Effects of Short Day Cycles on Flowering Time and Nutritional Status of Vanda

Soraya Ruamrungsri1,3, Takonwan Sirisawad2, Kanokwan Panjama1,3, Nuttha Potapohn1 and Chaiartid Inkham1,2,3*

1Economic Flower Crop Research Cluster, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
2Science and Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand
3H.M. The King’s Initiative Centre for Flower and Fruit Propagation, Chiang Mai 50230, Thailand

In order to solve the problems of commercial orchid growers, who need to force Vanda flowering for both blooming on demand and uniformity, an appropriate environment to control flower development is a key factor. However, little research has been conducted on this topic. This research aimed to determine the effects of short day cycles on the flowering of Vanda ‘Manuvadee’, which usually has high productivity from September to February. Plants were grown under seven hour day lengths for three, six, and nine weeks before being moved to natural day length (approximately 12 hours/day), and then compared with plants cultivated only under a natural day length condition (control). All flowers were grown in an environment of 25 ± 2°C, 70–80% relative humidity. 21-21-21 (N-P2O5-K2O) fertilizer was sprayed weekly at an average of 0.44 g per plant. Results showed that forcing under seven hour day lengths for six and nine weeks could on average delay flowering to 22 and 38 days later than the control treatment, respectively. Forced plants had 100% of first flower opening within 55 days, while the control treatment group gradually opened and took approximately 101 days to anthesis. The nine-week short day treatment provided the most inflorescences, and better longevity was obtained with the six and nine-week short day treatments. Short day cycles decreased TNC concentration in leaves, but did not affect C/N balance at the ninth week; however, P and K concentrations increased.

Key Words: carbohydrate, orchid, photoperiodism, plant nutrition.

Introduction

Vanda, an epiphytic tropical orchid, is currently highly valued on the international market. These orchids usually have two or three flowering cycles a year. Young inflorescences appear beginning in June, open in September to October, and the next flowering ensues after the previous one has senesced. However, there is a lack of flowering during April and May, a period when there is high market demand. Exporters of cut Vanda face problems in controlling flowering, as well as uniformity, including when exported plants sometimes do not flower in the destination countries. Consequently, commercial Vanda growers need techniques to control Vanda flowering for uniformity of blooming on demand or off-season. However, flowering control techniques are limited, and there is a lack of research publications on the flowering physiology of this plant.

Photoperiodism, one of the most important factors for flowering (Arditti, 1992; Grove, 1995; Thomas and Vince-Prue, 1997; Hew and Yong, 2004; Glover, 2014), has shown positive results in many orchids, including V. ‘Ratchaburi Fuchs-Katsura’, where flowering was delayed under 8-hour day length conditions (Panjama, 2018a); however, various tropical orchids are classified as day-neutral plants (Hew and Yong, 2004). It was reported that photoperiod and temperature may regulate floral induction in many orchids (Goh and Arditti, 1985). Thomas and Vince-Prue (1997) suggested that flowering often occurs after receiving optimum cycles. In Cattleya, a short day (SD) period was a favorable factor for flower induction; it decreased the time to flowering, and enhanced some flower qualities, whereas long day periods (LD) promoted vegetative growth.
(Goh and Arditti, 1985; Lopez and Runkle, 2005). In addition, *Rhynchostylis gigantea* (Lindl.) Ridl. grown under a 10-hour SD cycle combined with 1,000 and 3,000 mg·L⁻¹ GA every ten days for three months, flowered earlier than the control group grown without the SD condition (Talee, 2008).

Non-structural carbohydrates (NSC) play an important role in plant metabolism, generating energy, transporting mineral nutrients and maintaining plant defense mechanisms. Starch and sucrose, which translocate from source to sink organs, are the main photosynthates (Hartmann and Trumbore, 2016; Santos et al., 2016). A photosynthetic product plays an important role for vegetative and reproductive growth and affects the proportion of carbon and nitrogen (C:N) levels in plants, which indicates the stage of vegetative or reproductive growth. Increases in the C:N ratio of the plant indicate a change from the vegetative to the reproductive stage (Zotz, 1999).

