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Promoting Effects of Paclobutrazol on the Productivity of Different Rice (*Oryza sativa* L.) Ecotypes Under Rainfed Lowland Condition

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Abstract

Rainfed lowland rice ecosystem occupies ~30% (~1.4 M ha) of the total rice production area in the Philippines. In this ecosystem, other rice varieties or ecotypes not bred for this ecosystem are also cultivated and may suffer from severe yield loss when drought occurs. Under such stress condition, paclobutrazol (PBZ) was reported to improve physiological processes and enhance yield of tillering crops by increasing the number of tillers and panicles. This study aimed to determine the effects of PBZ on the growth and yield response of different rice ecotypes under rainfed condition. The experiment was laid out in split-plot design with PBZ concentrations (0, 250, 500 ppm) as main plot applied at 14 days after transplanting. Rice ecotypes, namely rainfed lowland (PSB Rc14), irrigated lowland (NSIC Rc222), upland-special quality (Dinorado) and lowland-special quality (NSIC Rc216) as subplot, were arranged in randomized complete block design with three replications. Results showed that PBZ temporarily suppressed plant height of different rice ecotypes as they were able to recover resulting in increased plant height at maturity. PBZ also improved the tillering and tiller's ability to produce panicles resulting in a higher number of panicles at maturity. Other yield components, namely panicle length, number of spikelets and filled spikelets per panicle, especially percent filled grains, were also improved. Grain yield likewise increased with different optimum PBZ concentrations for each rice ecotype. A lower concentration (250 ppm) gave the highest grain yield for lowland rice ecotype while higher concentration (500 ppm) for special-quality rice.

Keywords*:* rainfed, paclobutrazol, ecotypes, tillers or panicles, grain yield

1. Introduction

Rainfed lowland rice ecosystem occupies around 36% (~52 M ha) of the total global rice area wherein more than 50% (~27 M ha) is vulnerable to drought stress (Global Rice Science Partnership, 2013). Drought stress occurs when soil moisture is inadequate to meet the water demand of the crop. It is a recurrent phenomenon and the major cause of low productivity in this ecosystem.

The major traits contributing to rice productivity under this ecosystem are yield potential (Kumar *et al*., 2010) and drought resistance (O'Toole, 1982). However, combining these traits remains very challenging for the breeders due to the complexity of both traits (Li and Gao, 2008). Yield trait is a quantitative trait controlled by different genes making this trait difficult to introgress. The same is true to drought resistance (Hao and Lin, 2010) which has different mechanisms (escape, avoidance, and tolerance) with different adaptive traits in each mechanism. In rainfed lowland rice ecosystem, farmers are also planting other varieties or other rice ecotypes aside from rainfed varieties. Some of the varieties commonly cultivated under rainfed condition are either high-yielding or special quality rice, which are bred for irrigated lowland ecosystem. Planting of high yielding variety can increase productivity in case of sufficient rainfall but may suffer from severe yield reduction when drought occurs. Cultivation of special quality rice, on the other hand, can increase profitability because it demands higher market price. There is also a type of rice that has potential in increasing the profitability of rainfed ecosystem and this is heirloom rice with export quality. However, these types of rice are not bred for rainfed ecosystem; hence, they may have lower productivity relative to rainfed rice variety.

In the absence of resistance or tolerance trait, plant performance under stress conditions can be still improved with application of plant growth regulators (PGRs). One of the most commonly used PGRs in improving plant response under stress condition including drought is paclobutrazol (PBZ). However, majority of the studies reported the positive effect only at the physiological level (Zhu *et al*., 2004; Somasundaram *et al*., 2009; Pal *et al*., 2016; Rezazadeh *et al*., 2016) because this PGR is also known as growth retardant (Duck *et al*., 2004). The reduction in growth usually affects the most important part which is the yield (Alvarez *et al*., 2012). This is the main reason why PBZ is not usually used in crop production despite its positive effect on different physiological processes. However, aside from improving physiological processes under drought stress, PBZ can also enhance the tillering capacity of tillering crops (Assuero *et al*., 2012; Plaza-Wüthrich *et al*., 2016). Thus, it can be a potential solution to increase the productivity of rice under rainfed condition. Therefore, this study aimed to determine the effect of PBZ on the growth and yield response of different rice ecotypes under rainfed condition.

2. Methodology

2.1 Time and Place of the Study

The study was conducted from June to October 2017 at Experimental Area of Crop Science, College of Agriculture, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines (15° 44’N, 120° 56’ E, 80 masl).

