

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF *STEVIA REBAUDIANA* BERTONI STEM CUTTINGS UNDER 3-INDOLEACETIC ACID (IAA) TREATMENT

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Stevia rebaudiana is a high-value crop because of its metabolites, such as steviol glycosides. However, it only produces a few seeds, resulting in low productivity. Therefore, an appropriate propagation method is required to enhance its productivity. This study aimed to assess stevia's morphological and physiological characteristics upon treatment with three doses of Indole-3-acetic acid (IAA) [mg/L] through stem-cutting propagation. The cuttings were treated with IAA at 0, 20, 40 and 60 mg/L by dipping them in the IAA solution for 1 minute, then planting in growth media (garden soil, organic compost and coco peat with a ratio of 2:1:1) and keeping them in the greenhouse. The survival percentage, root length and number, plant height, leaf number, total chlorophylls, and stomata length and number were assessed in the 3rd week. The application of 40 mg/L IAA resulted in a significant improvement in all parameters. However, IAA treatment also causes stomata abnormalities. This result suggested that stem cutting and IAA treatment significantly contribute to stevia propagation.

Key words: propagation, survival percentage, growth, stomata, chlorophyll

Stevia rebaudiana is a perennial plant belonging to the Asteraceae family. It originated in South America, particularly in Brazil and Paraguay (Peteliuk *et al.* 2021). Currently, stevia is cultivated in many regions, including Asia, Europe and North America (Lemus-Mondaca *et al.* 2012). The leaf mainly produces a sweet taste, as it contains more than 30 dif-

ferent steviol glycosides (SGs) (Peteliuk *et al.* 2021), including stevioside, rebaudioside A, rebaudioside C, and glucoside A. Steviosides are 300 times sweeter than sucrose or can sugar (Jahan *et al.* 2014). In addition, stevia leaf also contains volatile oil components, sterols, triterpenes, flavonoids, coumarins, and non-glycosidic diterpenes (Gauchan *et al.* 2014).

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In many countries, stevia is used as a sugar substitute in the food and beverage industry; for example, the cola company manufactures beverages containing stevia-based sugar (Peteliuk *et al.* 2021). Moreover, stevia is utilized in medicine because it has anti-microbial, anti-inflammatory, antioxidant, anti-carcinogenic, anti-hypertensive, anti-hyperglycaemic, anti-diarrheal, anti-tumour, and anti-diabetic potentials (Ghose *et al.* 2022). These benefits contribute to an increasing demand over time, prompting the high cultivation of stevia (Samuel *et al.* 2018). In Bandung, West Java, Indonesia, since 2010, a group of farmers have cultivated about 2 hectares and produced 500–600 kg of stevia leaves every two weeks after 40 days of planting. However, the production is still insufficient (Supyandi *et al.* 2018).

Despite that, propagation of this crop is challenging (Smitha & Umesha 2012), especially through its seeds. Unlike cuttings and *in vitro* culture, seed germination is more difficult because of the infertile seed (Singh *et al.* 2017) and small endosperm (Yadav *et al.* 2011). This results in heterogeneous plants with various sweetness levels and compositions (Kovylyayeva *et al.* 2007). Therefore, stem cutting or cutting propagation is preferentially used because it produces homogeneous plants. The growth of cuttings is influenced by many factors, including parental plant vigour, age, woodiness, cutting location, growth regulator, environment, nutrients, and genetic factors (Singh *et al.* 2015). Growth regulators significantly determine plant growth and development (Satpal *et al.* 2014). Among all the auxin types, a previous study reported that IAA particularly promotes the rooting of cuttings (Seran & Umadevi 2011). Therefore, we urge to evaluate the influence of IAA on the morpho-physiological characteristic of stevia stem cuttings and plant productivity. Different concentrations of IAA were applied to the stem cuttings to understand that idea, and several growth parameters were observed from the stem cuttings.

MATERIAL AND METHODS

Stevia plants were collected from Pandesari Village, Pujon District, Malang, East Java Province,

Indonesia. The stem-cutting method follows a previous study by Khalil *et al.* (2014). Cuttings were planted in November because it was the most suitable month for stem-cutting survival. The stem was cut from the upper part, measuring 13–14 cm, covering the leaf buds and excluding the lateral shoot.

The cuttings were treated with IAA (Sigma-Aldrich, Co) to induce rooting. The basal end of the cuttings (approximately 3.0 cm) was dipped in solutions containing different concentrations of IAA, including 20, 40 and 60 mg/L, for 1 min. Meanwhile, the control cuttings were dipped in water. The treatments were conducted late in the evening. After treatment, four cuttings were planted as deep as 5–7 cm in each polybag containing a soil mixture (consisting of garden soil, organic compost and coco peat with a ratio of 2:1:1). Cuttings were kept in the greenhouse and watered twice a day.

