Effects of Paclobutrazol on Growth, Yield and Water Use Efficiency of Rice (*Oryza sativa* L.) under Drought Stress Condition

Ronalyn P. Azarcon, Pacifico T. Vizmonte, Jr. and
Ace Mugssy L. Agustin*
Department of Crop Science
Central Luzon State University
Science City of Muñoz, Nueva Ecija 3119 Philippines
amlagustin@clsu.edu.ph

Date received: September 15, 2020
Revision accepted: March 3, 2022

Abstract

Paclobutrazol (PBZ) was reported to increase grain yield of different rice ecotypes via improved tillering ability under rainfed condition. In this study, the effects of PBZ on the shoot and root growth, and water use that might have contributed to higher yield under water-limited conditions were further examined. PSB Rc14 and NSIC Rc222 were grown in pots and subjected to continuously waterlogged (CWL) and drought (DR) treatments with soil moisture content maintained at 20% from 14 days after transplanting (DAT) until maturity. PBZ concentrations (0, 250 and 500 ppm) were sprayed at the onset of water treatments (14 DAT). Results showed that drought treatment significantly altered most of the parameters indicating successful imposition of the stress with the two varieties showing almost similar responses under the two water regimes, especially during maturity. PBZ application in CWL improved tiller number and yield components at a lower concentration and consequently increased grain yield. In drought, PBZ improved tiller number (34-39) starting at 35 DAT which contributed to a higher panicle number at maturity. Additionally, PBZ did not affect the shoot and root growth but reduced water use. Furthermore, the panicle number increased which could be linked to more grain numbers per plant leading to higher grain yields. These higher grain yield and lower water use improved water use efficiency, which required higher PBZ concentration (500 ppm). Overall, PBZ improved the panicle number contributing to a higher grain yield while reducing water use, thereby boosting water use efficiency of rice under drought stress condition.

Keywords*:* drought stress, paclobutrazol, number of tillers and panicles, grain yield, water use efficiency

1. Introduction

The rainfed rice ecosystem in the Philippines is composed of 1.4 million ha or ~30% of the total area devoted to rice (Philippine Statistical Authority, 2019). In the span of almost 20 years (2000-2019), the productivity of this ecosystem increased by only 0.90 t ha^{-1} with an average of 0.045 t ha^{-1} yr^{-1} or 45 kg ha^{-1} yr^{-1}. In an attempt to address this concern, many efforts have been done which included optimizing cultural practices, developing new varieties and applying chemicals or plant growth regulators (PGRs).

Optimizing cultural practices is centered on land preparation, planting method and seeding rate. Development of new varieties (high yielding combined with improved adaptive capacity and quality) is of major importance in all crops including rice. Since 2009, a total of 29 *Sahod Ulan* varieties have been developed by different institutions for rainfed cultivation (Philippine Rice Research Institute, 2022). However, due to the complexity of drought tolerance traits (Panda *et al*., 2021) as well as high-yielding trait (Li *et al*., 2021), breeding ideal rice varieties for the rainfed ecosystem remains a major challenge. In addition, parentals with superior drought adaptive mechanisms that can be used for breeding are very limited. More importantly, despite the numerous, newly released rainfed rice varieties, farmers are still planting other varieties like high-yielding or special quality rice varieties that are not bred for such an ecosystem. One of the problems in rainfed rice varieties is their low yield potential (trade-off is due to introgression of drought adaptive traits). Thus, with the presence of sufficient soil moisture which can be observed in the lowest toposequence, rainfed rice varieties will have lower yields compared with high-yielding ones. However, the latter, unlike the former, will suffer from severe yield loss when drought occurs.

In the absence of built-in resistance or tolerance, chemicals or PGRs can improve plant adaptation. An example is paclobutrazol (PBZ), which is known to improve the physiological response of plants under stress conditions including drought stress (Somasundaram *et al.*, 2009; Hajihashemi and Ehsanpour, 2013; Pal *et al*., 2016; Yooyongwech *et al*., 2017). PBZ was also found to have beneficial effect on tillering crops – improvement in the number of tillers and panicles; hence, increase in grain yield (Assuero *et al*., 2012; Plaza-Wuthrich *et al*., 2016; Magtalas *et al*., 2020).

As the rice plants develop new tillers, new nodal roots are also produced (Owusu-Nketia *et al*., 2018); thus, rice plants with more tillers may have more nodal roots. The nodal root is an important component of the root system that plays a critical role to access more water under water-limited conditions (Suralta *et al*., 2012; Owusu-Nketia *et al*., 2018). These led to a hypothesis that the increase in tiller number due to PBZ application produces more nodal roots resulting in higher crop water use. Therefore, this study aimed to evaluate the effects of PBZ on shoot growth, root system development and water use as well as the yield traits that might have contributed to high grain yield under water-limited conditions.

2. Methodology

2.1 Time and Place of the Study

A pot experiment study was conducted from December 2018 to April 2019 in an open field at the 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). The average daily solar radiation, minimum and maximum temperatures, relative humidity and pan evaporation were 23.5 MJ m^{-2} d^{-1}, 21.3 °C, 31.0 °C, 72% and 5.3 mm d^{-1}, respectively.

2.2 Experimental Design, Treatments and Plant Materials

The pot experiment was laid out using a split-split plot in a randomized complete block design (RCBD) with three replications. Water regimes, varieties and PBZ concentrations were assigned in the main plot, subplot and sub-subplot, respectively. Water regimes consisted of continuously waterlogged (CWL) as control and drought (DR) treatment maintained at 20% soil moisture content (SMC) that was imposed 14 days after transplanting (DAT). CWL had a water level of 3 cm above the soil surface whereas DR had water withheld until 20% SMC using the gravimetric method wherein both water regimes were maintained up to maturity. Rewatering was performed every other day to maintain the desired SMCs of the treatments.

The two varieties (PSB Rc14 and NSIC Rc222) that were used in the previous study by Magtalas *et al*. (2020) were also used in this study. PSB Rc14 is a rainfed lowland variety that is used as a drought-tolerant check in breeding, while NSIC Rc222 is an irrigated lowland variety that is widely cultivated even in the rainfed ecosystem.

Different PBZ concentrations of 0, 250 and 500 ppm were applied as foliar with the rate of ~3.0 mL per hill using a hand sprayer at 14 DAT, which was at the onset of the water treatments. The PBZ that was used in this study is PACLO 25 SC (250 g a.i. L^{-1}) manufactured by Fullong Chemicals.

2.3 Establishment and Plant Handling

Seeds of PSB Rc14 and NSIC Rc222 were oven-dried at 50 °C for three days and then stored for 24 h at room temperature. Thereafter, seeds were soaked for 24 h, incubated for another 48 h for pre-germination and were sown in a seedling tray. Seedlings were then transplanted at 21 days after sowing with three seedlings on a hill at the center of the pail. Each pail (32 x 28 cm [h x dm]) contained a mixture of 10 kg of dried silty soil (river silt) and 3 kg of dried clay loam soil and then water was added until 3 cm depth of water above the soil surface was attained. The soil in the pail was premixed with a fertilizer rate of 90-60-60 kg N-P_2O_5-K_2O ha^{-1}. Each pail was basally applied with 2.79 g of complete fertilizer (14-14-14) and top-dressed with 0.42 g of urea (46-0-0) at the panicle initiation stage. Plants were harvested when grains were 85-90% mature or straw-colored.