2.2 Experimental Design and Treatments

The experiment was arranged using split-plot in randomized complete block design (RCBD) with three replications. The PBZ was assigned to main plot while rice ecotypes as subplot. Three PBZ concentrations (0, 250, and 500 ppm) were used which were applied to plants using foliar application at 14 days after transplanting (DAT). Rice ecotypes, on the other hand, were composed of four different varieties cultivated in rainfed lowland, irrigated lowland, and upland. These are PSB Rc14, commonly used as tolerant check in breeding for drought tolerance under rainfed lowland; NSIC Rc222, high-yielding irrigated lowland variety; NSIC Rc216, widely cultivated modern special-quality rice under irrigated lowland; and Dinorado, traditional special-quality rice cultivated in upland.

2.3 Establishment and Maintenance

Seed conditioning was done before pre-germination by oven-drying the seeds for three days at 50 °C and then storing it for one day at room temperature. Raised seedbeds were prepared a day before sowing. The seeds were drilled into trenches (1.5 cm x 1 cm, depth x width) with 4 cm distance between trenches. Seeds were then covered with carbonized rice hull then covered with sacks to protect the seeds from pests and raindrops. Sacks were removed five days after sowing (DAS) (~5 cm tall seedlings). Fertilizer was applied at 10 DAT using urea (46-0-0) at a rate of 10 g m^{-2}. The 21-day old seedlings were transplanted at a distance of 20 cm x 20 cm with two to three seedlings per hill using straight method of transplanting. A total of 500 hills were transplanted per 20 m^2 (5 m x 4 m) subplot.

Supplemental irrigation was done from land preparation until 14 DAT to ensure proper establishment (land preparation, seedling production, transplanting, and recovery). Water with a depth of 3-5 cm was maintained from transplanting to 14 DAT. Thereafter, the field was drained and the source of irrigation was only the rainfall. Four-hundred twenty-nine grams of 14-14-14 (NPK) fertilizer was applied at 7 DAT; 300 g of 16-20-0, and 26 g of 46-0-0 was topdressed in each sub-plot at 21 and 35 DAT, respectively based on the recommended rate of 90-60-30 kg NPK ha^{-1}. Plants were harvested when the grains are 85-90% mature or straw-colored.

2.4 Data Gathering

2.4.1 Hydrological Parameters

Hydrological data such as rainfall, soil moisture content (SMC), and water table depth (WTD) were recorded throughout the experiment. Daily rainfall was recorded from the CLSU-Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) weather station located at ~1000 m west of the experimental area. The SMC (%) and WTD (cm) were monitored weekly from 14 DAT until harvesting using gravimetric method and piezometer, respectively.

2.4.2 Agronomic Parameters

To monitor growth of the plants at the early stage, plant height, number of tillers and leaf chlorophyll content were recorded from 14 to 49 DAT. Leaf chlorophyll content was measured using Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502Plus, Konica Minolta, Inc., Japan). Data such as days to 50% flowering, days to maturity, plant height, number and length of panicles, percent productive tillers, number of spikelets per panicle, number of grains per panicle, percent filled grains per panicle, 1000 grain weight at 14% moisture content (MC), plant biomass, harvest index (HI), and grain yield per hectare (t ha^{-1}) expressed at 14% MC were also recorded.

2.5 Statistical Analysis

Analysis of Variance (ANOVA) appropriate to split-plot RCBD was used in data analysis. Data such as percent filled grains and harvest index were transformed for analysis as suggested by Gomez and Gomez (1984). Comparison among means was done using Tukey or honestly significant difference at 0.05 level of significance. Pearson correlation was also performed to determine the relationship among agronomic traits. Analyses were done using Statistical Tools for Agricultural Research (STAR) v.2.0.1 developed by Biometrics and Breeding Informatics of Plant Breeding, Genetics, and Biotechnology Division (PBGBD) of International Rice Research Institute (IRRI).

3. Results and Discussion

3.1 SMC, WTD and Daily Rainfall of the Area

The experimental area was kept flooded (3-5 cm) from transplanting until 14 DAT. Thereafter, rainfall was the only source of soil moisture in the area. The cumulative amount of rainfall from transplanting to harvesting was 696.8 mm with an average of 6.77 mm d^{-1} (Figure 1). The range of rainfall during vegetative phase was 0-38.6 mm d^{-1}, 0-34.1 mm d^{-1} at reproductive phase, and 0-28.9 mm d^{-1} at ripening phase. Although there was only a maximum of three (3) days without rainfall, soil cracks starting at vegetative phase were observed indicating soil drying. The SMC and WTD ranges were 50-76% and 44-56 cm, respectively, at vegetative phase, whereas 28-61% SMC and 66-54 cm WTD at reproductive phase, and 28-72% SMC and 60-54 cm WTD at ripening phase. The lowest SMC (28-42%) occurred from the middle of reproductive phase up to the middle of ripening phase.

