

Effects of Day-length and Gibberellic Acid (GA₃) on Flowering and Endogenous Hormone Levels in *Rhynchostylis gigantea* (Lindl.) Ridl.

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Abstract

Effects of day-length and GA₃ on flowering and endogenous hormone levels during flowering process of *Rhynchostylis gigantea* (Lindl.) Ridl. were studied. Three-year-old plants were grown under natural condition and short-day (10 h of light) combined with GA₃ at 0 and 3,000 ppm, for 3 months. The results revealed that short-day could induce flower faster than natural condition around two weeks. Short-day and natural condition had effects on stem height and bush width, while GA₃ had effects only on number of leaves per plant. ABA in leaf seemed to decreased in all treatments except under NC-GA₃, there was a peak of ABA at 30 days. Furthermore, under SD, GA₃ seemed to decrease ABA in leaf. In shoot, ABA concentration was increased during 0-30 days in all treatments, after that GA₃ seemed to decrease ABA concentration under NC, while the ABA concentration kept constancy under SD. On the other hand, GA₃ seemed to boot t-ZR up in leaf under SD, but did not in shoot, where GA₃ seemed to drop t-ZR. The reducing ABA and increasing t-ZR in leaf and/ or shoot might be related to flower buds initiation and early flowering of *R. gigantea*, especially, under SD.

Keywords: *Rhynchostylis gigantea* (Lindl.) Ridl, Flowering, Day-length, Hormone

1. Introduction

Flowering is a process that plant changes from vegetative to reproductive phase, from leaf to flower (Boonyakiat, 2003; Taiz and Zeiger, 2007). This process is controlled by hormonal level (Ruamrungsri, 2004) and environmental condition (Hew and Yong, 2004). In orchids, factors controlled flowering process is less known. There are few informations available in this regards. In *Brassocattleya* Marella Koss, the percentage of flowering was reached 33%, when those plants were received GA₃ at 1,000 mg/l combined with more frequency of irrigation. On the other hand, when those plants were received GA₃ at 250 mg/l combined with low frequency of irrigation, percentage of flowering was 83%, but not found any floral bud initiation in *Cattleya* Eileen Holquin (Cardoso *et al.*, 2010). In *Dendrobium* Louisae, GA₃ alone had any effect on floral bud initiation, while GA₃ at 0.001M+BA 0.01M, floral buds were appeared earlier than BA alone, these floral buds were better development and reached 100% of flower (Goh, 1979). In *Dendrobium* Second Love, 2-year-old plants, were grown under 12 h of photoperiod. The amount of IAA and cytokinins on 15 days after treatment were about twice as much as those found from the beginning whereas the amount of ABA was less, about 1/3 of those found at the beginning. The amount of analyzed hormone in the leaves were less than those in the lateral buds (Campos and Kerbauy, 2004). On the other hand, there were reports on factors controlled flowering of *Rhynchostylis gigantea*. Plants grown under short-day of 10 h and night temperature at 18°C could give visible flower spike at average of 30.67 days after treatment, whereas those receiving 8 h light and night temperature at 18°C could give visible flower spikes at average of 34.50 days after treatment. Plants grown under those two conditions could give flower faster than those grown under natural condition (Theesoda, 2004). Talee (2008) reported that *R. gigantea* receiving

dark period at 14 h along with 1,000 and 3,000 ppm GA₃ application could give flower faster than natural condition for about 5-7 weeks.

It is an ideal to have orchid that can flower all year round, thus flowering process of some Thai native orchid requires to be understood. This research would determine endogenous hormone levels during floral transition of *R. gigantea* (Lindl.) Ridl.