The inflorescence of *Aranda ‘Noorah Alsagoff’* received 14C-assimilates from leaves along the stem indicating that the mature leaves of this orchid may have a storage function, while the fully expanded leaves may also be a major sink (Hew and Yong, 2004). The vegetative apical shoot competes with the inflorescence for assimilate supply. Upon removing the apical shoot, the flux of 14C-assimilates increased into the inflorescence. Conversely, the removal of the inflorescence led to distribution from tested leaves to the stem and roots. Talee (2008) reported that the starch concentrations of *R. gigantea* plants grown under SD conditions were less than those of plants grown under natural conditions. Carbohydrate use during the flowering period was reported to decrease after four weeks of SD treatments, increase during pre-inflorescence emergence, and then decrease again from flower blooming to senescence. Meanwhile, sugar concentrations increased at the beginning of treatments until inflorescence emerged and then slowly decreased during flowering until flower senescence. Similarly, the total non-structural carbohydrate (TNC) and decreasing sugar contents of *R. gigantea* (Lindl.) Ridl. *alba* leaves increased from the beginning of treatments to inflorescence emergence, then decreased until flower senescence (Watthanasrisong et al., 2010).

In addition, nitrogen (N) is a limiting factor of plant growth and development; a higher N content promotes better growth and quality, as reported in various orchid species (Poole and Seeley, 1978; Nashriyah et al., 2010). Phosphorous (P) is an energetic element that is relevant for all metabolic activities, and potassium (K) is involved in the mobility of elements and water, as well as stimulating enzyme activity for respiration and photosynthesis (Marschner, 2012). As well as influencing stem growth, an increased N level increases floral development, and the addition of P and K further increased both vegetative and reproductive growth (stem length, leaf production, inflorescence length, and flower size) in *Vanda ‘Miss Joaquim’* and *Aranda ‘Wendy Scott’* (Hew and Yong, 2004).

This study examined the effect of SD cycles on the flowering of *Vanda ‘Manuvadee’*, a strap-leafed *Vanda* hybrid, including the resulting TNC, N, P, and K levels, to better understand flowering induction and apply controls to flowering.

**Materials and Methods**

The experiment was conducted during June 2012 in Chiang Mai, Thailand. Natural conditions under 50% shade were 12 hour day length, 31°C and 25°C average day and night temperatures, respectively, and an average of 79% relative humidity. Three-year-old *Vanda ‘Manuvadee’* plants were grown in a greenhouse under a natural photoperiod as a control treatment (0W-SD). The treatment groups were grown under seven hour SD conditions for three, six, and nine weeks (3W-SD, 6W-SD, and 9W-SD) by covering the greenhouse with black cloth from 4 pm to am. Other conditions were 25 ± 2°C, and 70–80% relative humidity. After the SD treatments were completed, plants were transferred to grow under a natural photoperiod. During the experiment, plants were supplied weekly with 21-21-21 (N-P₂O₅-K₂O) fertilizer at an average of 0.44 g per plant by foliar feeding. Twenty plants per treatment were examined for flowering percentage, days to flowering, number of inflorescences and number of flowers, inflorescent length, longevities, and stem growth. The experimental design was a completely randomized design with four treatments.

Four plants from each treatment group were sampled in the morning for nutritional analysis at the ninth week after treatment (9 WAT) started. The detached stems, leaves, and inflorescences were washed under tap water, then deionized water, weighed before and after oven-drying at 70°C for 15 days, and then ground into fine powder. Each sample was digested by Kjeldahl method to determine total N content after modifying the indophenol method (Ohyama et al., 2004). Total P content was determined by ammonium molybdate method, and samples were digested by HNO₃ and HClO₄ for total K determination using an atomic absorption spectrophotometer (Ohyama et al., 1991). TNC analysis was by Nelson’s method (A.O.A.C., 1990). C/N balance was calculated by representing C concentration in millimoles of glucose and sucrose and total N was determined from millimolar concentrations of NO₃⁻ and NH₄⁺ (Zheng, 2009).

**Statistical analysis**

The experiment was performed in a completely randomized design. Results were expressed as means of values measured from at least three replicates using Statistix 8 analytical software (SXW Tallahassee, FL, USA). Least significant difference at \(P < 0.05\) was used
to determine significant difference in plant growth, flowering data, and concentration of TNC, N, P and K.

Results

Plant growth

The stem height of the control treatment group increased the most, while those of the SD treatment groups decreased with more cycles of SD, i.e., the 9W-SD treatment had the least stem height increase, followed by the 6W-SD, while that of the 3W-SD was not different from the control. The numbers of leaves did not significantly differ between the SD treatments and the control treatment (Table 1), however the 3W-SD and the 6W-SD treatments were different.

Flowering time and flower quality

The flowering times of the 6W-SD and 9W-SD treatments were significantly later than the control, by 27 and 38 days, respectively. The 3W-SD treatment was approximately 12 days later, which was not significantly different from the control (Table 2). All SD treatments led to 100% blooming of the first flower within 50–55 days, while those in the 0W-SD treatment bloomed gradually over 101 days.

