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Effects of Paclobutrazol on the Growth of Marang (*Artocarpus odoratissimus*) Seedlings

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ABSTRACT

Shorter trees are ideal in tree orchards and allow easy harvesting, thus increasing fruit yields per unit area. In the Philippines, marang has been considered a potential crop to help food security. However, the crop can grow very tall and make fruit harvesting tedious. This study was conducted to determine if paclobutrazol (PBZ) at concentrations of 0, 250, 500, and 1000 ppm, applied as a media drench, could regulate the growth of marang at the seedling stage. Results showed that paclobutrazol treatment reduced the leaf size of 110-day-old seedlings by almost four-fold compared to the non-PBZ-treated group (control). The stomatal density of the leaf in 250, 500, and 1000 PBZ-treated seedlings was 8.51, 8.49, and 8.11 mm^{-2} compared to only 5.62 mm^{-2} in the control, respectively. Closed stomata in 250, 500, and 1000 ppm PBZ-treated seedlings were 2.78, 2.49, and 3.24 mm^{-2} compared to 1.33 mm^{-2} in the control, respectively. The 250 ppm PBZ treatment increased the chlorophyll index (489.08) in the marang leaf compared to the control with 256.58. Seedling shoot length decreased five-fold and root length by 42.35-52.23% in response to the paclobutrazol treatment. The control seedling fresh weight (18.33 g) was nearly five-fold heavier than the PBZ-treated seedlings. The shoot-root ratio of the control seedlings was three-fold higher than the PBZ-treated seedlings. On the other hand, PBZ treatments resulted in a three-fold increase in the root-to-shoot ratio. These results can appear promising in producing shorter marang trees with longer roots to ease horticultural management in orchards.

Introduction

Global changes in climate are a cause of tailoring horticultural management to the specific demands of crop productivity, as humankind should deliver a sustainable food supply to feed 9 billion people in 2050 (Leisner, 2020; Raza et al., 2019). One way of alleviating the effects of climate change on crop production is to grow climate-change-resilient crop species. Among the potential crop species is marang (*Artocarpus odoratissimus*). This crop grows fast and tall in marginal and hilly lands and produces fruits without requiring specific inputs (Pustadan, 2022). In addition, it is an underutilized crop in the Philippines (Sales et al., 2011) with several economic and pharmaceutical uses (Alvarado, 2023; Pustadan, 2022). The potential of marang to alleviate food security has become a subject of discussion in agricultural sectors in the Philippines (Pustadan, 2022). Moreover, the

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National Research Council of the Philippines (NRCP) in November 2022 called for researchers to contribute technologies that can assist in formulating a holistic production technology to develop the marang industry (Pustadan, 2022).

The marang fruit is delicious and has medicinal properties (Noorfarahzilah et al., 2017). The inedible portion of the fruit eventually becomes waste and has various potential uses (Alvarado, 2023). Marang starts to bear fruits 4-5 years after seed planting. The tree can grow as tall as 20-25 m (Noorfarahzilah et al., 2017), making harvesting operations tedious, especially when marang fruit trees get old. A shorter crop variety may then address this concern. However, breeding a new variety of perennial fruit trees requires ample time and resources.

The application of a plant growth regulator (PGR) such as paclobutrazol (PBZ) to regulate the size of trees is a low-cost solution for this matter (Desta and Amare, 2020). PBZ is a classic PGR that controls the growth of fruit trees (Bausher and Yelenosky, 1986; Valle and de Almeida, 1989; Kasran, 1994; Nafees et al., 2010; Ramos and Acedo, 2016). PBZ blocks the oxidation of ent-kaurene and decreases GA biosynthesis (Protacio et al., 2000; Desta and Amare, 2021). As a result, stem elongation falls behind, resulting in shorter plants. Planting shorter crop varieties has several advantages in crop production, such as higher yield per unit area via high-density planting, ease of employing cultural management practices such as pruning, and less effort during harvest.

