Optimization of Potato Cuttings of Granola Kembang Cultivars with the Application of Auxin and Paclobutrazol for Tuber Production

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Abstract. Every year the need for potatoes continues to increase, both for the industry as processed potatoes and vegetable potatoes. One of the important things in potato cultivation is the use of potato seeds, quality seeds are the key to potato crop productivity. The purpose of this study was to determine the optimal concentration of different auxin and paclobutrazol on the growth of cuttings and the production of potato tubers (tubers for seeds). This study used a Randomized Block Design (RBD) with two treatment factors, namely the concentration of auxin (Rootone F) with three levels and the concentration of paclobutrazol (three levels), and repeated three times. The results showed that additional treatments were able to inhibit plant height at 42 DAP (Day After Planting). The concentration of 200 mg L^{-1} auxin and 100 mg L^{-1} Paclobutrazol were more effective on tuber production. The combination of 200 mg L^{-1} auxin and 100 mg L^{-1} Paclobutrazol treatment resulted in an average number of tubers 12 tubers per plant and tuber classification based on tuber weight obtained an average number of bulbs grade S 6.83, grade M 2.33, and grade L 0.5.

Keywords: Improve seed quality, *in vitro* culture, micropropagation, *Solanum tuberosum* L., tissue culture.

1 Introduction

Potato (*Solanum tuberosum* L.) is the main staple food and is the 4th largest crop grown worldwide [1]. In Indonesia, potatoes are widely used as vegetable potatoes and industrial potatoes. Potato is a plant that is used as a source of carbohydrates and has the potential to diversify food so potato development in Indonesia has a priority. The main obstacle to increasing potato production is the procurement and distribution of quality potato seeds that

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are not continuous, adequate and in the seedling program, the use of pathogen-free or quality seeds capable of producing optimally in tropical countries such as Indonesia, the main obstacle to increasing potato production is the availability and distribution of quality potato seeds due to limited supply [2–4]. The success of potato production lies in the consistent availability of quality potato seeds [5, 6].

The formation of potato tubers is influenced by the balance between stimulating and inhibitory hormones contained in these plants. This comparison can be made by increasing the pusher, reducing the inhibitor, or a combination of both. Auxin is generally known as a vegetative growth-promoting hormone so that plants are encouraged to continue producing lateral organs [7], while to stimulate the rooting process, growth regulators belonging to the inhibitor or retardant group can be used which work to divert carbon and nutrient assimilate to tubers [8]. One example of an auxin growth regulator is Rootone F which is a trademark [9], while according to Desta and Amare [10], a growth regulator included in the inhibitor group is paclobutrazol which has the property of reducing tissue metabolism and can inhibit vegetative growth due to inhibition of gibberellin synthesis and increase the content of cytokinins in stems [11, 12].

Giving auxin and paclobutrazol will form the maximum tuber. Different research was conducted to determine the application of auxin and paclobutrazol on the growth and production of potato cuttings tubers, therefore it is necessary to research the application of different concentrations of auxin and paclobutrazol to optimize the growth of cuttings and increase potato tuber production. This study aims to determine the effect of different treatments (auxin and paclobutrazol) from the control on the production of potato tubers (*Solanum tubero*sum L.) granola Kembang cultivar.

2 Materials and methods

This research was conducted at the Pujon screen house, Batu, Malang with an altitude of 1.200 m above sea level, coordinate map: S7°51'29.2428" and E112°27'51.696". The time of implementation was from July 2017 to November 2017. This research was conducted using a randomized block design with two treatment factors, namely the concentration of auxin (Rootone F): R1 (100 mg L^{-1}); R2 (200 mg L^{-1}); R3 (300 mg L^{-1}); and concentration of Paklobutrazole: P1 (50 mg L^{-1}); P1 (100 mg L^{-1}); and P2 (150 mg L^{-1}). There were nine treatment combinations and one control (not given auxin and paclobutrazol) and each was repeated three times.