2.4 Data Gathering

2.4.1 Cumulative Water Use

Total water use (L) at evapotranspiration level was gathered by measuring all the water applied into the pail from transplanting to harvesting using a graduated cylinder. The water was supplied every other day to maintain the desired SMCs until the termination of the experiment.

2.4.2 Shoot and Root Growth Measurements

To monitor the effects of PBZ and drought stress starting at the early growth stage, plant height and number of tillers were recorded from 14 DAT (during the onset of water treatments and PBZ application) until maturity. Data such as shoot dry weight, nodal root number, lateral root number, root dry weight, root:shoot ratio and water use were measured during the vegetative, reproductive and ripening growth phases.

During harvesting in each phase, the shoots were cut at the base and oven-dried at 70 °C for three days before recording the dry weight. After which, the root system was collected and gently washed. All nodal roots per plant were counted manually while lateral root number, regardless of the length or type, was estimated by sampling 1-cm length at the top, middle and bottom of five randomly selected nodal root samples. All the roots were then oven-dried at 70 °C for three days. The number of panicles, percent productive tillers, grain number per plant, grain yield per plant (expressed at 14% MC), total grain yield and water use efficiency (WUE) (WUE = grain yield/cumulative weight of water evapotranspired) (Bouman *et al*., 2007) were recorded at maturity stage.

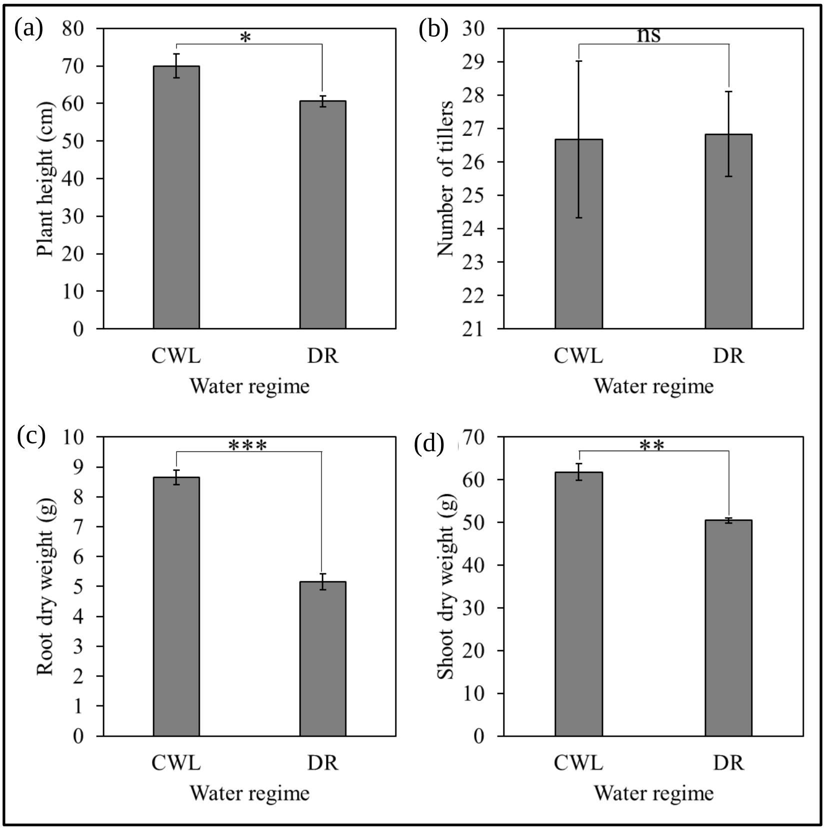
2.5 Statistical Analysis

Analysis of variance appropriate to split-split-plot RCBD was used for data analysis. Comparison among means was done using the least significant difference (LSD) at a 0.05 level of significance. Pearson correlation was also performed to determine the relationship among agro-morphological traits or parameters. All analyses were performed using Statistical Tools for Agricultural Research (STAR) software version 2.0.1 developed by the International Rice Research Institute.

3. Results and Discussion

3.1 Drought Treatment Imposition

The 20% SMC was reported to induce mild drought stress that led to reduced shoot dry matter production (Suralta *et al*., 2012). In this study, similar results were obtained wherein the average plant height and dry matter production (shoot and root) of the two varieties were reduced by the drought treatment at 20% SMC when compared with CWL treatment (Figure 1). The 20% SMC treatment reduced plant height, root dry weight and shoot dry weight by 13, 40 and 18%, respectively. This suggested the effectiveness of the 20% SMC as drought treatment.



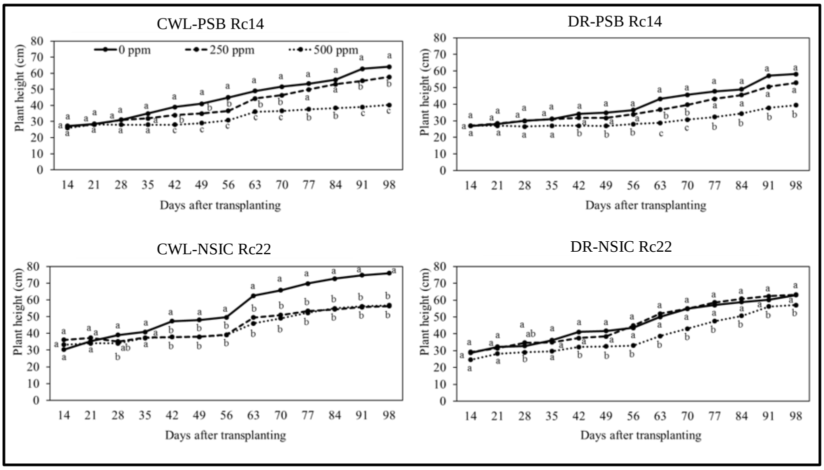
*, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ level, respectively; ns – not significant based on T-*t*est. Values are the average of the two varieties. Error bars denote SE ($n = 6$).

Figure 1. Plant height (a), number of tillers (b) and dry matter production (c and d) of test varieties under CWL and DR conditions

3.2 Shoot Growth

3.2.1 Plant Height

PBZ was previously shown to reduce the height of several crops (Benjawan *et al*., 2007; Ghosh *et al*., 2010; Alvarez *et al*., 2012; Kamran *et al*., 2018a). At 14 DAT, during the onset of water regimes and PBZ foliar application, the plant height of PSB Rc14 and NSIC Rc222 in CWL and DR conditions showed no significant differences indicating the uniformity of experimental plant materials before treatment application (Figure 2). At 28 DAT or 14 days after PBZ application, the initial effect of PBZ on plant height was observed wherein PBZ reduced plant height in both water regimes but only in NSIC Rc222. A more obvious manifestation of the effect of PBZ on plant height was noticed at 42 DAT (28 days after PBZ application) where both varieties were negatively affected in both water regimes. The reduction in plant height was observed until maturity with increasing differences between treated and untreated plants. Moreover, PBZ application of 250 ppm showed a significant reduction in plant height of both varieties under CWL, while a higher PBZ concentration of 500 ppm under DR condition. These results indicated that a higher PBZ concentration can further reduce the plant height under drought stress condition.



