Effect of Paclobutrazol on Growth and Development of *Curcuma alismatifolia* Gagnep. Grown Off-season

Kriangsuk Boontiang*, Benjawan Chutichudet and Prasit Chutichudet

Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44150

* Corresponding author. E-mail address: kriangsuk.b@msu.ac.th

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Abstract

A study on the effects of paclobutrazol on growth and development of *C. alismatifolia* Gagnep. grown in the off-season was conducted on an open field. A comparison was done between plants grown under sunlight plus 2 hrs. extra night light without paclobutrazol (Control 1) and plants grown under sunlight source without paclobutrazol (Control 2). This experiment was done by a completely randomized design with 6 treatments and 10 replications (10 plants/ replication). The results demonstrated that plants grown with paclobutrazol treatments were significantly affected in both height and peduncle length. The 600 mg/ L paclobutrazol treatment was non-significant difference in terms of leaf area, chlorophyll content in leaf, non-structural carbohydrate content in rhizome, pseudo-stem, peduncle and inflorescence, and anthocyanin content in coma bract compared to control 1. Earliest flowering parameters; the day to inflorescence apparent (day 47.2) and first flower opening (day 47.2) were found in plants grown under sunlight + 2 hours night-break without paclobutrazol. The most delay inflorescence apparent (day 62.9) and first flower opening (day 94.1) were recorded under sunlight source without paclobutrazol.

Keywords: off-season *Curcuma alismatiflolia* Gagnep., paclobutrazol, vegetative growth, physical development, chemical compound

Introduction

In the tropical climate zone, Thailand is the landmark area for production of cut flowers and other ornamental crops which are consumed on top 9th of market share in Aalsmeer Flower Auction, Netherland (Prabhakarn, 2013). *Curcuma alismatifolia* Gagnep. is one of the most popular varieties of cut flowers and potted plant exporting from Thailand. Soraya (2013) reported that its exportation value was up to 64 million US$ per year and it was ranked on seconded of market races comparing to orchids (Suthira, Kriangsuk, Prasit, & Pirot, 2012). Actually, this plant is initial grown and its inflorescence occurs in the rain season (from June-August), while upper parts of all green leave, pseudo-stem and inflorescence are turn downed which the total nonstructural carbohydrate are accumulated into the rhizome to be dominancy under the ground throughout winter and summer (dormant period). Therefore, off-season production of ornamental *Curcuma* is highly required due to the market needs for year-round decoration. Phatchari, Sawit, and Soraya (2010) reported the technique to breaking dormancy of the rhizome of *C. alismatifolia* Gagnep. to force off-season flowering for open field cultivation by using a night break treatment. Additionally, Sarawut et al. (2012) recommended that supplementing a night break treatment supported sufficient photosynthesis of off-season flowering of *C. alismatifolia* Gagnep. should be done under the greenhouse. However, construction of greenhouse and installation of illumination electrical system for a night break treatment in order to cultivate off-season flowering of *C. alismatifolia* Gagnep. are fully burdened cost to farmers.

The objective of this study was to determine the effect of paclobutrazol on physical growth and development, and chemical compound in various parts of *C. alismatifolia* Gagnep. grown off-season in the open field, based on low cost cultivation.

Materials and Methods

1. Plant material: Rhizomes of *Curcuma alismatifolia* Gagnep. cv. Kimono Pink (potted *Curcuma*) were selected as plant material in this study. The trial was initially performed by select the rhizome which was ranked size in 1.5-1.7 centimeters. Each rhizome was attached with 4 storage roots. All selected rhizomes were stored in a storage room, operating temperature at 15 ºC and relative humidity (RH) at 70 % ambient conditions for 4 mounts to delay the germinate activity. Immediately after storage, the rhizomes were soaked into tap water for 3 days (daily water changes) and were placed in a planting bed, filled with sand and rich husk ash at a rate of 1:1 in order to stimulate germination. Germinated rhizomes were transplanted (one rhizome per one pot) in size of 6 x 11 inches plastic bag filled with soil, sand and rich husk ash at a rate of 1:1:1. Off-season cultivation of *C. alismatifolia* Gagnep. cv. Kimono Pink was carried out from September to December, 2015 at an open field of Department of Agricultural Technology, Faculty of Technology, Mahasarakham University.

