Scientific Research Publishing

American Journal of Plant Sciences, 2019, 10, 1789-1796
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

In Situ Clonal Propagation of Stevia (*Stevia rebaudiana*, Bertoni) Using Hormones

Elmer V. Galo

College of Agriculture, Western Mindanao State University, Zamboanga City, Philippines
Email: elmergalo74@gmail.com

How to cite this paper: Galo, E.V. (2019) *In Situ* Clonal Propagation of Stevia (*Stevia rebaudiana*, Bertoni) Using Hormones. *American Journal of Plant Sciences*, **10**, 1789-1796.
https://doi.org/10.4236/ajps.2019.1010126

Received: August 16, 2019
Accepted: October 20, 2019
Published: October 23, 2019

Abstract

The sweet taste of Stevia leaves makes it a potential substitute for table sugar which can be used to sweeten foods and beverages. However, the limited planting materials become a constrained to large production; hence the experiment aims to investigate the different part and methods of propagation for stevia specifically use of different rooting hormones. The experiment was laid out in 3 × 4 factorial arranged in Randomized Complete Block Design. It consists of 3 types of cutting (shoot tips, intermediate stem and basal stem part) and four kinds of commercial hormones (miracle gro, rootech gel, NAA and control). Results showed that the highest percentage survival of stevia was obtained from shoot tips (93.92%) which differed statistically from those intermediate (91.00%) and basal stem cuttings (85.51%). On the other hand, basal stem cutting significantly has the lowest percent survival. Results revealed that shoot tip cuttings treated with Rootech Gel developed roots early (6.92 days), with most number of roots (13.70), longer roots (3.33 cm), and 96.38% survival.

Keywords

Clone, *In Situ*, Propagation

1. Introduction

Stevia rebaudiana (Bert.) is an emerging sugar alternative and anti-diabetic plant in the Philippines. It is commonly known as "honey leaf" in Indian market [1], "sweet leaf" in the USA, "sweet honey leaf" in Australia, and "sweet herb in Paraguay [2]. It is a tender perennial herb that belongs to Asteraceae family and usually grown as annual. *Stevia* is 300 times sweeter than sugar and is non-glycemic and calorie free [3]. The sweetness of the leaves is due to its stevioside content. Although it is very sweet, it is non-glycemic hence it will not affect

blood sugar levels. This is beneficial to about 20 percent of the Philippines population who have diabetes [4].

The sweet taste of *Stevia* leaves makes it a potential substitute for table sugar. *Stevia* can be used to sweeten foods and beverages and is a potential export winner [5].

The demand and production of this plant in the Philippines would likely increase due to its various uses and consumption especially as the population becomes increasingly diabetic [6]. However, there are some drawbacks on the production and utilization of this plant. Poor seed germination, unavailability or lack of planting materials and limited production technology (such as nutrient requirement and proper period of harvesting) and few product development derived from *Stevia* are major limiting factors in production and utilization of *stevia*.

Stevia can be propagated by stem cuttings as the cheapest way to multiply the plant. However, propagation by cuttings utilizes only the shoot tips while the lower parts of the stem are not used as planting materials. This limits the number of explants obtained from a single plant; hence, a suitable alternative method for plant production is to utilize stem cuttings from the lower parts of the stem as planting materials to obtain fast production of rooted stem cuttings or planting material.

2. Materials and Methods

The experiment was conducted at Western Mindanao State University College of Agriculture, San Ramon, Zamboanga City from August 2018 to January 2019. The study used Stevia (*Stevia rebaudiana* Bertoni) mother plants as sources of experimental materials purchased from a private nursery in Cagayan de Oro City. Commercial rooting hormones such as naphthalene acetic acid, Rootech gel and Miracle Gro were purchased from agricultural outlets.

The experiment was laid in 3 × 4 factorial arrangement in Randomized Complete Block Design (RCBD), replicated three times. Factor A consist of 3 types of cutting (shoot tip, intermediate stem and basal stem) while Factor B consist of three rooting hormones (Miracle Gro, Rootech Gel and NAA) and a control. Miracle Gro contained 0.1% indole-3 Buteric Acid (IBA) [7], Rootech Gel contained 0.55% IBA and 0.0054 Vitamin B_1 [8], and NAA contained 0.10% alpha naphthalene acetic acid and 0.09% B_1 [9]. The said rooting hormones were selected base on their availability in the community.