The available literature has no information on the effects of paclobutrazol on the vegetative growth of marang. This study aimed to determine if paclobutrazol could regulate marang seedling growth and evaluate how various PBZ concentrations can affect growth and morphological characteristics in the seedlings.

Materials and Methods

The experiment was conducted in a randomized complete block design (RCBD) with four treatments and three replications in a farm situated at Lantapan, Bukidnon, Philippines, September to December 2023. Each plot hosted ten seedlings. Healthy marang seeds of standard size were collected from a local cultivar in Lantapan, Bukidnon, Philippines. The seeds were soaked in tap water for 16 h. The water was drained and the seeds were wrapped inside a piece of moistened cloth (cotton). Pre-germinated seeds had their radicles emerging. They were selected and sown at a depth of two inches in growth media comprised of topsoil, rice hull, and chicken dung inside 6” x 10” x 0.002” polyethylene bags. Different concentrations (0, 100, 200, and 1000 ppm) of paclobutrazol were prepared by adding the required volume of Greenfast® (25% paclobutrazol) with five liters of tap water. Different concentrations of paclobutrazol were then applied as a growth media drench treatment at 500 mL per polyethylene bag. The seedlings were placed and positioned under direct sunlight for 110 days. To prevent seedling dehydration, irrigation was applied at 250 mL of water per seedling bag, three times a week.

Data collection

All data were collected 110 days after the PBZ treatment application or when 50% of the leaves from the control treatment had started to form sinuses and lobes.

Seedling emergence (Mitchell and Vogel, 2012) was determined using the formula below:

$$\text{Seedling emergence (\%)} = \frac{\Sigma \text{ seedlings emerged}}{\text{Number of seeds sown}} \times 100$$

Seedling height (Susilo et al., 2019) was determined by measuring the length from the stem at ground level to the apical leaf. The formula below was then used for determining the average seedling height.

$$\text{Seedling height (cm)} = \frac{\Sigma \text{ seedling height (cm)}}{\text{Number of seedlings}}$$

Length of the longest root was referred to as root length (Wright et al., 1989) and was measured using a ruler. The average root length was determined using the equation below.

$$\text{Root length (cm)} = \frac{\Sigma \text{ longest root length (cm)}}{\text{Number of seedlings}}$$

The number of leaves (Santos et al., 2016) was determined by counting the total number of leaves developed per seedling. The formula below was then used for computing the average number of leaves developed per seedling.

$$\text{Number of leaves developed per seedling} = \frac{\Sigma \text{ number of leaves}}{\text{Number of seedlings}}$$

Fully expanded leaf length in each seedling was measured using a ruler. Measurements were made according to Liu et al. (2017). The average leaf length was determined using the formula below.

$$\text{Leaf length (cm)} = \frac{\Sigma \text{ length of leaf (cm)}}{\text{Number of seedlings}}$$

Fully expanded leaf width in each seedling was measured using a ruler, following instructions by Liu et al. (2017). The average leaf width was determined using the formula below.

$$\text{Leaf width (cm)} = \frac{\Sigma \text{ width of leaf (cm)}}{\text{Number of seedlings}}$$

Leaf chlorophyll index was measured using the FieldScout CM 1000 (Spectrum Technologies Inc, 360 Thayer Court, Aurora, IL 60,504). The FieldScout CM 1000 was pointed to the youngest fully expanded leaf at a distance of 18 inches to measure the chlorophyll index.

Stomatal density per unit leaf area was determined by microscopic examinations at 40x magnification. The youngest fully expanded leaf of a seedling was collected from each plot at 11 a.m. A nail polish was then applied to the adaxial part of the leaf. After drying, a Scotch® transparent tape was placed on top of the nail polish in the leaf. The Scotch® transparent tape was then gripped carefully and immediately mounted on a microscope slide for microscopic examinations. The number of open and closed stomata in each leaf was then counted and the values were expressed as mm^2.

