IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 14, Issue 6 Ser. II (June 2021), PP 01-07
www.iosrjournals.org

Growth Response of Five Cultivars Stevia (*Stevia rebaudiana* Bertoni) Using Commercial Fertilizers as Basic Media In Vitro

Eka Darma Gati, Suseno Amien, Noladhi Wicaksana
Department of Agronomy, Faculty of Agriculture, Padjadjaran University, Indonesia
Jln. Raya Jatinangor Km 21, Sumedang, West Java, Indonesia 45363

Abstract:
Background*: Conventional propagation of seedling stevia is limited by parent material. Propagation by seeds that are difficult due to low of germination rate. Propagation through tissue culture can be applied as an alternative for propagation stevia and can produce last of seedling and in short-time in suitable media. Generaly, culture media is quite expensive, so it is necessary to find an alternative types of culture media. This study aimed to evaluate the growth response of five stevia cultivars on culture media containing commercial fertilizers.*
Materials and Methods*: A Randomized Block Design (RBD) was used in this experiment with two factors and replicated three times. The first factor consisted of five culture media DKW, MS, AB Mix, Grandasil, and Growmore. The second factor consisted of Stevia cultivars i.e. Kanada, Bogor, Garut, Tawangmangu, and Pengalengan.*
Results and Conclusion*: The result showed that the best responses to the character number of shoots (28.9 shoots), shoot height (10.47 cm), and number of leaves (136.6 leaves) were obtained on DKW medium compared to MS, AB Mix, Grandasil D, and Growmore medium. The commercial fertilizer (AB Mix, Grandasil D, and Growmore) can be used as basic medium in vitro. All cultivar have same responses in number of shoots, shoot height, and number of leaves on all medium.*
Key Word*: Stevia, Cultivars, Basic Medium, Commercial Fertilizers, In Vitro.*

Date of Submission: 05-06-2021 Date of Acceptance: 19-06-2021

I. Introduction

Public health awareness continues to increase by consuming healthy food ingredients such as natural sweeteners derived from stevia. The International Association for Stevia Research (2004) has stated an increasing trend in the consumption of sweeteners made from stevia in various parts of Asia, followed by Europe and America. Reports and Data (2020) have stated that stevia's global market value will increase from $ 578,64 million in 2019 to $ 1.010,77 million in 2027, or an increase of 8%. Following Imarc Group (2021), the stevia market will increase from $ 590 million in 2020 to $ 900 million in 2025 in all stevia derivative products such as sweeteners and antioxidants.

In Indonesia, sweeteners need is very high compared to national production, so the Indonesian government imported from other countries. According to the Director-General of Agro-Industry of the Ministry of Industry Indonesia (Ramli, 2020), the national sugar production is around 2.1 million tons, much smaller than the national demand of about 5,8 tons, so it is necessary to import approximately 3.7 million tons. The Central Statistics Agency or BPS (2018) states that it predicted that the national sugar demand would continue to experience an increase in the deficit in the following years. Stevia can be an alternative substitute for sugar cane sweeteners to meet national needs. However, stevia's cultivation and development in Indonesia are still very limited, so it needs research and innovation on this plant.

Conventional propagation of stevia (cuttings and seeds) has several obstacles to undertaking on a large scale. According to Djajadi (2013), the limited number of seeds is one of the stevia cultivation obstacles because it is through propagation through cuttings. Meanwhile, seed propagation is complicated because the seeds produced are difficult to germinate, making it difficult to implement on an industrial scale (Mishra et al., 2010). Tissue culture technology can be an alternative to produce large numbers of seeds in a relatively short time, disease-free, uniform, and identical to its parents (Tamura et al., 1984; Singh, 2015). One stevia tissue culture method that can be developed is the multiplication of stevia shoots and roots (Huda et al., 2007). The growth and development of stevia greatly influence several factors, namely plant growth regulators (PGR), the media, and the explants used. IAA and BA respectively are a type of auxin and cytokinin that can induce shoot and stevia's root growth. A study conducted by Aziz and Al-Taweel (2019) reported that IAA at low concentrations

could cause more than 80% roots. A study conducted by the Pusat Penelitian dan Pengembangan Perkebunan (2015) said that BA could trigger stevia shoots.

