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The Potential of Biotic Elicitors to Increase Isoflavone Production (Pachyrhizus erosus L.) Callus

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ABSTRACT

Abstract The isoflavones contained in the Pachyrhizus plant (*Pachyrhizus erosus* Linn) have multiple functions, including serving as raw materials for the health/pharmacology or cosmetic industries. Isoflavones are beneficial as antioxidants, and cholesterol reducers and are capable of neutralizing free radicals and delaying aging. Tissue culture techniques are widely used to produce secondary metabolite compounds. Isoflavones can be made in vitro through callus production by tissue culture. By using elicitation techniques with the addition of various glucose concentrations to the culture media (media Murashige and Skoog or Vacin and Went), it is expected to increase the quantity and quality of callus and the content of secondary metabolites (Isoflavones) in Pachyrhizus callus. The research aims to increase the production of Isoflavones in Pachyrhizus callus by adding elicitors with various concentrations of glucose. The research method used a completely randomized factorial design. Factor I: two levels of basic media: M1 (Murashige and Skoog Media) and M2 (Vacin and Went Media). Factor II: Addition of Glucose concentrations with seven levels: 0%, 5%, 10%, 15%, 20%, 25%, 30%. The plant material used was the young leaves of Pachyrhizus. The results of the study showed that (1) all formed callus exhibited compact quality; (2) the addition of 30% glucose concentration resulted in the best growth of callus; (3) the highest Isoflavone content (0.15%) was found in the callus of *Pachyrhizus erosus* L. due to the treatment with both MS and VW media with 20% Glucose Elicitor

1. Introduction

Pachyrhizus erosus L. is a tuberous plant with economic potential and is known to contain nutrients such as vitamin C, Vitamin B complex, fibre, and various minerals. Due to its nutritional content, it is widely used in the food, pharmaceutical, and cosmetic industries. One of the bioactive compounds found in Pachyrhizus is an isoflavone, which has health benefits such as anti-inflammatory properties and potential anti-cancer effects. Isoflavones are also known for their

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antioxidant and antimicrobial properties, and they are commonly used as cholesterol-lowering agents. The increasing use of natural antioxidants is supported by epidemiological studies, which have shown that consuming natural antioxidants can reduce the risk of cardiovascular diseases and cancer. The antioxidant activity in plants is attributed to various metabolites, including flavonoids, isoflavones, anthocyanins, coumarins and carotenoids [1].

Consuming Pachyrhizus offers several benefits, including maintaining skin health and removing dead skin cells. Pachyrhizus is rich in vitamin C, which nourishes the skin. It is also widely used as an ingredient for face masks that refresh and brighten the skin [2]. According to Jaiswal, Chauhan and Lee [3], Pachyrhizus is known for its skin-lightening properties, and it has been traditionally used by communities for generations. Currently, Pachyrhizus is extensively utilized to enhance both health and beauty. In the beauty industry, Pachyrhizus is used to reduce facial wrinkles, as an anti-aging agent, to fade dark spots, and to lighten the skin. However, the natural production of isoflavones from Pachyrhizus is still limited in both quantity and quality.

Therefore, efforts are needed to increase the production of isoflavones from Pachyrhizus. With the advancement of biotechnology, the production of secondary metabolites can be achieved using tissue culture techniques. One of the strategies used to increase the content of secondary metabolites in callus is through elicitation, a process of inducing or stimulating plants to produce higher levels of secondary metabolites, including isoflavones, by using elicitors [4]. Elicitors can be categorized into two groups: abiotic elicitors, which include inorganic chemical compounds such as heavy metals, and biotic elicitors, which are organic compounds such as carbohydrates, proteins, and volatile compounds [4].