Cuttings were assessed after three weeks. The parameters measurement includes survival percentage, root length [cm], root number, plant height [cm], leaves number, chlorophyll content [mg/L], and stomata (stomata number, length and abnormalities). Stem cutting with a root length of 1 cm or longer was considered rooted.

Total chlorophyll content was measured according to Latifa *et al.* (2019) using a spectrophotometer UV-Vis. Approximately 0.1 grams of leaf samples were ground using mortar and pestle and homogenized in 15 mL of 80% Acetone. Subsequently, the homogenized sample was filtered using filter paper and transferred into a test tube. Absorbance at $\lambda 663$ and $\lambda 645$ was measured to count the samples' chlorophyll *a*, *b* and total chlorophylls, and 80% acetone was as a blank. Total chlorophyll was calculated as follows = $(8.02 \times \lambda 663) + (20.2 \times \lambda 645)$.

The stomatal observation was done using two types of microscopes, microscope CX21 OLYMPUS and optilab for stomatal number and abnormalities with a magnification of 1,000 $\times$, and Scanning Electron Microscope (SEM) INSPECT S50 for stomatal length with a magnification of 10,000 $\times$.

The statistical was a randomized block design method with One-Way ANOVA using IBM SPSS v.25. The data were obtained from 6 replicates with four cuttings for each. The significance of differences among treatments was further evaluated us-

ing Duncan's Multiple Range Test (DMRT) with a $p < 0.05$. The results were expressed as mean $\pm$ SD.

RESULTS

The results showed that the survival percentage of stem cuttings treated with various plant growth regulator concentrations was higher than stem cutting without plant growth regulator treatment (control). While control resulted in a 50% survival percentage, each concentration of IAA exhibited a 70 % survival percentage (Figure 1).

Root development on stem cuttings varied among treatments depending on the plant growth regulator concentration. Figure 2 and Table 1 show that IAA concentration significantly influences root development. Interestingly, 40 mg/L IAA exhibited the longest root and the highest root number, 1.3-fold and 2.3-fold, better than the control. In contrast, 60 mg/L IAA resulted in the shortest roots and the lowest root number. Meanwhile, 20 mg/L IAA possessed a moderate root length and number. The result showed that 40 mg/L IAA was most appropriate for promoting root development since it influenced the highest plant height and leaf number. The lowest plant height and leaf number were observed from the cuttings treated under 20 mg/L IAA (Figure 3 and Table 1).

As chlorophyll metabolism is regulated by auxin, various concentrations of IAA significantly affect the chlorophyll content. The result in parallel also showed that the highest chlorophyll *a*, *b*, and total was obtained from cuttings treated with 40 mg/L IAA. The lowest chlorophyll content was found in

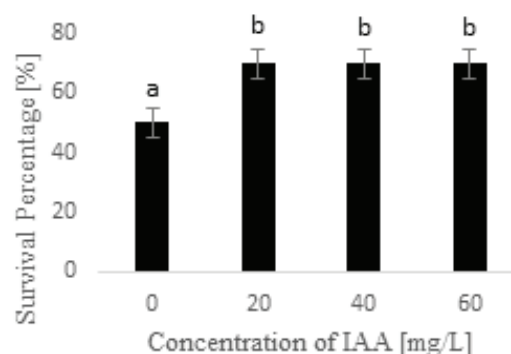


Figure 1. Effect of Indole acetic acid (IAA) on survival percentage of stevia stem cuttings. The letters (a, b, c, d) indicate the significant difference in value ($P < 0.05$).



Figure 2. Phenotype of stevia root after IAA treatment for three weeks. (A) control plant; (B-D) stevia treated with 20, 40 and 60 mg/L IAA, respectively. IAA – see Figure 1.

untreated cuttings. Compared to untreated cuttings, chlorophyll content significantly increased by 1.6-fold and decreased by 1.4-fold when treated with 40 and 60 mg/L IAA, respectively (Table 2).

The upper part of the leaf (adaxial) contains a sinuous epidermis wall with less than five stomata [units/mm²]. In contrast, the lower part (abaxi-

T a b l e 1

Effect of IAA on the morphological characteristic of stevia stem cuttings

Concentration of IAA [mg/L]	Parameters			
	Root length [cm]	Root number	Plant height [cm]	Leaf number
0	11.6 $\pm$ 0.7 ^c	4.8 $\pm$ 2.7 ^{ab}	14.0 $\pm$ 1.5 ^a	9.0 $\pm$ 0.9 ^a
20	9.4 $\pm$ 0.7 ^b	6.7 $\pm$ 1.2 ^b	13.6 $\pm$ 0.9 ^a	11.7 $\pm$ 1.7 ^b
40	14.8 $\pm$ 1.7 ^d	10.8 $\pm$ 1.6 ^c	16.9 $\pm$ 0.9 ^b	18.2 $\pm$ 1.8 ^c
60	5.9 $\pm$ 2.6 ^a	4.0 $\pm$ 2.4 ^a	14.6 $\pm$ 1.6 ^a	12.0 $\pm$ 1.4 ^b

Note: the letters (a, b, c, d) indicate the significant difference in value ($P < 0.05$). IAA – see Figure 1.

