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Changes in Endogenous Abscisic Acid, *trans*-Zeatin Riboside, Indole-3-Acetic Acid Levels and the Photosynthetic Rate during the Growth Cycle of *Curcuma alismatifolia* Gagnep. in Different Production Seasons

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Summary

Off-season (OS) cropping of *Curcuma alismatifolia* Gagnep. in Thailand results in a decline in plant growth and flowers quality compared to regular season (RS) cropping. This may be due to the distinct climatic conditions in OS such as short day length, low temperature, water stress etc. which induce changes in some endogenous hormones and alter plant growth and development. The experiments in two cropping season (RS and OS) were conducted to examine the changes to endogenous hormones i.e. abscisic acid, *trans*-zeatin riboside and indole-3-acetic acid, during the growth cycle. The result indicated that OS cropping brought about a decrease in plant height and flower quality but significantly stimulated the formation of rhizomes compare with RS cropping. The levels of ABA in leaves, old rhizomes and old storage roots were higher and consistently increased during off-season cropping. The maximum concentration of ABA was up to 2.2 μg gDW^{-1} in leaves, 1.4 μg gDW^{-1} in old rhizomes, 1.0 μg gDW^{-1} in old storage roots, 2.3 μg gDW^{-1} in inflorescence, 0.9 μg gDW^{-1} in new rhizomes, and 0.9 μg gDW^{-1} in new storage roots. The levels of ABA in RS cropping were relatively constant throughout growth stages and mostly less than 0.1 μg gDW^{-1}. The levels of *t*-ZR were also slightly higher in leaves, old rhizomes, old storage roots, inflorescence and new rhizomes in OS than RS cropping. The increase of *t*-ZR in new rhizomes may cause the larger number of new rhizomes in OS cropping (4.8 per plant). The concentration of IAA in leaf exudates was similar between the two growth seasons except in the final stage. Therefore, it could be concluded that OS conditions induced a decrease in the photosynthetic rate, and increases in ABA and *t*-ZR concentrations in various organs at different growth stages, leading to depressed shoot growth and an increase in rhizome numbers.

Key words. ABA – *Curcuma alismatifolia* – IAA – off-season cropping – *t*-ZR

Introduction

Curcuma alismatifolia Gagnep. is a mono-cotyledonous perennial, a member of the ginger family (*Zingiberaceae*) originating from tropical and subtropical areas of northern Thailand and Cambodia (Apavatjrut et al. 1999). It has become popular as a cut flower, potted flower and garden plant, with colourful, long-lasting and repeated inflorescences. More than 400,000 plants with a value of 1.3 million Euros are sold each year by commercial auction in The Netherland (Flower Council of Holland 2009). The cropping of *Curcuma* in regular season (RS) production in Thailand usually starts from April to May by planting a rhizome with several storage roots. Shoots sprout within 4–8 weeks after planting. The flowering occurs from July to August, during the rainy season and the shoots become senescent from prior to a period of dormancy in the cool-dry season (winter) during November to December (Ruamrungsri et al. 2001). The rhizomes with storage roots are harvested and stored during the dormancy period until the next planting season. The off-season (OS) production of *Curcuma* is used by growers to increase their income, because the price for RS crops is usually the cheapest of the year. Although *Curcuma* plants can be cultivated in wide areas including tropical and temperate regions, OS cropping may reduce the quality of flowers or rhizomes. It is postulated that changes in environmental conditions such as temperature, humidity, photoperiod and light intensity in different seasons affect the rhythm of the growth cycle and plant growth. The growth patterns and quality of crops may be influenced by modifications of endogenous developmen-

tal programs through changes in the levels of plant hormones and metabolites (Thomas and Vince-Prue 1997) and photosynthesis (Riberiro et al. 2009). Endogenous phytohormones generally play an importance role in regulating plant development and metabolism. Several phytohormones, such as ABA, CKs, IAA, GAs and ethylene, have also been implicated in the tuberization of potatoes (Rodriguez-Falcon et al. 2006) and stem swelling in tulips (Nishijima et al. 2005). Rietveld et al. (2000) reported that auxin caused a rapid elongation of the stalk in tulips under low temperature. In *C. roscoeana* and *C. alismatifolia*, the application of a plant growth retardant reduced plant height and flower stalk length (Thohirah et al. 2005). Khuankaew et al. (2009b) reported that a GA_3 solution increased shoot height in *Curcuma* plants. The present study aimed to compared the levels of endogenous hormones in various parts of *curcuma* during cultivation between off-season cropping and regular season cropping, in relation to plant growth patterns and to clarify the possible role of ABA, *t*-ZR and IAA in the regulation of plant growth and development.

Materials and Methods

Abbreviations

ABA: abscisic acid, CK: cytokinin, DHZR: dihydro-zeatin riboside, DW: dry weight, FW: fresh weight, GA_3: gibberellic acid, IAA: indole-3-acetic acid, OS: off-season, PVP: polyvinylpolypyrrolidone, RS: regular season, *t*-ZR: *trans*-zeatin riboside.

Plant materials and experimental conditions

Stubbed rhizomes of *C. alismatifolia* 'Chiang Mai Pink' 1.8–2.5 cm in diameter and with 4 storage roots were purchased from a commercial nursery (Ubonrat Garden, Chiang Mai, 2006). They were stored at 15 °C and 70 % RH until used (4 months for OS). One rhizome was planted in a 6 x 12 inch plastic bag containing a mixture of soil: rice husk: rice husk charcoal (ratio 1:1:1) and 7.5 g of 16-16-16 (N-P_2O_5-K_2O) fertilizer was applied twice a month from after the first leaves fully expanded until the flowering stage. Then 7.5 g of 13-13-21 (N-P_2O_5-K_2O) fertilizer was supplied twice a month from the flowering stage until the rhizome-forming stage. Each pot was irrigated daily with 450 ml of tap water throughout the growth periods. Plants were grown in two seasonal cultivations; i.e. 1) regular season (RS) crops in which plants were cultivated from 22 July 2006 and rhizomes harvested on 18 January 2007 and 2) off-season (OS) crops in which plants were cultivated on 22 November 2006 and rhizomes harvested on 22 May 2007 (details see in Table 1). To examine the changes in endogenous hormonal levels (ABA, *t*-ZR and IAA), plant materials were analyzed at 6 stages of growth; i.e. 1) sprouting, 2) 5–10 cm shoot length, 3) fully expanded leaves in the first shoot, 4) the 1st floret opening, 5) the last flower senescence and 6) dormancy (aboveground parts dried). For RS cropping, the dates were as follows; 1) Aug 11, 2) Aug 18, 3) Aug 30, 4) Sep 22, 5) Nov 3 and 6) Jan 18. For OS cropping, the dates were 1) Dec 20, 2) Dec 27, 3) Jan10, 4) Feb 15, 5) Mar 22 and 6) May 22. Growth and development were measured in terms of plant height, leaf fresh weight (FW), rhizome FW, storage roots FW, flower quality and rhizome yield.