Adding biotic elicitors to the tissue culture media can stimulate the plant's defence response and trigger the production of secondary metabolites, including isoflavones [5]. For the pharmaceutical industry, biosynthesis of secondary metabolites using tissue culture techniques has been of significant interest and importance. By using callus, production can be less affected by seasonal changes that often influence secondary metabolite production [6]. The advantages of callus culture include the ability to produce metabolites with higher concentrations compared to those taken directly from conventional propagation. This is because callus culture provides a controlled supply of nutrients and metabolic processes, resulting in better yields [7]. Researchers have reported that callus induced from leaf explants cultured on a medium containing 2.0 mg/L picloram and 0.5 mg/L 6-benzyl adenine (BA) in Murashige and Skoog (MS) media had the highest accumulation of active metabolites, specifically Maac. In vitro, shoots were regenerated on media containing a combination of TDZ and α-Naphthalene acetic acid (NAA). This reliable protocol for mass production of flavonoid-type secondary metabolites using S. flavescens callus culture has shown promising results [8].

In their research, Indarwati *et al.,* [8] reported that manipulation of MS Media with the addition of sucrose elicitors in callus culture successfully increased the anthocyanin content in dragon fruit callus. The addition of sucrose elicitor to MS media resulted in callus formed from young shoots containing the highest anthocyanin content (0.14%) compared to other treatments, while the addition of 5% carbohydrate elicitor callus only contained anthocyanin ranging from 0.06% to 0.09%.

Additionally, Shofiyani [9] adds that tissue culture is an alternative technology that can be used to produce secondary metabolites. Tissue culture methods can be employed to increase the content of metabolic compounds in callus by manipulating the levels of components in the media or adding precursor compounds. Another alternative is to enhance cell productivity maintained in various aseptic artificial media [10]. The utilization of in vitro culture technology, which was previously used for plant breeding and propagation, is now directed toward producing large

quantities of secondary metabolites in a short period. This technology simultaneously addresses the issue of limited land availability while preserving biodiversity balance by avoiding excessive exploitation of germplasm as a source of natural medicines.

In this research, the objective is on the content of isoflavone metabolites in Pachyrhizius erosus L. callus, which will be increased by modifying the basic media and using glucose elicitors. Glucose is a type of sugar that can be utilized as an energy source in plant metabolism. Adding glucose supplements to tissue culture media (MS and VW) is intended to meet the energy needs during callus growth. The research is conducted in vitro, using Pachyrhizus callus as the research subject.

2. Methodology

2.1 Place and Time

For The research was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Wijaya Kusuma University, Surabaya. The research began in early February and lasted until May 2023.

2.2 Materials and Equipment

i. Materials: The materials used in this research include young leaf explants of Pachyrhizus plants (*Pachyrhizus erosus* L), basic media MS and VW, glucose, 70% and 90% alcohol, Clorox, Betadine, aluminium foil, and plastic wrap.
ii. Equipment: The necessary equipment for the research includes a Sartorius balance, autoclave, Laminar Air Flow (LAF) oven, pH meter, tween, scalpel, Erlenmeyer flask, measuring glass, measuring pipette, dropper pipette, petri dish, spatula, culture tubes, and magnetic stirrer.

2.3 Research Method

The research was conducted using a completely randomized factorial design with 2 factors. Factor I include various types of basic media with 3 levels, and factor II includes 7 levels of fructose concentration. Each treatment was replicated 3 times, and each replication consisted of 5 samples. The treatments are as follows:

i. Factor I: Types of Culture Media: (M1) MS Media; (M2) VW Media
ii. Factor II: Glucose Addition Concentration (0%, 5%, 10%, 15%, 20%, 25%, 30%)

2.4 Implementation: Sterilization of Equipment

The used equipment was wrapped in brown paper and sterilized in an oven at 121°C for 30 minutes. Culture tubes were sterilized using an autoclave at 17 psi for 30 minutes. Media Composition: The composition of MS culture media (Table 1) and VW media (Table 2) [8]:

i. Planting: The leaf explants are sterilized with 5%, 10%, and 15% Clorox solutions, each supplemented with one drop of Tween 20. Then, they are cut into approximately 1 cm pieces and soaked in Betadine before being planted in culture tubes containing the respective media according to the treatments.

ii. Incubation: After planting, the tubes are placed on an incubation rack, which consists of incubation stages. The incubation process involves observing and monitoring the callus growth.

