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*Original Article*

# Effect of paclobutrazol on patumma cv. Chiang Mai Pink under water stress

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**Abstract**

The effects of paclobutrazol on physiological and biochemical changes in Patumma cv. Chiang Mai Pink during water stress were investigated by using 0 (control) and 1,500 ppm (treatment) of paclobutrazol. Paclobutrazol was applied once as soil drench. Two weeks later, water withholding was started for 40 days. Soil water content, relative water content (RWC) in the leaves, plant height, dry weight/plant, chlorophyll a and b contents, and proline and malondialdehyde (MDA) contents in leaves were evaluated every 10 days for 40 days after the start of water withholding. The results showed that the dry weight and RWC in the plants treated with paclobutrazol were higher, while the reduction of plant height, proline, and MDA contents were lower than the control during water deficit. This study indicates that paclobutrazol increased the tolerance of Patumma plants to water stress.

**Keywords:** *Curcuma alismatifolia* Patumma cv. Chiang Mai Pink, paclobutrazol, water stress, proline

## 1. Introduction

Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl-4,4-dimethyl-2-(1H-1,2,4 triazol-1-yl)pentan-3-ol] is a well-known plant growth retardant. Many ways of its application to plants have been done, for example, spraying on leaves or stems and on soil drench. The latter way seems to be more effective than the other ways (Willium and Edgerton, 1983). Paclobutrazol functions by inhibiting cytochrome P-450, which mediates oxidative dimethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellins biosynthetic pathway (Fletcher *et al.*, 2000). From this function, paclo-butrazol has long been used to reduce plant height for potted plant production, particularly ornamental plants (Beattie *et al.*, 1990; Fletcher *et al.*, 2000). Using 1-4 mg a.i./pot did not reduced the height of *Curcuma* spp. (Zingiberaceae) (Sarmiento and Kuehny, 2003). An increase in the paclobutrazol concentration to 35 mg a.i./pot reduced plant foliage height and flower stem length, without affecting inflorescence length and delaying the production of *C. alismatifolia* (Zingiberaceae) (Pinto *et al.*, 2006). Paclobutrazol has been used to induce water stress tolerance in some plants such as *Arachis hypogaea* L. (Leguminosae, Papilioideae) and *Phillyrea angustifolia* L. (Oleaceae) (Fernandez *et al.*, 2006; Sankar *et al.*, 2007). The induction of water stress tolerance results from some biochemical changes, which induces the chlorophyll a and chlorophyll b contents in potato leaf (Tekalign and Hammes, 2004). The proline content also increased with increasing water stress tolerance (Jaleel *et al.*, 2007 and Srivastav *et al.*, 2010). When plants are subjected to water stress, proline is synthesized from glutamic acid to act as osmoprotectant for keeping the water balance in cells and the outer environment (Delauney and Verma, 1993). Higher water stress tolerance is also caused from an increase of antioxidants, a decrease of electrolyte leakage, and from lipid peroxidation, which is well known as an indicator to evaluate cell membrane damage (Gopi *et al.*, 2007; Jaleel *et al.*, 2007; Srivastav *et al.*, 2010). Some physiological changes, such as reduction of plant height and leaf size, increase of leaf thickness and stomata density are also known to occur (Sankhla *et al.*, 1985; Burrows *et al.*, 1992).

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In Thailand, Patumma (*Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink, Zingiberaceae) has been grown as a commercial cut flower in the north and produced along with rhizomes. Recently, this plant has become popular as a flowering pot plant to decorate many places, for example inside and surrounding buildings, gardens. A characteristic of ornamental pot plants is that they should be compact, therefore, paclobutrazol is used for this objective. Moreover, northern Thailand has a very hot and dry season from March-May. Although there is irrigation in nurseries, overall it is not sufficient resulting in water shortage for the plants. Since paclobutrazol can be used to reduce plant height and induce drought tolerant plants, the purpose of this study was to investigate the effects of paclobutrazol on some physiological and biochemical changes under water stress in Patumma. The results from this investigation can be used to produce this plant in a more economical way.

