Optimization of Sterilization Techniques and Effects of Coconut Water for the Induction of Shoots of Stevia (Stevia rebaudiana Bertoni)

by Nanang Dwi Wahyono

Submission date: 10-Nov-2021 08:07AM (UTC+0700)
Submission ID: 1698286967
File name: r_the_Induction_of_Shoots_of_Stevia_SteviarebaudianaBertoni.pdf (5.88M)
Word count: 3749
Character count: 19181

ISBN : 978-623-96220-1-5

Optimization of Sterilization Techniques and Effects of Coconut Water for the Induction of Shoots of Stevia (*Stevia rebaudiana* Bertoni)

N D Wahyono[1] and N Hasanah[2] N Nurprahastani[3]

[1]Agribussines Management Politeknik Negeri Jember

[2]Department of Animal Husbandry Agriculture, Politeknik Negeri Jember

[3]Cultivation of Plantation Crops

*Corresponding author: nanang_d_wahyono@polije.ac.id

Abstract. The advantage of stevia is that it doesn't bring teeth, has a low-calorie content and is suitable for diabetics, and is safe to use. In this study, the explants used were derived from the stem segment of the stevia plant. The results showed that shoots had a contamination percentage of 30-40% and the treatment experienced 10% browning in the T1M1, T2M3 and T3M1 treatments. and the percentage of shoot growth produced was the same from all treatments, namely 60%. While the fastest shoot emergence time at 8 days after planting (HST) was found in the sterilization technique treatment using 70% alcohol for 1 minute and the use of 0 ml / L coconut water (T1M1). The treatment that had the highest number of shoots was the sterilization technique using 70% alcohol for 1 minute and using coconut water 0 ml / L coconut water (T1M1) as many as 2.9 shoots.

1. Introduction

Plant is a source of natural sweetener which has a sweetness level of 200-300 times sweeter than cane sugar. Stevia can provide a solution for consumers who cannot or should not consume sugar / cane sugar, for example diabetics, because of course stevia sugar is safer than synthetic or artificial sweeteners. The advantages of stevia are that it does not cause pockets on teeth, has low calorie content and is suitable for diabetics, and is safe for use. Stevia plants can be propagated sexually and vegetatively, but the most proven one is vegetative propagation. Generative propagation by seed is difficult because of its very low germination capacity. Vegetative propagation of stevia plants can be done using tillers, stem cuttings, and tissue culture. This study aims to determine the use of sterilization techniques and proper coconut air concentration in the stevia induction process.

2. Methods

ResearchThe research was carried out at the Jember State Polytechnic Tissue Culture Laboratory in January 2020 - March 2020. This study used a completely randomized design (CRD) consisting of 2 factors, namely differences in sterilization techniques, namely T1 = 70% alcohol for 1 minute, T2 = NaOCl 5% + 2 drops of tween 20 for 1 minute, T3 = NaOCl 5% + 2 drops of tween 20 for 1 minute +

ISBN : 978-623-96220-1-5

$HgCl_2$ 0.01% + 2 drops of tween 20 for 30 seconds and using the concentration of coconut air, namely: M1 = 0 ml / L, M2 = 50ml / L, M3 = 100 ml / L, M4 = 150 ml / L.

3. Research and Materials

Toolstools used are erlenmeyer, dissecting set, measuring cup, pH-meter, pipette, autoclave, laminar water flow, petri dishes, culture bottles, culture racks, bunsen, plastic pipettes, analytical scales, stoves, pans, magnetic stirrers, stirrers, beakers, hot plates, rulers, cameras, stationery.

The materials used are stevia plant seeds, sterile distilled water, 70% alcohol, tween 20, 5% NaOCl, $HgCl_2$ 0.01%, bactericides and fungicides, agar - agar, sugar, aluminum foil, stock solution AH, NaOH, HCl, water. coconut.