Another factor that influences the development of stevia culture is the culture media. The media contains several essential components such as macronutrients, micronutrients, vitamins, PGR, carbon sources, organic substances, and compacting substances that significantly influence the success of culture (Priyadarshan, 2019). Some culture media that are often used in tissue culture research include (Murashige and Skoog, 1962), Woody Plant (WPM) (Lloyd and McCown, 1980), Driver Kuniyuki Walnut (DKW) (Driver and Kuniyuki, 1984), and several other media. Hafiizh and Ermayanti (2019) have stated that DKW media can provide a very good response compared to MS, Chu (N6) media (Chu et al., 1975), and WPM because it contains certain higher macro and micronutrients and is equipped with higher vitamins. However, culture media is generally quite expensive, so it is necessary to find economical alternative types of culture media.

One of the nutrient sources of culture media can come from commercial inorganic fertilizers. Several types of commercial inorganic fertilizers that are often used are Growmore, Grandasil D, and AB Mix. These commercial fertilizers have been widely researched and can respond to cultures including potatoes (Nuraini et al., 2014), bananas (Prabowo et al., 2018), Dendrobium and Cattleya orchids (Hardianti and Lita, 2019), and various other plants. However, few attempts to see the response to the growth and development of explants on alternative culture media, so research is needed research on this subject. This study aims to determine the stevia culture's response to various stevia cultivars and culture media.

II. Material And Methods

This experiment was carried out at the Tissue Culture Technology Laboratory, Faculty of Agriculture, Padjadjaran University, Indonesia which was held in August - December 2020.

Study Design: The experiment used an experimental method with a factorial randomized block design (RBD) consisting of two factors.

Study Location: Tissue Culture Technology Laboratory, Faculty of Agriculture, Padjadjaran University, Indonesia (Jalan Raya Jatinangor, Jatinangor District, Sumedang Regency, West Java Province, Indonesia)

Study Duration: August - December 2020.

Sample size: 75 samples.

Sample size calculation: This experiment consisted of a treatment combination of 5 media and 5 cultivars of stevia with 3 repetitions so that 75 samples were observed.

Subjects & selection method: The first treatment was 5 culture media consisting of DKW, MS, AB Mix, Growmore (32-10-10), and Grandasil D (20-15-15). The second treatment was 5 cultivars of stevia consisting of Kanada, Bogor, Garut, Tawangmangu, and Pengalengan.

Procedure methodology

This research was conducted in several stages, namely:

1. Preparation of tools and materials. The tool to be used is sterilized using an autoclave at a temperature of 121 ^{o}C with a pressure of 1 atm for 20 minutes. The materials to be used are prepared by making stock solutions to make it easier for smaller dosing on DKW, MS, and AB Mix media.
2. Making culture media. The available stock media (DKW and MS) were dissolved with distilled water with 30 g / l added sugar and an additional 1.5 ppm BA and 0.15 ppm IAA. While AB Mix media was formulated by taking 5 ml each from stock A and B as well as additional vitamin DKW, 30 g / l sugar, and PGR substance (BA 1.5 ppm and IAA 0.15 ppm). Growmore and Grandasil D media were formulated by taking 1 g / l plus vitamin DKW, sugar 30 g / l, and PGR substance (BA 1.5 ppm and IAA 0.15 ppm). All media are conditioned at pH 5.7-5.8. The finished culture media is poured into a jam bottle as much as 40 ml/bottle. The cutlery media was sterilized using an autoclave at a temperature of 121 oC at a pressure of 1 atm for 20 minutes.
3. Explant planting. Sterile culture medium was planted with explants of stevia shoots with 1 cm explant length in the LAF. The bottles planted with explants are closed using plastic warping and secured with rubber.

