



Research article

Effects of cytokinin and paclobutrazol application time on growth and yield of G2 potato (*Solanum tuberosum* L.) Medians cultivar at medium altitude in Indonesia

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Abstract

Many efforts have been made to optimize the growth of potato plants at medium altitude, including the addition of the growth regulators cytokinin and paclobutrazol, which regulate the balance of sources and sinks. This study examined the effect of the application times of cytokinin and paclobutrazol on the growth and yield of G2 potato at low and medium altitude. An experiment was carried out in the Ciparanje Experimental Garden, Jatinangor, at 685 m above sea level in Inceptisol soil. The experiment used a randomized complete block design with the following treatments: no cytokinin or paclobutrazol (control), only cytokinin applied at 30 d after planting (DAP), paclobutrazol applied at 30 DAP, combinations of cytokinin applied at 20 DAP, 30 DAP and 40 DAP and paclobutrazol applied at 40 DAP, 50 DAP and 60 DAP. The concentrations were 100 ppm for both cytokinin and paclobutrazol. The results showed that the application of cytokinin at 30 DAP and paclobutrazol at 40 DAP produced the highest numbers of G2 seed tubers (Class S<40 g: 84.36 ± 8.93) but resulted in a statistically comparable tuber weight per plant (349.25 ± 68.65 g) compared with the control (283.33 ± 31.47 g). High tuber weights per plant were obtained with the application of only cytokinin at 30 DAP (450.28 ± 13.90 g), the combination of cytokinin at 20 DAP + paclobutrazol at 40 DAP (483.33 ± 47.14 g) and the combination of cytokinin at 30 DAP + paclobutrazol at 50 DAP (478.72 ± 42.76 g).

Introduction

In tropical countries such as Indonesia, Malaysia and the Philippines, the main obstacle to increasing potato production is the availability and distribution of quality seed potatoes because the supply has not been continuous or sufficient due to potato cultivation being limited to the highlands (Hamdani et al., 2018; Gonzales et al., 2016). However, highland potato plantations cause environmental damage, which is why it is necessary to expand plantations to lower lands. Indonesia has a vast amount of land available at medium altitude with very good

prospects for seed potato production; nevertheless, there are many obstacles to overcome, especially the high temperatures (Duaja, 2012; Hamdani et al., 2018; Suradinata et al., 2019).

Temperatures can reach 35°C during the day and 24°C at night on the medium-altitude plains in Indonesia, which inhibit the growth of potatoes. High temperatures stimulate an increase in the hormone gibberellin, which delays and slows the formation process. As a result, vegetative and generative growth of potato plants is often stunted or slow (Duaja, 2012; Hamdani et al., 2018). One of the efforts to optimize potato growth in these areas involves the addition of plant growth regulators (PGRs). PGRs are expected to help overcome environmental obstacles and regulate growth balance to ensure an optimal number and size of potatoes as seed tubers (Hamdani et al., 2018; Dewi

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and Darussalam, 2018). This process utilizes cytokinin or ethylene as PGRs (Abelenda and Prat, 2013; Mubarok et al., 2019a; Rosniawaty et al., 2020) and paclobutrazol as an anti-gibberellin (Hatamifar and Samani, 2017).

A cytokinin is an adenine-derived compound that contributes to the regulation of cell division and morphogenesis and it is also used to stimulate the formation of shoots, influence cell metabolism and stimulate dormant cells to encourage cell division. Benzyl amino purine (BAP) is one example of a cytokinin (Aryakia and Hamidoglu, 2010; Mubarok et al., 2020). The cytokinin needs to be applied to potato plants to accelerate growth in the early vegetative phase during the leaf development stage, followed by the use of inhibiting hormones to accelerate the growth and development of tubers and to focus their energy on tuber formation (Hamdani et al., 2016).

