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ENHANCING GROWTH AND ALOIN PRODUCTION OF *ALOE VERA* L. PLANTLETS BY SUCROSE AND YEAST EXTRACT ELICITOR DOSAGES

MARIA THERESIA DARINI[1], DIDIK INDRADEWA[2], AZIZ PURWANTORO [3] & DJA'FAR SHIDDIEQ[4]

[1]Agriculture Faculty of Sarjanawiyata Tamansiswa University, Yogyakarta, Indonesia

[2,3,4]Agriculture Faculty of Gadjah Mada University,Yogyakarta, Indonesia

ABSTRACT

The *Aloe vera* L. is an important plant to be cultivated due to aloin content and useful as antiinflamatory, antidiabetic and antichollesterol. The research aimed to enhance the growth, and anloin production of *Aloe vera* through elicitation which has been conducted in the Agriculture Faculty Laboratory of Sarjanawiyata Tamansiswa University and Integrated Research and Testing Laboratory of Gadjah Mada University. The experiment was arranged with Completely Randomized Design (CRD) factorial with three replications. The first factor was sucrose dosages consisting of four levels, namely 0; 1.5; 3.0 and 4.5 % media. The second factor was yeast extract dosages consisting of four levels namely 0; 100; 200 and 300 ppm media. Growth age, viability, leaf area, fresh weight and dry weight of plantlets were measured 2 months after cultivation. Concentration of aloin was analysed with TLC methods. Variance analysis of all data were tested by using anova analysis α 5 % and followed by Duncan's Multiple Range Test at significan level of 5% of there was significantly different in variance. Analysis of correlation was colculated between dry weight and aloin production of planlets. The results showed that there were interactions of sucrose and yeast extract dosage source on the dry weight, aloin concentration and production, and there were no interactions on growth age, viability, leaf area and fresh weight of plantlets. The combination of without yeast extract and 4.5% sucrose gave the highest plantlet dry weight. The highest aloin concentration was obtained in no yeast extract and sucrose treatment and in no yeast extract combined with 4.5% sucrose . The highest aloin production in the plantlet were obtained in no yeast extract and 4.5% sucrose added in the medium induction, it was capable of enhancing 1.39 fold the aloin concentration compared with given sucrose 3.0% dosage. There were low correlation between fresh weight and dry weight, aloin concentration and aloin production. There was a high correlation between aloin concentration and production of plantlets.

KEYWORDS: Aloin, Elicitation, Growth, Sucrose, Yeast Extract

INTRODUCTION

The metabolism process which can induce a secondary metabolites pathway when it is added by compounds is called elicitation. Compounds which stimulate the process of plant elicitation are called elicitors. The elicitation could also be used to enhance the secondary metabolites synthesis of plant and could play an important role in biosynthetic pathway to enhance the production of commercial important compound. Elicitors are classified as physical or chemical, biotic or abiotic, and complex or defined depending on their origin and molecular structure. Biotic elicitors are molecules of either pathogen or host origin that can induce defence responses (Murthy et al. 2008; Angelova *et al.* 2010).

Aloe vera plant is intensively used as a basal element in preparation of medicine, cosmetic and food supplement. The Aloe plant produced aloe gel for herbal material source. Aloe gel is a clear gel substance produced by parechymatis cells, in the central part of the leaf. The main constituents of the gel aloe are antraquinones including the hydroxyathracene derivates, aloin A, aloin B, barbaloin, isobarbaloin and aloe emodin. The *Aloe vera* produces the secondary metabolites such as aloin, saponin, lignin, lectin and glucomannan. The aloin and saponin compounds have potential for the pharmaceutical industry as antibiotic, antiviral, antibacterial, antifungal, anti-inflammatory, antidiabetic, antiallergy, antileuchemic, anticancer and antineoplastic against some diseases (Naqvi *et al*. 2010; Acurero, 20..; Lambert *et al*, 2011; Hazrati *et al*. 2012). The most important secondary metabolites is not only for the medicine, cosmetic, and food supplement properties but also has the physiological role in (Acurero, 2008). Among the most active compounds which are produced by plant are alkaloid, essential oils, flavonoid, glucosinolates and phenolic in (Sawalha, 2010).

Recently the various tissue culture techniques are used to enhance the yield of secondary metabolites by trigger stress response like using elicitors. Enhancing of scondary metabolism can be done to add precursor, biotransformation, and environment change are the focus of the study on enhancement of secondary metabolites in (Sharma *et al*. 2011; Linden, 2012). Therefore, it in nesessary to do research activity of enhancing the growth and aloin concentration of *Aloe vera* explant through elicitation.

