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Morphological and Physiological Responses of Torch Ginger [*Etlingera elatior* (Jack) R.M. Smith] to Paclobutrazol Application

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Absract The effects of paclobutrazol (PBZ) on morphological and physiological responses of torch ginger was investigated. After drenching once stem height, leaf size, guard cell density, guard cell size and number of chloroplast were recorded for various periods of time. At the first month after PBZ treatment all concentrations gave significant different in stem height. The maximum height of torch ginger at 23.70 cm was obtained from control treatment without PBZ. Increaseing in concentrations of PBZ caused the decrement in stem height. At the second month after drenching the decrement in stem height was clearly observed. Treated-plants with high concentrations of PBZ (200, 300 and 400 $mg.L^{-1}$) had markedly slowed well in growth. The highest concentration of PBZ at 400 $mg.L^{-1}$ gave the lowest height of torch ginger at 17.65 cm. Guard cell density was not significantly different between PBZ-treated and non treated-plants but non treated-plants gave higher density than those from treated-plants. For guard cell size PBZ at concentration of 400 $mg.L^{-1}$ gave the highest width and length at 29.81 and 54.95 µm, respectively. This concentration of PBZ gave the highest number of chloroplasts at 31.67 cells as well.

Keywords: Torch ginger, *Etlingera elatior*, paclobutrazol, growth, guard cell, chloroplast

Introduction

Torch ginger [*Etlingera elatior* (Jack) R.M. Smith] which belongs to the Zingiberaceae (Mohamad *et al*., 2005; Abdelmageed *et al*., 2011) which is one of the most commonly known in the species of *Etlingera*. It is one type of native plants found in Thailand, Indonesia, Vietnam, Malaysia and other countries in Southeast Asia (Luachan *et al*., 2017). It is known as Bunga kecombrang or Honje in Indonesia, Bunga kantan in Malaysia (Abdelmageed *et al*., 2011) and Dala in Thailand. It is eaten as Thai salad. This species is also known as torch ginger or wax flower due to the strinking resemblance of the

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inflorescence to a flaming torch (Mohamad *et al*., 2005). Torch ginger is one of the 30 popular herbs that have a high demand in Malaysia and now being cultivated as a commercial scale in Australia, Thailand, Costa Rica and Malaysia (Lekawatana and Pituck, 1998; Abdelmageed *et al*., 2011) for cut flower. In nature, torch ginger is rhizomatous plant (Mendez *et al*., 2004). It grows up to 3 meter in height. Leaf length is up to 85 cm and purple in color when it is young. Inflorescence originates at the end of leaf stalk. The bracts have deep pink color. Size of inflorescence is approximately 10x10 cm with individual hairy green-red fruit. The utilization of torch ginger is still limited. Recently, it has been used as cut flower. But time required from planting to flowering is too long. Normally, it takes at least more than one year. For this plant the demand of markets also need pot plants for decorating. However, the size of plant is too high (from 75 cm upto 200 cm) (Sarmiento and Kuehny, 2003). So far, there are no growers plant it as pot plant.In order to create this type of plant for high demand of the market it should be developed by PBZ application. PBZ is plant growth regulator causing a slow growth rate of all kinds of plants, leading to a bushy type of those plants. The properties of this chemical is inhibiting gibberellin synthesis (Thohirah *et al*., 2005; Te-chato *et al*., 2009; Khan *et al*., 2009). Nowadays, many kinds of flowering plant can be successfully controlled canopy to be small or bushy characteristics, such as chrysanthemum (Gilbertz, 1992; Kucharska and Orlikowaka, 2008), orchid (Te-chato *et al*., 2009) and lilly (Zheng *et al*., 2012). However, there are no reports about the use of PBZ on controlling growth of torch ginger for production of pot plant and flowering plant.

Thus, the objective of this study was to investigate the effect of PBZ on morphological and physiological response in relation to growth of torch ginger for further benefit of growers in the future.

Materials and methods

Plant material: *In vitro* shoots of torch ginger raised on MS medium supplemented with 3 $mg.L^{-1}$ BA, 3% (w/v) sucrose and 0.8% (w/v) agar were used. Individual shoot was excised and transferred to rooting medium for root induction as described by Muangkaewngam (2016). Complete plantlets were acclimatized in pot containing composed, coconut fiber and cattle manure at ratio of 1:1:1. They were transferred to greenhouse and watered once a day with 500 ml per pot. After raising for one month when the plants reaching to the height of 15 cm they were ready to treat with various concentrations of PBZ.