However, the use of cytokinin alone is not enough. Retardants or growth inhibitors are also needed to inhibit and suppress the activity of gibberellins so that the energy can be focused on forming potato tubers (Nuraini et al., 2018). Suppressing gibberellin activity accelerates the formation of tubers and ultimately increases their production (Kolachevskaya et al., 2019). This process is one way of increasing the growth and yield of G2 potato seed tubers. However, retardants can affect the physiological properties of plants. Using the right timing for retardant application on certain types of plants produces satisfactory plant growth and development. For example, one commonly used retardant is paclobutrazol, which can inhibit stem elongation, cause stunted growth, inhibit the synthesis of gibberellins and accelerate the formation of potato tubers (Hamdani et al., 2016).

Potato plants have five phases of growth: sprouting initiation and emergence at 0–15 d, leaf development at 16–30 d, tuber initiation at 31–45 d, tuber filling at 45–90 d and maturity and harvesting at 91–120 d (Bijma et al., 2016). Changes that occur in the development phase of tubers include the formation of tuber peel, maximization of the dry weight of the tubers and the top of the plant becoming yellowish and dying. One problem in the field is that it is not yet known when it is best to apply cytokinin and paclobutrazol to achieve the best results. Therefore, the aim of this study was to examine the effect of cytokinin and paclobutrazol application times on the growth and yield of the G2 potato cultivar Median on medium-altitude land.

Materials and Methods

The experiment was carried out in the Jatinangor Experimental Garden, Sumedang, West Java, which has an altitude of 685 m above sea level and an Inceptisol soil. The experiment used a randomized complete block design with the following treatments: no cytokinin or paclobutrazol (control), cytokinin applied at 30 d after planting (DAP) without paclobutrazol, no cytokinin and paclobutrazol applied at 30 DAP, cytokinin applied at 30 DAP and paclobutrazol applied at 40 DAP, cytokinin applied at 40 DAP and paclobutrazol applied at 50 DAP, cytokinin applied at 20 DAP and paclobutrazol applied at 40 DAP, cytokinin applied at 30 DAP and paclobutrazol applied at 50 DAP and cytokinin applied at 40 DAP and paclobutrazol applied at 60 DAP. Each treatment was repeated three times for a total of 27 experimental units.

Each experimental unit consisted of 10 plants in polybags with a total of 270 polybags. The polybags were 50 cm × 50 cm and had

15 kg of growing medium mixed with compost. They were arranged with a spacing of 60 cm × 40 cm. Fertilizer was applied in accordance with the recommendations of the Lembang Vegetable Research Institute: 20 t/ha of manure totaling 600 g per plant mixed with growing media, 300 kg/ha of nitrogen fertilizer (46% N) applied at planting and at 30 DAP and 150 kg/ha of superphosphate-36 fertilizer (36% P₂O₅) and 100 kg/ha of KCl fertilizer (60% K₂O) given at the time of planting. First-generation potato seed tubers of the Medians cultivar (G1) with a weight of 20–30 g per tuber were planted at a depth of 5–7 cm. Furthermore, 3% carbofuran insecticide was spread around the seed tubers at 37.5 kg/ha to protect against insects and other soil pests.

An ultraviolet plastic shade was applied prior to planting by constructing a shade building with 30% shading. Benzyl aminopurine (BAP) was applied as a spray on potato plants at the time of treatment with a concentration of 100 parts per million (ppm). Paclobutrazol was also applied at the time of treatment with a concentration of 100 ppm and a spray volume of 15 mL per plant (Hamdani et al., 2009). Supplementary nitrogen fertilization was applied at 30 DAP. Pest and disease control were applied by spraying 80% mancozeb fungicide with a concentration of 2 g/L and deltamethrin insecticide with a concentration of 2 mL/L at 25 g/L, which was done in accordance with the intensity of pest and disease attacks. The plants were harvested at 89 DAP, after which the stems and leaves turned yellow and fell out and the skins were no longer peeling.

The data collected during the experiment were the endogenous gibberellin levels, chlorophyll content, plant height, leaf area, dry weight of plants, shoot and root ratio, number of tubers per plant, weight of tuber per plant and percentage of tuber seeds. The endogenous gibberellin and chlorophyll contents were analyzed according to the method described by Okamoto et al. (2009) and Hendry and Grime (1993), respectively. The endogenous gibberellin, chlorophyll content, plant height, leaf area, dry weight of plants and shoot and root ratio were analyzed at 75 DAP. The number of tubers per plant, tuber weight per plant and percentage of tuber seed tubers were analyzed at harvest.