Hormone analysis

ABA analysis. The extraction, purification and quantification of ABA were performed as described by Walker-Simmons et al. (2000). Plants were separated into leaves, rhizomes, storage roots, inflorescence, new rhizomes and

Table 1. Environmental conditions during the experimental period, considering the variation in macroclimate between regular season (RS) production and off-season (OS) production. Macroclimate refers to meteorological data recorded with an automatic Multiple Cropping Center: MCC, Faculty of Agriculture, Chiang Mai University weather station: Air temperature (T air max, T air min and T air mean), relative humidity (RH max, RH min and RH mean), rain fall (Rf) and sunshine duration.

	Month	T air			RH			Rain fall (mm)	Sunshine duration (h)
		max (°C)	min (°C)	mean (°C)	max (%)	min (%)	mean (%)		
RS	Jul	32.9	24.2	27.9	86.5	67.3	77.3	119.0	13.0
	Aug	33.2	23.7	27.7	90.9	64.8	79.0	205.0	12.1
	Sep	33.3	23.9	27.9	89.0	66.2	78.3	156.0	12.7
	Oct	32.4	22.1	26.5	92.2	59.5	75.9	93.0	11.6
	avg.	32.9	23.5	27.5	89.7	64.5	77.6	143.1	12.4
OS	Nov	29.8	18.5	23.3	92.3	54.4	74.1	59.4	11.1
	Dec	30.9	15.2	21.9	91.9	43.8	69.0	0.0	10.9
	Jan	32.7	15.7	23.0	80.2	30.4	56.7	0.0	11.4
	Feb	30.9	13.8	21.1	89.8	36.8	64.0	0.0	11.0
	avg.	31.1	15.8	22.3	88.6	41.4	65.9	14.86	11.1

avg. = average

new storage roots, washed with water and weighed (FW). Then parts were frozen in liquid nitrogen. They were freeze-dried, weighed, ground into a fine powder and stored at −20 °C prior to analysis. Next, 100 mg dry weight (DW) of each sample was extracted with 10 mL of extracting solvent (100 % methanol with 500 mg L^{-1} citric acid monohydrate and 100 mg L^{-1} BHT: butyl hydroxyl toluene) overnight in the dark at 4 °C. The crude extract supernatant was adjusted to 70 % methanol by diluting 200 µL of sample with 800 µL of 62.5 % methanol. Each diluted extract was filtered through a Sep-Pak® C18 cartridge (Water, Milford, Mass U.S.A.) that had been washed twice with 2 mL of 100 % and 1 mL of 70 % methanol, respectively. The filtrate was evaporated overnight using a speed-vacuum concentrator, dissolved in absolute methanol, and refrigerated at 4 °C until it was assayed. An internal ABA standard was occasionally added to parallel samples to re-calculate % recovery after ELISA. The rate of recovery of ABA was 62 %.

CK (*t*-ZR) analysis. Samples were prepared as in the ABA analysis except that the extraction process was modified as follows (Potchanasin et al. 2009). A total of 100 mg DW of sample powder was extracted with 50 mL of cold 80 % methanol overnight at 4 °C. The extract was filtered through a G-4 glass-inter-filter (max. pore size 10–16 µm). The filtrate was evaporated dry at 40 °C, and the residue was re-dissolved in 12 mL of 0.01 M ammonium acetate (pH 7.5) and frozen at −20 °C overnight. After thawing, the extracts were centrifuged at 22,000 rpm for 25 min at 4 °C. The supernatant was purified by passing it through a pre-conditioned column filled with about 10 mL of PVP followed by a Sep-Pak cartridge. The CK was eluted directly from the removed Sep-Pak with 4 mL of 30 % methanol in 0.01 M acetic acid, evaporated overnight, re-dissolved in 0.2 mL of methanol and refrigerated at 4 °C until it was assayed for CK. The rate of recovery of CK ranged between 77 and 80 %.

The measurements of ABA and CK (*t*-ZR) were carried out by competitive ELISA, using Phytodetek test kits (monoclonal antibodies) (Agdia, Inc. Elkhart, IN.). The *t*-ZR values were designated as *t*-ZR equivalents.

Leaf diffusible IAA. Leaf exudates were collected from the cut basal end of leaves for the quantification of IAA, as described by Gruber and Bangerth (1990) with some modifications. All the leaves were cut off from the shoots and the cut basal end of leaves were placed into falcon test tubes (one plant per tube), which contained 5.0 mL of 0.10 M phosphate buffer at pH 6.20. The tubes were placed in sealed ice boxes and incubated in the dark at 22 °C, and 100 % RH for 20 h. Then, the leaves were removed and the tubes were kept at −20 °C until used for the IAA analysis.

