CODE-01

INDUCTIONS CALLUS VARIOUS CULTIVARS STEVIA (*Stevia rebaudiana* Bertoni M.) BY THE ADDITION OF VARIATION THE CONCENTRATIONS OF *2,4 Dichlorophenoxyacetic Acid* (2,4-D) ON MEDIUM DRIVER, KUNIYUKI,WALNUT (DKW)

Wiwi Indri Anti [1)], Tia Setiawati [2)], Suseno Amien [3)]
Padjadjaran University, Jl. Raya Bandung – Sumedang Km 21 Jatinangor 45363, Indonesia
Email:
wiwi_indri_anti20@yahoo.co.id[1)]
suseno2011@gmail.com[2)]
tiarasakimura@yahoo.com[3)]

Abstract

The current sugar production decreased due to weather anomalies, and many food and beverage industries using sugar farmers. Stevia sugar has the opportunity to fill the shortfall in domestic production of sugar cane.*Stevia rebaudiana* Bertoni M. is one source of sweetening plant. Stevia leaves contain steviosida. Stevia is cultivated by vegetative means one of them with tissue culture.This research aims to determine the concentration of 2,4-D is most effective to induce callus on stevia leaf cultivars explant Bogor, Garut and Tawangmangu and determine the best cultivar of Stevia in response to grow callus. This research was performed using random pattern full factorial design with two factors. The first factor was the concentration of 2,4-D (D) consisting of 4 degrees of the factor are 0 ppm (d_0); 1 ppm (d_1); 2 ppm (d_2) and 3 ppm (d_3) and the second factor is a cultivar of Stevia (S) which consists of three levels of factors namely Stevia cultivar Bogor (s_1), Stevia cultivar Garut (s_2) and Stevia cultivar Tawangmangu (s_3). The basic Media used is medium of DKW. Observed, the parameters of observation among others the wet weight of the kalus (mg), the time appears kalus (HST), the color and texture callus. The weight of wet callus analyzed using ANOVA, while time appears callus and the color and texture callus analyzed in descriptive. The result showed that the concentration of 2,4-D, cultivars and interaction between two factors influencing the weight of wet callus. Response growth callus it is best obtained on Stevia cultivars of bogor by the addition of 1 ppm 2,4-D, with time appears callus (HSI) of 7 days, a measure of callus best namely scale 16 according to clay model scales and having the weight of wet callus best of 0,734 grams. Crumb textured and thick yellow color.

Keywords : Medium of DKW, *Stevia rebaudiana* BertoniM., *2,4 Dichlorophenoxyacetic Acid* (2,4-D).

INTRODUCTION

Sweeteners is one food that his needs are always increasing. This sweetener is used as a sweetener food, beverage and pharmaceutical products. In Indonesia, sweeteners can be divided into two types: natural sweeteners and synthetic sweeteners. Natural sweetener derived from sugar cane, palm, and palm sugar beet, while the synthetic sweetener that has been known and used, such as saccharin and cyclamate. There are constraints in the supply of the sweeteners is the decline in the production of sugar cane and the lack of sugar cane plantations. According to the Departement of Agriculture in 2012, the total demand of sugar

for household consumption in Indonesia increased to 1.76%, while the difference between production and demand of sugar is only by 1.21% (MoA, 2012). When viewed from the availability of sugar to the needs of the industry average over the last 5 years, the need for the sugar industry each year ranged from 1.6 to 1.7 million tons, so Indonesia over the next 3 years remained require between 700000-800000 tons of sugar imports every year, so we can conclude the sugar production in Indonesia is low.

Synthetic sweeteners such as saccharin and cyclamate are widely used, but lead to cancer, so its use is limited and very strictly regulated [2]. Stevia plant is very potential to be developed as a natural sweetener, sugar cane companion and substitute of sugar synthetic and safe for consumption. The advantages of stevia as a sweetener has sweetness 200-300 better than of sugar cane, obtained of stevia leaf extract containing diterpene glycoside and stevia also has a very low calorie [7].

