Chapter 6

Effects of night interruption and PGRs application on the changes in some endogenous hormones in *Curcuma alismatifolia* Gagnep.

6.1 Introduction

Curcuma alismatifolia off-season cropping, under short day length and low night temperature, in Thailand was speculated to induce the changes in some endogenous hormones, particularly, ABA and *t*-ZR accumulation in various organs and partially led to depressing of shoot growth and flower quality as demonstrated in **'chapter 3'**. Both environmental conditions, day length and temperature, were limiting factors for different growth and development in this plant.

Nevertheless, previous study in **'chapter 4'** has proved the possible roles of photoperiods, which strongly affected in hormonal changes, and storage organs formation rather than the temperature in **'chapter 5'** by the short day length induced ABA in aboveground organs and *t*-ZR in underground organs. This finding could also partly described the early reports that night interruption to substitute long day conditions, causing a considerable decreased in ABA levels in leaves and *t*-ZR levels

in old rhizomes which resulted in the reduction of spike length and new storage roots number (Ruamrungsri *et al*., 2007).

Gibberellins (GAs) was generally classified as regulator hormones. It influenced various plant developmental process, including stem elongation, germination, dormancy, flowering and senescence. Its role was also antagonistic with the growth inhibitor hormone as abscisic acids (ABA). In *C. alismatifolia*, GA_3 application was reported to delay sprouting and flowering, but it enhanced the plant height, leaves elongation and flower stalk length when compared with untreated plant (Khuankaew *et al*., 2008a; Khuankaew *et al*., 2008b; Khuankaew *et al*., 2009; Kuehny *et al*., 2002).

Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4-(1H)-pyridinone) is an aquatic herbicide (Fig. 6.1) (Vencill, 2002) whose mode of action at the molecular level has not been satisfactorily elucidated. It has been claimed that this compound is an inhibitor of the enzyme phytoene desaturase which converts phytoene to phytofluene in carotenoid synthesis pathway (Bartels and Watson, 1978; Fong and Schiff., 1979). Similar mode of action has been reported for a pyridasinones herbicide norflurazon (Eder, 1979; Vaisberg and Schiff, 1976).

Treating plants with fluridone, may result in accumulation of phytoene with photo bleaching phenomena. Carotenoids and chlorophylls are essential constituents of photosynthetic membranes; they play a role as accessory pigments for harvesting light (Oelmuller, 1989). The existed evidence that the carotenoids are the main precursor for ABA biosynthesis indirect pathway in plant has been proposed.

Therefore, the inhibition of carotenoid biosynthesis can lead to blocking the production of ABA (Zeevaart and Creelman, 1988). Application of fluridone allows the germination to occur in dormant embryo of *Halianthus annuus* with the drastic reduction of ABA (Le Page-Degivry and Garello, 1992) and inhibits the accumulation of ABA in *Vincia faba* under the drought stress condition (Popova and Riddle, 1996). Drenching fluridone to the soil just before exposure to long day condition has been reported to bleach newly expanding leaves and reduce bulb size, but has no effect on the development of bulb scales with a decreased ABA levels in bulb, buds of the bulbs, leaf blades and root of *Aullium wakegi* (Yamazaki *et al.*, 1999).

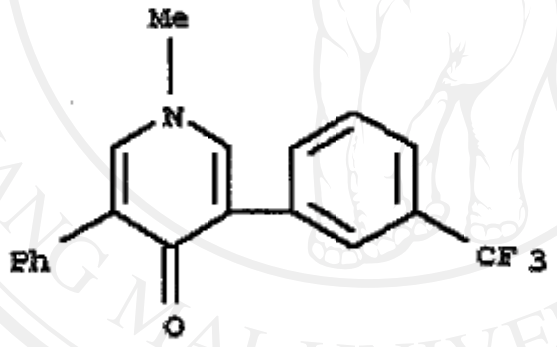

Figure 6.1 Molecular structure of fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4-(1H)-pyridinone). Me: methyl group, Ph: Phenyl group and CF3: trifluoromethyl group.

Due to the more profitable in commercial production, reducing the growth inhibitor (ABA) concentration in leaves and old rhizomes for the off season cropping

might be the appropriate technique to enhance the growth, flower quality and rhizomes yields in this plant.

In the present work, it was aimed to investigate the effects of night interruption with gibberellins and fluridone applications on the changes of some endogenous hormone levels in *Curcuma* to stimulate its growth.

6.2 Materials and methods

6.2.1 Plant materials and experimental conditions

Stubbed rhizomes of *C. alismatifolia* cv.'Chiang Mai Pink' were planted in the same technique and condition as described in **'Chapter 4'** . The study was conducted on November 2007 to February 2008 (off-season cropping) at Chiang Mai University. The experimental design used in this study was a split plot design with natural light and night interruption as main plots and four applications of plant growth regulators (PGRs) by soaking rhizomes before planting as sub plot; i.e. 1) No PGRs application, 2) GA_3 100 mg L^{-1}, 3) Fluridone 10 μM and 4) GA_3 plus fluridone. The treatment combination between natural light and no PGRs application represented as control treatment. There were three replications for each treatment in this study. Night interruption treatment was during 20.00 - 22.00 for 2 h using 18 Watt fluorescent lamp (SL*18R Reflector, Philip, The Netherland) as a supplemental light source (Fig. 6.2). The cultural practices and other maintenances on plant samples were the same as described in the experiment of **'Chapter 4'.**

Plant growth, in terms of plant height, total leaf area, number of leaves, leaf dry weight (DW), number of shoots per cluster, flower quality and rhizome yield, were measured at 12 WAP. Endogenous hormones (ABA and CK) in leaves, old rhizomes and storage roots were analyzed at the flowering stage (12 weeks after planting: WAP). These protocols were the same as explained in **'chapter 3'**.