Variables observed included plant height, chlorophyll content, number of tubers per plant and, tuber classification based on weight. Observational data were analyzed using Analysis of Variance (ANOVA), to determine whether there was an interaction between factors and the influence of each factor and, HSD-Tukey comparison test was carried out if the treatment seemed to have a significant effect on the results [13, 14]. To determine the difference in the effect between the control and treatment combinations, it was analyzed using the orthogonal contrast test. Presentation of data using tables and figures.

3 Results and discussion

3.1 Results

3.1.1 Plant height

The control and the treatment were significantly different to plant height at 35 DAP and 42 DAP with the control mean value higher than the treatment means, namely 39.78 cm with 32.06 cm and 76.67 cm with 44.58 cm. This proves that the application of paclobutrazol at 30 DAP was able to inhibit the growth of plant height. Plant height with inhibited treatment at 35 DAP (Table 1). The treatment of auxin concentration separately was significantly different to plant height at 7 DAP and 14 DAP. The highest mean value at 7 DAP and 14 DAP, respectively 6.02 cm and 9.61 cm, while at 21 DAP plant height growth was not significantly different from all auxin concentrations. This indicates that auxin acts early in vegetative growth.

Table 1. Effect of auxin and paclobutrazol on plant height.

Tratment	**Plant height (cm) at various ages of observation (DAP)**						
	0 DAP	**7 DAP**	**14 DAP**	**21 DAP**	**28 DAP**	**35 DAP**	**42 DAP**
Orthogonal contrast							
Control	1.92 a	4.24 a	6.50 a	11.50 a	22.67 a	39.78 b	76.67 b
Treatment	2.05 a	5.39 a	8.89 a	14.52 a	22.88 a	32.06 a	44.58 a
Auxin							
100 mg L^{-1}	1.91 a	4.73 a	8.17 a	13.94 a	22.33 a	31.93 a	47.30 a
200 mg L^{-1}	2.03 a	5.43 ab	8.90 ab	14.17 a	22.96 a	32.48 a	43.85 a
300 mg L^{-1}	2.21 a	6.02 b	9.61 b	15.44 a	23.33 a	31.78 a	42.59 a
HSD 5 %	0.31	0.83	1.05	2.09	3.99	6.29	9.88
Paclobutrazol							
50 mg L^{-1}	2.13 a	5.83 a	9.28 a	14.87 a	23.59 a	32.00 a	46.85 a
100 mg L^{-1}	2.03 a	5.36 a	8.71 a	14.15 a	23.78 a	32.63 a	46.30 a
150 mg L^{-1}	1.99 a	5.00 a	8.69 a	14.54 a	21.26 a	31.56 a	40.59 a
Interaction	ns	ns	ns	ns	ns	ns	ns
HSD 5 %	0.31	0.83	1.05	2.09	3.99	6.29	9.88

Note: (ns) = not significant. The number which is followed by similar alphabet in the same column is not significantly different based on Tukey test on level of 5 %.

3.1.2 Chlorophyll content

The combination treatment of auxin and paclobutrazol concentrations was significantly different from the content of chlorophyll a, chlorophyll b and, total chlorophyll (Table 2). The average value of giving auxin and paclobutrazol concentrations to the highest total chlorophyll content was found in the application of 200 mg L^{-1} auxin and 150 mg L^{-1} paclobutrazol concentrations, which was 43.30 mg L^{-1}. It is suspected that the greater the concentration of auxin and paclobutrazol given, the higher the chlorophyll content in potato plants.

Table 2. Effect of auxin and paclobutrazol on chlorophyll content.

Treatment		**Chlorophyll content (mg L^{-1})**		
		Chlorophyll a	**Chlorophyll b**	**Toral chlorophyll**
Orthogonal contrast				
Control		25.48 a	12.02 a	37.45 a
Treatment		25.71 a	13.14 a	38.80 a
Interaction				
Auxin	Paclobutrazol			
100 mg L^{-1}	50 mg L^{-1}	25.57 bc	12.87 bc	38.40 c
100 mg L^{-1}	100 mg L^{-1}	23.68 b	11.21 b	34.84 b
100 mg L^{-1}	150 mg L^{-1}	27.06 c	14.99 d	41.99 d

Continue on the next table.

Table 2. Continue.