2.5 Variables

The following parameters of calyx growth are observed:

i. Callus Quality: Visual observation is conducted once a week, and the callus is scored as follows:
   1 = No callus formation
   2 = Compact callus
   3 = Friable callus
ii. Callus Quantity: Visual observation is also conducted once a week, and the callus is scored as follows:
   1 = No callus formation
   2 = Few calluses (< 1 times the size of the explant
   3 = Moderate callus (1-2 times the size of the explants)
   4 = Abundant callus (> 2 times the size of the explants)
iii. Secondary Metabolite Content in callus: Destructive analysis is conducted on the callus when they are 8 and 12 weeks old. Gas Chromatography is used for the analysis of secondary metabolites. The plant material is extracted using absolute alcohol before the gas chromatography analysis.

Table 1
Composition Of MS Media [10]

Components	Amount (mg/l)
1. Macro-nutrients	
KNO3	1900
H4NO3	1650
CaCl2 2H2O	440
MgSO4 7H2O	370
KH2PO4	170
2. Micro-nutrients	
$MnSO_4 7H_2O$	22.3
$ZnSO_4 7H_2O$	8.6
H3BO	6.2
Kl	0.83
CuSO4 5H2O	0.025
NaMoO4 . 2H2O	0.25
CaCl2 . 6H2O	0.025
FeSO4 . 7H2O	27.8
NaEDTA . 2H2O	37.3
3. Vitamins	
Mio-inositol	8100
Thamin HCl	0,1
Nikotinik acid	0,5
Piridoksin HCl	0,5
Glisin	2
4. Glucose 30.000 (+ Based on the treatments)	

Table 2
Composition of VW Media [10]

Components	Amount (mg/l)
1. Macro-nutrients	
(NH4)2 SO4	500
KNO3	525
MgSO4.7H2	250
KH2PO4	250
2. Micro-nutrients	
Fe3 Tartrat	28,0
Mn SO4.4H2O	7,5
Carbohydrate Glucose 20.000 (+ based on the treatments)	

2.6 Data Analysis

Obtained observational results are subjected to statistical analysis using analysis of variance (ANOVA). If significant differences are found, the analysis will be followed by the Least Significant Difference (LSD) test at 5% significance level (5% BNT).

3. Results

3.1 Callus Quality

The analysis of callus quality revealed significant interactions at 21 and 28 days after planting (DAP) and significant differences in the single factor of glucose addition concentration at 56 and 84 DAP.

From Table 3, it can be observed that during the first week of observation, all the explants did not form a callus (score 1). However, starting from the second week and onwards, the explants planted in the bottles began to form callus. The change in callus quality indicates the growth of the planted material and serves as one of the observation parameters. The size of the callus increases depending on the rate of cell division. Cell division is influenced by various endogenous and exogenous factors. Cell metabolism in tissue culture affects cell/callus growth and the synthesis of various secondary metabolites.

Table 3
Results of Callus Quality Observation Formed under Different Glucose Addition Treatmentsin MS and VW Media

