

THE EFFECT OF SHOOT EXPLANT TYPES OF ELEVEN STEVIA (*Stevia rebaudiana* Bertoni) ACCESSIONS ON *IN VITRO* GROWTH

PENGARUH JENIS EKSPAN PUCUK TERHADAP SEBELAS AKSESI STEVIA (*Stevia rebaudiana* Bertoni) PADA PERTUMBUHAN *IN VITRO*

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ABSTRACT

Explants play an important role in the propagation system of stevia (*Stevia rebaudiana* Bertoni). A Completely Randomized Design (CRD) was used in this experiment with factorial pattern consisting of two factors, namely three types of explants (shoot tip, first node, and second node) and eleven accessions of stevia namely a₁ (Bogor), a₂ (Garut), a₃ (Canada), a₄ (Tawangmangu), a₅ (STG1), a₆ (SBG 4), a₇ (SBG 10), a₈ (SGB 2), a₉ (BR5), a₁₀ (SGR 7.5), a₁₁ (TR 3.5). The results showed that shoot tip explant was the best explant than first node and second node for the number of shoots (18.11 shoots), number of leaves (93.49 leaves) and wet weight (3.56 grams). The best accession of shoot height was a₁₀ (SGR 7.5) (19.95 cm), the highest number of shoots was a₇ (SBG 10) (21.87 shoots), the highest number of leaves was a₇ (SBG 10) (138.00 leaves), the heaviest wet weight was a₇ (SBG 10) (3.56 grams), the highest leaf chlorophyll content was a₁₀ (SGR 7.5) (0.63 µg mL⁻¹). Accessions with the fastest root initiation time at the rooting stage was a₁₁ (TR 3.5) (4.00 DAC), the highest number of roots was a₁₀ (TR 3.5) (27.11 roots), the best root length was a₂ (Garut) (4.51 cm). Information on the best explant types and stevia accessions in the *in-vitro* multiplication stage can be used as the basis for stevia breeding programs in Indonesia.

Key words : Accession, Explant, *In vitro*, Shoot tip, *Stevia rebaudiana*

ABSTRAK

Eksplan berperan penting dalam sistem perbanyakan Stevia (*Stevia rebaudiana* Bertoni). Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan pola faktorial yang terdiri dari dua faktor yaitu tiga jenis eksplan (ujung pucuk, ruas pertama, dan ruas kedua) dan sebelas aksesori stevia yaitu a₁ (Bogor), a₂ (Garut), a₃ (Canada), a₄ (Tawangmangu), a₅ (STG1), a₆ (SBG 4), a₇ (SBG 10), a₈ (SGB 2), a₉ (BR5), a₁₀ (SGR 7.5), a₁₁ (TR 3.5). Hasil penelitian menunjukkan bahwa eksplan ujung pucuk merupakan eksplan terbaik dibandingkan buku pertama dan kedua untuk jumlah tunas (18,11 tunas), jumlah daun (93,49 daun) dan berat basah (3,56 gram). Aksesori terbaik pada tinggi pucuk adalah a₁₀ (SGR 7.5) (19,95 cm), jumlah pucuk tertinggi a₇ (SBG 10) (21,87 pucuk), jumlah daun terbanyak a₇ (SBG 10) (138,00 helai daun), bobot basah terberat a₇ (SBG 10) (3,56 gram), kandungan klorofil daun tertinggi adalah GR 7,5 (0,63 µg mL⁻¹). Aksesori dengan waktu inisiasi akar tercepat pada tahap perakaran adalah TR 3,5 (4,00 HST), jumlah akar terbanyak adalah TR 3,5 (27,11 akar), panjang akar terbaik adalah a₂ (Garut) (4,51 cm). Informasi

jenis eksplan dan aksesi stevia terbaik pada tahap multiplikasi *in-vitro* dapat dijadikan dasar program pemuliaan stevia di Indonesia.