Fresh seedling weight was measured by weighing the whole seedling using a pre-calibrated digital weighing scale. The average fresh biomass was then determined using the formula below.

$$\text{Fresh weight (g)} = \frac{\Sigma \text{ fresh weight (g)}}{\text{Number of seedlings}}$$

Statistical analysis

Data were subjected to the analysis of variance using the Statistical Analysis for Agricultural Research (STAR 2.0.1) software (http://bbi.irri.org/products). Comparison of mean values followed the least significant difference (LSD) test.

Results

Seedling emergence

Seedling emergence of marang was 76.67%, 76.67%, 93.33%, and 73.33% at concentrations of 0, 250, 500, and 1000 ppm, respectively (Fig. 1). Based on statistics, these figures were significantly comparable (P≤0.05). It has also been observed that the seed cotyledons of marang in different concentrations of PBZ remained attached to the emerged seedlings 110 days after sowing (Fig. 2).

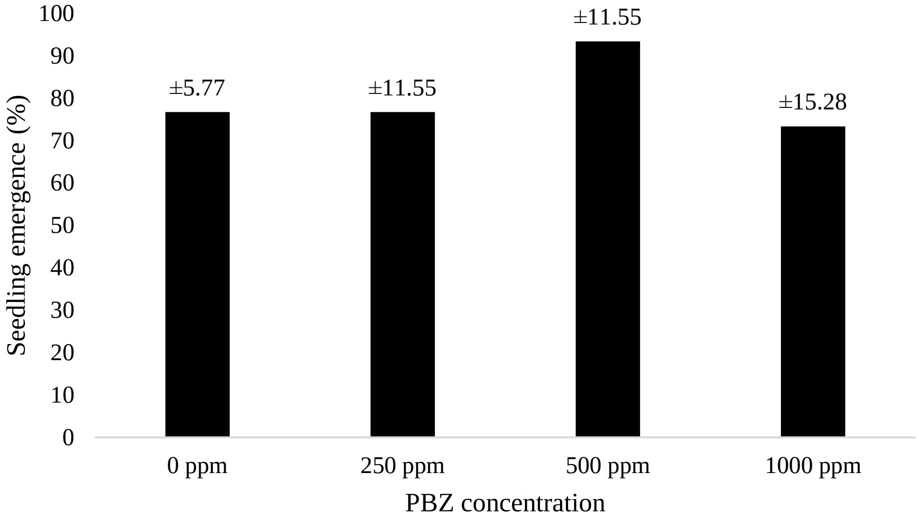

Fig. 1. Emergence percentage of marang seedlings at 0, 250, 500, and 1000 ppm PBZ treatments. cv (%)= 13.66. ns: mean values are not significantly different (P≤0.05) by the LSD test.

Fig. 2. Emerged marang seedlings at 0, 250, 500, and 1000 ppm PBZ treatments. Note the cotyledons (pointed by yellow arrows) still attached to the 110-day-old seedlings

Leaf production

Leaf count per seedling at 110 days after treatment was not significantly affected by the different concentrations of PBZ. As shown in Table 1, the number of leaves produced in marang seedlings was 7.67, 8.13, 8.27, and 8.13 in response to PBZ treatment at concentrations of 0, 250, 500, and 1000 ppm, respectively.

Table 1. Marang seedling leaf morphology at 0, 250, 500, and 1000 ppm PBZ treatments.

PBZ concentration	Number of leaves	Leaf width (cm)	Leaf length (cm)
0 ppm	7.67 ± 0.31	9.08 ± 1.75^{a}	15.24 ± 2.27^{a}
250 ppm	8.13 ± 0.76	2.35 ± 0.24^{b}	4.25 ± 0.52^{b}
500 ppm	8.27 ± 0.64	2.23 ± 0.30^{b}	4.12 ± 0.51^{b}
1000 ppm	8.13 ± 0.12	2.37 ± 0.24^{b}	4.35 ± 0.35^{b}
cv (%)	6.01	22.08	17.17
Significance	ns	**	**

ns: mean values within a column are not significantly different. **- mean values within a column followed by the same letter as superscript are not significantly different ($P \leq 0.01$) by the LSD test.