The percentages of tuber seed tubers were classified into three groups: S (< 40 g), M (40– 90 g) and L (90–120 g). All treatments were repeated three times. Plant samples were taken from four plants per replication to analyze all of the plant characteristics. Therefore, the number of samples was 12 plants per treatment with three replications.

Statistical analysis

A two-factor analysis of variance was conducted to analyze the data, followed by Duncan's multiple range test to compare differences among treatments. The difference was considered significant at $p < 0.05$.

Results

Endogenous gibberellin content and chlorophyll content

The endogenous gibberellin content of the potato plants was observed with paclobutrazol applied at different times. The cytokinin application at 30 DAP and the control conditions resulted in high values

of gibberellin content. Paclobutrazol application at 60 DAP resulted in a low gibberellin content compared to the early paclobutrazol treatment (Table 1). However, it should be noted that the data on gibberellin content were collected without replications and hence they were not statistically compared. The statistical analysis showed that the application times of cytokinin and paclobutrazol significantly affected the leaf chlorophyll content. The chlorophyll content was significantly improved compared to the control by the application of cytokinin at 20 DAP + paclobutrazol at 30 DAP and by cytokinin at 20 DAP + paclobutrazol at 40 DAP (Table 1).

Plant height, leaf area, dry weight and shoot-root ratio

The results showed that the application of cytokinin and paclobutrazol resulted in a reduction in plant height. The highest plant height occurred in the untreated plants (control), which was not significantly different from the results with cytokinin at 40 DAP + paclobutrazol at 60 DAP. The application of paclobutrazol at 30 DAP without cytokinin or with cytokinin at 20 DAP resulted in the lowest plant height (Table 3). The highest leaf area and plant dry weight were found with cytokinin at 30 DAP + without paclobutrazol, but the results were not significantly different from the control group. No significant difference was found among treatments for the shoot-root ratio (Table 2).

Number and weight of tubers per plant

The tuber number per plant and tuber weight per plant were considered as the yield components of the potato plants. The application of cytokinin at 30 DAP and paclobutrazol at 40 DAP resulted in

higher numbers of tubers per plant compared to cytokinin at 30 DAP + paclobutrazol at 50 DAP, but the result was not significantly different from the control group. The application of cytokinin and paclobutrazol improved the weight of potato tubers per plant, but some of the treatments (paclobutrazol at 30 DAP + without cytokinin, paclobutrazol at 30 DAP + cytokinin at 20 DAP and paclobutrazol at 40 DAP with cytokinin at 30 DAP) did not improve the tuber weight per plant, with the results being comparable to those of the control (Table 3).

Tuber seed-size classification

The statistical analysis showed that the application of cytokinin at 30 DAP and paclobutrazol at 40 DAP produced the highest number of class S tuber seeds, and they were significantly bigger than in the control group. However, it did not have an effect on the class M and L tuber seeds (Table 4). G1 potato tubers were used in this experiment, which produced a higher number of S-sized potato tubers. This indicated that potato tuber classification could be easily achieved since S-class tubers are expected to produce a higher number of second-generation potato seed tubers (G2).

Discussion

Cytokinin plays a role in budding stimulation, with its main activity being to promote cell division (Karjadi and Buchory, 2008). However, to achieve the desired results, the use of cytokinin alone is not enough. The activity of gibberellins needs to be inhibited and suppressed by paclobutrazol, which can reduce the gibberellin content. The results showed that the gibberellin content of paclobutrazol-treated plants was mostly low (0.00110–0.0210%), as shown in Table 1.