The number of inflorescences per plant was significantly increased in the 9W-SD treatment compared to control, but it was not different for plants under the 3W-SD and the 6W-SD treatments. The numbers of flowers per plant of the 6W-SD and 9W-SD treatments, averaging 16.65 and 16.90 flowers per plant, respectively, were significantly higher than the control at 13.45 flowers per plant. However, the inflorescence sizes (data not shown) of each treatment were not significantly different, and were within the range of 45–47 cm spike lengths, 15–16 cm stalk lengths, and approximately 10 cm for flower diameters (Fig. 1). Moreover, the inflorescences of plants grown under 6W-SD and 9W-SD conditions had about 32 days longevity, and lasted longer than both the control and the 3W-SD treatments, for 28.1 and 27.4 days, respectively (Table 2).

Total nitrogen, phosphorous and potassium concentrations

In the ninth week, N concentrations in leaves of the SD treatments were 4.69–5.60 mg g⁻¹DW in all treat-
ments. N concentrations in *Vanda* grown under SD conditions were significantly different between treatments (Table 3). It was interesting that P concentrations in leaves increased in plants grown under 6W-SD, but not significantly different from 9W-SD. However, K concentrations were found to be higher in leaves than in stems; the highest value observed was in the 9W-SD treatment for both leaves and stems (Table 3).

**Total non-structural carbohydrate**

The TNC concentrations in leaves of *Vanda ‘Manuvadee’* were significantly different among treatments, but were not significantly different in the stems (Table 4). The control treatment, grown under the natural day length, had the highest TNC concentration at 94.90 mg·g⁻¹DW, and the 9W-SD treatment had the lowest at 72.85 mg·g⁻¹DW, while 3W-SD and 6W-SD did not significantly differ from either control or 9W-SD treatments. C/N balance in leaves was rather low; however, they were not significantly different among treatments.

**Correlation between parameters**

Positive correlations were found between the days to flower opening and the number of inflorescences as well as the number of flowers per plant and inflorescence longevity for short day cycles (Table 5). TNC concentrations had negative correlations with the days to flower opening and increasing number of leaves. However, TNC had positive correlations to increased stem height, while K concentration had positive correlations with the days to flower opening and number of inflorescences.

**Discussion**

The flowering times increased by increasing the short day cycles (Table 2), indicating that the SD cycle treatments affected delayed flowering. The SD condition is unfavorable when flowers may not initiate or develop to anthesis, but flowering was induced and then proceeded until blooming after transfer of the plants to natural conditions. The delaying period was beneficial in controlling flowering time by SD cycles. In addition, the SD treatments could induce 100% flower opening within a short period, about half the time of the control, which brought about flowering uniformity that may benefit commercial production. This may involve plants with a behavioral response to confront environmental conditions.
stress; plants could produce flowers rapidly whenever receiving the right factors (Thomas 1993; Hew and Yong, 2004). Similarly, Wang (1995) reported that when non-spiking Phalaenopsis plants in darkness treatments were transferred to a high enough light intensity most of them flowered in a shorter period.

Flower induction and development is sponsored by photosynthates (Hew and Yong, 2004), which can be investigated with TNC concentrations. In thick-leaved orchids, leaves are storage organs (Hew and Yong, 2004). Data showed that SD treatments decreased TNC accumulation in the leaves, and it decreased more with more SD cycles. Decreasing TNC accumulation may involve lower rates of photosynthesis or photosynthetic accumulation by plants under SD treatments compared to those under natural day lengths. On the other hand, photosynthates of plants under SD treatments may be used in other activities, such as vegetative growth or stress resistance. Similarly, Phalaenopsis plants without flowering had lower activities in CO₂ uptake for CAM photosynthesis than those with inflorescences (Ota et al., 1991). Lower TNC concentrations when there were more SD treatment cycles resulted in stem height decreasing. An increase in stem heights correlated with TNC concentration in leaves at nine weeks, which means the TNC concentration in the 0W-SD treatment resulted in the highest growth rate. However, the numbers of leaves were negatively correlated to TNC concentrations in stems, because most of the carbohydrates were used for leaf formation, and so remained lower in stems.