2. Experimental design: A Completely Randomized Design was arranged in 6 treatments and 10 replications as follows:

Treatment 1: Plant materials were grown under sunlight plus 2 hrs. of extra night light treatment (23.00 pm.-01.00 am.) without paclobutrazol (control 1). Artificial light source was an illuminated electricity system supplementing with 100 W incandescent lamps. Each lamp was set up at 1.5 meters height above the ground (4 lamps per one square meter). Additional night break was continuously illuminated at week 3 after transplanted until true flowers were in full bloom.

Treatment 2: Plant materials were grown under sunlight source without paclobutrazol (control 2).

Treatment 3: Plant materials were grown under sunlight source with 200 ml/L 15% paclobutrazol treatment.

Treatment 4: Plant materials were grown under sunlight source with 400 ml/L 15% paclobutrazol treatment.

Treatment 5: Plant materials were grown under sunlight source with 600 ml/L 15% paclobutrazol treatment.

Treatment 6: Plant materials were grown under sunlight source with 800 ml/L 15% paclobutrazol treatment.

Different concentrations of paclobutrazol were poured one time into planting media at week 3 after transplanted as a volume of 200 mL per one plant.

3. Determination of vegetative growth and physical development: Recorded data were taken at week 6, 8 and 10 after transplanted as follows: shoot length, number of new shoot, leaf number per one plant, total leave area using a CI-203 Handheld Leaf Area Meter, date of inflorescence occurs, date of true flower blooming, peduncle length, inflorescence length, inflorescence diameter, number of fertile bract, and number of coma bract.

4. Determination of chemical compound: Recorded data were taken on day 90 after transplanted as follows;

4.1 Chlorophyll content in leaf: Fresh leaves were ground and treated with 10 mL of 50 % methanol in a glass tube, kept in dark condition for 24 hrs. Liquid chlorophyll was filtered using a Whatman's cellulose

filter paper grade 5. 2 mL of purified chlorophyll were harvested by 12,000 revolutions per minute centrifugation at 4 ºC for 5 minutes. Purified chlorophyll was analyzed at 665 and 625 nanometers absorption inspector photometer using UV-visible spectrophotometer series V-325-XS. Chlorophyll content was determined using method described by Madison and Anderson (1963).

4. 2 Total non-structural carbohydrate content in rhizome, pseudo-stem, leaf, peduncle and inflorescence: Various parts of plant material were washed with distilled water, immediately air dried and placed at 60 ºC in a hot air oven for 72 hours. Each dried sample was ground in a laboratory mortar grinder. 0.2 gram of ground sample was poured to a 250 mL Erlenmeyer flask, mixed with 40 mL of 0.2 N H_2SO_4 and 60 mL of distilled water, controlled pH at 7 with buffer solution. Liquid sample was filtered using a Whatman's cellulose filter paper grade 5. 1 mL of filtered sample was poured into a 15 mL test tube with 1 mL of Nelson's alkaline copper reagent. A test tube was wrapped in aluminum foil and kept in boiled water for 20 minutes. All samples were analyzed at 540 nanometers absorption inspector photometer using UV-visible spectrophotometer series V-325-XS. Non-structural carbohydrate content was determined comparing to D-glucose standard curve.

4.3 Total anthocyanin content in coma bract: Total anthocyanin content was extracted according to the method of Giusti and Wrolstad (2005). 2 grams (fresh weight) of coma bract of plant material were collected for anthocyanin extraction with 20 mL of 50% methanol using homogenizer. A sample was filtered using a Whatman's cellulose filter paper grade 5. 0.4 mL of liquid anthocyanin was poured into a 15 mL test tube with 0.4 mL of 0.025 M buffer of Potassium chloride (pH 1). Purified anthocyanin was harvested by 5,000 revolutions per minute centrifugation for 10 minutes and was analyzed at 515 and 700 nanometers absorption inspector photometer using UV-visible spectrophotometer series V-325-XS. Total anthocyanin content was determined comparing to total monomeric anthocyanin pigment.

5. Statistical analysis: The collected data were statistically analyzed using the Statistix Computer Grogram, Version 8. Analysis of variance and statistically significant differences were compared using LSD ($P<0.05$).

