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Paclobutrazol Improves The Agronomic Performance and Micromorphological Profile of Five Local Black Rice (*Oryza sativa*) Varieties in Central Java Indonesia

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Abstract. Local black rice (*Oryza sativa* L.) is one of the biodiversity of rice in Indonesia, which has tall stature and long cultivation period, easy to lodge, and requires more intensive care. Paclobutrazol (PBZ) is a plant growth inhibitor compound that could reduce plant height and accelerate plants entering the generative phase. This study aimed to determine the effects of PBZ concentration on the agronomic character and micromorphological profile of five local black rice varieties. This research was conducted experimentally using a completely randomized factorial design. The first factor was the local black rice variety (Brebes, Klaten, Magelang, Pati, and Pekalongan), while the second was PBZ concentration (0, 100, 200, and 300 ppm) with three replications. PBZ spraying was conducted in the fourth week after planting. The data obtained were analyzed using ANOVA and HSD test (α=5%). The results showed that the concentrations of PBZ 300 ppm produced the heaviest 100 grains. The Klaten variety had wide abaxial stomata, the Magelang variety had high abaxial stomata density, while the Pati variety had long abaxial stomata and heaviest 100 grains. The interaction between the Brebes variety and PBZ 0 ppm produced high adaxial stomatal density, the Klaten variety, and PBZ 100 ppm have vast adaxial stomatal and high abaxial trichome density. The Pati variety and PBZ 0 ppm had the most tillers, while the Pati variety and PBZ 100 ppm had long adaxial stomata and the most grains per panicle. The study's critical findings are that PBZ gives different micromorphology improvements and increases agronomic performance in local black rice.

Key words: agronomy, black rice, micromorphology, paclobutrazol

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INTRODUCTION

Black rice (*Oryza sativa* L.) variety local is one of Indonesia's most diverse rice among white rice, red rice, and brown-reddish (Nurhidayah & Umbara, 2019). Black rice has seeds colored purple and concentrated until black because it contains high anthocyanins in aleurone, endosperm, and pericardium (Ryu et al., 1998; Yoshimura et al., 2012). Cultivation of local black rice by farmers until the moment this still a little compared with varieties of paddy other because of age, relative cultivation long, low yields, and very liked by birds (Putri et al., 2017). Warman et al. (2016) stated that part of great black rice in Indonesia is locally not yet experienced breeding, so that has more minor character, profitable like having age older plants and more posture high. A character this cause local black rice varieties easily fall when the wind hits and need more intensive care.

Paclobutrazol (PBZ) is a compound from a group working triazole as a blocker growth plant. PBZ could shorten phase vegetative and accelerate the flowering process because capable of increasing ABA and cytokinin concentrations in shoots plants (Upreti, et al. 2013). PBZ could hinder the work of enzyme cytochrome P450-dependent oxygenase, which obstructs oxidation ent-Laurent becomes sour ent-kauronics and biosynthesis of gibberellins (Soumya et al. 2017). The properties of PBZ that can hinder growth vegetative and improve results harvest can be utilized to advance the productivity of plant black rice varieties locally.

Hardiyati et al . (2018) reported that PBZ 100–300 ppm can increase the micromorphology profile of rice stomata density on gogo varieties

Inpago Unsoed 1. Based on Maulana et al. (2018), a PBZ concentration of 50-150 ppm can hinder growth high in rice varieties Stump Hideung, Situ Bagendit, and Purple Pandan. PBZ with a concentration of 150 ppm produces amount puppies most rice. Pan et al. (2013) stated that adding PBZ could increase rice yields by increasing the grain per panicle and grain contained. Application PBZ expected could reduce tall plants, age plants and get increased results grain local black rice varieties. The aim of the study is for knowing the influence of the concentration PBZ variety of local black rice in Central Java and the interaction among concentration and variety of black rice to profile micromorphology and character agronomy.

