

The Role of Paclobutrazol on Production Strategy of *Curcuma alismatifolia* Gagnep. for Off-Season Marketing

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Abstract

From September 2017 through February 2018, this open field research was conducted with 6 random treatments and 10 replications. Plants grown under sunlight source with paclobutrazol (PBZ) treatments were compared to those grown under sunlight plus 2 h night break (without PBZ) (control 1) and those grown under sunlight source (without PBZ) (control 2). The results showed that PBZ treatments lowered both height and peduncle length substantially. In comparison to control 1, the 600 mg/L PBZ treatment led in non-significant changes in physical growth and anthocyanin content in coma bract and fertile bract. Plants grown under sunshine + 2 h night break without PBZ had the shortest number of days to inflorescence appearance (day 46) and first flower opening (day 72). Plants cultivated under control 2 conditions had the largest delay in inflorescence emergence (day 61) and first flower opening (day 92). These findings will aid in the low-cost cultivation of *C. alismatifolia* cultivated in the open field during the off-season.

Keywords: *Curcuma alismatifolia* Gagnep., Low-cost strategy, Off-season marketing, Open field cultivation, Paclobutrazol

Introduction

Thailand is a major producer of flowers and other ornamental crops in the tropical climatic zone [1,2], with the Aalsmeer Flower Auction in the Netherlands [3] ranking Thailand ninth in market share. Thai floriculture's production area was projected to be 3,100 hectares in 2014, with an export value of \$38.1 million US dollars. The market value in 2014 was 13.7 % significantly higher than previously reported [4]. *Curcuma alismatifolia* Gagnep. called "Patumma" or "Siam Tulip" is one of the most popular varieties of flowers exported from Thailand. Its market value was ranked 2nd after orchids [5]. The 3 main market demands are that this plant can be used as cut flowers, potted plants, and its rhizomes can be grown for landscape gardening [6]. The normal season for cultivation of this plant involves initial growth in the early rainy season with its inflorescence occurring through the rainy season (June-August). Total non-structural carbohydrate (TNC) from green leaves, pseudo-stem, and inflorescence in the upper part of the plant is translocated down to the rhizome which remains dormant throughout the long period of winter until summer (September-April). Therefore, off-season production of *C. alismatifolia* is required due to the market demand for year-round flowers. A technique for breaking dormancy of the rhizome was reported [7], with a night break treatment to improve photosynthesis in *C. alismatifolia* grown off-season under the greenhouse [8]. However, construction of greenhouses and installation of electrical illumination systems for a night break treatment to cultivate off-season flowering of *C. alismatifolia* is cost burdens to the farmers. A low-cost productive technique for off-season cultivation of *C. alismatifolia* in the open field demonstrated that physical growth and development of plants grown under sunlight with paclobutrazol (PBZ) were comparable to those were grown under sunlight plus 2 h night break without PBZ [9]. Therefore, they were significantly different compared to those grown under sunlight source only.

In light of the aforementioned considerations, the goal of this study was to assess the impact of PBZ on *C. alismatifolia*'s off-season production strategy in order to satisfy the high occasional demand of flower markets.

Materials and methods

Plant materials

Rhizomes of *C. alismatifolia* Gagnep. cv. Kimono Pink (potted cultivar) were selected for off-season cultivation. Preparation of plant materials was performed following a described method [9]. Rhizomes used in this work were 1.5 - 1.7 cm in diameter with 4 storage roots. All selected rhizomes were stored under ambient conditions at 15 °C and relative humidity (RH) at 70 % for 4 months to delay the germination activity. Immediately after storage, the rhizomes were soaked in tap water for 3 days (daily water changes) and were placed in a planting bed, filled with sand and rich husk ash at a ratio of 1:1 to stimulate germination. Germinating rhizomes were transplanted (1 rhizome per 1 pot) into 6×11 inches of plastic bags filled with soil, sand, and rich husk ash at a ratio of 1:1:1. Off-season cultivation of *C. alismatifolia* was carried out in the open field from September 2017 to February 2018 at a research station in the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Thailand.