Research Design

study used a completely randomized design (CRD) which consisted of two factors. The first factor is the difference in sterilization techniques with 3 types of techniques, namely:

T1 = 70% alcohol for 1 minute
T2 = 5% NaOCl + 2 drops of tween 20 for 1 minute
T3 = 5% NaOCl + 2 drops of tween 20 for 1 minute + $HgCl_2$ 0.01% + 2 drops of Tween 20 for 30 seconds

while the second factor is the use of coconut water with 4 kinds of treatments, namely:

M1 = MS +2 ppm BAP + 0 ml / L coconut water
M2 = MS +2 ppm BAP + 50 ml / L coconut water
M3 = MS +2 ppm BAP + 100 ml / L coconut water
M4 = MS +2 ppm BAP + 150 ml / L coconut water The

data obtained were analyzed using analysis of variance (ANOVA), there was a significant difference between the DMRT further test (Duncan's Multiple Distance Test) with a level of 5%.

4. Results and Discussion

4.1. Contamination

In in vitro culture, the initiation phase is the first phase which aims to obtain explants that are free of microorganisms and produce the initiation of new growth. Initiation is the stage of taking explants from the parent plant which will be propagated by tissue culture. It is at the initiation stage that the contamination problem is the main limiting factor that is often encountered. Things that must be considered in the sterilization process include the type of sterilization material, the concentration of the sterilization material, and the time of immersion (Kumar, 2001).

The following is a graph of the proportion of stevia explant contamination produced in each treatment.

ISBN : 978-623-96220-1-5

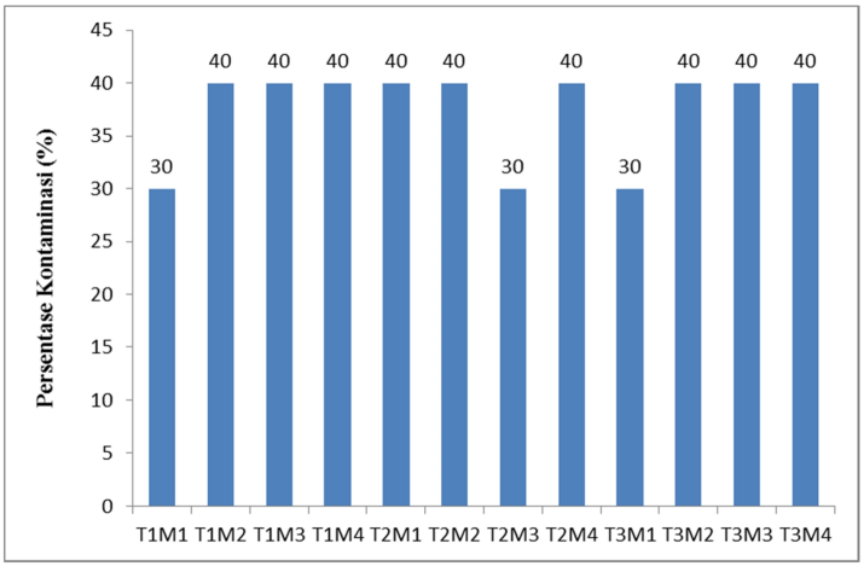

Figure 1 Contamination Percentage

Graph Graph 4.1 shows that the proportion of contamination on stevia explants ranges from 30-40%. Contamination in this stevia explant is a fairly large contamination because the percentage figure is almost half of the 100% figure. The following explants are contaminated.

Figure 2. Bacterial contamination, around the explants and the media there is mucus

Figure 3. Fungal contamination, around the explants and the media there is a white hyphae.

