ISSN 2460 - 5506

PROSIDING
KONSER KARYA ILMIAH
TINGKAT NASIONAL TAHUN 2018
" Peluang dan Tantangan Pembangunan Pertanian Berkelanjutan di Era Global dan Digital"
Kamis, 13 September 2018 | Fakultas Pertanian & Bisnis UKSW

THE EFFECTS OF GIVING 2,4 DICHLOROPHENOXYACETATE AND BENZILADENIN ON EXPLANT GROWTH OF STEVIA (*Stevia rebaudiana* BERTONI)

Andreas Petra Wibiantoro[1)], Maria Marina Herawati[2)]
[1]Student of Faculty of Agriculture and Business, Universitas Kristen Satya Wacana
Author email: 512014019@student.uksw.edu
[2]Lecturer of Faculty of Agriculture and Business, Universitas Kristen Satya Wacana
Correspondent Author email: marinakartika@gmail.com

ABSTRACT

Stevia rebaudiana Bertoni. is a perennial shrub of the family Asteraceae from Paraguay. Stevia produces a diterpene glycoside called steviol glycosides. Glycosides from stevia are low-calorie sweeteners and have a low glycemic index so it does not affect blood sugar levels. Increasing contents of steviol glycosides can be done by tissue culture methods. The addition of plant growth regulator as 2,4-Dichlorophenoxyacetate (2,4-D) and Benziladenine (BA) in MS medium agar is expected to increase the growth of stevia shoot explants. Treatments of the research are combination of 2,4-D with a concentration of 2 and 3 mg. L-1 and Benziladenine at a concentration of 1; 1.5, 2, and 3 mg. L-1. Growth regulators are given to explants with inductions time of 21 days. Results of the research showed that combinations of 2,4-D 2 mg.L-1 and BA 1 mg.L-1 can get the highest number of grown explants at 88%. The shoots produced from treatment with combinations of 2,4-D 2 mg. L-1 and BA 3 mg. L-1 are 2,04 shoots per explant.

Keywords: *Stevia, 2,4-Dichlorophenoxyacetate, Benziladenin,*

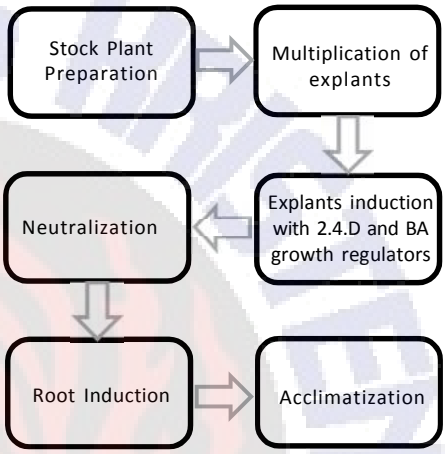
INTRODUCTION

Stevia is a plant from family Asteraceae. (Soejarto, 2002). According to Mantoro, (2013) the type of stevia that has big developed is Stevia rebaudiana because it can produce secondary metabolites with a type of diterpenes glycoside compound called steviol glycoside. The glycosides that produced by stevia do not contain calories and contain a nearly zero glycemic index. Because of this, steviol glycosides do not affect blood sugar levels so it is safe for diabetics (Jeppesen *et al*., 2002; Gregersen *et al*., 2004). The highest content of steviol glycosides is found in the shoots that are still growing. Steviol glycosides will accumulate until the plant entering to generative phase. Steviol glycosides are produced in the leaves and stored in glandular trichomes so that the density of glandular trichomes will be directly proportional to the number of steviol glycosides stored (Jain, 2014).