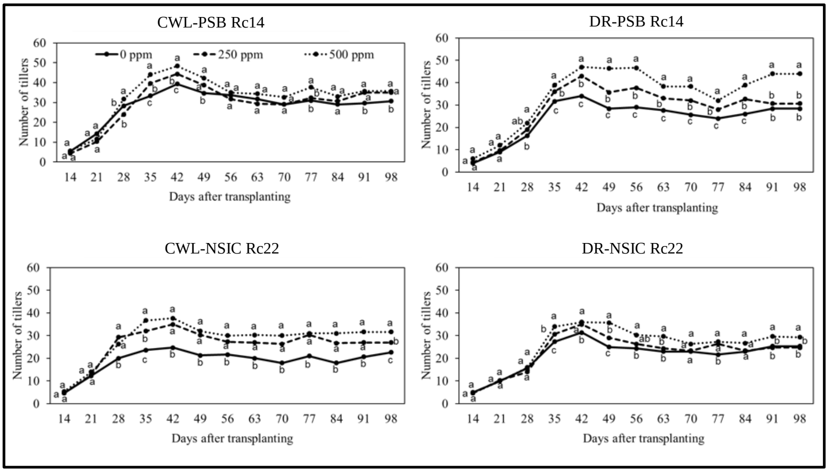
Means within days after transplanting with the same letter are not significantly different at 5% level by LSD.

Figure 2. Plant height of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions from 14 to 98 DAT

The negative effects of PBZ on plant height were also reported in several studies. Foliar application of 50 g a.i. PBZ ha^{-1} in black rice at early tillering and panicle initiation stages caused plant height reduction at maturity (Alvarez *et al*., 2012). Additionally, soil drenching of 1.0-1.5 g a.i. PBZ m^{-1} of canopy diameter in two-year-old *Jatropha curcas* resulted in smaller plant height compared with untreated plants (Ghosh *et al*., 2010). Similarly, soil drenching of 100-400 ppm of PBZ at the seedling stage of okra led to reduced height (Benjawan *et al*., 2007). Likewise, seed soaking in 200-400 ppm of PBZ induced an increasing reduction in height of corn (Kamran *et al*., 2018b). According to Plaza-Wüthrich *et al*. (2016), the reduction in plant height due to PBZ application was the result of the shortening of internodal length linked to the decreased level of endogenous GA.

3.2.2 Number of Tillers

PBZ can improve the tillering capacity of crops (Assuero *et al*., 2012; Plaza-Wüthrich *et al*., 2016). Similar to height measurement at 14 DAT (during the onset of water regimes and PBZ foliar application), there were no significant differences observed in the number of tillers in both varieties under both water regimes (Figure 3).



Means within days after transplanting with the same letter are not significantly different at 5% level by LSD.

Figure 3. Number of tillers of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions from 14 to 98 DAT

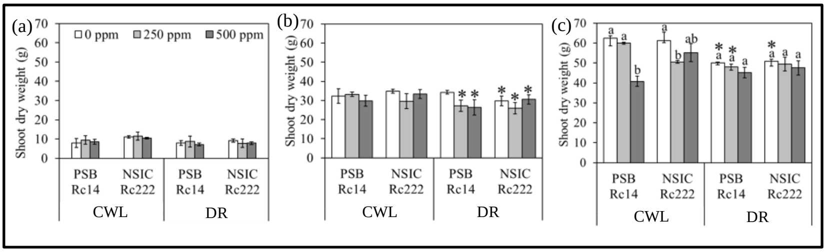
The recorded number of tillers of both varieties in two water regimes at 14 DAT was four to six per plant, which continued to increase and reached the maximum at 42 DAT (PSB Rc14: 48 and NSIC Rc222: 38) but showed decreasing trend until 63 DAT and maintained until maturity. The effect of PBZ on the number of tillers was first manifested at 35 DAT or 21 days after PBZ application in which there were more tillers in PBZ-treated plants (250 and 500 ppm) relative to the untreated control. The difference between treated and untreated plants was increasing as the plants reached the peak of the maximum tillering stage. Application of 250 ppm PBZ increased the number of tillers in both varieties under two water regimes at 42 DAT. On the other hand, a higher PBZ concentration of 500 ppm caused further increase in the number of tillers of PSB Rc14 except NSIC Rc222. At this stage, untreated PSB Rc14 under CWL had 39 tillers per plant while PBZ-treated with 250 and 500 ppm had 44 and 48 tillers, respectively. Untreated PSB Rc14 under DR had 34 tillers per plant while 43 and 47 for plants treated with 250 and 500 ppm, respectively. On the other hand, untreated NSIC Rc222 under CWL had

25 tillers while 44 and 47 for plants treated with 250 and 500 ppm of PBZ, respectively. Untreated NSIC Rc222 plants under DR had 31 tillers per plant while 35-36 tillers for plants treated with 250-500 ppm of PBZ. In both PBZ treatments under both water regimes, the number of tillers of PSB Rc14 was significantly higher than that of NSIC Rc222. In other words, PSB Rc14 had a higher tillering ability and was more responsive to PBZ than the NSIC Rc222, which suggested that PBZ effects on tiller production varied with variety.

Moreover, the above differences were observed until maturity but with slight changes. Both PBZ concentrations of 250 and 500 ppm produced a significantly higher number of tillers than untreated plants under CWL while only PBZ concentration of 500 ppm under DR condition at maturity. This demonstrated that a lower PBZ concentration (250 ppm) was needed to increase the tiller number of rice under favorable condition whereas a higher PBZ concentration (500 ppm) under water-limited condition. This increase in the number of tillers with PBZ application is in agreement with the results obtained in the previous study (Magtalas *et al.*, 2020) that used different rice ecotypes grown under rainfed condition and with the results reported in other plant species such as black rice (Dewi *et al.*, 2016), teff (Plaza-Wüthrich *et al.*, 2016) and sugarcane (Liu *et al.*, 2017). Furthermore, in studying the molecular mechanism controlling height and tillering in rice, Liao *et al.* (2019) also observed an increase in tiller number by PBZ application. The mechanism involved inhibition of SLENDER RICE 1 (SLR1) by GAs resulting in stem elongation and degradation of MONOCULM 1 (MOC1); hence, a decrease in tiller number. Thus, PBZ application that inhibited GAs would increase MOC1 level, thereby increasing tiller number. Assuero *et al.* (2012) proposed a model wherein PBZ prevents GAs biosynthesis which lowers stem elongation as well as carbon investment in growth. This contributes to photoassimilate accumulation and shorter phyllochron resulting in earlier and more tiller development.

3.2.3 Shoot Dry Weight

For the aboveground or shoot dry weight, PBZ application showed a significant difference only at the ripening phase in CWL although there was a tendency to reduce shoot dry weight with increasing PBZ under DR (Figure 4). Under CWL, a higher PBZ concentration significantly reduced shoot dry weight in PSB Rc14 while a lower PBZ concentration in NSIC Rc222. Plant height (Abebe *et al.*, 2019) and the number of tillers (Kondhia *et al.*, 2015) in rice have a positive relationship with the shoot dry weight; in other words, taller plants with more tillers have higher shoot dry weight. In this study, the observed contrasting effects of PBZ in plant height and number of tillers consequently did not influence shoot dry weight. This finding is similar to the result of Mactal and Canare (2015) wherein the application of 250-1,000 ppm of PBZ at 19 DAT did not affect the dry matter yield of traditional rice varieties. For the effect of drought, a significant difference was observed in the reproductive and ripening phases. In general, DR reduced shoot dry weight of treated and untreated plants in the reproductive phase but only untreated plants in the ripening phase. The result in the ripening phase was due to the reduced shoot dry weight of PBZ-treated plants under CWL condition. Thus, no significant difference was found between CWL and DR conditions for PBZ-treated plants.



