Saudi Journal of Biological Sciences (2017) **24**, 1505–1512

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Effects of water-deficit stress and paclobutrazol on growth, relative water content, electrolyte leakage, proline content and some antioxidant changes in *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink

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Received 2 May 2015; revised 3 September 2015; accepted 9 September 2015
Available online 14 September 2015

KEYWORDS

Curcuma alismatifolia Gagnep. cv. Chiang Mai Pink;
Water stress;
Paclobutrazol

Abstract Effects of water-deficit stress and paclobutrazol (PBZ) on the physiological and biochemical changes in *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink (Zingiberaceae) were investigated. One hundred rhizomes were grown for 30–35 days and then divided into the following 4 treatments: (1) well-watered, (2) not watered, (3) well-watered and treated with 1500 ppm PBZ being applied once to the soil, and (4) not watered but treated with 1500 ppm PBZ. After 50 days of growth, watering was withheld for 30 days. After water stress was initiated, plant height, plant fresh weight, soil water content, relative water content (RWC), electrolyte leakage (EL), proline content, vitamin C and E content, as well as the activities of catalase (CAT) and superoxide dismutase (SOD) in the leaves were determined every 10 days. The results showed that water-deficit stress decreased plant height and plant fresh weight, whereas this stress and PBZ did not result in a decrease in these parameters. Water stress reduced RWC, but induced EL and proline content in the leaves. However, the leaves showed opposite results when PBZ was added to the treatments. Some antioxidants such as vitamin C, vitamin E, and the activities of CAT and SOD were induced in the leaves by PBZ. Moreover, the content of vitamin C, vitamin E and CAT activity were higher in relation to water-deficit stress and PBZ treatments. This indicates that PBZ induced a number of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.09.017

some physiological and biochemical adaptations (maintaining growth and RWC, decreasing EL and proline content, increasing the vitamin C and vitamin E levels, and CAT and SOD activities) that enable the *Curcuma* plant to tolerate drought.

1. Introduction

Curcuma alismatifolia Gagnep. cv. Chiang Mai Pink (Zingiberaceae), or Patumma in the Thai language, is an economically important plant species in Thailand. It is an ornamental plant with a variety of shapes and colors of the bracts and is popular for cut-inflorescence and ornamental purposes in plots and gardens (Songhongsa, 2014). Potted plants can be moved easily and are commonly used as a form of decoration in many places, both inside structures and surrounding buildings and gardens (Fig. 1). To make potted plants shorter, plant growth regulators are needed. Paclobutrazol or PBZ [(2RS,3RS)-1-(4-chlorophenyl-4,4-dimethyl-2-(1H-1,2,4 triazol-1-yl)pentan-3-ol] is a plant growth retardant which blocks three steps in the terpenoid pathway for the production of gibberellins (Fletcher et al., 2000). One of the main roles of gibberellins in plants is the stimulation of cell elongation. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate, resulting in stems with the same number of leaves and shorter internodes (Pinto et al., 2006; Francescangeli and Zagabria, 2008). For these reasons, PBZ is used to reduce plant height for potted plant production in several species (Pinto et al., 2006; Francescangeli and Zagabria, 2008; Hua et al., 2014; Wanderley et al., 2014). PBZ has been reported as an ameliorated compound when plants are subjected to salinity or water-deficit stresses. This occurs as a result of the reduction of malondialdehyde (MDA), electrolyte leakage (EL) and

