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Chlorophyll Fluorescence Parameter as a Tool in Selecting Heat-tolerant Summer-flowering Chrysanthemum (*Dendranthema* ×*grandiflorum*)

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Abstract. **Chrysanthemum 'Bai Tian Xing', 'Huang Ching Chin', 'Pink Pearl', and 'NCHU-001' plants were preheated at 35 °C for 24 hours to induce heat tolerance. The recently fully expanded leaves were detached, kept in a moist Ziploc bag, and then subjected to 35, 40, 45, 47.5, 50, 52.5, 55, 60, or 65 °C for 20 minutes. After dark-acclimatized at room temperature for 30 minutes, leaves were measured for Fv/Fm value with a chlorophyll fluorescence parameter. Results showed that 'Bai Tian Xing' had the highest critical (T_{crit}) and midpoint temperature (T_{mid}). Mean T_{crit} and T_{mid} were shown to be 47 and 50 °C, respectively, and T_{mid} gave greater distinguishment of Fv/Fm value among cultivars. Plants of four cultivars were acclimatized at 15 to 40 °C for 3 days and 35 °C being the most effective temperature to induce a heat-tolerant response in chrysanthemum. Required inducing time to reach a stable leaf Fv/Fm value ranged from 4.6 to 11.1 hours among cultivars. All cultivars had similar required time to reach visible bud between summer and autumn crops (except NCHU-001), but all had delayed flowering in the summer crop. There is a negative linear relationship between flowering heat delay and leaf Fv/Fm value (R^2 = 0.93). Progenies from reciprocal crossing of 'Bai Tian Xing' × 'NCHU-001' and 'Huang Ching Chin' × 'Pink Pearl' were also subjected to treatments for Fv/Fm measurements and observed for time to flowering in the summer crop. All combinations showed negative linear relationship between time to flowering and leaf Fv/Fm value (R^2 = 0.70–0.87). Two plants, 109-W001Y and 109-W003Pi, showed early flowering habit and good flower performance under heat conditions were selected. All four cultivars and the two selected lines were measured for photosynthetic parameters under day/night temperatures of 35/30 or 25/20 °C in growth chambers. All cultivars and lines showed decreased net photosynthetic rate and dark respiration rate under 35/30 °C when compared with 25/20 °C. Relatively higher net photosynthetic rate and lower dark respiration rate in 'Bai Tian Xing', '109-W001Y', and '109-W003Pi' under 35/30 °C, when compared with the other three cultivars, might have contributed to better flowering performance in the summer.**

Chrysanthemum (*Dendranthema* ×*grandiflorum*) is an important flower crop around the world (AstuteAnalytica 2022). As global warming and higher energy cost become an issue for precise greenhouse production, it might be crucial to select chrysanthemum cultivars with better heat tolerance to fit in summer or warmer-sites production requirements. For conventional chrysanthemum cultivars, the critical photoperiod is 12 h or less for reproductive growth and 14 h or more for best vegetative growth (Cockshull 1985). However, longer critical photoperiodic or day-neutral breeding lines exist (Anderson and Ascher 2001). Apart from photoperiod, flower initiation and development for chrysanthemum are temperature dependent. The optimum average daily temperature for the most rapid flowering was ca. 19 to 23 °C (Karlsson et al. 1989; Pearson et al. 1993). The base and maximal temperatures for flowering have not yet been defined, while flowering was delayed at 10 °C or 25 to 30 °C, as compared with those at 16 to 20 °C (Karlsson et al. 1989). Heat stress is known to cause delayed flowering and decreased flower size in chrysanthemum (Nakano et al. 2013). Heat-tolerant cultivars are currently available for summer production in greenhouses or under tropical field conditions (Wang et al. 2008).

Membrane stability, as revealed by electrolyte leakage, could be a selective index for heat tolerance in chrysanthemum (Wang et al. 2008). Later, Wang and Yeh (2013) showed that early selection of chrysanthemum seedlings using relative injury value as an index is feasible to obtaining plants that flowered earlier in the summer. However, assessing heat tolerance by measuring electrolyte leakage is still labor-consuming and destructive.

Chlorophyll fluorescence is an intriguing tool that can assess photosynthetic performance and monitor plant response to the environmental stress (Adams and Demmig-Adams 2004). The excess energy intercepted by a leaf is diffused in forms of heat and fluorescence (Murchie and Lawson 2013). The Fv/Fm (potential photochemical efficiency of open photosystem II, PS II) was used successfully for screening for traits, based on basic physiological capacity, related to stress tolerance, including heat-tolerant wheat cultivars (Sharma et al. 2012, 2014). Janka et al. (2015) showed that both irradiance and temperature have significant adverse effect on Fv/Fm in chrysanthemum 'Coral Charm' at temperatures over 28 °C. Bahrami et al. (2019) showed that grain yield loss caused by heat in barley was inversely correlated with Fv/Fm. Bhattarai et al. (2021), however, demonstrated that Fv/Fm was only negatively related to heat injury index (thermal injury symptoms) but not to tomato field yield, whereas electrolyte leakage was negatively related to actual field yield. Therefore, proving that Fv/Fm value is useful in discriminating chrysanthemum cultivars with different heat sensitivity of flowering is necessary before applying in the breeding program.