Means with the same letter/s within rice variety are not significantly different at 5% level by LSD. Means under DR condition with asterisk (*) are significantly different from the CWL counterpart at 5% LSD. Error bars denote SE (*n* = 3).

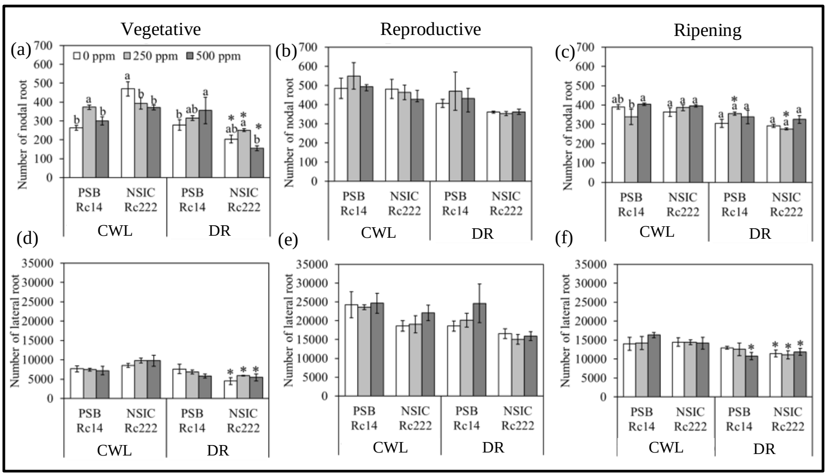
Figure 4. Shoot dry weight of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions at different growth phases – vegetative (a), reproductive (b) and ripening/maturity (c)

3.3 Root Growth

3.3.1 Nodal Root Production

As rice plants produce new tillers, the new tillers also produce nodal roots (Owusu-Nketia *et al.*, 2018); hence, more tillers mean more nodal roots. The nodal root is one of the key root traits in water-limited condition because it can contribute to access greater soil volume to absorb more water (Suralta *et al.*, 2012). In the present work, PBZ application showed significant nodal root differences at vegetative and ripening phases (Figures 5a to 5c). In the vegetative phase, improvement in nodal root number was only observed in PSB Rc14 regardless of PBZ concentrations in both water regimes – a lower PBZ concentration of 250 ppm increased nodal root number under CWL, while a higher PBZ concentration of 500 ppm under DR condition.

Between water regimes, no significant difference was identified indicating that PSB Rc14 can maintain nodal root number despite the negative effects of drought stress. On the other hand, NSIC Rc222 obtained a reduced nodal root numbers with PBZ application under CWL while no significant effects under DR condition. A significant difference was observed between CWL and DR conditions with lower nodal root number under stress condition. Previous studies on drought (Suralta *et al*., 2010, 2012, 2015) showed similar results in which drought stress reduced nodal root number in different rice genotypes in the vegetative phase.



Means with the same letter/s within rice variety are not significantly different at 5% level by LSD. Means under DR condition with asterisk (*) are significantly different from the CWL counterpart at 5% LSD. Error bars denote SE (n = 3).

Figure 5. Number of nodal roots (a-c) and lateral roots (d-f) of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions at different growth phases

In the ripening phase, although ANOVA exhibited significant differences among PBZ concentrations, no significant difference among means was noted by the LSD test. However, there was a tendency to improve the nodal root numbers with PBZ application. To understand whether the number of tillers was associated with the number of nodal roots, the trends between the two traits were compared. The result showed that the observed trend in nodal root number did not match the trend of the number of tillers as supported by their moderately weak relationship (r^2 = 0.37). This demonstrated that PBZ application in rice at 14 DAT improved the number of tillers without affecting nodal root number.

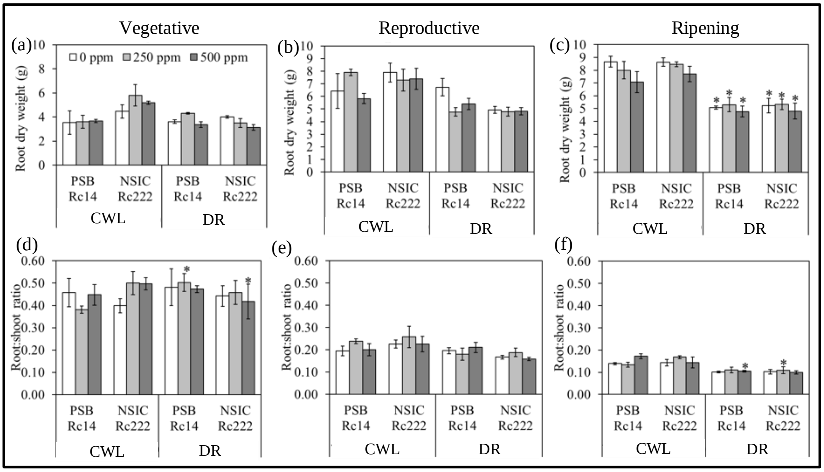
3.3.2 Lateral Root Production

To determine the effects of PBZ on lateral roots, the total lateral root number was estimated by counting from the nodal roots. However, no significant differences were observed among PBZ concentrations in both water regimes (Figure 5d to 5f) although DR stress significantly reduced lateral root number in NSIC Rc222 during vegetative and ripening phases while there was no effect in PSB Rc14 for the entire growth duration. The observed decrease in lateral root number of NSIC Rc222 can be ascribed to the reduction in S-type lateral root number instead of L-type lateral roots as drought stress reduced S-type but increased L-type number (Suralta *et al.*, 2015). S-type lateral roots are short, slender and non-branching but normally numerous, while L-type lateral roots are generally long, thick and capable of a higher order of branching (Yamauchi *et al.*, 1996; Kawai *et al.*, 2017).

3.3.3 Root Dry Weight and Root:Shoot Ratio

The absence of significant effects of PBZ application in nodal and lateral root numbers were also noted in root dry weight (Figures 6a to 6c). Root dry weight ranged from 3.13-5.80 g per plant in the vegetative phase, 4.77-7.90 g in the reproductive phase and 4.77-8.67 g in the ripening phase. Drought stress condition significantly reduced the root dry weight but in the ripening phase only. The root dry weight in the ripening phase in PSB Rc14 under CWL was 7.07-8.67 g per plant while only 4.77-5.30 g under DR stress condition. For NSIC Rc222, 7.70-8.63 g per plant under CWL while only 4.80-5.33 g under DR condition. These non-distinct significant effects of PBZ in root dry weight (Figures 6a to 6c) and shoot dry weight (Figure 4) consequently resulted in not significantly different root:shoot ratio of PBZ-treated plants of both varieties in two water regimes (Figures 6d to 6f). In terms of phenological development, root:shoot ratio showed a decreasing trend from vegetative to ripening phase wherein root:shoot ratio in vegetative phase ranged from 0.38 to 0.50, 0.16-0.26 in reproductive phase and 0.10-0.17 in ripening phase. This was because of the greater increase in shoot dry weight relative to root dry weight.