Table 1 Effect of cytokinin and paclobutrazol on gibberellin and chlorophyll content

Application time	GA ₃ content (mg/g fresh weight)	Chlorophyll content (index)
Control	0.0512	27.94 ± 4.42 ^{ab}
Cytokinin at 30 DAP + Without paclobutrazol	0.0621	24.80 ± 7.00 ^a
Without cytokinin + Paclobutrazol at 30 DAP	0.0210	33.06 ± 3.72 ^{bc}
Cytokinin at 20 DAP + Paclobutrazol at 30 DAP	0.0194	35.14 ± 3.56 ^c
Cytokinin at 30 DAP + Paclobutrazol at 40 DAP	0.0161	33.10 ± 9.04 ^{bc}
Cytokinin at 40 DAP + Paclobutrazol at 50 DAP	0.0141	26.34 ± 2.09 ^{ab}
Cytokinin at 20 DAP + Paclobutrazol at 40 DAP	0.0139	35.88 ± 6.77 ^c
Cytokinin at 30 DAP + Paclobutrazol at 50 DAP	0.0125	28.62 ± 2.70 ^{abc}
Cytokinin at 40 DAP + Paclobutrazol at 60 DAP	0.0110	23.06 ± 2.74 ^a
Coefficient of variation (%)	-	11.5

DAP = days after planting

Mean values ± SD in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$).

Table 2 Effect of cytokinin and paclobutrazol on plant height, leaf area, plant dry weight, shoot and root ratio

Application time	Plant height (cm)	Leaf area (cm ²)	Plant dry weight (g)	Shoot-root ratio
Control	83.67 ± 4.33 ^d	6423.60 ± 1137.04 ^{cd}	38.09 ± 8.42 ^{abc}	11.88 ± 6.52 ^a
Cytokinin at 30 DAP + Without paclobutrazol	74.67 ± 6.01 ^c	7609.20 ± 1413.21 ^d	45.26 ± 9.20 ^c	14.62 ± 9.29 ^a
Without cytokinin + Paclobutrazol at 30 DAP	53.44 ± 1.90 ^a	4969.53 ± 573.59 ^{ab}	34.85 ± 3.86 ^{ab}	9.67 ± 6.10 ^a
Cytokinin at 20 DAP + Paclobutrazol at 30 DAP	55.00 ± 1.46 ^a	5515.65 ± 711.24 ^{abc}	33.35 ± 3.62 ^a	9.50 ± 3.82 ^a
Cytokinin at 30 DAP + Paclobutrazol at 40 DAP	70.89 ± 0.51 ^{bc}	5463.36 ± 643.66 ^{abc}	43.44 ± 6.42 ^{bc}	9.43 ± 4.08 ^a
Cytokinin at 40 DAP + Paclobutrazol at 50 DAP	70.56 ± 6.54 ^{bc}	6115.73 ± 980.49 ^{bc}	38.96 ± 6.00 ^{abc}	11.83 ± 8.77 ^a
Cytokinin at 20 DAP + Paclobutrazol at 40 DAP	66.06 ± 4.59 ^b	5584.95 ± 210.47 ^{abc}	36.17 ± 3.51 ^{abc}	11.61 ± 4.05 ^a
Cytokinin at 30 DAP + Paclobutrazol at 50 DAP	71.89 ± 2.46 ^{bc}	4598.58 ± 377.11 ^a	36.00 ± 3.56 ^{abc}	13.21 ± 9.60 ^a
Cytokinin at 40 DAP + Paclobutrazol at 60 DAP	77.78 ± 3.67 ^{cd}	5572.88 ± 691.95 ^{ab}	37.31 ± 0.79 ^{abc}	14.17 ± 3.53 ^a
Coefficient of variation (%)	1.94	8.93	8.94	13.05

Mean values ± SD in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$).