The observation in the image above is one of the explants that has been contaminated by fungi and bacteria. In bacterial contamination, symptoms such as around explants and the media contain mucus, while in fungal contamination the symptoms are such as white hyphae. This statement is supported by Shonhaji (2014), namely the contamination that often occurs in plant tissue cultures containing two types of contamination by bacteria and contamination by fungi. To distinguish the type of fungal or bacterial contamination, it can be seen from the physical characteristics that appear on explants and culture media. When exposed to bacterial contamination, mucus appears, this is because the bacteria directly attack the tissues of the plant body itself. Meanwhile, if contaminated by fungi or fungi, a fungal hyphae will appear on the affected plants and can usually be characterized by the presence of white to gray lines (like threads).

ISBN : 978-623-96220-1-5

The contamination of the stevia explants was suspected due to sterile conditions at the time of planting. This is also supported by Kristina, DKK (2017) stated that the tissue culture process requires sterile conditions. If the conditions are contaminated, the culture will die or be damaged. The components most susceptible to contamination by microorganisms are growing media and explants.

Contamination is also caused during planting, exploration is not paying attention to the acceptability of both hands, the stage tools at the time of sterilization which cause contamination to spread quickly and rapidly in breeding and contaminated explants will not grow properly and will experience explant death because the nutrients and substrate in the media have been used. taken for breeding. This is supported by the statements of Doods and Roberts (1983) and Gunawan (1988) that growth media and explants can be contaminated by microorganisms because both can function as good subsrates for the growth of microorganisms including bacteria and fungi.

4.2. Browning

The occurrence of browning is a physiological setback of an explant which is often found in in vitro culture which ultimately inhibits the development of an explant.

In this study, the treatment that experienced browning occurred in only 3 treatments, namely the T1M1, T2M3 and T3M1 treatments by 10%. While the other treatments did not experience browning.

Browning on the explants is thought to be caused because the explants were injured during planting so that the explants turned brown. This is supported by the statement of Sri Hutami (2008), namely the explant tissue culture that turns brown (browning) or black (blackening) at any time after isolation which in turn can inhibit growth and eventually cause tissue death.

Browning stevia explants showed brown explants and did not grow. This is supported by Mariska's (2003) statement that browning is a character that can inhibit the growth and development of explants that change the color of the explants to black / brown. This occurs because of changes caused by physical or biochemical effects (injury or disease).

4.3. Percentage of shoot growth. Shoot

formation in in vitro culture is often a parameter in a culture. In this study, all explants could respond to each treatment.

The following is a graph of the proportions of the stevia explant plants produced in each treatment.

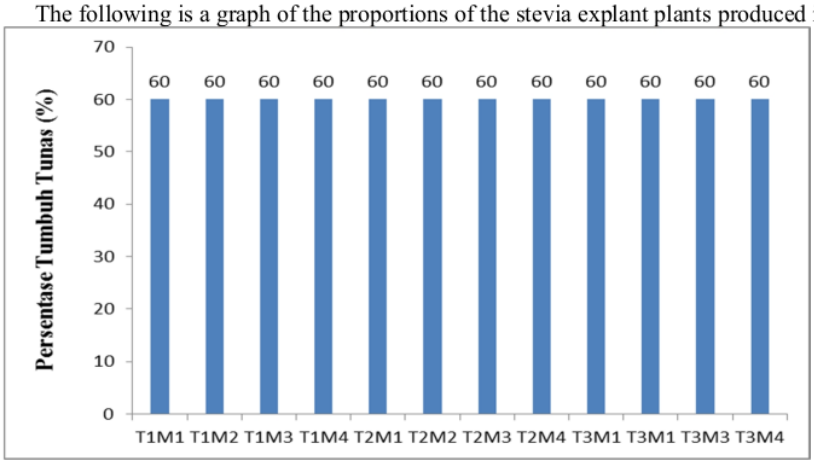

Figure 4. Shoot Growth Percentage Graph The

Graph shows that, the proportion of shoot growth on stevia explants is the same, namely 60%. The growth of this stevia shoot has a proportion that reaches 100%. This is thought to be using sterilization techniques and growth regulators to provide an optimal growth response so that the proportion of shoot

ISBN : 978-623-96220-1-5

growth is uniform. Growth regulators given through stevia explants, namely cytokinins in the form of coconut water. According to Pisesha (2008), the statement that the cytokinins contained in coconut water from small missiles can support plant growth.