Experiment I Effect of PBZ on growth and morphological characteristics

Plants at height of 15 cm after 1 month of transfer were applied by drenching with PBZ at concentrations of 0, 100, 200, 300 and 400 $mg.L^{-1}$. After drenching plant height was measured every month for 4 months. At month 4 leaf size were recorded and statistically compared. Completely randomized design (CRD) was employed to distinguish the difference among treatments and mean among treatments was separated by Duncan's multiple range test (DMRT). Each data was presented as the mean of four replications. Each replication consisted of 3 pots.

Experiment II Effect of PBZ on physiological responses

In this experiment, 4-month-old plants after drenching with various concentrations of PBZ were used. Leaves of 2^{nd} node from shoot tip of controlled and PBZ-treated plants were sampling and collected for examining guard cell characteristics. Epidermal layer from 3 positions proximal, middle and distal of the leaf samples was peeled off mounted on glass slide and observed under the compound microscope. Guard cell size, density and number of chloroplasts in guard cell were recorded and statistically compared. CRD was employed to distinguish the difference among treatments and mean among treatments was separated by DMRT. Each data was presented as the mean of four replications from each position.

Results

Experiment I Effect of PBZ on growth and morphological characteristics

Application of all concentrations of PBZ caused the decrement in stem height of torch ginger at the first month after drenching, but significantly different was not clearly observed. Only the controlled treatment (without application of PBZ) gave the highest stem height at 23.70 cm significantly different ($p \leq 0.01$) from those obtained from PBZ-treated plants. However, all concentrations of PBZ-treated plants gave non significantly different in stem height. At the second month after drenching with different concentrations of PBZ (200, 300 and 400 $mg.L^{-1}$) gave clearly significantly different in stem height ($p \leq 0.01$). The highest concentration of PBZ at 400 $mg.L^{-1}$ gave the lowest growth of torch ginger (17.65 cm). Similar results were also found after raising torch ginger for 3 and 4 months. All plants exposed to PBZ at concentrations of 300 and 400 $mg.L^{-1}$ had stem height far lower than the other treatments (Table 1, Figure 1). When analyzing the LD_{50} values at different concentrations, the values for 50 percentage of growth reduction (LD_{50}) of torch ginger were 510.44, 428.77, 326.55 and 279.19 $mg.L^{-1}$ respectively (Figure 3).

Table 1. Effect of various concentrations of PBZ drenching on height of torch ginger after raising for various periods of time.

Concentrations of PBZ ($mg.L^{-1}$)	Height (cm) at different times of raising (months)			
	1	2	3	4
0	23.70a (100)	29.85a (100)	41.75a (100)	53.65a (100)
100	16.70b (70.46)	20.77b (69.58)	30.97b (74.18)	36.17b (67.42)
200	16.57b (69.91)	19.42c (65.06)	25.00b (59.88)	31.58b (58.86)
300	15.95b (67.30)	18.00c (60.30)	20.77c (49.75)	22.54c (42.01)
400	14.88b (62.78)	17.65c (59.13)	19.95c (47.78)	21.25c (39.61)
F-test	**	**	**	**
C.V.%	20.68	22.80	23.65	31.02

** significantly different at $p \leq 0.01$

Means followed by the same letter (s) within each column are not significantly different according to DMRT

