Growth and Anatomical Responses of Gogo Rice Plant (*Oryza sativa* L.) Var. Inpago Unsoed 1 to Paclobutrazol Application

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Abstract. The improvement of gogo rice production in dryland is constrained by its extraordinary height, which leads to falling, and its long lifespan. The shorter gogo rice plant can be obtained through growth inhibition using paclobutrazol. Gogo rice plant var. Inpago Unsoed 1 was used during the study. An experimental study was performed using factorial completely randomized design. The main factor was four paclobutrazol concentrations (0, 100, 200, and 300 ppm). The sub-main factor was paclobutrazol application time after planting (three, four, and five weeks). The results showed that the interaction between paclobutrazol concentration and application time was highly significantly affecting plant height. The most appropriate treatment resulting in optimum plant height was the combination of 100 ppm paclobutrazol applied four weeks after planting. Interaction between paclobutrazol concentration and application time also significantly affect stem diameter, leaf width, and stomata number in the lower leaf epidermis. However, there was no significant effect of paclobutrazol observed on stomata size. The significant result of the combination of paclobutrazol concentration and the application time was also supporting tissue thickness. The thickest tissue was obtained by 300 ppm of paclobutrazol applied five weeks after plantation.

1. Introduction

Gogo paddy is among paddy varieties grown in a dry land, which is grown once a year at the beginning of the rainy season. The paddy production is still low due to its extraordinary height and long lifespan. Therefore, it has several disadvantages, such as easily fall and shed off, low rice production, and intolerant to drying.

Inpago Unsoed 1 rice is a result of breeding effort between Poso and Mentik Wangi varieties developed by Plant Breeding and Biotechnology Laboratory, Agriculture Faculty of Jenderal Soedirman University Purwokerto, Central Java, Indonesia. The Poso variety is chosen because of its high production, tolerant to drying, and resistant to blast disease. Mentik Wangi is selected for its aromatic parental, which is also well adapted to Banyumas areas. Inpago Unsoed 1 has high production, sticky, and tolerant to drying. However, this variety shows faster growth, which might cause to fall easily and shed off [1]. The ability of the breeder to differentiate precisely and fastly does not help to select the variety, which is not easy to fall [2]. Plants with high bending capability tend to fall compared to plant with a low bending ability [3]. There are several factors which hold plant from falling down, such as stem bark thickness, stem diameter, level of internode covering by leaf stem and, lignin density [4].

Short and hardly falling plants can be obtained through the application of inhibited plant growth regulator, e.g., Paclobutrazol. Paclobutrazol is a retardant capable of inhibiting cell and stem sub-
apical internode elongation. Paclobutrazol might shorten the internode of maize, which results in the hardly falling plant [5]. Paclobutrazol has been used to reduce the high of Acasia mangium seedling [6].

It is reported that 200 ppm paclobutrazol has decreased the height of the geranium plant, but increased the number of the shoot [7]. Paclobutrazol with Prehexadion-Ca and strangulation are proven to accelerate flowering and increase the number of flowers and fruits on the mandarin citrus plant [8]. Also, it is observed that 8 mg/L paclobutrazol effectively inhibits the growth of the bean plant, and increases the number of pods and grains [9].

Paclobutrazol can enhance dry flower length, flower diameter, and the number of flowers per inflorescence of the clove. The addition of 150 g/L sugar and 5 ppm paclobutrazol can induce micro potato tubers [10].

Paclobutrazol treatment increases the root length, although it decreases the stem length and leaf area in Arachnis hypogaea plant [11]. The other research shows that the vascular bundles of paclobutrazol treated plants were narrower in density compared to that of control in Arachnis hypogaea plant [12]. A combination of 125 ppm paclobutrazol in 6 WAP (weeks after planting) results in an insignificant effect on plant height, numbers of chlorophyll content, and weight of tuber yield [13]. It is suggested that multiple foliar application of paclobutrazol is effective in promoting flowering in Namdokmai-sitong mango, inducing early flowering, and an increasing percentage of flowering shoots [14].

Paclobutrazol (C15H20CINO3, the molar mass of 293.79 g mol) is a plant growth regulator antagonist to gibberellin. Its activity inhibits gibberellin biosynthesis and internodes growth leading to development of strong stem, improvement of root growth, shortening of flowering time, and reduction of plant sensitivity to drying. Moreover, Paclobutrazol can increase the chlorophyll content and plant resistance to bacteria and mold. It has been noted that paclobutrazol belongs to the triazole family of phytohormone, which effectively prevents a plant from drying stress, chilling heat, and UV radiation [15].

