

## Research Article

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# Paclobutrazol and benzylaminopurine improve potato yield grown under high temperatures in lowland and medium land

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**Abstract:** Potato is one of the most important vegetable crops in the world. In a tropical country such as Indonesia, potato is cultivated in highland that has low temperature. However, the extensification and intensification of potato cultivation cause environmental problems in the highland. Soil erosion is one of the common problems resulting from potato cultivation that applies heavy tillage. To prevent environmental problems, the land expansion for potato cultivation is carried out in both the medium and lowland. High temperature in both medium and lowland results in the limitation of plant growth and yield. Therefore, the present study aimed to evaluate the effect of plant growth regulators (PGRs), namely paclobutrazol and benzylaminopurine (BAP) application on the growth and yield of potatoes grown under high temperature in both lowland and medium land. A split-plot design was used in this experiment with the main plot as growing altitude, i.e., low and medium land, and the PGR treatments as the subplot, i.e., control, paclobutrazol at  $100 \text{ mg L}^{-1}$ , BAP at  $50 \text{ mg L}^{-1}$ , and a combination of paclobutrazol at  $100 \text{ mg L}^{-1}$  and BAP at  $100 \text{ mg L}^{-1}$ . The result showed a reduction in plant growth and yield in potatoes grown in lowland compared to those in medium land. The application of paclobutrazol and BAP improved the number, the weight of tuber, and its starch content. The result indicated that the application of paclobutrazol and BAP could be used to solve an environmental limitation for potato cultivation in both lowland and medium land.

**Keywords:** altitude, potato, plant growth regulator, yield, plant growth

## 1 Introduction

Potato (*Solanum tuberosum* L.) is an economic commodity with high demand in many countries, including Indonesia, due to its importance as raw material for numerous processed food products. Potatoes contain high carbohydrates, calcium, potassium, phosphorus, vitamins (C, B1, B3, B6), and amino acids [1]. Potato production in Indonesia has gradually increased every year, and its current production reached 2.33% in 2019 [2].

In Indonesia, the land can be separated into three levels based on its altitude, namely lowland (0–400 m above sea level [asl]), medium land (400–700 m asl), and highland (>700 m asl) [3]. Environmental conditions such as temperature and soil properties affect potato production and yield. Hlisnikovský et al. [4] stated that potato is a crop sensitive to soil–climate conditions. In Indonesia, potato production is commonly found in highlands with low temperature. However, massive potato production expansion in the highland negatively impacts the environment, i.e., soil erosion. The erosion rate due to potato cultivation varies from 3.34 to 223.11 tons per hectare per year [5]. Therefore, there is a need to shift potato cultivation from highland to medium and lowland.

Although the extensification and intensification of potato cultivation cause soil erosion in highlands, the development of potatoes in lowland and medium land must be supported by heat-tolerant cultivars, such as Median cultivar. The “Median” cultivar has high plant adaptability under high temperatures to  $35^\circ\text{C}$  in medium land, as evidenced by the larger diameter and number of tubers than other cultivars [6]. The use of heat-tolerant cultivars is expected to improve potato production in high-temperature areas, such as medium and lowland.

The temperature has an important effect on potato growth and development. High temperature increases gibberellin biosynthesis, inhibits the initiation of tuber by breaking potato dormancy, and increases the number

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of stems or stolon [7,8]. To improve tuber initiation, gibberellin biosynthesis should be reduced using plant growth regulators (PGRs), like paclobutrazol.

Paclobutrazol is an inhibitor of gibberellin biosynthesis and abscisic acid catabolism through its interference with ent-kaurene oxidase activity in the ent-kaurene oxidase pathway [9]. Several studies showed that the application of paclobutrazol at the beginning of planting increases the quality and yield of potatoes up to 108% under high temperatures around 28–35°C and also increases the percentage of stolon forming and the number of potato tubers in medium land [10,11].

Cytokinin is another plant hormone that triggers the tuberization in potatoes [12] and increases the number of stolons [13,14] and starch synthesis by inhibiting the starch hydrolysis and then supporting the growth of potato tuber [15]. One of the important cytokinins is benzylaminopurine (BAP) [14]. Since paclobutrazol and cytokinin have a positive impact on potato growth and development under high temperatures growing conditions more than 35°C, the study on the combination of paclobutrazol and BAP becomes very important. However, there is still limited study regarding that topic. Therefore, this study was conducted to determine the combined effect of paclobutrazol as an inhibitor of gibberellin biosynthesis and BAP as a tuberization modulator on the growth, yield, and yield quality of potatoes grown under high temperature in medium and lowland.