2. Materials and Methods

Rhynchostylis gigantea (Lindl.) Ridl. plants with the age of three-year-old were randomly selected and the total of 120 plants were used in this experiment. The experiment was factorial (2x2) in RCBD, factor A: Natural condition (NC) and short-day (SD) (10 h of light), while factor B: 0 and 3,000 ppm of GA₃ application. GA₃ was applied at 10 days after treatment and 10 days interval for 9 times, 200 µl of 3,000 ppm GA₃ was injected at the base of the leaf locating above the last year blooming stalk. For short-day condition, plants were hanged on rails where black cloth was covered above. The black cloth was opened at 7 am and closed at 5 pm for 3 months. After that, all plants were transferred to shaded house and grown under natural condition. This experiment was conducted during June 2009 to March 2010 at Orchid Nursery, Mae Hia Agricultural Centre for Research, Demonstration and Training, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University.

Leaves and shoots of each treatment were harvested at 0, 30, 60 and 90 days after treatment. Samples were immediately grounded in liquid nitrogen, and stored at -20°C until used. The endogenous hormone levels Abscisic acid (ABA) and trans-Zeatin Riboside (t-ZR) were extracted according to Naphrom and Sringarm (2009). The measurement of ABA and t-ZR were carried out by ELISA test Kit, Phytodetek® ABA Test Kit Catalog number: PDK 09347/0096 and Phytodetek® t-ZR Test Kit Catalog number: PDK 09348/0096 were used. The levels of endogenous hormones were then calculated.

Plants growth and development were records i.e. stem height, bush height and width, number of leaves per plant, leaf length and thickness. All data were analyzed, and the difference was compared by Least Significant Difference (LSD).

3. Results and Discussions

3.1 Percentage of Visible Flowering

The experiment was conducted on June 2nd 2009. Plants grown under NC-GA₃ and those grown under NC+GA₃ could give visible flower shoot on September 30th and 20th 2009, respectively; whereas those grown under SD±GA₃ could give visible flower shoot on September 11th 2009, which was 19 days earlier than those grown under NC±GA₃. All plants grown under SD could give visible flower shoot at 100% on October 20th 2009 (Data not show). This result was similar to the report that *R. gigantea* could give flower shoot faster after exposed to dark period of 14 and 16 h combined with night temperature of 18°C, which was faster than dark period of 14 and 16 h combined with night temperature of 20°C (Theesoda, 2004). While those plants grown under dark period of 14 h along with GA₃ application at 1,000 and 3,000 ppm could give flower shoot about 5-7 weeks faster than those of natural condition (Talee, 2008). GA₃ at 0.001M+BA 0.01M, floral buds were occurred earlier than those of BA or GA₃ alone, as found in *D. Louisae* (Goh, 1979). These results indicated that short day might be a favorable factor of flower initiation in *R. gigantea*.

3.2 Flowering Shoot Development and Flowering

Take to the consideration, flower development was accounted from the experiment set-up day, which was June 2nd, 2009. First visible flower shoot was appeared at 111 days after treatment, this occurrence was found on those plants grown under SD±GA₃, which was earlier than those plants grown under natural condition. Flowering of plants grown under natural condition appeared later than those grown under other conditions, however they took short time from first flowering to full flowering, only 7 days, which was shorter than those of other conditions. The blooming period (from 10% of flowering to 100% of flowering) of all treatments was about 21 days (Table 1).

3.3 Endogenous Abscisic Acid (ABA) Analysis

Plants were grown under different day-length i.e. NC and SD combined with two levels of GA₃ application at 0 and 3,000 ppm for 3 months. It was found that, different patterns of ABA level in leaf and shoot were found. The amount of ABA in leaf of plants grown under different growing condition was seemly decreased almost all treatments except NC+GA₃, the highest amount of ABA level was found at 30 days after treatment and declined to the same levels as the others of 60 and 90 days after treatment (Figure 1A). The increasing amount was found in condition with GA₃ application, but under SD, GA₃ decrease ABA level in leaf.

In shoot, ABA level was sharply increased at 30 days after treatment for all treatments (Figure 1B). Under NC-GA₃, the increasing amount of ABA was found until at 60 days after treatment and then dropped. In the contrary, under NC+GA₃, ABA decreased until 90 days after treatments, while under SD±GA₃, ABA were kept constancy. It seems that GA₃ dropped ABA level in shoot under NC.

