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Research Article

Plant Growth Regulators (PGRs) Study for Root and Bud Induction on Stem Cuttings of *Stevia rebaudiana*

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ABSTRACT
The Stevia plant is used as a low calorie natural sweetener known as"the sweet herb of Paraguay". Stevia leaves a higher level of sweetness than cane sugar. Stevia can be propagated by stem cutting techniques. Soaked of stem cuttings in Plant Growth Regulators (PGRs) auxin can promote the growth and development of Stevia. Research was conducted to determine the effect of commercial PGRs (Rootone-f) and natural PGRs from Shallot extract. This study used a complete randomized design with different concentrations, both Rootone-f (0, 100, 200, 300, 400 and 500 mg/L) and shallot extract (0, 0.5%, 1%, 1.5% and 2%). Observations were made after 6 weeks (42 days) of planting on several growth parameters (height of plant, number or buds, number of leaves, number of roots and length of roots). Commercial PGRs showed a significant effect on growth parameters. While natural PGRs of shallot showed a significant effect on height of growth parameters. The recommended concentration of commercial PGRs for Stevia rebaudiana Bertoni M. stem cuttings *was 300 mg/L.*

Keywords: *Auxin; Shallot Extract; Stem Cuttings; Stevia; Rootone-f.*

Introduction

Stevia is a plant used as a natural sweetener without calories known as "*the sweet herb of Paraguay*". It was Asteraceae originating from South America. This Genus has 200 species. Among these species, only Stevia is used as a sweetener. Stevia is shrub which height of about 1 m, including annual plants that have a life concentration of 2-4 years or even more depending on the care and can be harvested 6-7 times per year [1]. Stevia can grow with temperatures of 20^0-40^0 C, with an altitude of 700-1500 meters above sea level and can grow on almost all types of soil [1].

Stevia leaves have a higher sweetness level that was 200-300 times higher sucrose, non calories and non-carcinogenic [2]. The sweet taste caused by the content of steviol glycosides which were diterpene glycoside compounds [3]. In plant cultivation, the familiar method for Stevia cultivation was vegetative propagation by shoot cutting. This method was traditionally method which simple and easy procedures. It can used by cutting organ plant such as stems, roots, and leave which growth it on medium nursery before being transferred to the field [4]. The success of this method was influenced by growth regulators [5].

The presence of growth regulators

can stimulate the growth of cuttings roots, which caused the roots growth better and the root system affects the overall plant growth [6], [7]. It can help plants absorb nutrients from the soil, prevent leaf fall and improve the process of photosynthesis [8]. Growth regulators used were Indole Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Naphtalene Acetic Acid (NAA) and Rootone-f [9], [10].

Rootone-f is a widely used commercial brand that is known to be effective in stimulating rooting, because it contains Indole Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Naphtalene Acetic Acid (NAA) [11]. It plays a role in accelerating cell elongation in root tissue. While IBA and NAA play a role in the formation of advanced roots such as root hair and bud growth [12]. Plant growth regulator commercial (Rootone-f) and *Allium cepa* extracts are compounds whose mechanism of action such as auxin, which at appropriate concentrations can increase division, cell elongation and cell differentiation in the form of bud elongation [13]. In addition, they are easy to get in the market at low prices. Thus, they are necessary to conduct on the growth of *Stevia rebaudiana* Bertoni M. cuttings.

Materials and methods

1. Stevia rebaudiana shoot cultures

High quality stevia cuttings, woody shoots and free of pests and diseases are selected as the starting material. The cuttings are about 8 cm long and are cut at an angle to reduce transpiration. The mother tree from which the cuttings are taken should be about 6 months old. After cutting, each cutting is left with 3-4 leaves for optimal propagation [14]. This method ensures that the resulting cuttings have a high chance of developing into strong and healthy stevia plants.

Table 1. Effect of commercial PGRs concentration on stem cutting of *Stevia rebaudiana* (42 days).

Concentration of commercial PGRs (mg/L)	Height of plant (cm)	Number of buds	Number of leaves	Number of roots	Lenght of roots (cm)
Kontrol	5,88^{a}	0,25^{a}	2^{a}	5^{a}	4,5^{a}
100	6,25^{a}	2^{ab}	11^{ab}	7^{ab}	4,38^{a}
200	6,63^{a}	2^{ab}	11^{ab}	13^{bc}	4,8^{a}
300	**8,63^{c}**	3^{ab}	11^{ab}	**24 ^{d}**	**9,75^{b}**
400	7,63^{ab}	4^{b}	12^{b}	17^{c}	6,5^{a}
500	7,13 ^{ab}	4^{b}	17^{b}	15^{c}	4,68^{a}

Note. significant differences at 5%.

2. PGR application and culture conditions

Rootone-f was applied by soaking the base of the cuttings in a solution of the specified concentration, prepared according to the treatment protocol. The cuttings were then planted in the prepared planting media with moist soil conditions. Daily watering was performed with 600 ml of water in each polybag [15].