Kata kunci: Aksesi, Eksplan, *In vitro*, *Stevia rebaudiana*, Ujung pucuk

INTRODUCTION

Plant tissue culture technique is advantageous over traditional propagation as it can be used to multiply novel plants, such as those that have been genetically modified or bred through plant breeding methods. The conventional propagation methods of stevia are not always suitable for large scale cultivation due to less availability of planting material at a time. Stevia has self-incompatibility which causes a low germination rate (Raina et al., 2013). Furthermore et al. (2011) reported that vegetative propagation of stevia using stem cuttings has drawbacks because the number of stem cuttings produced from stock plants is limited. Therefore, propagation of stevia using tissue culture is an alternative way to meet the supply of seeds on a large scale at rapid time.

The large scale cultivation of stevia plants is important for the human health because of its medicinal properties and the presence of sweetener compounds on its leaves. These compounds can be a substitute for sucrose in a wide variety of products. Some researchers reported that stevia has different medicinal properties including anti-diabetic (Ucar et al., 2018), anti-hypertensive and immuno-stimulating (Misra et al., 2015), and anti-inflammatory (Chagas-Paula et al., 2015).

In vitro techniques is one of the reliable source used for commercial plantlet production of stevia. Type and source of explants play an important role for the successful application plant tissue culture technique. All plant parts can be used as

explants because they have totipotent properties (Deshmukh & Ade, 2012). Shoot tip and nodal segment explants have been used as starting material for producing various plants such as Tomato (Banu et al., 2017), *Pogostemon erectus* (Dogan et al., 2016). Alhady (2011) obtained the highest shoot multiplication rate of stevia from single stem node segment cultured on medium supplemented with BA. Singh et al. (2017) reported there was different efficiency on average number of shoot by culturing shoot-tip and nodal segments explants of stevia.

Genotype also plays an important role in *in-vitro* culture. The same explant type can produce different plant growth in different plant genotypes (Smith, 2013). Naranjo et al. (2016) reported that the genotype showed a significant effect on the embryogenic response of stevia. This study aimed to evaluate a suitable and efficient protocol for rapid *in-vitro* propagation, as well as to evaluate the best explant types and stevia accessions at the growth stage in *in-vitro*.

MATERIAL AND METHODS

The research was carried out in the Laboratory of Plant Breeding, Faculty of Agriculture, Universitas Padjadjaran from September 2020 to June 2021. A Completely Randomized Design (CRD) was used in this experiment with factorial pattern consisting of two factors. The first factor were the eleven stevia accessions i.e., a₁ (Bogor), a₂ (Garut), a₃ (Canada), a₄ (Tawangmangu), a₅ (STG 1), a₆ (SBG 4), a₇ (SBG 10), a₈ (SGB 2), a₉ (BR 5), a₁₀ (SGR 7.5), and a₁₁ (TR 3.5). The

second factor were the explant types i.e., shoot tip, first node, and second node.

The explants were existed from plantlets that had been cultured previously at culture room of Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. Size of explants was 1 cm with two leaves. The experiment consisted of two stages, the shoot multiplication stage and the rooting stage. In the multiplication stage, each explant were cultured on Driver and Kuniyuki Walnut (DKW) (Driver and Kuniyuki, 1984) supplemented with 30 g L⁻¹ sucrose, 1.5 ppm BA and 0.15 ppm IAA. At the root stage, 0.35 ppm IAA was added without BA and IAA.

Before adding the gellifying agent, pH was adjusted to 5.8 and sterilized in an autoclave at 121 °C and 1 atm for 20 min. Then, the medium was heated and stirred used a magnetic stirrer. After the solution was homogeneous, it was poured into culture bottles as much as 10 mL per bottle.

Explants in culture bottles were stored in a culture room with a temperature of 26-28°C and relative humidity of 59-66%. The lamps used are TL lamps (Tubular Lamp) with an average of 40 watts m² or ± 1000 lux. The culture room was routinely sprayed with 70% alcohol twice a week to avoid contamination.

Observed parameters on the root stage included number of roots (pcs), root length (cm), plant height (cm), number of shoots (pcs), number of leaves (sheet), shoot height (cm), and chlorophyll content (µg mL⁻¹) which were recorded four weeks after planting for shoot multiplication stage and eight weeks after planting for rooting stage. The data obtained were processed using R Studio software version 1.4.1106 to analyze the variance (Analysis of Variance). Significant differences in treatment were

tested using the Scott-Knott test with a confidence level of $p < 0.05$.