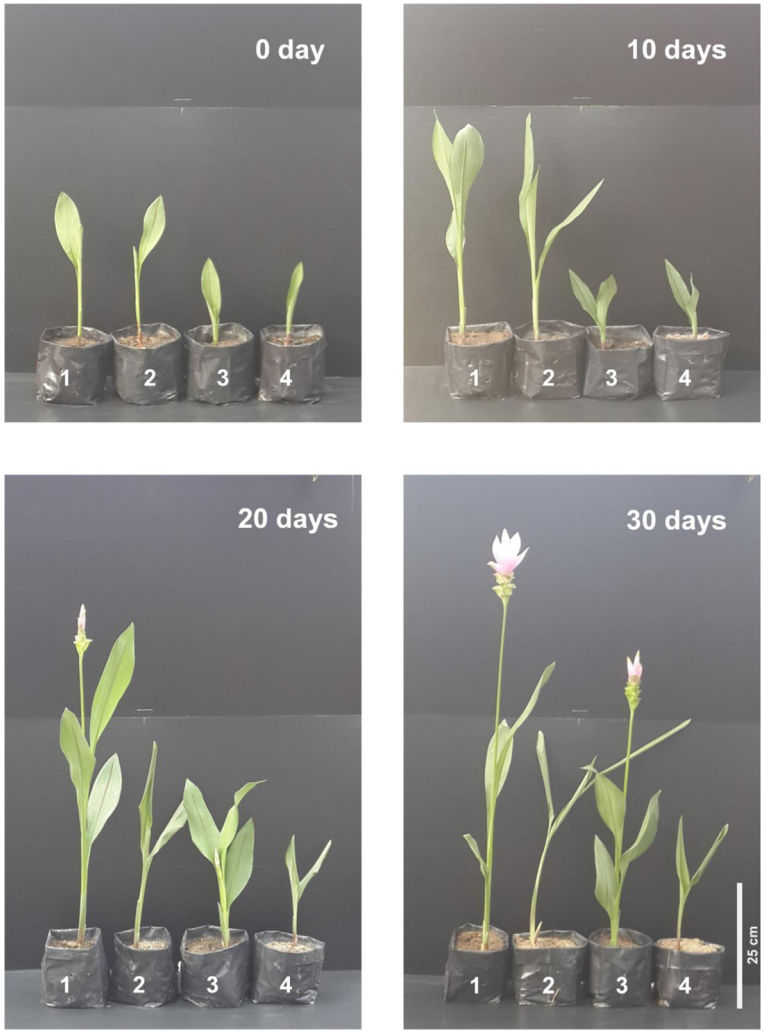

Figure 1 Treatments of *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink; (1) well-watered, (2) no water, and (3) well-watered + 1500 ppm PBZ and (4) no water + 1500 ppm PBZ after withholding water for 0–30 days.

induction of the relative water content, proline and the antioxidant system (Fernandez et al., 2006; Sankar et al., 2007; Srivastav et al., 2010; Jungklang and Saengnil, 2012). Antioxidants such as vitamins C and E, and the activities of antioxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD), are increased by PBZ, which in turn provides stress tolerance to plants (Somasundaram et al., 2009; Srivastav et al., 2010). PBZ reduces height and induces water stress tolerance in *C. alismatifolia* Gagnep. cv. Chiang Mai Pink (Jungklang and Saengnil, 2012). Our study provides further information on how PBZ induces water stress tolerance in this plant. We have investigated the effects of water-deficit stress and PBZ on growth, relative water content, electrolyte leakage, proline content and certain antioxidants in the leaves of this cultivar. The results from this investigation provide more knowledge on how PBZ ameliorates water stress in this plant.

2. Materials and methods

Experiments were carried out in a nursery with 30 ± 5 °C temperature, 20,000 lx light intensity and 65 ± 5% relative humidity at the Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand from 1 January 2013 to 31 July 2014. All experiments were done following a completely randomized design. All data obtained were subjected to a one-way ANOVA and the differences were compared by least significance difference (LSD) test. Each data point was the mean average of three replicates (3 pots/replicate). Comparisons with P values < 0.05 were considered significantly different.