## 2 Materials and methods

### 2.1 Experimental design and plant preparation

A split-plot design was used in this experiment, with the land altitude as the main plot, consisting of medium land (670 m asl) and lowland (300 m asl). The subplot was PGR, consisting of control, paclobutrazol at 100 mg L<sup>-1</sup>, BAP at 50 mg L<sup>-1</sup>, and paclobutrazol at 100 mg L<sup>-1</sup> + BAP at 50 mg L<sup>-1</sup>. In total, there were eight combination treatments and replicated three times, forming 24 experimental units. Each experimental unit consisted of 10 plants in polybags, thus there were 240 polybags in total. The first generation potatoes (G1) with a weight of 20–30 g per tuber were planted at a depth of 5–7 cm in a polybag containing a growing medium of soil and chicken manure

with a ratio of 2:1 (v:v). Polybags were arranged with a spacing of 60 cm × 40 cm, so the plant density was 4.17 plants per m<sup>2</sup>. Fertilizer was applied in accordance with the recommendations of the Lembang Vegetable Research Institute, i.e., urea (46% N) for about 300 kg ha<sup>-1</sup> that split into two application times, i.e., 150 kg ha<sup>-1</sup> at planting time and the rest at 30 days after planting (DAP). In addition, SP36 150 kg ha<sup>-1</sup> and KCl 100 kg ha<sup>-1</sup> were also applied by using the side application method at the planting date. Pest and disease control were applied by spraying 80% mancozeb fungicide and deltamethrin insecticide with a concentration of 2 g L<sup>-1</sup>, which was done in accordance with the intensity of pest and disease attacks. The plant was harvested at 85–90 DAP, as indicated by the yellowing stems and leaves, and the tuber is not easily peeled off [12].

### 2.2 PGR applications

BAP solutions were prepared by dissolving the required amount of BAP (Sigma Aldrich 98%) in a 0.5 M KOH and then diluted with distilled water + 0.1% Tween 20 to make the desired concentrations of BAP, whereas paclobutrazol (Goldstar 250 g L<sup>-1</sup>) solutions were prepared by dissolving the required amount of paclobutrazol in distilled water + 0.1% Tween 20 to make the desired concentration of paclobutrazol. BAP solutions were sprayed on the potato plants at 30 DAP with a concentration of 50 mg L<sup>-1</sup>. Paclobutrazol was also applied by spraying to the plants at 40 DAP with a concentration of 100 g L<sup>-1</sup>. The combination of BAP and paclobutrazol was sprayed at 30 DAP for BAP 50 mg L<sup>-1</sup> and at 40 DAP for paclobutrazol 100 g L<sup>-1</sup>. All of PGRs were sprayed with a volume of 20 mL per plant.

### 2.3 Data collections

During plant growth and development, observations on plant height, number of tubers per plant, weight of tuber per plant, gibberellin, and chlorophyll content were taken. Plant height (measured from the stem base to the apical tip), dry weight, and shoot and root ratio were analyzed at 75 DAP, whereas the number of tubers per plant and tuber weight per plant were analyzed at the harvesting date.

## 2.4 Gibberellin and chlorophyll content analysis

Gibberellin and chlorophyll contents were analyzed at 75 DAP. Endogenous gibberellin was analyzed according to the method described by Okamoto *et al.* [16] by using gas chromatography-mass spectrometry analysis. The procedure was composed of four major steps, namely extraction, fractionation, derivatization, and detection. Chlorophyll content was estimated by using the soil plant analysis development (SPAD) index. The SPAD index was determined according to the method described by Yamamoto *et al.* [17], which was calculated from 20 readings per leaf sample by using a chlorophyll meter (SPAD-502; Minolta Corp.).

## 2.5 Plant dry weight

The analysis of plant dry weight was carried out based on the method described by Puvanitha and Mahendran [18]. The whole plant used as a sample was dried out in a hot air oven at 80°C until constant weight.

## 2.6 Shoot and root ratio analysis

The shoot and root ratio was estimated according to the method described by Rogers *et al.* [19]. Sample preparation was done by cutting the plant into two parts. The cutting point was the stem base that became an interconnected part of the shoot lowest part, and the upper root part. The cleaned samples were put in a paper bag and dried in an oven at 70–80°C until constant weight.

## 2.7 Starch content and reducing sugar analysis

The starch content analysis was analyzed according to the association of official analytical chemists method described by Nurdjanah *et al.* [20]. Reducing sugar analysis was prepared according to the method described by Freitas *et al.* [21]. The starch content and reducing sugar were estimated using an Orion AquaMate 8000 UV-vis Spectrophotometer (Thermo Scientific, USA) at 490 and 600 nm, respectively.

