EFFECT OF AUXINS ON PROPAGATION OF HONEY CROP: *STEVIA REBAUDIANA* (BERTONI) BERTONI.

S. Javad*, S. Basharat, S. Shaheen, and A. Aftab

Postal Address: Dept. of Botany, Lahore College for Women University, Lahore.
Corresponding Author E-mail: zif_4@yahoo.com

ABSTRACT: The present study was conducted to optimize the green house conditions for Propagation of *Stevia rebaudiana* cuttings. *Stevia rebaudiana* belonging to the family Asteraceae, is a native to the Brazil and Paraguay. Its leaves produced stevioside, an a caloric natural sweetener. The sweetness of stevioside was 150 -300 times greater than sucrose. Seeds of Stevia were very small in size. Production of Stevia through seeds was not beneficial because seeds were not viable for germination due to their infertility. In present study different concentrations of Naphthalene acetic acid (NAA) and Indole Butyric acid (IBA) were used to study their effect on the rooting of *Stevia rebaudiana* cuttings. Results showed that highest number of roots per cutting were observed to be 34.9 from 3% NAA solution, showing maximum root length, root number, shoot length and number of nodes per cutting. Cuttings of *Stevia rebaudiana* responded well in NAA treatment as compared to the IBA treatment. It was concluded that Naphthalene acetic acid was a better choice for enhanced rooting and vegetative propagation of *Stevia rebaudiana* through stem cuttings.

Key words: *Stevia rebaudiana*, Cuttings, auxins, rooting.

(Received 18-05-2015 accepted 15-12-2015).

INTRODUCTION

Sugar is a necessary ingredient of human food. The main source of sugar is sucrose i.e., table sugar. Some artificial sweeteners are also available in the market but it is found that these have high health risks (Richman *et al*., 2005). Sucrose increases the chances of diabetes as well as obesity. People with obesity have a higher chance of developing a number of health problems, like diabetes, heart arrest, cancers and problems related to liver and gall bladder (Pinheiro and Oliveria, 2005).

Natural sweetener may be a good alternative with low calories. A lot of research has been done on *Stevia rebaudiana* which has proved it as the best alternative for sugarcane because it makes stevioside which is 200 -300 times sweeter as compared to table sugar (sucrose) and has zero calories with no side effects (Dacome *et al*., 2005). *Stevia rebaudiana* belongs to the areas like Paraguay and Brazil where climate is tropical and average annual rainfall is 1500-1800mm. *Stevia rebaudiana* can tolerate temperature up to a range of -6 to 43˚C and pH of soil upto 6.5 to 7 (Oddone, 1999). This sweet compound is able to pass through the digestive system without any chemical break down, hence Stevia is beneficial for the people who wish to reduce the sugar content from their blood (Thiyagarajan and Venkatachalam, 2012).

Seeds of Stevia are very small in size and infertile and production of Stevia through seeds is not beneficial. The seed germination of *Stevia rebaudiana* is very poor which creates problems with its establishment as a crop. Therefore, plant tissue culture or stem cuttings may be used as an alternative for propagation of this plant. As the former technique, being expensive is difficult to opt on large scale, so the successful method may be to propagate Stevia through cuttings (Lateef and Osman, 2012 and Savita *et al.*, 2004). Cuttings enable one to clone selected genotypes and test the effect of treatment on genetically identical individual. Hence, it can minimize the variation in intrinsic properties among individual plants (Lapierre, 2001).

During the present study, an elaborative experiment was carried out to optimize the effect of auxin on rooting of *Stevia rebaudiana* cuttings as well as on the vigor of developing shoots.

MATERIALS AND METHODS

The cuttings were collected from green house of Lahore College for Women University, Lahore. The apical portion of the cutting with a length of 10 to 12 cm having 6 leaves per cutting was selected. Good quality sand (small particle size) was filled into small sized plastic pots.

Stock solutions of auxins were prepared in the strength of 1 mg/ 1 ml separately. Growth regulator formulations were made for all the three auxins in the percentages as 0.1%, 0.5%, 1%, 2%, 3%, 4% and 5%, separately. Combinations of these auxins were also made. Cuttings were dipped in the solution for 30 seconds. Afterwards these cuttings were directly planted in the

pots filled with rooting media (sand) covered with plastic sheet and placed in green house. Temperature of green house was set at 36°C with proper aeration. Plantlets were placed in trays containing water to avoid wilting. Plantlets were regularly checked for water requirement and were irrigated with distilled water according to the need. Sometimes only spraying of water was enough. Cuttings were observed daily to check freshness as well as emergence of new shoots. After twenty days of the planting into sand, the rooted cuttings were carefully taken out and sand was removed and roots were slightly covered with sand and shifted into the clay and manure with 1:1 ratio and were allowed to grow for another thirty days. Then these cuttings were taken out, dipped in water to release the soil particles. Observations of root parameters were recorded which included number of roots per cutting, root length, fresh root weight and dry root weight. Roots were dried in oven at a temperature of 40°C for 6 hours. Other observations related to shoot parameters included number of leaves per cutting, number of branches per cutting, length of internode and rooting percentage.