2.1. Plant materials

One hundred rhizomes of *C. alismatifolia* Gagnep. cv. Chiang Mai Pink were produced by a horticultural farmer in Chiang Mai Province and were sterilized by applying a fungicide, 3% Captane 50, for 30 min. The rhizomes were soaked in tap water for 3 days and the water was changed daily. The rhizomes were then sown in plastic bags (15 × 20 cm) containing 1000 L of mixed soil which consisted of mold, husk, dung and dried leaves at a ratio of 1:1:1:1. Every two days, 200 ml of water was applied. After 30–35 days of growth, the plants were 3–5 cm high and were divided into 4 treatments: (1) watered every 2 days [well-watered], (2) not watered, and (3) well-watered and treated with 1500 ppm PBZ once to the mixed soil, and (4) not watered but treated with 1500 ppm PBZ. After 50 days of growth, watering was withheld for 30 days. After water stress was initiated, 4 individuals in each group were selected every 10 days for 30 days to determine plant height, plant fresh weight, relative water content (RWC), electrolyte leakage (EL), proline content, vitamin C and E content, and the activities of catalase (CAT) and superoxide dismutase (SOD) in the leaves.

2.2. Plant height, plant fresh weight and soil water content

The height of plant was measured from the stem where it adjoins with the ground to the end of the longest leaf. This part was then weighted as the fresh weight. The middle area of the mixed soil was collecting by a spoon of 2.5 cm in diameter. The soil was weighted as fresh weight (FW) and then dried at 80 °C for 48 h as dry weight (DW). The soil water content (%) was calculated using the following formula: ([FW − DW]/DW) * 100.

2.3. Relative water content

Relative water content was carried out according to the procedure of Barrs and Weatherley (1962) and Gonzalez and Gonzalez-Vilar (2001) with slight modifications. Leaf disks of 2.5 cm diameters were excised from the middle of the lowest leaf blades for RWC assays. After fresh weight (FW) determination, the disks were floated in distilled water for 4 h. The turgid samples were quickly blotted dry prior to the determination of the turgid weight (TW). The dry weight (DW) of the samples was determined after being oven-dried at 80 °C for 24 h. RWC was calculated using the following formula: RWC (%) = (FW − DW)/(TW − DW) * 100.

2.4. Electrolyte leakage

Leaf disks of 2.5 cm in diameter were excised from the lowest leaves for EL determination according to the modified methods of Mao et al. (2007). After being rinsed 3 times for 2–3 min with de-ionized water, 5 pieces were put into 20 ml of de-ionized water in test tubes and shaken every 5 min for 30 min. Conductivity was measured using a conductivity meter. Total conductivity was obtained after keeping the test tubes boiling for 15 min and electrolyte leakage was expressed as percentage of total conductivity.

2.5. Proline content

Proline content was determined according to the modified methods of Bates et al. (1973) and Ghoulam et al. (2002). Leaf blade material (300 mg FW) was homogenized in 5 ml of 40% methanol. One milliliter of the homogenate was mixed with 1 ml of acid-ninhydrin reagent and put in glass tubes. After 1 h in boiling water, the tubes were placed in an ice bath to stop the reaction. Then 5 ml of toluene was added to the tubes. The absorbance of the upper phase (supernatant) was spectrophotometrically determined at 528 nm. The proline content was determined using a standard curve.

2.6. Vitamin C content

Vitamin C (ascorbic acid) content was quantitatively determined using the 2,6-dichlorophenolindophenol dye method as described by AOAC (1995) and Deepa et al. (2006) with slight modifications. Fresh samples (3 g) of the lowest leaf were homogenized with 10 ml of 3% metaphosphoric acid using a pinch of acid-washed quartz sand. The extract was centrifuged at 3000*g* for 20 min at 4 °C. Two milliliters of supernatant were titrated against standard 2,6-dichlorophenolindophenol dye which had already been standardized against standard ascorbic acid. The results were expressed as mg 100 g^{-1} on a fresh weight basis.