T a b l e 2

Effect of IAA on the total chlorophylls of stevia stem cutting

Concentration of IAA [mg/L]	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophylls
Control	8.50 ^d	8.50 ^d	16.99 ^d
20	9.77 ^c	9.61 ^c	19.37 ^c
40	15.13 ^a	15.43 ^a	30.56 ^a
60	11.29 ^b	11.07 ^b	22.35 ^b

Note: the letters (a, b, c, d) indicate the significant difference in value ($P < 0.05$). IAA – see Figure 1.



Figure 3. Morphological appearance of stevia under IAA treatments. (A) control plant; (B-D) stevia treated with 20, 40 and 60 mg/L IAA, respectively. IAA – see Figure 1.

al) contains sinuous walls and a stroke cuticle with more than 15 stomata [units/mm²] under the treatment of 40 mg/L IAA, which is 3-fold higher than the control (without treatment). However, adding 20 and 60 mg/L IAA showed almost no effect on

the stomata number and significantly no difference from the control (Figure 4A). Stevia is of anisocytic stomata type, where the guard cell is surrounded by three unequal-neighbouring cells of different sizes.

The results indicated a non-significant difference in the stomata length between 40 and 60 mg/L IAA but a significant difference compared to 20 mg/L IAA and control (Figure 4B). The longest stomata length was observed from the abaxial part of the stevia leaves treated with 40 mg/L (Figure 5), followed by 60 and 20 mg/L IAA. Moreover, the microscopic observation showed that the stevia's stomata type is kidney-shaped guard cells with an outer ledge over the stomatal pore and external periclinal cell walls (Figure 6 and 7A), but abnormal stomata were found in the cuttings treated with 60 mg/L IAA (Figure 7B). The abnormal stomata were composed of protruded periclinal cell walls, resulting in the deformation of guard cells. The highest number of stomata was obtained at 40 mg/L IAA, while the smallest

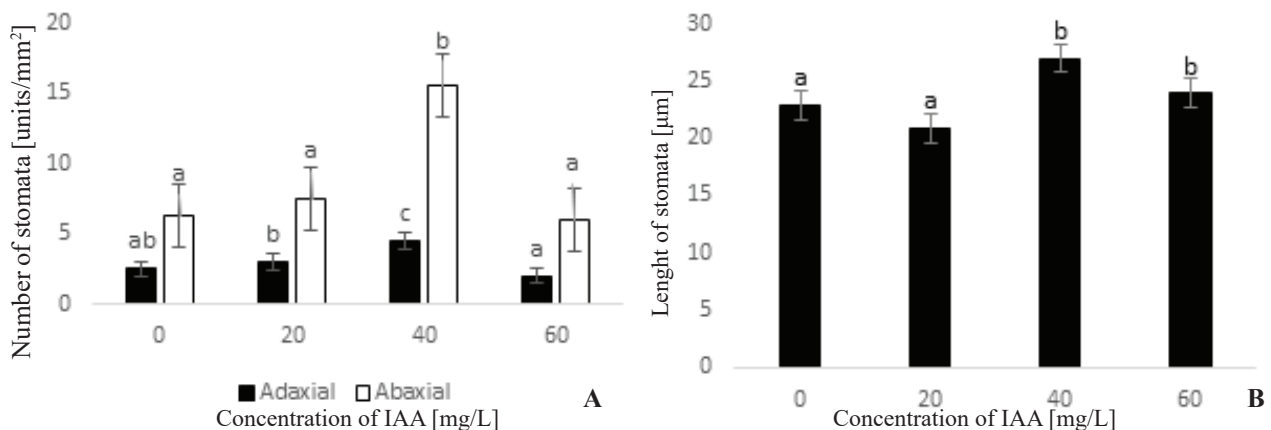


Figure 4. Effects of IAA on stomata number (A) and length (B) on stevia stem cutting. The letters (a, b, c, d) indicate the significant difference in value ($P < 0.05$). IAA – see Figure 1.