Treatment	Scores for callus quality on different Days After Planting (DAP)											
	7	14	21	28	35	42	49	56	63	70	77	84
MS/0%	1.00	1.13	1.96c	19.6c	2.00	2.03	2.06	2.10	2.13	2.13	2.13	2.17
MS/5%	1.00	1.56	2.06a	2.06a	2.06	2.10	2.10	2.13	2.17	2.17	2.17	2.20
MS/10%	1.00	1.50	2.00b	2.00b	2.00	2.03	2.06	2.10	2.17	2.17	2.17	2.20
MS/15%	1.00	1.50	2.00b	2.00b	2.03	2.06	2.10	2.13	2.17	2.17	2.17	2.20
MS/20%	1.00	1.56	2.00b	2,00b	2.03	2.06	2.10	2.13	2.20	2.17	2.17	2.23
MS/25%	1.00	1.33	2.00b	2.00b	2.03	2.03	2.06	2.13	2.13	2.17	2.20	2.23
MS/30%	1.00	1.26	2.00b	2.00b	2.00	2.03	2.06	2.10	2.13	2.17	2.27	2.30
VW/0%	1.00	1.00	2.00b	2.00b	2.00	2.03	2.06	2.40	2.13	2.13	2.20	2.17
VW/5%	1.00	1.03	2.00b	2.00b	2.00	2.06	2.06	2.10	2.13	2.13	2.20	2.20
VW/10%	1.00	1.00	2.00b	2.00b	2.00	2.06	2.06	2.10	2.13	2.13	2.17	2.20
VW/15%	1.00	1.00	2.00b	2.00b	2.00	2.06	2.10	2.13	2.17	2.17	2.17	2.20
VW/20%	1.00	1.00	2.03b	2.00b	2.00	2.06	2.10	2.13	2.17	2.17	2.17	2.20
VW/25%	1.00	1.10	2.03b	2.00b	2.00	2.06	2.10	2.13	2.17	2.17	2.20	2.23
VW/30%	1.00	1.00	2.00b	2.00b	2.00	2.03	2.03	2.10	2.13	2.17	2.20	2.20
LSD 5%	NR	NR	0.0029	0.029	NR	NR	NR	NR	NR	NR	NR	NR

Note: The numbers accompanied by the same letter indicate no significant difference
NR : No Real Difference
LSD (Least Significant Difference)

All observed callus showed compact callus quality (score 2). Compact callus is characterized by a dense and hard texture. The quality of callus is influenced by the addition of supplementary substances (plant growth regulators, elicitor compounds), the type of explant, and the conditions of the tissue culture environment [11]. The compact callus structure displayed characteristics such as being a dense collection of small cells with large vacuoles, less visible nuclei, and high starch content. Morphologically, the texture is compact, difficult to separate, and the colour of the callus is dominantly lighter.

The results of callus growth observation up to week 4 (28 days after subculture/planting) showed that the treatments had a significant effect on callus quality. However, in the subsequent observations up to 84 days after subculture, the treatments had the same effect on callus growth. The callus quality scores ranged from 2.17 to 2.23, indicating a tendency towards compact callus. For the following observations, it was found that the tested treatments did not significantly affect the callus quality of Pachyrhizus. Additionally, a study on Nyamplung callus tissue culture by Latif, Warnita and Mayerni [12] indicated that compact-textured callus is considered better as it can accumulate more secondary metabolites compared to fragmented and intermediate callus. Similarly, Junairiah *et al.,* [13] stated that compact callus is the most suitable for subculture and secondary metabolite production.

Table 4
The quality of callus formed due to the single-factor treatment of different types of media and various concentrations of glucose elicitor

Treatment	Age (Days After Planting - DAP)				
	7	56	63	77	84
Glucosa Concentration					
0%	1.08	2.10b	2.13b	2.13a	2.17c
5%	1.28	2.12ab	2.15ab	2.18abc	2.20bc
10%	1.27	2.10b	2.13b	2.17bc	2.20bc
15%	1.25	2.13a	2.17a	2.17bc	2.20bc
20%	1.28	2.13a	2.17a	2.18abc	2.22b
25%	1.22	2.12ab	2.15ab	2.20ab	2.23ab
30%	1.13	2.10b	2.13b	2.23a	2.27a
BNT 5%	NR	0.020	NR	0.054	0.044
type of Medium					
MS	1.41a	2.11	2.15	2.19	2.22
VW	1.02b	2.11	2.15	2.18	2.21
LSD 5%	0.126	NR	NR	NR	NR

Note: Numbers followed by the same letter indicate no significant difference
NR : No. Real Difference
LSD (Least Significant Difference)

Table 4 The effect of individual treatments on callus quality growth showed that the addition of glucose concentration resulted in callus with a compact texture, where the addition of 30% glucose tended to produce the highest quality score (2.27), significantly different from the other tested treatments. Shows, all the treatments on the 84-day observation showed callus quality towards compact (with a score around 2). It is suspected that with further observations, the quality score will increase towards a score of 3 (friable callus).

