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Time of Flowering and Quality of Flowers in Thai Commercial Chrysanthemums as Affected by Photoperiod and high night temperature

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Abstract

Chrysanthemum is a facultative short-day plant with the tendency to flower late when night temperatures are high. Therefore, year-round cultivation of high-quality chrysanthemums in Thailand is not desirable. Some day-neutral chrysanthemum cultivars are insensitive to heat-delay phenotype. Consequently, the discovery of these key characteristics through the planting of many cultivars can be the key to enhancing the breeding programme. Eleven Thai chrysanthemum cultivars were selected and planted under various conditions, including an average day length of 11.47 hours (SD), long days with incandescent light night break (LD1), and long days with incandescent light night break with a night temperature of 30.23 °C (LD2). The number of leaves on the main stem at flowering, the length of the main stem at flowering, the diameter of the terminal flower, the number of LD leaves, the ratio of flowering to nonflowering plants, and the thermostability of cell membrane were measured and analysed. By removing the light integral factor, all cultivars in this study were able to flower in both SD and LD conditions. The effects of different photoperiods and night temperatures on flowering time and floral quality varied amongst cultivars. Each cultivar, especially 'Kaewglom', exhibited a unique pattern of sensitivity, which benefits the selection for a new breeding programme to create new day-neutral and heat-tolerant cultivars more suitable for the cut flower market.

Keywords: Chrysanthemum, cell membrane thermostability, heat tolerance, day neutral

Introduction

Because of its considerable export value, chrysanthemum is a globally economically important ornamental plant. The auction on the flower market in the Netherlands ranks it among the best in the world (Nissim-Levi *et al*., 2019). This flower is a facultative short-day plant (SDP) that requires less than 13.5 hours of daily photoperiod to flower (Park and Jeong, 2020). Therefore, chrysanthemum production is restricted and depends on the environment. Light supplement (day-length extension or night interruption) or black cloth (night prolong) treatment is used to adjust the optimal flowering time for cut flower production (Belay *et al*., 2021). Due to the fluctuation in temperature during treatment, the procedure controlling flowering time is both expensive and detrimental to flower quality.

In Thailand, chrysanthemums cannot be grown adequately during the summer, which is the peak season for this flower's demand (Chomchalow, 2004). In addition to environmental control, breeding day-neutral (DN) and heat-tolerant chrysanthemums is an excellent solution to the production problem. The day-neutral chrysanthemum exists, but it is relatively rare, and its characteristics are not yet appropriate for marketing as cut flowers (Anderson, 2007). Interestingly, day-neutral chrysanthemums also demonstrate heat tolerance (Cockshull, 2019). This information highlights the possibilities of improving day-neutral and heat-tolerant chrysanthemum breeding in the near future.

Although there were numerous breeding initiatives to improve chrysanthemum characteristics in Thailand and some DN varieties were established (Krasaechai and Senawong, 2003) additional DN and heat-tolerant cultivars are still required to improve the phenotype for the market (Nakano *et al*., 2015; Mekapogu *et al*., 2022). Unfortunately, information regarding the flowering time and heat tolerance of chrysanthemum cultivars in Thailand is still unclear (Krasaechai and Senawong, 2003) due to the random import of commercial types from Europe, the United States, and Japan and the breeding of varieties to fulfil market demand (Komkris, 1964; Krasaechai and Senawong, 2003). To facilitate chrysanthemum breeding in Thailand, information on each existing cultivar is essential, as the response of chrysanthemums to environmental conditions is immensely complicated and may vary among species. This study intends to present a flowering time and heat tolerance analysis of each eleven commercially available chrysanthemum cultivar in Thailand under different light and high night temperature conditions. And it will benefit the selection of cultivars for a new breeding programme.