IAA analysis. The extraction and purification of IAA was performed as described by Koshita et al. (1999). Briefly, all (approximately 5 ml) of the thawed samples were adjusted to pH 2.5 by adding 4 M acetic acid, then extracted in diethyl ether, and subjected to solvent partitioning. The diethyl ether fraction was collected and evaporated dry with N_2 gas. The dried sample was dissolved in 200 µL of 50 % methanol containing 0.10 M acetic acid. The samples were further purified by high performance liquid chromatography (HPLC): column SC-150 (150 x 4.6 mm), PRONTOSIL 120-5-C18, H 5.0 µm, BISCHOFF Chromatography; flow rate 1.0 mL min^{-1}; mobile phase was solvent A; 0.1 M acetic acid in water, solvent B; 0.1 M acetic acid in methanol; gradient profile; 0–12 min 45–85 % of B, 12–15 min 85–100 % of B, 15–18 min 100–45 % of B and 18–22 min 45–45 % of B. Under these conditions, the retention time of IAA was 7.10 min as quantified from the calculated peak area related to a standard curve. The limit of detection and limit of quantification was 1 ng mL^{-1} and 6.25 ng mL^{-1}, respectively. The recovery rate ranged from 67 to 70 %.

Photosynthesis physiological variables

Measurements of leaf gas exchange in fully expanded leaves were carried out between 10.00 and 12.00 AM at the flowering stage (12 WAP) for both RS and OS production. The evaluation was performed on a clear day without clouds. Physiological variables, i.e. the photosynthetic rate (A), stomatal conductance (gs), stomatal resistance (rs), transpiration rate (E), Photosynthetical active radiation: P.A.R. on leaf surface (Q leaf) and leaf surface temperature (T leaf), were analyzed at the flowering stage by using a leaf gas chamber analyzer (Model LCA4, ADC Hoddessdon, Herts, England). Chlorophyll fluorescence (Fv/Fm) was measured at the flowering stage by using an exciting chlorophyll fluorescence meter (Handy-PEA Type, Hansatech Model, UK).

Statistical analysis

Data were analyzed for statistical significance using Statistic 8 analytical software (SXW Tallahassee, FL.). Student's *t*-test was used to determine significant differences in growth, photosynthesis (with 10 replicates per treatment), and hormones (3 replicates, 4 plants per replicate).

Results

Comparison of climatic conditions between RS and OS cultivation periods

The macroclimates are shown in Table 1, and were significantly different between the RS and OS seasons. Air temperature (T air) and rain fall (Rf) were lowest in December to February during OS cultivation. The minimum temperature (T min) in OS was 13.8 °C in February, about 8 °C lower than that in RS, 22.1 °C in October. Average day length during RS was 12.4 h, significantly longer than during the OS period, 11.1 h (Table 1).

Comparisons of photosynthesis between RS and OS cultivations

At the flowering stage, the net photosynthetic rate (*A*) was higher in RS plants, 8.19±0.89 µmol m^{-2} s^{-1}, than in OS plants, 5.78±0.20 µmol m^{-2} s^{-1} (Table 2). Both stomatal conductance (*gs*) and the transpiration rate (*E*) were significantly higher in OS. P. A. R. on leaf surface (*Q leaf*) was significantly lower in OS plants, 570±19 µmol m^{-2} s^{-1}, than RS plants, 1130±50 µmol m^{-2} s^{-1}. Leaf surface temperature (*T leaf*) and *Fv/Fm* values were also significantly lower in OS leaves than RS leaves. A negative correlation

Table 2. Physiological variables related to the photosynthesis of *Curcuma* plants 'Chiang Mai Pink' as affected by RS and OS: net photosynthetic rate (A), stomatal conductance (gs), stomatal resistance (rs), transpiration rate (E), P.A.R. on leaf surface (Q leaf), leaf surface temperature (T leaf) and Chlorophyll fluorescence (Fv/Fm) at 10.00–12.00 am.

Physiological variation[1)]		Season		t-test at P< 0.05
		Regular season	Off-season	
A	(μmol m^{-2} s^{-1})	8.19 ± 0.89	5.78 ± 0.20	*
gs	(mol m^{-2} s^{-1})	0.06 ± 0.00	0.08 ± 0.00	*
rs	(mol m^{-2} s^{-1})	17.70 ± 1.28	12.20 ± 0.52	*
E	(mol m^{-2} s^{-1})	1.92 ± 0.08	2.42 ± 0.06	*
Q leaf	(μmol m^{-2} s^{-1})	1130.00 ± 47.30	570.00 ± 18.80	*
T leaf	(°C)	39.30 ± 0.14	35.50 ± 0.06	*
Fv/Fm		0.80 ± 0.00	0.75 ± 0.01	*

[1)]Data are means (n = 10, one plant per replicate) ± SE.

Table 3. Correlation between physiological variables of *Curcuma alismatifolia* 'Chiang Mai Pink' related to photosynthesis: net photosynthetic rate (A), stomatal conductance (gs), stomatal resistance (rs), transpiration rate (E), P.A.R. on leaf surface (Q leaf) and leaf surface temperature (T leaf). The linear regression and correlation coefficient R are presented at P>0.05. Data were calculated from means at the flowering stage.

Parameter (X axis *vs.* Y axis)	Regression equation	R	Correlation test at P<0.05	n
A *vs.* E	y = −0.03x + 2.39	−0.22	*	20
A *vs.* Q leaf	y = 62.2x + 415.5	0.47	*	20
A *vs.* T leaf	y = 0.41x + 34.53	0.49	*	20
A *vs.* gs	y = −0.00x + 0.08	−0.22	ns	20
A *vs.* rs	y = 0.48x + 11.57	0.27	ns	20
Q leaf *vs.* T leaf	y = 0.01x + 32.21	0.95	*	20

vs. = *versus*

was found between *A* and *E* (R = −0.22) while a positive correlation was found between *A* and *Q* leaf (R = 0.47), *A* and *T leaf* (R = 0.49), *Q leaf* and *T leaf* (R = 0.95). There was no correlation between *A* and *gs* or *rs* (Table 3).

