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Effects of Paclobutrazol Application and Soil Mulching on Flower Induction and Physiological Conditions in Durian Trees (*Durio zibethinus* Murr.)

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Abstract

In durian trees, flower induction is required to guarantee a consistent supply of durian fruit. Paclobutrazol (PBZ) is used extensively to stimulate flowering, and artificial drought stress may stabilize its effectiveness. We investigated the effects of PBZ application combined with drought stress on flower induction. We applied PBZ at three concentrations (0 [control], 1,000, and 1,500 ppm) on 9-year-old durian trees in an orchard in central Thailand 12 days after two groundcover treatments had been established (mulching versus bare ground). Then, we observed flower induction, soil water conditions, and the physiological conditions of the durian trees. Drought stress was confirmed in mulched trees: they had lower soil water potential, lower stomatal conductance, and very low leaf water potential (−0.9 MPa) compared to trees with bare ground. In both mulched and bare-ground trees, PBZ application did not induce flowering. Conversely, compared to the bare-ground treatment, mulching delayed flower bud emergence and the flowering date and reduced flower-cluster density. Therefore, we propose that strong drought stress, regardless of PBZ administration, inhibits flower induction in durian trees.

Discipline: Horticulture
Additional key words: flower induction, leaf water potential, mulching, soil water potential, stomatal conductance

Introduction

Durian (*Durio zibethinus* Murr.) is one of the most important fruit crops in Southeast Asia. The global trade of durian reached approximately 515,000 tons in 2016, with Thailand being the top exporter, accounting for more than 82% of exports from 2015 to 2017 (Altendorf 2018). In Thailand, the durian harvest season lasts from April to September, with monthly production decreasing in October, and the off-season continues until March (Higuchi et al. 2012). The price of durian fruit declines during the peak harvest period. Thus, price stabilization through the control of fruit production, such as producing early crops (Subhadrabandhu & Ketsa 2001), would benefit durian producers and vendors.

Flower induction drives fruiting. Both environmental stress and chemical treatment can induce flowering in durian. Among stressors, drought stress is necessary to induce flowering in this species (Chandraparnik et al. 1992, Subhadrabandhu & Ketsa 2001, Paull & Duarte 2012). Among treatments, paclobutrazol (PBZ) is widely used in Southeast Asia to induce flowering in fruit trees (Hasan & Karim 1989, Chandraparnik et al. 1992, Blaikie et al. 2004, Tri et al. 2011, Upreti et al. 2013). PBZ is a triazole plant growth regulator that inhibits gibberellin biosynthesis (Desta & Amare 2021). Its impact is well known among durian producers (Chandraparnik et al. 1992, Hasan & Karim 1989, Tri et al. 2011), although its effectiveness is inconsistent because it is influenced by weather conditions (Chandraparnik et al. 1992).

Cotreatment with drought stress and PBZ may stabilize the effectiveness of PBZ. According to Chandraparnik et al. (1992), the effectiveness of PBZ benefits from a 10- to 14-day period of drought stress. In

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citrus crops, mulching with moisture-permeable plastic sheets has been applied to enhance drought stress artificially for boosting the Brix value (Xuemei et al. 2017, Iwasaki et al. 2019). Thus, we hypothesized that PBZ application combined with soil mulching using moisture-permeable plastic sheets would stabilize the effect of PBZ on flowering in durian.

In this study, we aim to evaluate the effect of PBZ application combined with drought stress on flower induction in durian. We applied PBZ to 9-year-old durian trees in an orchard in central Thailand with or without soil mulching using moisture-permeable plastic sheets and observed flower induction along with the soil water and physiological conditions of the durian trees.

Materials and methods

1. Study site and plants

The experiment was conducted from August 2007 to January 2008 at a commercial orchard in Chanthaburi Province, Thailand (12.60°N, 102.28°E). In the orchard, 9-year-old ‘Monthong’ durian trees were planted in 10-m-wide, 50-cm-high mounted rows with 10-m spacing. We selected 24 healthy trees for the experiment. Figure 1 shows the temperature and precipitation at the study site. The hourly temperature was monitored using a data logger (TR-52; T&D Corporation, Nagano, Japan) and temperature sensor (TR-5106; T&D Corporation) attached to a force-ventilated radiation shield at 2-m height, and precipitation was recorded using a rain gauge (DAVIS 7852M; Davis Instruments Corporation, Hayward, CA, USA) with an event data logger installed (HOBO CO-UA-003–6; Onset Computer Corporation, Bourne, MA, USA).