According to [8] vegetative clones of stevia in Indonesia is shorter than the vegetative period in the region of origin. It is caused by the condition of Indonesian tropical climate has a long 12 hour day, so it is not profitable in the cultivation of stevia because it reduces production of stevia leaves, to get stevia seeds that can adapt to the tropical climate conditions in Indonesia and has a long vegetative period is by tissue culture techniques [9]. Plant tissue culture is a technique insulating parts of plants, such as tissues, organs, or embryos, and cultured on artificial medium is sterile so that the parts of the plant is able to regenerate and differentiate into complete plants [12].

Induction of Callus is stage of tissue culture methods that stimulate cell division continuously from certain plant parts such as leaves, shoots, roots, stems, and so on, by using growth regulators to form a mass of cells (callus). Growth regulators are widely used for callus induction was 2.4-D. The results showed that administration of 2,4-D at a concentration of 1.0 mg / l to 3.5 mg / l to induce callus on leaf explants of groundnut [6]. DKW medium commonly used to induce callus and shoots, because of the various studies that have been done, planting explants on DKW medium speed time appears on ekplan cultured callus [6].

Based on the above statement, so can be several hypotheses are growth regulating substances of different 2,4-D concentrations on DKW medium (Driver, Kuniyuki and Walnut) has an influence on the growth of callus from leaf explants of stevia (stevia cultivars Bogor, Garut and stevia cultivars Tawangmangu cultivars stevia), there is a concentration of 2,4-D best for callus formation Stevia (Stevia rebaudiana Bertoni M.) cultivar of Bogor, cultivar of garut and cultivar of Tawangmangu. and there is certain Stevia has the best response to form callus

The purpose of this study is to get growth regulators 2,4-D is best to induce callus from leaf explants of stevia and obtain cultivars of stevia that have the best response to form callus. This study is expected to provide useful information regarding the use of plant growth regulators 2,4-D in inducing callus from leaf explants of stevia, so get a lot of seeds stevia useful as a natural sweetener. This

study was conducted in September-November 2013 in the Tissue Culture Laboratory of the Department of Agriculture Faculty of Agriculture, Padjadjaran University Jatinangor-Sumedang, West Java-Indonesia.

MATERIALS AND METHOD

The experiment was conducted in September 2013 until November 2013 at the Tissue Culture Laboratory of the Department of Agriculture Faculty of Agriculture, Padjadjaran University Jatinangor . The materials used in tissue culture studies is that, aluminum foil , alcohol (70 % and 90 %) , sterile distilled water, chlorox , leaf explants of Stevia (*Stevia rebaudiana* Bertoni M.) , 0.1 N HCl (Hydrochloric Acid), 0.1 N NaOH (sodium hydroxide), Medium DKW , 2,4-D (2,4–Dichlorophenoxyacetic acid) 0 ppm, 1 ppm, 2 ppm, and 3 ppm . The design used in this study was a complete randomized design (CRD) factorial consisting of two factors . The first factor is the concentration of 2,4 - D (D) consisting of a four level concentrations , namely : d0 = 0 ppm ; d1 = 1 ppm ; d2 = 2 ppm ; d3 = 3 ppm . The second factor is the cultivar variation stevia (S) which consists of three levels cultivars namely: s1 = Stevia Bogor and s2 = stevia Garut ; s3 = Stevia Tawangmangu .

Experiment Procedur

Stevia leaf explants were sterilized in 1 % HgCl solution, 1 % bactericide solution , 1% fungicide solution and 1 % chlorox respectively for three minutes. Planting is done in the Laminar Air Flow. Eksplan used Stevia leaf is the central part with the size of 0.5 cm x 0.5 cm. Treatment of growth regulators 2,4-D consists of 0 ppm, 1 ppm, 2 ppm and 3 ppm. Explants grown in culture bottles containing 10 ml of medium DKW equipped with concentrations of 2,4-D treatment. Each treatment consisted of three units of culture bottles. Culture medium acidity was adjusted to pH 5.7 to 5.8 with 0.1 M KOH or HCL. Explants that have been planted, saved in the culture room with white fluorescent intensity , ambient temperature was maintained approximately 22^{o}C and 70 % relative humidity. Observations were made every day for 4 weeks after planting does explants. Callus color, texture callus, and time appears callus analyzed descriptively, whereas callus fresh weight data were analyzed using ANOVA. If there is a real difference , then continued using the Duncan test level of 5 %.