4.4. The emergence of shoots The

formation of shoots on the explants is an initial phase of growth of the explants. Shoots are part of a newly grown plant (Raharja and Wiryantha, 2003).

The results of variance for parameters when searching for shoots on stevia explants can be seen in table 4.1 below.

Table 1. Sidik Analysis of Optimization Variety of Sterilization Techniques and Effects of Coconut Water for the Induction of Stevia Shoots on the emergence of shoots

Source of diversity	Db	JK	KT	Fhit	Notation	F Table	
						5%	1%
Deuteronomy	4	192.85	48.21	0.48	ns	2.58	3.78
Treatment	11	1683.41	153.04	1.52	ns	2.01	2.68
T	2	915.60	457.80	4.56	*	3.21	5.12
M	3	272.55	90.85	0.90	ns	2, 82	4, 26
T x M	6	495.27	82.54	0.82	ns	2.31	3.24
Gallat	44	4422.15	100.50				
Total	59	98.41Significantly					

Note: (*)different, (**) Different very real, (ns) Different not

Above table 1, it can be denied that the treatment of sterilization technique (T) has an effect on when it materializes, the interaction between the sterilization technique (T) and coconut water (real M) does not have a significant effect on when the shoot is manifested, and the treatment coconut water (M) has no significant effect on the alarm time.

Following are the results of the 5% DMRT test for the sterilization technique (T) treatment is presented in table 2 below.

Table 2. Results of the Advanced Test of the Effect of the T Factor on the Appearance of the Stevia Explant Shoots Using the DMRT 5% Advanced Test

Treatment	Average(HST)
T1	14.4 b
T2	22.2
T3	23.1 a

Description: The numbers followed by the same letter indicate not significantly different in the DMRT level of 5%.

The results of the 5% DMRT further test on the sterilization technique (T) treatment showed the fastest shoot emergence speed on the stevia explants with the sterilization technique (T1) treatment with a mean of 14.4 days after planting (HST). While the slowest shoot emergence rate was found in treatment (T3) with a mean of 23.1 days after planting (DAT). In the sterilization technique (T2 and (T3), the sterilization technique used was 5% NaOCl + 2 drops of Tween 20 for 1 minute and 5% NaOCl + 2 drops of Tween 20 for 1 minute +$HgCl_2$ 0.01%+ 2 drops tween 20 for 30 seconds. Whereas in the sterilization technique (T1), the sterilization technique used was only 70% alcohol for 1 minute. The difference in the speed of shoot formation in the stevia explants at the induction of each treatment was probably due to the different sterilization techniques used. The following is a graph of the mean when determining shoots for each treatment.

ISBN : 978-623-96220-1-5

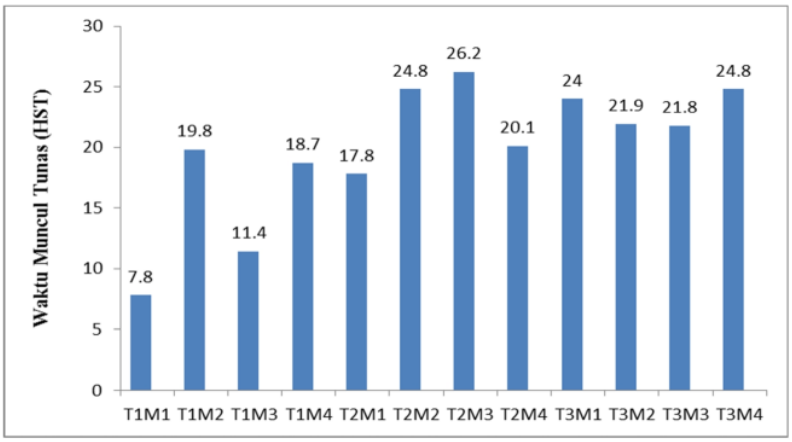

Figure 5. Graph of MeanAppearance Time in DAS

Sprout Data on the graph, show that the actual stevia explants were first counted on the 8th day after planting and explants appeared shoots ranged from 8 to 26 days after planting (DAT).