Leaf length and width

Leaf size of marang seedlings treated with PBZ at various concentrations was significantly reduced compared to non-PBZ treated (control) (Table 1). In comparison with the non-PBZ-treated (control), the leaf width of seedlings in 250, 500, and 1000 ppm PBZ treatments was reduced by 74.12%, 75.44%, and 73.90%, respectively. Likewise, the leaf length of seedlings in 250, 500, and 1000 ppm PBZ treatments was reduced by 72.11%, 72.97%, and 71.46%, respectively.

Chlorophyll index

The chlorophyll index of marang leaves at different concentrations of PBZ is shown in Figure 3. The results showed that the marang leaves at 250 ppm PBZ treatment had the highest chlorophyll index (489.08). In contrast, the chlorophyll index of non-PBZ-treated (control) was the lowest (256.58). Seedlings treated with PBZ (500 ppm and 1000 ppm) had leaves with a chlorophyll index of 363.08 and 369.08, which did not vary significantly with the non-PBZ-treated plants (control).

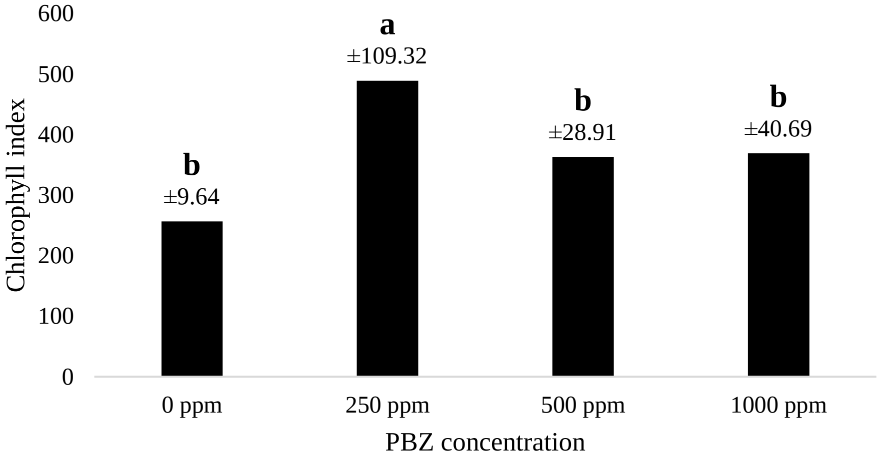

Fig. 3. Chlorophyll index of marang leaves at 0, 250, 500, and 1000 ppm PBZ treatments. cv (%) = 15.81. Mean values followed by the same letter as superscript are not significantly different (P≤0.05) by the LSD test.

Stomatal density

The stomatal density in the leaf of marang seedlings was influenced by the PBZ treatment (Table 2). The number of stomata per mm2 of leaf in response to the PBZ treatment at concentrations of 250, 500, and 1000 ppm was 8.51, 8.49, and 8.11, respectively. The stomatal density of marang leaf in PBZ treatments at concentrations 250, 500, and 1000 ppm increased by 51.42%, 51.07%, and 44.31%, respectively, compared to the non-PBZ-treated plants (control).

Also, there were more closed stomata in the leaves of PBZ-treated seedlings than in the non-PBZ-treated plants (control) (Table 2). In comparison with the non-PBZ-treated seedlings (control), the leaves of PBZ-treated seedlings at concentrations of 250, 500, and 1000 ppm had more closed stomata per mm2 by 109.02%, 87.22%, and 143.61%, respectively.

The number of open stomata per leaf was not significantly affected by the different concentrations of PBZ (Table 2).

Shoot length

The shoot length in non-PBZ-treated seedlings (control) was the highest in value (Fig. 4 and Table 3). All paclobutrazol treatment concentrations significantly reduced the shoot length in marang seedlings five-fold, compared to non-PBZ-treated (control) seedlings (Table 3).