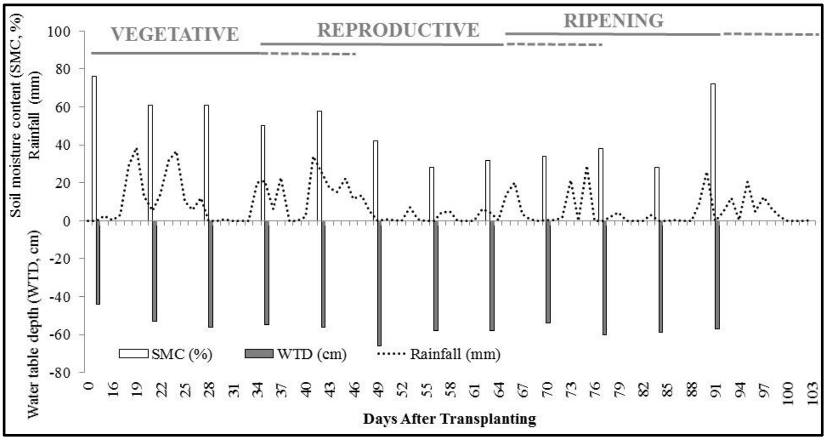

Figure 1. Weekly SMC (%) and WTD (cm), and daily rainfall (mm) of the area from transplanting to harvesting. Broken lines at growth phases represent differential duration of phenological stages among rice ecotypes.

3.2 Plant Height, Number of Tillers, and Leaf Chlorophyll Content of Different Rice Ecotypes at Different Paclobutrazol Concentrations from 14 to 49 DAT

3.2.1 Plant Height

Growth rate especially at the early growth stage is an important characteristic of rice under rainfed condition to compete against weeds. Plant height (cm) of different rice ecotypes showed an increasing trend from 14 to 49 DAT with rapid increase in height observed at the early stage of growth specifically from 14 to 28 DAT (Figure 2).

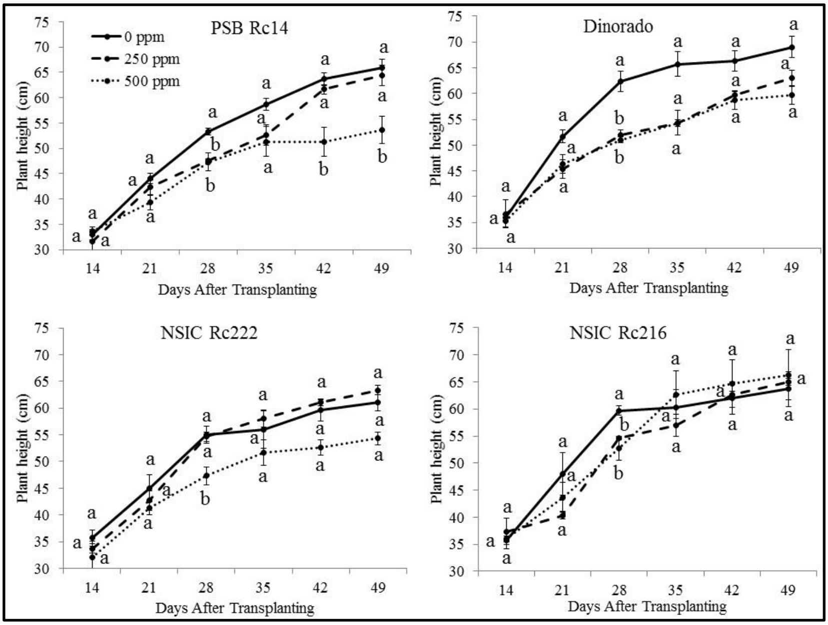

Figure 2. Plant height of different rice ecotypes at different PBZ concentrations from 14 to 49 DAT under rainfed condition. Means within each sampling point with the same letter are not significantly different at 5% level by HSD. Error bars denote SE (n = 3).

At 28 DAT, however, plant height of different rice ecotypes was reduced due to the effect of PBZ. In general, PBZ application either 250 or 500 ppm (except 250 ppm in NSIC Rc222) caused significant reduction in plant height. The PBZ is known as a growth retardant and widely used in many ornamental plants to control height, thereby improving aesthetic value (Duck *et al*., 2004). However, in this study, except for PSB Rc14, the effect of PBZ in rice height was only short-term as no significant difference was observed after 28 DAT.

Plant heights at 49 DAT were 54-66 cm for PSB Rc14; 60-69 cm for Dinorado; 54-63 cm for NSIC Rc222; and 64-66 cm for NSIC Rc216. This result indicates that 250-500 ppm PBZ application in different rice ecotypes at 14 DAT under rainfed condition temporarily suppressed plant height and the plants were able to recover after few weeks.