The experiment was carried out in randomized complete block design. There were three treatments which comprised of control also. The data generated was analyzed using one way ANOVA i.e., analysis of variance. The means of treatments were compared to check significance by applying Duncan's New Multiple Range test at 5 % level of significance. COSTAT computer software was used for statistical analysis (Anonymous, 2009).

RESULTS AND DISCUSSION

The purpose of this study was to check the effect of auxins i.e., IBA and NAA on rooting and shooting capability of *Stevia* rebaudiana. Fig 1 clearly shows the tendency that on increasing the concentration of NAA up to 3% in the rooting solution, number of roots per cutting, root length as well as rooting percentage increased. Maximum number of roots (34.9 roots per cutting) were obtained from 3% NAA rooting solution (Fig 3). Figure 1 also supported the statement that maximum root length (6cm) was obtained from cuttings treated with 3%NAA solution. While considering the effect of NAA on shooting capability of *Stevia rebaudiana*, Figure 2 shows that shoot length and number of nodes were higher in lower concentrations of NAA. Maximum values of shoot length and number of nodes were obtained from cuttings treated with 3% NAA solution (Fig 4) whereas on increasing the NAA percentage in the rooting media, shoot parameters showed a negative trend.

Figure 5 and 6 show the effect of IBA on rooting and shooting capability of *Stevia rebaudiana* cuttings respectively. It was clear from the results that maximum rooting percentage (90%) was obtained from cuttings treated with 1% IBA solution. Number of roots and root length was also found to be maximum in the same treatment of IBA (Figure 7). Figure 8 shows that maximum shoot length and number of nodes,in cuttings treated with 1% IBA solution was observed (Fig 4).

Text Figure 9 simply shows a comparison of NAA (3%) and IBA (1%) treatments for growth parameters of number of roots, root length and shoot length. It showed that NAA was a better choice for cost effective and better propagation of *Stevia rebaudiana* cuttings.

Vegetative propagation was the technique which was widely used in the field of forestry, agriculture and horticulture for growing choicest plants. Targeted better varieties were selected from natural populations (Hartmann *et al.,* 1990). The root formation was a critical step in vegetative propagation and hence if roots do not develop on cuttings, losses occur to crops. Now new treatments for rooting have been reported observing the effect of plant growth regulators on rooting capability of plants (Haissig and Davis, 1994). Rooting was not a single step process, but it was an advanced process consisting of a number of steps, each step was well regulated by different factors (Kevers *et al.,* 1997).

In a study Wiesmann and Epstein (1988) noticed that all the stages of rooting were linked directly or indirectly, with changes in endogenous auxin concentrations and later on similar results were reported (Heloir *et al.,* 1996). Auxins have also proved their effectiveness for initiation of rooting in a number of woody species.

On the application of auxins to the cuttings, the increase in the rooting response was observed with enhanced sprouting. This indirect effect of auxin on sprouting highlighted the role of materials produced in the roots, which were basically responsible for sprouting. There may be a number of reasons for increase in length of cutting's roots, treated with plant growth regulators; it may be due to boost of metabolites at the site of use of auxin, cell enlargement, synthesis of new amino acids and cell division (Madan *et al.,* 2010).

Plant growth was controlled by two main factors, i.e., genetic and environmental, which had obvious effects on biosynthesis of auxins, their metabolism, transport, and signaling pathway (Han *et al.,* 2009). Auxins control cell division as well as cell elongation. Auxins also affect certain stages of differentiation (Davies, 2004). Auxins basically take the control of transcription of a few genes inside the nucleus which otherwise were responsible for the elongation of cell wall. Auxin receiver was a protein, namely TIR1, that is localized in the nucleus and acts as transcriptional promoter. Plant response to auxins depends upon the presence and abundance of this receptor protein (Dhermasiri *et al.,* 2005).