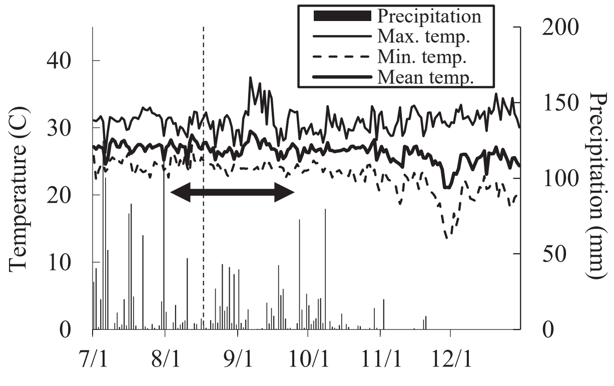


Fig. 1. Air temperature (maximum, minimum, and mean) and precipitation during the experiment
The double-ended arrow indicates the mulch treatment period (August 3 to September 26). The vertical dashed line indicates the date of paclobutrazol treatment (August 15).

2. Experimental design

We applied three concentrations of PBZ (0 [control], 1,000, or 1,500 ppm) 12 days after two groundcover treatments (bare ground or mulching). A total of six treatments were established: bare ground with PBZ control (B-PC) for four trees, bare ground with 1,000 ppm PBZ (B-P1000) for three trees, bare ground with 1,500 ppm PBZ (B-P1500) for five trees, mulching with PBZ control (M-PC) for four trees, mulching with 1,000 ppm PBZ (M-P1000) for four trees, and mulching with 1,500 ppm PBZ (M-P1500) for four trees.

As the mulching treatment, six moisture-permeable plastic sheets (OS-20, 0.22 mm thickness, 2 m width; Taniguchi Sangyo Co., Ltd., Osaka, Japan) were arranged in parallel to cover the 10-m-wide raised rows (Fig. 2). The mulching application began on August 1, 2007, and was completed on August 3, 2007. All mulching materials were removed on September 26.

For the PBZ treatments, PBZ solution was sprayed on all leaves of the target trees on August 15, when the second flush of leaves after the previous fruit harvest had fully expanded (Chandraparnik et al. 1992, Tri et al. 2011). Each tree received 20 (P1000 treatment) or 30 g (P1500 treatment) of PBZ.

3. Measurements and observations

(1) Soil water conditions

Soil water potential (SWP) was measured at a soil depth of 20 cm using a 30-cm tensiometer (Eastern Agritek Co., Ltd., Rayong, Thailand) to monitor daily changes in soil water conditions. Tensiometers were placed at 1, 2, and 3 m away from the trunk (under the canopy) along the mound slope. The tensiometers for the mulching treatments (M-PC, M-P1000, and M-P1500)

Fig. 2. Experimental trees with mulch treatment (left) and bare ground (right)

were removed on September 26 when the mulch was removed.

(2) Physiological conditions of trees

To assess the effects of drought stress on the physiology of the trees, we measured the temporal changes in leaf water potential (LWP) and stomatal conductance. Using a pressure chamber (Model 670; PMS Instrument Company, Albany, OR, USA), we measured the LWP of three leaves on one tree randomly selected from each treatment every 2 h from 6:00 to 18:00 on sunny days: August 3 (the date when mulching was completed), September 25 (53 days after mulching began), and October 19 (23 days after the mulch cover was removed). For each tree, the mean LWP of the three leaves was used as the representative LWP.

We also measured stomatal conductance with photosynthetic active radiation (PAR) for one tree randomly selected in each treatment using a leaf porometer (LI-1600; Li-COR, Inc., Lincoln, NE, USA) on two sunny days: August 3 and September 25. Data were collected every 2 h from 8:00 to 18:00 on August 3 and at 8:00, 10:00, 13:00, 15:00, and 17:00 on September 25. However, because of equipment failure, data for M-P1000 and M-P1500 could not be collected at 8:00 on August 3.

To assess the impact of drought stress on vegetation growth, we measured shoot elongation and leaf formation in all 24 trees. Two shoots 30 cm-40 cm in length were randomly selected from each tree and marked with tags. The shoot lengths and numbers of leaves were recorded monthly from August to December.