The commercial growth regulator used was Rootone-f, which were consisting of 6 treatments for different concentrations, such as: 0 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, (400 mg/L and 500 mg/L and repeated for 4 times. Natural growth regulator used was *Allium cepa* extract which consists of 5 treatments, such as; 0%, 0.5%, 1%, 1.5% and 2%. *Stevia* stems were cut along 8 cm from the mother plant, then soaked in a solution containing Rootone-f and *A. cepa* extract of various concentrations for 15 minutes. Grow it on medium nursery. Then, stem cutting growth was observed every day, including: height of plant, number or buds, number of

leaves. While the number of roots and length of roots were observed at the end of the observation. All unit were observation for 6 weeks (42 days) (Table 1).

3. Data analysis

Quantitative experimental methods were used in this study. Using a completely randomized design (CRD) factorial. Then the results of the study were analyzed using the Annova test and honestly significantdifference (HSD), test with significant differences at 5%.

Result and Discussion

Stevia stems were cut along 8 cm from the mother plant, then soaked in a solution containing Rootone-f and *Allium cepa* extract of various concentrations for 15 minutes. Grow it for 6 weeks (42 days) on medium nursery. Then, stem cutting growth is observed every day, including: height of plant, number or buds, number of leaves. While the number of roots and length of roots were observed at the end of the observation.

Based on the results of the analysis of variance (ANOVA) test with significant differences at 5%, it was found that Rootone-f had an effect on the parameters height of plant, number or buds, number of leaves, number of roots and length of roots (Table 1). The best concentration to increase the number of roots, root length and plant height was 300 mg/L. However, there was no significant difference between the control and treatment in the number of buds and number of leaves. The role of Rootone-F at appropriate concentrations can accelerate the plant growth process, but in high concentration it can inhibit growth and development of shoots and sometimes cause death [16]. This in line with the fact that auxin at optimal concentrations can help root growth, but conversely concentrations that exceeding the optimal limits can inhibit growth [17].

In this study, the formation of roots and buds on cuttings stem can be seen in Figure 1. The growth of roots on cuttings stem method is a determinant of the success of this method [18]. The effect of Rootone-F dose treatment is due to the content of four types of synthetic auxins, namely 2-methyl-1-naphthalenecetamide (0.13%), 1-naphthalenecetamide (0.067%), 2-methyl-1-naphthalene and indole-3-butyrate (0.057%), Synthetic auxins such as IAA (3-indoleacetic acid), NAA (1-naphtyl-acetic acid) spur cuttings growth through accelerated cambium cell division and differentiation of phloem and xylem, stimulating the initiation of root formation and root tissue differentiation [19].

(a) (b) (c)

Figure 1. (a) Stem cutting of *Stevia rebaudiana,* (b) Shoots of *Stevia rebaudiana* added commercial PGRs at 42 days, (c) Roots of *Stevia rebaudiana* added commercial PGRs at 42 days.

The increase in height growth of stem cuttings of *Stevia* is caused by the NAA contained in Rootone-f which stimulates cell elongation. Cell elongation

occurs when the plasticity of the cell wall stretches, causing water to enter the cell wall by osmosis so that the cell experiences elongation and differentiation in the form of segment [19], [20]. In addition, NAA can stimulate root cell division so that the root system grows better, thus plant physiological activities such as water and nutrient absorption by protoplast increase, which is followed by the root cell elongation process [18].

Shallots contain auxin which can stimulate root growth in stem cutting [20]. Based on the results of the analysis of variance (ANOVA) test with significant differences at 5%, it was found that shallot extract had an effect on the parameters height of plant and number of leaves. But it does not affect the number of roots and root length Table 2, shows that the best extract concentration for plant height parameters and number of leaves was 1%. Previous research shows that using the same concentration of auxin contained in shallots affects stem elongation, growth, differentiation of xylem and phloem tissue, and root formation [21].

Table 2. Effect of *Allium cepa* extract concentration on stem cutting of *Stevia rebaudiana* (42 days).

Concentration of *Allium cepa* (%)	Height of plant (cm)	Number of buds	Number of leaves	Number of roots	Lenght of roots (cm)
Kontrol	6,7[a]	2	11[a]	19	4
0,5	7,2[ab]	2	11[a]	18	5,1
1	8[b]	1	15[b]	18	4,4
1,5	6,8[a]	2	13[ab]	17	4,4
2	6,9[a]	2	15[b]	20	3,6

Note. significant differences at 5%.

Shallots extract had no effect on root induction and root length of *Stevia* (Table 2). It happens because the concentration used was not appropriate. 50% shallot extract was no effect on growth of *Syzygium aquenum* [22]. However, in different studies the information obtained show that a concentration of 60% is best concentration for cutting *S. Aqueum* stem [23]. Shallot contained auxin and gibberellin which composed of riboflavin and thiamin can stimulate root initiation in stem cutting, lateral roots in root development [24]. The types of endogenous auxin contained in shallot are IAA, NAA and 2,4 D [25]. These plant growth regulators are non-nutrient organic compounds that in certain amounts actively stimulate or inhibit plant growth and development [22].

Conclusion

Commercial PGRs used affect all growth parameters. The recommended concentration of Commercial PGRs for *Stevia rebaudiana* Bertoni M. stem cuttings is 300 mg/L. While natural PGRs of shallot showed a significant effect on height of parameters.

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