2.7. Vitamin E content

Vitamin E (α-tocopherol) content was quantitatively determined using the method described by Contreras-Guzman

and Strong (1982) with slight modifications. Fresh samples (3 g) of the lowest leaf were placed in test tubes. To each tube, 20 ml of absolute ethanol was added and the tubes were closed tightly and immersed for 30 min in a water bath at 85 °C. After the solution was allowed to cool, 20 ml of heptane was added and the tubes were shaken for 5 min. Then, 20 ml of 1.25% Na_2SO_4 was added, the tubes were shaken again for 2 min, and the contents were allowed to separate into layers. Tocopherols remained in the heptane layer (upper phase). A volume of 0.5 ml of 0.1% α-tocopherol in ethanol was processed in the same way as described above as a standard. Next, 2.5 ml of the supernatant heptane layer was mixed with 5 ml of complexing reagent. All tubes were shaken vigorously for 2.5 min, the mixture was allowed to settle, and 3 ml of the lower phase was mixed with 0.5 ml of absolute ethanol. The solution was spectrophotometrically determined at 545 nm. The results were expressed as mg 100 g^{-1} on a fresh weight basis.

2.8. Extraction of samples for determination of antioxidative enzyme activity

Our method was slightly modified from those of Manoranjan and Dinabandhu (1976) and Goth (1991). Two hundred milligrams of the lowest leaf samples was homogenized with 10 ml of 0.1 M phosphate buffer at a pH value of 6.8 and then centrifuged at 2 °C for 15 min at 17,000*g* in a refrigerated centrifuge. The clear supernatant was used as the enzyme source and was kept on ice during the experiment.

2.8.1. CAT assay

0.1 ml of the extracted sample was diluted in 0.1 ml of 0.1 M phosphate buffer at a pH value of 6.8 and then incubated in 1.0 ml of the substrate (65 µmol/l hydrogen peroxide in 60 mmol/l sodium potassium phosphate buffer, pH 7.4) at 37 °C for 1 min. The enzymatic reaction was terminated with 1.0 ml of 32.4 mmol/l ammonium molybdate and the yellow complex of molybdate and hydrogen peroxide was spectrophotometrically determined at 405 nm with 3 blanks. The substances in each blank are described as follows: blank I contained 1.0 ml substrate, 1.0 ml molybdate and 0.2 ml of the sample, blank II contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml buffer, and blank III contained 1.0 ml buffer, 1.0 ml molybdate, and 0.2 ml buffer. The activity of CAT (kU/L) was then calculated from the formula: A(sample) − A (blank I)/A(blank II) − A(blank III) * 271.

2.8.2. SOD assay

(Sigma kit) 20 µL of the extracted sample was added into the samples and they were put onto blank II plates. Distilled water was also put onto the blank I and blank III plates. Two hundred microliters of working solution were put onto every plate. Twenty microliters of the enzyme working solution was put into the sample and the blank I plates. Twenty microliters of dilution buffer was put onto the blank II and blank III plates. The plates were incubated at 37 °C for 20 min. The solution was spectrophotometrically tested at 450 nm using a micro plate reader. SOD activity was calculated from the inhibition rate of oxygen changing to the superoxide anion radical as: {[(A blank I − A blank III) − (A sample − A blank II)]/(A blank I − A blank III)} * 100.

3. Results and discussion

As shown in Fig. 1 and Table 1, 1500 ppm PBZ reduced plant height by 50% compared to the non-treated plants. Water limitation for 10–30 days reduced the plant height and plant fresh weight in the plants that were subjected to water-deficit stress. This period of stress did not reduce these parameters in the PBZ-treated plants under water-deficit stress (Table 1). These results indicate that one PBZ-application to the soil of 3–5 cm high plants was effective for height reduction, but was unable to affect biomass during water-deficit stress. PBZ controls height as a cell and internode elongation inhibitor. It retards plant growth by inhibition of gibberellin biosynthesis (Fletcher et al., 2000; Hua et al., 2014; Wanderley et al., 2014). Burrows et al. (1992) reported that PBZ reduced the height of *Chrysanthemum* cv. Lillian Hoek, but increased leaf thickness by adding layers of palisade and spongy mesophyll cells.