It was reported by Wang *et al.* (2002), during flower shoot development of *Phalaenopsis hybrida*, free ABA in leaf was slightly increased as compared to dormant stage, whereas, free ABA level in buds decreased along with the emergence of flowering shoot and could not be detected during inflorescence development. In *D. Second Love*, ABA level was decreased during flower initiation process (Campos and Kerbauy, 2004). In orchid hybrid *Aranda Deborah*, ABA could not stimulate any floral initiation (Goh, 1977). In the present study, the decreasing of ABA in shoot after 30 days, under NC+GA₃ might be related to flower initiation and earlier flowering. This might be due to the amount of GA₃ is higher than the ABA which caused flower initiation and early flowering.

3.4 Endogenous Trans-Zeatin Riboside (t-ZR) Analysis

The endogenous t-ZR in both leaf and shoot were increased as found at 30 days after treatments (Figure 2). In leaf, under NC, the amount of t-ZR slightly increased. However, under SD±GA₃ application, t-ZR level was sharply increased at 30 days and declined afterward at 60 days and seemly be stable until the end of the treatment (Figure 2A). Under SD, GA₃ application seemed to booths the amount of t-ZR in leaf up at 30 days, abundant ZR in leaf might be related to floral initiation and early flowering. It was reported that flower bud initiation of *R. gigantea* was found on 30 days after those plants were grown under short-day condition (Talee, 2008), in this study, it was found that amount of t-ZR in leaf was increased under SD+GA₃ application at 30 days after treatment. It could be suggested that flower bud initiation of *R. gigantea* might be related to the increasing of t-ZR in leaf. In *D. Second Love*, cytokinins, (9R) Z and Z were increased to a certain amount during flower buds swelling and development (Campos and Kerbauy, 2004). In *P. hybrida* cv. Taisuco Snow, it was found that Z was slightly decreased, while ZR was increased during flower bud initiation (Chou *et al.*, 2000). In *Miltoniopsis* Eileen and *Mil. Akatsuka*, inflorescences were emerged earlier than control, when those plants were treated with GA₃ (Matsumoto, 2006). However, GA₃ alone did not simulate any flower bud in *D. Louisae* (Goh, 1979). Cytokinins, 6-Benzylaminopurine, BA, stimulated floral buds and floral buds development in *Aranda Deborah* (Goh, 1977).

However, amount of t-ZR in shoot did not show similar pattern (Figure 2B). The increasing in t-ZR was found only under SD-GA₃, whereas in other treatments only slightly changes were found. The changes of t-ZR in shoot, might not be related to flower initiation and early flowering as in leaf, where the relation of GA₃ and t-ZR would be further studied.

3.5 Vegetative Growth and Development of *Rhynchosytilis gigantea* (Lindl.) Ridl.

Vegetative growth and development of *R. gigantea* were recorded. It was found that day-length had effects on stem height and bush width (Table 2). Whereas, GA₃ application had effect only on number of leaves per plant. Plant grown under SD had greater stem height and bush width, 7.13 cm and 36.78 cm, respectively, which was greater than those grown under NC, 6.28 cm and 33.88 cm, respectively. GA₃ application reduced number of leaves per plant. Interaction effects between day-length and GA₃ application were found on stem height, bush height and width, number of leaves per plant and leaf length. Plants grown under SD+GA₃ had the greatest stem height than those plants grown under NC±GA₃. On the other hand, plants grown under SD+GA₃ had greater bush height as well as plants grown under NC-GA₃, those were significantly different from NC+GA₃. For bush width, plants grown under SD±GA₃ and NC-GA₃ had the greatest bush width than those plants grown under NC+GA₃. Plants grown under NC-GA₃ had the greatest number of leaves per plants, that was greater than those of other conditions. For leaf length, it was found that plants grown under SD±GA₃ and NC-GA₃ had the greatest leaf length than the plant grown under NC+GA₃. Plants grown under SD+GA₃ could give significantly better vegetative growth and development in terms of stem height than those of NC±GA₃. NC+GA₃ application or natural condition gave less growth in terms of stem height, 5.87 cm and 6.24 cm, respectively.