Tissue culture is a method of clonal propagation in plants. The advantage of using tissue culture methods is producing uniform seeds in large quantities (Lestari, 2008). Tissue culture can be used as a stevia cultivation method to increase the productivity of secondary metabolites. The used of growth regulation substances in the form of auxins and cytokines expected to trigger shoot growth on stevia explants better. The shoot that has grown in vitro basically requires the addition of growth regulating substances. At each stage, the shoot growth has different concentration and type of growth regulating agent given. In shoot culture, there are several types of auxin that are often given, namely NAA, IAA, and IBA. The type of auxin that is rarely used in shoot growth is 2,4-D because 2,4-D compounds will be more intended to callus formation in plant tissues (Winata, 1988). Other growth regulators used are cytokines one of them namely benzyl adenine (BA). Benzyl adenine is often used in the growth and multiplication of shoots because BA has an activity that induces shoot growth greater than kinetin (Zaer and Mapes, 1982). In shoot production in vitro, cytokines will play a greater role than auxin to shoots production in vitro (Winata, 1988). However, in the production of secondary metabolites can be used a combination of auxin and cytokines (Herawati, 2015).

METHOD

This research was conducted from March to June 2018 at the Plant Tissue Culture Laboratory of the Faculty of Agriculture and Business at the Universitas Kristen Satya Wacana. The stages of the study included preparation of stock plants, multiplication of shoots, induction by 2,4-D and BA plant growth regulators (PGR), neutralization, root induction, and acclimatization. The variables observed in this study were the number of growing buds and the percentage of explants grown. Data analysis was carried out in a descriptive parametric manner.



Picture 1 Flow Chart of *Stevia rebaudiana* Bertoni shoot culture

RESULT AND DECISION

The growth of shoots in vitro is influenced by the addition of plant growth regulators in the MS medium. In this study, the explants used in the form of stevia shoots were grown in MS medium with the addition of 2,4-D at a concentration of 2 and 3 mg. L^{-1} and also addition of BA at concentration 1; 1.5; 2; and 3 mg. L^{-1}.

Picture 2. The results of S. Rebaudiana shoot culture in vitro with the addition of Plant Growth Regulator for 21 days

Based on observations of explant growth on MS media and the addition of 2,4-D and BA combination obtained and the percentage of live plantlets after induction with a combination of PGR for 21 days (Table 1.) and the total shoots grew on explants (Table 2.)

Table 1 Percentage of plantlet lives that produced from shoot culture by incubation with combination beetwen 2,4-D and BA treatment for 21 days

Treatment		% Planlet life at the time of the PGR application for 21 days
2,4 – D ($mg.L^{-1}$)	BA ($mg.L^{-1}$)	
2,0	1	88
	1,5	60
	2	76
	3	80
3,0	1	76
	1,5	68
	2	64
	3	80

Explant response after planted in the induction medium it is indicated that application 2,4-D of 2 $mg.L^{-1}$ and BA of 1 $mg.L^{-1}$ can produce the highest percentage of explant growth. BA is a growth regulator of cytokines which has a role as a trigger for cell division, cell division, and morphogenesis (Herawati, 2016). Therefore, when 2,4-D used at 2$mg.L^{-1}$, BA aplication not needed in high quantities. Application 2,4-D 3 $mg.L^{-1}$ can produce a high percentage of live explants when combined with BA on 3$mg.L^{-1}$. This is because the toxic properties of 2,4-D can be inhibited by explants with morphogenesis and rapid cell division. In addition, dead explants is also influenced by the growth of molds. Explants that do not immediately form buds and leaves will lose on the competition with molds and than going to die.

The result of explants induction indicated that on combination threatment 2,4-D 2 $mg.L^{-1}$ dan BA 3 $mg.L^{-1}$ was able growth bud on all explant with the average shoots were grown on explants are 2.04 shoots per explant. Other results stated that the treatment of 2,4-D of 3$mg.L^{1}$ that combined on BA with a concentration of 1,5 and 3 $mg.L^{-1}$ was also able to grow buds on all explants, but the average shoots produced at explant were lower than treatment on 2,4-D with a concentration of 2 $mg.L^{-1}$. The use of 2,4-D must be in low concentrations or about in the range of 0.88 to 3.1 $mg.L^{-1}$ because it can inhibit plant growth and also even kill plants (Wattimena, 1992). All explants that grew on induction media were 3 $mg.L^{-1}$ 2,4-D and 1,5 $mg.L^{-1}$ BA was able to form buds, but the average number of shoots that grew was 1,40 shoots per explant. The combination of 2,4-D 3 $mg.L^{-1}$ and BA 3 $mg.L^{-1}$ was also able to grow shoots on all living explants. However, the average total shoots grew by 1.99 shoots per explant, which means it is still lower than the treatment of 2,4-D 2 $mg.L^{-1}$ combined with BA 3 $mg.L^{-1}$. 2,4-D is an auxin group growth regulator.