RESULTS AND DISCUSSION

Callus Induction

Callus formation was induced from a particular part of the plant growth regulators by providing a lot of ZPT has used for callus induction are a combination of auxin and cytokinin (Zulkarnain, 2004). 2,4-D is the most commonly used plant growth regulator on callus culture because of the strong activity to stimulate the process of cell de-differentiation, organogenesis and keep pressing the callus growth. Activity 2-4-D robust and optimal is due to the carboxyl groups are separated by carbon or carbon and oxygen (Budiyati, 2002). Growth and morphogenesis in vitro influenced the interaction and balance between growth regulators were

added in the medium and growth hormone produced by the plant cells endogenously by cells cultured (Shahid and Natalini, 2007)

Time Appear Callus

Indicator of growth in vitro culture is the initial formation of callus explants.

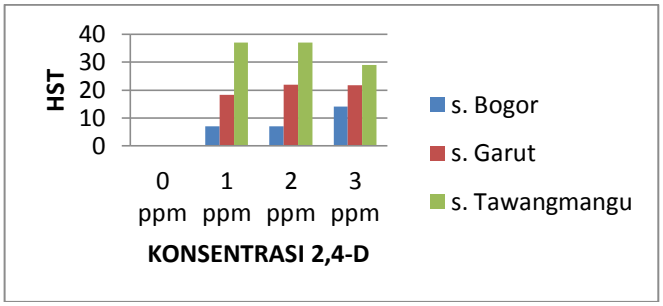


Figure 1.1. Graph of mean time appears callus on leaf explants *Stevia rebaudiana* Bertoni M

Figure 1.4, shows that the three cultivars stevia , the addition of 2,4 - D at concentrations of 1, 2, and 3 ppm can induce the formation of callus from leaf explants of stevia. Without 2,4-D, did not show the callus formation because to a combination auxine hormone in the media and outer have not been able to meet the needs of the explants to induce callus. Explants are likely to have a low endogenous auxin content, so it still requires additional exogenous auxin is much more to the culture medium. The results of the study, the Bogor cultivars showed that stevia with 1 ppm 2,4-D and 2 ppm of 2,4-D, which is able to induce callus fastest growing after 7^{th} days after planting. Addition of auxin at low concentrations would stimulate callus formation. While the longest callus induction was obtained in stevia of cultivar of tawangmangu with 1 ppm 2,4-D and 2-D is 2.4 ppm at 30^{th} days after planting. This is presumably due to a combination of hormone concentration given in the explants not callus induction, so inhibiting the growth of callus on explants .

In the study, the same concentration of the 1 and 2 ppm of 2,4-D on different stevia cultivar, produce callus induction different time, a different response is influenced by the concentration of growth regulators granted, explants endogenous hormone levels, needs different tissue explants to induce callus,interaction between endogenous hormones and exogenous hormones on ekplan on different media and genotypic differences between cultivars Bogor with stevia stevia Tawangmangu cultivars and physical state of the environment or culture where grown too morphology of explants

Texture and Color of Callus

Indicators to growth of explants cultured in vitro such as color and texture callus describe the visual appearance of callus so it can be seen callus that still has cells that are actively or dead. Callus tissue resulting from an explant typically elicits different colors.

Table 1.4 Percentage Texture Callus

Cultivars	Texture Callus	
	Crumb (%)	Compact (%)
k_1 (Stevia Bogor)	25	0
k_2 (Stevia Garut)	8,33	16,67
k_3 (Stevia Tawangmangu)	16,67	0

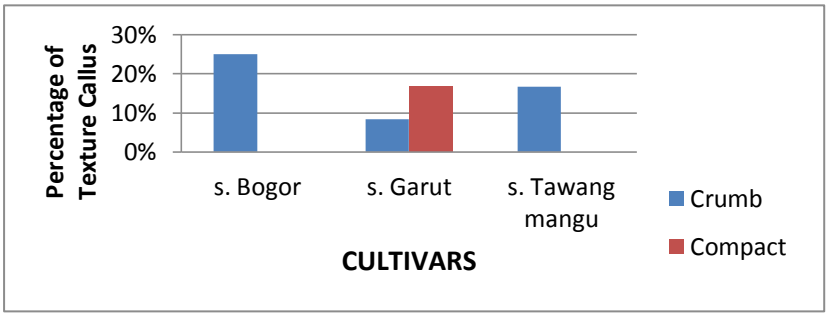


Figure 1.2. Graph of *Stevia rebaudiana* callus texture Bertoni M.