Table 3 Effect of cytokinin and paclobutrazol on tuber number and tuber weight

Application time	Tuber number per plant	Tuber weight per plant (g)
Control	8.75 ± 2.18 ^{ab}	283.33 ± 31.47 ^a
Cytokinin at 30 DAP + Without paclobutrazol	10.17 ± 1.80 ^{ab}	450.28 ± 13.90 ^c
Without cytokinin + Paclobutrazol at 30 DAP	10.64 ± 2.36 ^{ab}	373.89 ± 10.52 ^{abc}
Cytokinin at 20 DAP + Paclobutrazol at 30 DAP	9.08 ± 2.75 ^{ab}	328.03 ± 82.02 ^{ab}
Cytokinin at 30 DAP + Paclobutrazol at 40 DAP	14.83 ± 5.11 ^b	349.25 ± 68.65 ^{ab}
Cytokinin at 40 DAP + Paclobutrazol at 50 DAP	10.11 ± 2.31 ^{ab}	402.00 ± 27.54 ^{bc}
Cytokinin at 20 DAP + Paclobutrazol at 40 DAP	11.17 ± 3.69 ^{ab}	483.33 ± 47.14 ^c
Cytokinin at 30 DAP + Paclobutrazol at 50 DAP	7.39 ± 2.84 ^a	478.72 ± 42.76 ^c
Cytokinin at 40 DAP + Paclobutrazol at 60 DAP	9.56 ± 1.89 ^{ab}	408.11 ± 68.76 ^{bc}
Coefficient of variation (%)	3.83	3.02

Mean values ± SD in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$).

Table 4 Effect of cytokinin and paclobutrazol on percentage of tuber seed classification

Application time	Percentage of tuber seed classification		
	S (< 40 g)	M (40–90 g)	L (91–120 g)
Control	53.57 ± 7.79 ^{ab}	27.00 ± 9.53 ^{ab}	14.56 ± 4.78 ^{ab}
Cytokinin at 30 DAP + Without paclobutrazol	57.58 ± 6.05 ^{ab}	34.13 ± 9.96 ^b	11.17 ± 0.67 ^{ab}
Without cytokinin + Paclobutrazol at 30 DAP	64.71 ± 9.33 ^{bc}	23.02 ± 9.90 ^{ab}	8.57 ± 7.50 ^{ab}
Cytokinin at 20 DAP + Paclobutrazol at 30 DAP	65.97 ± 9.86 ^{bc}	19.73 ± 8.28 ^{ab}	14.30 ± 6.08 ^{ab}
Cytokinin at 30 DAP + Paclobutrazol at 40 DAP	84.36 ± 8.93 ^c	9.62 ± 6.85 ^a	6.02 ± 5.10 ^a
Cytokinin at 40 DAP + Paclobutrazol at 50 DAP	55.10 ± 12.19 ^{ab}	32.23 ± 7.89 ^{ab}	12.67 ± 8.70 ^{ab}
Cytokinin at 20 DAP + Paclobutrazol at 40 DAP	60.42 ± 12.48 ^{abc}	26.89 ± 7.56 ^{ab}	12.68 ± 6.26 ^{ab}
Cytokinin at 30 DAP + Paclobutrazol at 50 DAP	38.74 ± 10.74 ^a	37.40 ± 7.02 ^b	23.86 ± 8.67 ^b
Cytokinin at 40 DAP + Paclobutrazol at 60 DAP	64.31 ± 4.53 ^{bc}	23.01 ± 5.71 ^{ab}	12.68 ± 6.50 ^{ab}
Coefficient of variation (%)	3.84	12.11	13.23

Mean values ± SD in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$).

Earlier application of cytokinin and paclobutrazol resulted in a higher chlorophyll content due to the increase in cytokinin enhancing chloroplast differentiation and chlorophyll biosynthesis, while also preventing the degradation of chlorophyll. Therefore, early application of cytokinin increases the chlorophyll content (Wilkinson and Richard, 1991). The application of paclobutrazol can increase the leaf chlorophyll content by diverting the gibberellin pathway, in which certain compounds are formed, such as phytol, which is a precursor of chlorophyll formation. Researchers have reported hormonal regulation of the chlorophyll content in plants; for example, gibberellin and cytokinin inhibit chlorophyll degradation, while ethylene promotes chlorophyll degradation (Mubarok et al., 2019b, c).

The use of anti-gibberellin compounds is known to increase the cytokinin levels in potato leaves, soybeans and *Dianthus caryophyllus* (Youssef and Abd El-Aal, 2013). The increase in chlorophyll and delayed senescence in potato leaves that were given paclobutrazol were closely related to the effect of paclobutrazol in stimulating endogenous cytokinin hormones. Increased cytokinin spurs chloroplast differentiation and chlorophyll biosynthesis while also preventing chlorophyll degradation. In addition, endogenous cytokinin can delay senescence at the cellular and tissue levels.