Observation characters include:

1. The number of shoots per bottle was measured every week up to 8 weeks after planting.
2. Shoot height (cm) was measured after 8 weeks.
3. The number of leaves per bottle is counted after 8 weeks.

Statistical analysis

Data were analyzed using JMP Pro SAS version 14 (SAS Institute Inc.). The data that has been collected will be analyzed through analysis of variance (ANOVA) with a confidence level of 95% ($\alpha = 5\%$).

III. Result

Number of Shoots

The number of shoots is one of the main characters to determine the response to growth and stevia multiplication development. Based on the results of the analysis of variance (ANOVA) in Table 1, it shows that the treatment of culture media with very significant criteria (α = 1%) and does not differ significantly in stevia treatment cultivars, and there is no interaction between media and stevia cultivars.

Table no 1: ANOVA of shoot number at 8 weeks after planting.

Source	DF	Mean Square	*F*	*P value*
Media (M)	4	1949.347	20.304	0.0001**
Stevia cultivars (K)	4	169.513	1.766	0.151^{ns}
M*K	16	96.097	1.001	0.472^{ns}
Replication (R)	2	8.44	0.088	0.916^{ns}
Error	48	96.009		
Total	75			

Note: *: Significantly different at the level of α = 5%
**: Significantly different at the level of α = 1%
ns: Not significantly different at the level of α = 5%

The Tukey HSD test in Table 2 shows that the best response to the number of shoots on the eight weeks after planting the DKW culture medium (28.93 shoots) was significantly different in all other media treatments. DKW media is thought to be very suitable for stevia shoot multiplication because it can produce quite many shoots compared to other media. Figure 1 shows that the highest number of shoots at 1 to 8 weeks was found on DKW media. Ajijah reported that DKW media is recommended for primary callus and somatic embryo formation in cocoa because it contains high levels of various nutrients[21]. A study conducted by Hafiizh and Ermayanti[16] that DKW medium gave a better response to the number of shoots due to the higher content of nickel (Ni) and nutrient content of Zn and Cu so it is thought to be a factor that triggers more shoot formation than WPM, MS, and Chu (N6) media..

Table no 2: The average number of shoots in the treatment of media types and stevia cultivars after 8 weeks after planting.

Media	No. of Shoot
MS	13.67b
DKW	**28.93a**
AB Mix	7.87bc
Growmore	1.60c
Grandasil D	1.33c
Stevia Cultivars	
Kanada	8.60a
Bogor	12.00a
Tawangmangu	10.20a
Garut	6.933a
Pengalengan	15.67a

Note: The numbers followed by different letters on the same line are significantly different in the Tukey HSD test at the level of α = 5%.

Figure no 1: The average increase in the number of shoots based on the culture media treatment