3.2.2 Number of Tillers

Similar to the observation in plant height, significant differences in number of tillers at 28 DAT among PBZ concentrations were observed. However, the effect of PBZ was more stable on number of tillers than on plant height because of the significant difference on number of tillers that was observed up to 49 DAT (except for NSIC Rc216). The PBZ concentrations that produced the highest number of tillers for PSB Rc14, NSIC Rc222, and Dinorado at 28 DAT were also the same concentrations that gave highest number of tillers until 49 DAT. Thus, from 28 to 49 DAT, highest number of tillers was produced with 250 ppm PBZ for PSB Rc14 and NSIC Rc222 while 500 ppm for Dinorado (Figure 3).

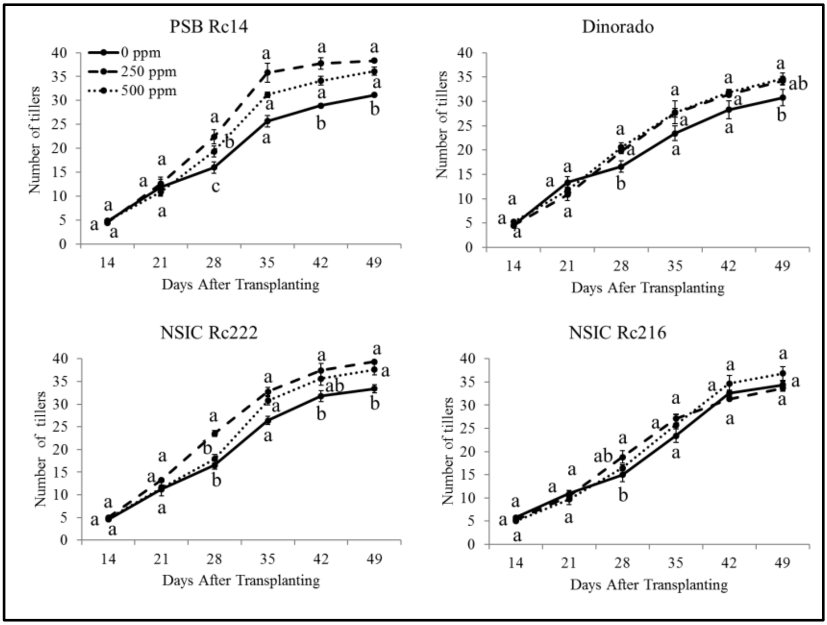

Figure 3. Number of tillers of different rice ecotypes at different PBZ concentrations from 14 to 49 DAT under rainfed condition. Means within each sampling point with the same letter are not significantly different at 5% level by HSD. Error bars denote SE (n = 3).

At 49 DAT, 250 ppm PBZ increased number of tillers from 31 to 38 in PSB Rc14 and from 33 to 39 in NSIC Rc222, while 500 ppm PBZ increased number of tillers from 31 to 35 in Dinorado. Also, NSIC Rc216 produced 37 tillers per plant with 500 ppm but not significantly higher than the 34 tillers of untreated plants. The application of PBZ on tillering crops advances the onset of tiller production (Assuero *et al*., 2012); hence, resulting to the increase in the number of tillers (Plaza-Wüthrich *et al*., 2016). These results indicate that PBZ application increases number of tillers of different rice ecotypes at 28 DAT under rainfed condition. Additionally, relative to plant height, the effect of PBZ on number of tillers is more stable.

3.2.3 Leaf Chlorophyll Content

Application of 250-500 ppm PBZ to different rice ecotypes at 14 DAT under rainfed condition did not improve leaf chlorophyll content (Figure 4). The trends of leaf chlorophyll content in all rice ecotypes were almost similar.