(3) Flower induction

To determine the status of flower induction, the dates of flower bud emergence and flowering, as well as flower-cluster density, were recorded for all 24 trees. We observed the flower bud emergence on the main branches of each tree every 5 days and recorded the date when we confirmed the first flower bud at the *khaai plaa* stage, which is a local term for the first developmental stage of flower buds in durian trees (Kozai & Higuchi 2011). Every day, the main branch of each tree was checked for flowers and the date when the first flower appeared was recorded. We further determined the flower-cluster density on three randomly selected main branches with perimeter lengths ranging from 20 to 40 cm in each tree on December 7. We recorded the number of flower clusters and the branch length and calculated the number of flower clusters per 1 m of branch as the flower-cluster density.

4. Statistical analysis

All analyses were carried out using R version 3.5.2 (R Core Team 2018).

Shoot elongation and leaf formation from September onward were calculated by taking the respective values in August as the baseline. A generalized linear mixed model (GLMM; R package “lme4”; Bates et al. 2015) was used to evaluate the effects of mulching treatment, PBZ application, and their interaction on these values. The data were presumed to be gamma distributed with a log-link function, and each tree was assigned as a random effect. We used the shoot length or leaf number data in each month after September as response variables and set the August data as an offset term.

In terms of flower induction, the date when the first flower bud emerged among all trees was considered day 0 and the number of elapsed days from that date was counted for all other trees. For the date of flowering, the date when the first flower appeared among all trees was designated day 0 and the number of elapsed days from that date was counted for the other trees. Then, we analyzed the effects of mulching treatment, PBZ application, and their interaction on the numbers of elapsed days for flower bud emergence and flowering using a generalized linear model (GLM), and the data were presumed to be Poisson distributed with a log-link function.

Regarding flower-cluster density, the effects of mulching treatment, PBZ concentration, and their interaction were evaluated using a GLMM. The response variable, i.e., the number of flower clusters, was presumed to be Poisson distributed with a log-link function, and the length of the main branch was set as an offset. The data from the three observed branches in each tree were nested within that tree, and each tree was assigned as a random effect.

For all GLMs and GLMMs, the significance of each factor, i.e., the fixed effect, was determined using analysis of deviance (type II) with the R package “car” (Fox & Weisberg 2019). Then, the differences in a response variable among treatments were evaluated using Tukey’s method based on general linear hypotheses with the R package “multcomp” (Hothorn et al. 2008).

Results

1. SWP

The SWP was lower in all mulching treatments (M-PC, M-P1000, and M-P1500) than in the bare-ground treatments (B-PC, B-P1000, and B-P1500) regardless of the PBZ treatment (Fig. 3). During the mulching period, the SWP in the mulching treatments decreased and reached −0.08 MPa by mid-September. The SWP tended to be lower at 1 m from the trunk than at 2 m or 3 m. The SWP in the bare-ground treatments began to decrease

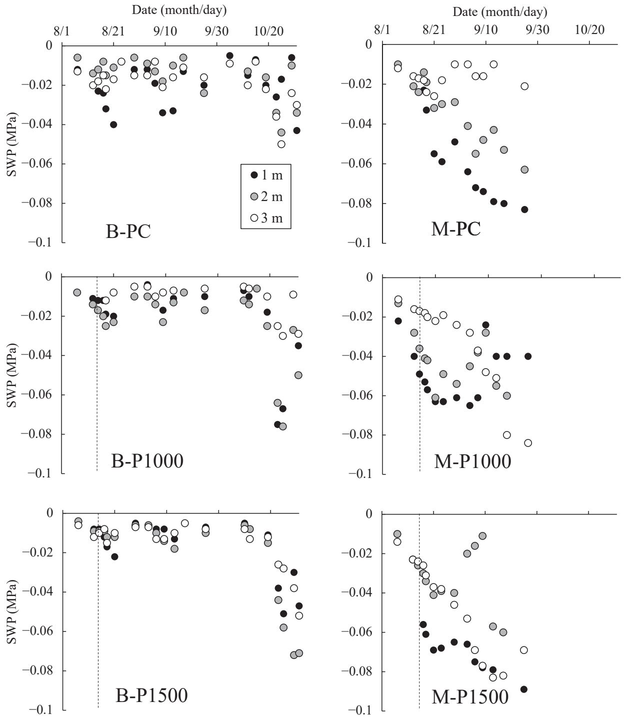


Fig. 3. Temporal changes in soil water potential (SWP) at 1, 2, and 3 m from the trunk in each treatment

B-PC, bare-ground control; B-P1000, bare ground with 1,000 ppm paclobutrazol (PBZ); B-P1500, bare ground with 1,500 ppm PBZ; M-PC, mulching control; M-P1000, mulching with 1,000 ppm PBZ; M-P1500, mulching with 1,500 ppm PBZ. The vertical dashed line indicates the date of PBZ application (August 15).

rapidly toward the end of October and reached approximately −0.08 MPa.