Cytokinin maintains the integrity of the tonoplast membrane, thus blocking the activity of enzymes that degrade chlorophyll (chlorophyllase), which ensures that the cytoplasm can change several aspects of cellular metabolism (Taiz and Zeiger, 2006). This includes the continuous absorption and translocation of solute to the parts in need to maintain tissue freshness. This phenomenon may be due to gibberellin, cytokinin, abscisic acid and chlorophyll having the same precursors, namely geranylgeranyl pyrophosphate, which is part of the

gibberellin pathway (Taiz and Zeiger, 2006).

The increase in chlorophyll was due to the diversion of the reaction of the geranylgeranyl pyrophosphate precursor compound. This was caused by the application of paclobutrazol, which should form ent-kaurenoic acid. This process was inhibited by paclobutrazol so that phytol pyrophosphate formed instead, which is a precursor compound for chlorophyll synthesis. This caused an increase in total leaf chlorophyll. Gibberellins have the same precursor compound as cytokinin: isophentenyl pyrophosphate (Chaney, 2004). Inhibition of the gibberellin synthesis by paclobutrazol impacts cytokinin synthesis. Endogenous cytokinin plays a role in stimulating chlorophyll synthesis and causes an increase in chlorophyll content.

The effect of paclobutrazol on the reduction of leaf area was detected in only the treatments without cytokinin. A similar result was reported in Mariana and Hamdani (2016). Another study reported that paclobutrazol application could reduce the leaf area index (Burrow, et al., 1992). Earlier application of paclobutrazol results in a smaller leaf size, which also means that the plants without paclobutrazol treatment will always have a bigger leaf area.

The application of cytokinin with or without paclobutrazol did not increase the leaf area of potato plants. The current study showed that the application of cytokinin was not able to stimulate leaf division and enlargement. Taiz and Zeiger (2006) stated that cytokinin in leaves stimulates the division and enlargement of young leaf cells to their normal size, which in turn increases leaf area (Taiz and Zeiger, 2006).

The application of cytokinin, paclobutrazol or their combinations significantly affected the reduction in plant height except for cytokinin at 40 DAP + paclobutrazol at 60 DAP. Aryakia and Hamidoghly (2010) stated that cytokinin could play a role in growth stimulation, which

affects the cell division, induction and production of potato plants, especially for shoot development. Cytokinin can stimulate shoot development of the upper part of the potato plant, while paclobutrazol can accelerate potato tuber initiation. Therefore, cytokinin can stimulate upper vegetative growth at the beginning of planting, and then after optimal growth, it is stopped by paclobutrazol to stimulate the formation and initiation of potato tubers. The inhibition makes plants reach their formation phase faster since energy is accumulated to grow branches and roots and thus also decreasing the time needed to form potato tubers (Nuraini et al., 2016; Hamdani et al., 2018).

Cytokinins are adenine-derived compounds and play a role in the regulation of cell division and morphogenesis by stimulating the formation of shoots, influencing cell metabolism and stimulating dormant cells, and their main activity is to spur cell division (Karjadi and Buchory, 2008). According to Karjadi and Buchory (2008), cytokinin tissue culture plays a role in spurring cell or tissue division for explants and stimulates the development of shoots.

According to Lienargo et al. (2014), the use of paclobutrazol as a retardant shortens plant segments. Paclobutrazol inhibits the synthesis of gibberellins, which play a role in cell elongation. Gibberellin synthesis inhibitors still allow plant cells to divide, but the new cells do not experience elongation. Paclobutrazol is used to regulate plant growth patterns and to balance vegetative and generative growth in order to suppress competitive resource use (Serly et al., 2013).