## 2.8 Statistical data analysis

All obtained data were represented as mean values  $\pm$  SE of three replicates. For the statistical data analysis, data were subjected to the analysis of variance and then followed by Duncan Multiple Range Test (DMRT) at  $p < 0.05$ .

## 3 Results

### 3.1 Gibberellin content and chlorophyll content index (CCI)

The statistical data analysis showed no significant interaction effect between growing altitude and PGR factor on gibberellin content. Table 1 shows that the gibberellin content in potatoes grown on the medium land was significantly smaller ( $0.0087 \mu\text{g g}^{-1}$  DW) than in the lowland ( $0.0346 \mu\text{g g}^{-1}$  DW). The decreased gibberellin content was detected in all PGR-treated plants in both locations. The control plant had the significant highest gibberellin content among other treatments ( $0.0409 \mu\text{g g}^{-1}$  DW) (Table 1).

The results of statistical analysis showed that there was no significant interaction effect between growing altitude and PGRs' application on chlorophyll content of potato plants (Table 1). Growing location factor has a significant effect on the CCI of potato leaves, i.e., potato plants grown in the lowland had significantly higher CCI than those grown in the medium land. In addition to the growing altitude factor, the PGR application significantly affected the CCI. The application of paclobutrazol, solely

**Table 1:** Independent effect of paclobutrazol and BAP on gibberellin content and CCI of potato grown in medium and lowland

| Treatment               | Gibberellin content ( $\mu\text{g g}^{-1}$ DW) | CCIs (SPAD indexes)      |
|-------------------------|------------------------------------------------|--------------------------|
| <b>Growing altitude</b> |                                                |                          |
| Medium land             | $0.0087 \pm 0.00012\text{b}$                   | $38.51 \pm 0.09\text{a}$ |
| Lowland                 | $0.0346 \pm 0.00015\text{a}$                   | $53.28 \pm 0.60\text{b}$ |
| <b>PGR</b>              |                                                |                          |
| Control                 | $0.0409 \pm 0.00023\text{c}$                   | $40.60 \pm 0.39\text{a}$ |
| Paclobutrazol           | $0.0089 \pm 0.00015\text{a}$                   | $50.62 \pm 0.53\text{b}$ |
| BAP                     | $0.0109 \pm 0.00022\text{b}$                   | $42.34 \pm 0.70\text{a}$ |
| BAP + paclobutrazol     | $0.0080 \pm 0.00012\text{a}$                   | $50.11 \pm 0.57\text{b}$ |

The mean followed by the same letter was not significantly different based on the Duncan Multiple Range Test (DMRT) at 95% level.

or combined with BAP, caused a significantly higher CCI than control and BAP-treated plants (Table 1).

was a significant increase of plant dry weight in BAP-treated plants (37.40 g) compared to control plants (27.13 g).

### 3.2 Plant height and plant dry weight

There was a significant interaction effect between growing altitude and PGR application on the potato plant height. The potato height in the lowland was higher than those growing in the medium land in all PGR treatments. PGR application did not show any significant improvement of plant height in the medium land. In contrast, the application of BAP significantly increased plant height compared to other treatments in the lowland (Table 2).

Statistical analysis showed a significant interaction between altitude and PGR application on the dry weight of the potato plant (Table 2). The potato plant in the lowland has a higher dry weight than those in the medium land, irrespective of PGR treatment. The PGR application has no significant effect on plant dry weight when applied in medium land. While for plants grown in lowland, there

### 3.3 Shoot and root ratio, number of tubers per plant, and weight of tuber per plant

In terms of shoot and root ratio, there was no significant interaction between growing altitude and PGR application. Table 3 shows that the growing altitude affected the shoot and root ratio, i.e., plants in the lowland produced a lower shoot and root ratio than those in the medium land. Apart from the growing altitude difference, the independent effect of PGR application was significant on the shoot and root ratio. The combination treatment of paclobutrazol and BAP produced the highest shoot and root ratio with a value of 6.32, and this result was significantly different from others (Table 3).