Table 2. Stomata density per mm2 adaxial portion of marang leaf at 0, 250, 500, and 1000 ppm PBZ treatments.

PBZ concentration	Stomatal count per mm^2 of leaf	Closed stomata	Open stomata
0 ppm	5.62 ± 0.74^b	1.33 ± 0.46^b	4.30 ± 1.04
250 ppm	8.51 ± 0.76^a	2.78 ± 0.48^a	5.73 ± 1.20
500 ppm	8.49 ± 0.66^a	2.49 ± 0.26^a	5.99 ± 0.44
1000 ppm	8.11 ± 1.35^a	3.24 ± 0.73^a	4.88 ± 2.08
cv (%)	9.47	18.34	19.78
Significance	**	**	ns

ns- treatment means within a column are not significantly different. **- mean values followed by the same letter as superscript are not significantly different (P≤0.01) by the LSD test.

Root length

Non-PBZ-treated (control) seedlings had the longest roots (Table 3). The root length of seedlings treated with 250, 500, and 1000 ppm of PBZ decreased by 42.35%, 50.46%, and 52.23%, compared to the control, respectively (Table 3).

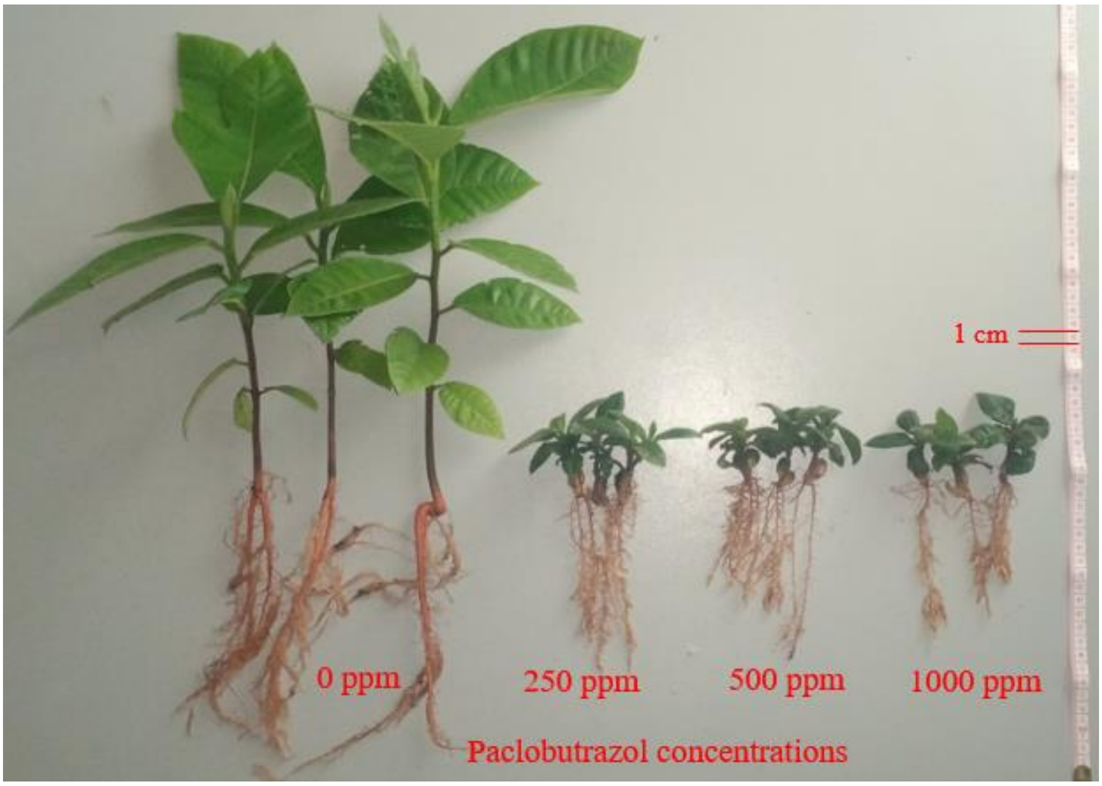

Fig. 4. Marang seedling growth 110 days after 0, 250, 500, and 1000 ppm PBZ treatments.