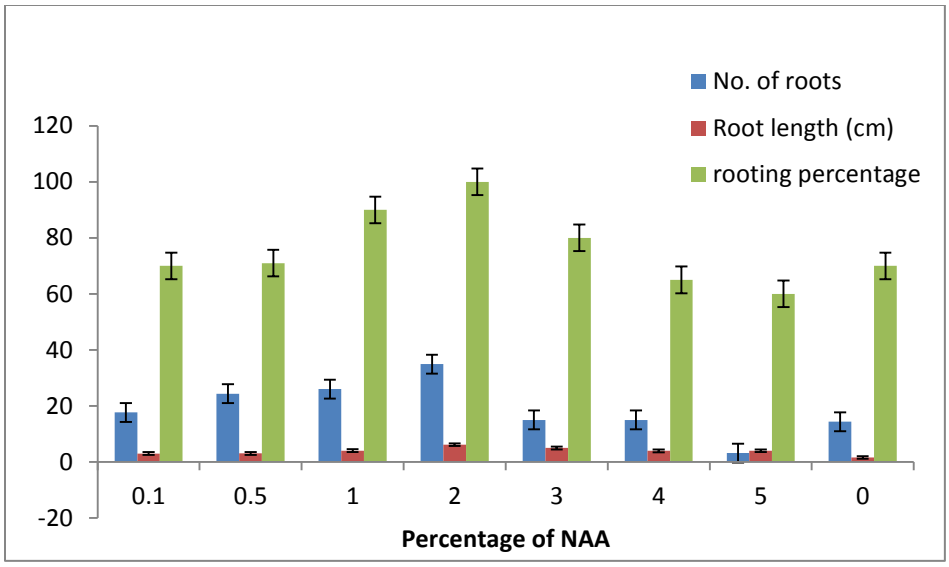

Figure 1: Effect of NAA on rooting parameters in *Stevia rebaudiana* Bertoni

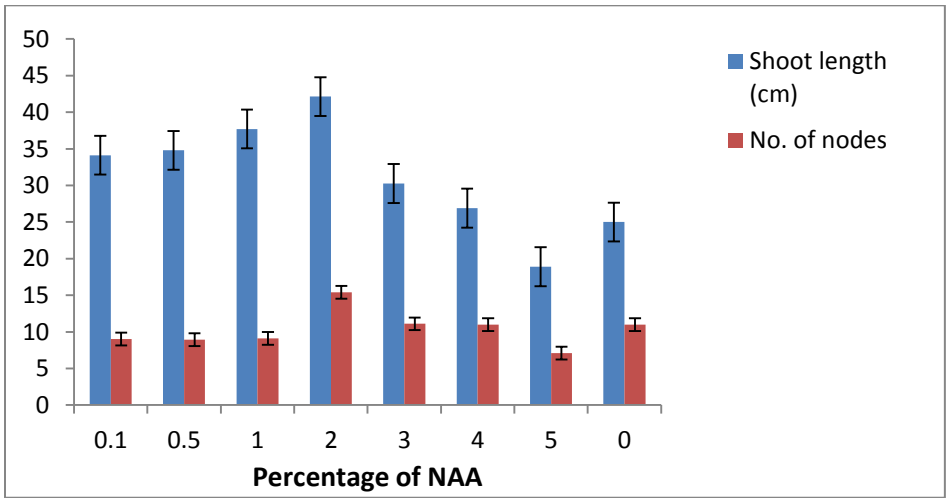

Figure 2: Effect of NAA on shooting parameters in *Stevia rebaudiana* Bertoni

Fig 3: Rooting of *Stevia rebaudiana* Bertoni cuttings treated with 3% NAA solution

Fig 4: Shooting of *Stevia rebaudiana* Bertoni cuttings treated with 3% NAA solution