METHODS

Plant materials and experimental design

The research was used five local black rice varieties in Central Java. The materials used in the research consist of seeds of black rice varieties Brebes, Pekalongan, Klaten, Magelang, and Pati. This research was carried out in greenhouses and laboratories at Plant Structure and Development, Faculty of Biology, Universitas Jenderal Sudirman, from October 2020 until March 2021. Treatment of PBZ with concentrations of 0, 100, 200, and 300 ppm. Stage preparation study is seed paddy soaked in water for 48 hours, then sown for two weeks. Next up, planted seeds of black rice in growing media land that had been added fertilizer compost. Treatment or spraying of PBZ has done 28 days after the plant. Sample leaves used as fresh preparation is leaf fifth from end plants. Method study held with method experimental with design test design random complete (CRD) factorial. The first factor is the varieties of local black rice, while the second is the PBZ concentration.

Paradermal section on stomata and trichome characters

The replica method was used for observing the stomatal and trichomes density of the leaves. The steps were as follows: (1) Cleaning the abaxial and adaxial surfaces of the leaves, (2) Applying the nail polish and leaving it for 30 minutes to be dried (3) Dried spreads were attached with transparent tape and flattened, (4) The transparent tape was peeled and removed slowly from the leaves surfaces, then attached to the object-glass, (5) Flattening and labeling with a description of the plant type, and (5) Observing the types of stomata and trichomes as well as their size and density by using a light microscope with same magnification (400x). The paranormal section was done using the quantitative (size and density) of trichomes and stomata cells. Stomata and trichomes density were obtained from the following calculation: stomata/trichomes density = total stomata/trichomes per area of the field of view (mm2) (Samiyarsih et al., 2020).

$$density = \frac{\text{total of stomata/trichomata}}{\text{area (mm2)}}$$

Embedding methods for leaf anatomy in transverse section

Micromorphology profile was observed using the Embedding method (Samiyarsih et al., 2019) with minor modification. The nail polish is transparently smeared on both sides leaves. After the nail polish dry, taken by being carefully, put it on top object glass, dripped with water, and closed it with cover glass, next with labeling. S*quare* micrometer and eyepiece mounted on the tube lens oculars, preparations observed with 400× magnification. The length and width of the stomata are multiplied by the results calibration micrometer eyepiece. Embedding methods for leaf anatomy in transverse section The observed anatomical profiles include cuticle thickness, epidermis thickness, and palisade ratio. The 5th leaf from the shoot bud was taken and cut into a 1 cm2 piece. It was then subjected to fixation in FAA solution (FAA: 10% formalin, 5% acetic acid, 50% ethyl alcohol, and 35% distilled water) for 24 hours. The preparation of leaf anatomy was based on the embedding method and the staining was done using safranin (1%) in 70% alcohol. The transversal section using to observations of the thickness of the cuticle, epidermis, and palisade ratio (cell/mm). The palisade ratio was obtained by counting the total palisade cell per area of the field of view (mm2). Observations were conducted by using a binocular microscope, Olympus CH-20, at 400x magnification. Measurement of anatomical profiles was done using a calibrated ocular micrometer (Samiyarsih et al., 2020).

Agronomic Characters

Observation of character agronomy started the moment black reentered the generative period. Counting quantity and weighing weight grain per panicle conducted when paddy enters time to harvest. One panicle was chosen randomly on

every clump and calculated the number of whole grains. In comparison, the resulting offspring in one clump paddy was estimated at 73 days after the plant (DAP) or beginning phase generative.

Data analysis

Data observations were analysed using SPSS 16.0., with analysis test variance (ANOVA) and further test [U1] honestly significant difference (HSD) with level 5% significance. Weight of 100 grains containing weighed use scales analytic with units of grams (g).