However, the flowering delay led to a carbohydrate accumulation period, which extended as the period of SD lengthened (6W-SD and the 9W-SD) and increased the numbers of inflorescences, numbers of florets per plant, and longevity of inflorescences (Table 2). Short day cycles resulted in a positive correlation between flowering times and number of inflorescences, number of flowers per plant and inflorescence longevity. Although carbohydrate plays an important role in the process of floral development, the highest inflorescence longevity was obtained from SD treatments. This result suggests that plants may adjust the starch degradation pattern under short day conditions. When plants confront shorter day-lengths, starch is slowly degraded during the night, to ensure the photosynthates availability for plant productivity (Gibon et al., 2004; Graf and Smith, 2011; Scialdone and Howard, 2015). Additionally, TNC concentrations had a negative correlation with the days to flower opening. These results clearly indicate that lower TNC concentration in the 6W-SD and the 9W-SD treatments extended the period of flower opening and inflorescence longevity. Besides the long period of carbohydrate accumulation, the better productivity may be because flowering was delayed and occurred at times when the conditions were optimal for flowering development and growth. In the ninth week, the amounts of carbohydrates for those treatments were more than the amounts of nitrogen, which showed their reproductive stage (Hew and Yong, 2004). However, C/N balance was not different among treatments, although the control tended to have the highest C/N balance.

N, P, and K are components of organic and inorganic substances, which are synthesized for metabolism and plant growth. N concentrations in both leaves and stem were not significantly different (Table 3). This confirmed the role of N in growth and metabolism, even an adequate N is important and has had a positive interaction with P and K in optimal flowering of Cattleya, Phalaenopsis and V. ratchaburi (Sheehan, 1960; Wang, 2000; Panjama, 2018b). P concentrations in leaves from the SD treatments were higher than the control. This may be due to plants grown under SD treatments using energetic substances for metabolism less than those in the non-SD treatment. The P compounds of SD treatments remained higher than in control treatments, while those in stems were not different. A similar result was found in the Rinchostele maculate orchid, P value in old leaves was decreased during plant flowering (Jiménez-Peña et al., 2018). Phosphorus is an essential element, and is an inorganic substance for energy metabolism and also the transfer of carbohydrates in leaf (Marschner, 2012). However, these results did not vary by cycles of SD. K concentrations were higher in leaves and stems. At the ninth week, plants from of the 9W-SD treatment had the highest K concentrations. This may be related to the plants under SD conditions for nine weeks having no flowering activity, while the other treatments did. Therefore, there was less transportation of nutritional substances and water, stimulation of enzyme activities for respiration, and a lower rate of photosynthesis, as previously discussed.

The results showed that a 7-hour photoperiod could depress and delay flowering of Vanda ‘Manuvadee’.

The delay durations depended on the number of cycles in the SD conditions. In this experiment, the first flower open was delayed in plants grown under the 6W and 9W-SD treatments. Moreover, 100% of the SD treatment flower openings were shorter than those of the non-SD treatment, and the longer SD cycles resulted in more inflorescences and higher longevities. Delayed flowering correlated with low photoassimilate accumulation, and SD treatments tended to have increased P and K concentrations. These results were useful for controlling flowering on demand and uniform flower production.

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**Literature Cited**

Arditti, J. 1992. Fundamentals of Orchid Biology. John Wiley & Sons, New York.

Association of Official Analytical Chemists (A.O.A.C.). 1990. Official Methods of Analytical Association of Official Analytical Chemists Inc., Virginia.

Gibon, Y., O. E. Bläsing, N. Palacios-Rojas, D. Pankovic, J. H. M. Hendriks, J. Fisahn, M. Höhne, M. Günther and M. Stitt. 2004. Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of adp-glucose pyrophosphorylase in the following light period. Plant J. 39: 847–862.

Glover, B. 2014. Understanding Flowers and Flowering. Oxford University Press, Oxford.

Goh, C. J. and J. Arditti. 1985. Orchidaceae. p. 309–336. In: A. H. Haley (ed.). Handbook of flowering. CRC press, Boca Raton, Florida.

Graf, A. and A. M. Smith. 2011. Starch and the clock: the dark side of plant productivity. Trends Plant Sci. 16: 169–175.

Grove, D. L. 1995. *Vandaceae* and *Ascosciaceae* and their combinations with other genera. Timber Press, Oregon.

Hartmann, H. and S. Trumbore. 2004. The physiology of tropical plants. Academic Press, MA, USA.

Hew, C. S. and J. W. H. Yong. 2004. The physiology of tropical orchids in relation to the industry. The World Scientific Publishing, Singapore.