2. Physiological conditions of trees

On August 3 (the day when the mulching treatment was completed) and October 19 (23 days after mulch was removed), the LWP did not differ across treatments (Fig. 4). However, on September 25, 53 days after mulching began, the LWP from 10:00 to 16:00 was significantly lower in mulched trees than in those in bare ground regardless of PBZ treatment.

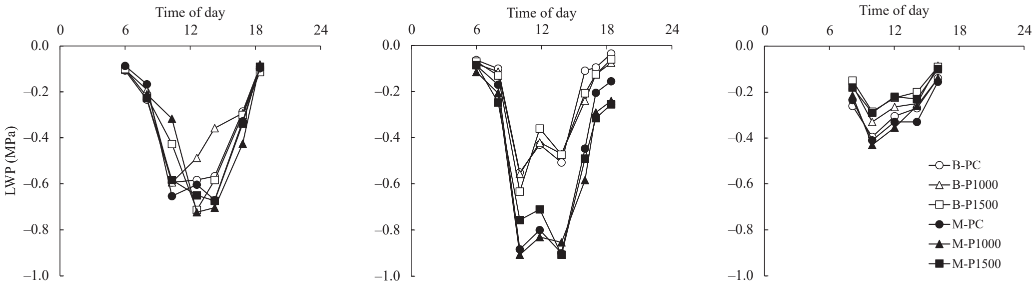
On August 3, stomatal conductance varied with PAR (Fig. 5), but there were no differences among treatments. However, on September 25, from 10:00 to 14:00, the temporal change in stomatal conductance differed from that in PAR between groundcover treatments, and stomatal conductance was lower in mulched trees than in those in bare ground. In addition, at 10:00, stomatal conductance tended to be higher at lower PBZ concentrations in mulched trees. A similar trend was observed in the bare-ground treatment.

After the removal of mulch (i.e., in November and December), the mean shoot length ratio was significantly higher in trees in the mulching treatment than in the bare-ground treatment in November ($P = 0.028$) and December ($P = 0.018$; Fig. 6 and Table 1). There were no significant differences in the leaf number ratio across treatments in any month (Fig. 6 and Table 1).

3. Flower induction

The earliest emergence of flower buds was observed on October 25 in two trees from the B-P1500 treatment (Table 2). In all mulched trees, flower buds emerged 20.0 to 22.5 days after October 25 and mulching significantly ($P < 0.001$) influenced bud emergence (Table 3). Moreover, the mean date of flower bud emergence was delayed, albeit nonsignificantly, with decreasing PBZ concentration across the three bare-ground treatments.

The first flower was observed on December 15 on a tree receiving the B-P1500 treatment. Flowering in the mulching treatments occurred 9.0-13.8 days after



August 3 September 25 October 19

Fig. 4. Diurnal changes in leaf water potential (LWP) in each treatment on August 3, September 25, and October 19

B-PC, bare-ground control; B-P1000, bare ground with 1,000 ppm paclobutrazol (PBZ); B-P1500, bare ground with 1,500 ppm PBZ; M-PC, mulching control; M-P1000, mulching with 1,000 ppm PBZ; M-P1500, mulching with 1,500 ppm PBZ

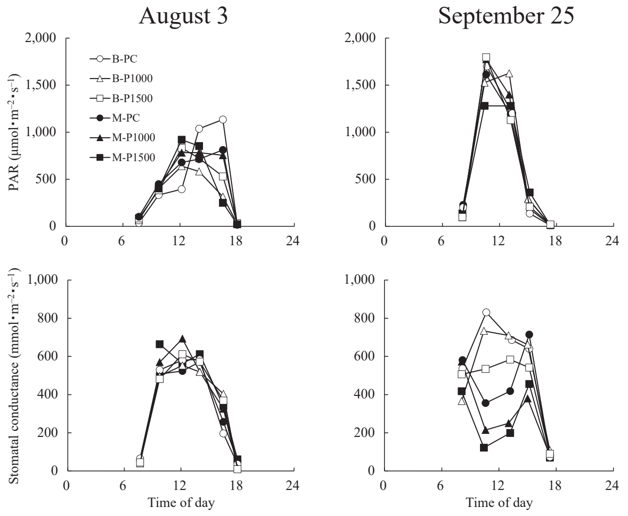


Fig. 5. Diurnal changes in photosynthetic active radiation (PAR) and stomatal conductance in each treatment on August 3 and September 25