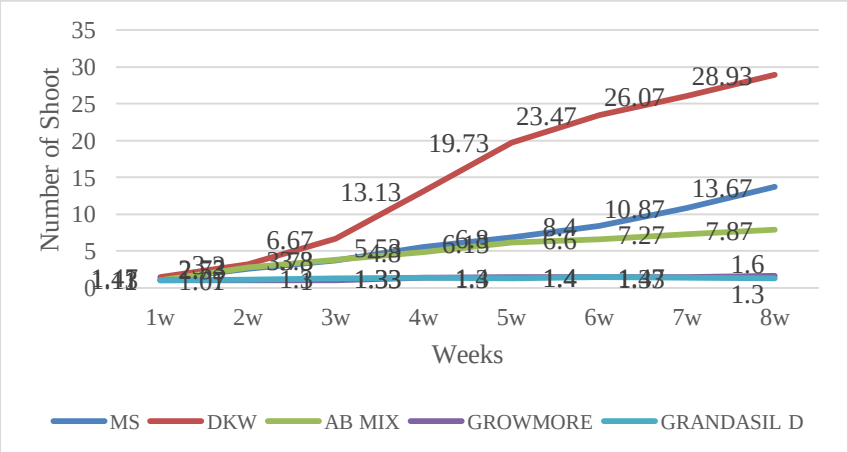
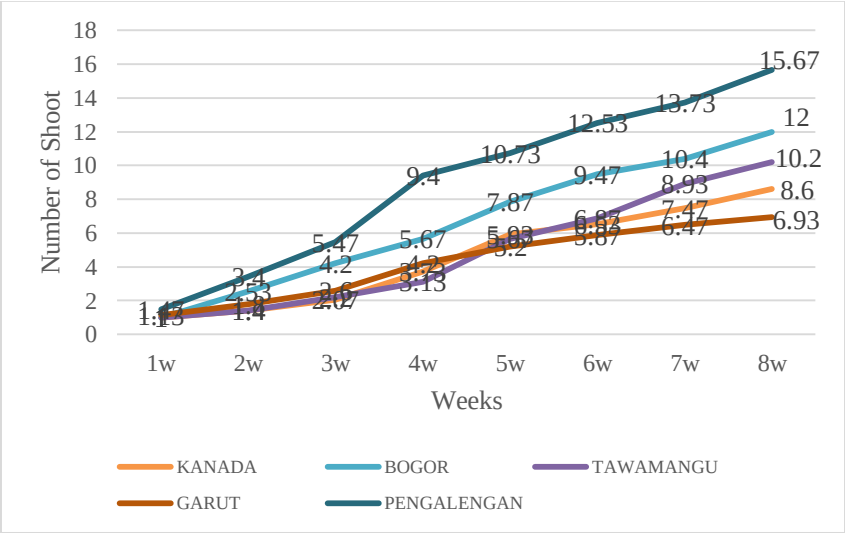


Figure no 2: The Average Increase in the Number of Shoots Based on the Treatment of Stevia Cultivars



The content of PGR in the media affects triggering the growth of explant shoots. BA that poured into the media can give a positive response by increasing the number of culture shoots. According to Sairkar *et al.*, the media added with BA in a specific concentration could trigger the growth of shoots of stevia culture in MS media[22]. Other media such as Grandasi D and Growmore have a low response because the concentration of fertilizers used is so tiny that they cannot meet the needs of culture explants. A study conducted by Hasanah *et al.*, reported that the use of a concentration of 1 g/l in Growmore and Grandasil D media in dendrobium orchid plantlets was considered less than optimal, and a concentration of 2 g/l gave a more significant response to plantlet development because it was related to nutritional adequacy[23].

The best response to the number of shoots in the stevia cultivar treatment in Table 2 was found in Pengalengan cultivars that were not significantly different in all cultivar treatments. The treatment of stevia cultivars showed that Pengalengan had more shoots than other cultivars. Pengalengan cultivars showed the best increase in the number of shoots at two weeks to 8 weeks (Figure 2). Based on the results of research conducted by Amien *et al.*, that the growth response between the stevia cultivars tested (Bogor, Garut, and Tawangmangu) showed different responses to the same treatment in media[24]. The difference in response to the number of shoots is thought to be caused by existing genetic backgrounds. Genetic differences can cause varied responses even within the same plant species. Tiwari *et al.*, stated that there were significant differences in response to the ten soybean cultivars on the tested culture media[25].

Shoot height of explants

Table no 3: ANOVA of shoot height at 8 weeks after planting.