Treatment		Chlorophyll content (mg L^{-1})		
		Chlorophyll a	Chlorophyll b	Toral chlorophyll
200 mg L^{-1}	50 mg L^{-1}	26.74 c	14.53 cd	41.22 d
200 mg L^{-1}	100 mg L^{-1}	27.69 d	15.27 e	42.91 d
200 mg L^{-1}	150 mg L^{-1}	27.92 d	15.44 e	43.30 e
300 mg L^{-1}	50 mg L^{-1}	23.58 b	10.79 a	34.33 b
300 mg L^{-1}	100 mg L^{-1}	22.12 a	9.90 a	31.98 a
300 mg L^{-1}	150 mg L^{-1}	27.02 c	13.29 cd	40.26 cd
HSD 5 %		2.05	1.91	2.70

Note: The number which is followed by similar alphabet in the same column is not significantly different based on Tukey test on level of 5 %.

3.1.3 Number of tuber per plant

The variable of the number of tubers produced is one of the important parameters. The combination of auxin and paclobutrazol treatments was significantly different from the number of tubers. The average value of giving concentrations of auxin and paclobutrazol to the highest number of tubers was found in the application of 200 mg L^{-1} auxin and 100 mg L^{-1} paclobutrazol concentrations, which was 12 and the lowest was in the application of 200 mg L^{-1} auxin and 50 mg L^{-1} paclobutrazol concentrations, which was 3 (Table 3).

Table 3. Effect of auxin and paclobutrazol on number of tubers.

Treatment		Number of tubers
Orthogonal contrast		
Control		6.00 a
Treatment		6.56 a
Interaction		
Auxin	Paclobutrazol	
100 mg L^{-1}	50 mg L^{-1}	10.00 f
100 mg L^{-1}	100 mg L^{-1}	7.00 de
100 mg L^{-1}	150 mg L^{-1}	5.00 bc
200 mg L^{-1}	50 mg L^{-1}	3.00 a
200 mg L^{-1}	100 mg L^{-1}	12.00 g
200 mg L^{-1}	150 mg L^{-1}	8.00 e
300 mg L^{-1}	50 mg L^{-1}	4.00 b
300 mg L^{-1}	100 mg L^{-1}	6.00 cd
300 mg L^{-1}	150 mg L^{-1}	4.00 b
HSD 5 %		1.08

Note: The number which is followed by similar alphabet in the same column is not significantly different based on Tukey test on level of 5 %.

3.2 Tuber seed-size classification

Figure 1 shows a bar chart for grading tubers by weight, showing that the combination of 200 mg L^{-1} auxin and 100 mg L^{-1} paclobutrazol had the highest average grade S and M grade compared to other treatments with an average grade S of 6.83 and grade M of 2.33. The potato tubers produced in this experiment were G1 potatoes and produced more S size potato tubers. This indicates that the classification of potato tubers can be easily achieved because class S tubers are expected to produce more second-generation potato tubers.