The number in parentheses showed percent of control

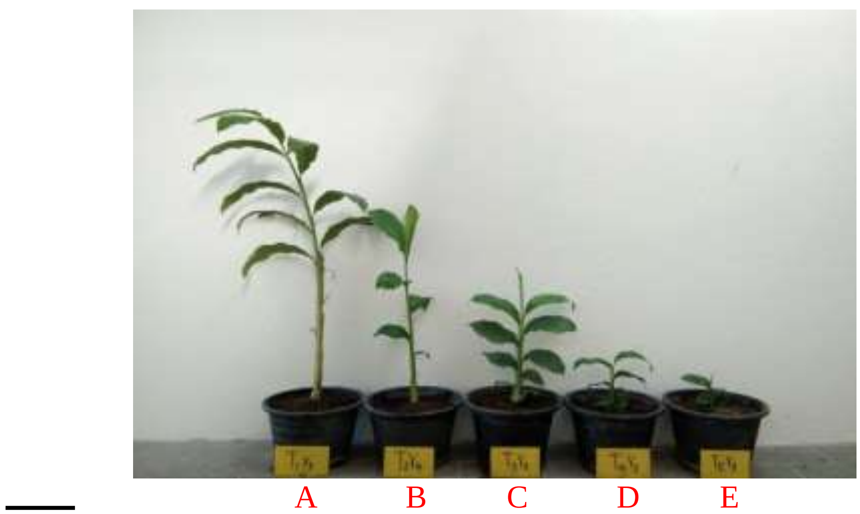

Figure1. Height of torch ginger after drenching once with various concentrations of PBZ and raising for 4 months (A) control 0 $mg.L^{-1}$ PBZ, 100 $mg.L^{-1}$ PBZ (B), 200 $mg.L^{-1}$ PBZ (C), 300 $mg.L^{-1}$ PBZ (D), 400 $mg.L^{-1}$ PBZ (E) (bar = 10 cm)

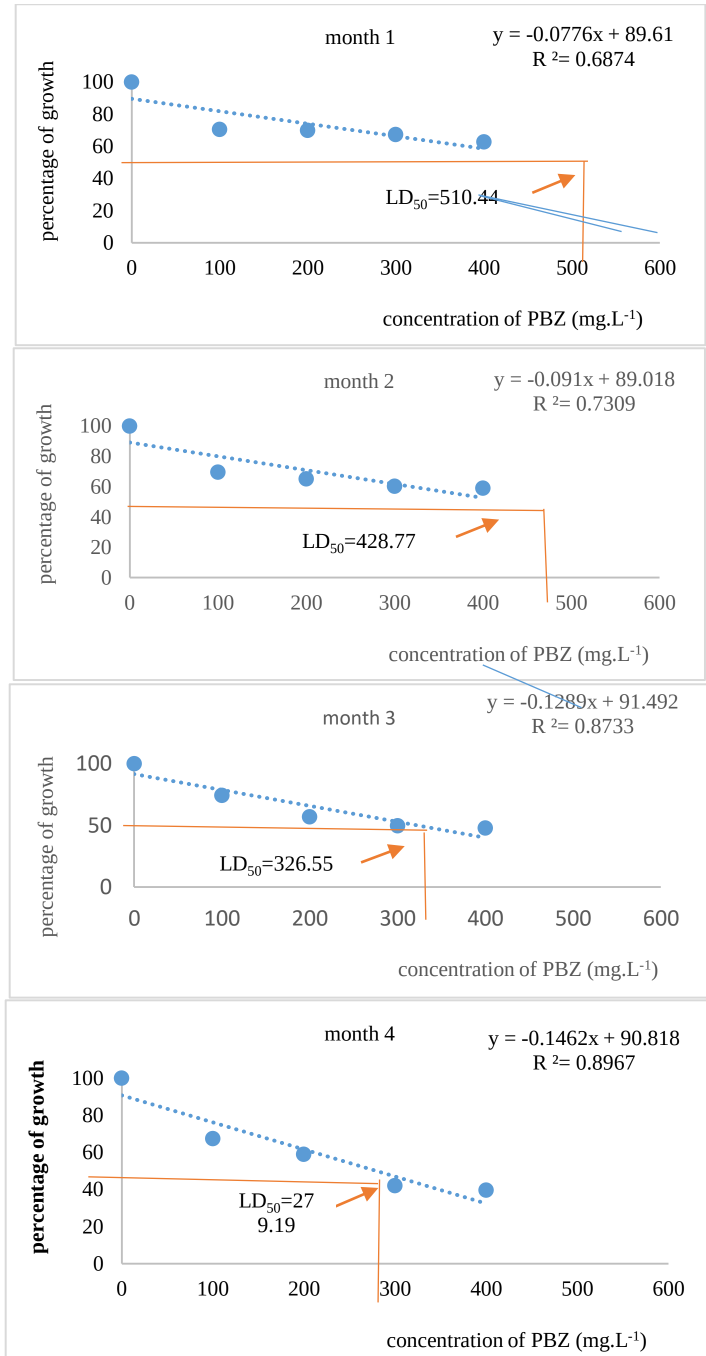

Figure 2. The growth reduction rate of 50% (LD_{50}) of torch ginger drenching with various concentrations of PBZ and raising in the pot for 1-4 months

After 4 months of of raising, PBZ-treated plants had different leaf size with controlled plants. The results were clearly shown that the controlled treatment (without application of PBZ) gave significant bigger leaf size ($p \leq 0.05$) (6.80 cm in width and 23.67 cm in length) than treated plants. However, PBZ at high concentration up to 200 mg.L^{-1} gave non significant decrement in leaf size ($p \leq 0.05$) with the lower concentrations (100 mg.L^{-1}) and control. Higher concentrations of PBZ than this gave significant different decrement in leaf size. The highest concentration of PBZ at 400 mg.L^{-1} gave the lowest leaf size of torch ginger but was not significantly different with 300 mg.L^{-1}. The width and length of leaf obtained from 400 mg.L^{-1} PBZ were 5.37 and 12.05 cm, respectively (Table 2).