RESULTS AND DISCUSSION

Micromorphological Profile

Analysis result variety shows that interaction among concentration PBZ with varieties black rice used _ take effect significant ($p<0.05$) to length and width as well as adaxial stomatal density. Size length and width and abaxial stomatal density by significant ($p<0.05$) only influenced by the variety of black rice used. Density trichomes side abaxial influenced by interaction Among concentration PBZ with varieties black rice by significant ($p<0.05$).

Adaxial Stomata Width and Length

The average result of measurement of the length and width of the adaxial stomata (Table 1 and Table 2) shows the longest stomata found in the V4K2 treatment of 25.6±1.39 µm. The V2K2 interaction resulted in the mean adaxial stomata size of 15.2±1.39 µm. In the V3K4 interaction, it has the average size of the smallest stomata length and width, namely 14.4±2.4 µm and 8±1.39 µm. Interaction among concentration PBZ with varieties black rice on research this cause change size length and width and adaxial stomatal density. PBZ could inhibit the elongation process of cells in tissue leaf because it disturbs the synthesis of gibberellins (Soumy et al., 2017). The delayed elongation cells in tissue leaf could cause a decrease in the size of long and wide surface leaves that impact changing stomata density (Subantoro, 2014; Sumadji & Purbasari, 2018).

Changes follow changes in stomatal density in stomata size. Stomata with tall and inclined density have to size small width compared with dense stomata low (Juairiah, 2014).

Adaxial Stomata Density

Average adaxial stomatal density is the highest found in the V1K1 interaction, as much as 37.67±5.51 stomata per mm^2 large leaves, while the average density Lowest found in V4K2 is 19.33±2.08 stomata per mm^2 large leaves (Table 3). Observation results show that adaxial stomatal density compares backward with size, length, and width. Stomata density will be small if the size, length, and width are big. According to Hafiz et al. (2013), magnitude stomata density is affected by stomata size. The more oversized the score density, the smaller the stomata size. The same thing was stated by the Goddess et al. (2015); stomata size can be affected by stomata density. The bigger the size, length, and width of the stomata, the score density will make the smaller, and vice versa.

Based on results measurement, Pati varieties have the longest stomata, i.e., 22 µm, whereas varieties Klaten have the largest stomata width, 14.6 µm. Varieties Magelang has the smallest length and width of stomata compared to fourth varieties other, namely 16.2 µm and 10 µm (Table 6). When compared with adaxial stomata, abaxial stomata size has a larger average size small. Difference size will take effect to level density.

Rochman & Hamida (2017) stated that stomata size could influence score density. The bigger stomata size then will lower the score, which thickness is correlated positively with the production plant. According to Guest et al. (2018), the number of abaxial stomata in terrestrial plants is more than in adaxial stomata. The number and size of stomata are mutual, which correlates with plants with a large number of stomata tend to have smaller sizes.

Table 1. Average Length of Adaxial Stomata (µm)

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	16 ± 2.77 ab	17.6 ± 3.67 ab	17.6 ± 5.54 ab	24.8 ± 7.72 b
Klaten (V2)	17.6 ± 2.77 ab	21.6 ab	20.8 ± 3.67 ab	20 ± 1.39 ab
Magelang (V3)	20 ± 1.39 ab	16 ± 2.77 ab	16.8 ± 4.16 ab	14.4 ± 2.4 a
Pati (V4)	20 ± 1.39 ab	25.6 ± 1.39 b	18.4 ± 1.39 ab	18.4 ± 3.67 ab
Pekalongan (V5)	18.4 ± 1.39 ab	19.2 ± 2.40 ab	20 ± 4.50 ab	21.6 ± 2.40 ab

Description: Numbers to follow with the same letter, no different significance at the test level of 0.05.