RESULT AND DISCUSSION

Shoot Multiplication Stage

There was no significant effect of the interaction between explant type and stevia accessions shoot height, number of shoots, number of leaves, and chlorophyll content in shoot multiplication stage, so required the analysis to be carried out independently.

Based on Table 1, the highest of stevia shoot height was a₁₀ (SGR 7.5), and the lowest of shoot heights were a₁(Bogor), a₂ (Garut), a₅ (STG1), a₆ (SBG4), a₈ (SGB 2). The significant differences in shoot height occurred due to differences in the growth response of each genotype (Smith, 2013). The appearance of stevia can be seen in Figure 1.

The treatment of explant types e₁ (shoot tip), e₂ (first node), and e₃ (second node) showed that not significantly different in shoot height (Table 1). This is in line with the experiment Singh et al. (2017), that the shoot tip and node explant treatments showed the same shoot height results, namely 2.32 cm for the shoot tip explant treatment and 2.00 cm for the node explant treatment. Another experiment was carried out by Hossain et al. (2008), the treatment of shoot tip and node explants also showed the same shoot height of 6.38 cm at the shoot tip treatment and 5.83 cm at the node treatment. These results indicated that the endogenous hormones contained in the three explant types (shoot tip, first node, and second node) didn't significantly affect for the shoot height of stevia.

The highest number of shoots of stevia were a₇, a₉, a₁₀ (SBG 10, BR 5, GR 7.5) (Table 1). The lowest number of

shoots of stevia were a₁, a₂, a₅ (Bogor, Garut, STG 1). The highest number of shoots of explant type treatment was e₁ (shoot tip). Treatments e₂ and e₃ (first node and second node) produced the no significant different for number of

shoots. This is in line with the experimental results Hossain et al. (2017), the shoot tip explant treatment and the node explant treatment showed that significantly different in stevia.

Table 1. Independent effect of stevia accession and explant types on shoot height, number of shoots, number of leaves, and chlorophyll content in shoot multiplication stage

Accession	Characteristics							
	Shoot Height (cm)		Number of Shoots (pcs)		Number of Leaves (sheet)		Chlorophyll Content (µg mL ⁻¹)	
a ₁ (Bogor)	7.97	d	9.27	d	43.07	d	0.16	e
a ₂ (Garut)	6.75	d	12.07	d	54.67	d	0.15	e
a ₃ (Canada)	11.50	c	13.33	c	68.00	c	0.17	e
a ₄ (Tawangmangu)	13.01	b	15.33	c	76.47	c	0.26	d
a ₅ (STG 1)	7.53	d	11.13	d	61.20	d	0.15	e
a ₆ (SBG 4)	8.78	d	14.67	c	85.60	c	0.17	e
a ₇ (SBG 10)	13.47	b	21.87	a	138.00	a	0.31	d
a ₈ (SGB 2)	8.35	d	14.27	c	77.60	c	0.17	e
a ₉ (BR 5)	15.68	b	20.47	a	95.60	b	0.53	b
a ₁₀ (SGR 7.5)	19.95	a	21.20	a	105.60	b	0.63	a
a ₁₁ (TR 3.5)	11.27	c	17.27	b	86.80	c	0.39	c
Explant types								
e ₁ (Shoot Tip)	11.42	a	18.11	a	93.49	a	0.30	a
e ₂ (First Node)	11.31	a	14.75	b	76.89	b	0.281	a
e ₃ (Second Node)	11.15	a	13.75	b	73.05	b	0.261	a

Note: The mean values followed by the same alphabet are not significantly different based on the Scott Knott Test at the level p<5%

Differences in response of explant types may occur due to the differences in the balance of endogenous hormones in plant tissues (Baghel & Bansal, 2017). There is a different ratio of endogenous phytohormones in the shoot tip and node. Shoot tip explants were better than node segment explants for shoot multiplication because a higher cytokinin to auxin ratio founded at the shoot tip (Baghel & Bansal, 2017; Naranjo et al., 2016). Cytokinins stimulate plant cell division and play a role in released lateral bud dormancy, lateral shoot growth, and cell cycle control (Baghel & Bansal, 2017). The combination of

endogenous and exogenous hormones added to the media, namely BA 1.5 ppm and IAA 0.15 ppm, contributed to shoot tip explants than node explants.