Source	DF	Mean Square	*F*	*P value*
Media (M)	4	163.377	12.819	0.0001**
Stevia cultivars (K)	4	22.242	1.745	0,156^{ns}
M*K	16	18.225	1.430	0.168^{ns}
Replication (R)	2	4.06	0.319	0.729^{ns}
Error (E)	48	12.745		
Total	75			

Note: *: Significantly different at the level of α = 5%
**: Significantly different at the level of α = 1%
ns: Not significantly different at the level of α = 5%

Shoot height is one of the important characteristics related to the growth and development of stevia culture. Based on results of the analysis of variance on Table 3, it shows that the treatment of culture media with very significant criteria (α = 1%) and does not differ significantly in stevia treatment cultivars, and there is no interaction between media and stevia cultivars.

Table 4 shows that the DKW media gave the best response to shoot height (10.47 cm) and was significantly different from other treatments. DKW media gave the best response due to the higher nutrient content, including phosphorus (P). The P content in DKW media was higher than other media[26]. The nutrient P plays a vital role in the preparation of cell protoplasm that plays a role in forming energy (adenosine

triphosphate / ATP) in the process of photosynthesis [27]. The nutrient P has an enzyme activator role that plays a role in cell and tissue metabolism[28].

Table no 4: The mean shoot height of explants (cm) in the treatment of media and cultivars at 8 weeks after planting.

Media	Height (cm)
MS	6.67b
DKW	**10.47a**
AB Mix	4.03bc
Growmore	2.58c
Grandasil D	2.85c
Stevia Cultivars	
Kanada	3.36a
Bogor	5.85a
Tawangmangu	6.15a
Garut	4.94a
Pengalengan	6.31a

Note: The numbers followed by different letters on the same line are significantly different in the Tukey HSD test at the level of α = 5%.

Number of Leaves

Table no 5: ANOVA number of leaves at 8 weeks after planting.

Source	DF	Mean Square	*F*	*P value*
Media (M)	4	44629.433	15.797	0.0001**
Stevia cultivars (K)	4	3280.733	1.161	0.340^{ns}
M*K	16	1890.167	0.669	0.809^{ns}
Replication (R)	2	1368.120	0.484	0.619^{ns}
Error (E)	48	2825.176		
Total	75			

Note: *: Significantly different at the level of α = 5%
**: Significantly different at the level of α = 1%
ns: Not significantly different at the level of α = 5%

Leaves are important organs that play a role in the photosynthesis process to produce energy (Shipunov, 2020). Leaves play a role in the process of gas exchange and absorption of nutrients through the respiration process. Based on results of the analysis of variance on Table 5. it shows that the treatment of culture media with very significant criteria (α = 1%) and does not differ significantly in stevia treatment cultivars, and there is no interaction between media and stevia cultivars.

The Tukey HSD test in Table 6 shows that the treatment of DKW media gave the highest leaf number response (136.60), which was significantly different from other media. Grandasil D media (6.40 leaves) showed the lowest leaf number response and was not significantly different in AB Mix (43.40) and Growmore (7.47) media. The treatment of stevia cultivars showed that the Pengalengan cultivar had the highest number of leaves (75.60), which was not significantly different from other cultivars.

Table no 6: The mean number of leaves in the treatment of media types and stevia cultivars at 8 weeks after planting.

Media	No. Leaves
MS	77.13b
DKW	**136.60a**
AB Mix	43.40bc
Growmore	7.47c
Grandasil D	6.40c
Stevia Cultivars	
Kanada	44.60a
Bogor	47.07a
Tawangmangu	63.33a
Garut	40.40a
Pengalengan	75.60a

Note: The numbers followed by different letters on the same line are significantly different in the Tukey HSD test at the level of α = 5%.

DKW media has the highest number of leaves than other media, indicating that DKW media contains essential nutrients sufficient for the growth and development of culture explants, including leaf formation. The content of N and K in the media plays a role in forming new tissues, respiration, photosynthesis, transpiration, and other metabolic processes, so that explant growth is more optimal[30]. Besides, leaf formation is also influenced by genetic factors. According to Graham, the development of culture explants micropropagation in the micropropagation process is controlled by many factors, including genetic variation, presence of mutated

tissue, types and sources of explants, types of culture media, types and concentrations of PGR substance, microclimate, and duration of culture[31].