The combination of sterilization technique using 70% alcohol for 1 minute with a coconut air concentration of 0 ml / L (T1M1) has the lowest mean, this shows that in this treatment explants appear shoots faster than the others, which is about 8 days after planting (DAS), while other treatments were around 11-26 days after planting (DAT). This means that the longer the explants grow, the current level of the shoots is slow, conversely, the faster the explants grow, the better the current rate of sprouting. So that the explants that have an average day to grow shoots smaller (earlier) have a fast response in absorbing the nutrients provided in the medium.

In tissue culture, coconut water contains cytokinin ZPT. Cytokinins themselves are ZPT which are important in regulating cell division and morphogenesis. Some kinds of cytokinins are natural cytokinins (for example kinetin and zeatin) some are synthetic cytokinins. Natural cytokinins are produced in actively growing tissues, especially in roots, embryos and fruits. The cytokinins produced in the roots are then transported by xylem to target cells in the stem (Gunawan, 2008). Coconut water is an organic compound that is often used in tissue culture engineering applications. This is because coconut water is natural water that contains high levels of K and Cl. In addition, coconut water contains sucrose, fructose and glucose (Netty, 2002).

4.5. Number

Number of explant shoots, the number of shoots counted when explant shoots are 1-1.5 cm in size. The number of shoots was counted from the shoots that emerged from the axillary buds or side shoots on each explant. Shoot growth on each explant is varied. The difference in shoot growth may be due to nutrient uptake for different explants for regeneration such as shoot growth and development. Another possibility is the adaptation of explant growth and development in a small environment such as a culture bottle.

ISBN : 978-623-96220-1-5

The results of variance for the number of shoots parameters can be seen in table 4.3 below.

Table 3. Variety Fingerprint Analysis and Optimization Techniques Sterilization Effect of Coconut Water For Plants Induction of Stevia to Total Tunas

source of diversity	Db	JK	KT	Fhit	Notation	Ftabel	
						5%	1%
Deuteronomy	4	96 407	24 102	86 192	**	2,584	3,778
Treatment	11	10 805	0.982	3,513	**	2.014	2.680
T	2	4790	2395	8565	**	3209	5123
M	3	0061	0020	0072	ns	2816	4261
T x M	6	5955	0992	3549	**	2313	3243
Gallat	44	12.304	0.280				
Total	59	119,516					

Description: (*) DIFFERENT Real, (**) DIFFERENT Very Real, (ns) Not significantly different

Based on table 3 it can be ignored that the treatment of the sterilization technique (T) has a very significant effect on the number of shoots and the treatment interaction between the sterilization technique (T) and coconut water (M) has a very significant effect on the number of shoots. Meanwhile, coconut water treatment (M) had no significant effect on the number of shoots.

The following are the results of the 5% DMRT test for the combination of sterilization technique (T) treatments presented in table 4.4 below.

Table 4. Results of the Advanced Test of the Effect of the T Factor on the Number of Shoots Using the DMRT Advanced Test 5%

Treatment	Average Number of Shoots
T1	2,6 a
T2	2,3 a
T3	1,9 b

Description: The numbers followed by the same letter show no significant difference in DMRT test at 5% level.