Materials and Methods

Plant materials

The eleven cultivars of commercial chrysanthemum in Thailand were selected with the collaboration of Maejo University's Office of Agricultural Research and Extension in Chiang Mai, Thailand. All cultivars included 'Mona', 'Khao sai kaew', 'Puma', 'Old rose', 'Banyen', 'Muangson', 'Som lychee', 'Rumet', 'Khao plai chompu', 'Khaoson', and 'Kaewglom'. There were variants in petal colour as follows: white, white with a green centre, white, pink orange, dark pink, purple, yellow with an orange centre, yellow, white with a pink tip, white, and light green (Figure 1). All plantlets utilised in this study were derived from tissue culture of shoot tips. Following rooting, the seedlings were brought to the greenhouse for one week of acclimatization. After thirty days, identical plants were chosen for two experiments. The first experiment was an evaluation of the cell membrane thermostability (CMT) in which plants were employed directly. For the second experiment, the flowering time, each plant was placed in a six-inch pot containing loam soil, manure, and rice husk in equal amounts.

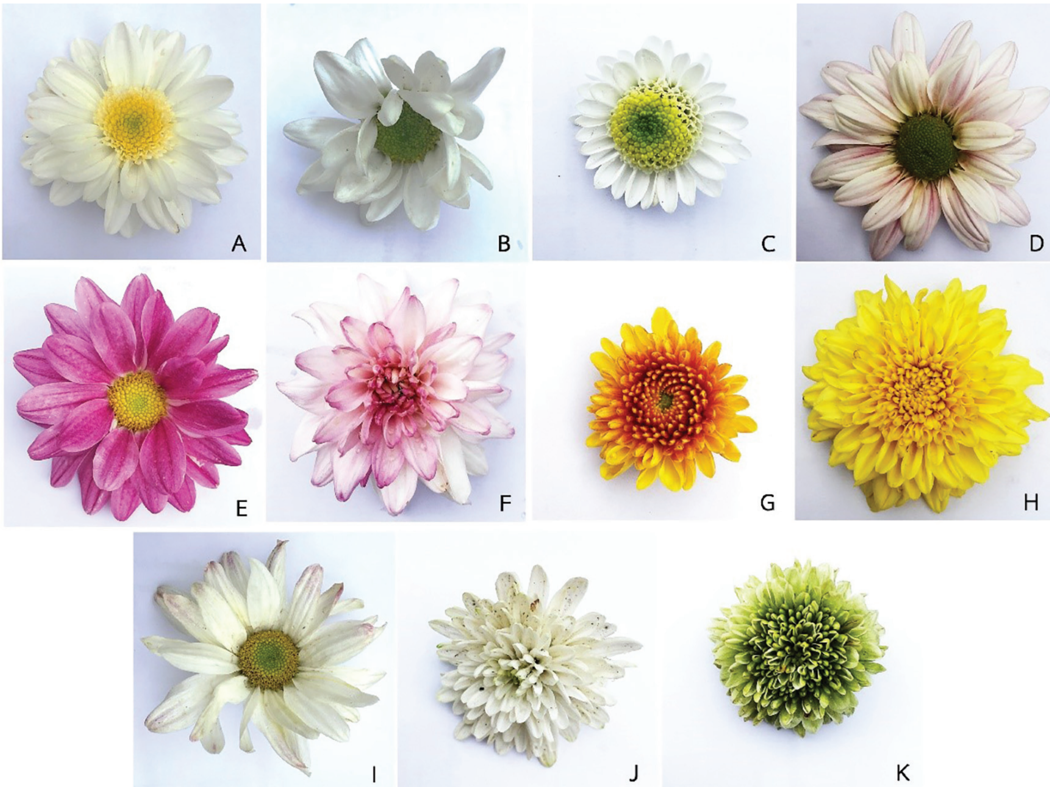

Figure 1 The selected cultivars in this study: 'Mona' (A), 'Khao sai kaew' (B), 'Puma' (C), 'Old rose' (D), 'Banyen' (E), 'Muangson' (F), 'Som lychee' (G), 'Rumet' (H), 'Khao plai chompu' (I), 'Khaoson' (J), and 'Kaewglom' (K)