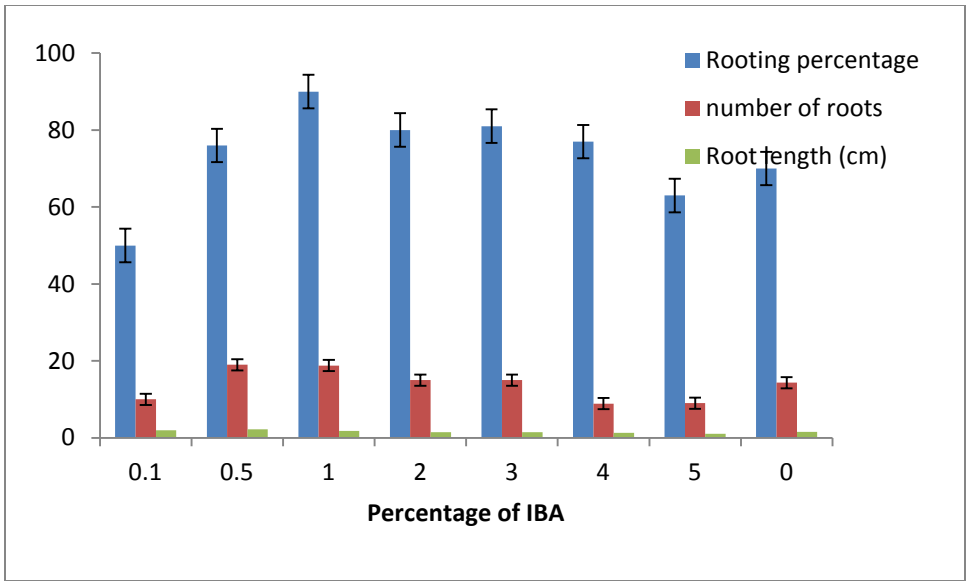

Figure 5: Effect of IBA on rooting parameters in *Stevia rebaudiana*

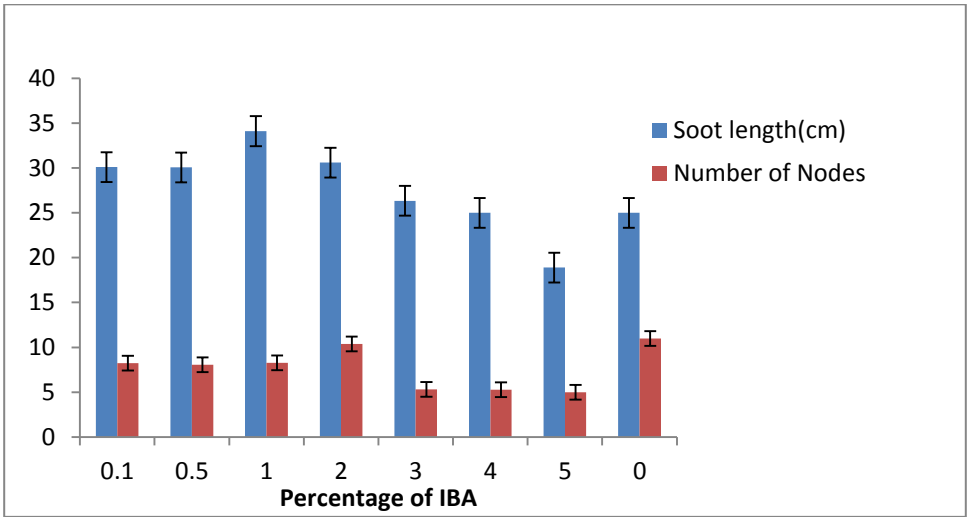

Figure 6: Effect of IBA on shooting parameters in *Stevia rebaudiana*

Fig 7: Rooting of *Stevia rebaudiana* Bertoni cuttings IBA solution

Fig 8: Shooting of *Stevia rebaudiana* treated with 1% Bertoni cuttings treated with 1% IBA solution

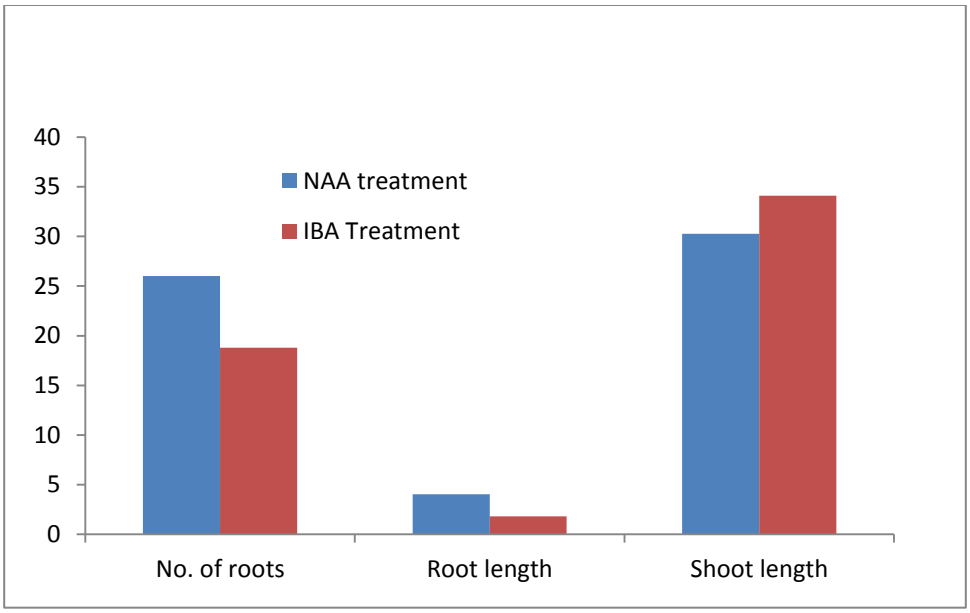

Figure 9: Comparison of NAA and IBA for rooting and shooting of *Stevia rebaudiana* Bertoni.