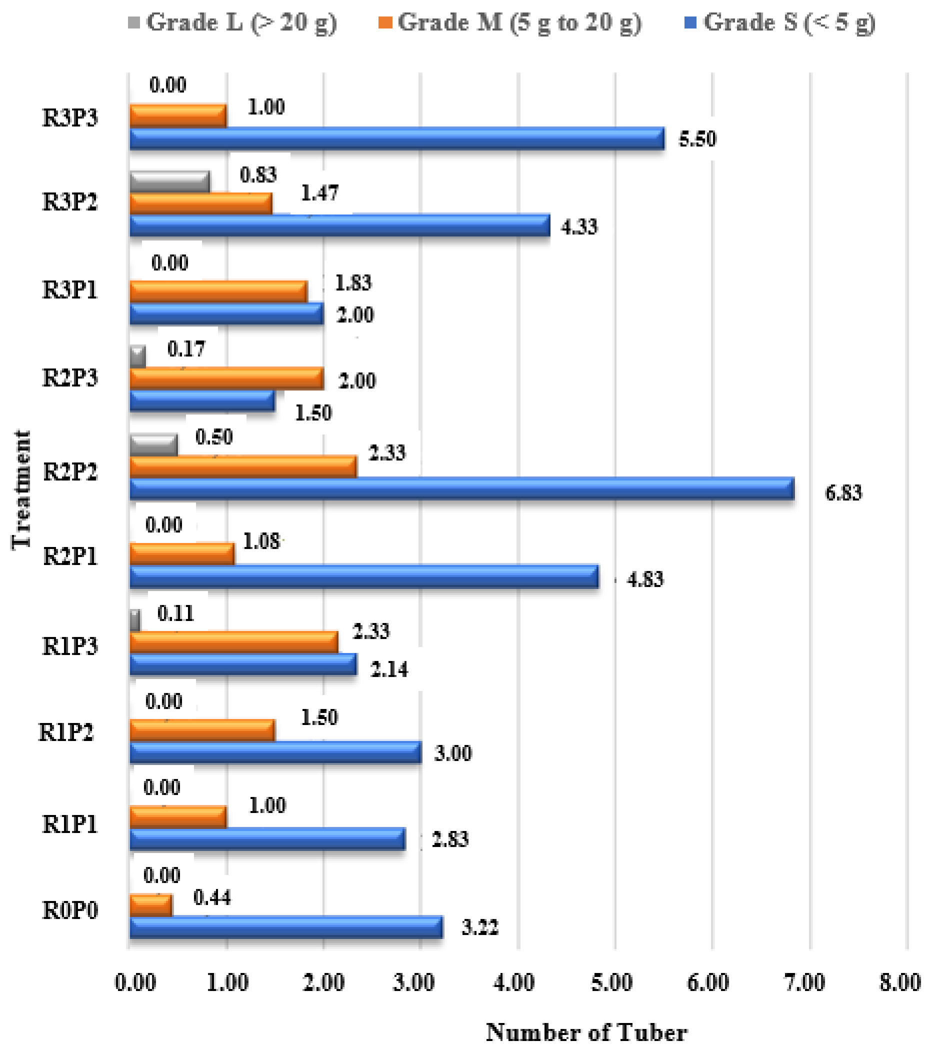

Fig. 1. Classification of tubers based on weight**.**

Note:
R0P0: Without auxin and paclobutrazol (control)
R1P1: 100 mg L^{-1} auxin + 50 mg L^{-1} paclobutrazol
R1P2: 100 mg L^{-1} auxin + 100 mg L^{-1} paclobutrazol
R1P3: 100 mg L^{-1} auxin + 150 mg L^{-1} paclobutrazol
R2P1: 200 mg L^{-1} auxin + 50 mg L^{-1} paclobutrazol
R2P2: 200 mg L^{-1} auxin + 100 mg L^{-1} paclobutrazol
R2P3: 200 mg L^{-1} auxin + 150 mg L^{-1} paclobutrazol
R3P1: 300 mg L^{-1} auxin + 50 mg L^{-1} paclobutrazol
R3P2: 300 mg L^{-1} auxin + 100 mg L^{-1} pacloburazol
R3P3: 300 mg L^{-1} auxin + 150 mg L^{-1} paclobutrazol

3.3 Discussion

Auxins have an important role in plant growth and development processes such as cell division and enlargement, control of apical dominance and fertilization [15]. However, to achieve better crop yields, the use of auxin alone is not sufficient. The activity of gibberellins needs to be inhibited and suppressed with paclobutrazol, which can reduce the content of gibberellins so that plants can focus on generative growth. The results showed that the application of paclobutrazol could inhibit plant height at 42 DAP, as shown in

Table 1. This is following the report of Karmenila *et al.* [16], three potato varieties gave paclobutrazol 75 mg L^{-1} and 125 mg L^{-1} experienced plant height suppression.

Potato cuttings with a weekly application of auxin continued to increase in height, this was because auxin was included in ZPT which could affect plant growth and worked in the early vegetative stages. However, after the application of paclobutrazol at 30 DAP, the height of the control plants was much higher than that of the treated plants. This is presumably because paclobutrazol can inhibit plant growth due to inhibition of gibberellin synthesis. Paclobutrazol affects the isoprenoid pathway and alters plant hormone levels by inhibiting gibberellin synthesis and increasing cytokinin and consequently reducing stem elongation [10]. This is by the report of Nuraini *et al.* [2] potato plants with the application of paclobutrazol at 30 DAP had the smallest plant height.