Stem cuttings were obtained from mother plants early in the morning. Stem cuttings were cut according to treatments: 1) shoot tips; 2) middle stem; and, 3) basal stem with at least two nodes per cutting. The cuttings were dipped in their respective rooting hormone per treatment prior to planting. The cuttings were planted to a germination try consist of mixture of 1:1:1:1 ratio of garden soil, sawdust, vermicast and carbonized rice hull. The seedlings were covered with rooting bed to minimized adverse effects of environmental factors such as wind, heavy rain and pest on seedling viability. Watering using mist sprayer was done

every morning or as often as necessary. The experiment was monitored regularly for insect pest, diseases and weeds. Data on number of days to rooting, number of root, length of root, number of leaves and percent survival were taken.

The data were analyzed using analysis of variance (ANOVA) to determine the significant effects of the treatments. Differences among treatment means were analyzed using Tukey's HSD (Honestly Significant Difference) test.

3. Results and Discussion

Table 1 shows that the number of days to rooting of stevia was highly influenced by the independent and interaction effects of both factors employed. It revealed that shoot tip of stem cuttings of stevia highly exhibited the earliest rooting of 7.93 days which differed significantly from the intermediate stem cuttings which took 8.51 days while the basal stem cuttings had delayed rooting of 9.14 days.

Among the rooting hormones, stem cuttings applied with Rootech Gel significantly rooted early at 6. 92 days and differed significantly from those treated with Miracle Gro and NAA which formed roots more or less at the same duration of 7.88 and 7.82 days respectively. The Control had significantly delayed rooting of 11.47 days.

The early rooting of shoot tip stem cuttings of stevia can be due to the highly meristematic tissues of the shoot tips. According to [10] shoot tips have actively dividing cells, contain hormones like auxins which induce cell division and cell elongation, thus, results in early rooting of stem cuttings. The intermediate stem had earlier rooting compared to the basal which had delayed rooting. The delayed rooting of the basal stem cuttings may be attributed to the older tissues and lesser meristematic activity of differentiated cells already. It was reported by [11], that although the basal cuttings have accumulated more stored food which can be utilized for growth and rooting of stem cuttings, however plants differ in their response to other various factors.

Results also showed that Rootech Gel highly induced the early rooting of cuttings. According to Technoflora Plant Product Ltd, Rootech Gel contains 0.55% NAA and 0.0054 Vitamin B_1 which are essential for rooting of stem cuttings. It was further reported by [12], that using hormones of the auxin family induced the roots to develop quickly, resulting in early development of roots. Vitamin B_1 contained in Rootech Gel.

Inter-action effects of both factors showed that the use of shoot tip stem cuttings treated with Rootech Gel had significantly induced the early rooting of stevia cuttings (Table 1). This may be attributed to the highly meristematic tissues of shoot tips and as pointed out by [10] that the apex as also the loci of hormone production; hence lead to early rooting of shoot tips stem cuttings.

The number of roots of stevia in response to types of stem cutting and kinds of rooting hormone is presented in Table 1. However, this parameter was highly influenced by the rooting hormones. The types of stem cuttings failed to affect the number of roots. No significant interaction effects between these two factors were noted.

Table 1. Number of days to rooting, number of roots and root length (cm) of stevia as influenced by types of stem cuttings and rooting hormones.

