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1 **Plant Growth Regulators (PGRs) Study for Root and Bud Induction on Stem** 2 **Cuttings of *Stevia rebaudiana***

3
4 **Ike Apriani^{1*}, Sonia Adiba¹, Dawani¹, Tito Nurseha¹, Awalul Fatiqin², Yessy Velina³**

5 ¹Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Raden
6 Fatah Palembang, South Sumatera, Palembang, Indonesia.

7 ²Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Palangka
8 Raya, Central Kalimantan, Palangka Raya, Indonesia.

9 ³Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan.

10
11 *Email: ikeapriani_uin@radenfatah.ac.id

13 **Abstract**

14 The *Stevia* plant is used as a low calorie natural sweetener known as "the sweet herb of
15 Paraguay". *Stevia* leaves a higher level of sweetness than cane sugar. *Stevia* can be propagated
16 by stem cutting techniques. Soaked of stem cuttings in Plant Growth Regulators (PGRs) auxin
17 can promote the growth and development of *Stevia*. Research was conducted to determine the
18 effect of commercial PGRs (Rootone-f) and natural PGRs from Shallot extract. This study used
19 a complete randomized design with different concentrations, both Rootone-f (0, 100, 200, 300,
20 400 and 500 mg/L) and shallot extract (0, 0.5%, 1%, 1.5% and 2%). Observations were made
21 after 6 weeks (42 days) of planting on several growth parameters (height of plant, number or
22 buds, number of leaves, number of roots and length of roots). Commercial PGRs showed a
23 significant effect on growth parameters. While natural PGRs of shallot showed a significant
24 effect on height of growth parameters. The recommended concentration of commercial PGRs
25 for *Stevia rebaudiana* Bertoni M. stem cuttings was 300 mg/L.

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

28 **Introduction**

29 *Stevia* is a plant used as a natural sweetener without calories known as "the sweet herb
30 of Paraguay". It was Asteraceae originating from South America. This Genus has 200 species.
31 Among these species, only *Stevia* is used as a sweetener. *Stevia* is shrub which height of about
32 1 m, including annual plants that have a life concentration of 2-4 years or even more depending
33 on the care and can be harvested 6-7 times per year [1]. *Stevia* can grow with temperatures of
34 20⁰-40⁰ C, with an altitude of 700-1500 meters above sea level and can grow on almost all

35 types of soil [1].

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

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

48 Rootone-f is a widely used commercial brand that is known to be effective in stimulating
49 rooting, because it contains Indole Acetic Acid (IAA), Indole-3-Butyric Acid (IBA),
50 Naphtalene Acetic Acid (NAA) [11]. It plays a role in accelerating cell elongation in root
51 tissue. While IBA and NAA play a role in the formation of advanced roots such as root hair
52 and bud growth [12]

53 Plant growth regulator commercial (Rootone-f) and *Allium cepa* extracts are compounds
54 whose mechanism of action such as auxin, which at appropriate concentrations can increase
55 division, cell elongation and cell differentiation in the form of bud elongation [13]. In addition,
56 they are easy to get in the market at low prices. Thus, they are necessary to conduct on the
57 growth of *Stevia rebaudiana* Bertoni M. cuttings.

58

59 **Materials and methods**

60 *1. Stevia rebaudiana* shoot cultures

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

67 *2. PGR application and culture conditions*

68 Rootone-f was applied by soaking the base of the cuttings in a solution of the specified

69 concentration, prepared according to the treatment protocol. The cuttings were then planted
70 in the prepared planting media with moist soil conditions. Daily watering was performed with
71 600 ml of water in each polybag [15].

72 The commercial growth regulator used was Rootone-f, which were consisting of 6
73 treatments for different concentrations, such as: 0 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, (400
74 mg/L and 500 mg/L and repeated for 4 times. Natural growth regulator used was *Allium cepa*
75 extract which consists of 5 treatments, such as; 0%, 0.5%, 1%, 1.5% and 2%. *Stevia* stems
76 were cut along 8 cm from the mother plant, then soaked in a solution containing Rootone-f and
77 *A. cepa* extract of various concentrations for 15 minutes. Grow it on medium nursery. Then,
78 stem cutting growth was observed every day, including: height of plant, number of buds,
79 number of leaves. While the number of roots and length of roots were observed at the end of
80 the observation. All unit were observation for 6 weeks (42 days) (Table 1).