3.2 Quantitative Analysis of Callus

The analysis of variance showed a significant interaction in callus quantity from 21 to 49 days after planting. However, in the subsequent observation at 56 days after planting, there was no longer any interaction between the two tested factors on callus quantity. The average score values of callus quantity are presented in Table 5.

Table 5 shows that the addition of 30% glucose to MS media tends to result in a higher quantity of callus, although not significantly different.

Table 5
Results of callus quantity observation formed by the addition of Glucose in VW and MS media

Treatment	Age (Days After Planting - DAP)										
7		14	21	28	35	42	49	56	63	70	77	84
MS/0%	1.00	1.13	1.93ab	2.00c	2.30f	3.30bc	3.30c	3.30	3.33	3.43	35.3	3.67
MS/5%	1.00	1.56	2.23a	2.30abc	2.80def	3.87a	3.87a	3.87	3.87	3.87	3.87	3.87
MS/10%	1.00	1.50	2.10cb	2.23abc	2,80def	3.80a	3.80ab	3.80	3.87	3.87	3.87	3.87
MS/15%	1.00	1.50	2.10ab	2.23abc	2.70def	3.63ab	3.80ab	3.80	3.87	3.87	3.87	3.87
MS/20%	1.00	1.56	2.23a	2.53a	3.37ab	3.73ab	3.87a	3.87	3.87	3.90	3.93	3.93
MS/25%	1.00	1.33	2.13ab	2.50a	3.53a	3.53ab	3.87a	3.87	3.87	3.90	3.93	3,93
MS/30%	1 . 0	1.26	2.83b	2.07bc	2.40f	3.10c	3.87a	3.90	3.97	3.97	4.00	4.00
VW/0%	1.00	1.03	2.10ab	2.13bc	2.53a	3.33bc	3.50c	3.50	3.53	3.53	3.53	3.67
VW/5%	1.00	1.00	2.16ab	2.17bc	2.70def	3.13c	3.53c	3.73	3.80	3,80	3.80	3.83
VW/10%	1.00	1.03	2.10ab	2.30ab	3.10abc	3.14c	3.53c	3.60	3.67	3.70	3.76	3.76
VW/15%	1.00	1.00	2.06ab	2.10bc	2.93bcde	3.70ab	3. 70b	3.70	3.70	3.70	3.76	3.80
VW/20%	1.00	1.00	2.06ab	2.27abc	3.00bcde	3.67ab	3.67b	3.67	3.67	3.70	3.76	3.80
VW/25%	1.00	1.10	2.03ab	2.43ab	2.93bcde	3.63ab	3.70b	3.73	3.80	3.87	3.90	3.93
VW/30%	1.00	1.00	2.00ab	2.27abc	3.23abc	3.73ab	3.77ab	3.80	3.87	3.87	3.90	3.93
LSD 5%	NR	NR	0.364	0.362	0.512	0.432	0.139	NR	NR	NR	NR	NR

Note: Numbers accompanied by the same letter indicate no significant difference
NR : No. Real Difference LSD (Least Significant Difference)

The effect of the single-factor treatment on callus quantity in observations at 56 to 84 days after planting is presented in Table 6. Table 6 represents the results of the single-factor treatment, where the addition of 30% glucose (G7) resulted in the highest score for callus quantity (3.95), but was not significantly different from the addition of 20% (G5) and 25% (G6) glucose elicitors. Meanwhile, the most suitable media is MS (M1). The culture medium is one of the determining factors for the success of tissue culture techniques. Several formulations of culture media can be used to optimize the growth of plant materials (plantlets).