Table 2. Average Width of Adaxial Stomata (µm)

Varieties Paddy Black	Stomata Length (µm)	Stomata Width (µm)	Axial Stomata Density (stomata/mm ^{2} large leaves)
Brebes	18.8 ab	12 a	38.50 b
Klaten	21.4 b	14.6 b	33.33 ab
Magelang	16.2 a	10 ab	39.25 b
Pati	22 b	14 b	27.17 a
Pekalongan	18.8 ab	13 b	32.75 ab

Description: Numbers to follow with the same letter mean no different significance at the test level of 0.05

Table 3. Average Adaxial Stomata Density (stomata/mm^2 large leaves)

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	9.6 ab	10.4 ± 1.39 ab	12 ± 2.40 ab	13.6 ± 2.77 ab
Klaten (V2)	12.8 ± 3.67 ab	15.2 ± 1.39 b	12 ± 2.40 ab	13.6 ± 1.39 ab
Magelang (V3)	12 ab	12.8 ± 1.39 ab	10.4 ± 1.39 ab	8 ± 1.39 a
Pati (V4)	13.6 ±1.39 ab	13.6 ± 1.39 ab	11.2 ± 2.77 ab	12.8 ± 1.39 ab
Pekalongan (V5)	10.4 ±1.39 ab	12 ± 2.40 ab	13.6 ± 3.67 ab	14.4 ± 2.40 b

Description: Numbers to follow with the same letter mean no different significance at the test level of 0.05

Table 4. Average Length, Width, and Density of Abaxial Stomata

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	37.67 ± 5.51 b	28.67 ± 4.04 ab	27.67 ± 5.67 ab	20 ± 11.27 a
Klaten (V2)	29.33 ± 6.66 ab	21.67 ± 1.16 ab	26 ± 2.65 ab	21.67 ± 2.52 ab
Magelang (V3)	28.33 ± 2.52 ab	26.67 ± 5.13 ab	30.33 ± 9.29 ab	32.67 ± 3.21 ab
Pati (V4)	30.33 ± 2.08 ab	19.33 ± 2.08 a	22.33 ± 2.52 ab	32.33 ± 9.07 ab
Pekalongan (V5)	28.67 ± 2.08 ab	24.67 ± 4.73 ab	23.33 ± 6.51 ab	24.33 ± 0.58 ab

Description: Numbers to follow with the same letter mean no different significance at the test level of 0.05

Abaxial Stomata Density

The results of the calculation of the average abaxial stomatal density (Table 4), varieties Magelang has the highest average stomata density of 39.25 stomata per mm^2 area leaves, while the density Lowest found in the Pati variety of 27.17 stomata per mm^2 large leaves. Based on the results mean calculation, abaxial stomata have more thickness than the adaxial layer. The same thing was also reported by Pathare et al . (2020), surface stomata density abaxial on some species of plant from the family Poaceae taller than surface adaxial. Stomata could be seen from the difference, which amounts more on the surface adaxial the stomata. Papuangan et al . (2014) stated that on the surface adaxial, a thickly layered cuticle hinders the excessive transpiration process. Besides that, a cuticle will cover the stomata on the surface. As a result, the stomatal density in the adaxial is lower than the stomata density section abaxial in all leaves of local black rice.

The difference in size, length, width, and abaxial stomatal density caused by different varieties of black rice, is used. Differences in arrangement genetics are the leading cause of other characteristics between varieties. According to Barus et al. (2018), different arrangement genetics could occur in species same plant but different types. When the gene is expressed, then will cause other or diverse characteristics in plants good on morphology or physiology.

Besides factor genetics, factors environment is essential in determining the phenotype of plants (Juairiah, 2014). According to Mighty et al. (2017), factors that can influence plants' stomata density include intensity of light, temperature, humidity, and CO2 concentration in the air. Imaningsih (2006) stated that CO2 concentration in the air, water stress, and temperature could influence stomata size and density in rice varieties.