Applications of auxin and paclobutrazol with higher concentrations resulted in higher chlorophyll content at concentrations of 200 mg L^{-1} auxin and 150 mg L^{-1} paclobutrazol. This is presumably because potato plants that have been applied to paclobutrazol have increased cytokinins. Cytokinins can enhance chloroplast differentiation and chlorophyll biosynthesis, while also preventing chlorophyll degradation [2], and this cannot be separated from the role of paclobutrazol. Paclobutrazol can increase leaf chlorophyll content by diverting the gibberellin pathway, where certain compounds are formed, such as phytol, which are precursors for chlorophyll formation [2].

Plants that were given paclobutrazol showed the color of the leaves and dark green stems, which indicated a fairly high chlorophyll content. Paclobutrazol delays aging and prolongs the green period in *Camelina sativa* (L.) Crantz [17] by increasing endogenous cytokinin levels and promoting chlorophyll formation and, increasing the activity of certain antioxidant enzymes [10]. The application of paclobutrazol with the highest concentration of 150 mg L^{-1} increased the total chlorophyll in the leaves. This was due to the diversion of the reaction of the precursor compound geranyl pyrophosphate, which was caused by the application of paclobutrazol which was supposed to form ent-kaurenoic acid but formed geranyl pyrophosphate which is the precursor compound for chlorophyll synthesis [2].

The application of auxin combined with paclobutrazol showed the best results among other treatments on the number of tubers parameters. The combination of the two resulted in the highest number of tubers per plant, which was 12.00 (Table 3). The role of auxin is very large for plant growth, which regulates shoot formation and differentiation time and/or growth response both quantitatively and qualitatively [18]. Auxin can stimulate the growth and development of the upper shoots of potato plants, while paclobutrazol can accelerate the formation or initiation of potato tubers. Therefore, the application of auxin at the beginning of planting can stimulate vegetative growth optimally, then after optimal growth is stopped by the application of paclobutrazol to stimulate the generative phase, namely the formation of potato tubers. This inhibition makes the generative phase of potato plants faster because energy is accumulated for the formation of branches and roots and thus also reduces the time needed to form potato tubers [19]. Most potato tubers were produced in the combination treatment of 200 mg L^{-1} auxin and 100 mg L^{-1} paclobutrazol. This is in line with the report of Karmenila *et al.* [16] that three potato varieties given paclobutrazol 125 mg L^{-1} gave higher tuber yields than those without paclobutrazol, and Pertiwi *et al.* [20] stated that the application of paclobutrazol at 39 DAP to 42 DAP with a concentration of 67.5 mg $plant^{-1}$ significantly increased the number of potato tubers.

The combination treatment of 200 mg L^{-1} auxin and 100 mg L^{-1} paclobutrazol showed good results compared to other treatments for the number of tuber classes. This treatment can produce tubers with the most class S, which is 6.83. This is following the report of Pertiwi *et al.* [20] that the application of 67.5 mg of paclobutrazol $plant^{-1}$ resulted in the highest number of tubers with the smallest class (< 80 g). In potato seedlings, the number of tubers that can be produced is an important indicator. The larger the potato tubers, the less the number of tubers that can be produced, so class S is an indicator of the success of potato seed tuber production. In addition, according to Nuraini *et al.* [2] the size of potato seed tubers belonging to class S is a suitable size to increase production because shoots appear slowly, but the root system is faster.

4 Conclusion

One factor in accelerating plant growth is by giving it growth regulators and combining them with growth inhibitors will induce generative growth. In conclusion, the application of auxin to potato cuttings can promote the growth of potato plants properly so that the vegetative phase can be optimal. To accelerate the generative phase of potato plants, paclobutrazol was given at 30 DAP to shorten the cultivation time. The combination of 200 mg L^{-1} auxin treatment and 100 mg L^{-1} paclobutrazol produced the most class S potato tubers so it was very helpful in potato seed production.

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