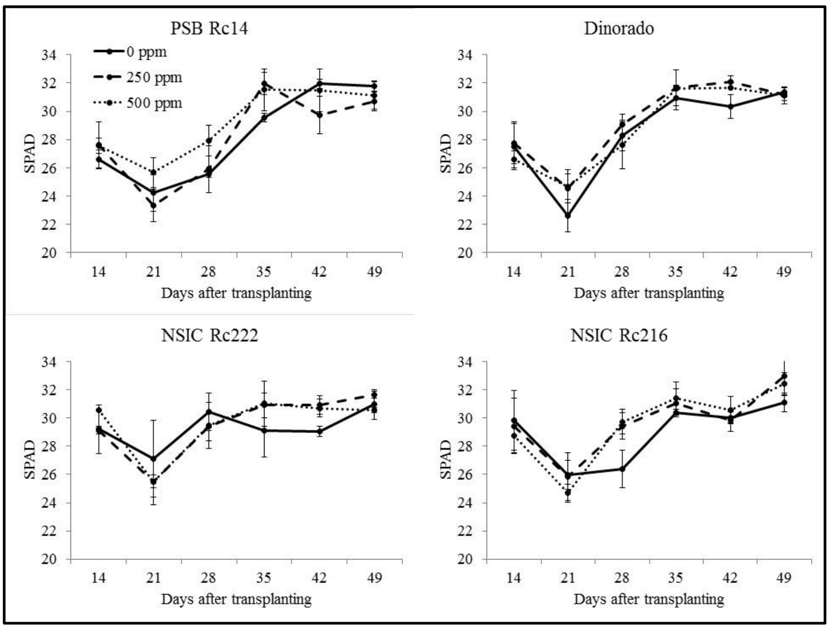

Figure 4. Leaf chlorophyll content of different rice ecotypes at different PBZ concentrations from 14 to 49 DAT under rainfed condition. Error bars denote SE (n = 3).

Leaf chlorophyll content decreased from 14 to 21 DAT, which might be due to the depletion of nutrients from complete fertilizer applied at 7 DAT. The increasing leaf chlorophyll content from 21 to 49 DAT might be due to top-dressing of Nitrogen-containing fertilizer. Leaf chlorophyll content of the different rice ecotypes applied with different PBZ concentrations range from 31 to 33 SPAD value at 49 DAT. Although it was previously reported that PBZ can improve leaf chlorophyll content in many plants, some studies reported that PBZ has no effect in leaf chlorophyll content. PBZ application of 26-60 ppm did not affect SPAD readings of ornamental chili (França *et al*., 2018). Additionally, an earlier experiment on PBZ application in two traditional rice varieties (same location and season) showed that 250-1000 ppm PBZ had no effect on leaf chlorophyll content (Mactal and Canare, 2015). This might be due to the low photosynthetic activity of crops because of low solar radiation during wet season.

3.3 Agronomic Parameters at Maturity

Previous studies have reported different effects (negative and no effect) of PBZ on plant height because PBZ effects might depend on concentration (Mactal and Canare, 2015) and varieties (Detpitthayanan *et al*., 2019). Mactal and Canare (2015) found that high PBZ concentration of 1000 ppm applied at 19 DAT was effective in reducing height of the two traditional rice varieties (Elon-elon and Palawan red) at harvest and that lower concentration of 250 and 500 ppm did not affect plant height. In the study of Detpitthayanan *et al*. (2019), application of 100 ppm PBZ at vegetative phase did not reduce the height of all rice varieties or lines tested. In this study, on the other hand, PBZ application increased plant height of the different rice ecotypes under rainfed condition at maturity stage (Figure 5a). PBZ-treated plants of PSB Rc14 and NSIC Rc222 produced comparable heights which were significantly taller than the untreated plants (0 ppm). The application of 500 ppm PBZ produced tallest plant in Dinorado but comparable to 250 ppm. Lastly, both PBZ concentrations (250 and 500 ppm) increased plant height in NSIC Rc216 but the tallest height was observed at lower concentration (250 ppm). The results indicate that although PBZ application reduced the height of the rice ecotypes at the early growth stages (Figure 2), it increased plant height at maturity stage. The recovery and increase might be due to the reported improvement in physiological processes (i.e. photosynthesis, anti-oxidant activities, membrane stability, etc.) associated with PBZ application (Soumya *et al*., 2017) although not measured in this study.