Table 3. Shoot and root lengths of marang seedlings at 0, 250, 500, and 1000 ppm PBZ treatments.

PBZ concentration	Shoot length, SL (cm)	Root length, RL (cm)	SL: RL ratio	RL: SL ratio
0 ppm	22.33 ± 5.01^{a}	22.67 ± 1.53^{a}	1.00 ± 0.30^{a}	1.06 ± 0.28^{b}
250 ppm	4.33 ± 0.58^{b}	13.07 ± 1.60^{b}	0.33 ± 0.03^{b}	3.04 ± 0.27^{a}
500 ppm	3.43 ± 0.21^{b}	11.23 ± 2.31^{b}	0.31 ± 0.05^{b}	3.26 ± 0.53^{b}
1000 ppm	3.17 ± 0.29^{b}	10.83 ± 2.75^{b}	0.31 ± 0.09^{b}	3.47 ± 1.08^{b}
cv (%)	28.16	14.91	30.28	23.73
Significance	**	**	**	*

*- mean values within a column followed by the same letter as superscript are not significantly different ($P \leq 0.05$) by the LSD test. **- mean values followed by the same letter as superscript are not significantly different ($P \leq 0.01$) by the LSD test.

Seedlings fresh weight

The fresh weight of seedlings was significantly affected by the different concentrations of paclobutrazol (Table 4). Non-PBZ-treated (control) seedlings were heavier, nearly five-fold, compared to the PBZ-treated seedlings (Table 3). The fresh weight of seedlings treated with 250, 500, and 1000 ppm PBZ decreased by 78.18%, 79.98%, and 79.98%, compared to the control, respectively (Table 4).

The shoot weight of the control seedlings (14.33 g) was also heavier, seven-fold, than those of PBZ-treated seedlings (Table 4). The shoot of seedlings treated with 250, 500, and 1000 ppm PBZ weighed only 2 g.

In terms of root weight, the non-PBZ-treated seedlings were heavier two-fold, compared to the PBZ-treated seedlings (Table 4). The root weight of seedlings treated with 250, 500, and 1000 ppm PBZ decreased by 50%, 58.25%, and 58.25%, compared to the control, respectively (Table 4).

Table 4. Fresh weight of marang seedlings at 0, 250, 500, and 1000 ppm PBZ treatments.

PBZ concentration	Seedlings fresh weight (g)	Shoot weight, SW (g)	Root weight, RW (g)	SW: RW ratio	RW: SW ratio
0 ppm	18.33 ± 5.13^{a}	14.33 ± 4.16^{a}	4.00 ± 1.00^{a}	3.57 ± 0.29^{a}	0.28 ± 0.02^{a}
250 ppm	4.00 ± 0.00^{b}	2.00 ± 0.00^{b}	2.00 ± 0.00^{b}	1.00 ± 0.00^{b}	1.00 ± 0.00^{b}
500 ppm	3.67 ± 0.58^{b}	2.00 ± 0.00^{b}	1.67 ± 0.58^{b}	1.33 ± 0.58^{b}	0.83 ± 0.29^{b}
1000 ppm	3.67 ± 0.58^{b}	2.00 ± 0.00^{b}	1.67 ± 0.58^{b}	1.33 ± 0.58^{b}	0.83 ± 0.29^{b}
cv (%)	37.27	40.95	31.13	16.92	22.05
Significance	**	**	*	**	**

*- mean values within a column followed by the same letter as superscript are not significantly different ($P \leq 0.05$) by the LSD test. **- mean values within a column followed by the same letter as superscript are not significantly different ($P \leq 0.01$) by the LSD test.