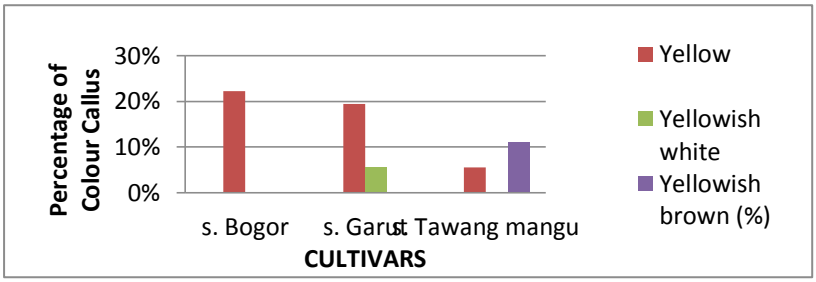
Table 1.5, explaining that the callus which has the highest crumb texture found in stevia Bogor cultivars by 25% of the combined treatment, whereas callus which has a compact callus texture found in stevia Garut cultivars by 8.22%, while to cultivar Tawangmangu stevia growing callus just crumb textured with a percentage of 16.67%. In general, callus growing had type II is callus crumbs. In general, growing callus which is had type II callus crumbs. Tomes in Sutjahjo (1994), explains there are two kinds of callus formed in the in vitro culture a plant, which is (1) embryogenic callus and (2) non-embryogenic callus. Embryogenic callus is a callus having the potential to regenerate into plants through organogenesis or embryogenesis. While the non-embryogenic callus is a callus having slightly ability or have not the ability to regenerate into plants. According to Green et al. (1984) in Sutjahjo (1994), callus type-I has a compact structure, opaque and relatively slow growth is desired types in vitro selection of plants. otherwise less compact callus, crumbs and rapid growth of so-called type-II of callus. Research shows that all treatments were formed callus, callus dominant textured crumb. Callus formation textured crumb according Andaryani (2010), spurred by the presence of endogenous auxin hormone produced internally by the explants formed callus has grown. Giving 2,4-D effect on the texture of the callus formed, in general, 2,4-D helps the growth of callus and callus produce crumb texture, this is reinforced by research conducted by the beautiful and Ermavitalini (2013) on leaves callus induction Nyamplung , which produce callus crumb texture with addition of 2,4-D.

A B C

Gambar 1.3. Texture and Color Callus
Description: A. Callus stevia of Bogor cultivar; B. Callus stevia of Garut cultivar; C. Callus stevia Tawangmangu of cultivar

Table 1.5. Percentage Color Callus

Cultivars	Color Callus		
	Yellow (%)	Yellowish white (%)	Yellowish brown (%)
k_1 (Stevia Bogor)	22,22	0	0
k_2 (Stevia Garut)	19,44	5,56	0
k_3 (Stevia Tawangmangu)	5,56	0	11,11



Gambar 1.4 Graph color callus of *Stevia rebaudiana* Bertoni M.

Research shows, the average callus a bright yellow, so it has a fast growing callus size of large. brownish Color of callus contained in almost all treatments formed callus. brownish color from callus (browning) due to metabolism of phenolic compounds to be redundant, which is often induced as a result of the sterilization process explant [1].

Wet weight of callus

Growth is characterized by the irreversible increase in weight, so that measurements can represent the wet weight of callus growth variables callus derived from leaf explants Stevia rebaudiana Bertoni M. [10]. Physiologically wet weight consists of two content which is water and carbohydrates. wet weight of Callus high caused by the water content. Wet weight produced very depend on the speed of split itself these cells, reproduce itself and proceed with the growing to callus [1]. Based on ANOVA, a combination of various cultivars stevia and various concentration of 2,4-D effect on wet weight of leaf callus Stevia rebaudiana Bertoni M., since ($P \leq 0.05$). Likewise, the interaction of both factors.