Treatments	No. of Days to Rooting	No. of Roots	Root Length (cm)	No. of Leaves	Percent Survival
Type of Cuttings (A)					
Shoot Tip	7.93 [c]	11.42	3.31 [a]	3.86 [b]	93.92[a]
Intermediate Stem	8.51 [b]	11.17	2.39 [c]	4.67 [a]	91.00[b]
Basal Stem	9.14 [a]	10.63	3.05 [b]	4.54 [a]	85.51[c]
F-Test A	**	ns	**	*	**
Rooting Hormones (B)					
Control	11.47 [a]	8.69 [d]	2.39 [c]	4.07	81.95[d]
Miracle Gro	7.88 [b]	11.70 [b]	2.92 [b]	4.26	93.48[b]
Rootech Gel	6.92 [c]	13.70 [a]	3.33 [a]	4.72	96.28[a]
NAA (0.09%)	7.82 [b]	10.19 [c]	3.01 [b]	4.37	88.86[c]
F-Test B	**	**	**	ns	**
F-Test A × B	**	ns	ns	ns	**
% CV	6.3	9.89	8.9	13.22	8.9

Means within the same column followed by a common letter are not significantly different at 5% level of significance based on Tukey's HSD test (**—highly significant, ns—not significant).

It was found that the number of roots of stevia plants regardless of types of stem cuttings were more or less similar which ranged from 10.63 to 11.42 roots per cutting.

Results however showed that regardless of types of stem cuttings, those treated with Rootech Gel significantly developed the most number of 13.70 roots/cuttings. This was followed by those applied with Miracle Gro (11.70 roots) which was significantly higher than cuttings treated with NAA 4ppm with a mean of 10.19 roots. The Control obtained significantly the least number of 8.69 roots per cutting.

The most number of roots developed in stem cuttings applied with Rootech Gel may be due to indole butyric acid (IBA) and Vitamin B_1 contents compared to the rest of the rooting hormones. Based on the manufacturer's label it contains 0.55% IBA and Vitamin B_1. The hormone IBA belongs to the group of auxins which induces cell division and cell elongation and consequently induced root formation hence more number of roots. According to [13] NAA is an auxin type similar to IBA which induced the development of more leaves, longer and more roots and higher dry weight of roots.

The Miracle Gro ranked second with 11.70 roots. This rooting hormone contains 0.10% IBA of concentration that of Rootech Gel. The treatment NAA on the other hand contained 0.10% Alpha Naphthalene Acetic Acid and 0.09% vitamin B_1. According to [14], IBA has shown to produce a higher number of roots compared to other auxins and IBA is the most commonly used auxin for root formation [15].

The mechanism of IBA action can be attributed to its conversion into IAA through a similar process to B-oxidation of fatty acids. The conversion of IBA to IAA then suggests that IBA works as a storage sink for IAA in plants [16]. However, [17] suggested that IBA is not converted to IAA but acts as an auxin on its own. Furthermore, according to [18] and [19], IBA can be synthesized from IAA by a microsomal membrane preparation from maize seedlings and by an enzyme preparation from Arabidopsis seedlings in the presence of acetyl-CoA and ATP. These results only show that IBA works in similar manner or IAA which can induce cell division, cell enlargement and to some degree cell differentiation in combination with other substances like Vitamin B_1.

The length of root in response to rooting hormones and types of cuttings and showed that this parameter was highly affected by the independent effects of both factors but no interaction effects were observed (**Table 1**). Among the types of cuttings, the shoot tip cuttings developed the longest roots with a mean of 3.31 cm, which differed from the rest of types of stem cuttings. This was followed by the basal cuttings with a mean of 3.05 cm and the shortest root length was observed from intermediate cutting with a mean of 2.39 cm.

Among the rooting hormones, Rootech Gel significantly exhibited longest root of 3.33 cm but differed from the rest of the treatments. However, cuttings treated with NAA had relatively longer roots (3.01 cm) but statistically similar with cuttings treated with Miracle Gro which developed root length of 2.92 cm the shortest root length of 2.39 cm was observed in Control which differed significantly from the rest of treatment.

Studies of [13] revealed that the longest roots of stevia was obtained from treated with 500 ppm IBA. This result was contrary to the current commercial hormones like Rootech Gel and Miracle Gro whose IBA content is about 10% - 55% only. Similar results was obtained by [20] in rose cuttings where maximal number of roots and root length and healthier seedlings were recorded by using 500 ppm IBA. Rootech Gel however contained higher concentrate of IBA (0.55%) than Miracle Gro (0.10%) and (0.09%), respectively.