Shoot-to-root ratio

The non-PBZ-treated (control) seedlings had a higher shoot-to-root ratio compared to the PBZ-treated seedlings (Tables 3 and 4). The shoot-to-root ratio of the control seedlings in terms of length (Table 3) and weight (Table 4) was three-fold higher than the PBZ-treated seedlings.

Root-to-shoot ratio

The non-PBZ-treated (control) seedlings had a lower root-to-shoot ratio compared to the PBZ-treated seedlings (Tables 3 and 4). The root-to-shoot ratio of the PBZ-treated seedlings in terms of length (Table 3) and weight (Table 4) was three-fold higher than the control seedlings.

Discussion

Seedling emergence

Paclobutrazol has reportedly been used in crop production to protect plants against the adverse effects of abiotic stresses (Desta and Amare, 2021; Dinler et al., 2021; Solichatun et al., 2021; Pal et al., 2016; Soluklui et al., 2014) which in turn could lead to high plant survival. In this study, however, the seedling emergence of marang from the control treatment and those treated with different PBZ concentrations were comparable. This observation is notable because the seedlings were grown under conditions without stress. Thus, the potential effects of PBZ to enhance seedling emergence were not determined in this study.

In this present investigation, we also found that seed cotyledons remained intact and completely attached to the emerged PBZ-treated marang seedlings 110 days after treatment. In contrast, seed cotyledons were detached from the emerging marang seedling, a common phenomenon during seedling development. This finding indicates that PBZ alters the seedling growth of marang. The cotyledons act as storage organs that provide nutrients to the developing seedlings (de Vogel, 1980). However, once the plant has produced enough true leaves for photosynthesizing and providing the photoassimilates needed for growth and development, the cotyledons can be shed off from the plant (de Vogel, 1980). In this case, the plant has progressed from the seedling stage to the mature stage of growth, with adult leaves taking over. Our observation suggests that the various PBZ concentrations applied as a drench to potted growth media before seed sowing can extend the juvenile phase of a marang plant.

Leaf morphology, chlorophyll index, and stomatal density of marang in response to PBZ concentrations

Photosynthetically active mature leaves are considered the primary source of photoassimilates for supporting various physiological processes in plant growth and development (Heldt and Piechulla, 2021). The production of leaves in plants is regulated by several factors, including the application of growth regulators (Meshram et al., 2022). In this study, the number of leaves produced by a 110-day-old marang seedling was statistically comparable among treatments (Table 1). However, the leaf size of seedlings was altered by the PBZ treatment. All concentrations of PBZ significantly reduced the length and width of leaves of marang seedlings at 110 days (Table 1). This result indicates that the various concentrations of PBZ inhibit the leaf size and expansion of marang, thus confirming previous research on other crops such as *Syzygium myrtifolium* (Roxb.) Walp. (Nazarudin et al., 2014), *Syzygium campanulatum* (Nazarudin et al., 2007), *Ficus benjamina* L. (LeCain et al., 1986), and tomato (Rahman et al., 1989).

Seedlings with broader leaves have a larger surface area that would capture more light than smaller ones. However, capturing excessive light may also lead to photoinhibition (Shomali et al., 2023). Therefore, marang seedlings with smaller leaf sizes as a result of paclobutrazol treatment would be advantageous, especially during high irradiance and drought conditions (Wang et al., 2019; Tozer et al., 2015; Meier and Leuschner, 2008; Niinemets et al., 2006). The photosynthetic efficiency of a plant leaf is also dependent on its chlorophyll content. Chlorophyll is a green pigment contained in chloroplasts wherein photosynthesis takes place. In the present study, the leaves of seedlings in the 250 ppm PBZ treatment had a higher chlorophyll index than the non-PBZ-treated seedlings (Fig. 3). Our findings were similar to previous research in that paclobutrazol treatments increased chlorophyll content in crops such as basil (Filho et al., 2022), cucumber, squash, melon, and watermelon seedlings (Flores et al., 2018). The chlorophyll content in leaf tissue reportedly increases by applying paclobutrazol (Desta and Amare, 2021; Tesfahun, 2018), which supports the current research. Paclobutrazol increases cytokinin synthesis (Desta and Amare, 2021) as a hormone that prevents chlorophyll degradation in leaf tissue (Taiz and Zeiger, 2010).

