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PACLOBUTRAZOL AND CYTOKININ REGULATION ON CULM GROWHT OF BLACK RICE (*Oryza sativa* L. "Cempo Ireng")

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Abstract *– Rice is a staple food for the majority of Asians; nevertheless, black rice has not been commonly consumed among them. The components of the yield will be determined by the allocation of photosynthate on rice and the tall phenotype of black rice, which is a significant contributing factor to lodging. The growth inhibitor paclobutrazol prevents the manufacture of gibberellin, which results in the tall phenotype of black rice. The endogenous cytokinin content is high at the beginning of the grain filling and then rapidly decreases after that. The purpose of the study was to compare the effects of paclobutrazol and cytokinin on culm height, internode length, culm diameter, lodging resistance, culm parenchyma cell arrangement, and flag leaf structure. Throughout the inquiry, Cempo Ireng black rice cultivar seeds were employed. The seeds were planted in plastic containers that held 10 kg of soil for growth medium and organic fertilizer, and a random block pattern was used for the experiment's design. At 10 weeks following sowing, paclobutrazol was sprayed as a foliar application at concentrations of 0, 50, and 100 ppm. By injecting the kinetin diluted in 0.8 percent agar gel at a rate of 10-5 M in the flag leaf internode lacuna twice, separated by two days, two weeks after anthesis. Eight replications and six combinations were made. DMRT and the significant different were used in the statistical analysis of the mean comparisons (P=0.05). Plant height and internode length were decreased by paclobutrazol (100 ppm), although internode diameter was raised and lodging resistance was induced. The organization of the parenchyma cells in the culm (100 ppm packlobutrazol) changed, and the cells were small and lacked intercellular gaps.*

Keywords: *black rice, cultivar, culm anatomy, paclobutrazol.*

Abstrak *– Sebagian besar penduduk Asia mengkonsumsi beras sebagai makanan pokok, sebaliknya beras hitam belum banyak dikonsumsi di antara mereka. Beras hitam yang dicirikan oleh fenotipe tinggi merupakan faktor penyumbang utama terhadap rebah dan alokasi fotosintat pada beras akan menentukan komponen hasil. Paclobutrazol merupakan penghambat pertumbuhan yang menghambat biosintesis giberelin penyebab tingginya fenotipe beras hitam. Kandungan endogen sitokinin tinggi pada awal pengisian biji-bijian dan setelah itu, kadarnya menurun tajam. Penelitian ini bertujuan untuk mengetahui pengaruh paclobutrazol dan sitokinin terhadap tinggi batang, panjang ruas, diameter batang, ketahanan rebah, susunan sel parenkim batang dan struktur daun bendera. Benih padi hitam kultivar Cempo Ireng digunakan selama penelitian ini, benih disemai dalam wadah plastik dengan kapasitas 10 kg tanah sebagai media tumbuh dan pemupukan pupuk organik, desain percobaan menggunakan Rancangan Acak Kelompok. Paclobutrazol pada tingkat 0; 50 dan 100 ppm diterapkan sebagai aplikasi daun pada 10 minggu setelah tanam. Kinetin yang diencerkan dalam gel agar 0,8% digunakan dengan kecepatan* 10^{-5} *M yang diaplikasikan pada dua minggu setelah pembungaan dengan cara disuntikkan ke dalam kekosongan ruas daun bendera pada dua kali aplikasi dengan interval dua hari. Ada 6 kombinasi dan 8 ulangan. Analisis statistik perbandingan rata-rata dilakukan dengan menggunakan DMRT dan perbedaan nyata (P=0,05). Paclobutrazol (100 ppm) mengurangi tinggi tanaman dan panjang ruas, tetapi meningkatkan diameter ruas dan menyebabkan tahan rebah. Sel-sel parenkim batang (packlobutrazol 100 ppm) berubah susunannya dan sel-selnya kompak tanpa ruang antar sel.*