Principal activities of Paclobutrazol on gibberellin biosynthesis inhibition is by blocking caurenoat formation from caurene. Paclobutrazol also minimizes the effect of abscisic acid, ethylene and indole acetic acid (IAA) in plant cell division and elongation.

Therefore, paclobutrazol application on gogo rice plant Inpago Unsoed 1 is expected to produce shorter and sturdy rice plants. It is less understood as how the growth factor influences the stem or leaf anatomy of the rice plant. A strong stem, good plant performance, balance ratio between rice leaf and stalk is required characters in growing new rice varieties. The present research aimed to understand the growth and anatomical responses of Inpago Unsoed 1 gogo rice plant to paclobutrazol application.

2. Methods

The research was done in the greenhouse of Structure and Development Laboratory, Faculty of Biology Unsoed from December 2015 to March 2016. The factorial treatments were arranged in accordance with a completely randomized design. The main factor was paclobutrazol dosage, consisted of 0, 100, 200, and 300 ppm. The second factor was interval of paclobutrazol application, which was 3, 4, and 5 weeks after planting. The treatment combinations were replicated three times. The measured variables were plant height, leaf width, stoma density, stoma size, stem diameter, and thickness of supporting tissues on stem. Data were analyzed statistically using F-test with 0.05 significance level. Statistically significant F-values were followed by least significance difference (LSD) test.

3. Results

F-test of paclobutrazol concentration and its application times showed a highly significant effect on leaf width and plant height. LSD-test showed that paclobutrazol concentration of 300 ppm combined with paclobutrazol application in 5 weeks after planting reduced plant height up to 41.3 cm (Table 1).
Table 1. LSD test on the effect of Paclobutrazol dosage with different application times on plant height and leaf size.

| Treatment | Mean Height (cm) | Mean Leaf Width (cm) |
|-----------|------------------|----------------------|
| P0W1      | 101.3 a          | 1.43 e               |
| P0W2      | 65.3 cd          | 1.67 cd              |
| P0W3      | 54.0 de          | 1.83 abc             |
| P1W1      | 49.0 de          | 1.77 bcd             |
| P1W2      | 87.7 ab          | 1.63 d               |
| P1W3      | 79.0 bc          | 1.67 cd              |
| P2W1      | 64.7 cd          | 2.00 a               |
| P2W2      | 48.0 de          | 1.83 abc             |
| P2W3      | 81.3 bc          | 1.80 bcd             |
| P3W1      | 64.0 cd          | 1.90 ab              |
| P3W2      | 47.7 de          | 1.77 bcd             |
| P3W3      | 41.3 e           | 1.80 bcd             |
| LSD 0.05% | 19.29673         | 0.186311             |

Notes: The values followed by the same word mean statistically not different on 95% significance level.

Variance analysis showed that the induction of different concentrations of paclobutrazol has a significant effect on stomata number per mm2 leaf width in the lower epidermis of the leaf. Meanwhile, there was no significant effect on the number of stomata in the upper epidermis of the leaf. Increasing paclobutrazol concentration raised the number of stomata in the lower epidermis of the leaf for all application times (Table 2).

Table 2. LSD test on the effect of paclobutrazol dosage on stoma number in the lower epidermis of the leaf

| Paclobutrazol dosage (ppm) | Number of stomata |
|----------------------------|--------------------|
| 0 (control)                | 10.6 b             |
| 100                        | 16.3 ab            |
| 200                        | 14.9 b             |
| 300                        | 24.4 a             |
| LSD 0.05%                  | 8.4667183          |

Notes: The values followed by the same word mean statistically not different on 95% significance level.

The application of paclobutrazol concentration and application times did not significantly affect stoma size in both leaf epidermis (Figure 1).
Interaction between paclobutrazol concentration and its application times also had a significant effect on the thickness of supporting tissue and the stem diameter. The thickest supporting tissue (30.83 µm) was gained by applying 200 ppm paclobutrazol three weeks after planting (Table 3).