Taken together, PBZ improved the number of tillers without affecting the root traits as well as root:shoot ratio. This finding is similar to the result of Pinto *et al*. (2005) in potted *Zinnia elegans* which revealed that 50-100 mg a.i. of PBZ did not affect the root dry weight. Interestingly, other studies obtained different results wherein PBZ application negatively affected the root system of banana (Chang *et al.*, 2019) and okra (Bashir *et al.*, 2021), while in other works, PBZ improved root system of corn (Kamran *et al.*, 2018b) and rice seedlings (Huang *et al.*, 2019).



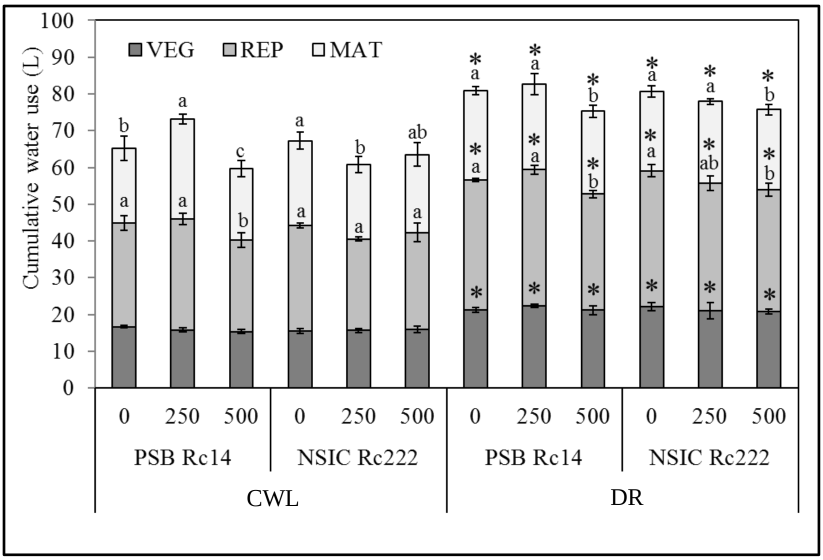
Means with the same letter/s within rice variety are not significantly different at 5% level by LSD. Means under DR condition with asterisk (*) are significantly different to its CWL counterpart at 5% LSD. Error bars denote SE (*n* = 3).

Figure 6. Root dry weight (a-c) and root:shoot ratio (d-f) of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions at different growth phases

3.3.4 Cumulative Water Use

Despite no distinct significant effects of PBZ in the root system (i.e. root dry weight, nodal root number and estimated lateral root number) and shoot biomass (although there was a decreasing numerical value with PBZ application), application of PBZ caused a reduction in cumulative water use at the reproductive and ripening phases in both varieties under the two water regimes (Figure 7). The trend of cumulative water use under DR condition was higher than CWL, and only higher PBZ concentration (500 ppm) can reduce the water use of both varieties. Although PBZ can increase stomatal conductance and improve transpiration rate under DR condition (Pal *et al.*, 2016), the overall water use at the plant level is also affected by biomass – that is, lower biomass generally requires less water (Agustin and Cadiz, 2015). Thus, the decreasing numerical trend of shoot biomass with PBZ application (Figure 4) might have contributed to the lower cumulative water use with PBZ application (500 ppm).

Collectively, PBZ improved tiller number (Figure 3) without affecting the root system (nodal and lateral roots and root dry weight) (Figures 5a to 5f and 6a to 6c) while reducing plant water use under DR condition (Figure 7). On the other hand, in this pot experiment wherein upward movement of water (evaporation [E] and transpiration [T]) was the only source of water loss, the DR condition caused a significant increase in the water use (mean of 79 L in DR versus 65 L in CWL). Although other studies observed higher ET in flooded soil (4.29^{-1} mm d in flooded rice field versus 3.81 mm d^{-1} in aerobic rice field) (Alberto *et al*., 2010), there is a tendency that lower SMC has a higher evaporation (600 g d^{-1} in 23% SMC vs. 585 g d^{-1} in 38% SMC) (Harris and Robinson, 1916) or transpiration (12.4 μmol m^{-2} s^{-1} 15% in SMC versus 10.3 μmol m^{-2} s^{-1} in CWL) (Suralta *et al*., 2012).



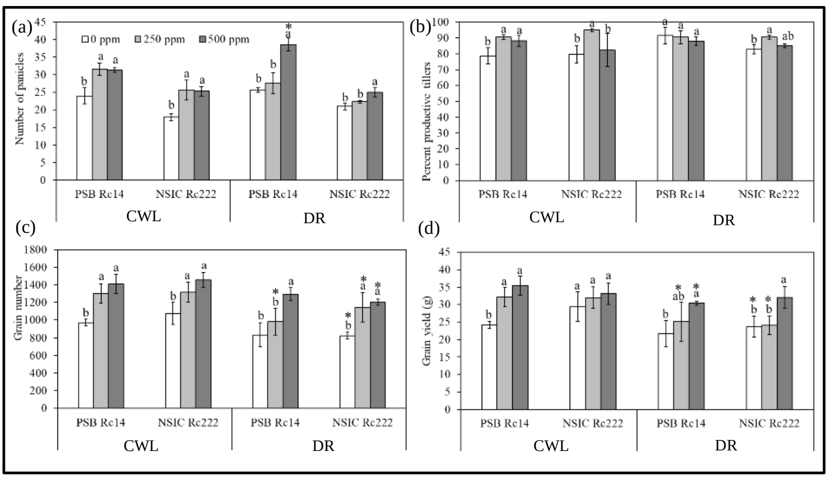
Means with the same letter within rice variety are not significantly different at 5% level by LSD. Means under DR with asterisk (*) are significantly different from the CWL counterpart at 5% LSD. Error bars denote SE (*n* = 3).

Figure 7. Cumulative water use at different growth phases (vegetative [veg], reproductive [rep] and maturity [mat]) of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions

3.4 Yield Traits

3.4.1 Number of Panicles and Percent Productive Tillers

Application of PBZ showed significant increase in the number of panicles in both varieties in two water regimes (Figure 8a). Under CWL, 250-ppm PBZ produced a significantly higher number of panicles than untreated control but with no further increase with higher PBZ concentration (500 ppm). Under DR condition, 250-ppm PBZ was not significantly higher than the untreated plants whereas 500 ppm showed a significant increase in the number of panicles. This suggested that a lower PBZ concentration was effective in increasing the number of panicles under CWL, while a higher PBZ concentration was needed under DR stress condition. This trend was similar to the trend in the number of tillers. Panicles developed from tillers, and were termed productive tillers. Application of PBZ at 14 DAT established improvement in the number of tillers as early as 28 DAT or before panicle initiation which was recorded between 28-35 DAT. At 35 DAT, the relationship of the tiller number to the panicle number was already strong (r^2 = 0.65**) and improved to very strong at 49 DAT up to flowering (r^2 = 0.81**-0.85**) (Table 1). This supported that the observed production of more tillers due to PBZ application at the early growth stage, which occurred before the panicle initiation stage, contributed to the increase in the number of productive tillers or panicles.



