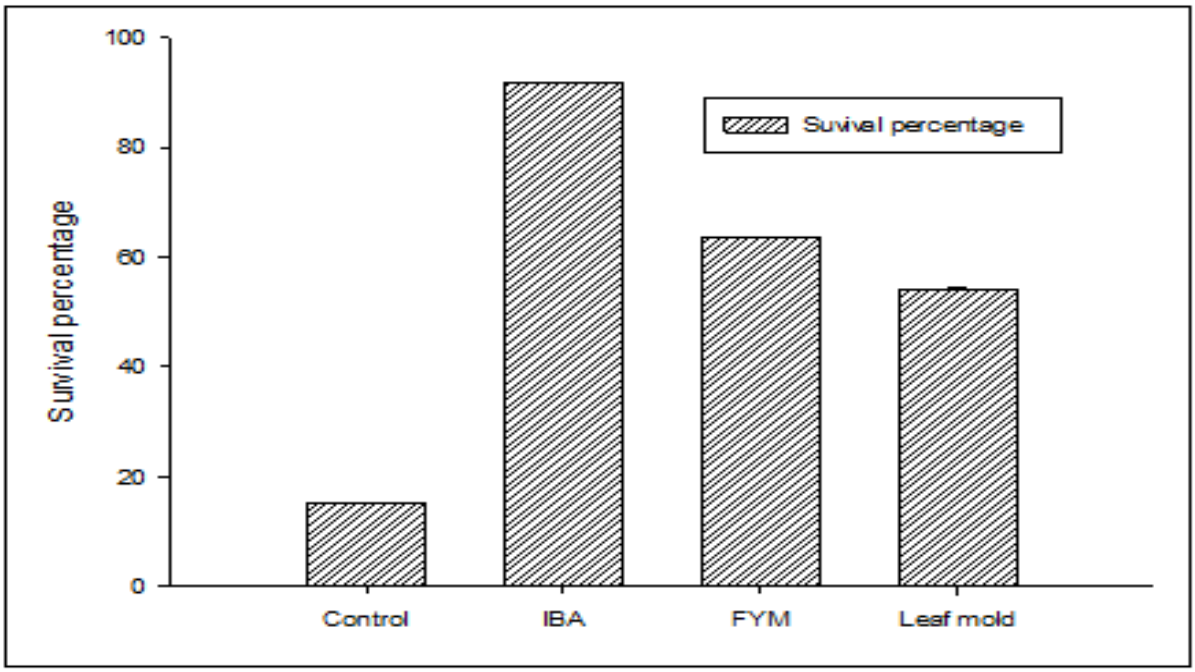

Fig. 1. Survival percentage of stevia as affected by different media amendments.

Days to Spike emergence

The statistical analysis of data revealed that highly significant result were contributed by all treatments approximately (Fig 3). Minimum days to spike emergence was taken by the treatment T_1 (35.25) days, treated with IBA followed by T_3 (39) days and T_2 (42.25) days. While the higher number of days to spike emergence were taken by T_0 (47) days.

Number of inflorescence plant^{-1}

The statistical analysis of Fig 3 revealed that highly significant result of number of infloresence plant^{-1}

were contributed by all treatments approximately. Maximum number of inflorescence (9.50) plant^{-1} were recorded in T_1 which was statistically higher than the other treatments (T_2:6.25, T_3:4 and T_o: 2.25).

Root length (cm)

The statistical analysis of Fig 4 showed that media amendments had significantly influenced root length of stevia plant. Maximum root length was recorded in T_1 (10cm), followed by T_2 (7.63cm) and T_3 (7.10cm), while minimum was recorded in T_o (0.7cm).

Fresh weight of root (cm)

The mean data presented in Fig 4 revealed that maximum value fresh weight was recorded in treatment T_1 (5.23g) followed by T_2 (3.60g) and T_3 (3.20g) while minimum value of fresh weight was observed in treatment T_o (0.02g).