6.2.2 Statistical analysis

Data were analyzed for statistical significance using Statistic 8 analytical software (SXW Tallahassee, FL, USA). Student's *t*-test was used to determine significant differences between the means in growth (with ten replicates per treatment) and plant hormone (three replicates, three plants per replicate) parameters.

Figure 6.2 Night interruption treatment during 20.00 - 22.00 for 2 h using fluorescent lamp 18 Watt (SL*18R Reflector, Philip, The Netherland) as a light source.

6.3 Results

6.3.1 Plant growth and development

Plant growth

Supplemental lighting had no significant effect on plant height, total leaves area or the number of leaves of *Curcuma* plant (Table 6.1). However, the different PGRs applications significantly effected on those parameters. The GA_3 application produced the tallest plant (51.18 cm) with slender and frail characteristic, while the fluridone application gave the greatest in total leaves area and the number of leaves (339.03 cm^2 and 4.83 leaves per plant), but they were not different from that of the control (Table 6.1 and Fig. 6.3).

Thus, the interaction of lighting and PGRs application were significantly affected in those parameters, night interruption with GA_3 application gave the greatest plant height (62.50 cm), but its interaction with GA_3 plus fluridone conferred the lowest total leaves area (238.09 cm^2). The interaction between supplemental lighting and fluridone application gave the highest number of leaves (4.83 leaves $plant^{-1}$) (Table 6.1 and Fig. 6.3).

Table 6.1 Plant height (cm), total leaves area (cm^2) and number of leaves in *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at first floret opening stage (12 WAP)

PGR	Height			Total leaves area			No. of leaves		
	Lighting		Average[1/]	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	37.84	34.08	**35.78^{b}**	380.28	280.02	**330.15^{a}**	4.17	4.33	**4.25^{a}**
GA	39.87	62.50	**51.18^{a}**	250.02	337.47	**293.75^{ab}**	3.00	3.67	**3.33^{b}**
Fluridone	37.97	34.32	**36.14^{b}**	341.49	336.58	**339.03^{a}**	4.83	4.83	**4.83^{a}**
GA+Fluridone	27.70	45.63	**36.67^{b}**	288.91	238.09	**263.50^{b}**	2.33	4.33	**3.33^{b}**
Average[2/]	**35.75^{a}**	**44.13^{a}**		**315.17^{a}**	**298.04^{a}**		**4.29^{a}**	**3.58^{a}**	
LSD *at* $p < 0.05$									
Lighting	ns			ns			ns		
PGR	*			*			*		
Lighting x PGR	*			*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference.

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference. *, significant, ns = not significant

Flower quality

At flowering stage, both lighting and PGRs application treatments significantly affected on flower quality parameters, in the terms of stalk length, spike length, the number of green bract, pink bract and the percentage of flowering (Table 6.2 and 6.3). Plants were grown under the night interruption treatment had significantly greater in all flower quality parameters (37.46 cm, 11.61 cm, 6.72 bracts, 10.10 bracts and 83.33%, respectively) than those the natural condition (30.87 cm, 8.83 cm, 6.45 bracts, 9.83 bracts and 50%, respectively) (Table 6.2, 6.3 and Fig. 6.3).

GA_3 and GA_3 plus fluridone treated plants produced the longest stalk length (44.39 cm), while those of only fluridone application had the longest spike length (11.31 cm), but it was not different from that of the control (11.09 cm) (Table 6.2). GA_3 application gave the highest the number of green bracts (7.00) and pink bracts (10.33), but they were not different when compared with control (6.89 and 11.06, respectively). However, GA_3 plus fluridone treated plants had the lowest the percentage of flowering (33.33%) (Table 6.3).

There was a significant interaction between lighting and PGRs application on the flower quality; where the night interruption treatment with GA_3 application gave the longest stalk length (44.39 cm), its interaction with control conveyed the longest spike length (12.37 cm). The night interruption with no PGRs treatment produced the highest number of green bracts and pink bracts, 7.44 and 11.78, respectively (Table 6.3).

Table 6.2 Flower quality in *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGR at first floret opening stage

PGR	Stalk length (cm)			Spike length (cm)		
	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	29.83	29.24	**29.54[b]**	9.82	12.37	**11.09[a]**
GA	-[3/]	44.39	**44.39[a]**	-[3/]	12.07	**9.04[c]**
Fluridone	31.90	35.39	**33.64[b]**	10.68	11.94	**11.31[a]**
GA+Fluridone	-[3/]	40.77	**40.77[a]**	-[3/]	10.07	**10.07[b]**
Average[2/]	**30.87[b]**	**37.46[a]**		**8.83[b]**	**11.61[a]**	
LSD *at* $p < 0.05$						
Lighting	*			*		
PGR	*			*		
Lighting x PGR	*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference.

[2/] Means with the same letter within column were not significant difference at $P < 0.05$ by least significant difference.

[3/] Delayed flowering

*, significant, ns = not significant

Table 6.3 Flower quality (number of green bract, pink bract and % flowering) in *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at first floret opening stage (12 WAP)

PGR	No. of green bract			No. of pink bract			% Flowering		
	Lighting		Average[1/]	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	6.33	7.44	**6.89[a]**	10.33	11.78	**11.06[a]**	100	100	**100**
GA	-[3/]	7.00	**7.00[a]**	-[3/]	10.33	**10.33[a]**	-[3/]	100	**100**
Fluridone	6.56	6.44	**6.50[b]**	9.33	8.89	**9.11[b]**	100	100	**100**
GA+Fluridone	-[3/]	6.00	**6.00c**	-[3/]	9.40	**9.40[b]**	-[3/]	33.33	**33.33**
Average[2/]	**6.45[b]**	**6.72[a]**		**9.83[b]**	**10.10[a]**		**50**	**83.33**	
LSD *at p< 0.05*									
Lighting	*			*			-		
PGR	*			*			-		
Lighting x PGR	*			*			-		