Plant growth and development

OS plants sprouted about 3 weeks later than RS plants. The height of RS plants reached a maximum at 58.5±1.3 cm 40 days after planting (DAP) and remained constant thereafter, but that of OS plants reached only a maximum at 27.3±1.0 cm 86 DAP (Fig. 1A and Fig. 2A). The rate of increase was 1.60 cm d^{-1} in RS and 0.36 cm d^{-1} in OS. The first floret opening was at 60 DAP when the plant produced 3–4 fully expanded leaves in RS, but 90 DAP in OS. Flower quality (spike length, stalk length, number of green bracts and number of pink bracts) was better in RS than OS at stage 4 (Table 4 and Fig. 1A). During this period, the fresh weight of RS leaves increased at 0.43 g $d^{-1}$and reached a maximum at 39.5±1.2 g, then declined. The fresh weight of OS leaves increased continuously at a rate of 0.31 g $d^{-1}$from stages 1 to 5 and a reached maximum of 30.9±2.4 g at 120 DAP (stage 5), then the leaves became senescent at stage 6, approximately 180 DAP (Fig. 2B). A sharp decline in the FW of old rhizomes was observed in OS plants, from 4.36±0.2 g at stage 3 (50 DAP) to 3.3±0.1 g at stage 6 (183 DAP), a rate of −0.01 g d^{-1} (Fig. 2C). In addition, the FW of old storage roots in OS plants dropped from19.6±0.6 g at stage 4 (86 DAP) to 1.9±0.6 g at stage 5 (122 DAP), a rate of −0.5 g d^{-1} (Fig. 2D). During flowering (stage 4), the formation of new rhizomes was initiated in both RS and OS plants by swelling of the basal pseudo stem. OS plants showed a significantly higher number of new rhizomes per plant (2.1±0.2 in RS and 4.8±0.4 in OS) and FW of new rhizomes (11.6±0.8 g in RS and 17.3±1.5 g in OS), although the diameter did not change (2.22±0.0 cm in RS and 2.1±0.1 cm in OS), compared with RS plants (Table 4). The rate of production was 0.04 g d^{-1}in RS and 0.04 g d^{-1}in OS (Fig. 2E).

New storage roots had formed at 90 DAP in OS plants versus 110 DAP in RS plants. Their fresh weights reached a steady level at 120 DAP in OS plants with a growth rate of 0.4 g d^{-1}, while in RS plants, they increased from 120 to 180 DAP at 0.3 g d^{-1} (Fig. 2F). The number and fresh weight of new storage roots increased in OS, but less than in RS, at harvest (stage 6) (Table 4 and Fig. 1B).

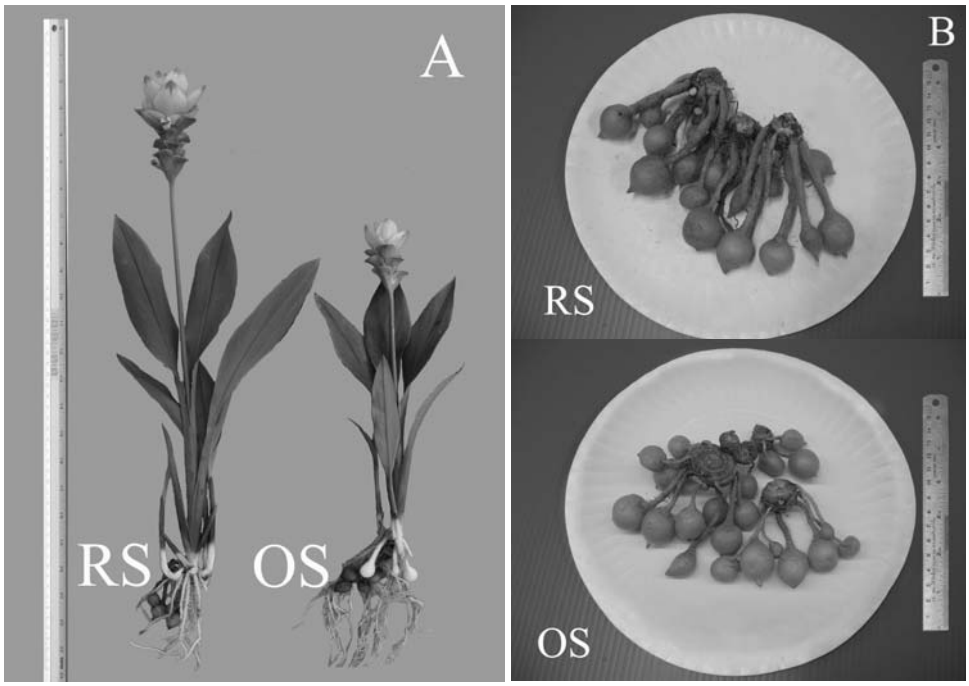


Fig. 1. Plant height and flower quality (A) and rhizome yields (B) of *Curcuma alismatifolia* 'Chiang Mai Pink' grown in the regular season (RS) and off-season (OS) at the flowering and dormancy stage, respectively.

Comparison of the levels of endogenous plant hormones between RS and OS plants

ABA concentrations. The ABA concentrations in leaves, old rhizomes and old storage roots were higher at stage 3 to 6 in OS plants than RS plants (Fig. 3A–C). The ABA concentration in RS leaves reached a maximum of 1.1 μg g^{-1} DWat stage 3, and declined thereafter. In contrast, those in OS leaves peaked of 2.2±80 μg g^{-1} DW at stage 4, and sharply declined thereafter (Fig. 3A). ABA concentrations in RS rhizomes were relatively constant at about 0.5 to 0.6 μg g^{-1} DW through all stages. Those in old rhizomes of OS plants were initially low at sprouting; however, they gradually increased and were higher than in RS plants from stage 3 until stage 6. The maximum was 1.3 μg g^{-1} DW (Fig. 3B). The ABA concentration in old storage roots in RS plants were about 0.5 to 0.6 μg g^{-1} DW, except for stage 3. The concentrations in OS plants continuously increased after stage 4 and reached a maximum of 0.1 μg g^{-1} DW at stage 6 (Fig. 3C). A decline in

Table 4. Flower quality and rhizome yields in *Cucuma alismatifolia* Gagnep. grown in different seasons at the flowering and dormancy stage, respectively.