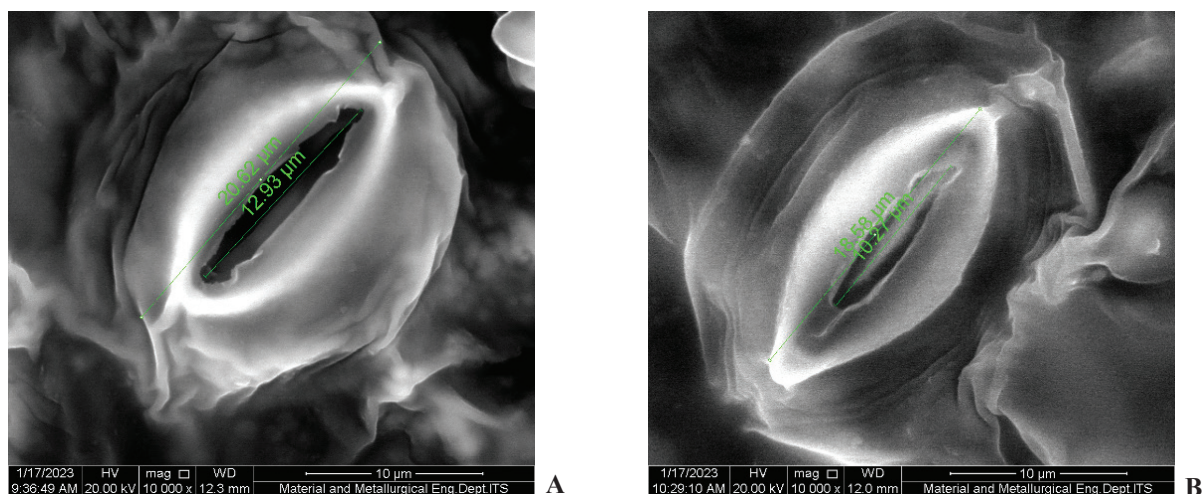


Figure 5. Stomatal appearance observed using a Scanning Electron Microscope (SEM) with a magnification of 10,000×. (A) Stomata of the untreated plant (control); (B) Stomata of stevia treated with 40 mg/L IAA. IAA – see Figure 1.

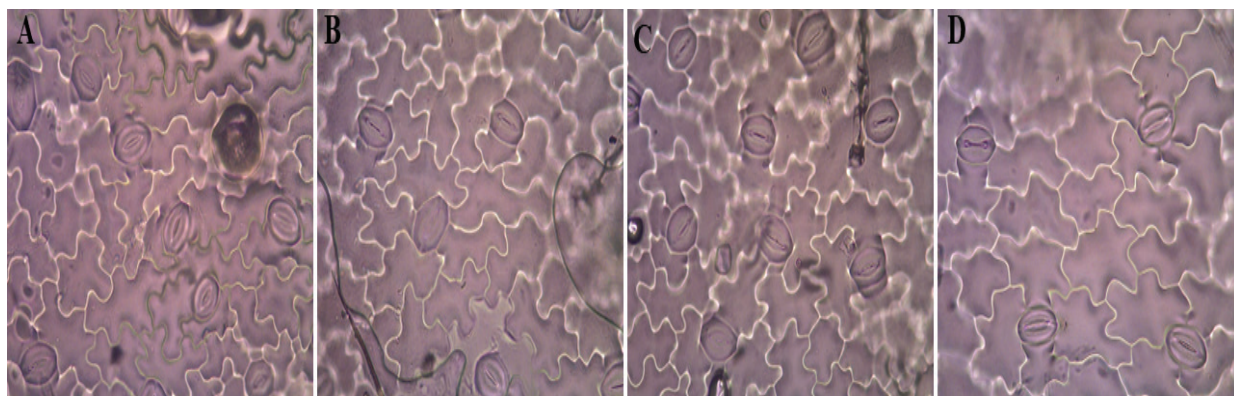


Figure 6. Stomatal appearance observed using a light microscope with a magnification of 1,000×. (A) Control; (B, C, D) Stomata of stevia treated with 20, 40 60 mg/L IAA. IAA – see Figure 1.