B-PC, bare-ground control; B-P1000, bare ground with 1,000 ppm paclobutrazol (PBZ); B-P1500, bare ground with 1,500 ppm PBZ; M-PC, mulching control; M-P1000, mulching with 1,000 ppm PBZ; M-P1500, mulching with 1,500 ppm PBZ

December 15 (Table 2), significantly (P = 0.002) later than flowering in the bare-ground treatments (Table 3). Regarding the effect of PBZ, the mean date of flowering was earlier, albeit nonsignificantly, in trees receiving PBZ than in those not receiving PBZ treatment in the bare-ground treatments.

Flower-cluster density was influenced significantly (P <0.001) by the groundcover treatments (Table 3). Flower-cluster density was significantly lower in the mulch treatments, ranging from 0.3 to 0.6, than in the bare-ground treatments (Table 2).

Discussion

As a result of the mulching-induced reduction in SWP (Fig. 3), 55 days after beginning mulching, the LWP decreased drastically (Fig. 4). Mulching also decreased stomatal conductance (Fig. 5). These data indicate that the mulched trees were under drought stress.

Flower bud emergence and flowering were delayed and flower-cluster density was reduced in mulched trees (Table 2). The LWP of −0.9 MPa during soil mulching (Fig. 4) indicated that the trees were under severe drought stress. According to Paull & Duarte (2012), mild drought stress is necessary to induce flowering in durian. However, our findings imply that severe drought stress delays flowering and reduces the number of flowers, similar to ‘Valencia’ oranges (Melgar et al. 2010).

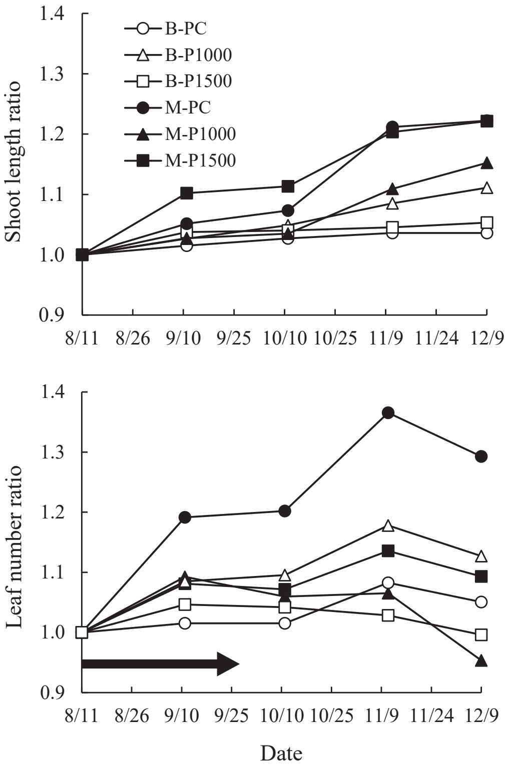


Fig. 6. Changes in shoot length and leaf number ratios during the experiment

The arrow indicates the mulch treatment period (August 3 to September 26). Shoot length and leaf number ratios were determined as the growth rate compared to data determined on August 11.

B-PC, bare-ground control; B-P1000, bare ground with 1,000 ppm paclobutrazol (PBZ); B-P1500, bare ground with 1,500 ppm PBZ; M-PC, mulching control; M-P1000, mulching with 1,000 ppm PBZ; M-P1,500, mulching with 1,500 ppm PBZ

In contrast to our hypothesis, under the severe drought stress induced by mulching, PBZ treatment did not enhance flower induction (Table 2). Moreover, even in the bare-ground treatments, the effect of PBZ was not stable. The mean dates of flower bud emergence and flowering were earlier in trees treated with PBZ than in untreated trees, but the difference was not significant. The variation in the effect of PBZ within each bare-ground treatment may have been due to rainfall during the experiment, as suggested by Chandraparnik et al. (1992). The durian trees received approximately 50 mm of rainwater during the 7 days following PBZ application, and another 137 mm of rain fell in the subsequent 7 days (Fig. 1).