The highest number of leaves was a₇ (SBG 10) (Table 1). The lowest number of leaves were a₁, a₂, a₅ (Bogor, Garut, STG 1 respectively). The highest number of leaves of explant type treatment was e₁ (shoot tip). Treatments of e₂ and e₃ (first node and second node) showed that no significant difference for number of leaves appeared. This is in line with the experiment Rock-Okuyucu et al. (2016), the number of nodes produced from shoot tip explants was more

than node explants. Meanwhile, the number of stevia nodes is directly proportional to the number of leaves (Ermayanti et al., 2017). So, the more nodes number, the more leaves in the shoot explants.

The highest chlorophyll content of stevia leaves was a_7 (SBG 10). The lowest chlorophyll content of stevia leaves were a_1 ,

a_2 , a_3 , a_5 , a_6 , a_8 (Bogor, Garut, Canada, STG 1, SBG 4, GBS 2 respectively) (Table 1). Treatment of shoot explant types showed a same chlorophyll content result. This proves that the endogenous hormones contained in the three explant types (shoot tip, first node, and second node) did not affect the character of stevia's chlorophyll content.

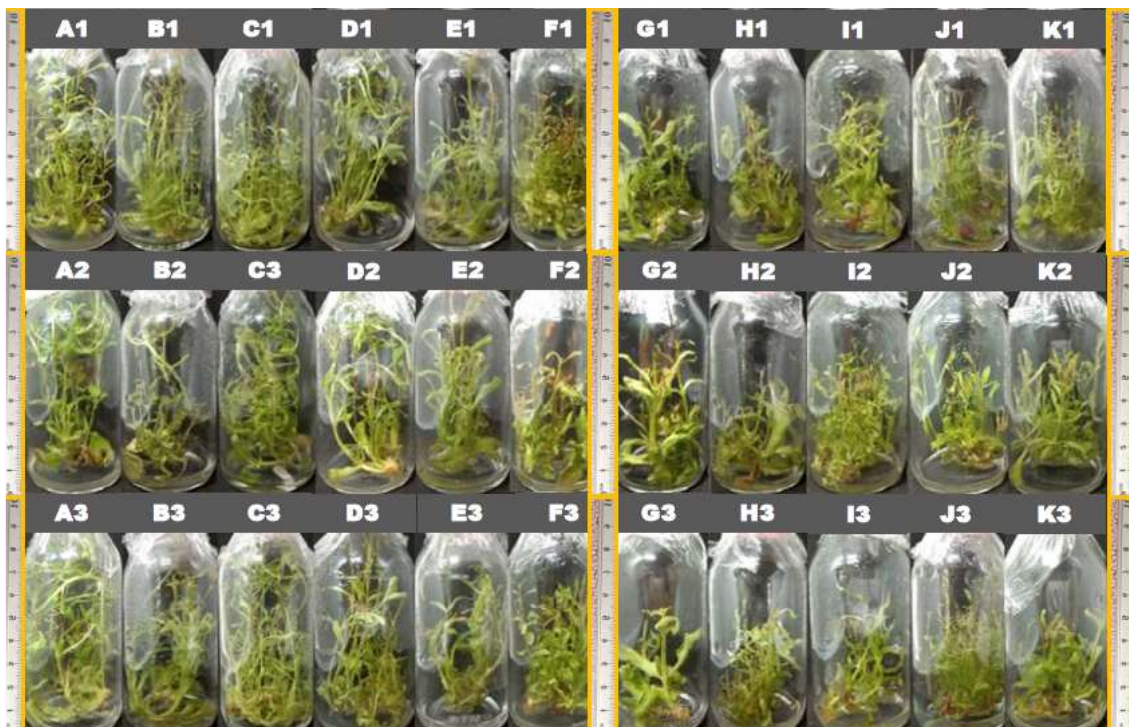


Figure 1. Stevia shoot height at the stage of shoot multiplication. A) a_{10} (GR 7.5); B) a_4 (Tawangmangu); C) a_7 (SBG 10); D) a_9 (BR 7.5); E) a_3 (Canada); F) a_{11} (TR 3.5); G) a_1 (Bogor); H) a_2 (Garut); I) a_5 (STG 1); J) a_6 (SBG 4); K) a_8 (SGB 2). In the code numbers 1, 2, and 3, respectively, the treatment of shoot tip explants, first node, and second node are shown. Ruler = 10 cm