The number of stevia leaves in response to the types of stem cuttings and rooting hormone is shown in **Table 1**. Results revealed that this parameter was significantly affected by the types of stem cuttings but the rooting hormones failed to influence the number of leaves. No significant interaction effects were observed.

It was found that intermediate stem cuttings developed the highest number of leaves (4.67) followed by the basal cuttings with a mean of 4.54 leaves, while the shoot tip cuttings have significantly the least number of (3.86) leaves. Regardless of types of stem cuttings and hormone treatments, all rooted cuttings developed a more or less similar number of 4.0 to 4.7 leaves per cutting. The most number of leaves that developed from the intermediate and basal stem cuttings may be due to the two shoots that arise at the nodal axils of the intermediate and basal stem cuttings. Each shoot formed fully developed leaves which were higher than that of the shoot tip because the shoot tip has only one growing point the leaves

arise only from the tip of the stem cuttings.

The percent survival of stevia as influenced by types of stem cutting and rooting hormones is shown in the Table. Statistical analysis on these data revealed that this parameter was highly influenced by the independent and interaction of both factors employed.

Results showed that the highest percentage survival of stevia was obtained from shoot tips (93.92%) which differed statistically from those intermediate (91.00%) and basal stem cuttings (85.51%). On the other hand, basal stem cutting significantly has the lowest percent survival.

Comparison among the rooting hormones revealed that stem cuttings treated with Rootech Gel significantly exhibited the highest survival of 96.28% which differed from the rest of the treatments. This was followed by those stem cuttings treated with Miracle Grow with a mean of 93.48. The lowest percent survival was observed in cuttings treated with NAA. Differences among treatment means on percentage survival were highly significant.

Interaction effects of both factors revealed that the use of shoot tips treated with Rootech Gel significantly exhibited the highest survival of 98.33% but relatively similar to those shoot tip cuttings regardless of rooting hormone which ranged from 83.33% to 98.17%.

The high percent survival of stevia shoot tip stem cuttings regardless of rooting hormones may be due to its meristematic activity, and its hormone production at the apex which induce cell division, cell enlargement that leads to survival and growth. Likewise, these results are best supplied by the studies of [21] which revealed that shoot tip cuttings for the propagation of stevia gave the best results and high percentage survival. Moreover, [11] reported that shoot tip stem cuttings with three nodes, have demonstrated significantly higher values on number of shoots, most number of leaves/branch and high percentage survival.

4. Conclusions

Generally it was observed that the use of shoot tip stem cuttings treated with Rootech Gel is the best method for *in situ* clonal propagation of stevia for early rooting of stem cuttings with more developed number of roots, leaves and for high percentage survival;

Results showed that the highest percentage survival of stevia was obtained from shoot tips (93.92%) which differed statistically from those intermediate (91.00%) and basal stem cuttings (85.51%). On the other hand, basal stem cutting significantly has the lowest percent survival.

It can be concluded that the shoot tip is the best propagating part stevia plant and Rootech Gel shows the best result among the rooting hormones.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Rayaguru, K. and Khan, M.K. (2008) Post-Harvest Management of Stevia Leaves: A Review. *Journal of Food Science and Technology*, **45**, 391-397.

[2] Kinghorn, A.D. (1987) Biologically Active Compounds from Plants with Reputed Medicinal and Sweetening Properties. *Journal National Production*, **50**, 1009-1024. https://doi.org/10.1021/np50054a002

[3] Jitendra, M., Monika, S., Ratan, S.D., Priyanka, G., Priyanka, S. and Kiran, D.J. (2012) Micropropagation of an Anti-Diabetic Plant—*Stevia rebaudiana* Bertoni, Natural Sweetener in Hadoti Region of South-East Rajasthan, India. *ISCA Journal of Biological Sciences*, **1**, 37-42.

[4] Castillo, R.R. (2018) The Philippines Is a Diabetes 'Hotspot'. Philippine Daily Inquirer.

[5] PhilStar (2017) Potential of Stevia in the Philippines.