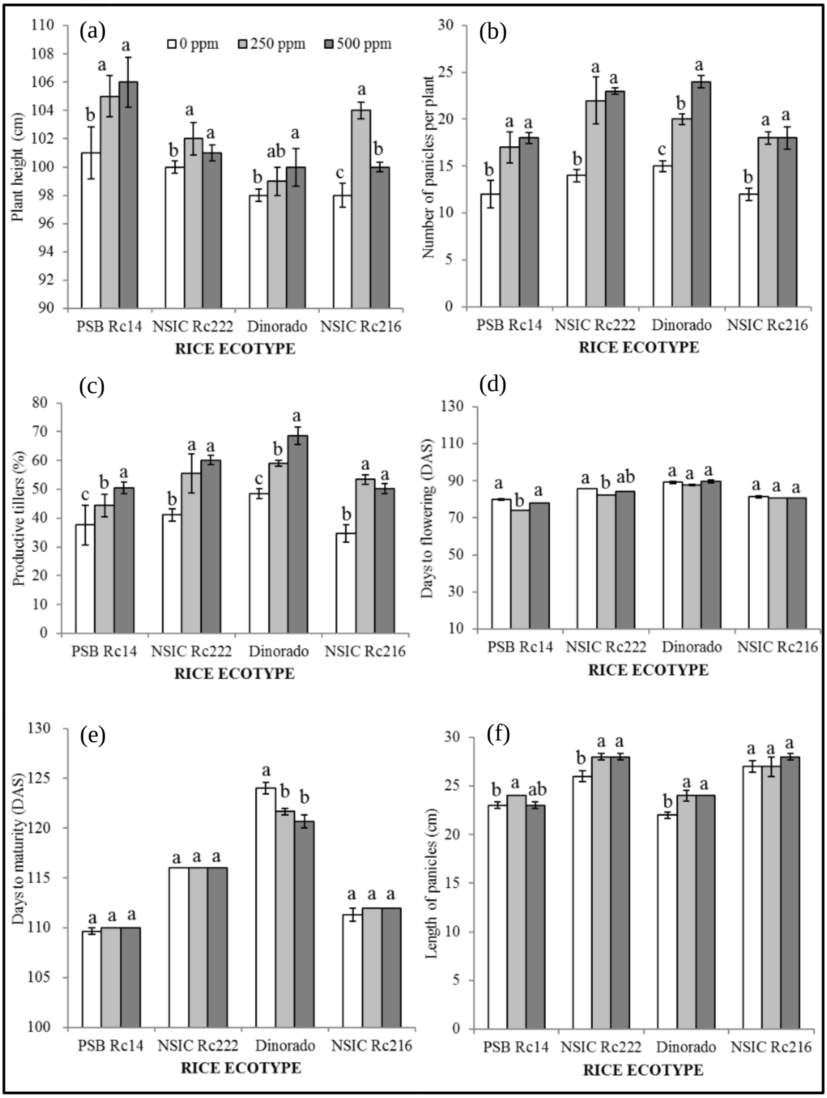

Figure 5. Plant height (a), panicle number per plant (b), productive tillers (%) (c), days to flowering (d), days to maturity (e), and length of panicles (f) of different rice ecotypes at different concentrations of PBZ under rainfed condition.

Panicle is one of the yield components of rice which is produced from tillers; hence, there is a higher chance of developing more panicles when there are more tillers. The increase in number of tillers at the early growth stage due to PBZ application contributed to the development of more panicles as shown by significant increase in number of panicles of all rice ecotypes with PBZ application (Figure 5b). In all rice ecotypes, PBZ application regardless of concentration increased the number of panicles. In modern rice ecotypes (PSB Rc14, NSIC Rc222, and NSIC Rc216) 250 and 500 ppm PBZ produced comparable number of panicles per plant but significantly higher than 0 ppm. For the traditional variety (Dinorado), there was increasing number of panicles with increasing PBZ concentration. This result suggests that PBZ applied at 14 DAT or at tillering stage can increase number of panicles of different rice ecotypes under rainfed condition. This finding is in agreement to the result of Plaza-Wüthrich *et al*. (2016) wherein PBZ application increased number of panicles of tillering crops under water-limited condition (but not under well-watered condition). It is also interesting to note that PBZ application improved percent productive tillers or the ability of the tillers to bear panicles (Figure 5c). For irrigated lowland ecotype (NSIC Rc222 and Rc216), application of 250 ppm PBZ improved percent productive tillers but there was no further increase with higher PBZ concentration (500 ppm PBZ). On the other hand, in rainfed lowland ecotype PSB Rc14 and upland Dinorado, there was increasing trend with increasing PBZ concentrations; thus, highest percent productive tillers was observed with 500 ppm PBZ. Hence, the increase in number of panicles with PBZ application was due to the production of more tillers and more importantly due to improved ability of the tillers to produce panicles.

For the days to flowering, significant differences were only found in two rice ecotypes (PSB Rc14 and NSIC Rc222) wherein 250 ppm PBZ induced earlier flowering (Figure 5d). However, this early flowering did not contribute to earlier maturity of these rice ecotypes. On the other hand, 250-500 ppm PBZ shorten maturity of Dinorado by two to three days (Figure 5e). The maturity of PSB Rc14 was 110 DAS, NSIC Rc222 was 116 DAS, Dinorado was 121-124 DAS, and NSIC Rc216 was 111-112 DAS.

Likewise, PBZ application increased length of panicles of the rice ecotypes except for NSIC Rc216 (Figure 5f). PBZ concentrations of 250 and 500 ppm increased the length of panicles in NSIC Rc222 and Dinorado but were of comparable effects while 250 ppm PBZ only was found effective in increasing length of panicles in PSB Rc14.