**Table 3.** LSD test on the effect of paclobutrazol dosage on the thickness of stem diameter and supporting tissue in paddy.

| Treatment combination | Stem thickness (diameter µm) | Supporting tissue thickness (µm) |
|-----------------------|-----------------------------|---------------------------------|
| P0W1                  | 2066.7 de                   | 19.17 cd                        |
| P0W2                  | 2333.3 cd                   | 25.00 abc                       |
| P0W3                  | 2650.0 cd                   | 21.67 bcd                       |
| P1W1                  | 3033.3 bc                   | 23.33 abcd                      |
| P1W2                  | 1250.0 f                    | 16.67 d                         |
| P1W3                  | 1966.7 def                  | 20.83 bcd                       |
| P2W1                  | 2366.7 cd                   | 30.83 a                         |
| P2W2                  | 4740.0 a                    | 20.83 bcd                       |
| P2W3                  | 1466.7 ef                   | 16.67 d                         |
| P3W1                  | 3423.3 b                    | 19.17 cd                        |
| P3W2                  | 2583.3 cd                   | 20.00 cd                        |
| P3W3                  | 4383.3 a                    | 28.33 ab                        |
| BNT 0.05%             | 737.7978                    | 8.1888379                       |

Notes: The values followed by the same word mean statistically not different on 95% significance level.
The thickness of supporting tissue might be caused by paclobutrazol that prevents cell division processes. This mechanism has caused the divided cell in the stem cortex to become thick as a result of deposit in supporting tissues, sclerenchyma (Figure 2).

![Image of supporting tissue cross-section](image)

**Figure 2.** Cross-section of supporting tissue on the stem (400x) (A. Control, B. P2W1) [16].

### 4. Discussion

The highest plant was shown by the control plants (no paclobutrazol). Considering the effect of paclobutrazol activities, which were only two weeks after use, the use of the growth factor five weeks after planting provided a short period for plant vegetative growth before the generative phase was reached. Considering that rice spikelet is a site for the rice seed to grow, a shorter spikelet would presumably minimize rice production. Therefore, the optimum length of rice spikelet could be a prerequisite to maximize rice production. The optimal plant height (87.7 cm) was reached by the plant treated with 100 ppm paclobutrazol four weeks after planting (Table 1). This result agreed with [4], demonstrating falling-down paddy plant has 0–1 index and plant high less than 90 cm.

The application of a higher paclobutrazol concentration closed to the generative phase gradually reduced leaf size. The application of 200 ppm paclobutrazol at three weeks after planting resulted in a leaf width of 2.00 cm, whereas 300 ppm paclobutrazol produced narrower leaf width (1.77 cm). The detailed results are summarized in Table 1. This data suggest that paclobutrazol as a retardant was effective in reducing the growth of Inpago unsoed 1 gogo paddy. Activities of paclobutrazol in inhibiting gibberellin production prevent caurene oxidation from becoming caurenic acid, which leads to a decrease in cell division rate [16].

The average number of stomata in the lower leaf epidermis was 11 stomata/mm² leaf. Inducing 200 ppm of paclobutrazol in five weeks after planting increased the number of stomata (27.7 stomata/mm² leaf). On upper leaf epidermis, although statistically not different, the average number of stomata increased by increasing the concentration of paclobutrazol applied. This could be due to the way the experiments were conducted. The study was performed inside a greenhouse with routine watering, so the paddy did not need to adapt through exceeding transpiration, even though gogo paddy is more tolerant of drying or chilling heat. The present result is different from that of [17], who reported that the increasing size of stomata on *Chrysanthemum* sp. was induced by application of 100 ppm paclobutrazol.

Paclobutrazol concentration, application times, and their interaction significantly affected stem diameter. The greatest stem diameter was reached by the plant treated with 200 ppm paclobutrazol four weeks after planting. The results suggested that presumably, the plant still had sufficient time for its vegetative growth before entering its generative phase. When entering the generative phase (week nine), the plant has five weeks for vegetative growth to become more prominent. Our results are similar to that of [18, 19], who have concluded that paclobutrazol application had increased stem thickness and diameter on a sunflower. The thickening of the stem was caused by increasing the cell volume to side direction.
5. Conclusion

It can be concluded that 1) the application of paclobutrazol concentration and its application times significantly affect plant height, with the optimal height, was reached on 200 ppm and application at four weeks after planting; 2) paclobutrazol application increased leaf width and number of stomata in lower epidermis, but it does not affect stomata size; 3) paclobutrazol application improved stem diameter and supporting tissue thickness.

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