Consideration of the relative water content in the leaves is probably the most appropriate measure of plant water status for the physiological consequences of cellular water deficit. As shown in Table 2, PBZ kept relative leaf water content higher than the non-treated plants when subjected to water stress for 30 days. This is in agreement with the findings of Jungklang and Saengnil (2012) who reported that PBZ had the ability to maintain leaf water content for 30–40 days of water-deficit stress. In many plant species, relative water content in the leaves ranged from between 88% and 95% in fully turgid transpiring leaves and to about 30–40% in severely desiccated and dying leaves, depending on the species (Trouhton, 1969; Gonzalez and Gonzalez-Vilar, 2001; Schlemmer et al., 2005). In most crop species, typical leaf RWC at around the initial wilting is at about 60–70%. PBZ kept water turgidity in our leaves during drought stress conditions and indicates that this substance enhances cell turgidity.

After 30 days of withholding water, the PBZ treated-plants had lower levels of electrolyte leakage (EL) compared to the untreated-plants. Under water-deficit conditions, EL of the untreated plants was 2–2.5 times greater than the PBZ-treated plants (Table 2). EL is an indicator of membrane injury. From our results, it is clear that PBZ plays an important role in protecting cell membrane damage that results from water-deficit stress. Srivastav et al. (2010) reported that mango seedlings treated with 1500 ppm of PBZ enhanced the capacity to limit cell membrane damage during salt stress. Jungklang and Saengnil (2012) showed that our *Curcuma* specimens treated with 1500 ppm of PBZ decreased the malondialdehyde (MDA) level when compared to the untreated PBZ plants. MDA has been reported as a secondary oxidative product of lipid peroxidation. This mechanism has resulted from the active oxygen species (AOS), which can adversely affect the breakdown of macromolecules such as proteins and phospholipids (Finaud et al., 2006). Phospholipids are a component of the cell membranes. When these macromolecules break down, cell membrane injury occurs. EL and MDA contents are used as markers to estimate cell membrane injury under water-deficit stress conditions.

Drought for 30 days produced the highest proline content in our *Curcuma* leaves (Table 2). This result is similar to those of the studies in which proline accumulation was lower in the PBZ-treated *Curcuma* than in the un-treated plants (Jungklang and Saengnil, 2012). Osmotic adjustment is a factor of

Table 1 Effects of paclobutrazol (PBZ) on plant height, plant fresh weight and soil water content in *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink during 0–30 days of water being withheld.

	Treatment	Withholding water			
		0 day	10 days	20 days	30 days
Plant height (cm)	Well-watered	32.5 ± 1.7a	48.5 ± 1.3a	72.9 ± 2.2a	80.3 ± 3.9a
	No water	36.7 ± 2.7a	41.2 ± 0.6b	41.4 ± 1.8b	66.0 ± 2.0b
	Well-watered + 1500 ppm PBZ	16.6 ± 0.7b	18.2 ± 0.9c	18.7 ± 1.2c	28.8 ± 3.6c
	No water + 1500 ppm PBZ	17.1 ± 2.3b	18.9 ± 3.7c	22.5 ± 2.2c	23.5 ± 0.9c
F-test		*	*	*	*
Plant fresh weight (g)	Well-watered	5.8 ± 0.6a	14.6 ± 1.2a	34.8 ± 10.2a	75.7 ± 20.4a
	No water	5.1 ± 0.1a	9.9 ± 1.1b	7.9 ± 1.8b	17.6 ± 2.9b
	Well-watered + 1500 ppm PBZ	2.9 ± 0.7b	4.2 ± 0.5c	6.3 ± 1.8b	8.7 ± 1.1c
	No water + 1500 ppm PBZ	3.5 ± 0.5b	3.9 ± 0.7c	7.7 ± 1.6b	8.5 ± 1.8c
F-test		*	*	*	*
Soil water content (%)	Well-watered	27.6 ± 1.4	27.7 ± 3.0a	30.0 ± 1.4a	30.3 ± 1.1a
	No water	26.7 ± 3.1	12.8 ± 2.1b	5.0 ± 1.5b	3.3 ± 1.1b
	Well-watered + 1500 ppm PBZ	28.0 ± 2.6	30.1 ± 2.7a	30.8 ± 1.1a	30.6 ± 1.9a
	No water + 1500 ppm PBZ	27.7 ± 1.4	17.9 ± 0.6b	6.3 ± 1.9b	4.8 ± 0.6b
F-test		ns	*	*	*

The data are expressed as the means of three replications ± standard deviation (SD). *F*-test, ns: no significant difference; *: significant difference at $p < 0.05$. Mean sharing with different letters in a single column of the same parameter is considered significantly different by the least significance difference (LSD) test.