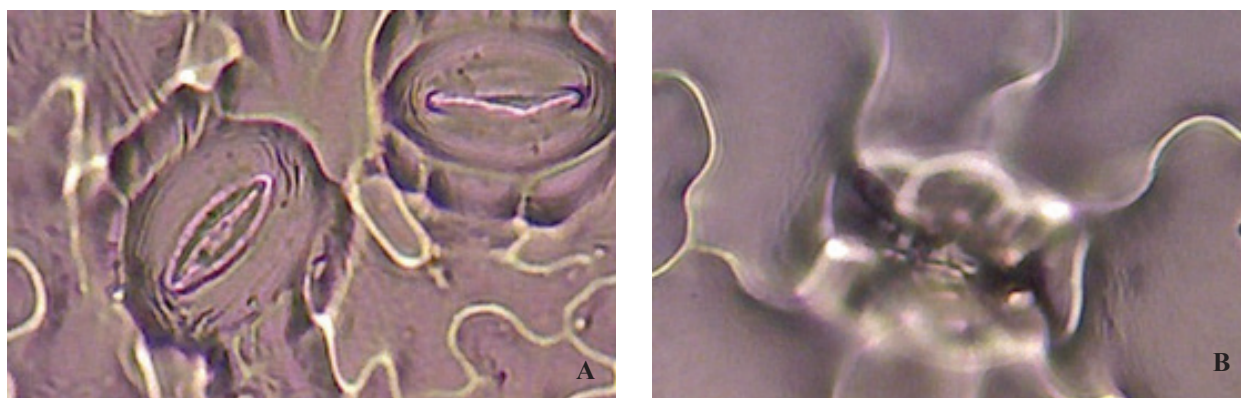


Figure 7. Stomatal appearance observed using a light microscope with a magnification of 1,000×. (A) Normal stomata of untreated plant (Control); (B) Abnormal stomata of stevia treated with 60 mg/L IAA. IAA – see Figure 1.

number was 60 mg/L IAA. The number of stomata on the abaxial side was higher than on the adaxial side for all IAA concentrations, including control.

DISCUSSION

Taking all the data together, we suggest that IAA is involved in a few metabolic pathways of stevia,

improving stevia growth and development. The main pre-requisite of successful stem cutting is the ability of the plant to form a new root system (Hartmann *et al.* 1997). The survival rate of stem cuttings depends on various factors such as age, location of stem cuttings, environment, growth regulator, nutrients, and genetic factors (Yoon *et al.* 2021). Among them, the growth regulator is an important factor in the growth and development of stem cuttings (Seran & Umadevi 2011). One of the most well-known growth regulators is auxin or IAA, which is important for stimulating root growth (Ogunyale *et al.* 2014). Our result showed that IAA treatments for three weeks resulted in a 70% stevia survival rate. Align with this result, a previous study by Khalil *et al.* (2014) reported that stevia stem cuttings dipped in 500 and 1,000 mg/L IBA for 90 days showed a survival percentage of up to 11.1 and 33.3%, respectively. Meanwhile, the survival percentage of stevia treated with 500 and 1,000 mg/L NAA was 2.66% and 22.2%.

In this study, the survival percentages of treated plants were higher than those of control plants. The high survival percentage might be attributed to the fact that auxin induces hydrolysis and mobilization of nutritional factors to the application site, thereby promoting root initiation in stem cutting (Nanda *et al.* 1970). Auxin promotes starch breakdown into soluble sugar bulk, which is transported quickly to the cutting base for root regeneration (Li *et al.* 2009). This root primordium acts as a metabolic sink, drawing on the nutritional reserves of the cutting for their growth and development (Das *et al.* 1997). Upon formation, the root starts absorbing water and nutrients for plant survival (Pooja & Sadatulla 2022).

Besides, the high survival percentage is also affected by the stevia part chosen as the cutting stem, which is the top part (Aini *et al.* 2010). The top stem has high auxin content and rapid stem tissue regeneration. Thus, the ability to form new roots and shoots is high in parallel.

A high survival rate was also supported by other factors, such as the season and the growth medium (Kassahun & Mekonnen 2012). Khalil *et al.* (2014) reported that November, the rainy season is the best month for stevia survival. During the rainy season, the transpiration rate is low, which, in turn, pre-

vents water loss. Thus, the plants can survive longer (Swarts *et al.* 2018).

Furthermore, plant growth regulators, especially auxins, are indispensable for root formation (Keeley *et al.* 2004). The root formation at the basal of stem cuttings is an important component of development that supports the survival and growth of cuttings (Kaur *et al.* 2002). As shown in Table 1, the highest root length and number was found in stevia treated with 40 mg/L IAA. In line with this result, a previous study by Khalil *et al.* (2014) found that 500 mg/L and 1,000 mg/L NAA for 90 days increase the root length. Additionally, Smitha and Umesa (2012) demonstrated that the root number was improved after adding 500 mg/L, 1,000 mg/L and 2,000 mg/L IBA for 60 days. An increasing IAA concentration rapidly promotes root emergence by enhancing root formation compared to the control (Khan *et al.* 2021). However, an excessive amount of IAA decreases the root length and number. This is because auxins degrade cuttings' basal tissues, interfere with plant hormonal balance, damage the cells, and inhibit bud growth and shoot development (Aghdaei *et al.* 2019). Moreover, auxins play essential functions in the acidification apoplast and the activation of key wall-loosening proteins (Depuydt *et al.* 2016).