Table 1.6. The influence of 2,4-D and cultivar of Stevia on Average Wet weight of Callus

Cultivars	2,4-D			
	0 ppm	1 ppm	2 ppm	3 ppm
Bogor	0,081a	0.669b	0.378a	0.298a
	A	B	A	A
Garut	0.086a	0.297a	0.244a	0.264a
	A	A	A	A
Tawangmangu	0,060a	0.101a	0.131a	0.15a
	A	A	A	A

Remarks: The numbers followed by the same letter (lowercase horizontal and uppercase vertical direction) were significantly different according to Duncan's Multiple Range Test 5%

Based on the results of Duncan's multiple range test level 5%, the effect of 2,4-D and cultivars stevia to the average wet weight of callus interaction effect is significantly different. Addition 1 ppm of 2,4-D significantly affect callus wet weight and concentration is best for callus growth. The highest callus wet weight was 0.734 g. Cultivars that provide the best response to establish callus and significant effect on wet weight of callus is stevia cultivars Bogor. Based on Duncan's multiple range test, ZPT and cultivars that produce results significantly different on the wet weight of callus was stevia Bogor cultivars with addition of 1 ppm of 2,4-D. therefore, the stevia kultivar Bogor with addition 1 ppm 2,4-D is the best treatment in the form callus and significant effect on the callus wet weight.

CONCLUSION

The addition of 2,4-D on DKW medium, giving effect on callus growth and callus fresh weight of leaf ekplan on three cultivars of stevia (Stevia cultivar Bogor, stevia cultivar Garut and stevia cultivar Tawangmangu). The best concentration of 2,4-D for callus formation from leaf explants of stevia cultivars from Bogor, stevia cultivar Garut and stevia cultivar Tawangmangu is 1 ppm. Of the three cultivars that provide the best response in the formed of callus from stevia cultivars Bogor, with tthe time appears callus which is seven days after planting, the best callus size is 16 by clay scale models and has a wet weight of 0.734 grams best callus. Textured crumb and dark yellow

ACKNOWLEDGEMENT

I would like to thank Mr. Suseno Amien and Mrs Tia Setiawati who has guided me. Finally, we would like to thank my parents for their love and encouragement.

REFERENCE

[1] Andaryani. 2010. *Various studies use concentrations of BAP and 2,4-D Against Callus Induction of Jatropha (Jatropha curcas L.).*University of Sebelas Maret Surakarta.

[2] Budiarso, Irwan T. 2008. *Chemical carcinogens and Mikokarsinogen.* Department of Health R.I. Jakarta.

[3] Budiyati, R. 2002. *Callus growth Leaf Stalk Purwoceng (Pimpinella alpine Kds) in MS medium (Murashige and Skoog) with the granting of 2,4-D and BAP*. Thesis. Diponegoro University, Semarang.

[4] Deptan. 2012. Cane. http://www.deptan.go.id/pusdatin/admin/PUB/Outlook/ outlook_komoditas_bun.pdf (Accessed on January 15, 2014)

[5] Ermavitalini. 2013. *Somatic Soybean Embryo growth response (Glycine Max) Varieties Against Stress Argomulyo and Willis NaCl.* Biology. Institute of Technology Sepuluh Nopember.

[6] Lizawati. 2009. *Know Stevia sweetener without calories roebaudiana material.* News Research and Development Department of Agriculture 4 (2) :1-3.

[7] Maudy E. and Paimin, Fendy R. 1992. *Stevia cultivation*. Trubus. No.. 274 Year XXIII, p. 22-23.

[8] Mubiyanto, Bambang Odang. 1990. *Analysis of Plant Growth Stevia rebaudiana Bertoni on Three High Places*. Faculty of Graduate Studies. Bogor Agricultural University.

[9] Mugiono, 2001. *With Mutation Breeding Techniques. National Nuclear Energy Agency*. Center for Education and Training, Jakarta.

[10] Ruswaningsih, F. 2007. *Effect of Ammonium Nitrate and BAP concentration on the growth shoots of Artemisia annua L. explants in culture in vitro*. Thesis Faculty of Agriculture UNS. Surakarta.

[11] Sutjahjo, S. H. 1994. *Induction of Somaclonal to Diversity Tension Direction Against Aluminum Toxicity in Maize*. Dissertation, Graduate Program, IPB. 139.

[12] Winata, L. l987. *Tissue Culture Technique*. PAU Bogor.252 p.

[13] Zulkarnain. 2009. *Tissue Culture*. Bumi Akasara. Jakarta.