Table 2 presents information about the best combination of treatments on wet weight in shoot multiplication stage. The best combination on wet weight characters were all accessions in shoot tip (e_1) and first node (e_2) types, but the results were varied in second node type (e_3). The result showed that the wet weight of the plant was related to the number of leaves. The number of leaves supports the process of photosynthesis, which produces

photosynthate for plant growth. The increase in the number of leaves and the number of shoots will be in line with the wet weight increase of the plant (Naranjo et al., 2016).

The highest number of roots of stevia was a_{11} (TR 3.5) (Table 3). The least number of roots of stevia were a_4 , a_5 , a_7 , a_8 (Tawangmangu, STG 1, SBG 10, SGB 2 respectively) which were not significantly different for the number of roots. In this

experiment, exogenous auxin used in the medium was IAA 0.35 ppm. Auxin is known for its ability in the process of root formation. It is related to the role of auxin in stimulating cell division (Naranjo et al.,

2016). Besides influenced by endogenous hormone, different genotype gave different responses to the hormone given.

Table 2. Interaction of stevia accessions treatment with shoot explant types on average wet weight (grams) in shoot multiplication stage

Accession	Explant Types		
	e ₁ (Shoot Tip)	e ₂ (First Node)	E ₃ (Second Node)
a ₁ (Bogor)	3.28 a A	2.97 a A	2.38 b B
a ₂ (Garut)	2.78 a A	2.60 a A	2.35 a B
a ₃ (Canada)	3.05 a A	3.02 a A	2.71 a A
a ₄ (Tawangmangu)	3.06 a A	2.67 a A	2.64 a B
a ₅ (STG 1)	3.20 a A	2.12 b A	2.09 b B
a ₆ (SBG 4)	2.87 a A	2.81 a A	2.69 a B
a ₇ (SBG 10)	3.56 a A	3.35 a A	3.20 a A
a ₈ (SGB 2)	2.95 a A	2.85 a A	2.69 a A
a ₉ (BR 5)	2.96 a A	2.85 a A	2.72 a A
a ₁₀ (SGR 7.5)	3.33 a A	2.93 a A	2.93 a A
a ₁₁ (TR 3.5)	2.99 a A	2.70 a A	2.60 a B

Noted: The mean values followed by the same alphabet (capital in the vertical direction and lower case in the horizontal direction) were not significantly different based on the Scott Knott Test at the level $p < 5\%$.

Meanwhile the results also showed that there was no significant difference between the treatment of shoot tip explants, first and second nodes. This indicated that endogenous auxin in different explant types did not affect the number of the roots of stevia. It is caused by auxin transport occurs basipetal through the stem so that the highest concentration of auxin is in the basal part. (Hu et al., 2017) stated that the auxins is synthesized in young leaves and shoot apical meristems. While the distribution of endogenous auxin is controlled by polar

auxin transport (Hu et al., 2017; Singh et al., 2017).

The longest of stevia root length was a₂ (Garut) (Table 3). The shortest roots lengths of stevia were a₁, a₃, a₄, a₇, a₈, a₉ (Bogor, Canada, Tawangmangu, SBG 10, SGB 2, BR 5 respectively). Treatment of shoot explant types e.i e₁ (shoot tip), e₂ (first node), and e₃ (second node) showed a non significant root length values, it showed that endogenous auxin in each shoot explant type did not affect

the root length of stevia. The mechanism of auxin action in influencing the elongation of plant cells is that certain proteins contained in the plasma membrane are stimulated by auxin so that H⁺ ions are pumped into the cell wall. This H⁺ ions activates certain enzymes and breaks some of the

hydrogen cross-links in the cellulose molecular chains that make up the cell wall. Plant cells then elongate due to water entering by osmosis. After the elongated cell, the cell grows by synthesizing the cytoplasmic cell wall material (Smith, 2013).