The measurement of leaf number, plant height, and chlorophyll content of *S. rebaudiana* treated with several concentrations of exogenous auxin indicate that different concentrations exhibit different responses to plant growth. This study shows that the plant growth regulator concentration significantly affects plant growth. The highest plant height was observed in *S. rebaudiana* treated with 40 mg/L compared to all treatments, suggesting that 40 mg/L is the best concentration for *S. rebaudiana* growth. Consistent with these results, Leyser (2018) and Zhao (2018) reported that IAA plays an important role in elongating the hypocotyl, the epicotyl, and the other organs responsible for plant height. Moreover, applying 40 mg/L IAA resulted in the highest leaf number and higher chlorophyll content than other treatments. This may indicate that more leaf number relates to more chlorophyll and photosynthesis rates. Reinhardt *et al.* (2003) and Bayer *et al.* (2009) demonstrated that leaf primordium initiation is correlated with auxin accumulation resulting from

PIN 1-mediated polar auxin transport, which in turn, increases the leaf number (Wang & Guo 2015). Modifying auxin concentration influences photosynthesis and respiration, influencing the cellular energy budget for biosynthesis and proliferation (Tivendale & Millar 2022).

Stomata serve central roles in plant development by controlling carbon dioxide assimilation during photosynthesis and regulating the turgor pressure of the guard cell (Chen *et al.* 2022). Stomata can be found on both leaf surfaces, abaxial and adaxial, but the abaxial side has a higher number of stomata than the adaxial side. A low number of stomata [<5 units/mm²] on the adaxial side is beneficial to reduce transpiration. Our result revealed that stevia treated with 40 mg/L IAA had the highest stomata number. These results were supported by Khudhur & Omer (2015), reporting that auxin can affect stomatal characteristics, including stomata number (adaxial and abaxial) and length in *Dalbergia sissoo* stems cuttings treated in 100, 300 and 500 mg/L IAA. Auxin participates in leaf development by coordinating asymmetrical cell differentiation and division of the guard cell mother cell (GMC) and two subsidiary mother cells (SMC) (Livanos *et al.* 2015). This indicated that increasing the division of GMC can improve the stomata number.

The stevia plant has an anisocytic stomata type. Stevia, treated with high IAA (60 mg/L IAA), exhibited abnormal stomata with irregular shapes in the mesophyll. Similarly, Xuan *et al.* (2011) reported that *Bougainvillea spectabilis* Willd possessed abnormal anisocytic stomata. Abnormal stomata are characterized by the irregular shape of epidermal cells and the undulate pattern of anticlinal walls. In contrast, kidney-shaped normal cells contain a polygonal shape of epidermal cells and the straight patterns of anticlinal walls.

CONCLUSIONS

This study suggested that an appropriate concentration of IAA improves the growth and development of *S. rebaudiana* Bertoni from stem cuttings. Unlike 20 and 60 mg/L IAA, 40 mg/L IAA resulted in a promising growth phenotype: high surviv-

al percentage, long root, high plant, high root and leaf number, high chlorophyll content, and stomata length and number.

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REFERENCES

- Aghdaei, M., Nemati, S.H., Samiee, L., and Sharifi, A. (2019). Effect of rooting medium, cutting type, and auxin on rooting of Pepino (*Solanum muricatum* Aiton) cutting. *Applied Ecology and Environmental Research*, 17(5), 10357–10369. DOI:10.15666/aeer/1705_1035710369.
- Aini, N.A.S., Guanah, V.S. and Ismail, P. (2010). Effects of cuttings positions and growth regulators on rooting ability of *Gonystylus bancanus*. *African Journal of Plant Science*, 4(8), 290–295. DOI:10.5897/AJPS.9000079.
- Bayer, E.M., Smith, R.S., Mandel, T., Nakayama, N., Sauer, M., Prusinkiewicz, P., and Kuhlemeier, C. (2009). Integration of transport-based models for phyllotaxis and midvein formation. *Genes and Development*, 23(3), 373–384. DOI: 10.1101/gad.497009.
- Chen, S., Wang, D., Sheng, X., Zhang, C., Li, W., Xiao, N., Wang, L., and Lu, Z. (2022). Comparison of stomatal structure and distribution between ovule and leaves in *Ginkgo biloba*. *Forests*, 13, 1–11. DOI:10.3390/f13111801.
- Das, P., Basak, U. and Das, A. (1997). Metabolic changes during rooting in pre-girdled stem cuttings and air layers of *Heritiera*. *Botanical Bulletin of Academia Sinica*, 38, 91–95.
- Depuydt, S., Praet, S.V., Nelissen, H., Vanholme, B., and Verbeeck, D. (2016). How plant hormone and their interactions affect cell growth. In Rose, R.J. (Ed.) *Molecular Cell Biology of the Growth and Differentiation of Plant Cells*, CRC Press: Boca Raton, pp. 176–198. DOI:10.1201/b20316-14.
- Gauchan, D.P., Dhakal, A., Sharma, N., Bhandari, S., Maskey, E., Shrestha, N., Gautam, R., Giri, S., and Gurung, S. (2014). Regenerative callus induction and biochemical analysis of *Stevia rebaudiana* Bertoni. *Journal of Advanced Laboratory Research in Biology*, 5(3), 41–45.
- Ghose, A.K., Abdullah, S.N.A., Hatta, M.A.Md., and Wahab, P.E.M. (2022). In vitro regeneration of *Stevia rebaudiana* Bertoni and evaluation of the impacts of growth media nutrients on the biosynthesis of steviol glycosides (SGs). *Agronomy*, 12, 1–24. DOI:10.3390/agronomy12081957.
- Hartmann, H.T., Kester, D.E., Davies, F.T., and Geneve, R.L. (1997). *Plant Propagation: Principles and Practices*. 6th ed. New Jersey: Prentice Hall. ISBN:978-0-13-261488-7.
- Jahan, M.T., Islam, M.R., Roy, P.K., Mamun, A.N.K., and Islam, M.A. (2014). In vitro clonal propagation of *Stevia rebaudiana* Bertoni through node and shoot tip culture. *Nuclear Sciences and Applications*, 23, 61–65.
- Kassahun, B.M., Mekonnen, Sa. (2012). Effect of cutting position and rooting hormone on propagation ability of stevia (*Stevia rebaudiana* Bertoni). *The African Journal of Plant Science and Biotechnology*, 6(1), 5–8.