Yields	Production period		t-test at P<0.05
	Regular season	Off-season	
Flower quality			
Spike length (cm)	16.1 ± 0.48	10.7 ± 0.33	*
Stalk length (cm)	52.1 ± 1.81	28.5 ± 0.69	*
No. of green bracts	9.40 ± 0.40	6.80 ± 0.37	*
No. of pink bracts	13.0 ± 0.32	9.60 ± 0.75	*
Rhizomes yields			
New rhizomes			
Total numbers	2.06 ± 0.17	4.75 ± 0.40	*
Fresh weight (g)	11.6 ± 0.80	17.3 ± 1.50	*
Diameter (cm)	2.22 ± 0.04	2.10 ± 0.07	ns
New storage roots			
Total numbers	9.88 ± 0.86	13.9 ± 1.13	*
Fresh weight (g)	55.2 ± 4.77	72.0 ± 7.06	ns
Diameter (cm)	2.32 ± 0.06	2.10 ± 0.05	*

Data are means (n = 10, one plant per replicate) ± SE.

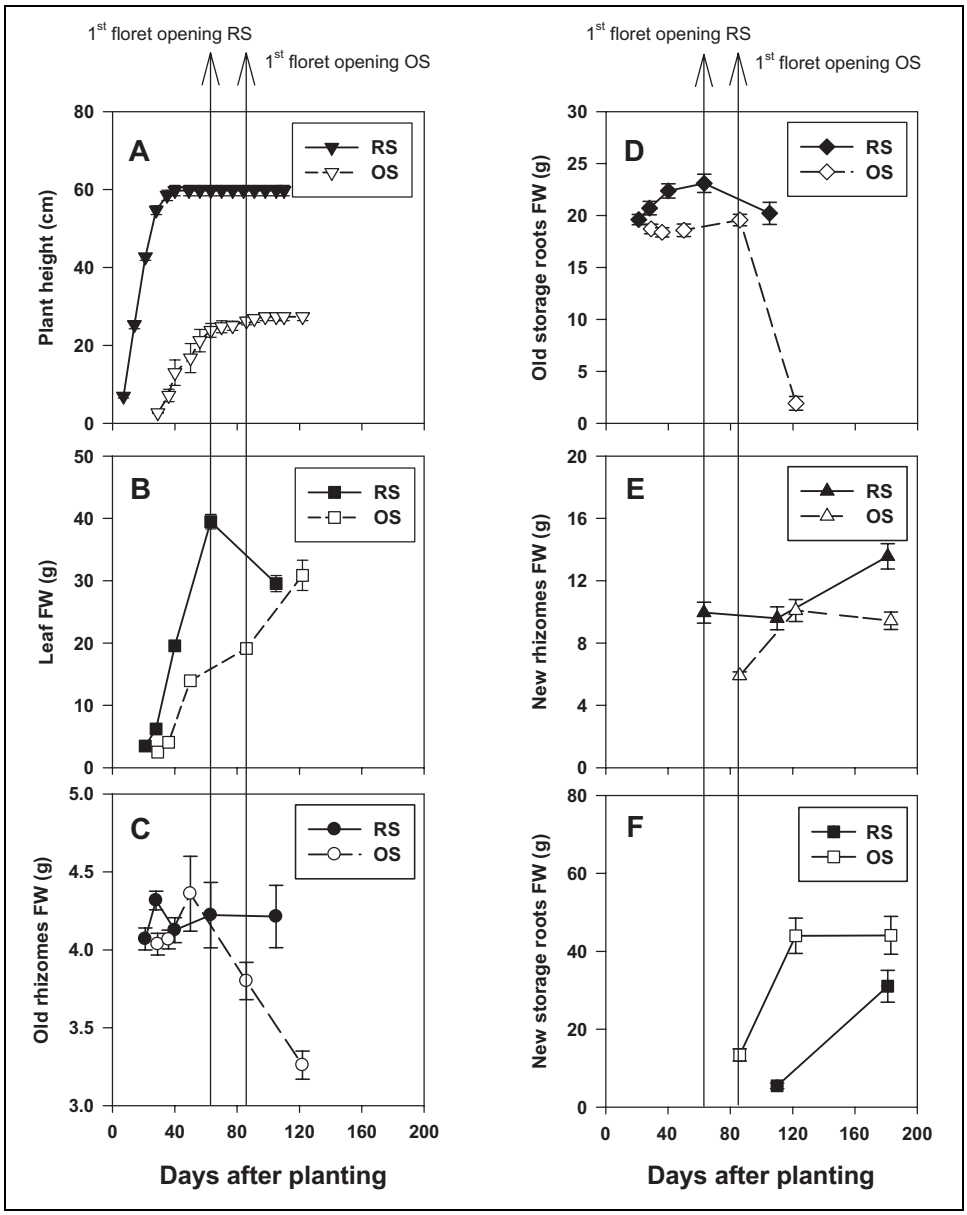


Fig. 2. Changes in plant height (A), leaf FW (B), old rhizomes FW (C), old storage roots FW (D), new rhizomes FW (E) and new storage roots FW (F) during the growth cycle of *Curcuma alismatifolia* 'Chiang Mai Pink' in the regular season (RS, solid symbols) and off-season (OS, open symbols). Days after planting (DAP) indicate the six growth stages for RS: stage 1 (21 DAP), 2 (28 DAP), 3 (40 DAP), 4 (63 DAP), 5 (105 DAP) and 6 (181 DAP), and for OS: stage 1 (29 DAP), 2 (36 DAP), 3 (50 DAP), 4 (86 DAP), 5 (122 DAP) and 6 (183 DAP). Data are means (n = 3, 4 plants per replicate) ± SE. Arrows indicate the 1st floret opening stage.

the ABA concentration in RS inflorescence was observed at stage 5. On the other hand, a strong increase from 1.2 to 2.2 μg g^{-1} DW was found in OS inflorescence at stage 5 (Fig. 3D). The ABA concentrations in new rhizomes and new storage roots were higher in OS plants than RS plants (Fig. 3E, F).

t-ZR concentrations. *t*-ZR concentration were significantly higher in OS leaves than RS leaves at stages 1, 3, 4 and 5 (Fig. 4A). The same finding was also made in old rhizomes (Fig. 4B). *t*-ZR levels in old storage roots were significantly higher in OS than RS at stages 1, 2 and 5 (Fig. 4C). From stage 4 to 6, the *t*-ZR levels in inflorescence were also higher in OS than RS plants (Fig. 4D). A decline in *t*-ZR concentrations in new rhizomes from stage 4 to 6 occurred in RS plants. The opposite was found in OS new rhizomes. A maximum was reached at 72.7±6.6 ng g^{-1} DW (Fig. 4E). From stage 5 to 6, *t*-ZR levels in new storage roots of OS were constant at around 30–40 ng g^{-1} DW in OS plants while they slightly declined in RS plants (Fig. 4F).