Table 2 Effects of paclobutrazol (PBZ) on relative water content (RWC), electrolyte leakage (EL) and proline content in the leaves of *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink during 0–30 days of water being withheld.

	Treatment	Withholding water			
		0 day	10 days	20 days	30 days
RWC (%)	Well-watered	88.3 ± 2.2	87.0 ± 3.3ab	87.5 ± 1.7a	87.8 ± 1.7b
	No water	86.2 ± 3.2	80.3 ± 1.4b	75.6 ± 2.9b	68.6 ± 1.6d
	Well-watered + 1500 ppm PBZ	91.2 ± 6.2	91.2 ± 4.5a	90.3 ± 0.7a	92.7 ± 0.8a
	No water + 1500 ppm PBZ	89.5 ± 4.1	83.5 ± 0.8b	75.5 ± 3.5b	71.9 ± 1.5c
F-test		ns	*	*	*
EL (%)	Well-watered	5.8 ± 0.6a	5.6 ± 0.3a	5.6 ± 1.0a	8.3 ± 1.4b
	No water	4.9 ± 0.5b	5.6 ± 0.6a	6.1 ± 0.6a	10.3 ± 1.8a
	Well-watered + 1500 ppm PBZ	4.9 ± 0.6b	4.9 ± 0.4b	4.9 ± 0.2b	6.8 ± 1.0c
	No water + 1500 ppm PBZ	4.1 ± 0.3c	4.6 ± 0.2b	4.8 ± 0.1b	4.1 ± 0.4d
F-test		ns	*	*	*
Proline (μmol/g FW)	Well-watered	0.1 ± 0.00	0.2 ± 0.01	0.2 ± 0.02	0.7 ± 0.06b
	No water	0.1 ± 0.01	0.3 ± 0.14	0.3 ± 0.05	1.1 ± 0.05a
	Well-watered + 1500 ppm PBZ	0.1 ± 0.01	0.2 ± 0.05	0.3 ± 0.05	0.6 ± 0.11b
	No water + 1500 ppm PBZ	0.1 ± 0.01	0.2 ± 0.04	0.3 ± 0.10	0.7 ± 0.07b
F-test		ns	ns	ns	*

The data are expressed as the means of three replications ± standard deviation (SD). *F*-test, ns: no significant difference; *: significant difference at $p < 0.05$. Mean sharing with the different letters in a single column of the same parameter is considered significantly different by the least significance difference (LSD) test.

physiological machinery when plants respond to water-deficit or salinity stresses. Proline is well-known as an osmotic regulator that can reduce osmotic damage (Slama et al., 2008; Surender Reddy et al., 2015). The accumulation of proline in our *Curcuma* leaves could possibly play a protection role apart from osmoregulation during drought stress. However, PBZ might act as a stress-ameliorating agent in this plant, as this plant does not need to accumulate the proline content in the leaves (Table 2). Previous studies have proved that proline accumulation was lower in tolerant plants when compared to sensitive plants during periods of salinity or drought stress (Jungklang et al., 2003; Turkan et al., 2005).

The vitamin C content increased in our leaves under water limitations of 20–30 days. Drought in PBZ treated-plants showed the highest levels of ascorbic acid when compared to other treatments (Table 3). Ascorbic acid is one of the most extensively studied antioxidants. A high level of endogenous ascorbic acid ensures plant protection from oxidative damage

Table 3 Effects of paclobutrazol on vitamins C and vitamin E contents and activities of catalase (CAT) and superoxide dismutase (SOD) in the leaves of *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink during 0–30 days of water being withheld.