Similar to the previous finding, there was no significant interaction effect between altitude and PGR

**Table 2:** Interaction effect of paclobutrazol and BAP application on plant height in medium and lowland

| Growing altitude            | Control            | Paclobutrazol       | BAP                | BAP + paclobutrazol |
|-----------------------------|--------------------|---------------------|--------------------|---------------------|
| <b>Plant height (cm)</b>    |                    |                     |                    |                     |
| <b>Lowland</b>              | 63.67 ± 4.25b<br>A | 62.00 ± 3.50b<br>A  | 80.67 ± 4.53b<br>B | 68.50 ± 3.39b<br>A  |
| <b>Medium land</b>          | 35.30 ± 1.59a<br>A | 32.73 ± 1.81a<br>A  | 36.50 ± 2.86a<br>A | 33.07 ± 0.85a<br>A  |
| <b>Plant dry weight (g)</b> |                    |                     |                    |                     |
| <b>Lowland</b>              | 27.13 ± 1.92b<br>A | 28.90 ± 1.93b<br>AB | 37.40 ± 1.43b<br>C | 33.50 ± 1.37b<br>BC |
| <b>Medium land</b>          | 12.27 ± 1.53a<br>A | 10.83 ± 0.64a<br>A  | 9.00 ± 0.29a<br>A  | 7.90 ± 1.07a<br>A   |

The mean followed by the same uppercase was not significantly different within the same row based on the DMRT at 95% level. The mean followed by the same lowercase was not significantly different within the same column of similar variable based on the DMRT at 95% level.

**Table 3:** Independent effect of paclobutrazol and BAP on shoot and root ratio, number of tubers per plant, and the weight of tuber per potato plant in medium and lowland

| Treatment               | Shoot and root ratio | Number of tubers per plant | Weight of tuber per plant (g) |
|-------------------------|----------------------|----------------------------|-------------------------------|
| <b>Growing altitude</b> |                      |                            |                               |
| Medium land             | 6.56 ± 0.24b         | 12.58 ± 1.07b              | 373.17 ± 14.12b               |
| Lowland                 | 4.06 ± 0.29a         | 7.67 ± 0.49a               | 120.00 ± 6.37a                |
| <b>PGR</b>              |                      |                            |                               |
| Control                 | 5.07 ± 0.41b         | 8.00 ± 0.17a               | 180.50 ± 11.32a               |
| Paclobutrazol           | 4.33 ± 0.28a         | 9.67 ± 0.68b               | 268.33 ± 5.47b                |
| BAP                     | 5.53 ± 0.36b         | 9.00 ± 0.26b               | 256.00 ± 10.55b               |
| BAP + paclobutrazol     | 6.32 ± 0.40c         | 9.83 ± 0.60b               | 261.50 ± 7.01b                |

The mean followed by the same letter was not significantly different based on the DMRT at 95% level.

application on the number of tubers per plant, while the effect of independent growing altitude and PGR application on tuber numbers was significant. Potatoes grown in the medium land had a significantly higher number of tubers than those grown in the lowland, with 12.58 and 7.67 tubers per plant, respectively. The application of PGR significantly increased the tuber numbers since the control had the lowest number of tubers per plant, about 8.00 tubers (Table 3).

No significant interaction effect between growing altitude and PGR application on the weight of tuber per plant was observed. In opposite, the independent effect of both growing altitude and the PGR factor showed significant results. Potato grown in medium land resulted in an increased tuber weight per plant compared to that grown in the lowland. Without PGR application, such a control plant had the significantly lowest tuber weight (Table 3).

### 3.4 Potato starch content and reducing sugar content

Statistical data analysis showed no significant interaction effect between growing altitude and PGR application on the starch content of the potato. However, the independent effect of both factors significantly affected the starch content. Potatoes grown in the medium land had a higher starch content, i.e., 1.88% higher than those harvested from the lowland. A similar finding, the PGR application significantly increased potato starch content compared to the control (Table 4).

In terms of reducing sugar content in potato tuber, no significant interaction was observed. The data showed

that there was a significant difference in the reducing sugar content of potato tubers as the effect of different growing locations. Potatoes grown in the medium land had lower reducing sugar content (0.74%) compared to those planted in the lowland (0.95%). Application of paclobutrazol significantly decreased reducing sugar content in potato, while BAP, solely or combined with paclobutrazol, did not significantly alter the reducing sugar content and was similar to control (Table 4).