Jiménez-Peña, N., L. I. Trejo-Téllez and P. Juárez-López. 2018. Concentration of macronutrients in organs of three wild *Rhynchostele* species in their natural habitat. Rev. Mex. Cienc. Agríc. 9: 971–980.

Lopez, R. G. and E. Runkle. 2005. Environmental physiology of growth and flowering of orchids. HortScience 40: 1969–1973.

Marschner, P. 2012. Marschner’s Mineral nutrition of higher plants. Academic Press, MA, USA.

Nashriyah, M., A. R. Shamsiah, S. Salmah, A. Maizatul, M. Y. Jamaliah and M. Mazleha. 2010. Growth and mineral content of *Mokara chark kuan* pink orchid as affected by allelopathic *Lantana camara* weed. World Acad. Sci. Eng. Technol. 48: 595–599.

Ohyama, T., M. Ito, K. Kobayashi, S. Araki, S. Yasuyoshi, O. Sasaki, T. Yamazaki, K. Sayoma, R. Tamemura, Y. Izuno and T. Ikarashi. 1991. Analytical procedures of N, P and K content in plant and manure materials using *H*<sub>2</sub>*SO*<sub>4</sub>–*H*<sub>2</sub>*O<sub>2</sub> Kjeldahl digestion method. Bull. Facult. Agric. Niigata Univ. 43: 111–120 (In Japanese with English abstract).

Ohyama, T., K. Tewari, S. Abdel-Latif, S. Ruanrungsri, S. Komiyama, M. Ito, A. Yamazaki, K. Sueyoshi and N. Ohtake. 2004. Direct analysis of *15N* abundance of Kjeldahl digested solution by emission spectrometry. Bull. Facul. Agric. Niigata Univ. 57: 33–40.

Ota, K., K. Morioka and Y. Yamamoto. 1991. Effects of leaf age, inflorescence, light intensity and moisture conditions on CAM photosynthesis in *Phalaenopsis*. J. Japan. Soc. Hort. Sci. 60: 125–132 (In Japanese with English abstract).

Panjama, K. 2018a. Molecular studies on *FLOWERING LOCUS T* gene in *Vanda* hybrid. Ph.D. Thesis. Niigata Univ., Niigata.

Panjama, K. 2018b. Nitrogen uptake and assimilation in *Vanda* hybrid. Ph.D. Thesis, Chiang Mai Univ., Chiang Mai.

Poole, H. A. and J. G. Seeley. 1978. Nitrogen potassium and magnesium nutrition of three orchid genera. J. Am. Soc. Hortic. Sci. 103: 485–488.

Santos, M. N. S., A. M. Mapeli and M. M. Tolentino. 2016. Carbohydrate metabolism in floral structures of *Lilium pumilum* in different development stages. Cienc. Rural 46: 1142–1144.

Scaldone, A. and M. Howard. 2015. How plants manage food reserves at night: Quantitative models and open questions. Front. Plant Sci. 6 (204).

Sheehan, T. J. 1960. Effects of nutrition and potting media on growth and flowering of certain epiphytic orchids. Proceedings of the Florida State Horticultural Society, 3rd World Orchid Conference: 211–218.

Talee, D. 2008. Effects of short day condition and gibberellic acid on off-season flowering of *Rhynchostylis gigantea* (Lindl.) Ridl. MS. Thesis. Chiang Mai Univ., Chiang Mai.

Thomas, B. 1993. The molecular biology of flowering. In: B. R. Jordan (ed.). Internal and external controls on flowering. Redwood Books, UK.

Thomas, B. and D. Vince-Prue. 1997. Photoperiodism in plants. Academic Press, Massachusetts.

Wang, Y. T. 1995. Phalaenopsis orchid light requirement during the induction of spiking. HortScience 30: 59–61.

Wang, Y. T. 2000. Impact of a high phosphorus fertilizer and timing of termination of fertilization on flowering of a hybrid *Moth* orchid. HortScience 35: 60–62.

Watthanasrisong, J., S. Ruanrungsri and N. Pothaporn. 2010. Effects of short day condition and low temperature on flowering of *Rhynchostylis gigantea* (Lindl.) Ridl. *alba*. Journal of Agricultural Research and Extension 27: 11–19.

Zheng, Z. L. 2009. Carbon and nitrogen nutrient balance signaling in plants. Plant Signal. Behav. 4: 584–591.

Zotz, G. 1999. What are back shoots good for? Seasonal changes in mineral, carbohydrate and water content of different organs of the epiphytic orchid, *Dimerandra emarginata*. Ann. Bot. 84: 791–798.