[1/] Means with the same letter within row are not significant difference at $p < 0.05$ by least significant difference. *, significant, ns = not significant

[2/] Means with the same letter within column are not significant difference at $p < 0.05$ by least significant difference. [3/] Delayed flowering

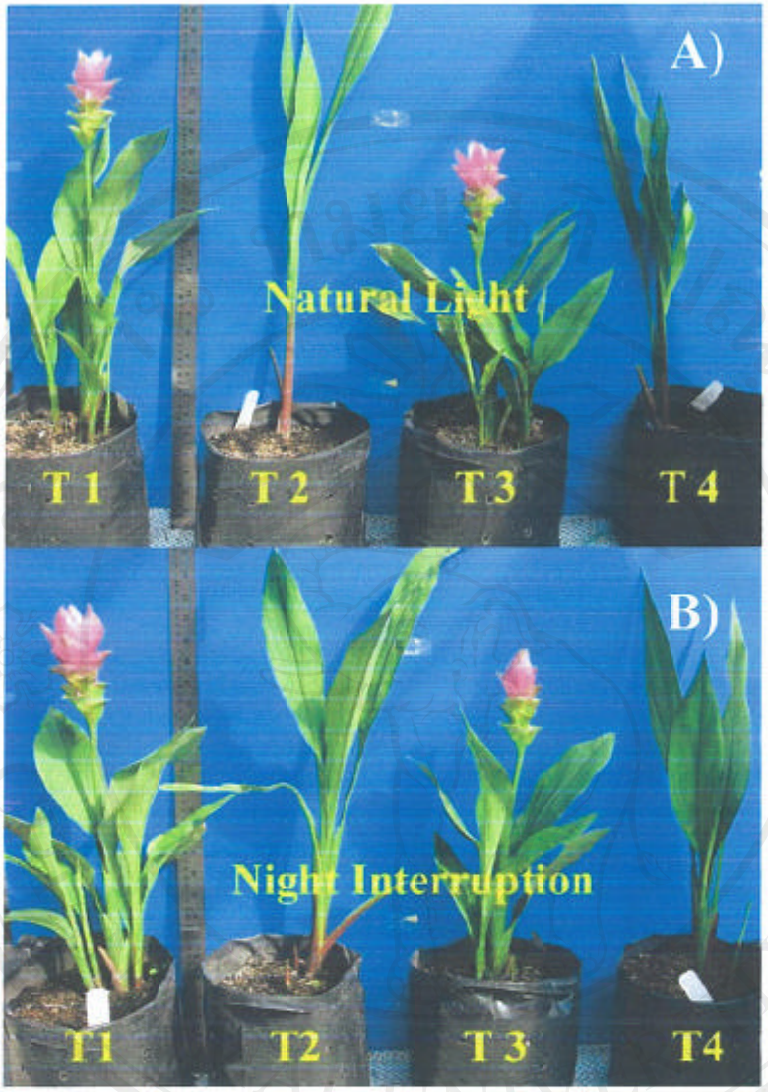

Figure 6.3 Plant growth and flower quality of *Curcuma* plants grown under the different lighting (Natural light: **A**, and Night interruption: **B**) and PGRs application (**T1**: No PGRs, **T2**: GA_3 100 mgL^{-1}, **T3**: Fluridone 10 µM and **T4**: GA_3 + Fluridone) at first floret opening stage (12 WAP).