The promotive effects of PBZ (250 and 500 ppm) was also observed in number of spikelets per panicle of PSB Rc14 and NSIC Rc222 but there might be a tendency to decrease with higher PBZ concentration (Figure 6a).

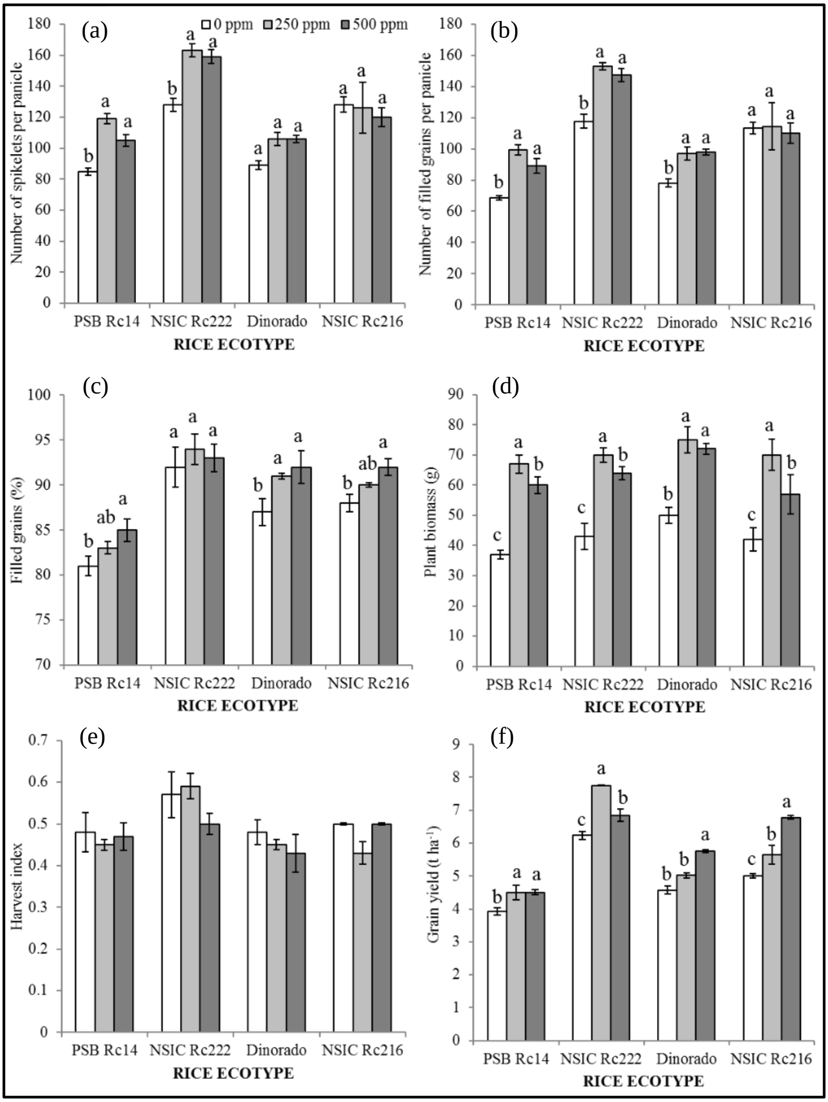

Figure 6. Number of spikelets per panicle (a), number of filled grains (b), percent filled grains (c), plant biomass (d), harvest index (e), and grain yield (f) of different rice ecotypes at different concentrations of PBZ under rainfed condition. Means with the same letter within rice ecotype are not significantly different at 5% level by HSD. Error bars denote SE (n = 3).

Similarly, the result was observed in number of filled grains per panicle except that Dinorado also showed significant improvement with PBZ application regardless of concentration (Figure 6b). In addition, PBZ application increased percent filled grains of PSB Rc14, Dinorado, and NSIC Rc216

(Figure 6c). These results indicate that PBZ application improves the most sensitive yield components of rice under drought stress, which is percentage of filled grains or spikelet fertility. This improvement was also observed in different types of rice under irrigated condition like special-quality rice when PBZ is applied at vegetative phase (Detpitthayanan *et al*., 2019), traditional rice (Mactal and Canare, 2015), and hybrid rice (Pal *et al*., 2013).