MATERIALS AND METHODS

The materials of research are *Aloe vera* seedling as explant material. The research was conducted at the tissue culture laboratory oh the Faculty of Agriculture, Sarjanawiyata Tamansiswa University and the Integrated Research and Testing Laboratory of Gadjah Mada University, from April – July 2013. The research was arranged in a Factorial Completely Randomized Design (CRD), with three replications. The first factor was yeast extract dosages consisting of four levels namely 0, 100, 200 and 300 ppm media; the second factor was sucrose dosages, namely 0, 15, 30, 45 % media. Four explants were plantlet in each bottle contained Morashige and Skoog media at 24 ^{0}C temperature and 16 houres radiation. Observation was done every day for the contaminated media. Contaminated media were cleaned and explants replanted on new media. After two month incubation, plantlet were examination for its growth components i.e. growth age, viability, leaf area, fresh and dry weight of plantlet and aloin con

Laboratory apparatus sterilization, preparing Morashige and Skoog Media two times of 1200 ml, sterilization and transfer explants on the culture media. Three bottles of each treatment were planted four explants. Observation was done every day for the contaminated media. Contaminated media were cleaned and explants replanted on new media. After two months incubation, plantlet were examined for its growth components i.e bud growth age, viability, leaf area, fresh and dry weight of plantlet, aloin concentration and production of plantlets.

Analysis of aloin in Thin Layer Chromatography: the leaf was dry weighted 5 mg, extracted with 2 ml of ethanol and centrifuged. Ethanol phase was discarded, 2 ml of ethanol was added into the residue and centrifuged. The latter steps were repeated three times. Filtrate was evaporated and then 500 µl of ethanol was added. A small drop of the mixture was placed on the silica plate using a microsyringe, and also the standard aloin was placed alongside it. The plate was placed in a chamber saturated mobile phase, and the solvent was allowed to rise until it almost reached the top of the plate. The plate was removed from the chamber and was air dried. Aloin density was determined using TLC scanner, at λ maximum 340 nm.

The data was analyzed in Analisys of Variance followed by Duncan's Multiple Range Test at the α 5 % if there was significantly different among treatments. Coeficient of correlation analysis was applied to calculated the relationship between variables.

RESULTS AND DISCUSSIONS

The results showed there were no interaction effects between sucrose and yeast extract dosages on bud growth age, viability, leaf area and fresh weight of plantlet (Tabel 1). There were interactions between sucrose and yeast extract dosages on dry weight, concentration and production of aloin (Tables 2, 3 and 4).

Bud Growth Age, Viability, Leaf Area and Fresh Weight of Plantlets

Yeast extract had no significant effect on the growth variabless, whereas sucrose did influence the date of bud growth age. Explants planted in media with sucrose 1.5 % to 4.5 % resulted in fast growth of bud in 6.42 days, while explants planted in media without sucrose in 8.00 days. Media with sucrose 4.5 % resulted in the highest leaves area of 13.49 cm^2, as reported by (Hartanto *et al*. 2010) that increased inductions microtuber *Gynura pseudochinea*, so reported by (Khoroussachi *et al*. 2011) that increased growth callus *Taxus brevifolia*. Yeast extract had no significant effect on leaf width The highest area of leaf 11.14 cm^2 was obtained if 300 $mg.L^{-1}$ yeast extract was used, while no yeast extract or 100 $mg.L^{-1}$ of yeast extract gave the shortest leaf. Yeast extract resulted in lower fresh weight, but the highest fresh weight was obtained when 300 $mg.L^{-1}$ of yeast extract was added 9.20 g.

Table 1: Bud Growth Age, Viability, Leaf Area and Fresh Weight of Plantlet

Treatment	Level	Variables			
		Bud Growth Age (day)	Viability (%)	Leaf Area Plantlet (cm^2)	Fresh Weight Plantlet (g)
Sukrosa Dosage (%)	0	8.00 a	64.58 b	8.61 b	8.19 b
	1.5	6.41 b	72.91 a	7.78 b	8.51 b
	3.0	6.28 b	75.00 a	11.51 b	10.90 a
	4.5	6.42 b	75.00 a	13.49 a	10.62 a
Yeast Extract Dosage (ppm)	0	8.25 p	58.33 q	9.06 q	8.50 q
	100	7.41 p	70.81 p	9.34 q	8.50 q
	200	7.50 p	77.80 p	9.31 q	8.90 q
	300	7.16 p	71.25 p	11.14 p	9.20 p
Interaction		(-)	(-)	(-)	(-)

Means within the same column followed by the same letters are not significantly different using

Duncan's Multiple range Test 0.05 probability level, (-) there are no interactions.