Leaf diffusible IAA concentrations. The IAA concentration in leaf exudates did not differ significantly between the cropping seasons at stages 1, 3 and 4. However, at stage 5, it was higher in RS than OS plants (Fig. 5). The maximum concentration of IAA in RS plants was 659±24 pg $leaf^{-1}$ 20 h^{-1}, while it was only 60±5.4 pg $leaf^{-1}$ 20 h^{-1} in OS plants (Fig. 5).

Correlation between concentration of endogenous hormones and plant growth

During the growth cycle (stages 1 to 6), negative correlations were found between plant height and ABA levels in leaves (R = −0.79) and old rhizomes (R = −0.94). Leaf FW also correlated with ABA levels in leaves (R = −0.81) and rhizomes (R = −0.67). Plant height correlated with the

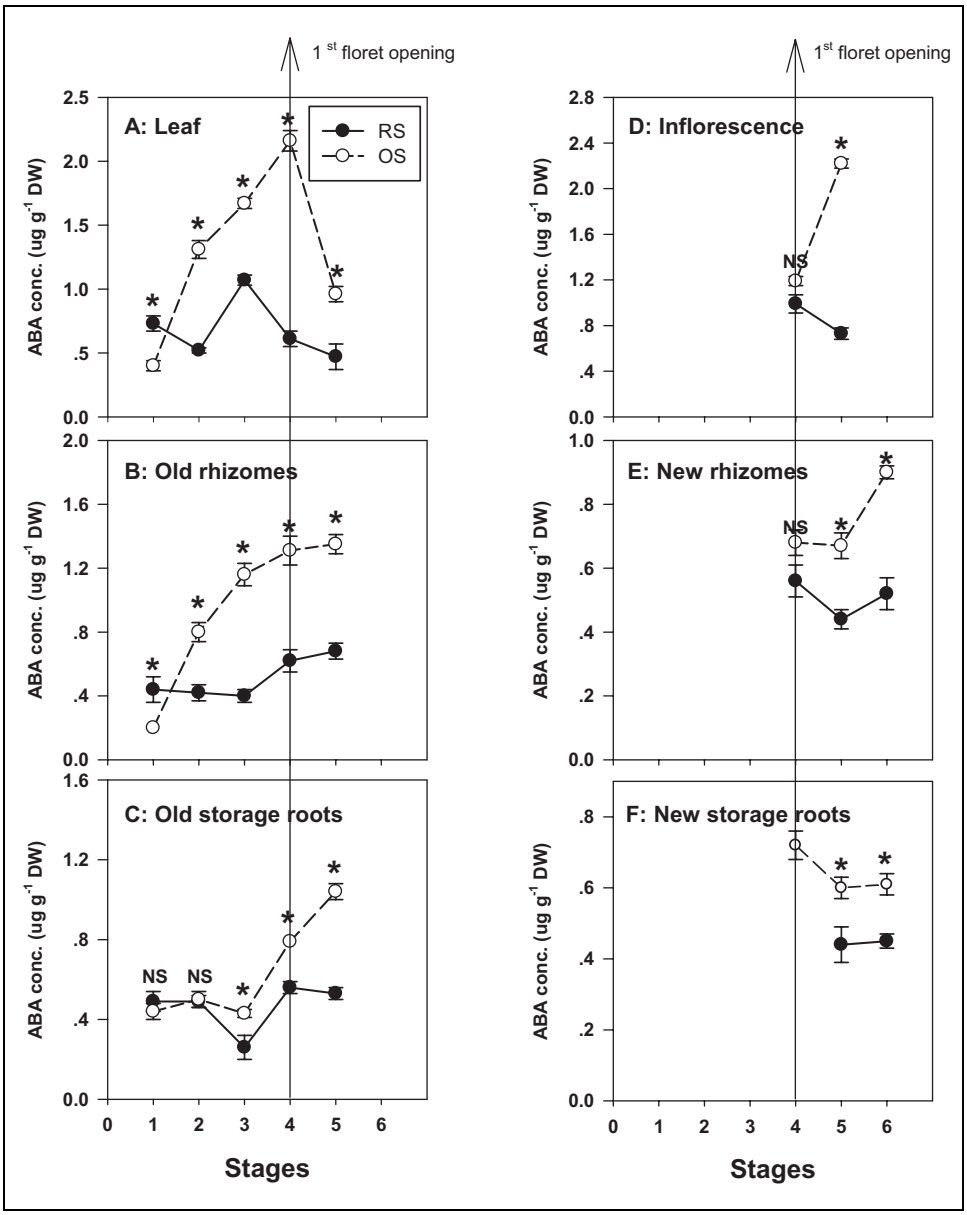


Fig. 3. Changes in endogenous ABA levels (μg g^{-1} DW) in leaves (A), rhizomes (B), storage roots (C), inflorescence (D), new rhizomes (E) and new storage roots (F) during the growth cycle of *Curcuma alismatifolia* 'Chiang Mai Pink' in the regular season (RS, solid circles) and off-season (OS, open circles). Data are means ± SE. (n=3). *, Significantly different at P<0.05 at the same stage. NS; not significantly different. Arrows indicate the 1st floret opening stage.

levels of ABA in leaves (R = −0.79). A positive correlation was found between endogenous ABA levels in leaves (R = 0.90) and in old rhizomes (Table 5). Positive correlations were also found between *t*-ZR in old rhizomes and number of new rhizomes (R = 0.66), and *t*-ZR in leaves and *t*-ZR in old storage roots (R = 0.81) (Table 5).