IV. Conclusion

The best responses to the character number of shoots (28.9 shoots), shoot height (10.47 cm), and number of leaves (136.6 leaves) were obtained on DKW medium compared to MS, AB Mix, Grandasil D, and Growmore medium. The commercial fertilizer (AB Mix, Grandasil D, and Growmore) can be used as basic medium in vitro. All cultivar have same responses in number of shoots, shoot height, and number of leaves on all medium.

References

[1]. International Association for Stevia Research. (2004). World Market of Sugar and Sweetener. https://www.uni-hohenheim.de/fileadmin/einrichtungen/stevia/downloads/World_Market_Sugar.

[2]. Report and Data. (2020). Stevia Market by Form (Powdered, liquid, and others) by Types (Reb A, Reb M, Reb D, and Others), by End Users, and by distribution Channel (online and offline), and Segment Forecasts, 2017 – 2027. [https://www.reportsanddata.com/report-detail/stevia-market].

[3]. Imarc Group. (2021). Stevia Market: Global Industry Trends, Share, Size, Growth, Opportunity, and Forecast 2021-2026. [https://www.imarcgroup.com/stevia-market]

[4]. Kompas. (2020). Impor Gula Masih Tinggi, Kemenperin Dorong Pembangunan Pabrik Gula Baru. kompas.com

[5]. Badan Pusat Statistik. (2018). *Distribusi Perdagangan Komoditas Gula Pasir Indonesia Tahun 2018*. https://www.bps.go.id/publication/2019/02/26/7385aa35981037653e744dd5/distribusi-perdagangan-komoditas-gula-pasir-di-indonesia-2018.html

[6]. Djajadi. (2014). Pengembangan Tanaman Pemanis Stevia rebaudiana (Bertoni) di Indonesia. *Perspektif* 13(1): 25-33.

[7]. Mishra, P., Singh, R., Kumar, U., Pakash, V. (2010). Stevia rebaudiana – A magical Sweetener. *Global Journal of Biotechnology & Biochemistry* 5:62-74.

[8]. 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.

[9]. Ahmed, A., Huda, M. N., Mandal, C., Alam, K. A., Reza, M. S. H., & Wadud, A. (2007). In Vitro Morphogenic Response of Different Explant of Stevia (Stevia rebaudiana). *Int. J. Agric. Res*. 2: 1006-1013.

[10]. Aziz, R. A., dan S. K. Al-Taweel. (2019). Effect of Plant Growth Retandants on Stevia (Stevia rebaudiana Bertoni) Acclimatization Produced In Vitro. *Plant Archives* 19(1): 1275-1284.

[11]. Pusat Penelitian dan Pengembangan Perkebunan. (2015). Perbanyakan In Vitro Stevia (Stevia rebaudiana Bertoni). *Info Tek Perkebunan* 7(5):19.

[12]. Priyadarshan, P. M. 2019. *Plant Breeding: Classical to Modern*. Kerala: Springer Nature Singapore Pte Ltd. DOI 10.1007/978-981-13-7095-3.

[13]. Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Planta*. 15: 473-497.

[14]. Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia by use shoot-tip culture. *Int. Plant Prop. Soc. Proc*. 30:421-427.

[15]. Driver, J., & Kuniyuki, A. (1984). In vitro propagation of Paradox walnut rootstock. *HortScience* 19:507-509.

[16]. Hafiizh, E. A., & Ermayanti, T. M. (2019). In vitro Multiplication of Stevia rebaudiana Bertoni Tetraploid on several Basal Media Containing BAP. Prosiding Seminar Nasional Agroteknologi 2019 Jurusan Agroteknologi UIN SDG Bandung 2 Maret 2019: 63-75.