[6] Marc, S. (2008) Concern Grows Over Increase in Diabetes Around World. The New York Times Washington, World Population Increasing over Diabetics.

[7] Miracle-Gro Lawn Product, Inc. (2014) Scotts Miracle-Gro Company. American Multinational Corporation, Marysville, OH.

[8] Technoflora Plant Product Ltd. (2017) Rootech Gel, Technoflora Plant Product Ltd., Mission, BC. http://www.regulatiry-into-technoflora.com

[9] Philippines Orchard Corp. (2012) Philippines Orchard Corporation, Dasmariñas MC, Makati City, Philippines.

[10] Adams, C.R., Bamford, K.M. and Early, M.P. (2008) Principles of Horticulture. 5th Edition, Butterworth-Heinemann, Oxford, 149-165.

[11] Kassahun, B.M. and Mekonnen, S.A. (2012) Effect of Cutting Position and Rooting. *The African Journal of Plant Science and Biotechnology*, **6**, 5-8.

[12] Abdullateef, R.A. (2012) Effects of Stem Cutting Types, Position and Hormonal Factors on Rooting in *Stevia rebaudiana* Bertoni. *Journal of Agricultural Science*, **4**, 49-57. https://doi.org/10.5539/jas.v4n1p49

[13] Ingle, M.R. and Venugopal, C.K. (2009) Effect of Different Growth Regulators on Rooting of Stevia (*Stevia rebaudiana* Bertoni) Cuttings. *Karnataka Journal of Agricultural* Sciences, **22**,455-456.

[14] Rout, G.R. (2006) Effect of Auxins on Adventitious Root Development from Single Node Cuttings of *Camellia sinensis* (L.) Kuntze and Associated Biochemical Changes. *Plant Growth Regulation*, **48**, 111–117. https://doi.org/10.1007/s10725-005-5665-1

[15] Goyal, P., Kachhwaha, S. and Kothari, S.L. (2012) Micropropagation of *Pithecellobium dulce* (Roxb.) Benth—A Multipurpose Leguminous Tree and Assessment of Genetic Fidelity of Micropropagated Plants Using Molecular Markers. *Physiology and Molecular Biology of Plants*, **18**, 169-176. https://doi.org/10.1007/s12298-012-0112-z

[16] Zolman, B.K., Martinez, N., Millius, A., Adham, A.R. and Bartel, B. (2008) Identification and Characterization of Arabidopsis Indole-3-Butyric Acid Response Mutants Defective in Novel Peroxisomal Enzymes. *Genetics*, **180**, 237-251. https://doi.org/10.1534/genetics.108.090399

[17] Ludwig-Müller, J. (2000) Indole-3-Butyric Acid in Plant Growth and Development. *Plant Growth Regulation*, **32**, 219-230. https://doi.org/10.1023/A:1010746806891

[18] Ludwig-Müller, J., Hilgenberg, W. and Epstein, E. (1995) The *in Vitro* Biosynthesis

of Indole-3-Butyric Acid in Maize. *Phytochemistry*, **40**, 61-68. https://doi.org/10.1016/0031-9422(95)00259-A

[19] Ludwig-Müller, J. (2007) Indole-3-Butyric Acid Synthesis in Ecotypes and Mutants of Arabidopsis Thaliana under Different Growth Conditions. *Journal of Plant Physiology*, **164**, 47–59. https://doi.org/10.1016/j.jplph.2005.10.008

[20] Elisabeta, S., Susaj, L. and Kallco, I. (2014) Effect of Different NAA and IBA Concentrations on Rooting of Vegetative Cuttings of Two Rose Cultivars. *Research Journal of Agricultural Science*, **44**, 121-127.

[21] Pigatto, G.B., Nunes, E., Gomes, J., De Cássia, T., Ferriani, A.P. and Deschamps, C. (2017) Effects of Indolebutyric Acid, Stem Cutting Positions and Substrates on the Vegetative Propagation of *Stevia rebaudiana* Bertoni. *Revista Colombiana De Ciencias Hortícolas*, **12**, 202-211. https://doi.org/10.17584/rcch.2018v12i1.6631