## 2. Materials and Methods

These experiments were carried out in a nursery at 30±2°C temperature and 62±2% relative humidity conditions at the Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, from 1 April 2006 to 30 April 2007. All experiments were done with a completely randomized design. All data obtained was subjected to one-way analysis of variance (ANOVA) and the differences were compared by least significance difference (LSD) test. Each data point was the mean of three replicates (3 pots/ replicate). Comparisons with $P$ values <0.05 were considered significantly different.

### 2.1 Plant materials

Rhizomes of Patumma, which were provided by a horticultural farmer in Chiang Mai Province, were sterilized by applying a fungicide, 3% Captane 50, for 30 minutes. The rhizomes were soaked in tap water for three days and the water was changed every day. The rhizomes were then sown in plastic bags (30 x 30 cm) containing mixed soil. Water and fertilizer (NPK, 15:15:15) were regularly applied. After the growth of a 10-15 cm long stem, paclobutrazol with concentrations of 0 and 1,500 ppm was applied once to the soil.

### 2.2 Paclobutrazol treatment

Two weeks after the paclobutazol application, a period of 40 days of water withholding was started. Three replicates were selected every 10 days for 40 days to determine soil water content, plant height, dry weight/plant, relative water content (RWC), chlorophyll a and b, and proline and malondialdehyde (MDA) content in the leaves.

#### 2.2.1 Soil water content

Soil samples were collected with a 4 cm in diameter spoon from 8 cm below the soil surface for fresh weight determinations. Dry weight was determined after oven-drying at 80°C for 24 hrs. The water content of the soil was calculated by subtracting the dry weight from the fresh weight.

#### 2.2.2 Relative water content

Leaf discs of 2.5 cm diameter were excised from the middle of the leafs for RWC assays. After fresh weight (FW) determination, the discs were floated in distilled water for 4 hrs. The turgid samples were quickly blotted dry prior to the determination of the turgid weight (TW). Dry weight of samples were determined after oven-drying at 80°C for 24 hrs. RWC was calculated according to Smart and Bingham (1974), using the following formula: RWC (%) = (FW–DW)/ (TW-DW) *100.

#### 2.2.3 Chlorophyll content

Chlorophyll content from Patumma leaf was extracted by dimethyl sulfoside (DMSO) and spectrophotometrically determined according to a modified Chappelle *et al.* (1992) method.

#### 2.2.4 Proline content

Proline content was determined according to the modified methods of Bates *et al.* (1973) and Ghoulam *et al.* (2002). Leaf material (300 mg FW) was homogenized in 5 ml of 40% methanol. One ml of the homogenate was mixed with 1 ml of acid-ninhydrin reagent and put in glass tubes. After 1 hr in boiling water, the tubes were placed in an ice bath to stop the reaction. Then 5 ml of toluene were 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.

#### 2.2.5 MDA content

MDA, an end product of lipid peroxidation, was determined according to a modified method of Heath and Packer (1968) and Velikova *et al.* (2000). The leaf sample was homogenized and reacted with trichloroacetic acid (TCA) and 2-thobarbituric acid (TBA). The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient of 155 mM $cm^{-1}$.

## 3. Results and Discussion

Water limitation for 0-40 days resulted in the reduction of water content in the soil. Less than 50% of the original soil moisture content was found 30 days after withholding water, which affected the relative water content (RWC) in the leaves. The RWC values decreased with increasing water stress (Table 1). The reduction of water content in Patumma leaves in both treatments indicated the

Table 1. Effects of water withholding (0-40 days) on soil water content, relative water content, plant height and plant dry weight in paclobutrazol treated Patumma cv. Chiang Mai Pink.