Table 4 shows the results of the 5% DMRT further test the effect of the T factor on the number of shoots formed in each treatment. The highest average was found in T1 treatment which reached 2.6 tuna. While the lowest average was found in the T3 treatment which reached 1.9 shoots. The T1 treatment is the treatment of sterilization techniques using 70% alcohol for 1 minute, the T2 treatment is the treatment of sterilization techniques using 5% NaOCl + 2 drops of tween 20 for 1 minute, while the T3 treatment is a sterilization technique using 5% NaOCl + 2 drops Tween 20 for 1 minute +$HgCl_2$ + 2 drops of Tween 20 for 30 seconds. Following are the results of the 5% DMRT test for the combination of sterilization technique (T) and coconut water (M) treatment are presented in table 4.5 below.

ISBN : 978-623-96220-1-5

Table 5. Result of Further Test the Effect of Factor T and M on the Number of Shoots Using the DMRT Advanced Test 5%

Treatment	Average Number of Shoots
T1M1	2.9 a
T2M4	2.7 ab
T1M3	2.7 ab
T3M2	2.6 abc
T1M4	2.5 abcd
T1M2	2.4 abcde
T2M1	2,3 abcdef
T2M3	2,1 bcdef
T2M2	1,9 cdef
T3M3	1,8 def
T3M1	1,7 ef
T3M4	1,6 f

Description: The numbers followed by the same letter show no significant difference in the level of DMRT test 5%.

Table 5 shows that the results of the 5% DMRT follow-up test had the effect of the T with M factor on the number of shoots formed in each treatment. The mean number of shoots in the sterilization technique using 70% alcohol for 1 minute and 0 ml / L coconut water (T1M1) was 2.9 tuna. While the lowest average number of shoots was found in the sterilization technique treatment using 5% NaOCl + 2 drops of tween 20 for 1 minute + $HgCl_2$ + 2 drops of tween 20 for 30 seconds and 150 ml / L coconut water (T3M4), namely 1.6 shoots.

The data in table 4.5 shows that the average number of shoots that appears is 2-3 shoots. Stevia explants can have a few or many shoots because the explants can absorb nutrients from coconut water. Cytokinins found in coconut water can help increase the number of tuna (Mandang, 1993). According to Pisesha (2008), the statement that the cytokinins contained in coconut water from small missiles can support plant growth. According to Kristina and Siti (2012), coconut water is a source of natural growth regulators, including the cytokinin group. Coconut water can spur the growth of tuna.

5. Conclusion

Based on the results of observations and discussions that have been obtained, it can be concluded as follows:

- The treatment of sterilization techniques provides a proportion of contamination to the induction of stevia plants ranging from 30-40% and the treatment that undergoes browning is only 3 treatments, namely T1M1, T2M3 and T3M1 of 10%. The proportion of plant growth produced by stevia plant induction was the same in the treatment of 60%. The treatment of the sterilization technique had a significant effect on the corrected parameters and the treatment of the sterilization technique had a very significant effect on the parameter of the number of shoots. The treatment of T1 sterilization technique gave the fastest shoot emergence rate, namely 14.4 days after planting (DAT). While the number of shoots produced by the effect of the T1 sterilization technique, the highest number of shoots was 2.9 tuna.
- The treatment of offering coconut water at the induction of stevia plants had no significant effect on the alarm parameters and the number of shoots.

Reference

[1] Gunawan, LW 2008. Tissue Culture Techniques. Laboratory of Tissue Culture of Essential Oil Producing Plants. In a LITTRO Special Edition. 5 (2). Research Institute for Medicinal and Aromatic Plants. Bogor

ISBN : 978-623-96220-1-5

Reference

[1] Gunawan, LW 2008. Tissue Culture Techniques. Laboratory of Tissue Culture of Essential Oil Producing Plants. In a LITTRO Special Edition. 5 (2). Research Institute for Medicinal and Aromatic Plants. Bogor

[2] Kumar N, Reddy MP. 2001. In Vitro Plant Propagation: An Overview. Journal of Forestry Science 27 (2): 61−72

[3] Kristina MO, D. Pandiangana, FE Kandou. 2017. Description of Types of Contaminants from Callus Cultures of *Catharanthus roseus* (L.) G. Don. Mipa Journal, Sam Ratulangi University, Manado. Pages 47-52. *https//Media.N Research.Com/Media/Publications/114048-ID-Descriptions-Jenis-Jenis-Kontaminan-Dari-Ku.Pdf:*. [March 6, 2020].