Means with the same letter within rice variety are not significantly different at 5% level by LSD. Means under DR condition with asterisk (*) are significantly different from the CWL counterpart at 5% LSD. Error bars denote SE (*n* = 3).

Figure 8. Number of panicles (a), percent productive tillers (b), grain number (c) and grain yield (g) per plant (d) of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions

The above findings conform with the previous study of Magtalas *et al.* (2020) wherein PBZ application improved the number of tillers and panicles of different rice ecotypes under rainfed condition. Similarly, in investigating the effect of GA in lodging and drought tolerance in small cereals, Plaza-Wüthrich *et al*. (2016) also uncovered that PBZ application produced more tillers and panicles in teff under water-deficit condition. In the studies mentioned, PBZ-treated plants that produced more tillers had also higher grain yield. In the present work, the number of panicles had a positive strong linear relationship with grain yield ($r^2 = 0.64^{**}$) (Figure 9a) suggesting that more panicles resulted in a higher grain yield. Furthermore, a significant difference in the number of panicles was found between water regimes but only in PSB Rc14 treated with 500-ppm PBZ. This may suggest that the number of panicles of the two varieties was generally maintained because of PBZ despite the negative effects of drought stress. Moreover, PBZ-treated plants showed significantly higher percent productive tillers than untreated control (except in PSB Rc14 under DR) (Figure 8b). This may suggest that in addition to the observed improvement in tillering ability with PBZ application, PBZ also improved the ability of the tillers to produce panicles.

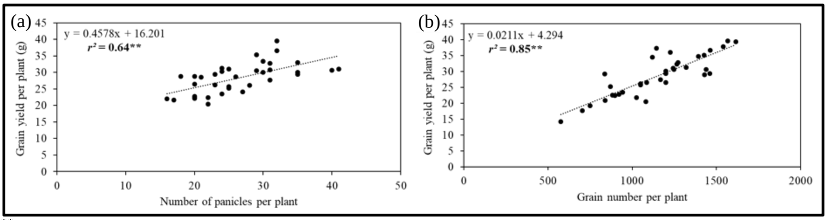
Table 1. Correlation coefficient of number of tillers and panicles of PSB Rc14 and NSIC Rc222 from 14 to 70 days after transplanting under CWL and DR conditions

Growth phase	Days after transplanting	Correlation coefficient (r^2)
Vegetative	14	0.22
	21	0.02
Overlap	28	0.29
	35	0.65^{**}
Reproductive	42	0.73^{**}
	49	0.81^{**}
	56	0.81^{**}
Overlap	63	0.81^{**}
	70	0.85^{**}

**significant at $p < 0.01$ level

3.4.2 Grain Number and Yield

Application of PBZ showed a significant increase in grain number of both varieties in two water regimes. Application of 250-ppm PBZ significantly improved grain number but without further increase with higher PBZ concentration, except in PSB Rc14 under DR wherein only higher concentration showed a significant increase (Figure 8c). Between varieties, NSIC Rc222 showed a significant reduction in grain number because of drought stress. Grain number showed a very strong positive linear relationship with grain yield ($r^2 = 0.85^{**}$) (Figure 9b). Thus, the application of PBZ also showed significant improvement in grain yield of both varieties in two water regimes (Figure 8d), except in NSIC Rc222 under CWL. Application of 250-ppm PBZ increased grain yield of PSB Rc14 under CWL without further increase with higher PBZ concentration. On the other hand, 250-ppm PBZ under DR condition was not significantly different from the untreated control, while the 500-ppm PBZ caused a significant increase in grain yield of both varieties. This demonstrated that a lower PBZ concentration improved grain yield under CWL, while a higher PBZ concentration was needed under DR condition.



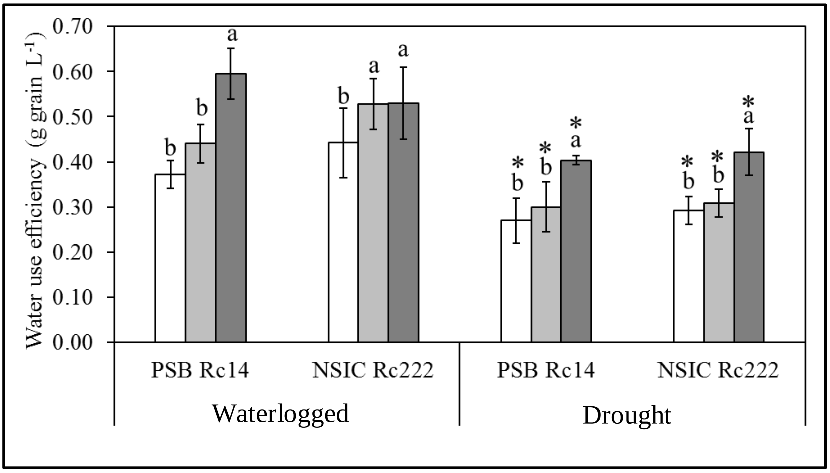
**significant at $p < 0.01$ level

Figure 9. Relationship of number of panicles per plant (a) and grain number per plant (b) to grain yield per plant of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions

Several studies have proven the usefulness of PBZ in improving crop performance under favorable and drought stress conditions. Under favorable condition, PBZ application increased the economic yield of *Camelina sativa* L. (Kumar *et al*., 2012), rice (Pan *et al*., 2013) and wheat (Pirahmadi *et al*., 2016). Under the water-limited condition, PBZ improved the grain yield of several crops such as maize (Bayat and Sepehri, 2012), teff (Plaza- Wüthrich *et al*., 2016) and different rice ecotypes (Magtalas *et al*., 2020). The observed improvement can be linked to the development of more tillers or panicles and enhancement of physiological processes. Other studies also reported the enhanced physiological processes of PBZ-treated plants under drought stress condition leading to improved stress adaptation (Fernandez *et al*., 2006; Somasundaram *et al*., 2009; Hajihashemi and Ehsanpour, 2013; Pal *et al*., 2016; Yooyongwech *et al*., 2017).

3.5 WUE

The general reduction in water use and improvement in grain yield with PBZ application significantly improved the grain WUE of both varieties in two water regimes (Figure 10). In general, a higher PBZ concentration of 500 ppm significantly improved WUE (except NSIC Rc222 under CWL wherein lower PBZ concentration exhibited a significant increase without further improvement with higher PBZ concentration). Similar to the trend observed in plant height, numbers of tillers and panicles especially grain yield and water use under DR condition, a higher PBZ concentration (500 ppm) was required to improve WUE. Both improvement in grain yield and reduction in water use under DR condition due to higher PBZ concentration improved WUE. This result conforms with the observation of Pal *et al*. (2016) wherein the application of PBZ enhanced the WUE of tomatoes under irrigated and water-deficit conditions. Also, Xia *et al*. (2018) observed an improvement in WUE of *Paeonia lactiflora* Pall with PBZ application.



Means with the same letter within rice variety are not significantly different at 5% level by LSD. Means under DR condition with asterisk (*) are significantly different from the CWL counterpart at 5% LSD. Error bars denote SE (n = 3).