Results

1. Vegetative growth: *C. alismatifolia* Gagnep. cv. Kimono Pink grown under sunlight source with 200, 400, 600 and 800 ml/ L paclobutrazol treatments were no significant differences occurred in plant height (23.0, 22.3, 22.3, and 22.0 centimeters) at week 6 after transplanted. Their shoot lengths were significant differences compared to the control 1 and 2 (24.7 and 24.4 centimeters). On week 8 and 10, the results showed plants grown under sunlight source with 600 and 800 ml/ L paclobutrazol treatments were no significant differences occurred in plant height (23.6 and 23.8 centimeters, and 23.3 and 23.4 centimeters). Both of paclobutrazol application methods (600 and 800 ml/ L paclobutrazol treatments) were significant difference compared to plants grown under sunlight source with 200 and 400 ml/L paclobutrazol treatments (25.4, 25.9 centimeters, and 24.8 and 25.2 centimeters). Therefore, the highest plant showed on week 8 and 10 was *C. alismatifolia* Gagnep. cv. Kimono Pink grown under sunlight source plus 2 hrs. night break treatment without paclobutrazol (27.4 and 30.0 centimeters). There were no significant differences occurred in number of new shoot and number of leaf. *C. alismatifolia* Gagnep. cv. Kimono Pink grown under sunlight source with 400 and 600 ml/L paclobutrazol treatments, at week 6, 8 and 10 were no significant differences

occurred in total area of leaf (9.3 and 9.5, 9.6 and 9.8, and 9.9 and 10.0 cm^2) compared to the control 1 (10.3, 10.6 and 10.6 cm^2). However, there were significant differences, compared to the other treatments (Table 1).

Table 1 Effect of paclobutrazol on vegetative growth of *Curcuma alismatifolia* Gagnep. cv. Kimono Pink grown off-season

Treatment	Vegetative growth (week after transplantation)											
	Plant height (cm)			No. of new shoot (shoot)			No. of leaf (leaf)			Total area of leaf (cm^2)		
	6	8	10	6	8	10	6	8	10	6	8	10
Sunlight source + 2 hrs. night break without paclobutrazol (control 1)	24.7a	27.4a	30.0a	1.0	1.1	1.7	2.3	3.1	3.2	10.3a	10.6a	10.6a
Sunlight source without paclobutrazol (control 2)	24.4a	26.6a	27.2b	1.0	1.1	1.3	1.8	2.7	3.1	7.6b	7.7b	7.8b
Sunlight source + 200 mg/L paclobutrazol	23.0b	25.4b	25.9c	1.0	1.1	1.4	2.1	2.9	3.0	8.7b	8.9b	8.9b
Sunlight source + 400 mg/L paclobutrazol	22.3b	24.8b	25.2c	1.0	1.1	1.6	2.0	2.9	3.0	9.3a	9.5a	9.6a
Sunlight source + 600 mg/L paclobutrazol	22.3b	23.6c	23.8d	1.1	1.2	1.6	1.9	2.9	3.4	9.8a	9.9a	10.0a
Sunlight source + 800 mg/L paclobutrazol	22.0b	23.3c	23.4d	1.0	1.1	1.2	1.9	2.6	3.1	8.2b	8.6b	7.9b
F-test	**	**	**	ns	ns	ns	ns	ns	ns	**	**	**
CV %	10.7	10.3	10.1	6.59	8.6	10.0	8.1	7.1	8.2	10.1	11.7	12.4

ns = non-significant difference, ** highly significant difference

Means value in the same column with different letters were significantly different ($p < 0.05$)