Table 2. Effect of various concentrations of PBZ on leaf size of torch ginger

Concentrations of PBZ (mg.L^{-1})	Width of leaf (cm)	Length of leaf (cm)
0	6.80a	23.67a
100	6.73a	23.30a
200	6.57a	22.27a
300	5.57b	13.80b
400	5.37b	12.05b
F-test	*	*
C.V.(%)	13.08	12.96

* significantly different at $p \leq 0.05$

Means followed by the same letter (s) within each column are not significantly different according to DMRT

Experiment II Effect of PBZ on physiological response

Density of guard cell and guard cell size

For the effect of various concentrations of PBZ on density of guard cell, controlled treatment (without application of PBZ) gave the highest result (2.67 cell/mm^2) but was not significantly different with the other treatments. In the case of guard cell size, the result showed that the controlled treatment gave significant bigger size ($p \leq 0.05$) at 21.61 µm in width and 44.77 µm in length than that obtained from PBZ-treated plants. Torch ginger applied with PBZ at concentrations of 200 and 300 mg.L^{-1} gave the biggest guard cell size. These concentrations of PBZ were significantly different ($p \leq 0.05$) with the other concentrations. The highest concentration of PBZ (400 mg.L^{-1}) gave the biggest guard cell size at 29.65 µm in width and 54.95 µm in length (Table 3).

Table 3. Effect of various concentrations of PBZ on density of guard cell and guard cell size of torch ginger

Concentrations of PBZ ($mg.L^{-1}$)	Density of guard cell ($cell/mm^2$)	Width of guard cell (μm)	Length of guard cell (μm)
0	2.67	21.61b	47.77b
100	2.33	21.75b	45.86b
200	2.33	20.38b	46.80b
300	2.00	29.65a	45.58b
400	2.05	29.81a	54.95a
F-test	ns	*	*
C.V.(%)	20.20	9.02	10.39

ns not significantly different
* significantly different at $p \leq 0.05$
Means followed by the same letter (s) within each column are not significantly different according to DMRT

A number of chloroplasts in guard cell

The controlled treatment (without application of PBZ) gave significant lowest number of chloroplast in guard cell (18.67 chloroplasts) ($p \leq 0.05$) in comparison with 300 and 400 $mg.L^{-1}$PBZ, but was not significantly different with 100 and 200 $mg.L^{-1}$ PBZ. Torch ginger which applied with the highest concentration of PBZ (400 $mg.L^{-1}$) gave the highest number of chloroplast (31.67 chloroplasts) in guard cell (Table 4 and Figure 3).

Table 4. Effect of various concentrations of PBZ on number of chloroplast in guard cell of torch ginger

Concentrations of PBZ ($mg.L^{-1}$)	Number of chloroplast (chloroplasts)
0	18.67c
100	19.00c
200	19.00c
300	23.00b
400	31.67a
F-test	*
C.V.(%)	23.29

* significantly different at $p \leq 0.05$
Means followed by the same letter (s) within each column are not significantly different according to DMRT

(a)

(b)

Figure 3. Number of chloroplasts in guard cell of torch ginger without (a) and with (b) treating with 400 mg.L^{-1} PBZ

Discussion

Paclobutrazol is one of growth retardant which inhibits kaurene oxidase and thus blocks the synthesis of gibberellic acid (Radimacher *et al*., 1984; Graebe, 1987). It is active as a growth retardant in broad spectrum of species (Dalziel and Lawrence, 1984) such as *Chrysanthemum morifolium* (Menhenett, 1984), *Curcuma roscoena, Curcuma alismatifolia* (Thohirah *et al*., 2005) and canola (Hua *et al*., 2014) etc. In this present study paclobutrazol played significant role on the torch ginger's height after pouring paclobutrazol at all 4 concentrations and times of application compared to non-treatment. Effective concentration affecting the decrease in height is different from species to species. In pot plant or floriculture, paclobutrazol (marketed as Cultar) at concentrations of 2 to 90 mg.L^{-1} was reported to be effective on height control when applied to the substrate for planting (Barret, 2001). However, our study revealed that paclobutrazol at 400 mg.L^{-1} (nearly four times) resulted in height inhibition to be 50% of original height. Time required for height reduction is generally 3-4 months after appliction. In torch ginger in this present study, it took about 4 months like that reported by Stefanini *et al.* (2002). Who reported that responses of paclobutrazol-treated plant was observed at 120 days after transplanting the seedlings.