Experimental design

A completely randomized design was arranged in 6 treatments and 10 replications (10 plants/replication) as follows:

Treatment 1. Plants were grown under natural sunlight with a 2 h night break (11:00 pm-01:00 am) without PBZ (control 1). The electrical illumination system was providing supplementary light from 100 W incandescent lamps. Each lamp was set up at 1.5 m height above the ground (4 lamps per square meter). Extra light treatment was continuously used from week 3 after transplanting until true flowers were in full bloom.

Treatment 2. Plants were grown under sunlight sources without PBZ (control 2).

Treatment 3. Plants were grown under sunlight sources with 200 mL/L 15 % PBZ (w/v in tap water) application.

Treatment 4. Plants were grown under sunlight sources with 400 mL/L 15 % PBZ application.

Treatment 5. Plants were grown under sunlight sources with 600 mL/L 15 % PBZ application.

Treatment 6. Plants were grown under sunlight sources with 800 mL/L 15 % PBZ application.

A single application of 200 mL of different concentrations of PBZ (0, 200, 400, 600 and 800 mL/L) was poured into planting media at week 3 after transplanting.

Determination of vegetative growth and physical development

The following data were recorded at weeks 6, 8 and 10 after transplanting; shoot length, number of new shoots, leaf number per plant, total leaf area (measured using a CI-203 Handheld Leaf Area Meter), date of inflorescence appearance, date of true flower blooming, peduncle length, inflorescence length, inflorescence diameter, number of fertile bracts, and number of coma bracts.

Determination of biochemical substances

The following data were recorded on day 90 after transplanting:

1. Chlorophyll content in the leaf: Liquid chlorophyll from a fresh leaf was extracted with 50 % ethanol. The absorption at 665 and 625 nm of purified chlorophyll was determined spectrophotometrically. Chlorophyll content was determined using the chlorophyll index method [10].

2. Total non-structural carbohydrate (TNC) content in rhizome, pseudo-stem, leaf, peduncle, and inflorescence was determined using the method of extraction of total available carbohydrates [11]. A dried sample from each part of the plant material was ground and digested with sulfuric acid (H₂SO₄). The digested sample was then reacted with Nelson's alkaline copper reagent. In all samples, absorption at 540 nm was measured spectrophotometrically. TNC content was determined by comparison with a D-glucose standard curve.

3. Total anthocyanin content in coma bract was extracted and measured according to [12,13] and absorption was analyzed spectrophotometrically at 515 and 700 nm. Total anthocyanin content was determined comparing to total monomeric anthocyanin pigment.

Statistical analysis

Data were statistically analyzed using the Statistics Computer Program, version 8. Analysis of variance and statistically significant differences were compared using Least-Significant Difference (LSD) ($p \leq 0.05$).

Results and discussions

For *C. alismatifolia* grown under sunlight source with 200, 400, 600 and 800 mL/L PBZ treatments, there were no significant differences in plant height at week 6 after transplanting. Their shoot lengths were significantly different compared to controls 1 and 2 (Table 1). At weeks 8 and 10, there were no significant differences in the heights of plants grown under sunlight source with 600 and 800 mL/L PBZ treatments. The highest plants (26.57 and 28.89 cm above the surface of planting media) at weeks 8 and 10 were grown under sunlight source plus 2 h night break treatment, without PBZ. There were no significant differences in the number of new shoots and number of leaves. Although there were no significant differences in the total area of leaf compared to the control 1 in plants grown under sunlight source with 400 and 600 mL/L PBZ treatment at weeks 6, 8 and 10; there were significant differences compared to other treatments.

For plants grown under control 1, there were significant differences in physical development. Significant differences occurred in the earliest flowering parameters, date of inflorescence appearance and date of true flower blooming (day 46 and 72 after transplanting). Inflorescence appearance in plants grown under sunlight source with 400, 600 and 800 mL/L PBZ treatment occurred on the same date as day 54 after transplant. The longest delay in the date of inflorescence appearance and true flower blooming was found in plants grown under control 2. Non-significant differences occurred in the length of peduncle in plants grown under sunlight source with 400 and 600 mL/L PBZ. Therefore, the length of peduncle found in both treatments was shorter than in controls 1 and 2 but was longer than plants grown under sunlight source with 800 mL/L PBZ. Inflorescence size on plants grown as control 1 was significantly different in its length and diameter (14.32 and 7.62 cm) as well as numbers of fertile bracts and coma bracts (10.50 and 8.57 bracts), compared to other treatments. The minimal size of inflorescence was found on plants grown under control 2 conditions (Table 2).