Figure 10. WUE of PSB Rc14 and NSIC Rc222 applied with PBZ under CWL and DR conditions

4. Conclusion and Recommendation

PBZ application in rice significantly reduced plant height starting at 42 DAT whereas it increased the number of tillers as early as 35 DAT; these were observed until maturity. However, despite the increase in the number of tillers, the nodal root number of PBZ-treated plants was generally comparable with the untreated plants under both CWL and DR conditions. Additionally, estimated lateral root number, root dry weight as well as shoot dry weight and root:shoot ratio were not affected by PBZ application. Water use in the reproductive and ripening phases was reduced with PBZ application. These demonstrated that PBZ improved the number of tillers of rice without affecting root growth and that PBZ reduced cumulative water use.

The established improvement in the number of tillers at the early growth stage due to PBZ application contributed to the increase in the number of panicles. This was supported by the strong positive linear relationship of the number of tillers to the number of panicles from 35 DAT up to flowering. Aside from increasing the number of tillers, PBZ application also boosted the percent productive tillers, suggesting that PBZ can also improve the ability of the tillers to produce panicles. The improvement in the number of panicles increased the grain number per plant. Among agronomic parameters evaluated, grain number had the highest correlation coefficient with a very strong linear relationship to grain yield; thus, grain yield increased with PBZ application. Furthermore, under drought stress condition, a higher PBZ concentration was required to alter the plant's response specifically to reduce the height and water use and to increase the number of tillers and panicles and grain yield. Thus, higher PBZ concentration improved water use efficiency under drought stress condition. This promotive effect of PBZ in grain yield while reducing plant water use could be a potential technology, particularly for a rainfed ecosystem wherein the major production constraint is drought stress.

The findings of this study offered a potential technology that can both save water and improve plant productivity, which could be highly useful particularly for rainfed ecosystem in which drought stress is the major limiting factor. However, the results should be further validated in an actual field condition with a higher plant population and with the use of a lysimeter to measure actual plant water use.

5. References

Abebe, T., Alamerew, S., & Tulu, L. (2019). Traits association and path coefficient analysis of yield and related traits in rainfed lowland rice (*Oryza sativa* L.) genotypes in North Western Ethiopia. African Journal of Plant Science, 13(1), 1-8. https://doi.org/10.5897/AJPS2018.1693

Agustin, A.M.L., & Cadiz, N.M. (2015). Relationship of agro-morphological traits to water use efficiency of irrigated lowland rice varieties under screenhouse condition. International Journal of Philippine Science and Technology, 8(2), 78-81.

Alberto, M.C.R., Wassmann, R., Hirano, T., Miyata, A., Hatano, R., Kumar, A., Padre, A., & Amante, M. (2010). Comparisons of energy balance and evapotranspiration between flooded and aerobic rice fields in the Philippines. Agricultural Water Management, 98(2011), 1417-1430. https://doi.org/10.1016/j.agwat.2011.04.011

Alvarez, R.D.C.F., Crusciol, C.A.C., Nascente, A.S., Rodrigues, J.D., & Habermann, G. (2012). Gas exchange rates, plant height, yield components, and productivity of upland rice as affected by plant growth regulators. Pesquisa Agropecuária Brasileira, 47(10), 1455-1461. https://doi.org/10.1590/S0100-204X2012001000007

Assuero, S.G., Lorenzo, M., Perez Ramirez, N.M., Velazquez, L.M., & Tognetti, J.A. (2012). Tillering promotion by paclobutrazol in wheat and its relationship with plant carbohydrate status. New Zealand Journal of Agricultural Research, 55(7), 347-358. https://doi.org/10.1080/00288233.2012.706223

Bashir, R.I., Hussain, R., Rasheed, S., Anwar, M., Awais, M., & Hassan, S. (2021). Seed invigoration with paclobutrazol improves seedling growth, physiological, biochemical attributes and fruit yield in okra. International Journal of Agriculture and Biology, 26, 277-286.

Bayat, S., & Sepehri, A. (2012). Paclobutrazol and salicylic acid application ameliorates the negative effect of water stress on growth and yield of maize plants. Journal of Research in Agricultural Science, 8(2), 127-139.

Benjawan, C., Chutichudet, P., & Chanaboon, T. (2007). Effect of chemical paclobutrazol on growth, yield and quality of okra (*Abelmoschus esculentus* L.) Har Lium cultivar in Northeast Thailand. Pakistan Journal of Biological Sciences, 10(3), 433-438.

Bouman, B.A.M., Lampayan, R.M., & Tuong, T.P. (2007). Water Management in Irrigated Rice: Coping with water scarcity. Los Baños, Philippines: International Rice Research Institute.

Chang, S., Wu, Z., Zeng, Q., Zhang, J., Sun, W., Qiao, L., & Shu, H. (2019). A method for prolonging culturing time of banana seedling in nursery shed. Advances in Bioscience and Biotechnology, 10, 119-131. https://doi.org/10.4236/abb.2019.105009

Dewi, K., Agustina, R.Z., & Nurmalika, F. (2016). Effects of blue light and paclobutrazol on seed germination, vegetative growth and yield of black rice *(Oryza sativa* L. 'Cempo Ireng'). Biotropia, 23(2), 84-95. https://doi.org/10.11598/btb.2016.23.2.478

Fernandez, J.A., Balenzategui, L., Baňon, S., & Franco, J.A. (2006). Induction of drought tolerance by paclobutrazol and irrigation deficit in *Phillyrea angustifolia* during the nursery period. Scientia Horticulturae, 107(3), 277-283.

Ghosh, A., Chikara, J., Chaudhary, D.R., Prakash, A.R., Boricha, G., & Zala, A. (2010). Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jatropha curcas*. Journal of Plant Growth Regulation, 29, 307-315.

Hajihashemi, S., & Ehsanpour, A.A. (2013). Influence of exogenously applied paclobutrazol on some physiological traits and growth of *Stevia rebaudiana* under in vitro drought stress. Biologia, 68(3), 414-420.

Harris, F.S., & Robinson, J.S. (1916). Factors affecting the evaporation of moisture from the soil. Journal of Agricultural Research, 7(10), 439-461

Huang, S., Luo, H., Ashraf, U., Abrar, M., He L., Zheng, A., Wang, Z., Zhang, T., & Tang, X. (2019). Seed treatment with paclobutrazol affects early growth, photosynthesis, chlorophyll fluorescence and physiology of rice. Applied Ecology and Environmental Research, 17(1), 999-1012. https://doi.org/10.15666/aeer/1701_9991012

Kamran, M., Cui, W., Ahmad, I., Meng, X., Zhang, X., Su, W., Chen, J., Ahmad, S., Fahad, S., Han, Q., & Liu, T. (2018a). Effect of paclobutrazol, a potential growth regulator on stalk mechanical strength, lignin accumulation and its relation with lodging resistance of maize. Plant Growth Regulation, 84, 317-322. https://doi.org/10.1007/s10725-017-0342-8

Kamran, M., Wennan, S., Ahmad, I., Xiangping, M., Wenwen, C., Xudong, Z., Siwei, M., Khan, A., Qingfang, H., & Liu, T. (2018b). Application of paclobutrazol affect maize grain yield by regulating root morphological and physiological characteristics under a semi-arid region. Scientific Reports, 8, 4818. https://doi.org/10.1038/s41598-018-23166-z