Table 5. Analysis Results Variety Character Agronomy Black Rice

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	2.00 ± 1.00 ab	1.00 a	2.67 ± 0.58 abc	1.67 ± 0.58 ab
Klaten (V2)	3.67 ± 1.16 bc	5.00 ± 1.00 c	2.67 ± 0.58 abc	3.00 ± 1.00 abc
Magelang (V3)	2.33 ± 1.16 ab	1.33 ± 0.56 ab	1.33 ± 0.58 ab	2.00 ± 1.00 ab
Pati (V4)	1.33 ± 0.58 ab	2.67 ± 1.16 abc	2.33 ± 0.58 ab	2.67 ± 0.58 abc
Pekalongan (V5)	2.33 ± 1.16 ab	3.33 ± 0.58 abc	2.00 ab	3.00 ± 1.00 abc

Description : * = significant ($p<0.05$), JA: Total tillers per clump, JG: Number grain per panicle, BG: Weight grain per 100 grains

Average density trichomes abaxial highest generated by the V2K2 interaction of 5.00±1.00 trichomes per mm^2 area leaves. V1K2 interaction produces density average trichomes lowest abaxial, i.e., one trichome per mm^2 area_leaves. Application PBZ cause change development of trichomes because it disturbs the synthesis of gibberellins (Desta & Amare, 2021). Gibberellins are essential in developing plants like germination seeds, elongation of hypocotyl, development of flowers, and initiation formation of trichomes (Pattanaik, et al. 2018).

PBZ could increase the production of cytokinins that will impact growing chlorophyll maturation and rate photosynthesis (Desta & Amare, 2021). In addition, PBZ can reduce the density of trichomes because it increases the rate of photosynthesis. Sutopo (2019) states mechanism for increasing interception light on the leaves besides adding large surface leaves is to reduce the number of trichomes. The cause is trichomes could reflect light and block sunlight from surface leaves.

Character Agronomy

Five local black rice in this research have different colors and characteristics (Figure 1). Based on agronomy results analysis of local black rice consequence addition paclobutrazol (Table 5), varieties of local black rice and concentration PBZ used by independent give influence significant ($p<0.05$) on weight grain per 100 grains. Interaction Among black rice varieties and PBZ concentration has a significant effect ($p<0.05$) on the number of tillers per clump and number of grains per panicle paddy black.

Influence Varieties to Weight Grain per 100 Grains

Differences in local black varieties used to take effect naturally to the average grain weight per 100 grains (Figure 1). They weigh the heaviest grain per 100 grains found in the Pati variety, namely 2.96 g, while the Brebes have the weight the lightest grain of 1.77 g. Dewi et al. (2017) state that diverse genetics causes different appearance in some varieties of black rice from