- Kaur, S., Cheema, S., Chhabra, B., and Talwar, K. (2002). Chemical induction of physiological changes during adventitious root formation and bud break in grapevine cuttings. *Plant Growth Regulation*, 37(1), 63–68. DOI: 10.1023/A:1020355505105.
- Keeley, K., Preece, J.E., Taylor, B.H., and Dami, I.E. (2004). Effects of high auxin concentrations, cold storage, and cane position on improved rooting of *Vitis aestivalis* Michx. Norton cuttings. *American Journal of Enology and Viticulture*, 55(3), 265–268. DOI:10.5344/ajev.2004.55.3.265.
- Khalil, S.A., Zamir, R. and Ahmad, N. (2014). Selection of Suitable Propagation Method for Consistent Plantlets Production in *Stevia rebaudiana* (Bertoni). *Saudi Journal of Biological Sciences*, 21, 566–573. DOI:10.1016/j.sjbs.2014.02.005.
- Khan, M.A., Wang, Y., Muhammad, B., Uddin, S., Saeed, A., Khan, D., Ali, M., Saeed, S., and Jia, Z. (2021). Morpho-physiological and phytohormonal changes during the induction of adventitious root development stimulated by exogenous IBA application in *Magnolia biondii* Pamp. *Brazilian Journal of Biology*, 24, 1–10. DOI:10.1590/1519-6984.255664.
- Khudur, S.A. and Omer, T.J. (2015). Effect of NAA and IAA on stem cuttings of *Dalbergia sissoo* (Roxb). *Journal of Biology and Life Science*, 6(2), 1–13. DOI:10.5296/jbls.v6i2.7445.
- Kovylyayeva, G.I., Bakaleinik, G.A., Strobyskina, I.Y., Gubskaya, V.I., Sharipova, R.R., Al'fonsov, V.A., Kataev, V.E., and Tolstikov, A.G. (2007). Glycosides from *Stevia rebaudiana*. *Chemistry of Natural Compounds*, 43, 81–85. DOI: 10.1007/s10600-007-0037-x.
- Latifa, R., Hadi, S. and Nurrohmah, E. (2019). The exploration of chlorophyll content of various plants in city forest of Malabar Malang. *Bioedukasi*, 17(2), 50–62. DOI: 10.19184/bioedu.v17i2.14091.
- Lemus-Mondaca, R., Vega-Galvez, A., Zura-Bravo, L., Ah-Hen L. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspect. *Food Chemistry*, 132(3), 1121–1132. DOI:10.1016/j.foodchem.2011.11.140.
- Leyser, O. (2018). Auxin signaling. *Plant Physiology*, 176(1), 465–479. DOI:10.1104/pp.17.00765.
- Li, S.W., Xue, L. and Feng, H. (2009). IBA induced changes in antioxidant enzymes during adventitious rooting in mung bean seedlings. *Environmental and Experimental Botany*, 66, 442–450. DOI:10.1016/j.envexpbot.2009.03.005.
- Livanos, P., Giannoutsou, E., Apostolakis, P., and Galatis, B. (2015). Auxin as an inducer of asymmetrical division generating the subsidiary cells in stomatal complexes of *Zea mays*. *Plant Signalling & Behavior*, 10, 1–15. DOI: 10.4161/15592324.2014.984531.
- Nanda, K.K., Anand, V.K. and Kumar, P. (1970). Some investigation on the use of auxins in vegetative reproduction of forest plants. *Indian Forester*, 96(3), 171–187.
- Ogunyale, O.G., Fawibe, O.O., Ajiboye, A.A., and Agboola, D.A. (2014). A review of plant growth substance: their forms, structure, synthesis, and function. *Journal of Advanced Laboratory Research in Biology*, 5(4), 152–168.
- Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K.B., and Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: functionalities, health benefits, and potential risks. *EXCLI Journal*, 20, 1412–1430. DOI:10.17179/excli2021-4211.
- Pooja, H.S. and Sadatulla, F. (2022). Effect of indole-3-butyric acid (IBA) on performance of Mulberry (*Morus alba* L.) sapling: A review. *International Journal of Environment and Climate Change*, 12(12), 791–797. DOI:10.9734/ijec/2022/v12i121515.
- Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. *Nature*, 426(6964), 255–260. DOI:10.1038/nature02081.
- Samuel, P., Ayoob, K.T., Magnuson, B.A., Wolwer-Rieck, U., Jeppesen, P.B., Rogers, P.J., Rowland, I., and Mathews, R. (2018). Stevia leaf to stevia sweetener: exploring its science, benefits, and future potential. *The Journal of Nutrition*, 148, 1186–1205. DOI:10.1093/jn/nxy102.
- Satpal, M., Rawat, S.S., Singh, K.K. (2014). Effect of various concentrations of IBA, type of cuttings and planting time on the rooting of cuttings of lemon (*Citrus limon* Burm.) cv. Pant Lemon-1 under valley conditions of Garhwal Himalaya. *International Journal of Current Research*, 6(12), 10974–10976.
- Seran, T.H. and Umadevi, T. (2011). Influence of Indole Acetic Acid (IAA) on the establishment of stem cuttings in lemon (*Citrus limon* L.). *Journal of Agricultural Research*, 49(4), 517–524. DOI:10.1093/jn/nxy102.
- Singh, M., Saharan, V., Dayma, J., Rajpurohit, D., Sen, Y., and Sharma, A. (2017). *In vitro* propagation of *Stevia rebaudiana* (Bertoni): An overview. *International Journal of Current Microbiology and Applied Sciences*, 6(7), 1010–1022. DOI:10.20546/ijemas.2017.607.122.
- Singh, V.P., Mishra, D.S., Mishra, N.K., and Rai, T. (2015). Effect of growing season, PGRs, and rooting media on survival of hardwood stem cuttings of lemon (*Citrus limon* Burm.) CV. Pant Lemon-1. *HortFlora Research Spectrum*, 4(4), 347–350.
- Smitha, G.R. and Umesha, K. (2012). Vegetative propagation of stevia (*Stevia rebaudiana* (Bertoni) Hemsl.) through stem cuttings. *Journal of Tropical Agriculture*, 50(1–2), 72–75.
- Supyandi, D., Sukayat, Y. and Hapsari, H. (2018). Farmer readiness for adopting stevia cultivation (a case study at District of Pasir Jambu, Regency of Bandung). *IOP Conf. Series: Earth and Environmental Sciences*, 142, 1–6. DOI: 10.1088/1755-1315/142/1/012069.
- Swarts, A., Matsiliza-Mlathi, B., Kleynhans, R. (2018). Rooting and survival of *Lobostemon fruticosus* (L) H. Buek stem cuttings as affected by season, media and cuttings position. *South African Journal of Botany*, 119, 80–85. DOI: 10.1016/j.sajb.2018.08.019.
- Tivendale, N.D. and Millar, A.H. (2022). How is auxin linked with cellular energy pathways to promote growth? *New Phytologist*, 233(6), 2397–2404. DOI:10.1111/nph.17946.
- Wang, J.J. and Guo, H.S. (2015). Cleavage of indole-3-acetic acid inducible28 mRNA by microRNA847 upregulates auxin signaling to modulate cell proliferation and lateral organ growth in *Arabidopsis*. *The Plant Cell*, 27(3), 574–590. DOI:10.1105/tpc.15.00101.
- Xuan, X., Wang, Y., Ma, S., and Ye, X. (2011). Comparisons of stomatal parameters between normal and abnormal leaf of *Bougainvillea spectabilis* Willd. *African Journal of Biotechnology*, 10(36), 6973–6978. DOI:10.5897/AJB10.2196.
- Yadav, A.K., Singh, S., Dhyani, D., and Ahuja, P.S. (2011). A review on the improvement of *Stevia (Stevia rebaudiana Bertoni)*. *Canadian Journal of Plant Science*, 9(11), 1–27. DOI:10.4141/cjps10086.
- Yoon, A., Oh, H.E., Kim, S.Y., and Park, Y.G. (2021). Plant growth regulators and rooting substrates affect growth and development of *Salix koriyanagi* cuttings. *Rhizosphere*, 20, 1–4. DOI:10.1016/j.rhisph.2021.100437.
- Zhao, Y. (2018). Essential roles of local auxin biosynthesis in plant development and in adaptation to environmental changes. *Annual Review of Plant Biology*, 69, 417–435. DOI:10.1146/annurev-arplant-042817-040226.

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