Kata kunci: *beras hitam, kultivar, anatomi batang, paclobutrazol.*

INTRODUCTION

Paklobutrazol [(2RS,3RS)-1-(4-chloro-phenyl) methyl-4,4-dimethyl (1h—1,2,4-trizol-1-yl) penten-3-ol] is a plant growht regulator, the most commonly used plant growth retardant , block the biosynthesis of gibberellin that acts by inhibiting the convertion of gibberellin precursor *ent*-kaurene to *ent*-kaurene acid, the early step of gibberellin biosynthesis pathway (Davies, 2004;Sinniah *et al.*, 2012). There are two approaches to reduce endogenous gibberellin content, i.e. by supress of gibberellin biosynthesis and enhancement of biosynthesis catabolism (Ashikari and Sakamoto, 2008). Yim *et al.*, (1997) reported that paklobutrazol (4,8 x 10^{-5}M was applied to the rice seedling) effectively decreased vegetative growth and increased chlorophyll content, but the number of vein in the leaf, the number of stomata per leaf and the lenght of guard cells were not affected by paklobutrazol. Paklobutrazol was applied as foliar spray inhibit plant growth, reduced culm internodes, retard the culm height by 20% (French *et al.*, 1990;Wahyuni *et al* (2012, but increased culm diameter, changed in parenchyma cell arrangement, and increased lodging resistance (Sinnia et al., 2012). Paklobutrazol application stimulated flowering off season mango cultivars Baladi AbuZaid (26.7%) and Baladi Burai (30.7%) by 60 and 70 days, respectively as compare to control (Rahim *et al*, 2011). Cytokinin is capable of promoting cell division (Roitsch and Ehneb, 2000).

The objectives of this experiment were to determine the effects of paclobutrazol and cytokinin on culm height, internodes lenght, culm diameter, the lodging and arrangement of the parenchyma cells of the culm.

MATERIALS AND METHODS

Rice (*Oryza sativa* cultivar Cempo Ireng) was the plant material used, and it was procured from a rice farmer in the Yogyakarta provincial district of Sleman. A random block design was chosen for the experiment's design. At 10 weeks after sowing or two weeks before anthesis, paclobutrazol was administered as a foliar treatment at concentrations of 50 and 100 ppm. The seeds were immersed in water for 24 hours at a time and then dried by air. A 3:1(w/w) ratio of organic and soil garden materials made up the medium. In the glass house, plants were planted in plastic jars with a 10 kilogram volume. As a liquid organic fertilizer, minerals nutrition were added. Each plastic container of seedlings was trimmed to 8 uniform seedlings at the age of 15 days. Every panicle's anthesis date was noted. The appearance of 50% of the anthers served as a reliable indicator of the anhesis date. Aquadest was used to dissolve the liquid form of paclobutrazol, and the resulting concentrations were 50 ppm and 100 ppm. Kinetin was employed as the cytokinin (BDH Chemical Ltd, Poole, England). The concentration of kinetoin was 10-5 M. At 15 days after panicle initiation, paclobutrazols were sprayed on the leaves' adaxial and abaxial surfaces. Using a 10 mL agar-filled syringe barrel and a 27 G x 12 gauge syringe needle, kinetoin was dissolved in a molten 0.8 percent (w/v) aqueous gel of agar. The kinetoin was then transferred to the needle and injected into the lacuna of the flag internode. A hole that was punched into the flag internode and covered with parafilm 1 cm below the panicle/culm junction allowed air inside the stem lacunae that had been displaced by the injection of agar gel to escape (American National, Greenwich, USA).

From the plant's root system to the panicle necknode, the culm height was calculated. Each internode's length was measured with a ruler, and the diameter of the culms was determined using a micrometer scale. Between the control plant and the plants treated with paclobutrazol, the lodging resistance was quantitatively compared. At the time of harvest, measurements of culm height, internode length, culm diameter, and lodging resistance were made (Sinniah et al.,2012).

By cutting the third internode of the cultivar Cempo Ireng into free-hand sections, the anatomical makeup of the rice stem was examined.

Statistical Analysis

Statistical analysis was performed according to methode given by (Gomez and Gomez, 1976). The data were analyzed analysis of variance with significant at P< 0.05. Significant differences among mean were determined using Duncan's Multiple Range Test (DMRT). All statistical analysis were performed using SPSS Software version 16 for window (SPSS Inc. Chicago, Illinois, USA).