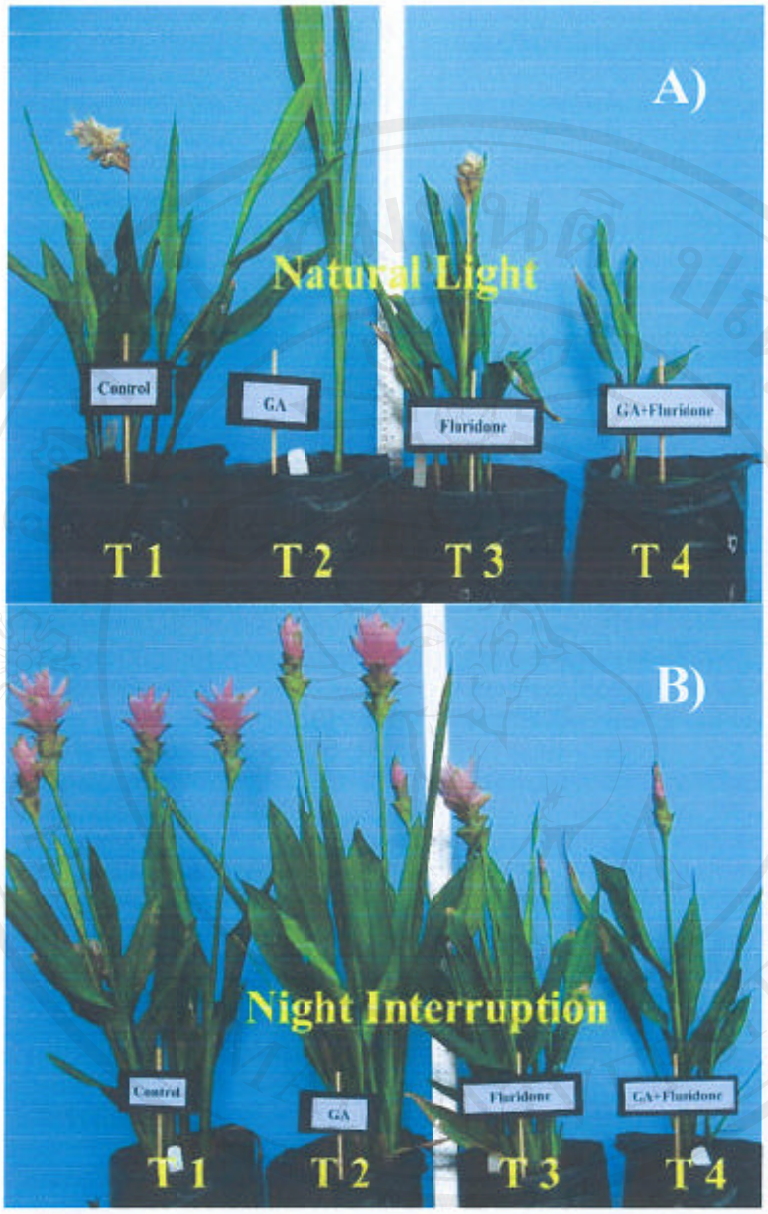

Figure 6.4 Plant growth and flower quality of *Curcuma* plants grown under the different lighting (Natural light: **A**, and Night interruption: **B**) and PGRs application (**T1**: No PGRs, **T2**: GA_3 100 mgL^{-1}, **T3**: Fluridone 10 µM and **T4**: GA_3 + Fluridone) at pre-dormancy stage (20 WAP).

New rhizomes and storage roots yields

At dormancy stage, lighting had no significant effect on the number of new rhizomes or storage roots, diameter or dry weight of new rhizomes or the length of new storage roots (Table 6.4 to 6.6). However, this treatment significantly reduced the diameter, dry weight of new storage roots from 1.86 to 0.57cm and 9.18 to 1.42 g, but promoted their length from 2.04 to 4.97 cm when compared with the natural light treatment (Table 6.5, 6.6 and Fig. 6.5).

PGRs application treatments had the influence on the new rhizomes and storage roots yields. Fluridone application caused the plant to have significantly higher in the number of new rhizomes (4.08) and storage roots (10.50), greater diameter of new rhizome (2.00 cm), dry weight of new rhizomes (6.51 g) and storage roots (8.43 g) than GA_3 + Fluridone application, but these were not different from no PGRs treatment (Table 6.4 to 6.6 and Fig. 6.5).

There were significant interactions between lighting and PGRs application on new rhizomes and storage roots yields. The natural light with no PGRs gave the highest number of rhizomes (4.50) and its interaction with fluridone application gave the greatest diameter of new rhizomes (2.47 cm) and the number, diameter and dry weight of new storage roots (15.83, 2.09 cm and 14.56 g, respectively). However, the night interruption treatment with fluridone application produced the best result in terms of dry weight of new rhizomes, while its interaction with GA_3 application gave the longest new storage roots length (Table 6.4 to 6.6 and Fig. 6.5).

Table 6.4 Number of new rhizomes and new storage roots of *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at dormancy stage (24 WAP)

PGR	No. of new rhizomes			No. of new storage roots		
	Lighting		Average	Lighting		Average
	Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	4.50	4.17	**4.33**[a]	6.83	8.33	**7.58**[ab]
GA	1.67	3.17	**2.42**[bc]	9.67	1.67	**5.67**[b]
Fluridone	4.17	4.00	**4.08**[ab]	15.83	5.17	**10.50**[a]
GA+Fluridone	1.17	2.00	**1.58**[c]	6.50	2.00	**4.25**[b]
Average	**2.86**[a]	**3.33**[a]		**9.71**[a]	**4.29**[a]	
LSD *at* $p < 0.05$						
Lighting	ns			ns		
PGR	*			*		
Lighting x PGR	*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference.

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference.

*, significant, ns = not significant

Table 6.5 Diameter of new rhizomes and storage roots (cm) of *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at dormancy stage (24 WAP)

PGR	Diameter of new rhizomes			Diameter of new storage roots		
	Lighting		Average	Lighting		Average
	Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	2.34	1.80	**2.07**[a]	1.59	0.98	**1.29**[a]
GA	1.94	1.66	**1.80**[ab]	1.81	0.35	**1.08**[a]
Fluridone	2.47	1.47	**2.00**[a]	2.09	0.51	**1.31**[a]
GA+Fluridone	2.05	1.18	**1.62**[b]	1.92	0.44	**1.18**[a]
Average	**2.20**[a]	**1.53**[a]		**1.86**[a]	**0.57**[b]	
LSD *at* $p < 0.05$						
Lighting	ns			*		
PGR	*			ns		
Lighting x PGR	*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference.

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference.

*, significant, ns = not significant

Table 6.6 Dry weight (DW) of new rhizomes and new storage roots (g) and length of new storage roots (cm) of *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at dormancy stage (24 WAP)

PGR	New rhizomes DW (g)			New storage roots DW (g)			New storage roots length (cm)		
	Lighting		Average[1/]	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	3.85	6.45	**5.15^{ab}**	6.89	1.01	**3.95^{b}**	2.02	5.48	**3.75^{a}**
GA	2.34	4.63	**3.48^{bc}**	7.39	1.33	**4.36^{b}**	1.91	6.06	**3.98^{a}**
Fluridone	5.94	7.08	**6.51^{a}**	14.56	2.31	**8.43^{a}**	2.38	4.25	**3.32^{a}**
GA+Fluridone	1.72	2.94	**2.33^{c}**	7.88	1.10	**4.47^{b}**	1.86	4.09	**2.98^{a}**
Average[2/]	**3.46^{a}**	**5.28^{a}**		**9.18^{a}**	**1.42^{b}**		**2.04^{b}**	**4.97^{a}**	
LSD *at* $p < 0.05$									
Lighting	ns			*			*		
PGR	*			*			ns		
Lighting x PGR	*			*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference. *, significant, ns = not significant

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference.