From addition of cytokinin influences shoot growth in shoot culture. Lestari, (2011) states

Table 2 Percentage of plantlet lives that produced from shoot culture by incubation with combination beetwen 2,4-D and BA treatment for 21 days

Treatment		% Shoots appear on explants	The average of total shoots grow on explants
2,4 – D ($mg.L^{-1}$)	BA ($mg.L^{-1}$)		
2,0	1,0	86%	1,60
	1,5	87%	1,33
	2,0	95%	1,55
	3,0	100%	2,04
3,0	1,0	84%	1,45
	1,5	100%	1,40
	2,0	94%	1,60
	3,0	100%	1,99

that the additions of growth regulators in plant tissue culture has different roles. Cytokinins are able to stimulate shoot formation. Auxins will be more needed if morphogenesis and organogenesis are puposed to callus and root formation.

CONCLUSION

From this study, it can be concluded that the use of plant growth regulators in the form of auxins and cytokines provides a different response to the culture of shoots on stevia explants. The addition of 2,4-D 2 mg.L ^{1} and BA 1 mg.L ^{1} on MS0 medium for stevia shoot culture can provide the highest percentage of living plantlets with 88%. Addition of 2,4-D 2 mg.L ^{1} and BA 3 mg.L ^{1} can grow buds in all live explants and the average shoots appeared the most were 2.44 shoots per explant.

ACKNOWLEDGMENTS

The author would like to thank because this research is part of internal compulsory research funded by Satya Wacana Christian University.

REFERENCES

Herawati. M.M, *et al*. 2015. Obtaining *Artemisia cina* Polyploidy Through Plant Growth Regulator Threatment in Shoot Culture. *Agrivita* 37:2.

Herawati. M.M. 2016. Peningkatan Hasil Artemisinin Melalui Poliploidisasi dan Kultur Teknik *Artemisia cina* Berg ex Poljakov. *Desertasi*. Gadjah Mada University:Yogyakarta.

Soejarto, D.D., 2002. *Ethnobotany of Stevia and Stevia rebaudiana*. In: Kinghorn, A.D. *Stevia*: *The Genus Stevia*. Taylor and Francis, London and New York, pp. 40–67.

Montoro.P., Molfetta.I.,ceccarini. L., Piacente.S., Pizza.C., Macchia.M. 2013. Determination of six steviol glycosides of *Stevia rebaudiana* (Bertoni) from different geographical origin. *Food Chem*. 15; 141(2):745-53.

Jeppesen, P., Gregersen, S., Gregersen, S., Alstrupp, K., & Hermansen, K. 2002. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: Studies in the diabetic Goto-Kakizaki (GK) rats. *Phytomedicine*. 9: 9-14.

Gregersen S, PB Jeppesen, JJ Holst & K Hermansen. 2004. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism.* 53(1): 73-76.

Jain. P, Sumita. K, dan Kothari .S.L. 2014. Biotechnology and Metabolic Engineering of *Stevia rebaudiana* (Bert.) Bertoni: Perspective and Possibilities. *International Journal of Life Sciences Biotechnology and Pharma Research*. 3 (3).

Zaer and Mapes. 1982. *Action of growth regeneration*. *In* Bonga and Durzan (*eds*.) *Tissue Culture in Forestry*. Martinus Nijhoff London. p. 231-235.

Winata, L. 1988. *Plant tissue culture techniques.* Plant tissue culture laboratory of the Inter-University Center (PAU) Bogor Agricultural University.

ooOoo