The first experiment: cell membrane thermostability

The eleven tissue-cultured chrysanthemum cultivars were utilised immediately after acclimatization using a methodology modified from Yeh and Lin (2003). Each sample contains ten leaf discs with a diameter of 8 mm. Leaf discs were placed in a test tube containing 17 ml of distilled water. The measurement was performed using seven replicates for each sample. All tubes were placed in a water bath at several temperatures for 30 minutes: 30, 35, 40, 45, 50, 55, 60, 65, and 70 °C. The control condition was 25 °C. The tubes were then incubated for 16 hours at 25 °C in the dark. After that, the first solution conductivity (EC1) of each sample was determined. Following the measurement, all samples were autoclaved at 121 °C for 15 minutes and then incubated at 25 °C for 24 hours. The second solution conductivity (EC2) was then measured with seven replicates for each sample. Using the following equations, the relative injury (RI) at 25 and 50 °C and the calibrated RI were determined.

RI at 25 and 50 °C (%) = (EC1/EC2) × 100

Calibrated RI (%) = {1− [1 − (EC1/EC2)]/[1 − ($EC1_{control}$/$EC2_{control}$)]} × 100

EC1, EC2, $EC1_{control}$, and $EC2_{control}$ refer to the first sample solution conductivity, the second sample solution conductivity, the first control solution conductivity, the second control solution conductivity, respectively.

The second experiment: evaluation of flowering time under different light and night temperature conditions

The experiment was conducted in a greenhouse in Thailand from January to March 2022, during the chrysanthemum growing season, with an average photoperiod of 11.47 hours (Time and Date AS, 2022), which did not exceed the chrysanthemum's critical photoperiod of 13.5 hours. To evaluate the flowering time in response to light period and high night temperature, seven replications of each treatment were conducted under three distinct conditions in a completely randomised design (CRD). The initial condition served as a control. During the growing season, without light or temperature treatment, the state was SD. The following was LD1. This long day condition comprised a 3-hour night interruption (8 to 11 PM) using tungsten lamps (Philips 32W, NL) to lengthen the photoperiod without increasing light integral. LD2 was the third condition. In addition to the 3-hour night interruption, the vertical heater (Xiaomi Inc., China) was used for six hours (11 PM to 5 AM) to enhance the night temperature. In Table 1, the information on each treatment is supplied. Neither growth regulator administration nor shoot pinching occurred during the trial. Since the purpose of this experiment was to examine the responses of plants to LD and high night temperature, plants were kept in each environmental condition until the end of the experiment. The flowering time data was collected throughout 12 weeks. Such information included the number of leaves on the main stem at flowering and the diameter of the terminal flower.

Table 1 The light and temperature conditions of each treatment

Treatment	Light integral of 3h photoperiod extension by tungsten bulbs (μmol s^{-2} m^{-1})	Average day temperature (°C)	Average night temperature (°C)
SD	N/A	26.84	17.79
LD1	6.14	26.61	18.11
LD2	5.89	26.42	30.23

Remarks: Abbreviations: SD, Short day condition without light or temperature treatment; LD1, Long day condition with a 3-hour night interruption; LD2, Long day condition with a 3-hour night interruption and a 6-hour hot wind treatment

Statistical analysis

This experiment was carried out with a completely randomised design (CRD). The factor variable was cultivar, with SD, LD1, and LD2 as the dependent variables. To assess how each cultivar responds to each environmental condition, one-way ANOVA and the TUKEY test (Assaad *et al*., 2014) were applied separately to determine the statistical significance between the mean response values of each cultivar to each condition. The results were then put in a single table for presentation purposes.