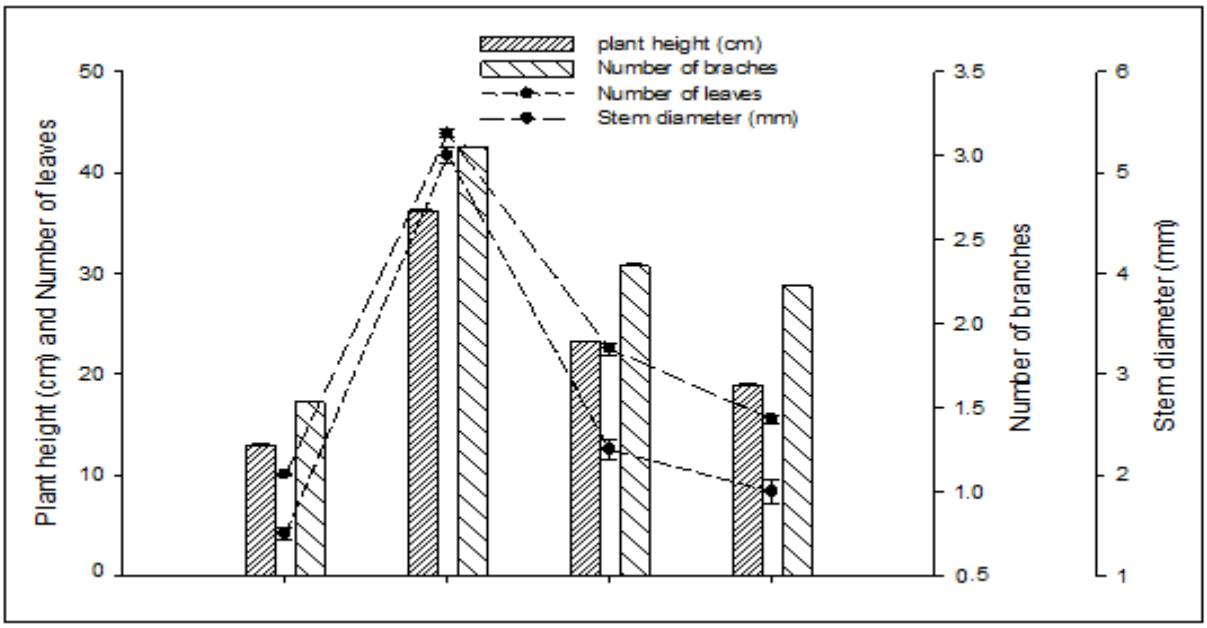

Fig. 2. Plant height, Number of leaves plant^{-1}, Number of branches plant^{-1} and stem diameter (cm) of stevia as affected by different media amendments.

Dry weight of root (cm)

The mean day pertaining in Fig 4 showed that higher value of dry root weight was recorded in treatment T_1 (0.96 g) followed by T_2 (0.35 g) and T_3 (0.28 g), whereas minimum was noted in treatment T_o (0.03g).

Discussion

Highest survival percentage in T_1 (IBA) might be due to better intake and storage of carbohydrates and other factors. These results are in agreement with Prizhmontas (1991) stated that *Prunus* cuttings treated with IBA had better survival rate that was associated with better rooting. Ahmad *et al.* (2002) also recorded similar results in Bougainvillea cuttings treated with IBA. Similarly, Alam *et al.* (2007) reported that application of IBA enhanced the survival percentage of Kiwi cuttings. Chandramouli (2001) reported that the increased shoot length may be due to the proper utilization of carbohydrates, nitrogen and other nutrients that has been provided by growth regulators Shivanna *et al.* (2006) also found similar results for the effect of IBA concentration on shoot length in Jeevanthi (*Leptadenia reticulata*). Although Pathak *et al.* (2002) found that chrysanthemum cuttings treated with IBA showed an increase in number of branches.