RESULT AND DISCUSSION

Culm height is important measure than plant height when lodging resistance is to be analysed (Yoshida, 1981). Results depicted in Table 1 indicated that the highest culm height was at control, i.e. 96.50 cm, but was not signifinat different with kinetin treatment. The shorted culm height was at 100 ppm paclobutrazol treatment that was 55,92 cm and signifinat different with other treatments. A-50 ppm paclobutrazol, 50 ppm paclobutrazol with kinetin and 100 ppm paclobutrazol with kinetin treatments were not signifinat different among them based on DMRT P<0.05 (Table 1). A-50 ppm paclobutrazol treatment reduced culm height by 28.39 (%), and that 100 ppm paclobutrazol by 35.99% compare to control plants. The rice culm is consist of a series of nodes and internodes which is enclosed within the sheath before heading (Yoshida, 1981). Results depicted in Table 1 indicated that the control plant had the highest culm height (88,17 cm) followed by kinetin treatment (88.17 cm) and those were not signifinat different. Paclobutrazol treatment at the level of 100 ppm had the shortest culm height (55.92 cm), and those were significant different with other treatments. The result was consistent with Na *et al.*, (2011),Wahyuni *et al.,* (2012) and Sinniah *et al.*, (2011). They found that paclobutrazol (100 ppm) applied at once as a foliar spray of rice leaf (*Oryza sativa* L.) at heading stage reduced plant height by 20%. Wahyuni *et al.,* (2012) and Sinniah *et al.*, (2011), used a modern rice which is short and stout culm. Other authors reported that paclobutrazol applied at level of 6.5 x10^{-5} M during the initial stage of rice development effective reduced culm by 82% (Yim *et al.*,(1997). Reducing of culm height caused by paclobutrazol treatments closely related to decrease level of gibberellin, it may be caused by blocking at any of three separate steps for the synhesis of gibberellin (Yim et al., 1997; Davies, 2004; Ashikari and Yamamoto, 2008; Sinniah *et al.*, 2011). Similar results with a significant decrease in culm height have been reported in several plants (Wieland and Wample,1985 in apple; Tsegaw *et al.*,2005 in potato; Vu and Yelonosky,1992 dan Yelonosky *et al.*,1995 in citrus; Frymire and Henderson-Cole, 1992 in **Pyracanthe**; Zhou and Haifu, 1993 in *Brassica napus*; Ruter, 1996; Hua *et al.*, 2014; Francescangali, 2009 in Iris x hollandica Tub.

Table 1. Effect of paclobutrazol and kinetin treatments on culm height of black rice (*Oryza sativa* L.)

No	Treatment	Culm Height (cm)
1	Control	95.60a
2	Kinetin	88.17a
3	50 ppm paclobutrazol	74.46a
4	50 ppm paclobutrazol + Kinetin	73.97b
5	100 ppm paclobutrazol	55.92c
6	100 ppm paclobutrazol + Kinetin	70.30b

Mean values of 6 replicates. Value followed by the same letters were not significantly different at P <0.05 based on DMRT.

Internode elongation is usually closely associated with growth duration (Yoshida, 1981). The reduce of culm height is closely associated with internode lenght (Yoshida, 1981). Only a few internodes at the top elongated, and the internodes lenght decrease toward to the base (Yoshida, 1981). As depicted in Table 2 indicated that kinetin treatment had the longest of the 1st internode elongated (6.02 cm), but those were not significant different with control and 100 ppm paclobutrazol combination with kinetin treatments. In contrast, the shortest of the 1st internode occur at 100 ppm paclobutrazol treatment (3.05 cm), and not significant different with 50 ppm paclobutrazol, 50 ppm paclobutrazol combination with kinetin, and 100 ppm paclobutrazol combination with kinetin treatments. The 2nd internode, the longest of the internode elongated occur at kinetin treatment (8.30 cm), but those were not significant different with other treatments, except that of 100 ppm paclobutrazol treatment (4.38 cm). For the 3st internode, the longest of the internode elongated occur at control (11.50 cm), significant different with paclobutrazol treatments. A-50 ppm paclobutrazol treatments is the shortest internode elongated (6.60 cm). The 4st internode, the longest of the internode elongated occur at control (17.98 cm), and it was significant different with other treatments and the shortest internode elongated performed by 100 ppm paclobutrazol treatment (9.87 cm). Finally, at 5st internode, at control plant consistanly have the longest of the internode elongated (20,58 cm) significant different with other treatments, and the shortest internode elongated occur at 100 ppm paclobutrazol treatment (9.17 cm) significant different with other treatments as shown in (Table 2). The control plant consistanly performed the longest internode lenght and the 100 ppm paclobutrazol treatment has the shortest internode elongated, the internode lenght in agreement with Yoshida (1991). The shortest internode elongated due to paclobutrazol treatment inhibited gibberellin synthesis (Davies, 2004; Siniah *et al.*, 2011), thus the levels of the endogenous gibberellin reduced (Rahim *et al.*,2011; Na *et al.*, 2011; Ashikari and Sakamoto, 2008). The cell division is not affected by paclobutrazol, but the new cells do not elongated (Setia *et al.*, 1995). The paclobutrazol at the level of 100 ppm was effectively reduce the culm internodes lenght of the rice culm (Wahyuni *et al*,. (2012); Siniah *et al.*, (2011). Similar results with a significant decrease in the culm internodes have been reported in several plants Wieland and Wample (1985) for an apple, (Hua *et al.*,2014) for canola; *Brassica napus* (Zhaou *et al.*,1993), and for citrus (Yelenosky *et al.*,1995).