Results and Discussion

Flowering time

The leaf number on main stem at flowering (Table 2) determines the flowering time. This number is more accurate than the number of days to flower (Adams *et al*., 2003). It represents the number of leaves a plant needs to generate flowers. The longer it takes to develop leaves, the longer it takes for the plant to flower (Adams *et al*., 2003). Interestingly, all eleven cultivars were able to flower under LD conditions. It is possible for a plant's photoperiod response to change because of alterations in environmental conditions or gene expression (Mizoguchi *et al*., 2007). Thus, SD plants that can produce flowers under LD could have DN response. Therefore, all cultivars exhibited a facultative SD response and the capacity to develop a DN response. Even though LD is an unfavourable condition, flowering may be triggered by an autonomous pathway in plants that tend to commence flowering at the appropriate time (Sumitomo *et al*., 2014). However, different types of night break lighting can produce diverse flowering responses (SharathKumar *et al*., 2021). This observation is intriguing and suggests that light integral rather than photoperiod may be the additional factor determining flowering in chrysanthemums. Furthermore, additional research is required to clarify this issue.

The SD-grown plant required the lowest leaves at flowering within all cultivars. Average leaf count ranged from 33.8 (‘Banyen’) to 47.4 (‘Khao sai kaew’) leaves (Table 2). Most cultivars flower faster under SD, LD1, and LD2 conditions, respectively, except for ‘Puma’, ‘Muangson’, and ‘Khaoson’ whose flowering time under SD and LD1 conditions did not differ significantly, and both conditions flower earlier than LD2. Since chrysanthemum is a facultative SD plant, LD may not be a significant restriction to flowering because it can still generate flowering despite the longer period. In addition, the high night temperature is an important environmental issue to consider. Additionally, night temperature is critical in regulating flowering time (Cho and Kim, 2021; Nakano *et al*., 2020). Consequently, the impact of flowering time delay is more obvious under LD2.

The response in ‘Kaewglom’ is remarkable. It flowered the quickest in the SD condition but did not differ significantly between the LD1 and LD2 conditions, which have different night temperatures. It indicates that ‘Kaewglom’ may not be affected by high night temperatures, making it a contender for a heat-tolerant cultivar. In this study, the photoperiod response of chrysanthemum varied across all cultivars examined.

Table 2 The number of leaves at flowering for 11 cultivars grown under different photoperiod and night temperature conditions

Cultivar	SD	CV (%)	LD1	CV (%)	LD2	CV (%)
‘Mona’	41.3 ± 0.41^{c}	0.99	44.8 ± 0.54^{b}	1.21	74.2 ± 0.48^{a}	0.65
‘Khao sai kaew’	47.4 ± 0.34^{c}	0.72	52.4 ± 0.35^{b}	0.67	90.7 ± 0.4^{a}	0.44
‘Puma’	45.2 ± 0.25^{b}	0.55	44.5 ± 0.40^{b}	0.90	64.8 ± 0.4^{a}	0.62
‘Old rose’	47 ± 0.35^{c}	0.74	52.4 ± 0.66^{b}	1.26	72.9 ± 0.57^{a}	0.78
‘Banyen’	33.8 ± 0.25^{c}	0.74	36 ± 0.27^{b}	0.75	75.2 ± 0.35^{a}	0.47
‘Muangson’	38.6 ± 0.3^{b}	0.78	38.7 ± 0.6^{b}	1.55	65.4 ± 0.35^{a}	0.54
‘Som lychee’	40.4 ± 0.37^{c}	0.92	49.4 ± 0.49^{b}	0.99	77.2 ± 0.48^{a}	0.62
‘Rumet’	43.8 ± 0.25^{c}	0.57	47 ± 0.59^{b}	1.26	73 ± 0.55^{a}	0.75
‘Khao plai chompu’	41.1 ± 0.27^{c}	0.66	44.8 ± 0.22^{b}	0.49	70 ± 0.31^{a}	0.44
‘Khaoson’	44.2 ± 0.58^{b}	1.31	44.4 ± 0.34^{b}	0.77	74.4 ± 0.47^{a}	0.63
‘Kaewglom’	38.5 ± 0.27^{b}	0.7	41.4 ± 0.39^{a}	0.94	41.8 ± 0.32^{a}	0.77