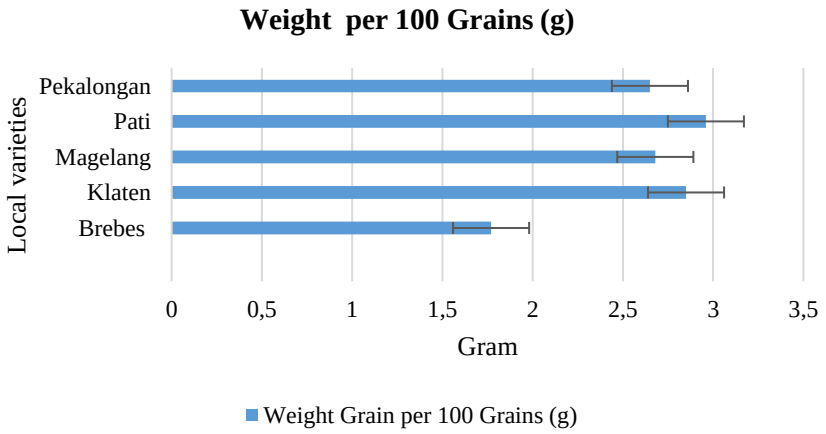

Figure 1. Influence Varieties to Weight Grain per 100 Grains

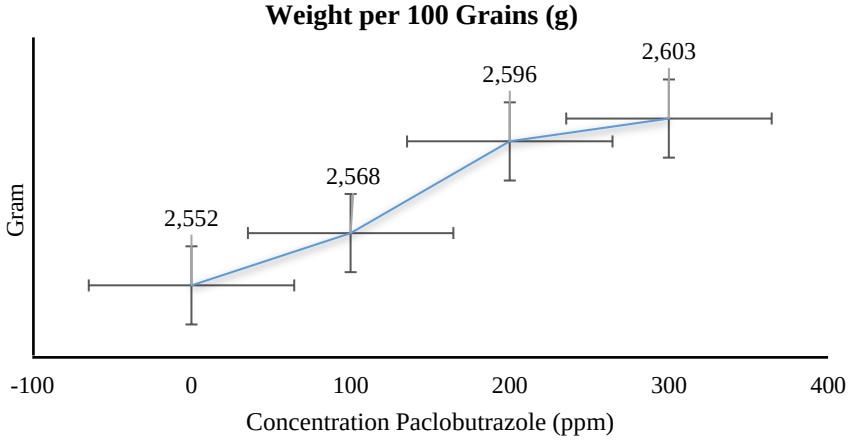

Figure 2. Influence PBZ to Weight Grain per 100 Grains

Figure 3. Five local black rice from different region in Central Java, A. Brebes, B. Pekalongan, C. Magelang, D. Pati, E. Klaten

West Java. Diversity genetics could be seen from different character morphology like the tall plant, the number of puppies productive, color stems colors and properties surface leaves, grain panicles, shapes and colors grain, and flowering. According to Please *et al* . (2019), though genotype has a role important in determining character descendants of a plant, the phenotype is influenced by interaction among genotype with the environment surrounding. Factor environment like altitude, rainfall, rain, humidity, and light intensity is essential in determining the diverse population of plants in a good area by morphology or physiology (Yusran & Maemunah, 2011).

Influence PBZ To Weight Grain per 100 Grains

The results of weighing the grain per 100 grains (Figure 2) with a concentration of 300 ppm produced the highest grain weight of 2.603 g, while the concentration of 0 ppm produced the lowest weight of 2.552 g. Based on observations, grain weight was positively correlated with increasing concentrations of PBZ. PBZ can increase the total chlorophyll count. As a result, the rate of photosynthesis will increase (Xia et al., 2018).

According to Wicaksono et al . (2019), applying retardants such as *chlormequat chloride* (CCC) to plants can inhibit the process of gibberellin formation, but the chlorophyll content in the leaves increases. This condition increases the photosynthetic process, and the resulting photosynthate will be distributed for filling rice grains. Harpitaningrum *et al* . (2014), the addition of PBZ causes a decrease in the rate of cell division and shifts the results of photosynthesis in the generative phase. As a result, the seeds become more extensive and heavier in weight.

Amount Tillers per Clump

The highest number of black rice tillers was produced by the interaction of V4K1, as many as 22.33±2.08 tillers, while the interaction of V5K4 produced the fewest tillers, namely 5.67±0.58 tillers. The interaction between the concentration of PBZ and black rice varieties tended to reduce the number of tillers per clump in the varieties of Brebes, Klaten, Pati, and Pekalongan (Table 6). Possibly caused by the concentration of PBZ applied too high. This report is supported by

Table 6. Total Tillers per Clump

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	10.67 ± 1.53 abcd	10.67 ± 1.16 abcd	11.00 ± 3.00 abcd	9.67 ± 2.08 abc
Klaten (V2)	12.33 ± 1.53 bcd	10.33 ± 0.58 abcd	8.33 ± 0.58 ab	7.00 ab
Magelang (V3)	12.00 ± 3.46 bcd	16.00 ± 4.36 def	18.67 ± 2.08 efg	19.00 ± 2.65 efg
Pati (V4)	22.33 ± 2.08 g	20.33 ± 2.52 fg	16.00 ± 1.00 def	14.67 ± 1.12 cdef
Pekalongan (V5)	13.00 ± 1.00 bcde	10.33 ± 1.16 abcd	7.33 ± 0.58 ab	5.67 ± 0.58 a