Table 2. Effect of paclobutrazol and kinetin treatments on internodes lenght of black rice (*Oryza sativa* L.).

Treatments	Culm lenght at different internodes (cm)				
	1st	2nd	3st	4th	5th
Control	5.00b	8.20b	11.50a	17.98a	20.58a
Kinetin	6.02b	8.30b	10.52a	13.52b	16.65b
50 ppm paclobutrazol	4.45ab	6.50ab	6.60b	10.13c	16.12bd
50 ppm paclobutrazol + Kinetin	4.53a	7.17ab	8.83ab	11.50bc	15.92bd
100 ppm paclobutrazol	3.05a	4.38a	6.67b	9.87c	9.17c
100 ppm paclobutrazol + Kinetin	3.98ab	5.95ab	8.80ab	10.44bc	13.12d

Mean values of 6 replicates. Value followed by the same letters in the same column are not significantly different at P <0.05 based on DMRT.

Paclobutrazol inhibit the gibberellin synthesis, thus reduce cell elongated, yet increase culm diameter. Results depicted in Table 3 indicated that the 1st and 2 nd internode diameter of control is not significant different with other treatments, but the biggest diameter occur at 100 ppm paclobutrazol treatment (5.39 mm) at 3st, similar pattern occur for the diameter of the 4th and 5th internode where the 100 ppm paclobutrazol has the highest culm diameter. In general, there is increase in culm diameter treated with high paclobutrazol level (Table 3). Results obtained were consistant with findings reported by (Wahyuni *et al.,* (2012); Siniah *et al.*, (2011); Na *et al.*, (2011); Frence *et al.*, (1990) on rice culm, and Tsegaw *et al.*,(2005) on potato. The diameter of culm is in agreement withYoshida (1981) who stated that the diameter of culm range from 2 to 6 mm.

Table 3. Effect of paclobutrazol and kinetin treatments on culm diameter of black rice (*Oryza sativa* L.).

Treatments	Culm diameter at different internodes (mm)				
	1st	2nd	3st	4th	5th
Control	5.95a	5.38a	4.35a	4.52a	3.15a
Kinetin	5.58a	5.39a	4.70ab	3.73a	3.23a
50 ppm paclobutrazol	5.93a	5.40a	4.88ab	4.57a	3.63ab
50 ppm paclobutrazol + Kinetin	6.10a	5.72a	4.80a	4.03a	3.65ab
100 ppm paclobutrazol	6.35a	6.23a	5.39b	4.58a	4.28b
100 ppm paclobutrazol + Kinetin	6.17a	5.73a	4.71ab	4.22a	3.72ab

Mean values of 6 replicates. Value followed by the same letters in the same column are not significantly different at P <0.05 based on DMRT.