## 4 Discussion

In general, plant growth and development was a natural process that was highly influenced by environmental conditions. A high-temperature condition caused an increase in gibberellin biosynthesis in leaves and shoots [22]. Our study reported that high-temperature conditions in lowland stimulated high endogenous gibberellin production in potato plants (Table 1). The increase in endogenous gibberellin positively improved the CCI potato leaves grown in lowland compared to medium land (Table 1). However, potato tuber formation in the lowland was inhibited due to high carbohydrate allocation for the shoot part [8,11,23], leading to the increase in plant height and plant dry weight (Table 2). The presence of high temperature in lowland could also decline the shoot and root ratio (Table 3), suggesting that the initiation of potatoes tuber is strongly influenced by an environmental factor, especially photoperiodicity and temperature [24]. High temperature in the growing area reduced potato tuber productivity through the decline of tuber yield. Potato requires a low temperature between 15 and 20°C to trigger tuber formation [25]. Moreover, the high temperature, photoperiodicity, and light radiation could trigger the increase in carbohydrate accumulation in potato tubers [21,26]. The present study also highlighted that the potato grown in lowland under high-temperature conditions had lower starch and high reducing sugar content than those in medium land (Table 4). A high temperature in the growing environment of potato plants decreases the photosynthesis rate, assimilation translocation to the tuber, and also conversion rate of starch to reducing sugar, leading to the inhibition of tuber formation and growth [23].

In addition to environmental conditions, potato productivity was influenced by endogenous gibberellin levels [27]. Gibberellin was one of the important plant hormones that play a role in photoperiod and regulation of potato tuber formation [28]. The role of gibberellin was to promote the synthesis of a hydrolytic enzyme such as  $\alpha$ -amylase that indirectly triggers shoot, root, and cell elongation by

**Table 4:** Independent effect of paclobutrazol and BAP on potato starch content and reducing sugar content in medium and low land

| Treatment               | Potato starch content (%) | Reducing sugar content (%) |
|-------------------------|---------------------------|----------------------------|
| <b>Growing altitude</b> |                           |                            |
| Medium land             | 13.44 $\pm$ 0.47b         | 0.74 $\pm$ 0.06b           |
| Lowland                 | 11.61 $\pm$ 0.89a         | 0.95 $\pm$ 0.10a           |
| <b>PGR</b>              |                           |                            |
| Control                 | 11.91 $\pm$ 1.71a         | 0.89 $\pm$ 0.09b           |
| Paclobutrazol           | 16.57 $\pm$ 1.55b         | 0.59 $\pm$ 0.07a           |
| BAP                     | 16.37 $\pm$ 1.02b         | 0.92 $\pm$ 0.03b           |
| BAP + paclobutrazol     | 16.24 $\pm$ 1.17b         | 0.88 $\pm$ 0.47b           |

The mean followed by the same letter was not significantly different based on the DMRT at 95% level.

transporting the auxin, even though it could inhibit plant growth at higher concentrations [29,30]. Paclobutrazol was a synthetic growth regulator that acted as an inhibitor of the gibberellin biosynthesis leading to the decrease in plant height and root architecture [31–33]. That argument was in accordance with our finding in Table 2, which showed the reduction of plant height as the presence of paclobutrazol. Interestingly, the inhibition of shoot growth resulted in the increase in the number of potato tubers and its weight (Table 3). Bridgemohan and Bridgemohan [34] reported that the inhibition of plant growth due to paclobutrazol increased photosynthate accumulation in potato tuber, which acted as a sink. The increase in photosynthetic activity was also caused by the increase in the chlorophyll content due to paclobutrazol treatment [33]. Increasing chlorophyll content was also reported in this study, i.e., paclobutrazol-treated plants had a higher CCI compared to control (Table 1). In addition, the application of paclobutrazol solely or combined with BAP increased the starch content in potato tuber (Table 4). Our findings are in accordance with Wu et al. [35], who stated that paclobutrazol treatment produced a higher carbohydrate content in the sink organ.

Not only the decline of endogenous gibberellins, but the application of paclobutrazol could also trigger a decrease in shoots growth due to the increase in abscisic acid that hindered root growth under drought stress conditions [36]. The cytokinin was used to cover the side effects of paclobutrazol, which inhibited cell division and degradation of chlorophyll by modulating the activity of antioxidant enzymes [37,38]. Cytokinin increased the size and activity of sink organs by increasing cell division and enlargement [37]. This study reported that the application of BAP in potato plants triggered an increase in plant height, plant dry weight, shoot and root ratio, and reduced sugar content (Tables 2–4). Cytokinin speeds up shoots' growth under high-temperature condition and then restores the root after reaching the average condition [39,40].

## 5 Conclusion

For most of the potato cultivars, the cultivation in medium and lowland could impede the plant growth and yield. However, the application of PGR was successful to improve these responses. The application of paclobutrazol at 100 mg L<sup>-1</sup> and BAP at 50 mg L<sup>-1</sup> and also a combination of both PGRs was evidently proved to gain the number of tubers per plant and the weight of

tuber per plant grown under high temperature in medium and lowland.

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**Data availability statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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