4. Conclusion

Short-day condition could give visible flower shoot earlier than those of natural condition. ABA in leaf decrease in all treatments except under NC-GA₃, there was a peak of ABA at 30 days. Furthermore, under SD, GA₃ decreased ABA in leaf. In shoot, ABA concentration was increased during 0-30 days in all treatments, after that GA₃ decreased ABA concentration under NC, while the ABA concentration kept constancy under SD. On the other hand, GA₃ boot t-ZR up in leaf under SD, but did not in shoot, where GA₃ seemed to drop t-ZR. It might be concluded that SD+GA₃ was a key factor affecting ABA and t-ZR in leaf and concern flower initiation

and flowering in *R. gigantea*. Day-length gave better growth in terms of stem height and bush width as found on plant grown under SD+GA₃ application.

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Table 1. The average number of days on each stage of flower development and senescence

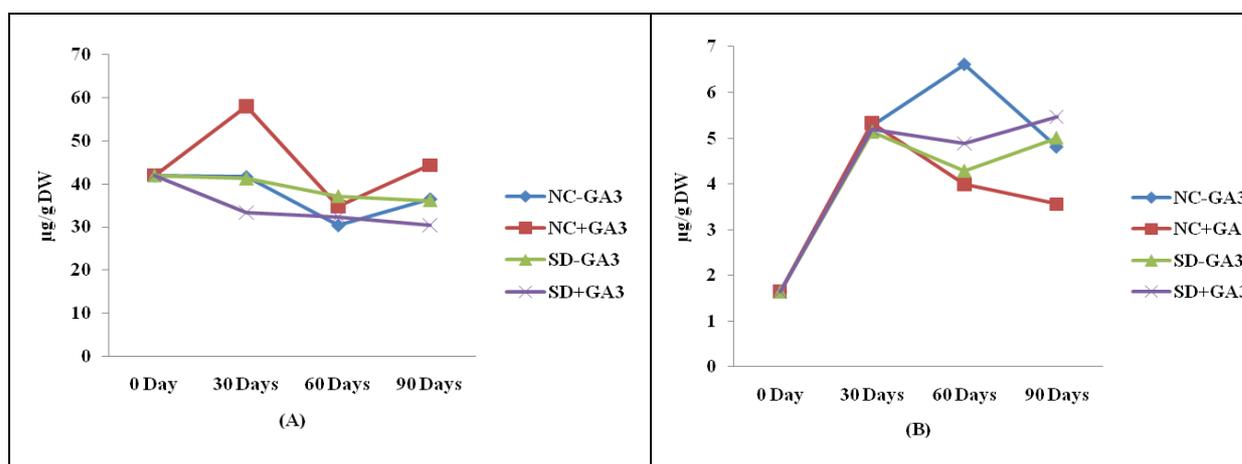
Flower development and Senescence					
Treatments	Date of initiation	Number of days to visible flower shoot (Days)	Number of days to first flowering (10%) (Days)	Number of days to full flowering (100%) (Days)	Percentage of Senescence at February 8 th 2010 (%)
NC-GA ₃	June 2 nd , 2009	130	255	262	76.72
NC+GA ₃		120	238	251	83.85
SD-GA ₃		111	229	242	92.86
SD+GA ₃		111	229	242	76.77

(NC-GA₃) Natural condition without GA₃ application; (NC+GA₃) Natural condition with GA₃ application; (SD-GA₃) Short-day without GA₃ application; (SD+GA₃) Short-day with GA₃ application.

Table 2. Vegetative growth and development of *Rhynchosyilis gigantea* (Lindl.) Ridl. after exposed to natural and short-day condition with or without GA₃ application