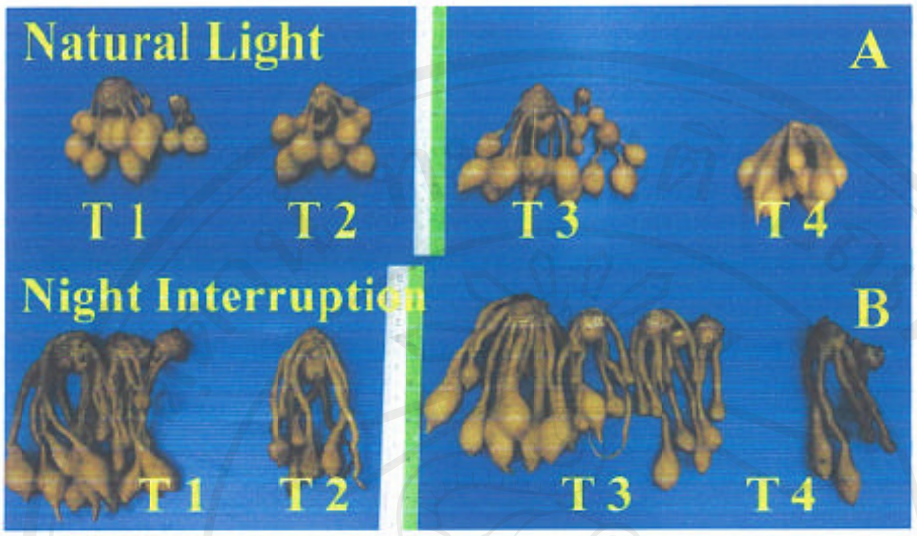

Figure 6.5 New rhizomes and storage roots of *Curcuma* plant grown under the different lighting (Natural light: **A**, and Night interruption: **B**) and PGRs application (**T1**: No PGRs, **T2**: GA_3 100 mgL^{-1}, **T3**: Fluridone 10 µM and **T4**: GA_3 + Fluridone) at dormancy stage (24 WAP).

6.3.2 Endogenous hormone concentrations

ABA concentrations

Lighting, PGRs application and their interaction had no significant effect on the ABA accumulations in leaves, old rhizomes or old storage roots of *Curcuma* plant (Table 6.7 and Fig. 6.6).

Table 6.7 ABA concentrations (ng gDW^{-1}) in leaves, old rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at first floret opening stage (12 WAP)

PGR	Leaves			Old rhizomes			Old storage roots		
	Lighting		Average[1/]	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	693.10	754.40	**723.74[a]**	825.70	792.70	**809.20[a]**	1046.00	1093.60	**1341.90[a]**
GA	757.50	1230.40	**993.97[a]**	814.80	1015.30	**915.00[a]**	965.00	1040.70	**1002.9[a]**
Fluridone	587.60	916.60	**752.11[a]**	740.80	922.10	**831.45[a]**	1151.40	1238.60	**1195.00[a]**
GA+Fluridone	876.50	1070.00	**973.27[a]**	1560.30	994.00	**926.57[a]**	964.90	1261.00	**1112.90[a]**
Average[2/]	**728.68[a]**	**992.86[a]**		**810.10[a]**	**931.03[a]**		**1031.80[a]**	**1158.50[a]**	
LSD *at* $p< 0.05$									
Lighting	ns			ns			ns		
PGR	ns			ns			ns		
Lighting x PGR	ns			ns			ns		

[1/] Means with the same letter within row were not significant difference at $p< 0.05$ by least significant difference.

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference. *, significant, ns = not significant