| | Paclobutrazol (ppm) | Water withholding | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 10 days | 20 days | 30 days | 40 days |
| Soil water content | 0 | 100 | 86.7±0.8 | 60.6±9.1 | 41.9±2.5 | 21.6±2.3 |
| (% of control) | 1500 | 100 | 87.2±1.3 | 64.8±3.8 | 43.0±3.2 | 26.3±2.8 |
| F-test | | | ns | ns | ns | ns |
| Relative water content | 0 | 100 | 96.9±2.1 | 92.2±1.7 | 81.9±1.4b | 73.3±0.6b |
| in leaves (% of control) | 1500 | 100 | 98.3±1.6 | 96.3±2.1 | 87.0±1.4a | 77.1±1.1a |
| F-test | | | ns | ns | * | * |
| Plant height | 0 | 100 | 118.8±6.4 | 130.9±12.1 | 156.9±5.5 | 158.3±3.2a |
| (% of control) | 1500 | 100 | 130.5±9.5 | 129.0±19.7 | 147.2±12.4 | 135.7±11.7b |
| F-test | | | ns | ns | ns | * |
| Plant dry weight | 0 | 100 | 141.4±24.5 | 122.1±3.1b | 143.6±19.1a | 121.8±2.9b |
| (% of control) | 1500 | 100 | 143.4±10.5 | 167.0±13.5a | 155.1±28.0a | 128.3±10.6a |
| F-test | | | ns | * | ns | * |

Data are the means of three replications ± standard deviation (SD) compared with the percent from the control (0 day). F-test, ns: not significant difference; *: significant difference at $p<0.05$. Mean sharing with the different letters in a column of the same parameter is significantly different by least significance difference (LSD) test.

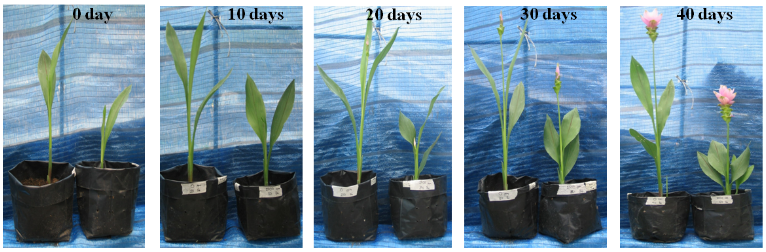


Figure 1. Patumma cv. Chiang Mai Pink plants treated with 0 (left) and 1,500 (right) ppm paclobutrazol after withholding water for 0–40 days.

reduction of water use efficiency in this plant. In fact, the 90-100% RWC causes closing of leaf stomata and a reduction in cellular development and growth. Levels of 80-90% RWC are correlated with changes in leaf tissue composition and some alterations in the rates of photosynthesis and respiration. The cessation of photosynthesis, increase in respiration, and proline and abscisic acid (ABA) accumulation occur when RWC is lower than 80% (Gonzalez and Gonzalez-Vilar, 2001). Water limitation has an impact on plant growth and development. The result of our study showed that 30-40 days after withholding water, the RWC in the leaves and dry weight per plant in the paclobutrazol treated plants were higher when compared to the control (Table 1). After 40 days of withholding water, the height of plant treated with 1,500 ppm paclobutrazol was 1.2 times lower than the control (Table 1 and Figure 1). Suppression of plant height by paclobutrazol occurs because the compound blocks three separate steps in the terpenoid pathway for the production of gibberellins (GA). One of the main roles of gibberellins in plants is the stimulation of cell elongation. When gibberellins production is inhibited, cell division still occurs, but the new cells do not elongate. This results in stems with the same numbers of leaves and internodes compressed into a shorter length (Fletcher *et al*., 2000; Taiz and Zeiger, 2006). Paclobutrazol can also be effective for obtaining sturdy plant and reducing plant height in several species without decreasing flowering quality (Klock, 1996; Mansuroglu *et al*., 2009; Currey and Lopez, 2010). Paclobutrazol did not effect on content of chlorophyll a and b in Patumma leafs (Table 2). This is in agreement with Wieland and Wample (1985) who reported that paclobutrazol did not affect chlorophyll content in 1-year-old 'dDlicious' apples. Other studies reported that this compound induces photosynthetic pigments with increasing leaf thickness due to an additional layer of palisade and spongy cells (Burrows *et al*., 1992; Jaleel *et al*., 2007).