In terms of plant biomass (Figure 6d), all rice ecotypes evaluated in this study showed positive response with PBZ application. The lower PBZ concentration (250 ppm) produced the heaviest plant biomass as there was a decreasing trend with higher PBZ concentration except for Dinorado that also showed comparable biomass in 500 ppm PBZ. The increase in biomass could be attributed to the increased number of tillers or panicles as shown by the strong positive linear relationship between these two parameters (Table 1). This result is similar to the studies of Plaza-Wüthrich *et al*. (2016) and Assuero *et al*. (2012) in which PBZ-treated plants had heavier biomass that could be due to higher number of tillers and panicles. Furthermore, PBZ did not affect harvest index of all rice ecotypes (Figure 6e). This implies that given the increase in biomass, there are no changes in partitioning of assimilates to the grain. Hence, the higher the biomass without change in harvest index may suggest higher grain yield as shown in Figure 6f. Thus, grain yield also increased in all rice ecotypes with PBZ application.

The increase in grain yield with PBZ application could also be attributed to other yield components particularly panicle number and length which resulted in increased number of filled grains (Table 1). The improvement in these yield components could be attributed to the other reported effects of PBZ which include improvement of physiological processes (i.e. photosynthesis, anti-oxidant activities, membrane stability, etc.) that are very important under water deficit condition (Soumya *et al*., 2017). Furthermore, result of grain yield also showed significant interaction between PBZ concentrations and rice ecotypes. The highest grain yield was obtained with lower PBZ concentration (250 ppm) for lowland rice (PSB Rc14 and NSIC Rc222) while higher PBZ concentration (500 ppm) for special quality rice (Dinorado and NSIC Rc216), suggesting that optimum PBZ concentrations vary with rice ecotype or variety. These findings were also observed by Plaza-Wüthrich *et al*. (2016) in which PBZ application increased grain yield of tillering crop, tef (*Eragrostis tef* [Zucc.] Trotter) under drought stress as well as under well-watered conditions. Interestingly, the grain yield of PBZ-treated tef under drought stress condition was higher than untreated plants under well-watered condition.

Table 1. Correlation coefficients of different agronomic parameters to grain yield

	DTF	MAT	PH	PN	PL	NFG	NS	PFG	BM	HI	PPT	GY
LC	0.02	-0.09	0.01	-0.01	0.08	-0.06	-0.07	0.00	0.08	-0.06	0.01	0.08
DTF		0.89^{**}	-0.52^{**}	0.25	-0.12	0.01	-0.10	0.47^{**}	0.12	0.10	0.44^{**}	0.19
MAT			-0.45^{**}	0.34^{*}	-0.24	-0.01	-0.10^{**}	0.43	0.26	0.04	0.50^{**}	0.15
PH				0.12	-0.06	-0.02	0.04	-0.29	0.29	-0.11	0.03	-0.07
PN					0.22	0.41^{*}	0.34^{*}	0.57^{**}	0.66^{**}	0.01	0.93^{**}	0.49^{**}
PL						0.86^{**}	0.85^{**}	0.57^{**}	0.15	0.24	0.06	0.77^{**}
NFG							0.99^{**}	0.66^{**}	0.27	0.39^{*}	0.25	0.84^{**}
NS								0.54^{**}	0.24	0.36^{*}	0.16	0.78^{**}
PFG									0.35^{*}	0.28	0.57^{**}	0.77^{**}
BM										-0.33	0.62^{**}	0.31
HI											-0.05	0.48^{**}
PPT												0.36^{*}

LC – leaf chlorophyll, DTF – days to flowering, MAT – maturity, PH – plant height, PN – panicle number, PL – panicle length, NFG – number of filled grains, NS – number of spikelet per panicle, PFG – percent filled grains, BM – biomass, HI – harvest index, PPT – percent productive tillers, GY – grain yield. * and ** represent significant levels at $p < 0.05$ and < 0.01, respectively.

4. Conclusion

This study examined the potential of PBZ foliar application in improving productivity of rainfed lowland rice ecosystem. Application of 250-500 ppm PBZ at early growth stage (35 DAS or 14 DAT) of different rice ecotypes temporarily reduced height and grew taller at maturity as compared to untreated plants. Additionally, PBZ increased number of tillers per plant at 28 DAT or 14 days after PBZ application, which might have contributed to higher number of panicles. The ability of these tillers to produce panicles was also enhanced by PBZ application. Similarly, PBZ improved length of panicles, number of filled grains, and percent filled grains. The improvement in these yield components contributed to the increased grain yield of different rice ecotypes. Furthermore, a lower PBZ concentration of 250 ppm produced the highest grain yield for lowland rice ecotype, PSB Rc14 (rainfed) and NSIC Rc222 (irrigated), whereas a higher concentration of 500 ppm for special-quality rice Dinorado (traditional upland) and NSIC Rc216 (irrigated lowland). This implies that optimum PBZ concentrations vary among rice ecotypes. Overall, PBZ has promotive effects on shoot growth and yield traits that contributed to the improved rice productivity under rainfed condition.

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