[17]. Chu, C.C., Wang, C.C., Sun, C.S., Hsu, K.C., Yin, K.C., Chu, C.Y., & Bi, F.Y. (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Scient. Sin*., 18: 659-668.

[18]. Nuraini, A., Rizky, W. H., & Susanti, D. (2014). Pemanfaatan Pupuk Daun sebagai Media Alternatif dan Bahan Pemanfaatan Pupuk Daun sebagai Media Alternatif dan Bahan Orgenik pada Kultur In Vitro Kentang (Solanum tuberosum L.) Kultivar Granola Orgenik pada Kultur in vitro Kentang (Solanum tuberosum L.) Kultivar Granola. Prosiding Seminar Nasional-Pengembangan Teknologi Pertanian-Politeknik Negeri Lampung: 189-196.

[19]. Prabowo, H., Samanhudi, Yuniastuti, E., & Yunus, A. (2018). Effects of media combination with concentration of AB-Mix nutrient on growth of banana shoots on in vitro. *Bulg. J. Agric. Sci*., 24 (3): 404–410.

[20]. Hardianti, O., & Soetopo, L. (2019). Pengaruh Konsentrasi Pupuk daun pada Media Anggrek *Dendrobium* dan *Cattleya* secara In Vitro. *Jurnal Produksi Tanaman* 7(5):881-888.

[21]. Ajijah, N. (2016). Pengaruh Komposisi Media Dasar dan Jenis Eksplan terhadap Pembentukan Embrio Somatik Kakao. *J.TIDP* 3(3): 127-134.

[22]. Sairkar, P., Chandravanshi, M. K., Shukla, N. P., Mehrotra, N. N. (2009). Mass Production of an Economically Important Medicinal Plant Stevia rebaudiana Using In Vitro Propagation Techniques. *Journal of Medical Plants Research* 3(4): 266-270.

[23]. Hasanah, U., Suwarni, E., & Sumadi. (2014). Pemanfaatan Pupuk Daun, Air Kelapa, dan Bubur Pisang Sebagai Komponen Medium Pertumbuhan Planlet Anggrek Dendrobium. *Biosaintifika* 6 (2): 161-168.

[24]. Amien, S., Aji, D.N., Mamluatul, T. 2020. Multiplikasi Cepat Tunas Tiga Aksesi Stevia Secara In Vitro. *Jurnal Kultivasi* 19(3): 1247-1253.

[25]. Tiwari, S., Shanker, P., & Tripathi, M. (2004). Effect of Genotype and Culture Medium on In Vitro Androgenesis in Soybean (Glycine max Merr.). Indian Journal of Biotechnology 3: 441-444.

[26]. Bell, R. L., Srinivasan, C., & Lomberk, D. (2009). Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cellular and Developmental Biology. *Plant* 45(6). 708–714. http://doi.org/10.1007/s11627-009-9196-8.

[27]. Jumin, H. B. (1992). *Ekologi Tanaman*. Jakarta: Rajawali.

[28]. Hanafiah, K. A. (2005). *Dasar-dasar Ilmu Tanah*. Jakarta: PT. Raja Grafindo Persada.

[29]. Shipunov, A. (2020). *Introduction to Botany*. North Dakota: Minot State University. 28-31. http://ashipunov.info/shipunov/school/biol_154/

[30]. Ruhnayat, A. (2007). Penentuan Kebutuhan unsur hara N P K untuk pertumbuhan Tanaman Panili (Vanilla planifolia Andrews). *Buletin Litro*. 18: 49-59.
[31]. Graham, J. (2005). *Fragaria Strawberry. In : Biotechnology of Fruit and Nut Crops. Biotechnology in Agriculture Series No. 29*. CAB International: Wallingford, UK: 456-474.

Eka Darma Gati, et. al. "Growth Response of Five Cultivars Stevia (Stevia rebaudiana Bertoni) Using Commercial Fertilizers as Basic Media In Vitro." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 14(6), 2021, pp. 01-07.