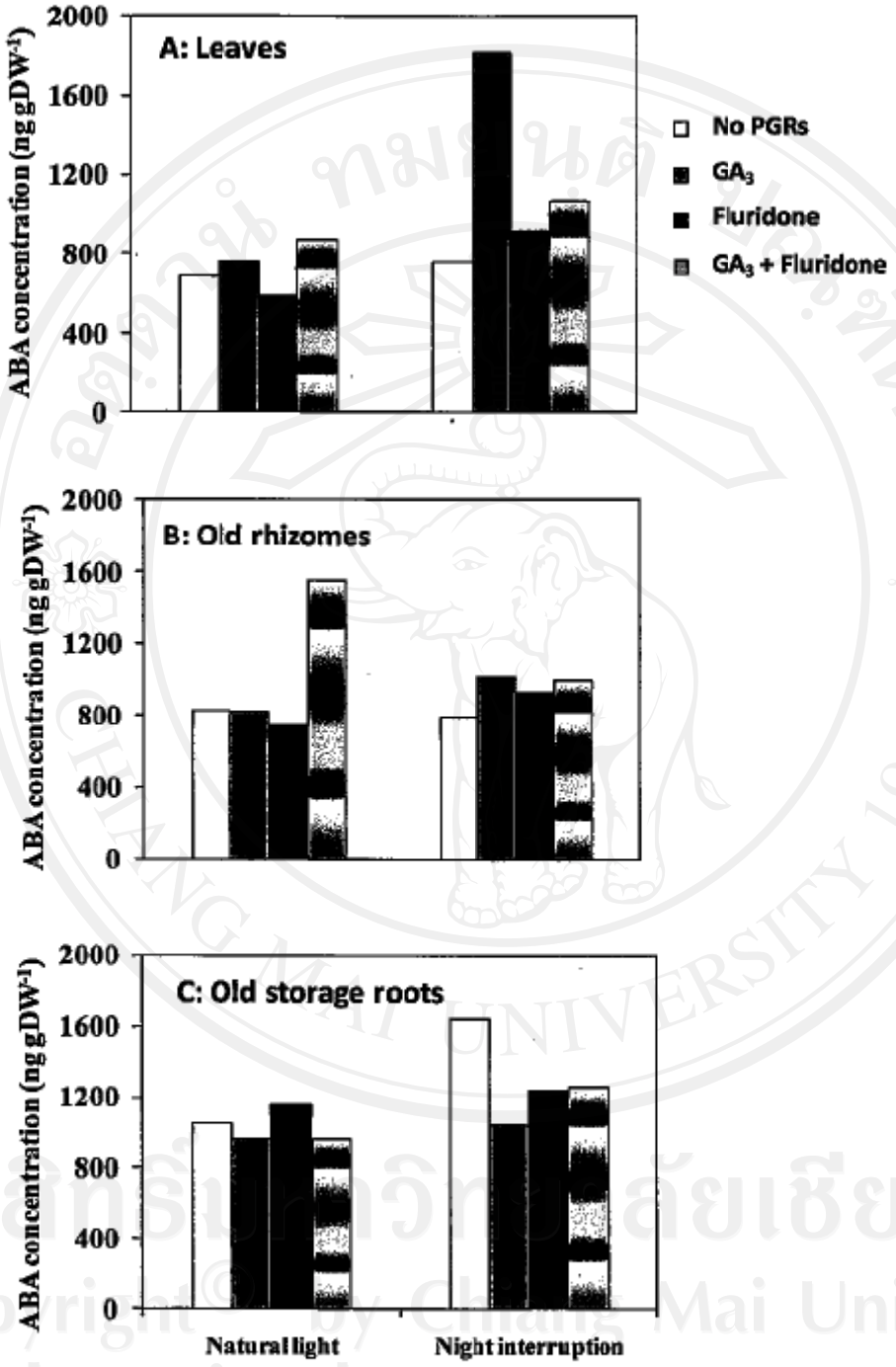

Figure 6.6 ABA concentrations (ng gDW^{-1}) in various organs of *C. alismatifolia* grown under the different lighting and PGRs at first floret opening stage (24 WAP).

***t*-ZR concentrations**

Lighting treatment had significantly influenced on *t*-ZR accumulation only in storage roots of *C. alismatifolia* plants. The night interruption significantly increased *t*-ZR concentration in old storage roots from 26.93 to 76.81 ng gDW^{-1}, while the PGRs application treatment significantly influenced on *t*-ZR concentration in all plant parts (leaves, old rhizomes and storage roots). Fluridone and GA_3 plus fluridone applications gave the highest *t*-ZR concentration in old rhizomes (51.56 and 45.65 ng gDW^{-1}). The GA_3 gave the highest *t*-ZR concentration in old storage roots (72.57 ng gDW^{-1}), while GA_3 plus fluridone gave the highest *t*-ZR concentration in leaves.

There were significant interactions among lighting and PGRs application on *t*-ZR concentration in all plant parts. The natural light with fluridone application gave the highest *t*-ZR concentration (65.78 ng gDW^{-1}) in old rhizomes (Table 6.8 and Fig. 6.7). Night interruption with GA_3 application gave the highest *t*-ZR concentration (131.36 ng gDW^{-1}) in old storage roots, but its interaction with GA_3 plus fluridone gave the highest *t*-ZR concentration (179.09 ng gDW^{-1}) in leaves (Table 6.8 and Fig. 6.7).

Table 6.8 *t*-ZR equivalent (ng gDW^{-1}) in leaves, old rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. grown under the different lighting and PGRs application at first floret opening stage (12 WAP)

PGR	Leaves			Old rhizomes			Old storage roots		
	Lighting		Average[1/]	Lighting		Average[1/]	Lighting		Average[1/]
	Natural light	Night Interruption		Natural light	Night Interruption		Natural light	Night Interruption	
No PGRs	57.44	41.07	**49.25[c]**	31.97	25.33	**28.65[b]**	79.24	35.86	**57.55[b]**
GA	37.22	61.33	**49.27[c]**	38.40	23.97	**31.19[b]**	13.78	131.36	**72.57[a]**
Fluridone	75.26	43.15	**59.20[b]**	65.78	37.34	**51.56[a]**	6.71	85.64	**46.17[c]**
GA+Fluridone	62.77	179.09	**71.97[a]**	27.10	64.20	**45.65[a]**	8.01	54.38	**31.19[d]**
Average[2/]	**58.17[a]**	**81.16[a]**		**40.81[a]**	**37.71[a]**		**26.93[b]**	**76.81[a]**	
LSD *at* $p < 0.05$									
Lighting	ns			ns			*		
PGR	*			*			*		
Lighting x PGR	*			*			*		

[1/] Means with the same letter within row were not significant difference at $p < 0.05$ by least significant difference. *, significant, ns = not significant

[2/] Means with the same letter within column were not significant difference at $p < 0.05$ by least significant difference.