The media with sucrose 3.0 and 4.5 % gave in the high fresh weight of 10.90 and 10.62 g, respectively. More over media with yeast extract 200 and 300 ppm showed high viability 77.80 % and 71.25%, whereas media with sucrose 1.5 to 4.5 % showed high viability 72.91, 75.00 and 75.00 % nonsignificantly, media no sucrose gave the least viability of 64.583 %. This result is simillar with (Broeckling *et al*. 2005) reported that Methyl Jasmonit and UV light could increase the growth of *Mediago truncatula*, as reported by (Hasanloo *et al.* 2008) as well that the addition of 3 mg. L^{-1} Picloram and Jasmonit Acid could increase the callus growth of *Silybrum marianum.*

Dry Weight of Plantlet

The highest plantlet dry weight of 2.82 g was obtained when no yeast extract given was combined with sucrose of 4.5 % (Table 2). This case.shows the addition of 4.5% sucrose elicitor act as an energy source to increas cell proliferation, thereby increasing dry weight plantlets. Likewise in (Morais *et al.* 2012) also found that the addition of sucrose 4.5 to 6.0 % increased the growth of brasil ginseng (*Panax glomerata*) explants. The best growth was obtained when 4.5 %. L^{-1} of sucrose was added to the medium in (4).

Table 2: Dry Weight of Plantlet (gram)

Extract Yeast Dosage (ppm)	Sucrose Dosage (%)				Means
	0	1.5	3.0	4.5	
0	2.53 d	2.67 c	2.74 b	2.82 a	2.69
100	2.59 d	2.70 b	2.73 b	2.72 b	2.70
200	2.52 d	2.58 d	2.71 b	2.54 d	2.58
300	2.48 e	2.73 b	2.73 b	2.72 b	2.65
Means	2.52	2.67	2.75	2.68	(+)

Means within the same column and row followed by the same letter are not significantly different using Duncan's Multiple Range Test at the α 5%, (+) there are interactions.

Reff in (Morais *et al.* 2012) stated that the addition of sucrose 1.5 % gave maximal growth of quince callus (*Cydonia oblonga* Mill), moreover found the best growth of garden herb (*Ocimun basilicum*) on the addition of sucrose 20 $g.L^{-1}$. The similar result also found, best growth of *Melissa officinale* callus obtained on the addition of sucrose 30 $g.L^{-1}$, while in reported that *Thymus vulgaris* callus grew best on sucrose dosage of 3.0 %.L^{-1}. Reff in (Kaswasara *et al.* 2010) reported that dry cell weight increased at the elicitor concentration of 24.6 $g.L^{-1}$, so as in (Li et al. 2011), found that cell dry weight increase up to 1.34 fold when Water Extract Cell Polysacharide was added to the medium at 20 $mg.L^{-1}$.

Aloin Concentration and Production of Plantlets

The higher aloin concentration in plantlets of 566.67 ppm and 513.73 ppm was obtained if neither yeast extract nor sucrose was applied and with sucrose 4.5% (Table 3). Treatment combination 200 $mg.L^{-1}$ yeast extract and 3.0 % sucrose, so that 300 ppm yeast extract and 1.5% sucrose, gave the lowest aloin concentration of 160.26 and 173.34 ppm, respectively. This result was similar to (Ramawat & Merillon, 1999), reff (Carloni, 2013) statement that stress will stimulate mRNA synthesis, producing enzyme which was involved in producing secondary metabolites. So as of (Rudrappa *et al*. 2006) by using biotic elicitor such as dry cell powder of microbial enhancing level peroxide enzyme. Peroxide enzyme is a multi-function enzyme, which is also involved in the secondary metabolite synthesis. According to (shidu, 2010), the formulation of culture medium influences the multiplication, growth and the production of secondary metabolites. It was supported by (Dao *et al*. 2011) that stress increased the production of peroxide enzyme which stimulates the production of secondary metabolites. The media with was sucrose 4.5% dosage of elicitor capable of enhancing 1.39 fold the aloin concentration compared with given sucrose 3.0 % dosage**.**

Table 3: Aloin Concentration of Plantlet (ppm)

Extract Yeast Dosage (ppm)	Sucrose Dosage (%)				Means
	0	1.5	3.0	4.5	
0	566.67 a	230.22 g	370.41 d	513.73 a	372.68
100	282.91 f	345.58 e	442.13 c	261.36 f	332.99

Table 3: Contd.,

200	419.95 c	289.82 f	160.26 h	340.57 e	302.65
300	228.52 g	173.34 h	364.02 d	490.90 b	314.19
Means	374.51	259.74	329.20	490.09	(+)

Means within the same column and row followed by the same letter are not significantly different using Duncan's Multiple Range Test at the α 5%, (+) there are interactions

Reff (Kaswasara *et al*. 2010) reported that yeast extract, salicyclic acid, ascorbic acid, and eugenol could induce and enhance the synthesis of glycyrrhizin. The addition of 53.62 gL^{-1} elicitor could increase the production of glycyrrhizin, 5.22 times higher in comparison to the control culture.