Table 3. Independent effect of stevia accession and explant types on the number of root, root length, plant height, number of shoots, and number of leaves in rooting stage

Accessions	Characteristics									
	Number of Roots (pcs)		Root Length (cm)		Plant Height (cm)		Number of Shoots (pcs)		Number of Leaves (sheet)	
a ₁ (Bogor)	16.89	c	1.96	c	16.57	A	7.88	a	44.11	a
a ₂ (Garut)	16.33	c	4.51	a	14.04	A	9.00	a	48.00	a
a ₃ (Canada)	19.11	b	1.75	c	15.61	A	8.77	a	54.44	a
a ₄ (Tawangmangu)	12.33	d	1.53	c	11.97	b	8.77	a	51.11	a
a ₅ (STG 1)	13.44	d	2.55	b	11.97	b	10.11	a	54.88	a
a ₆ (SBG 4)	16.89	c	3.18	b	12.17	b	10.77	a	56.88	a
a ₇ (SBG 10)	9.67	d	1.37	c	8.67	c	11.11	a	50.88	a
a ₈ (SGB 2)	11.89	d	1.23	c	8.54	c	8.33	a	37.66	a
a ₉ (BR 5)	15.56	c	1.66	c	8.86	c	9.66	a	50.22	a
a ₁₀ (SGR 7.5)	20.89	b	2.70	b	15.22	a	10.00	a	48.22	a
a ₁₁ (TR 3.5)	27.11	a	2.61	b	10.28	c	13.33	a	56.66	a
Explant types										
e ₁ (Shoot Tip)	16.69	a	2.12	a	13.18	a	9.39	a	49.90	a
e ₂ (First Node)	17.30	a	2.63	a	11.48	a	9.27	a	46.03	a
e ₃ (Second Node)	15.12	a	2.08	a	11.85	a	10.72	a	54.90	a

Noted: The mean values followed by the same alphabet are not significantly different based on the Scott Knott Test at the level p<5%

The highest plant height of stevia were a₁, a₂, a₃, a₁₀ (Bogor, Garut, Canada, GR 7.5 respectively) (Table 3). The lowest plant of stevia were a₇, a₈, a₉, a₁₁ (SBG 10, SGB 2, BR 5, TR 3.5 respectively). The appearance of the height of stevia can be seen in Figure 3.

The shoot explant types treatment i.e e₁ (shoot tip), e₂ (first node), and e₃ (second

node) showed the non significant plant height. That proves that the endogenous hormones contained in the three explant types (shoot tips, first node, and second node) did not affect the character of stevia plant height.