Maximum chlorophyll content in leaves was found in plants grown under control 1 conditions (68.34 mg.g⁻¹ dry weight). This result had no significant difference compared to the chlorophyll content in leaves found in those plants grown under sunlight source with 600 mL/L PBZ treatment (67.92 mg.g⁻¹ dry weight). A significant difference in TNC content in the rhizome, pseudo-stem, peduncle, and inflorescence occurred in plants grown in control 1 as well as in plants grown under sunlight source with 600 mL/L PBZ. However, this result was significantly different compared to other treatments (Table 3).

Table 1 Roles of PBZ on vegetative growth of *C. alismatifolia* Gagnep. cv. Kimono Pink grown off-season.

Treatment	Vegetative growth (week after planting)											
	Plant height (cm)			No. of shoot (shoot)			No. of leaf (leaf)			Total leaves area (cm ²)		
	6	8	10	6	8	10	6	8	10	6	8	10
Sunlight + 2 h night break, without PBZ (control 1)	24.36a	26.57a	28.89a	1.02	1.11	1.74	2.31	3.05	3.20	10.18a	10.57a	10.64a
Sunlight without PBZ (control 2)	24.29a	26.35a	26.90b	1.02	1.11	1.25	1.80	2.68	3.08	7.68b	7.52b	7.63b
Sunlight + 200 mg/L PBZ	22.32b	25.38b	25.81c	1.00	1.07	1.35	2.07	2.92	3.06	8.77b	8.74b	7.92b
Sunlight + 400 mg/L PBZ	22.26b	24.32b	25.76c	1.00	1.14	1.64	2.00	2.87	3.00	9.36a	9.55a	9.64a
Sunlight + 600 mg/L PBZ	22.24b	22.57c	24.08d	1.05	1.21	1.57	1.93	2.85	3.35	9.83a	9.92a	9.89a
Sunlight + 800 mg/L PBZ	21.87b	22.23c	23.74d	1.00	1.07	1.21	1.85	2.64	3.14	8.21b	8.62b	7.85b
F-test	**	**	**	ns	ns	ns	ns	ns	ns	**	**	**
CV (%)	11.84	10.33	10.05	6.59	8.6	9.69	8.09	7.12	8.15	10.05	11.74	12.44

ns = non-significant difference, ** significant difference

Mean values in the same column with different letters were significantly different ($p \leq 0.05$)

Table 2 Roles of PBZ on inflorescence qualities of *C. alismatifolia* Gagnep. cv. Kimono Pink grown off-season.

Treatment	Inflorescence qualities						
	Day to inflorescence appearance (day)	Day to 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 + 2 h night break, without PBZ (control 1)	46d	72d	25.49a	14.32a	7.62a	10.50a	8.57a
Sunlight without PBZ (control 2)	61a	92a	24.95a	13.96b	4.83b	8.34b	6.73b
Sunlight + 200 mg/L PBZ	58b	88b	23.82ab	13.66b	5.96ab	9.81a	8.21a
Sunlight + 400 mg/L PBZ	54c	84c	22.67b	13.41b	6.34a	10.07a	8.07a
Sunlight + 600 mg/L PBZ	54c	83c	21.95b	13.22b	7.52a	10.27a	8.50a
Sunlight + 800 mg/L PBZ	54c	82c	19.34c	11.07c	7.20a	10.64a	8.43a
F-test	**	**	**	**	**	**	**
CV (%)	8.72	9.87	10.83	8.46	10.02	11.84	9.14

ns = non-significant difference, ** significant difference

Mean values in the same column with different letters were significantly different ($p \leq 0.05$)