	Treatment	Water withholding			
		0 day	10 days	20 days	30 days
Vitamin C content (mg/100 g fresh weight)	Well-watered	4.1 ± 0.30b	4.2 ± 0.40c	4.3 ± 0.30d	5.3 ± 0.40d
	No water	4.1 ± 0.30b	4.6 ± 0.30c	5.2 ± 0.50c	6.4 ± 0.40c
		4.1 ± 0.10b	5.0 ± 0.20b	6.8 ± 0.10b	9.0 ± 0.15b
	No water + 1500 ppm PBZ	5.2 ± 0.30a	5.8 ± 0.30a	7.1 ± 0.40a	9.5 ± 0.50a
F-test		*	*	*	*
Vitamin E content (mg/100 g fresh weight)	Well-watered	0.6 ± 0.04b	0.6 ± 0.06b	0.6 ± 0.01c	0.6 ± 0.01b
	No water	0.6 ± 0.05b	0.6 ± 0.01b	0.8 ± 0.04b	0.4 ± 0.04c
		0.7 ± 0.02a	0.6 ± 0.02b	0.6 ± 0.03c	0.7 ± 0.03b
	No water + 1500 ppm PBZ	0.7 ± 0.01a	0.7 ± 0.06a	1.1 ± 0.07a	1.2 ± 0.05a
F-test		*	*	*	*
CAT activity (Kunit/L)	Well-watered	67.2 ± 6.60b	40.7 ± 1.12b	29.5 ± 3.80b	45.2 ± 3.91c
	No water	67.1 ± 6.62b	38.1 ± 0.72b	54.8 ± 2.13a	79.0 ± 3.91b
		93.3 ± 6.80a	49.7 ± 1.65a	56.5 ± 0.75a	85.8 ± 3.90b
	No water + 1500 ppm PBZ	94.4 ± 6.30a	51.6 ± 3.98a	57.5 ± 1.62a	144.5 ± 3.80a
F-test		*	*	*	*
SOD activity (% inhibition)	Well-watered	72.5 ± 1.90b	71.8 ± 0.25c	76.5 ± 2.46b	85.3 ± 0.63b
	No water	72.5 ± 1.90b	71.3 ± 1.03c	80.3 ± 0.52a	86.4 ± 0.48ab
		78.6 ± 1.50a	74.0 ± 0.86b	80.2 ± 0.36a	87.2 ± 1.44a
	No water + 1500 ppm PBZ	79.6 ± 0.90a	77.2 ± 1.52a	79.8 ± 0.66a	87.1 ± 0.41a
F-test		*	*	ns	*

The data are expressed as the means of three replications ± standard deviation (SD). *F*-test, ns: no significant difference; *: significant difference at $p < 0.05$. Mean sharing with the different letters in a single column of the same parameter is considered significantly different by the least significance difference (LSD) test.

that occurs due to abiotic stresses, such as from drought and salt exposure (Mittler, 2002). In plant cells, the most important reducing substrate for hydrogen peroxide (H_2O_2) scavenging is ascorbic acid, which is catalyzed by ascorbate peroxidase (Asada, 1999). Ascorbic acid has the ability to neutralize AOS, such as hydroxyl radical ($OH^{\cdot}$), superoxide radical (O_2^{-}), and fatty acid peroxyl radical ($LOO^{\cdot}$), against oxidative stress (Finaud et al., 2006). From our results, PBZ induces higher levels of endogenous ascorbic acid when the plants were subjected to water-deficit stress. This ability promotes plant tolerance during water stress conditions. This result is in agreement with Sankar et al. (2007) who reported that PBZ increased ascorbic acid content in *Arachis hypogaea* L. during conditions of water-deficit stress.