Kawai, T., Nosaka-Takahashi, M., Yamauchi, A., & Inukai, Y. (2017). Compensatory growth of lateral roots responding to excision of seminal root tip in rice. Plant Root, 11, 48-57. https://doi.org/10.3117/plantroot.11.48

Kondhia, A., Tabien, R.A., & Ibrahim, A. (2015). Evaluation and selection of high biomass rice (*Oryza sativa* L.) for drought tolerance. American Journal of Plant Sciences, 6, 1962-1972. https://doi.org/10.4236/ajps.2015.612197

Kumar, S., Ghatty, S., Satyanarayana, J., Guha, A., Chaitanya, B.S.K., & Reddy, A.R. (2012). Paclobutrazol treatment as a potential strategy for higher seed and oil yield in field-grown *Camelina sativa* L. Crantz. BMC Research Notes, 5, 137. https://doi.org/10.1186/1756-0500-5-137

Li, G., Tang, J., Zheng, J., & Chu, C. (2021). Exploration of rice yield potential: Decoding agronomic and physiological traits. The Crop Journal, 9(3), 577-589. https://doi.org/10.1016/j.cj.2021.03.014

Liao, Z., Duan, J., Yuan, K., Yu, C., Meng, X., Kou, L., Chen, M., Jing, Y., Liu, G., Smith, S.M., & Li, J. (2019). SLR1 inhibits MOC1 degradation to coordinate tiller number and plant height in rice. Nature Communication, 10, 2738. https://doi.org/10.10 38/s41467-019-10667-2

Liu, J., Li, S., Tan, F., Liu, X., He, Y., Wu, K., Xiong, F., & Liu, L. (2017). Effects of seed soaking with paclobutrazol on tillering and physiological characteristics of sugarcane seedlings. Asian Agricultural Research, 9(1), 65-69. https://doi.org/10.22004/ag.econ.257309

Mactal, A.G., & Canare, J.G., Jr. (2015). Lodging resistance and agro-morphological characteristics of Elon-elon and Palawan red sprayed with paclobutrazol. Journal of Agricultural Technology, 11(7), 1649-1667

Magtalas, M.P., Vizmonte, P.T., Jr., & Agustin, A.M.L. (2020). Promoting effects of paclobutrazol on the productivity of different rice (*Oryza sativa* L.) ecotypes under rainfed lowland condition. Mindanao Journal of Science and Technology, 18(2), 157-173.

Owusu-Nketia, S., Siangliw, J.L., Siangliw, M., Toojinda, T., Vanavichit, A., Ratsameejanphen, N., Ruangsiri, M., Sriwiset, S., Suralta, R.R., Inukai, Y., Mitsuya, S., Kano-Nakata, M., Nguyen, D.T.N., Takuya, K., & Yamauchi, A. (2018). Functional roles of root plasticity and its contribution to water uptake and dry matter production of CSSLs with the genetic background of KDML 105 under soil moisture fluctuation. Plant Production Science, 21(3), 266-277. https://doi.org/10.1080/1343943X.2018.14 77509

Pal, S., Zhao, J., Khan, A., Yadav, N.S., Batushansky, A., Barak, S., Rewald, B., Fait, A., Lazorovitch, N., & Rachmilevitch, S. (2016). Paclobutrazol induces tolerance in tomato to deficit irrigation through diversified effect on plant morphology, physiology and metabolism. Scientific Reports, 6, 39321. https://doi.org/10.1038/srep39321

Pan, S., Rasul, F., Li, W., Tian, H., Mo, Z., Duan, M., & Tang, X. (2013). Roles of plant growth regulators on yield, grain qualities and antioxidant enzyme activities in super hybrid rice (*Oryza sativa* L.). Rice, 6(1), 9. https://doi.org/10.1186/1939-8433-6-9

Panda, D., Mishra, S.S., & Behera, P.K. (2021). Drought tolerance in rice: Focus on recent mechanisms and approaches. Rice Science, 28(2), 119-132. https://doi.org/10 .1016/j.rsci.2021.01.002

Plaza-Wüthrich, S., Blösch, R., Rindisbacher, A., Cannarozzi, G., & Tadele, Z. (2016). Gibberellin deficiency confers both lodging and drought tolerance in small cereals. Frontiers of Plant Science, 7, 643. https://doi.org/10.3389/fpls.2016.00643

Pinto, A.C.R., Rodrigues, T.J.D, Leite, I.C., & Barbosa, J.C. (2005). Growth retardants on development and ornamental quality of potted 'Lilliput' *Zinnia elegans* Jacq.. Scientia Agricola (Piracicaba, Brazil), 62(4), 337-345.

Pirahmadi, L., Shekari, F., Javanmard, A., & Noori, K. (2016). Improvement of wheat (*Triticum aestivum* L.) yield by application of paclobutrazol. IOAB Journal, 7(5), 314-325.

Philippine Rice Research Institute (PhilRice). (2022). Rice varieties. Retrieved from https://www.phi lrice.gov.ph

Philippine Statistics Authority (PSA). (2019). OpenSTAT. Retrieved from http://opens tat.psa.gov.ph/

Somasundaram, R., Jaleel, C.A., Azooz, M.M., Abraham, S.S., Gomathinayagam, M., & Panneerselvam, R. (2009). Induction of drought stress tolerance by paclobutrazol and abscissic acid in gingelly (*Sesamum indicum*). Global Journal of Molecular Sciences, 4(2), 49-55.

Suralta, R.R., Inukai, Y., & Yamauchi, A. (2010). Dry matter production in relation to root plastic development, oxygen transport, and water uptake of rice under transient soil moisture stresses. Plant Soil, 332, 87-104 (2010). https://doi.org/10.1007/s11104-009-0275-8

Suralta, R.R., Lucob, N.B., & Perez, L.M. (2012). Shoot and root development in rice (*Oryza sativa* L.) genotypes during progressive drying in soils with varying moisture regimes. Philippine Journal of Crop Science, 37(2), 1-12.

Suralta, R.R., Lucob, N.B., Perez, L.M., Niones, J.M., & Nguyen, H.T. (2015). Developmental and quantitative trait loci analyses of root plasticity in response to soil moisture fluctuation in rice. Philippine Journal of Crop Science, 40(2), 12-24

Xia, X., Tang, Y., Wei, M., & Zhao, D. (2018). Effect of paclobutrazol application on plant photosynthetic performance and leaf greenness of herbaceous peony. Horticulturae, 4(5), 1-12.

Yamauchi, A., Pardales, J.R., Jr., & Kono, Y. (1996). Root system structure and its relation to stress tolerance. In: O. Ito, K. Katayama, C. Johansen, J.V.D.K. Kumar Rao, J.J. Adu-Gyamfi & T.J. Rego (Eds.), Dynamics of roots and nitrogen in cropping systems of the semiarid tropics (pp. 211-223). Tsukuba, Japan: JIRCAS.

Yooyongwech, S., Samphumphuang, T., Tisarum, R., Theerawitaya, C., & Cha-um, S. (2017). Water-deficit tolerance in sweet potato [*Ipomoea batatas* (L.) Lam.] by foliar application of paclobutrazol: Role of soluble sugar and free proline. Frontiers of Plant Science, 8, 1400. https://doi.org/10.3389/fpls.2017.01400