In tissue culture techniques, several media are often used in their implementation, but Murashige and Skoog (MS) and Vacin and Went (VW) media are relatively good because they provide a rich supply of macro and micronutrients, as well as vitamins for plant growth and development. In the in vitro propagation method, the growth and development of explants are highly influenced by the type of basal media and growth regulators. MS media are commonly used as the basal media for large-scale propagation of plant species. MS basal media are rich in minerals that stimulate callus growth [10]. The addition of certain substances or compounds is often done to induce cell suspension or callus formation.

The growth of callus culture is greatly influenced by the addition of supplementary compounds, growth regulators, explant types, and environmental conditions/media. The increase in callus weight indicates growth and is considered as one of the variables in callus growth observation [14]. The increase in callus quantity score depends on the rate of cell division.

Cell division is influenced by the availability of a source of energy in the culture media. The culture media contains macro and micro nutrients, vitamins, hormones, and carbohydrates as an energy source. Explants grown in the culture media, especially during the early stages of growth, may have a low rate of photosynthesis and, therefore, require additional carbohydrates as an energy source.

The addition of glucose is intended as an additional source of energy. Glucose is a good

carbohydrate source for providing energy in the process of callus growth and development [15]. Glucose is the primary source of energy utilized in cellular metabolic activities, as newly cultured plant materials or explants usually cannot produce their own energy or have a low rate of photosynthesis. Thus, the culture media should contain sufficient carbohydrates as an energy source. Besides serving as an energy source, glucose also functions as an osmotic pressure regulator in the media [16].

Table 6
The quantity of callus formed due to the influence of single-factor treatments, namelythe type of medium and the concentration of glucose elicitor

Treatment	Age (Days After Planting - DAP)			
Glucose Concentration	56	63	77	84
0 %	3.40 b	3.43 c	3.48 c	3.53 c
5 %	3.80 a	3.82 ab	3.82 ab	3.82 b
10%	3.70 a	3.77 b	3.78 b	3.82 b
15%	3.75 a	3.78 ab	3.82 ab	3.82 b
20%	3.77 a	3.77 b	3.82 ab	3.85 ab
25%	3.80 a	3.86 ab	3.88 ab	3.92 ab
30%	3.85 a	3.92 a	3.92 a	3.95 a
LSD :5%	0.171	0.149	0.135	0.106
type of Medium				
MS	3.77	3.77	3.77 a	3.86 a
BW	3.68	3.72	3.74 b	3.70 b
LSD : 5%	0.91	NR	0.072	0.056

Note : Numbers accompanied by the same letter indicate no significant difference.
NR : No Real Difference LSD (Least Significant Difference)

3.3 Isoflavone Content

The results of the analysis of isoflavone content at 56 and 84 days after planting (DAP) are presented in Table 7. The table shows that there is an interaction between the glucose concentration and the type of medium on the isoflavone content in Pachyrhizus callus. The average isoflavone content based on the laboratory analysis is presented in Table 7.

From Table 7, it can be observed that the average isoflavone content at 54 DAP is relatively low, but after reanalysis at 84 DAP, there is an increase in the average secondary metabolite content in the analysed callus. Treatment with 20% glucose elicitor, both in MS and VW media, resulted in higher isoflavone content (0.15%) compared to other treatments.

In their study, Mohaddab *et al.,* [17] explained that biotechnology in callus culture systems can alter the secondary metabolite synthesis pathway by increasing the production of phytochemicals in plants. This method offers an alternative approach to obtaining more secondary metabolites in callus compared to the parent plants. Furthermore, Chandran *et al.,* [18] also highlighted that secondary metabolites from plants can be effectively produced using tissue culture techniques. This application has several advantages, including independence from climate, soil, and geographical location, increased productivity with reduced production costs, and the avoidance of field maintenance expenses.