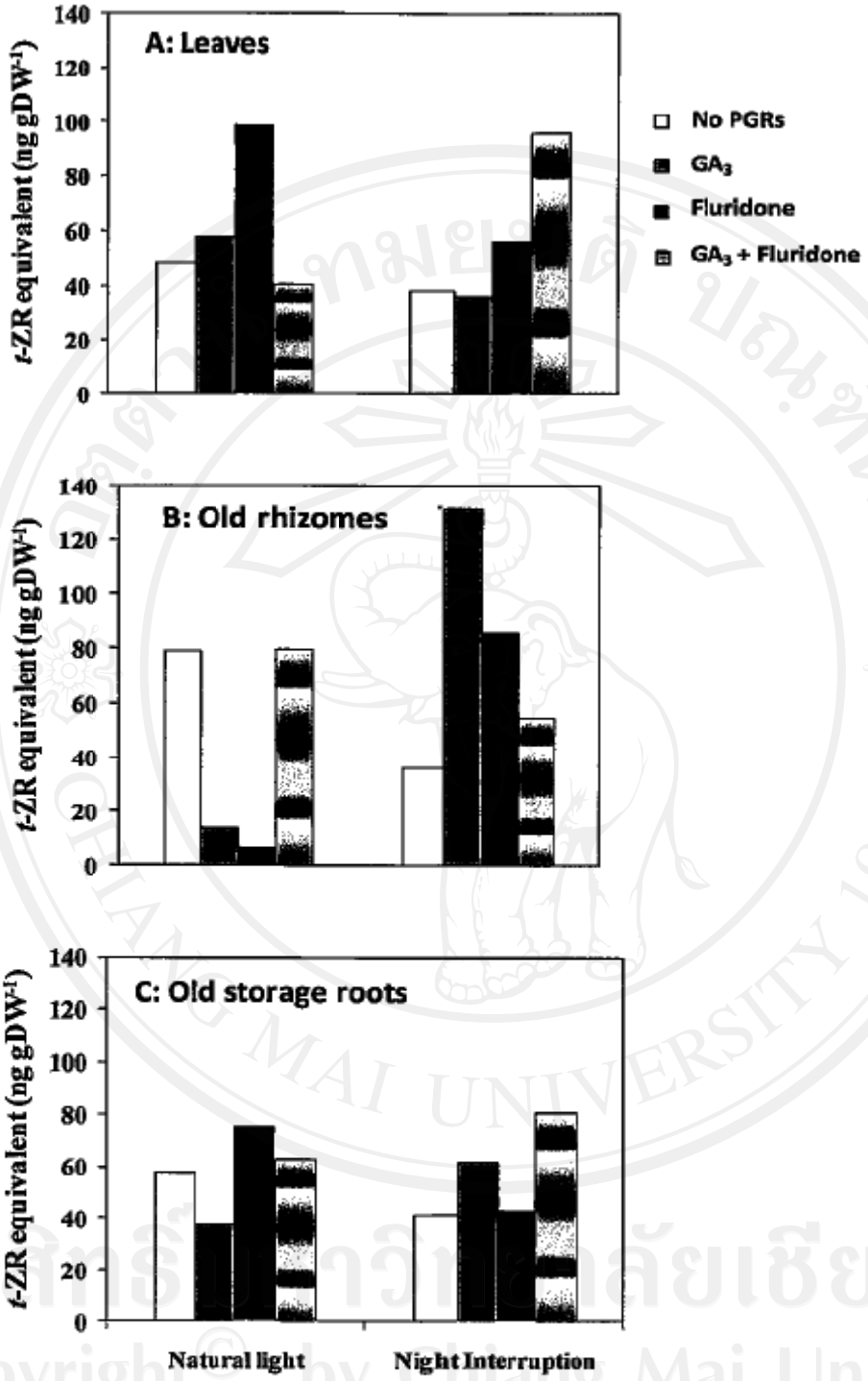

Figure 6.7 *t*-ZR equivalent (ng gDW^{-1}) in various organs of *C. alismatifolia* Gagnep. grown under the different lighting and PGRs at first floret opening stage (24 WAP)

6.4 Discussion

6.4.1 Plant growth and development

Lighting seemed to be more strongly affected on reproductive growth or flower quality parameters (Table 6.2 and Fig. 6.3) than those of vegetative growth (Table 6.1). Particularly, the effect of night interruption was so pronounced in enhancing flower quality, in the terms of stalk length, spike length, the number of green bracts and pink bracts and percentage of flowering. These effects were similar to those observed in plants grown in the off-season production with supplemental lighting as long day condition increased those parameters (Ruamrungsri *et al*., 2007). Photoperiods were well known in the control of flowering (Thomas and Vince-Prue, 1997) and the molecular mechanism in controlling this aspect was associated with the function of phytochrome and cryptochrome (Mockler *et al*., 2003). It had also been demonstrated that *C alismatifolia.* was sensitive to day length in flowering and it should be classified as a quantitative long day plant, since the long day condition by night break treatment could promote the flowering (Hagiladi *et al*., 1997a). However, the flower abortion was occurred when plant grown under short day and low light intensity condition (Chidburee, 2008). Thus, the decrease in percentage of flowering under natural light in this experiment might be expected to be the same reason as above.

Lighting was the major factor that affected new storage roots yields (diameter, dry weight and length), as night interruption clearly caused the reduction in both size

and dry matter accumulation, but it increased the length of storage roots. Comparable results were found in a previous study (Ruamrungsri *et al.*, 2007).

The PGRs applications affected on various physiological responses of *Curcuma* plant, especially GA_3 application which produced phenotypic changes in vegetative and reproductive growth as compared with no PGRs application. Stimulation of plant stem elongation by gibberellins was the basis for this hormone's discovery and their effect was used for biological assay of GAs (Ross, 1992). The GAs promoted stem elongation through both cell elongation and cell division had previously been reported (Huttly and Phillips., 1995). In this experiment, treated plants with GA_3 application were the tallest with slender and frangible slim stem characters. The result was similar to that observed in *C. alismatifolia* treated with GA_3 solutions of 100, 300 and 500 mg L^{-1} by drenching which caused an increase in plant height (Khuankaew *et al.*, 2008a; Khuankaew *et al.*, 2008b). The cell elongation by the effects of GAs was concerned with the increasing elasticity of cell wall and decreasing osmotic potential solution in cell (Ross, 1992). GAs promoted cell elongation by inducing enzymes that regulated cell wall expansion and loosening; i.e. expansin, xyloglucan trans- glycosylase/hydrolase (XET or XEH) and pectin methyl esterase (PME) (Stephen *et al.*, 2005). Thus, the above reasoning might possibly explained such night interruption and GA_3 interaction that brought about the tallest plant.