Vitamin E content increased in our leaves when water-deficit stress was increased for 20 days. The combination of water-deficit stress and PBZ revealed plants showed the highest levels of vitamin E when compared to the water-deficit stressed plants (Table 3). Vitamin E is a fat-soluble vitamin made up of several isoforms. Alpha-tocopherol is known to be the most active and abundant form (Fuchs et al., 2003). This compound is mainly localized on membranes (Mittler, 2002). Alpha-tocopherol has an important role in terminating the chain reaction of lipid peroxidation by intercepting intermediate $LOO^{\cdot}$ and donating hydrogen from its hydroxyl group. The product of this reaction is the α-tocopherol radical, which is poorly reactive and can be reduced back to α-tocopherol by ascorbic acid (Arora et al., 2002; Finaud et al., 2006). An increase of α-tocopherol content in the PBZ-treated leaves provided a higher tolerance against conditions of water-deficit stress.

The activities of catalase (CAT) increased in our leaves at 14 days after PBZ application. Water limitation for 30 days enhanced CAT activity compared to the well-watered conditions. The PBZ-treated plants experienced higher activities of this enzyme when compared to the untreated plants during 30 days of water-deficit stress conditions. However, the activity of SOD in PBZ-treated plants was kept high from the beginning to the end of the water-withholding period (Table 3). SOD and CAT are well-known antioxidative enzymes in cells, which can catalyze the poorly reactive oxygen species converting them to non-toxic substances. SOD constitutes the first line of defense against AOS. This enzyme removes O_2^{-} by catalyzing its dismutation, wherein one O_2^{-} is reduced to hydrogen peroxide (H_2O_2) and another is oxidized to oxygen (Alscher et al., 2002; Halliwell, 2006). H_2O_2 is generally known as a toxic substance. CAT is an enzyme that can convert H_2O_2 directly into water and oxygen. This enzyme is present in every cell and in particular on peroxisome (Mittler et al., 2004; Finaud et al., 2006). SOD and CAT play a significant role in defending against oxidative stress induced by abiotic stress in plant tissues (Mittler, 2002; Finaud et al., 2006). In our studies, PBZ increased the activities of SOD and CAT during water-deficit stress conditions. This compound can reduce damage in plants grown under water stress conditions by enhancing the activity of these antioxidative enzymes. PBZ minimizes the adverse effects of water-deficit stress by increasing the levels of the activities of antioxidative enzymes in many plants such as groundnuts, sesame seeds, mangos, and tomatoes (Percival and Salim AlBalushi, 2007; Sankar et al., 2007; Manivannan et al., 2008; Somasundaram et al., 2009; Srivastav et al., 2010; Mohamed et al., 2011).

Previous studies on ornamental plants have reported that PBZ could induce leaf thickness by adding layers of palisade and mesophyll cells (Burrows et al., 1992; Abdul Jaleel et al., 2007). In addition, the first to the third leaves of *C. alismatifolia* Gagnep. cv. Chiang Mai Pink were significantly thicker after 6 weeks of 1500 ppm PBZ application when compared to the non-treated plants (Uthaibutra et al., 2005). Based on these results, PBZ might enhance leaf thickness of *Curcuma*, which would improve the water requirements in their leaves. These may lead to an increase of RWC, a decrease of EL and finally the enhancement of the antioxidant system that might allow this plant to maintain its biomass and be effectively tolerant to water-deficit stress.

4. Conclusion

Jungklang and Saengnil (2012) reported that PBZ increased the tolerance of *C. alismatifolia* Gagnep. cv. Chiang Mai Pink plants under water-deficit stress conditions by reducing proline and MDA content. This result showed that PBZ induced the water stress tolerance of the plants by maintaining fresh weight and RWC, reducing EL and proline, enhancing the levels of vitamin C and E antioxidants, and by increasing the activities of the antioxidative enzymes, e.g. SOD and CAT, in the leaves.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

We are very grateful for the assistance of Miss Phornwisa Jaihaen, Miss Janyathip Pookjinda, Miss Nittaya Junsew, and Miss Warunee Jomkitichai as our assistant researchers, our colleague J.F. Maxwell and Mr. Russell Kirk Hollis for editing this manuscript, along with the Thai Government Fund for financial support.

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