The highest aloin production of plantlets of 0.66 mg was obtained if sucrose 4.5 % was applied in media (Table 4). The media with sucrose 4.5% dosage of elicitor capable of enhancing 1.20 fold the aloin production compared with given sucrose 3.0 % dosage. Treatment combinations of 200 to 300 $mg.L^{-1}$ yeast extract with 1.5 to 3.0 % sucrose, gave lower aloin production of 0.53 mg. This result is similar to (Ramawat & Merillon, 1999; Anonymous, 2010; Khorousschi *et al*., 2011; Sharm *et al*., 2011; Gago *et al*. 2014), reff (Carloni, 2013) statement that stress will stimulate mRNA synthesis, producing enzyme which was involved in producing secondary metabolites. So as of (Rudrappa *et al*. 2006) by using biotic elicitor such as dry cell powder of microbial enhancing level peroxide enzyme. Peroxide enzyme is a multi-function enzyme, which is also involved in the secondary metabolite synthesis. According to (Shidu, 2010), the formulation of culture medium influences the multiplication, growth and the production of secondary metabolites. It was supported by (Dao *et al*. 2011) that stress increased the production of peroxide enzyme which stimulates the production of secondary metabolites.

Table 4: Aloin Production of Plantlet (mg)

Extract Yeast Dosage (ppm)	Sucrose Dosage (%)				Means
	0	1.5	3.0	4.5	
0	0.53 d	0.53 e	0.55 c	0.66 a	0.55
100	0.55 c	0.57 c	0.58 b	0.57 c	0.57
200	0.56 c	0.53 d	0.53 d	0.59 b	0.55
300	0.57 c	0.53 d	0.53 d	0.58 b	0.56
Means	0.55	0.54	0.54	0.59	(+)

Means within the same and row column followed by the same letter are not significantly different using Duncan's Multiple Range Test at the α 5%, (+) there are interactions.

As also reported by (Jeong *et al.* 2013) that the addition of microbe, *Agrobacterium rhizogenesis* could increase biosynthesis secondary metabolite, while in (Rezaei *et al*. 2011) reported that 50 $mg.L^{-1}$ of salicyclic acid increased phenolic compound and taxol content in *Taxus baccata* callus. The work of (Lambert *et al*. 2011) affirmed that methyl jasmonate increased the production of saponin 6 fold, as (Li *et al.* 2011) also reported that the addition of WPS 20 mg. L^{-1} in the media could increase diosgenin content 2.85 fold. This result is in accordance with (Acurero, 2008) report that combination of growth substance 1 mg. L^{-1} 2.4. D and 5 mg. L^{-1} BA or 0.5 mg. L^{-1} 2.4.D and 0,1 mg. L^{-1} cinetina increased aloin production up to 45 µg. g^{-1} in callus of *Aloe vera* L., while in (Riedel *et al*., 2012) stated that the increase of phenolic acid in *Vitis viticola* callus was due to addition of elicitors such as Jasmonit Acid, Salicyclic Acid, Ethepon and Shikimic

Acid.

Correlation of Fresh Weight, Dry Weight, Concentration and Production Aloin Plantlet

A low coeficient correlation negatif - 0.240 was exist between fresh weight and aloin concentration, so that low coeficient correlation negatif – 0.068 between fresh weight and production plantlet. A low coeficient correlation positif 0.276 was exist between dry weight and aloin production plantlet, coeficient correlation higher 0.839 between concentration and production aloin planlets (Tabel 5).

Table 5: Correlation of Fresh Weight, Dry Weight, Concentration and Production Aloin

Correlation Between	Fresh Weight	Dry Weight	Aloin Concentrt	Aloin Production
Fresh weight (g)	1	0.284 0.287	- 0.240 0.370	- 0.068 0.802
Dry weight (g)	0.284 0.287	1	- 0.245 0.361	0.276 0.300
Aloin Concentrt (ppm)	- 0.240 0. 370	- 0.245 0.361	1	0.839** 0.000
Aloin Production (mg)	- 0.068 0.802	0.276 0.300	0.839** 0.000	1

*Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

CONCLUSIONS

- There were interaction effect between yeast extract dosage and sucrose on dry weight and aloin concentration in the plantlets. There were no interaction effect on bud growth age, viability, area leaf and fresh weight plantlet.
- The combination of 300 mgL^{-1} yeast extract with 4, 5 % *sucrose* gave the highest plantlet dry weight while highest aloin concentration was obtained if no yeast extract without sucrose, or no yeast extract combined with 4,5% sucrose respectively. The highest aloin production in the plantlet were obtained if no yeast extract and 4, 5 % sucrose added in the medium. The provision was sucrose 4.5% dosage of elicitor capable of enhancing 1.39 fold aloin concentration and 1.20 fold the aloin production compared with given sucrose 3.0 % dosage.
- A low correlation coefficient of 0.276 was exist between dry weight and aloin production plantlet, coeficient correlation higher 0.839 between plantlet concentration and production aloin plantlet.

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