2. Physical development: The results indicated that *C. alismatifolia* Gagnep. cv. Kimono Pink grown under sunlight source plus 2 hrs. night break treatment, without paclobutrazol was significant difference occurred in date of inflorescence appearance and date of true flower blooming (day 47.2 and 70.9 after transplanted). Date of inflorescence appearance in plants grown under sunlight source with 400, 600 and 800 ml/ L paclobutrazol treatments were occurred on day 54.0, 53.4 and 53.9, and date of true flower blooming were on day 82.7, 80.6 and 81.5. The most delay inflorescence appearance and true flower blooming was found in plants grown under sunlight source without paclobutrazol (day 62.9 and 91.1). There was no significant difference occurred in length of peduncle in plants grown under sunlight source with 400 and 600 ml/ L paclobutrazol treatments (22.8 and 21.9 centimeters). However, length of peduncle found in both treatments were higher than plants grown under sunlight source with 800 ml/L paclobutrazol (20.0 centimeters), but their length were shorter than those of control 1 and 2 (24.3 and 24.2 centimeters). Inflorescence size on plants grown under sunlight source plus 2 hrs. night break treatment, without paclobutrazol was significant difference occurred in its length and diameter (15.5 and 7.7 centimeters) as well as numbers of fertile bract and coma bract (11.5 and 9.6 bracts). Minimal size of inflorescence was found on plants grown under sunlight source without paclobutrazol (13.8 centimeters of length and 5.2 centimeters of diameter, and 8.3 of fertile bract and 7.4 of coma bract) (Table 2).

Table 2 Effect of paclobutrazol on physical development of *Curcuma alismatifolia* Gagnep. cv. Kimono Pink grown off-season

Treatment	Physical development						
	Date of inflorescence appearance (day)	Date of flower blooming (day)	Length of peduncle (cm)	Length of inflorescence (cm)	Inflorescence diameter (cm)	No. of fertile bract (bract)	No. of coma bract (bract)
Sunlight source + 2 hr. night break without paclobutrazol (control 1)	47.2d	70.9d	24.3a	15.5a	7.7a	11.5a	9.6a
Sunlight source without paclobutrazol (control 2)	62.9a	94.1a	24.2a	13.8b	5.2b	8.3b	7.4b
Sunlight source + 200 mg/L paclobutrazol	58.8b	88.0b	23.4a	14.5ab	7.2a	11.1a	9.2a
Sunlight source + 400 mg/L paclobutrazol	54.0c	82.7c	22.8b	14.6ab	7.4a	11.1a	9.1a
Sunlight source + 600 mg/L paclobutrazol	53.4c	80.6c	21.9b	15.2a	7.5a	10.7a	8.9a
Sunlight source + 800 mg/L paclobutrazol	53.9c	81.5c	20.0c	14.2ab	7.2a	10.6a	9.8a
F-test	**	**	**	**	**	**	**
CV %	8.7	11.0	10.8	7.4	10.7	11.8	9.4

ns = non-significant difference, ** highly significant difference

Means value in the same column with different letters were significantly different ($p < 0.05$)

3. Chemical compound: Maximum Chlorophyll content in leaf of *C. alismatifolia* Gagnep. cv. Kimono Pink was found in plant grown under sunlight source plus 2 hrs. night break treatment, without paclobutrazol (70.4 mg g^{-1} dry weight). This result had no significant difference compared to chlorophyll content in leaf found in that plant grown under sunlight source with 600 ml/L paclobutrazol treatment (70.2 mg g^{-1} dry weight). Significant difference in total nonstructural carbohydrate content in rhizome, pseudo-stem, leaf, peduncle and inflorescence were occurred in *C. alismatifolia* Gagnep. cv. Kimono Pink grown under sunlight source plus 2 hrs. night break treatment, without paclobutrazol (44.0, 44.6, 22.8 and 22.9 mg g^{-1} dry weight) as well as a treatment of plants grown under sunlight source with 600 ml/L paclobutrazol (43.8, 44.2, 22.7 and 22.4 mg g^{-1} dry weight). Additionally, significant difference in total anthocyanin content in coma bract was found in plants grown under sunlight source plus 2 hrs. night break treatment, without paclobutrazol (16.3 mg/100 g fresh weight) as well as a treatment of plant grown under sunlight source with 600 ml/L paclobutrazol (16.3 mg/100 g fresh weight). However, this result was significant difference compared to other treatments (Table 3).