Stomatal density in a leaf is also an essential feature of a leaf that affects photosynthesis. The stomata are where H_2O exits and where CO_2 enters. Both gases are substrates of photosynthesis to convert light energy to chemical energy (glucose). Higher stomatal density reportedly increased plant photosynthesis (Xiong et al., 2018; Tanaka et al., 2013). In our results, the PBZ treatment significantly increased the stomatal density per unit leaf area (Table 2). More stomata were closed in the leaves of PBZ-treated seedlings, which could indicate that photosynthesis started to decline during the sampling time (11:00 a.m.). In contrast, fewer stomata were closed in the leaf of control seedlings during the same period. However, since both non-PBZ-treated and PBZ-treated seedlings had similar numbers of open stomata during the same sampling time, their photosynthetic efficiency is also comparable.

Effects of PBZ concentrations on the growth of marang seedlings

Marang seedling growth in media treated with different concentrations of paclobutrazol decreased significantly in height and weight (Tables 3 and 4). PBZ inhibits gibberellin synthesis, which leads to precursor accumulation in the terpenoid pathway and facilitates abscisic acid synthesis (Soumya et al., 2017). Thus, plant growth declines in response to the PBZ treatment. The present study is the first report that paclobutrazol can reduce the growth of marang. These findings corroborate the reports of other researchers that paclobutrazol can reduce the growth of various crops. Abod and Jeng (1993) reported that paclobutrazol treatment reduced the growth of *Acacia mangium*. Abod and Jeng (1993) suggested that paclobutrazol has a more potent growth-inhibiting effect on *Acacia mangium* when sprayed or drenched into the soil. Similarly, soil drench application of paclobutrazol proved effective in reducing the growth of young pecan seedlings (Wood, 1984). If applied as a foliar spray, paclobutrazol at 50 ppm concentration was also effective in reducing the growth of oil palm under nursery conditions (Rahman et al., 2016).

The growth of PBZ-treated marang seedlings appeared more focused on the roots than the shoots, which confirms previous research by Abod and Jeng (1993), showing that PBZ affected the growth of *Acacia mangium* seedlings. Our findings come in association with plant capacity to deal with stressful conditions, specifically drought. Plant breeders usually consider longer roots as a potential trait of drought-tolerant varieties. Moreover, shorter plants are ideal in commercial orchards to facilitate farm management. With the application of PBZ, we produced shorter plants with longer roots within a shorter period with lower inputs.

Conclusions

This study showed that marang seedling growth can decline through paclobutrazol treatment. Regarding leaf size, such as width and length, paclobutrazol decreased those values almost four-fold compared to the control. The stomatal density of the leaf increased through PBZ treatment, but more closed stomata appeared during the same period compared to the control. Moreover, the leaves of marang seedlings in response to the 250 ppm PBZ treatment had the highest chlorophyll index. All paclobutrazol concentrations significantly reduced the shoot length of marang seedlings five-fold compared to the control. In addition, seedlings in the control treatment had the longest roots. The fresh weight of the control seedlings was also almost five-fold heavier than the PBZ-treated seedlings. The control seedlings had a higher shoot-to-root ratio, while paclobutrazol-treated seedlings had a higher root-to-shoot ratio. The results can assist management efforts to produce shorter marang trees with longer roots to facilitate crop harvest in the field. However, further work is also needed to demonstrate the efficacy of paclobutrazol treatment on the different stages of marang growth. Further investigations can determine whether the effect of PBZ on shortening the height of marang is reversible or irreversible by applying gibberellic acid or any growth regulators that stimulate plant height elongation.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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