Sambeka et al. (2012) showed that the application of paclobutrazol at a concentration of 125 ppm on leaves and roots at 42 DAP can inhibit the height and growth of potato plants, but the weights are significantly greater. This is caused by the smooth photosynthesis process, which produces high amounts of chlorophyll. Furthermore, Sambeka et al. (2012) stated that the application of paclobutrazol before 7 wk after planting suppressed vegetative growth and increased the potato yield, since in this phase, it was possible to inhibit the gibberellins contained in the apical meristem.

The application of paclobutrazol, cytokinin and their combinations at different plant stages did not affect the shoot-root ratio and the number of tubers per plant. The results of these treatments were not significantly different from the control. However, a significant effect of these treatment was observed in the tuber weight per plant with the application of paclobutrazol at the filling stage (40 DAP, 50 DAP and 60 DAP), as shown in Table 3. Duaja (2012) stated that if the vegetative phase is at its maximum point, growth will increase sharply due to decreased competition between plant parts in the distribution of assimilates. Therefore, the growth rate will continue to increase until reaching the maturation phase. This showed that higher weights are gained by applying paclobutrazol during the tuber-filling stage.

In addition, competition between plant organs utilizing photosynthesis also decreases, causing the growth rate to be higher than for an early paclobutrazol treatment, which resulted in higher amounts of production. This occurred because the number of potatoes per plant is directly proportional to the number of stolons formed. A high amount of gibberellin will spur a high formation rate of stolons since gibberellins can stimulate the development of new stolon cells. This was consistent with the observation of a higher gibberellin content in

potato plants that were given paclobutrazol at the beginning of the growth phase.

The use of paclobutrazol allows for a direct approach to growth control by inhibiting the synthesis of gibberellins in ent-kaurenoic acid oxidation (Mahoney et al., 1998). Paclobutrazol can effectively regulate plant growth by inhibiting growth and increasing the yield and quality of potato tubers (Esmaelpour et al., 2011). The application of paclobutrazol resulted in low potato-plant heights but provided the highest tuber weight per plant with the application of cytokinin at 20 DAP and paclobutrazol 40 DAP, as well as cytokinin at 30 DAP and paclobutrazol 50 DAP. The resulting mean weights \pm SD were 483.33 ± 47.14 g and 478.72 ± 42.76 g, respectively (Table 3). These results corresponded with previous study on potato plants (Hamdani et al., 2016).

The application of paclobutrazol at 30 DAP and its combination with cytokinin at 20 DAP resulted in a lower dry weight of plants. Wilkinson and Richard (1991) stated that paclobutrazol inhibits the activity and biosynthesis of gibberellins, thus inhibiting the cell elongation process, which ultimately shortens vegetative growth and indirectly diverts photosynthate to reproductive growth. Therefore, plants that are not inhibited by paclobutrazol will produce a higher dry weight. On the other hand, the application of paclobutrazol combined with cytokinin or cytokinin alone (but combined with cytokinin 20 DAP) did not affect plant dry weight. Cytokinin stimulates bud formation, influences cell metabolism, stimulates dormant cells and encourages cell division (Karjadi and Buchory, 2008), so the negative effect of paclobutrazol in inhibiting plant growth could be reduced by cytokinin application.

The application time for a combination of cytokinin and paclobutrazol did not have an effect on the percentage of class M and L tubers, but it affected S-class tubers. The size of potato seed tubers belonging to class S was a suitable size to increase production because the shoots appear slowly, but the root system propagates faster. The percentage of class S seed tubers was higher than for classes M and L. The high percentage of seed tubers with size S is suitable for programs for providing seed potatoes. Adiyoza et al. (2014) reported that potato farmers in Indonesia prefer to use seed potatoes of size S (< 40 g) rather than those weighing more than 40 g.

In conclusion, all the results showed that the application of paclobutrazol combined with cytokinin at the beginning of the leaf development stage (20 DAP) produced high chlorophyll content. The application of paclobutrazol during the tuber filling stage (40 DAP, 50 DAP and 60 DAP) combined with cytokinin improved the tuber weight per plant. However, the plant height, leaf area, dry weight, shoot and root ratio, tuber number per plant, and percentage of M and L seed tubers were not affected by the application of paclobutrazol, cytokinin or their combinations.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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