Table 3 Effect of paclobutrazol on chemical compound in various parts of *Curcuma alismatifolia* Gagnep. cv. Kimono Pink grown off-season

Treatment	Chlorophyll content in leaf (mg g^{-1} dry weight)	Total nonstructural carbohydrate content (mg g^{-1} dry weight)					Anthocyanin content in coma bract (mg/100 g fresh weight)
		Rhizome	Pseudo - stem	Leaf	Peduncle	Inflorescence	
Sunlight source + 2 hr. night break without paclobutrazol (control 1)	70.4a	44.0a	44.6a	11.9	22.8a	22.9a	16.3a
Sunlight source without paclobutrazol (control 2)	65.1c	39.9c	31.6c	11.6	18.4c	18.4c	13.3c
Sunlight source + 200 mg/L paclobutrazol	67.8cd	42.1b	42.1c	11.7	19.0b	20.0b	14.3b
Sunlight source + 400 mg/L paclobutrazol	68.6b	42.2b	43.4b	11.3	20.5a	20.4b	14.8b
Sunlight source + 600 mg/L paclobutrazol	70.2a	43.8a	44.2a	12.1	22.7a	22.4a	16.3a
Sunlight source + 800 mg/L paclobutrazol	68.3b	42.5b	42.9b	12.0	20.3b	20.5b	15.0b
F-test	**	**	**	ns	**	**	**
CV %	8.7	4.9	6.9	10.53	6.8	7.6	9.1

ns = non-significant difference, ** highly significant difference

Means value in the same column with different letters were significantly different ($p < 0.05$)

Discussion

Cultivation of off-season flowering in ornamental crops is an alternative approach to meet the market demand and resolve the problem of risk significant losses, which affects the farmer profits. Such farming productivity leads to significant in higher earning; event thought this concept necessitates technologies and intensive practices to support the quantitative growth and quality yield as well as seasonality products. In the present study, effects of paclobutrazol on growth and development of *C. alismatifolia* Gagnep. was compared to plants grown under sunlight plus 2 hrs. extra night light without paclobutrazol (fully burdened cost to farmers) (Control 1) and plants grown under sunlight source without paclobutrazol (Control 2). Cultivation period was carried out in off-season (September to December) in the open field. The results indicated that significant differences in plant height and length of peduncle were found in the control 1 and 2, compared to paclobutrazol treatments. Our results are agree with the findings of Koutroubas and Damalas (2015) who reported similar effects of paclobutrazol in terms of reduction in plant height and root length in fielded Sunflower due to the concentration of paclobutrazol applications. While the new shoot, number of leaf and total area of leaf of plants grown under sunlight source with 600 ml/L paclobutrazol treatment were no significant differences compared to plants grown under sunlight plus 2 hrs. extra night light without paclobutrazol. Therefore, those results were significant differences compared to other treatments. Burrows, Boag, and Stewart (1992) cited that proper concentration of paclobutrazol application could increase thicker

leaves and green chlorophyll supplement in leaves compared to untreated plants. In creased leaf thickness and green chlorophyll supplement were partly due to additional layer of palisade mesophyll. This finding show total chlorophyll content in leaf that refers to photosynthetic pigments in plants grown under sunlight source with 600 ml/L paclobutrazol treatment can gender equality be achieved with lower cost strategy. Additionally, plants grown under sunlight source with 600 ml/L paclobutrazol treatment showed no significant differences occurred in total nonstructural carbohydrate content in rhizome, pseudo-stem, peduncle and inflorescence, and anthocyanin content in coma bract, compared to extra night light illumination. However, these finding results were significant differences to plants grown under sunlight source without paclobutrazol. Our results are consistent with the studies of Upreti, Shivu Prasad, Reddy, and Rajeshwara (2014) who cited that paclobutrazol induced carbohydrates and sugars accumulation in plants, especially soluble sugars like sucrose, glucose and fructose in apical buds during floral initiation in mango. These results are in agreement with the finding of Jungklang and Saengil (2012) who reported that paclobutrazol can be used to increase the tolerance in *C. alismatifolia* Gagnep. to differential effects of climate change, due to this plant is an facultative short day ornamental that can be grown under ambient conditions in the winter, but it took long time to flower (Baloch, Munir, & Abid, 2013).

Conclusion

A concentration of 600 ml/L paclobutrazol treatment can be used to induce chlorophyll pigments, total nonstructural carbohydrates accumulation, and anthocyanin content in coma bract in *C. alismatifolia* Gagnep. grown off-season as well as supplementary lighting. Paclobutrazol application is recommended for low cost cultivation of *C. alismatifolia* Gagnep. grown off-season in the open field, without construction of protected condition and installation of illumination electrical system for a night break treatment. Cultivation of off-season of *C. alismatifolia* Gagnep. plants to meet the market demand for either year-round decoration or season's greeting should be done in further study.

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