However, flowering was not occurred when plant received GA_3 application under natural light condition (short day), but flowering was found in plant grown under night interruption condition. Generally, GAs imposed their effects on flowering

date in many species, either becoming earlier or being delayed flowering (Ben-Tal and Erner., 1999). GA_3 application in *C. alismatifolia* had been reported to delay flowering (Khuankaew *et al*., 2008a; Khuankaew *et al*., 2008b; Kuehny *et al*., 2002), but did not promote flowering. In the present case, the absent of flower bolting might be caused by the combining effects between delayed flowering in response to GAs and flower abortion in responses to short day length.

Fluridone application at 10 μM by soaking stubbed rhizomes before planting did not affect on plant photo bleaching phenomena, whereas the plant seemed to be healthy as untreated control plant. This application resulted in the greatest number of leaves. However, fluridone plus GA_3 gave the highest total leaves area, but did not differ from that of untreated plants.

Furthermore, the degree of striking visible effect, albino appearance in foliar tissue, was varied widely depending on the light intensity, herbicide concentration, the mode of treatment given or the nature of plant species (Stewart and Voetberg, 1987). It was difficult to extrapolate on the effect from one plant to another, especially since plants varied in their sensitivity to fluridone and large variation concentrations had been used. Treated plant with 10 μmoles fluridone in darkness caused a strong reduction of growth rate, the leaf area and also leaves became completely white in *Vicia faba* (Popova, 1995), while its concentration was 0.1 mM for barley (Stewart and Voetberg, 1987) and 100 mgL^{-1} (approximately 0.3 mM) for maize (Moore and Smith, 1985) without any visible damages of plants, except the observe leaves tissue bleaching.

Fluridone treated plants were also observed to produced the largest underground organs (new rhizomes and storage roots) and its interaction with natural light (short day) seemed to promote those organs (Table 6.5, 6.6 and Fig. 6.5). This result was consistent with the finding of Yamazaki et al. (1999) who reported that the effects of fluridone application could be specifically attributed to a reduction of bulb size by decreasing bulb fresh weight, but did not affect the development of bulb scales in *Allium wakegi* 'Araki'.

From the results above, it might be indicated that the regulation of new rhizomes and storage roots formation was controlled by the combined effect of short day and fluridone application. Nevertheless, the possible responses of plant to fluridone in blocking ABA accumulation with the inhibiting of bulbing phenomena could be considered to be similar processes to the finding in this experiment.

6.4.2 Endogenous hormone

Surprisingly that, both lighting and PGRs application did not affect the accumulation of endogenous ABA levels in any plant parts (leaves, old rhizomes and storage roots) (Table 6.7 and Fig.6.6). Similar result was also found in the interaction effects. The previous studies demonstrated that under the natural short day length in off-season cropping partly induced the accumulation of ABA in various organs of *C. alismatifolia* (**chapter 3**) and might confirm this response when the higher ABA concentration in plant grown under SDL condition was obtained in **'chapter 4'**. Some reports uncovered that the degradation of ABA was controlled by light passing

through the transduction pathway of phytochrome signals (Kraepiel *et al.*, 1994). Thus, it could be expected that the reduction of ABA accumulation under the night interruption would occur under the long day condition, but the present results found no differences in their levels in both lighting treatments. However, the night interruption tended to increase more ABA levels than those of the natural light; this might partly be concerned with plant was under stress by heat from supplemental lighting.

Fluridone application failed to block ABA accumulation in all parts of *Curcuma* plant (Table 6.7 and Fig. 6.6). Consistent with other reports; e.g. in alliums (Yamazaki *et al.*, 1999), lily (Kim *et al.*, 1994) and potatoes (Suttle and Hultstrand, 1994). Night interruption, as long day condition, was found to strongly regulate *t*-ZR accumulation in old storage roots (Table 6.8), but it was not involved in leaves or rhizomes. Nevertheless, the increase in CK was not parallel with the yield production. Similar to the findings of Wang *et al.* (2004), who reported that extending photoperiods could increase the endogenous cytokinin levels under the stress condition of creeping bent grass. However, these results above were consistent with the previous study in **'chapter 4'** that SDL condition also increased *t*-ZR in storage roots, and might be partly responsible for promoting the storage roots organs.

The fluctuation of *t*-ZR in various plant parts under different PGRs application treatments and their interactions with lighting factor were observed in this experiment (Table 6.8 and Fig. 6.7). The night interruption with GA_3 application was speculated to increase *t*-ZR in storage roots, and this might be concerned with causing the tallest plant.

Base on these findings, it was suggested that the greatest plant height when received the interaction between night interruption and GA_3 application might not caused by the reduction of ABA concentration in all plant parts, but probably due to the accumulation of *t*-ZR in old storage roots. Failing to block of ABA accumulation by furidone treatment might be due mainly to the ABA biosynthesis pathway did not accompany with carotenoids synthesis pathway in *Curcuma* plant, and the fluridone soaking absorbed by the stubbed rhizomes might be metabolized or transported to a site where it could not have any function or action. Nevertheless, the appropriate technique, optimum concentration or timing of application of this herbicide should be conducted and explored in further study.

6.5 Conclusion

Night interruption by supplemental lighting with 180 Watt fluorescent lamp from 20.00 to 22.00 under the short day conditions (winter) could promote flower quality, but it reduced the rhizomes yields. The 100 $mgL^{-1}$of GA_3 application by soaking stubbed rhizomes for 2 h before planting increased plant height, but was not suitable for flower production. The 10 µM of fluridone application promoted leaf growth and underground organs yields, but failed to reduce ABA concentration in all plant parts. However, night interruption with GA_3 application could promote aboveground organs (leaves and flower quality), but comparatively reduced underground yields. In addition, this treatment combination could not reduce ABA

accumulation in leaves, old rhizomes or storage roots, although it induced *t*-ZR accumulation only in old storage roots.