Remarks: Values are means ± SEM, n = 7 per treatment group. Means in a column without a common superscript letter differ (p<0.05) as analysed by one-way ANOVA and the TUKEY test

Cell membrane thermostability

Cell membrane thermostability (CMT) is a widely accepted assessment of a plant's heat tolerance. This method evaluates the electrolyte leakage from leaf materials when exposed to high temperatures (Anderson *et al*., 2016). In Taiwan, the United States, and South Korea (Anderson *et al*., 2016), chrysanthemum germplasm is screened for its heat tolerance phenotype using this technique. In this study, the CMT results of different cultivars were compared (Table 3). At 25 °C, the relative injury (RI) was not as severe. When the temperature was raised to 50 °C, the RI pattern started to become more diverse. The lowest was 'Kaewglom', while the highest was 'Khao sai kaew'. This information was connected with the flowering time data when 'Khao sai kaew' was the cultivar requiring more time to flower. Although 'Kaewglom' was not the cultivar with the shortest flowering time, the lack of a statistically significant difference in flowering time between LD1 and LD2 for this cultivar indicated that high night temperatures did not have the same effect on flowering time as they did for other cultivars. The CMT results confirm that 'Kaewglom' can flower under high night temperatures because it exhibits heat tolerance.

Table 3 The comparison of 11 chrysanthemum cultivars Relative Injury (%) at 25 and 50°C

Cultivar	Relative Injury (%)					
	25 °C	CV (%)	50 °C	CV (%)	Calibrated	CV (%)
'Mona'	14.4±0.19^{b}	1.32	65.2±0.57^{cd}	0.87	59.4±0.62^{cd}	1.04
'Khao sai kaew'	15.5±0.16^{a}	1.03	85.3±0.37^{a}	0.43	82.6±0.47^{a}	0.57
'Puma'	13.3±0.13^{c}	0.98	68.3±1.26^{bc}	1.84	63.5±1.43^{bc}	2.25
'Old rose'	14.5±0.12^{ab}	0.83	69.8±0.68^{b}	0.97	64.6±0.79^{bc}	1.22
'Ban yen'	14.2±0.24^{bc}	1.69	70.1±0.53^{b}	0.76	65.2±0.66^{b}	1.01
'Muang son'	13.7±0.09^{bc}	0.66	59.7±1.06^{e}	1.78	53.3±1.25^{e}	2.35
'Som lychee'	14.1±0.24^{bc}	1.70	62.6±1.09^{de}	1.74	56.4±1.39^{de}	2.46
'Rumet'	13.9±0.14^{bc}	1.01	59.9±0.09^{e}	0.15	53.5±0.18^{e}	0.34
'Khao plai chompu'	14±0.38^{bc}	2.71	70±0.91^{b}	1.30	65.1±1.15^{b}	1.77
'Khao son'	14.1±0.07^{bc}	0.50	68.3±1.41^{bc}	2.06	63±1.68^{bc}	2.67
'Kaewglom'	12.3±0.19^{d}	1.54	41±0.78^{f}	1.90	32.7±0.95^{f}	2.91

Remarks: Values are means ± SEM, n = 7 per treatment group. Means in a column without a common superscript letter differ ($p<0.05$) as analysed by one-way ANOVA and the TUKEY test

Flower size

The diameter of the flower can indicate the quality of the inflorescence. Multiple light conditions altered the size of flowers. Low light intensity and high temperature can reduce the size of chrysanthemum flowers (Nothnagl *et al*., 2004). This study measured the apical flower. Each cultivar had unique floral characteristics (Table 4). The flower sizes of 'Khao sai kaew', 'Old rose', 'Banyen', 'Muangson', and 'Khao plai chompu' varied greatly depending on the light treatment. Flower size was largest under SD, followed by LD1 and LD2. It suggests that longer photoperiod diminishes flower size and that high night temperatures amplify this effect.