Discussion

The lower Rf and average % RH (Table 1) and higher gs, lower rs and higher E (Table 2) induced stomatal opening in OS plants. The lower value of A under OS conditions was probably caused by the lower Q leaf and lower average T air. A similar response was found when low temperature treatment decreased photosynthetic activity in pea cultivars (Georgieva and Lichtenthaler 2006). The lower photosynthetic rate in OS apparently caused the reductions in plant height and flower quality (Table 4, Fig. 1A and 2A), because the daily gains in carbon derived from photosynthesis mostly accumulate in leaves as structural carbons and soluble sugars or starch, for using in plant growth (Khuankaew et al.2009a). However, the longer storage of rhizomes before planting was expected to have reduced food reserves and altered plant growth in OS. Interestingly rhizome yields were significantly higher in OS than RS, although shoot growth was strongly depressed, indicating the competition between aboveground (leaves and inflorescences) and underground organs. The new rhizomes and storage roots become the major sink in OS, since the developing of storage organs could act as a major sink for assimilates (Xu et al. 2008). The accumulation of ABA is generally considered to be a physiological response to adverse conditions; such as drought, cold, heat, or salinity (Zeevaart 1999). Under these conditions, levels rise and suppress the growth process (Kondrat' Eva et al. 2009). Although the roots of *Curcuma* plants were sufficiently watered in both seasons, the FW of leaves was

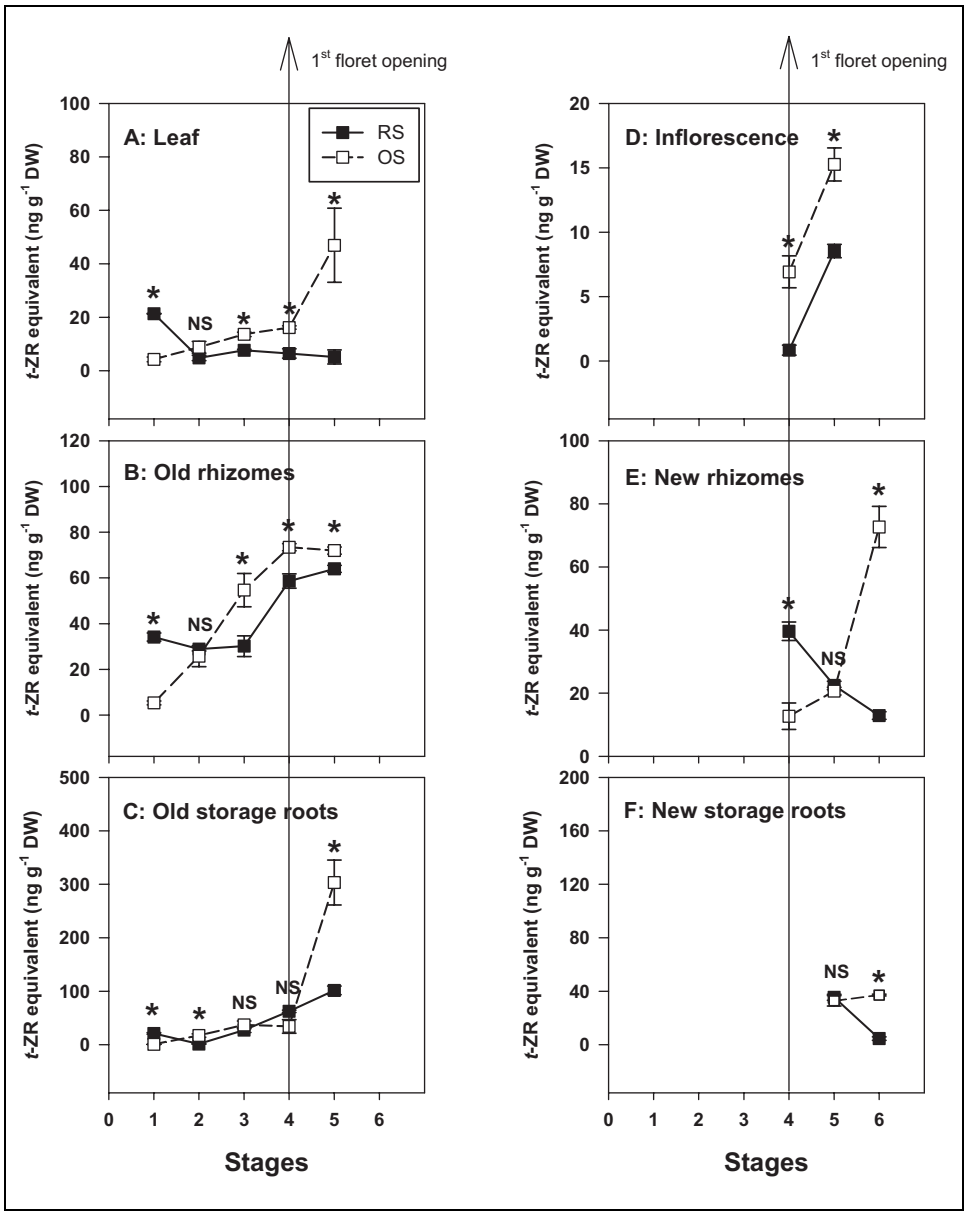


Fig. 4. Changes in endogenous *t*-ZR equivalent (ng g^{-1} DW) in leaves (A), rhizomes (B), storage roots (C), inflorescence (D), new rhizomes (E) and new storage roots (F) during the growth cycle of *Curcuma alismatifolia* 'Chiang Mai Pink' in the regular season (RS, solid squares) and off-season (OS, open squares). Data are means (n = 3, 4 plants per replicate) ± SE. *, Significantly different at P<0.05 at the same stage. NS; not significantly different. Arrows indicate the 1st floret opening stage.