This result suggests that the effect of paclobutrazol as a gibberellin biosynthesis inhibitor operates at the levels of leaf cell elongation, dry matter production, shoot elongation and other plant characteristics had the highest height. Also, as the concentration level of paclobutrazol increased, the height decreased and reached to the lowest at concentration when the old because paclobutrazol can inhibit cell division and the extension of cell length. Paclobutrazol has been tested with many plant species and giving the same results. When the concentration level of the substance increased, the height decreased accordingly. This was because paclobutrazol only had an effect on the extension of stalk, not for other parts of the plants. As a result, there were no abnormalities of the plants. Besides, there were various plant species that can be affected by paclobutrazol in the same way as torch ginger such as In addition to the impact on the height of the plant, paclobutrazol can also have an effect on the size of torch ginger's guard cells. The size of guard cells increased as the concentration level of paclobutrazol increased, and reached to the maximum at 400 mg.L^{-1} concentration. The size of guard cells was 29.81 μm (width) x 54.95 (length) μm. After considering the intensity of guard cells, it was found that there were no statistically differences. However, for the torch ginger's leaves which had not received paclobutrazol, there was the higher intensity of guard cells compared to those received paclobutrazol in every level of concentration, and reached to the lowest at 400 mg.L^{-1} concentration. The decrease in the number of guard cells can affect to the drought tolerance of plants. In other words, as the intensity of guard cells decreased, the amount of dehydration also decreased. This corresponds to the experiment on the implementation of paclobutrazol with *Curcuma alismatifolia* (Thohirah *et al*., 2005) under dehydrated condition in comparison with the plants that had not received paclobutrazol, it was found that the growth tendency of the plants received paclobutrazol was better under dehydrated condition (Barret, 2001; Stefanini *et al*., 2002). Moreover, there was less proline content in the plants received paclobutrazol than those had not received. Proline can help maintain water balance inside the cells with the external environment. When the plants are under dehydrated condition, they will accelerate the formation of proline in order to survive under dehydrated condition. However, the plants received paclobutrazol will generate less proline, indicating the increase in stress tolerance in plants (Jungklang and Saengnil, 2012). In addition, plants that were under stress condition due to dehydration had lower pigmentation rates in photosynthesis (Anjum *et al.*, 2011; Romero *et al*., 2017). However, in this study of torch ginger, it was found that the number of chloroplasts in torch ginger's leaves that received paclobutrazol was higher than those did not receive. As a result, torch ginger can grow well under stress condition due to dehydration. The number of chloroplasts increased to the maximum of 31.67 or 1.70 times at 400 mg.L^{-1} concentration as the concentration level of paclobutrazol increased, compared to those plants that had not received paclobutrazol. The number of chloroplasts and chromosomes number were correlated in direct variation (Junpugdee and Te-chato, 2010). In other words, the number of chloroplasts increased as the chromosomes number increased. Although the number of chloroplasts in torch ginger's leaves received paclobutrazol at 400 mg.L^{-1} concentration did not increase in double, the number was very close anyway. The number of chloroplast in guard cell can be used as an indicator of the increase in chromosome number. Which is considered a simple way. There are no complicated steps. Do not waste money. And use a short time.

Application of paclobutrazol caused the changes in growth, morphological and physiological characteristics of torch ginger. Growth in term of stem height, width of leaf and length of leaf had negative relation with the concentrations of paclobutrazol. Paclobutrazol at 279.19 mg.L^{-1} inhibited growth at 50%. This concentration was suggested to use for pot plant production of torch ginger. Guard cell size and number of chloroplast had positive relation with concentrations of paclobutrazol. Paclobutrazol at 400 mg.L^{-1} gave the highest guard cell size and number of chloroplasts. This concentration was suggested to use for mutation induction in torch ginger in the near future.

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