Leaves quantity per plant always play a vital role in photosynthesis which also shows an effect in gaining and increase yield. Research analysis for effect of IBA on number of leaves per plant showed highly significant results. The highest number of leaves might be due to availability of more minerals and nutrients like water and nitrogen, which encourages vegetative growth (Gilani *et al.*, 2018). These results

are confirmed with findings of Mehraj *et al.* (2013) found that IBA treatment enhance number of sprout buds, days to first sprout bud initiation, number of leaves, number of branches, and survival in Bougainvillea hardwood cuttings. Maximum value of diameter of stem, may be the result of proper nutrients level provided by IBA. Shahab *et al.* (2013) also stated that IBA applied to hardwood cuttings of Alstonia promotes stem diameter, number of roots, root diameter, sprout length. Thind *et al.* (2005) also found that IBA as a most appropriate plant growth regulator has proved to be best for successful survival of peach variety Shan-i-Punjab.

It also played a key role in better rooting and enhancing its vegetative characters.

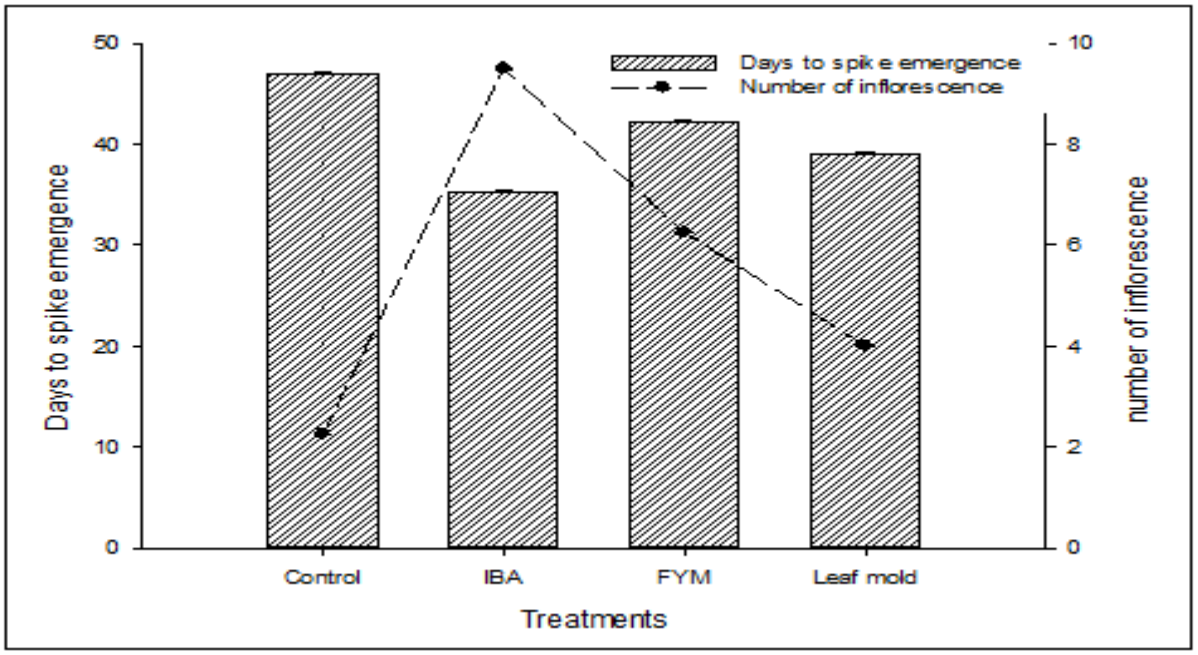

Fig. 3. Days to spike emergence and number of branches as affected by different media amendments.

Similarly IBA application has positive role in early flowering production in stevia plant. Similarly, Pathak *et al.* (2002) found that chrysanthemum cuttings when treated with IBA enhance flowering and produce more number of leaves. The relative number of inflorescence per plant It is renowned fact that auxin plays a significant role in initiation of roots in cuttings. Cell elongation in root was promoted by IBA which caused an increase in root length. Higher concentration of IBA and more consumption of the food materials may have caused an increase in roots length, which might be due to early development of the roots. Similar results have been described by Chalapathi *et al.* (2001);Debnath (2008) in stevia plant. Action of auxin activity is attributed that may have triggered hydrolysis and translocation of carbohydrates and nitrogenous ingredients at the base of cuttings. Which might have helped accelerating cell elongation and cell division in proper environment (Singh *et al.*, 2003).