[4] Kristina, NN, and SF Syahid. 2012. Effect of Coconut Water on In Vitro Shoot Multiplication, Rhizome Production, and Xanthorrhizoln Content of Temulawak in the Field. Littri's Journal. P.125-134. *http://perkebunan.litbang.pertanian.go.id/wpcontent/uploads/2013/01/perkebunan_jurnal-littri_Vol18312_6_NovaN.pdf*. [13 October 2017].

[5] Mandang, JP 1993. The Role of Coconut Water in the Tissue Culture of Chrysanthemum Plants (*Chrysanthemum morifolium* Ramat). Dissertation. Bogor Agricultural Institute. Bogor

[6] Mariska, I., and Sukmadjaja, D. 2003. *Propagation of Abaca Seeds through Tissue Culture*. Bogor: Research Institute for Agricultural Biotechnology and Genetic Resources.

[7] Netty, W. 2002. Optimal media for multiplication of kana shoots (Canna hibryda Hort.) With the addition of cytokinins. J. Bioscience and Indonesian Biotechnology. 2 (1): 27-31.

[8] Pisesha, PA 2008. Effect of IAA, IBA, BAP and Coconut Water Concentration on Poinsettia (Euphorbia pulcherrima Wild Et Klotzch) Root Formation In Vitro. Thesis. Department of Agriculture and Horticulture. Faculty of Agriculture. Bogor Agricultural Institute. 40 p.

[9] Queiroz C, Lopes MLM, Fialho E, Mesquita VLV. 2008. Polyphenol oxidase: characteristics and mechanism of browning control. Food Reviews International 24: 361−375.

[10] Sri Hutami. 2008. Review of Browning Problems in Tissue Culture. Journal of Agrobiogens. http://Biogen.Litbang.Pertanian.Go.Id/Terbitan/Pdf/Agrobiogen_4_2_2008_83.Pdf

[11] Shonhaji, A., Ruri Siti Resmisari, Andik Wijayanto. 2014. Effectiveness ofField *Acacia* Mangium WilldExplant Sterilization in Plant Propagation through Tissue Culture Techniques. Malang: Department of Biology, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University

Optimization of Sterilization Techniques and Effects of Coconut Water for the Induction of Shoots of Stevia (Stevia rebaudiana Bertoni)

ORIGINALITY REPORT

13%	8%	8%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	proceedings.polije.ac.id Internet Source	6%
2	Submitted to University of Duhok Student Paper	1%
3	Submitted to Sriwijaya University Student Paper	1%
4	Asmila Asmila, Zainuddin Basri, Ramal Yusuf, Hawalina Hawalina. "CALLUS INDUCTION OF CACAO CLONE SULAWESI 1 ON VARIOUS CONCENTRATIONS OF 2,4 -D AND COCONUT WATER VIA IN VITRO CULTURE", AGROLAND: The Agricultural Sciences Journal, 2018 Publication	1%
5	Mislah S. Harahap, Fauziyah Harahap, Syahmi Edi, Rosmayati. " In Vitro Multification of with Plant Growth Regulator ", Journal of Physics: Conference Series, 2020 Publication	1%

6	Submitted to University of Rajshahi Student Paper	1 %
7	Submitted to Marshall University Student Paper	1 %
8	repository.uin-malang.ac.id Internet Source	1 %
9	F Verawati, Asmarlaili, T Sabrina. " The influence of the composition of biodiversal fertilizers plant vegetative soybean plant ((L.) Merrill) in inceptisol ", Journal of Physics: Conference Series, 2018 Publication	1 %
10	ojs.unud.ac.id Internet Source	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		