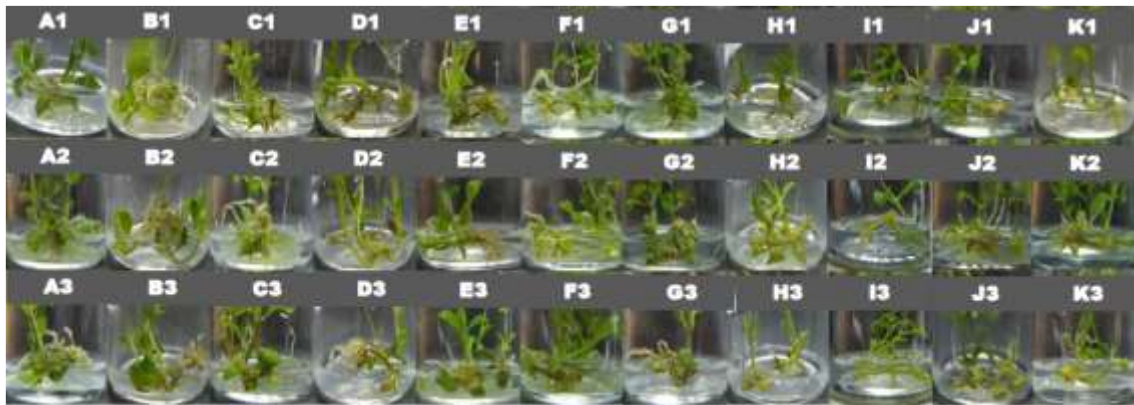


Figure 2. Stevia root at the rooting stage. A) a_{11} (TR 3.5); B) a_3 (Canada); C) a_{10} (GR 7.5); D) a_1 (Bogor); E) a_2 (Garut); F) a_6 (SBG 4); G) a_9 (BR 5); H) a_4 (Tawangmangu); I) a_5 (STG 1); J) a_7 (SBG 10); K) a_8 (SBG 2). In the code numbers 1, 2, and 3, respectively, the treatment of shoot tip explants, first node, and second node are shown. Culture bottle diameter= 5 cm.

All stevia accessions showed no significant difference for the number of shoots (Table 3). The explant types treatment namely e_1 (shoot tip), e_2 (first node), and e_3 (second node) also showed the same number of shoots. These results were different from the results obtained in the

shoot multiplication stage. It's because of the differences in the composition of the exogenous hormones added into the medium. In the multiplication stage, the added exogenous hormones were BA 1.5 ppm and IAA 0.15 ppm. Meanwhile, in the rooting stage, only 0.35 ppm IAA was added.

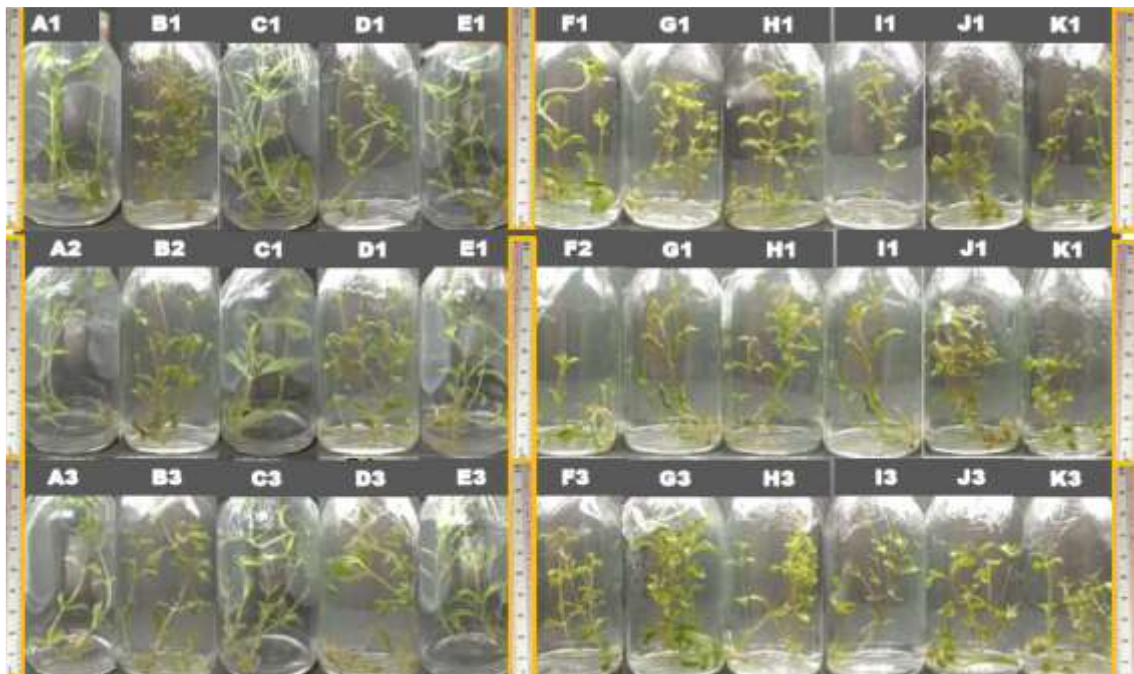


Figure 3. Stevia height at the rooting stage. A) a_1 (Bogor); B) a_2 (Garut); C) a_3 (Canada); D) a_{10} (GR 7.5); E) a_4 (Tawangmangu); F) a_5 (STG 1); G) a_6 (SBG 4); H) a_7 (SBG 10); I) a_8 (SGB 2); J) a_9 (BR 5); K) a_{11} (TR 3.5). In the code numbers 1, 2, and 3, respectively, the treatment of shoot tip explants, first node, and second node are shown. Ruler length = 10 cm