According to Arigony *et al.,* [19], the formulation of MS and VW media has been widely accepted as a medium for initiation and growth of explants in tissue culture. In line with Park *et al.,* [7], their research reported that cells utilize carbohydrates (sucrose or glucose) as an energy and biosynthesis source, including the biosynthesis of secondary metabolites. Furthermore, Shofiyani [9] reported in their study that sucrose, as a source of energy, plays a role in increasing callus growth

and secondary metabolite content in *Kaemferia galanga*. Treatment with sucrose concentrations of 30-40 g/L increased the Ethyl p-methoxycinnamate content from 1.18 to 2.01 times. Available sucrose carbon sources can be used for callus growth and the formation of secondary metabolites.

In Table 7, it is also evident that the addition of 25% and 30% glucose elicitors resulted in a decrease in Isoflavone content in *Pachyrhizus* callus. In tissue culture, the production of secondary metabolites is related to callus growth, but inhibition of tissue growth leads to an increase in the amount of added glucose. Adding carbohydrates at certain concentrations can increase the production of secondary metabolites; however, when their concentration is further increased, it induces osmotic stress. This hampers the formation of these secondary metabolites. This is consistent with the opinion of Hapsoro and Febrianie [20], who stated that in general, the growth and development of explant cells in vitro will increase with increasing sugar concentration. This increase occurs until the optimal point is reached and then decreases at higher sugar concentrations. Therefore, the addition of sugar to the medium should be done at the appropriate concentration.

Table 7
Average Isoflavone Content Formed in Callus with Glucose Addition to MS and VW Media (%)

Treatment	Isoflavone Content Formed in Callus			
Type of Media/ concentration	56 DAP		84 DAP	
MS/0%	0.04	f	0.06	d
MS/5%	0.06	ef	0.09	cd
MS/10%	0.08	de	0.10	bcd
MS/15%	0.11	abc	0.12	abc
MS/20%	0.12	ab	0.15	a
MS/25%	0.13	a	0.13	abc
MS/30%	0.12	ab	0.12	abc
VW/ 0%	0.08	de	0.10	bcd
VW/ 5%	0.09	cd	0.12	abc
VW/10%	0.10	bcd	0.12	abc
VW/15%	0.11	abc	0.14	ab
VW/20%	0.12	ab	0.15	a
VW/25%	0.13	a	0.13	abc
VW/30%	0.09	cd	0.11	bcd
LSD :5%	0.029		0.044	

Note: The numbers accompanied by the same letter indicate no significant difference
DAP : Days After Planting LSD (Least Significant Difference)

The addition of 20% glucose elicitor to both MS and VW media in *Pachyrhizus erosus* callus resulted in the production of secondary metabolite Isoflavone (0.15%), increasing by 150 % compared to the control in MS media and increasing by 50% in VW media. The use of MS media added with growth regulators is reported to trigger callus growth induction and increase the content of phenol and flavonoid content in *Cnidium officinale* callus [21].

4. Conclusions

The conclusion of this study is the quality of the formed callus tends towards compact callus. The highest quantity of callus was observed in the treatment with 30% glucose elicitor. The analysis of Isoflavone content in *Pachyrhizus erosus* callus showed variation. For the G1 treatment (5% glucose), the Isoflavone content ranged between 0.06% to 0.10%. The Isoflavone content increased

significantly in the G5 treatment (20% glucose) reaching 0.15%. However, the addition of 25% and 30% glucose elicitors (G6 and G7 treatments) decreased the Isoflavone content in *Pachyrhizus erosus* callus to a range of 0.11% to 0.12%. The addition of glucose elicitors to both MS and VW media resulted in the formation of *Pachyrhizus erosus* callus with the highest Isoflavone content of 0.15%. In tissue culture cultivation, the addition of glucose elicitor is recommended to increase the Isovlafon content in *Pachyrhizus erosus* callus.

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