After 20 days of withholding water, the treated plants

Table 2. Effect of water withholding (0-40 days) on physiological and biochemical changes in paclobutrazol treated Patumma cv. Chiang Mai Pink.

| | Paclobutrazol (ppm) | Water withholding | | | | |
|---|---|---|---|---|---|---|
| | | 0 day | 10 days | 20 days | 30 days | 40 days |
| Chlorophyll a content in leaves | 0 | 100 | 271.1±12.5a | 439.1±15.7a | 337.7±30.6 | 322.1±63.7 |
| (% of control) | 1500 | 100 | 201.6±34.8b | 329.7±30.1b | 316.0±78.1 | 271.7±49.6 |
| F-test | | * | * | * | ns | ns |
| Chlorophyll b content in leaves | 0 | 100 | 289.5±32.6 | 373.7±15.7 | 282.0±28.3 | 235.0±27.4 |
| (% of control) | 1500 | 100 | 244.7±39.5 | 359.0±49.4 | 349.8±53.4 | 246.8±40.4 |
| F-test | | ns | ns | ns | ns | ns |
| Proline content in leaves | 0 | 100 | 129.1±12.8 | 183.8±16.0a | 196.0±2.9a | 219.4±9.4a |
| (% of control) | 1500 | 100 | 115.6±12.2 | 155.1±11.1b | 169.7±18.5b | 184.3±10.8b |
| F-test | | ns | ns | * | * | * |
| MDA content in leaves | 0 | 100 | 127.1±11.8 | 174.8±17.0a | 212.3±35.1a | 341.3±147.6a |
| (% of control) | 1500 | 100 | 128.9±16.8 | 144.5±17.7b | 150.0±31.2b | 225.0±34.9b |
| F-test | | ns | ns | * | * | * |

Data are the means of three replications ± standard deviation (SD) compared with the percent from the control (0 day). F-test, ns: not significant difference; *: significant difference at $p<0.05$. Mean sharing with the different letters in a column of the same parameter is significantly different by least significance difference (LSD) test.

had 1.2 times lower levels of proline and MDA compared to the control plants (Table 2). Enhancement of proline with increasing time of water limitation occurred. Proline is an amino acid which is well known as an osmoticprotectant, which keeps the osmotic balance between plant cells and the outer environment when plants are subjected to water stress. It also functions as an antioxidant by keeping a suitable balance of $NAD^+$/NADH when there is water stress (Verma, 1999). The increase of proline in Patumma plants is one mechanism for water stress amelioration. Lower amounts of proline in the treated plants compared to the control imply that paclobutrazol treatment can induce water stress tolerance in the plants. Our results are similar to studies in which proline accumulation was lower in tolerant plants when compared to sensitive plants during salt and water stress (Jungklang *et al*., 2003; Turkan *et al*., 2005). Lipid peroxidation is a measure of the injury to cell membranes. Malondialdehyde, the cytotoxic product of this process, is considered a major TBA-reacting compound that indicates the magnitude of oxidation stress. In our experiment, the MDA content in the leaf of paclobutrazol-treated plants was lower than the control (Table 2). The reduction of lipid peroxidation in paclobutrazol-exposed Patumma plants was caused by paclobutrazol that increases the antioxidant potential system. As in other studies, water stress can be minimized by the application of paclobutrazol by increasing the antioxidant levels and activities of scavenging enzymes such as SOD, APX, and CAT in *Arachis hypogaea* L. (Sankar *et al*., 2007).

## 4. Conclusions

A concentration of 1,500 ppm paclobutrazol can be used to reduce plant height in Patumma cv. Chiang Mai Pink. Paclobutrazol can also increase relative water content, proline accumulation in leafs, dry weight of the plant, but can reduce MDA accumulation in the leaves. Paclobutrazol is optional to use for flowering pot plant production. This compound is recommended to commercially produce compact pot Patumma cv. Chiang Mai Pink plants.


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