Increase in root weight may be caused due to higher root length, more number of roots and root diameter. Where root length and diameter may be due to better intake and accumulation of carbohydrates, and other nutrients drawn from IBA. Results described by Ingle (2008) for rooting of *Stevia* showed that with increasing the concentration of IBA caused an increase in fresh weight of root, rooting percentage and root length. Ingle and Venugopal, 2009 reported that stevia cuttings treated with IBA has given more dry weight (0.290 g) of the roots per cutting. Higher dry root weight may be accredited to increase in length of root and in number of roots. Similarly, Farooqi *et al.* (1994) observed the similar effects in *Rosa damascena*. Instantaneously, encouragement of

rooting process with auxin, carbohydrate carriage from leaf to root increases, hence, it causes increase in dry root weight (Hartmann *et al.,* 2002). The increase in the number and length of roots of stevia stem cuttings submitted to auxin treatment may be related to a increase in the sensitivity of the basal tissue of the cutting, as well as to an improvement in the capacity for hydrolysis of reserves, necessary for root development (Osman *et al.*, 2013).

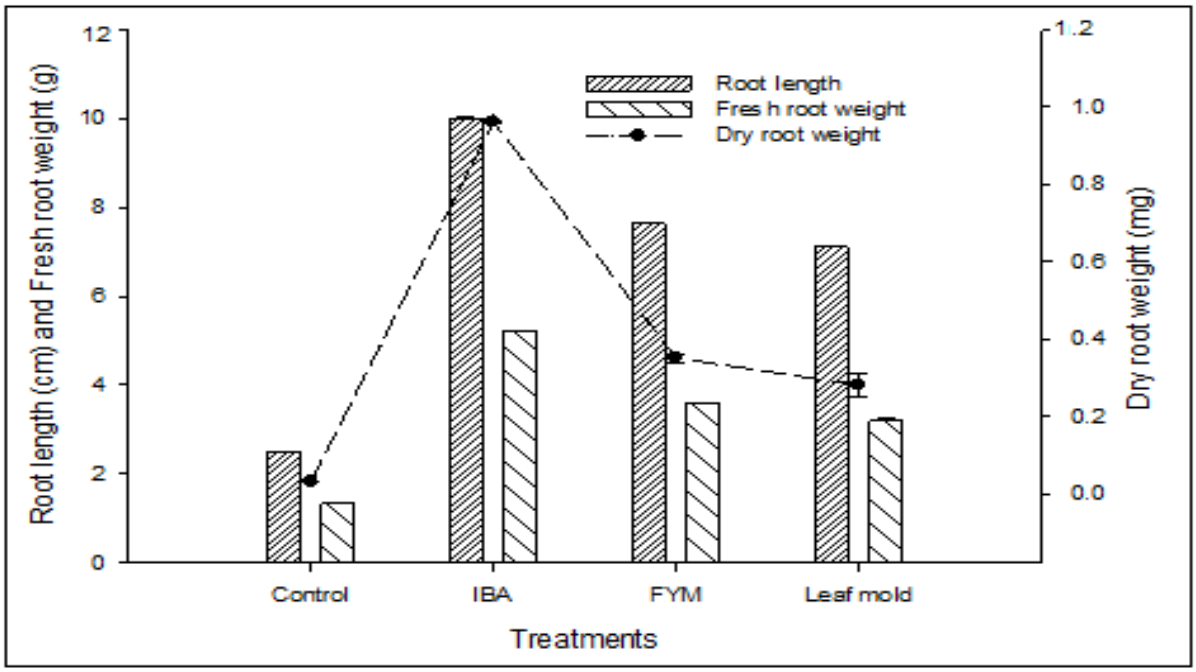

Fig. 4. Root length (cm), fresh root weight (g) and dry weight (cm) of stevia as affected by different media amendments.

Conclusions and recommendation

It also plays a key role in rooting, shooting and growth of stevia plant. On the basis of above results proper amount (150ppm) of IBA is recommended for the maximum growth and rooting of stevia cuttings under the agro-climatic conditions of Islamabad region.

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