The addition of higher exogenous IAA caused a significant decrease in the shoot's number because auxin suppresses shoot organogenesis (Hu et al., 2017). All stevia accessions showed no significant different for the number of leaves (Table 3). The explant type treatment were e₁ (shoot tip), e₂ (first node), and e₃ (second node) also showed the same number of leaves. These results are different from the results

obtained in the shoot multiplication stage, there was a significant difference in the explant type treatment. According to Hu et al. (2017), the decrease in the number of shoots was caused by the addition of exogenous auxin (IAA) without exogenous cytokinins. The decreasing number of shoots is in line with the decreasing number of branching so that the number of leaves also decreases (Ermayanti et al., 2017).

Table 4. Interaction of Stevia accession treatment with explant type on characters root initiation time (day after culture) in shoot multiplication stage

Accessions	Shoot Explant Types		
	e ₁ (shoot Tip)	e ₂ (first node)	e ₃ (second node)
a ₁ (Bogor)	6.67 a B	6.33 a B	6.00 a A
a ₂ (Garut)	6.33 a B	5.67 a B	5.67 a A
a ₃ (Canada)	5.33 a B	6.67 a B	9.33 a A
a ₄ (Tawangmangu)	7.33 a B	9.33 a B	6.00 a A
a ₅ (STG 1)	5.67 a B	14.3 a A	9.67 a A
a ₆ (SBG 4)	4.33 b B	5.00 b B	10.00 a A
a ₇ (SBG 10)	13.67 a A	6.67 a B	7.33 a A
a ₈ (SGB 2)	11.67 a A	9.00 a B	9.33 a A
a ₉ (BR 5)	9.00 a B	6.67 a B	7.00 a A
a ₁₀ (SGR 7.5)	7.00 a B	5.67 a B	4.00 a A
a ₁₁ (TR 3.5)	8.67 a B	4.00 b B	5.67 b A

Note: The mean values followed by the same alphabet (capital in the vertical direction and lower case in the horizontal direction) were not significantly different based on the Scott Knott Test at the level p<5%.

The best combination of treatments for the root initiation time was presented in table 4. Based on Table 4, the best combination of treatments for the root initiation time were a₆e₁, a₆e₂, and a₁₁e₂. Then, the combination of treatments with

the longest root emergence age was treatment a₁e₃, a₂e₃, a₃e₃, a₄e₃, a₅e₂, a₅e₃, a₆e₃, a₇e₃, a₈e₃, a₉e₃, and a₁₀e₃. The longest root emergence occurred in the second node explants. Root initiation is determined by the concentration of exogenous cytokinins and

auxins and their interactions with endogenous cytokinins and auxins (Singh et al., 2017).

Conclusion

1. Types of explants that grew best at the shoot multiplication stage were shoot tip explants for the characters of a number of shoots (18.11 shoots), number of leaves (93.49 leaves), and wet weight (3.56 grams). At the rooting stage, the explant type treatment gave the same results for all observation characters.
2. At the shoot multiplication stage, GR 7.5 gave the best response for shoot height (19.95 cm). SBG 10 gave the best response for the number of shoots (21.87). SBG 10 gave the best response for the number of leaves (138.00 leaves). GR 7.5 gave the best response for leaf chlorophyll content ($0.63 \mu\text{g mL}^{-1}$). All accessions with shoot tip explants gave the best response for wet-weight characters.
3. At the rooting stage, Bogor gave the best response for plant height (16.57 cm). Shoot tip explant of SBG 4, first node explant of SBG, and first node explant of TR 3.5 were the best combinations for the character of root initiation time in a row at 4.33 DAC; 5.00 DAC; and 4.00 DAC. TR 3.5 gives the best response for the number of roots characters (27.11 roots). Garut gave the best response for root length (4.51 cm).

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