In addition, our results on stomatal conductance

Table 1. Results of the analysis of deviance (type II test) of the shoot length and leaf number ratios

Explained variable	Month	Fixed effect*[1]	Df	Residual deviance	*P* value*[2]
Shoot length ratio	September	M	1	1.95	0.162
		P	2	1.81	0.405
		M × P	2	0.69	0.707
	October	M	1	1.63	0.202
		P	2	0.95	0.621
		M × P	2	1.45	0.484
	November	M	1	4.86	*0.028*
		P	2	0.21	0.899
		M × P	2	1.45	0.485
	December	M	1	5.57	*0.018*
		P	2	0.05	0.975
		M × P	2	1.22	0.542
Leaf number ratio	September	M	1	1.96	0.161
		P	2	0.12	0.940
		M × P	2	2.95	0.229
	October	M	1	1.06	0.302
		P	2	0.00	1.000
		M × P	2	3.80	0.150
	November	M	1	0.98	0.323
		P	2	1.66	0.436
		M × P	2	3.87	0.144
	December	M	1	0.23	0.629
		P	2	2.20	0.333
		M × P	2	4.74	0.094

*[1] M, mulching; P, PBZ concentration

*[2] Italicized numbers are significant (*P* <0.05).

Table 2. Date of flower bud emergence, date of the start of flowering, and flower-cluster density

Treatment	Flower bud emergence*[1]	Flowering*[2]	Flower cluster density*[3]
B-PC	11.3 ab	5.5 bcd	9.3 a
B-P1000	8.3 ab	3.3 cd	9.8 a
B-P1500	5.0 b	3.6 d	8.4 a
M-PC	20.0 ab	12.0 ab	0.5 b
M-P1000	22.5 a	9.0 ac	0.6 b
M-P1500	20.0 ab	13.8 a	0.3 b

*[1] Mean number of elapsed days from the date when the first flower bud emerged (October 25).

*[2] Mean number of elapsed days from the date when flowering began (December 15).

*[3] Mean number of flower clusters per 1 m of a main branch on December 7.

Different letters indicate significant differences among treatments (Tukey's test, *P* <0.05).

indicate that the metabolic process influenced by PBZ may have been inhibited. PBZ inhibits gibberellin biosynthesis, leading to the accumulation of precursors in the terpenoid pathway that are shunted to promote the genesis of abscisic acid (ABA; Desta & Amare 2021). Upreti et al. (2013) also reported that the application of PBZ to mango trees increased the ABA contents of buds and leaves and enhanced flower induction. ABA promotes stomatal closure (Trejo et al. 1993, Schroeder et al. 2001, Wang et al. 2023). In the present study, lower stomatal conductance was observed in trees that received higher concentrations of PBZ (Fig. 5). PBZ application may have led to stomatal closure, but its effects did not extend to flower induction.

Notably, removing the mulch in the mulching treatment enhanced shoot elongation compared to the bare-ground treatment (Fig. 6). Similar results have been reported in blueberry, where the number of vegetative

Table 3. Results of the analysis of deviance (type II) of the date of flower bud emergence, date of flowering, and flower-cluster density

Explained variable	Fixed effect*[1]	Df	Residual deviance	*P* value*[2]
Flower bud emergence	M	1	25.71	*<0.001*
	P	2	0.86	0.442
	M × P	2	1.41	0.271
Flowering	M	1	12.89	*0.002*
	P	2	0.51	0.609
	M × P	2	0.63	0.544
Flower cluster density	M	1	68.96	*<0.001*
	P	2	0.12	0.942
	M × P	2	1.84	0.399

*[1] M, mulching; P, PBZ concentration

*[2] Italicized numbers are significant (*P* <0.05).

bud breaks and the dry weight of new shoots increased as flower bud density decreased (Maust et al. 1999). Therefore, we hypothesize that severe drought stress inhibits flowering but reserves energy, resulting in significant elongation growth once drought stress subsides. This effect may be useful when growers want to prioritize vegetative growth rather than fruit production.

Overall, our findings indicate that severe drought stress induced by mulching inhibited flower induction in durian trees. Mulching also boosted shoot growth. PBZ did not induce flowering under drought stress caused by mulching, but its impact was also not so clear in the bare-ground treatment. Future research is required to identify the optimal environmental conditions for stabilizing the effect of PBZ.

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