Vegetative growth and development					
Vegetative growth	Day-length	GA ₃ application (ppm) ^{1/}		Average ^{3/}	LSD _{0.05}
		0	3000		
Stem height (cm)	Natural condition	6.60 ^{bc}	5.87 ^c	6.24 ^b	0.55
	Short-day condition	6.65 ^b	7.61 ^a	7.13 ^a	
	Average ^{2/}	6.63	6.74		0.78
	LSD _{0.05}	ns			
Bush height (cm)	Natural condition	17.97 ^a	15.57 ^b	16.77	ns
	Short-day condition	17.22 ^{ab}	18.94 ^a	18.08	
	Average ^{2/}	17.59	17.25		2.12
	LSD _{0.05}	ns			
Bush width (cm)	Natural condition	36.53 ^a	31.23 ^b	33.88 ^b	2.70
	Short-day condition	35.84 ^a	37.82 ^a	36.78 ^a	
	Average ^{2/}	36.14	34.53		3.81
	LSD _{0.05}	ns			
Number of leaves per plants	Natural condition	8.81 ^a	6.80 ^c	7.81	ns
	Short-day condition	8.19 ^b	8.15 ^b	8.17	
	Average ^{2/}	8.50 ^a	7.47 ^b		0.55
	LSD _{0.05}	0.37			
Leaf length (cm)	Natural condition	20.37 ^a	16.99 ^b	18.68	ns
	Short-day condition	19.53 ^a	20.32 ^a	19.93	
	Average ^{2/}	19.95	18.66		2.31
	LSD _{0.05}	ns			
Leaf thickness (cm)	Natural condition	0.37	0.39	0.38	ns
	Short-day condition	0.38	0.39	0.39	
	Average ^{2/}	0.38	0.39		ns
	LSD _{0.05}	ns			

^{1/}The mean in the vertical and horizontal are not significantly different at $p < 0.05$ by LSD; ^{2/} The mean in the horizontal are not significantly different $p < 0.05$ by LSD; ^{3/} The mean in the vertical are not significantly different at $p < 0.05$ by LSD; (ns) non-significance different; (*) Significantly different $p < 0.05$ by LSD

Figure 1. ABA level ($\mu\text{g/g DW}$) in leaf (A) and shoot (B) at 0, 30, 60 and 90days after Treatment

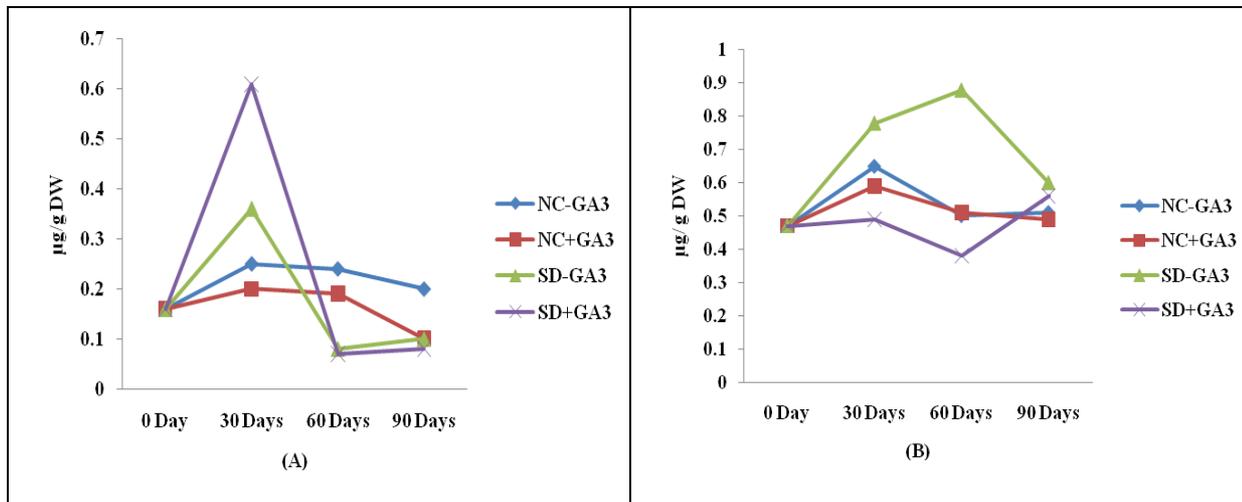


Figure 2. t-ZR level ($\mu\text{g/g DW}$) in leaf (A) and shoot (B) at 0, 30, 60 and 90 days after treatment