significantly lower in OS than RS, indicating a loss of water in OS plants which may have respond by raising levels of ABA. The significant correlations between plant height and leaf fresh weight and endogenous ABA levels in leaves suggested that the decrease of shoot growth in OS was not only caused by a low photosynthetic rate but due to the influence of a high level of ABA in shoot organs. Exogenous ABA has been report to inhibit shoot growth and stem elongation in tulips (Saniewski et al. 1990), and shoot elongation and floral development in Dutch iris (Doss et al. 1983). In our experiment, the OS plants produced more and heavier rhizomes than RS plants. The ABA levels in leaves and old rhizomes positively related to the number of new rhizomes and number of new storage roots, although it negatively correlated with leaf FW (Table 4). A low temperature and a high ABA content are considered favorable conditions for the formation of bulbs in plants (Okubo 2000). In addition, ABA was related to thickening and dry matter accumulation in tuberous roots of sweet potatoes (Nakatani and Komeichi 1991). Therefore, the significant increase in rhizome yields in OS plants might be caused by low temperature and the higher levels of ABA in rhizomes. In addition, the depression of shoot growth may reflect the sink-source relationship, with more photoassimilates transported to the underground parts under severe depression of shoot growth. The reduction of ABA concentrations in OS rhizomes at sprouting (stage 1) might be due to the longer storage at 15 °C before planting the same as in lily (Xu et al. 2006) and tulip (Geng et al. 2007). The ABA levels in old rhizomes were similar to those in leaves in both RS and OS plants (Fig. 3). The increase of ABA and *t*-ZR in old rhizomes from stage 2 to 6 may indicate that old rhizomes play the role in storing reserves of endogenous hormones in the growth cycle.

CKs are considered to be mainly synthesized in root tips and exported to other organs by the transpiration stream (Shashidhar et al. 1996; Hare et al. 1997). The

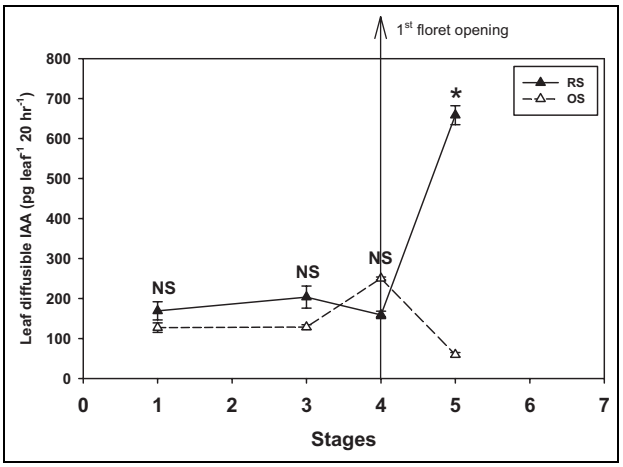


Fig. 5. Changes in leaf diffusible IAA (pg leaf^{-1} 20 h) during the growth cycle of *Curcuma alismatifolia* 'Chiang Mai Pink' in the regular season (RS, solid triangle) and off-season (OS, open triangle). Data are means (n = 10, one plant per replicate) ± SE. *, Significantly different at P<0.05 at the same stage. NS; not significantly different. Arrows indicate the 1st floret opening stage.

t-ZR levels in various organs were significantly higher in OS than RS plants (Fig. 4). There have been many reports that *t*-ZR is involved in root formation by controlling the rate of meristematic cell differentiation (Dello Ioio et al. 2007; Kyozuka 2007) and the formation of storage organs by creating a sink and regulation of the expression of genes implicated in assimilate partitioning and source-sink regulation (Roitsch and Ehneß 2000). Since old rhizomes and old storage roots are still alive, the increase of *t*-ZR in them and the positive correlation between *t*-ZR in leaves and old storage roots may speculate that these organs also functioned as temporary storage tanks for *t*-ZR. The positive correlation between new rhizome yield and the level of *t*-ZR in old rhizomes may support this hypothesis (Table 5).

Auxin has been implicated in tuber physiology but the level of endogenous IAA in buds was positively related to the termination of dormancy (Sorce et al. 2000) and would not be directly responsible for inhibiting sprouting in tuber potatoes (Sorce et al. 2009). That there are no differences in leaf diffusible IAA levels of OS and RS plants in this experiment suggests that the inferior shoot growth is not attributable to the decrease in IAA concentrations in shoots in OS plants (Fig. 5).

Conclusion

The unfavourable conditions in OS such as low temperature, short day length, water loss etc. induced a decrease in the photosynthetic rate, and increases in ABA and *t*-ZR concentrations in various organs at different growth stages. The response of ABA was much stronger than that of *t*-ZR and brought about decreases in plant height and flower quality but stimulated the formation of rhizomes in *C. alismatifolia*. The distinct factors involved in growth regulation via changes of endogenous hormones should be further studied.

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Table 5. Correlation between endogenous hormones in plant parts and some growth parameters (X value *vs.* Y value). The linear regression equation and correlation coefficient R are presented at P<0.05. Data were calculated from the means of growth parameters and hormone levels (n = 30) within six growth stages (1 to 6).

Parameter		Regression equation	R
X value	Y value		
ABA in old rhizomes	Plant height	y = −0.043x + 86.6	−0.94
	Leaf fresh weight	y = −0.013x + 43.5	−0.67
	No. of new rhizomes	y = 0.003x + 0.04	0.73
	Diameter of new storage roots	y = −0.00x + 2.52	−0.61
X value	Y value		
ABA in leaves	Plant height	y = −0.019x + 63.9	−0.79
	Leaf fresh weight	y = −0.008x + 40.0	−0.81
	No. of new rhizomes	y = −0.002x + 1.11	0.90
	No. of new storage roots	y = −0.006x + 5.29	0.87
X value	Y value		
t-ZR in old rhizomes	No. of new rhizomes	y = 0.173x − 8.04	0.66
X value	Y value		
t-ZR in leaf	*t*-ZR in old storage roots	y = 4.528x + 40.8	0.81

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