Conclusion: It was concluded from the present study that Auxin treatment to cuttings of *Stevia rebaudiana* Bertoni enhances the growth of both shoots and roots. Particularly, treatments of 2% NAA (Naphthalene acetic acid) gave enhanced rooting and better vegetative propagation of Stevia through stem cuttings. Data obtained during the present study may help to produce *Stevia rebaudiana* Bertoni in future on commercial scale.

REFERENCES

Anonymous, A. (2009) Cohort Software. 1986. Costat User's Manual Virgin 3.03. Berkley, California, USA.

Dacome, A. S., C. D. S. Cleuza, E. M. D. Cecila, D. F. A. Jose and C. D. C. Juliana. (2005). Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni. isolation and quantitative distribution by chromatographic, spectroscopic and electrophoretic methods. Process Biochem. 40: 3587-3594

Davies, P. J. (2004). Plant hormones: Biosynthesis, signal transduction, action! dordrecht, Kluwer Academic Publishers, The Netherlands

Dhermasiri, N., D. Sunethra and E. Mark. (2005). The F-Box Protien TIR1 is an auxin receptor. Nature, 435: 441-445

Haissig, B. E. and T. D. Davis. (1994). An historical evaluation of adventitious rooting research to Biology of adventitious root formation. New York, London: Plenum Publishing Corporation. 3: 275-331

Han, H., S. Zhang and X. Sun.(2009). A review on the molecular mechanism of plants rooting modulated by auxin. Afr. J. Biotech. 8(3): 348-353

Hartmann, H. T., D. E. Kester and F. T. Davies. (1990). Plant Propagation: Principles and Practices. Prentice-Hall, Englewood Cliffs, NJ 5th ed., p. 647

Heloir, M. C., C. Kevers, J. F. Hausman and T. Gaspar. (1996). Changes in the concentrations of auxins and polyamines during rooting of *in-vitro*-propagated walnut shoots. Tree Physiol. 16(5): 515-519

Kevers, C., J. E. Hausman, O. Faivre-Rampant, D. Evers and T. Gaspar. (1997). Hormonal control of adventitious rooting: progress and questions. J. Appl Bot Angew Bot.71: 71-79

LaPierre, L. M. (2001). Vegetative propagation of *Cecropia obtusifolia* (Cecropiaceae). Revista de Biol. Trop. 49(3-4): 973–976

Lateef, R. A. and M. Osman. (2012). Effects of stem cutting types, position and hormonal factors on rooting in *Stevia rebaudiana* Bertoni," J. of Agric. Sci. 4(1): 123-129

Madan, S., S. Ahmad, G. N. Singh, K. Kohli, Y. Kumar, R. Singh and M. Garg. (2010). *Stevia rebaudiana* (Bert.) Bertoni- A Review. Ind. J. of Nat. Prod. and Res. 1(3): 267-286

Oddone, B. (1999). How to grow Stevia, Guarani Botanicals, Inc.: Pawcatuck, Connecticut, 1-30

Pinheiro, M. V. S. and M. V. Oliveria. (2005). The effect of different sweeteners in low calorie yogurts- a review. Int. Dairy Tech. 58(4): 193-199

Richman, A., A. Swanson, T. Humphrey, R. Chapman, B. Mcgarvey, R. Pocs and J. Brindle. (2005). Functional genomics uncovers three glucosyl transferases involved in the synthesis of the major sweet glucosides of *Stevia rebaudiana*. Plant. J. 41: 56-67

Savita, S. M., K. Sheela, A. G. Sunanda, S. Sharan and Ramakrishna. (2004). *Stevia rebaudiana* – A functional component for food industry. J. Hum. Ecol. 15: 261-264

Thiyagarajan, M. and P. Venkatachalam. (2012). Large scale *in vitro* propagation of *Stevia rebaudiana* (Bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb. Ind. Crop. Prod. 37: 111-117

Wiesman, Z., J. Riov and E. Epstein. (1988). Comparison of movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean cuttings. Physiol. Plant. 74(3): 556-560.