81 3. Data analysis

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

85

86 Result and Discussion

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

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

102

103 **Table 1. Effect of commercial PGRs concentration on stem cutting of *Stevia rebaudiana***
 104 **(42 days).**

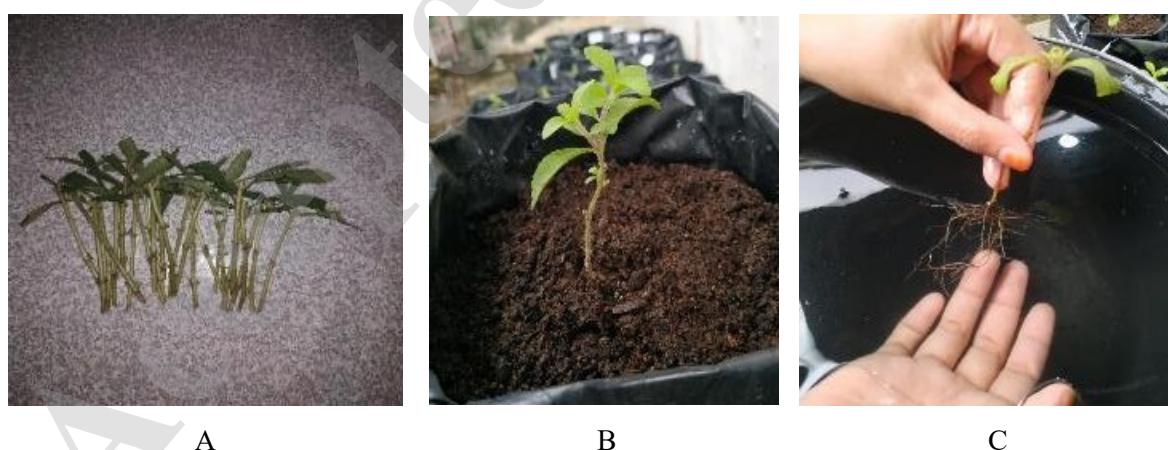
Concentration of commercial PGRs (mg/L)	Height of plant (cm)	Number of buds	Number of leaves	Number of roots	Length of roots (cm)
Kontrol	5,88 ^a	0,25 ^a	2 ^a	5 ^a	4,5 ^a
100	6,25 ^a	2 ^{ab}	11 ^{ab}	7 ^{ab}	4,38 ^a
200	6,63 ^a	2 ^{ab}	11 ^{ab}	13 ^{bc}	4,8 ^a
300	8,63^c	3 ^{ab}	11 ^{ab}	24^d	9,75^b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

105 Note. significant differences at 5%.

106

107 In this study, the formation of roots and buds on cuttings stem can be seen in Figure 1.
 108 The growth of roots on cuttings stem method is a determinant of the success of this method
 109 [18]. The effect of Rootone-F dose treatment is due to the content of four types of synthetic
 110 auxins, namely 2-methyl-1-naphthalenecetamide (0.13%), 1-naphthalenecetamide (0.067%),
 111 2-methyl-1-naphthalene and indole-3-butyrate (0.057%), Synthetic auxins such as IAA (3-
 112 indoleacetic acid), NAA (1-naphtyl-acetic acid) spur cuttings growth through accelerated
 113 cambium cell division and differentiation of phloem and xylem, stimulating the initiation of
 114 root formation and root tissue differentiation [19].

115



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

118

119 The increase in height growth of stem cuttings of *Stevia* is caused by the NAA contained

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

126

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

Concentration of <i>Allium cepa</i> (%)	Height of plant (cm)	Number of buds	Number of leaves	Number of roots	Length 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

128 Note. significant differences at 5%.

129

130 Shallots contain auxin which can stimulate root growth in stem cutting [20]. Based on
 131 the results of the analysis of variance (ANOVA) test with significant differences at 5%, it was
 132 found that shallot extract had an effect on the parameters height of plant and number of leaves.
 133 But it does not affect the number of roots and root length (Table 2).

134 Table 2 shows that the best extract concentration for plant height parameters and number
 135 of leaves was 1%. Previous research shows that using the same concentration of auxin
 136 contained in shallots affects stem elongation, growth, differentiation of xylem and phloem
 137 tissue, and root formation [21].

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

147

148 **Conclusion**

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

152

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156

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