Table 3 Roles of PBZ on biochemical substances in plant component of *C. 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 + 2 h night break, without PBZ (control 1)	68.34a	43.45a	42.36a	10.89	20.28a	20.39a	15.80a
Sunlight without PBZ (control 2)	63.51c	38.29c	38.61c	10.06	16.95c	16.44c	12.23c
Sunlight + 200 mg/L PBZ	64.28c	40.25b	40.10b	10.27	17.82b	18.00b	12.35b
Sunlight + 400 mg/L PBZ	66.36b	41.12b	41.84ab	10.38	18.18b	18.41b	13.08b
Sunlight + 600 mg/L PBZ	67.92a	42.58ab	42.29a	10.64	20.16a	20.25a	14.77a
Sunlight + 800 mg/L PBZ	66.03b	41.05b	42.13a	10.21	18.32b	18.34b	13.65b
F-test	**	**	**	ns	**	**	**
CV (%)	8.5	4.7	6.3	10.2	7.6	6.8	8.7

ns = non-significant difference, ** significant difference

Mean values in the same column with different letters were significantly different ($p \leq 0.05$)

Off-season production of floricultural crops is an alternative approach to meeting high demand and high market value. This strategy leads to higher earnings, which affects the farmers' profits and lessens the risk of significant losses. This study evaluated the roles of PBZ on quantitative growth and quality yield of *C. alismatifolia* in an open field experiment as a strategy for off-season cultivation. An off-season planting date was chosen in early December to promote plant growth and yield, and to meet a high season demand of flowers or for occasional celebration of valentine's greeting in February. The effects of PBZ on the growth and development of *C. alismatifolia* were evaluated by comparing plants grown under sunlight plus 2 h night break to support good quality of inflorescence in off-season planting [14] without PBZ (methodology that incurs the greatest cost to farmers), and plants grown under sunlight source without PBZ (regular cultivation). The results indicated that there were significant differences in plant height and the length of peduncle in controls 1 and 2 compared to PBZ treatments. The new shoot, number of leaves, total area, and chlorophyll content in leaves of plants grown under sunlight source with 600 mL/L PBZ treatment were not significantly different compared to plants grown under control 1. Therefore, those results were significantly different compared to other treatments. Our results agreed with the finding that in field-cultivated *Helianthus annuus* L. [15] and *Dendranthema x grandiflora* cv. Lilian Hoek [16], the effects of higher concentrations of PBZ include reduction in plant height and root length. On the other hand, our results showed that proper concentration of PBZ application can increase the chlorophyll content in leaves, compared to untreated plants. Higher chlorophyll content was partly due to an additional layer of palisade mesophyll [17,18]. Total chlorophyll content in green leaves shown in plants grown under sunlight source with 600 mL/L PBZ treatment was reflecting sufficient

photosynthesis [19-21]. Additionally, plants grown under sunlight source with 600 mL/L PBZ treatment showed no significant differences in TNC content in the rhizome, pseudo-stem, peduncle, and inflorescence, or of anthocyanin content in the coma bract, compared to control 1. However, there were significant differences compared to plants grown in control 2.

The flowering parameters: Number of dates of inflorescence appearance and first flower blooming in plants grown in sunlight source with PBZ treatment were delayed by 1 week compared to the plants grown in control 1. Therefore, the same parameters recorded in plants grown in sunlight source with PBZ treatment occurred 1 week earlier than those plants grown in control 2 (**Table 2**). Additionally, the vegetative growth and inflorescence qualities of plants grown in this study were comparable to the response of PBZ treatment for off-season cultivation [9], in which the planting date was set in late September to produce inflorescences during the high season and for occasional celebrations such as Christmas and New Year in late December to early January. Our results indicated that PBZ is a novel plant growth regulator acting as a stress protectant in *C. alismatifolia* Gagnep. grown under differential ambient conditions in the winter through early summer [22-26]. The present findings will be beneficial to the low-cost production strategy of *C. alismatifolia* for the off-season to meet the high occasional demand of flower markets.

Conclusions

PBZ application is recommended for strategic planning of low-cost cultivation of *C. alismatifolia* Gagnep. grown off-season in the open field to meet high market demand for high-value year-round flowers, without the construction of protected conditions or installation of electrical illumination systems for a night break treatment. A concentration of 600 mL/L PBZ treatment can be used to induce chlorophyll pigment, TNC accumulation, and anthocyanin content in coma bract in *C. alismatifolia* Gagnep. grown off-season.

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