Description: Numbers to follow with the same letter, no different significant at the test level of 0.05

Table 7. Total grain per panicle

Varieties Paddy Black	Concentration PBZ (ppm)			
	0 (K1)	100 (K2)	200 (K3)	300 (K4)
Brebes (V1)	10.67 ± 1.53 abcd	10.67 ± 1.16 abcd	11.00 ± 3.00 abcd	9.67 ± 2.08 abc
Klaten (V2)	12.33 ± 1.53 bcd	10.33 ± 0.58 abcd	8.33 ± 0.58 ab	7.00 ab
Magelang (V3)	12.00 ± 3.46 bcd	16.00 ± 4.36 def	18.67 ± 2.08 efg	19.00 ± 2.65 efg
Pati (V4)	22.33 ± 2.08 g	20.33 ± 2.52 fg	16.00 ± 1.00 def	14.67 ± 1.12 cdef
Pekalongan (V5)	13.00 ± 1.00 bcde	10.33 ± 1.16 abcd	7.33 ± 0.58 ab	5.67 ± 0.58 a

Description: Numbers to follow with the same letter, no different significant at the test level of 0.05

Assuero et al . (2012); PBZ concentration of 0.5-5.0 ppm can increase the number of wheat tillers. Liu et al . (2017) reported that PBZ with a concentration of 5 mg/L was the best for increasing the number of tillers in sugarcane. The mechanism of PBZ in the formation of the first child is that PBZ will suppress the elongation process of cells and increase carbohydrate non-structural. Enhancement concentration cytokinins that impact improving content chlorophyll work from photosystem II and cleavage cells in the meristem. The accumulation of photosynthesis consequences increases the photosynthesis rate, which affects the period. Finally, the son could develop more beginning, and the opportunity for increasing the number is also growing large (Assuero et al ., 2012).

Amount Grain per panicle

Calculation result amount of grain per panicle showing that V4K2 interaction resulted in the average number of most grain 101.67±11.02 grain per panicle. V2K0 interaction has a score average amount least grain 52.00 ± 1.73 grain per panicle (Table 7).

The resulting interaction Among varieties Brebes, Magelang, and Pati with enhancement concentration PBZ tend lower amount grain per panicle. This result is not following Pan et al . (2013), who reported that adding PBZ to rice could increase the grain per panicle and grain contained. Interaction PBZ with varieties the possibility could result in malai paddy being shortened. Hütsch & Schubert (2020) stated PBZ could disturb the development cob corn. Effect elongation inhibit in phase vegetative will also negatively impact phase generative, amounts the resulting seeds more. Prihantari et al. (2021) stated that the interaction between paclobutrazol and gibberellin was insignificant. Still, the treatment of 300 ppm paclobutrazol showed the best results in plant height inhibition. In comparison, treating paclobutrazol at 200 ppm was the best concentration to stimulate flowering and harvesting age.

The **novelty** of this research was to obtain the best dose of paclobutrazol in reducing the height of five local black rice varieties in Central Java. To improve the natural character of local black rice, which is easy to fall. The **advantage of this research** is to provide recommendations for the treatment of paclobutrazol in improving local black rice cultivation so that farmers' losses can be minimized. Another contribution to the biological sciences is the existence of anatomical, physiological and agronomic characteristics which are presented as scientific information from the latest research results.

CONCLUSION

This experiment revealed that the treatment of PBZ on five local black rice varieties improved the agronomic characters. The variation in the micromorphological profile is closely related to the local variety and the concentration of PBZ

given. Each local back rice variety would provide a different response to the same treatment of PBZ and vice versa. The recommended dose of PBZ at a specific concentration is needed to improve the agronomic character of local black rice. It significantly could reduce plant height and accelerate plants entering the generative phase.

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