Lodging is a common problem, especially in cereals and its can result in reduced yield. The characteristics of the local rice varieties wih tall phenotype usually is a major factor contributing to the lodging. The first and second internode lenght more than 4 cm reduce lodging resistance (Yoshida, 1981) and the culm diameter are a closely assosiated with lodging resistance. Paclobutrazol is generally used in many crops to reduce lodging through shortening of the culm. A tall variety such as cutivar Cempo Ireng has a greater bending moment than a short variety due to the greater culm lenght (Yoshida, 1981). Lodging usually caused by the bending of two lowest internodes that elongated more than 4 cm (Yoshida, 1981). During the vegetative stage, sink is the limiting factor for phosynthate storage, thus more starch accumulated in the stem (Yim *et al.*, 1997; Tsegaw *et al.*, 2005;Yang et al., 2006), and starch accumulated in the stem and sheath may induced the culm strenght (Yoshida, 1981). Paclobutrazol does not inhibit the rate of cell division, but inhibit rate of cell elongation (Setia *et al.*, 1995; Yim *et al.*, 1997), which ultimately results in reduction of vegetative growth, thus it is a commonly used to reduce lodging in cereals (Yim *et al.*, 1997; Jaleel *et al.*, 2009). The culm height reduced (Table 1), and that in internodes lenght (Table 2), and increaseing culm diameter (Table 3) caused by the treatments of the paclobutrazol, these components collectively increased the bending resistance (Sinniah *et al.*, 2011), thus, increase the lodging resistance. Results depicted in Table 4 indicated that the paclobutrazol treatment reduce the lodging resistance by 25%. Results were similar to that reported by Sinniah *et al.*, (2011); Wahyuni *et al.*, (2011), and French *et al.*, (1990). Na *et al.* (2011) reported that a *growth retardant* induce the breaking strenght by sebesar 37%. Other authors reported that paclobutrazol increased the minerals mobillization (Yelenosky *et al*, 1995) and a temporary starch allocated in the leaf is remobolized and then stored in the stem and it is used for stem growth (Yim *et al.*, 1997; Tsegaw *et al.*, 2005; Wang et al., 2006), thus increased culm strenght and stiffness (Yoshida, 1981).

Table 4. Effect of paclobutrazol and kinetin treatments on lodging of black rice (*Oryza sativa* L.).

Treatments	Lodging (%)
Control	25
Kinetin	6
50 ppm paclobutrazol	0
50 ppm paclobutrazol + Kinetin	0
100 ppm paclobutrazol	0
100 ppm paclobutrazol + Kinetin	0

Paclobutrazol treatment reduce in culm height of the black rice (*Oryza sativa* L. cultivar "Cempo Ireng") is closely assocaited with reduce internodes lenght, increase culm diameter, and may be changes in struture of parenchyme cells constituent of culm. Parenchyme cells generally round an equidimetal in shape, with intercellular spaces (Figure 1). Plant treated with pacloburazol at 100 ppm, featured changes in parenchyme cells arrangement, the cells were compacted with no intercellular spaces may result in greater strenght the culm. Similar observation reported by (Sinniah *et al.*, 2011) and Tsegaw *et al.*, 2005).

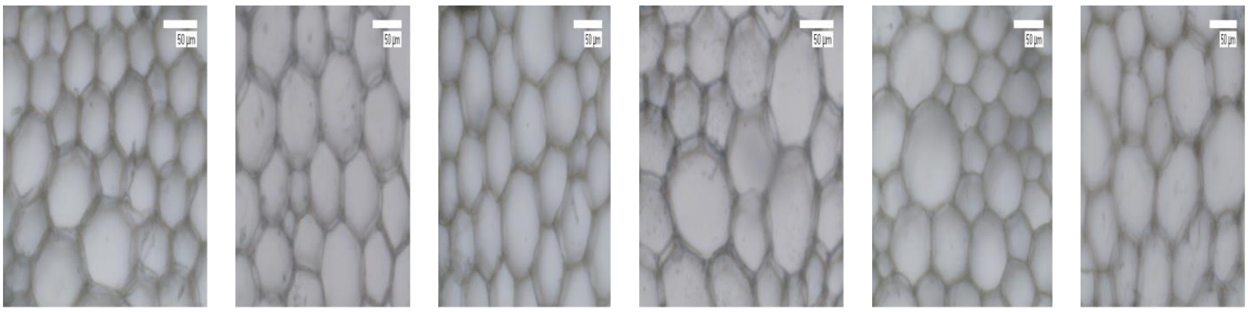
(a) (b) (c) (d) (e) (f)

Figure 1. Parencyma cell arrangement of 3rd internode for control (a), kinetin (b), and 50 ppm paclobutrazol (c) treatments, 50 ppm paclobutrazol with kinetin (d), 100 ppm paclobutrazol (e), and 100 ppm paclobutrazol with kinetin (f) treatments. Bars = 50 μm.

CONCLUSION

Based on results and discussion could be concluded that application of paclobutazol shorted the plant height, thicker the culm through enhance diameter and sharply reduced the lodging, modified the arrangement of the parencyme cells of the culm. On the other hand cytokinin application stimulate culm lenght and culmlenght at different intrnode contibute to the cell division.

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