The flower size of 'Som lychee' and 'Rumet' was largest under SD and reduced when treated with both LD conditions. Since there was no significant difference in flower size between LD1 and LD2, high night temperatures do not affect the flower size of 'Som lychee' and 'Rumet'. Intriguingly, the largest and smallest 'Kaewglom' flower sizes were identified in the LD2 and LD1 conditions, respectively. Still, the size found in the SD condition was not statistically different from either of the LD conditions. Unlike other cultivars, long photoperiods and high night temperature had no negative influence on flower diameter in this cultivar. In addition, neither photoperiod nor high night temperature impacted the size of 'Mona' and 'Puma' flowers. The reaction of various photoperiods and night temperature on flower size differs among cultivars, as previously demonstrated (Palai *et al*., 2018). These data indicate that flower sensitivity to light and temperature is encouraging. Although there were diverse reactions, photoperiod and temperature changes did not diminish the flower quality of some cultivars. It is interesting to use this information to develop a chrysanthemum cultivar that can yield flowers of high quality despite adverse environmental conditions.

Table 4 The Diameter of terminal flower for 11 cultivars grown under different photoperiod and night temperature conditions

Cultivar	Diameter (cm)					
	SD	CV (%)	LD1	CV (%)	LD2	CV (%)
'Mona'	6.55±0.0875	1.34	4.16±0.0545	1.31	6.28±2.17	34.55
'Khao sai kaew'	5.79±0.0665^{a}	1.15	5.11±0.0776^{b}	1.52	3.98±0.0658^{c}	1.65
'Puma'	4.26±0.115	2.70	4.33±0.0974	2.25	4.35±0.08	1.84
'Old rose'	6.88±0.0793^{a}	1.15	5.12±0.0547^{b}	1.07	4.8±0.0861^{c}	1.79
'Banyen'	6.02±0.0499^{a}	0.83	4.82±0.0647^{b}	1.34	4.05±0.0536^{c}	1.32
'Muangson'	7.74±0.169^{a}	2.18	5.86±0.0752^{b}	1.28	4.3±0.0634^{c}	1.47
'Som lychee'	6±0.0404^{a}	0.67	3.85±0.055^{b}	1.43	3.97±0.0572^{b}	1.44
'Rumet'	7.07±0.0562^{a}	0.79	4.74±0.0881^{b}	1.86	4.84±0.0455^{b}	0.94
'Khao plai chompu'	6.96±0.042^{a}	0.60	4.98±0.0213^{b}	0.43	4.57±0.0711^{c}	1.56
'Khaoson'	7.64±0.0773^{a}	1.01	3.93±0.0424^{c}	1.08	4.32±0.0756^{b}	1.75
'Kaewglom'	4.07±0.0293^{ab}	0.72	3.94±0.0413^{b}	1.05	4.14±0.0473^{a}	1.14

Remarks: Values are means ± SEM, n = 7 per treatment group. Means in a row without a common superscript letter differ (p<0.05) as analysed by one-way ANOVA and the TUKEY test

Conclusion

All eleven cultivars could be DN-responsive because each cultivar produced flowers in both SD and LD, despite their differing time to flower. The rapidity of flowering is proportional to the heat tolerance of the cultivar. 'Kaewglom' is outstanding. This cultivar exhibited heat tolerance based on its CMT value, which correlated with flowering time so that high night temperatures did not affect the flowering time. The ability of numerous cultivars in this study, such as 'Som lychee' and 'Rumet', to sustain flower size under high night temperatures is a positive sign. And 'Mona', 'Puma', and 'Kaewglom' maintain good size quality despite the long photoperiod and high night temperatures. The information gathered for each cultivar is highly valuable for selecting Thai cultivars in breeding programmes to improve the chrysanthemum's heat resistance and day neutrality.

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