Because thermotolerance at vegetative and reproductive stages is generally well-correlated in chrysanthemum (Wang et al. 2008), the present study examined heat tolerance in leaves taken from chrysanthemum progenies when young and using leaf Fv/Fm as an early selection indicator. Chrysanthemum cultivars with known difference in heat tolerance were investigated for short- to midterm Fv/Fm response as previously described (Sharma et al. 2012, 2014). Also, plants were grown in the greenhouse in the summer and autumn to investigate flowering heat delay. Second, crosses between cultivars were made and progenies, with heat tolerance unknown, were selected based on Fv/Fm value after heat treatments to verify the established criteria are valid. Last, selected plants and parental cultivars were measured for photosynthetic parameters under day/night temperatures of 25/20 and 35/30 °C.

Materials and Methods

Plant material. Cuttings of 'Bai Tian Xing' and 'Huang Ching Chin' were purchased from a local grower and 'Pink Pearl' and 'NCHU-001', bred by our laboratory, were harvested. Mother plants were weekly fertilized with water-soluble 20N–8.8P–16.6K fertilizer (Peters 20–20–20; Scotts, Marysville, OH, USA) at 1 g·L^{-1}. Night break was given from 2200 HR to 0200 HR by light-emitting diode (LED) bulbs (A21-NL/12W; Aprico, Taichung, Taiwan) supplying 2 ± 0.5 μmol·m^{-2}·s^{-1} photosynthetic photon flux density (*PPFD*) at canopy height. Cutting propagation was performed on mist beds inside a rain-proof net house. Mist was provided every 30 min for 45 s. The same night break was

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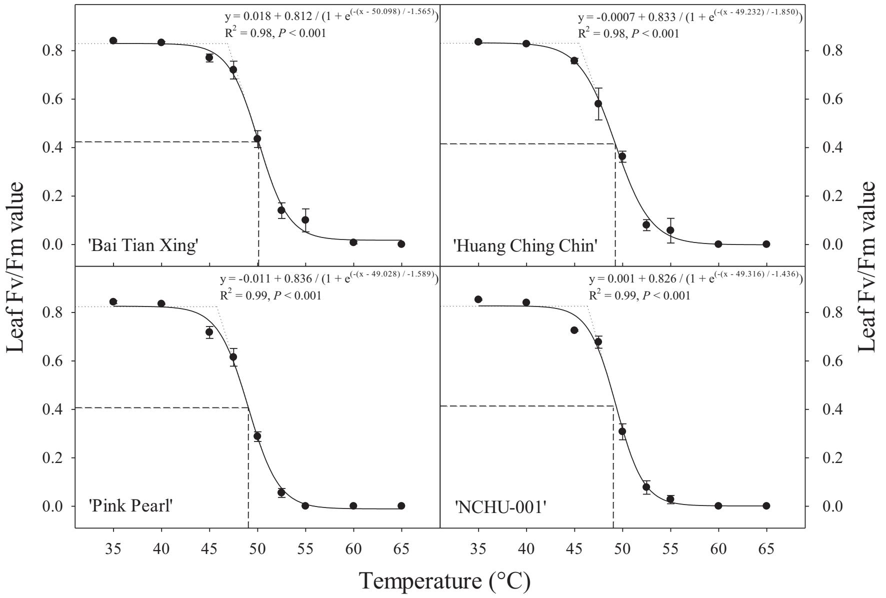

Fig. 1. Effect of heat stress temperature on leaf Fv/Fm value of three chrysanthemum cultivars and one line. Critical and midpoint temperature were drawn with dotted and short dashed lines, respectively. Critical temperature is the intersection of maximum Fv/Fm value by regression and slope around the inflection point of the S-curve. Bars indicate standard error of the mean (n = 4).

given during propagation and after potting but at 4 ± 2.5 μmol·m^{-2}·s^{-1} *PPFD*. Rooted cuttings were then planted in 9-cm diameter pots containing 3 peatmoss (Base substrate; Klasmann-Deilmann, Geeste, Germany): 1 perlite (Perligran® Premium; KNAUF, Oosterhout, Netherlands). Average noon time light intensity was 800 μmol·m^{-2}·s^{-1} *PPFD*. Plants were supplied with 3 g of 14N–4.8P–10.8K slow release fertilizer (Hi-Control® 14–11–13; JCAM-AGRI, Shizuoka, Japan) per pot at 3 d after potting and weekly fertilized with the water-soluble 20N–8.8P–16.6K fertilizer at 1 g·L^{-1}.

Determination of Fv/Fm value and heat tolerance inducing treatments. Plants were checked by measuring the most recently fully expanded leaves with a portable chlorophyll fluorescence meter (FP110; Photon Systems Instruments, Drasov, Czech Republic) before subjecting them to various temperature treatments. Leaves were dark-acclimatized with a leaf clipper for 20 min before measurements were taken. Plants with Fv/Fm values lower than 0.83 were excluded from subsequent experiments.

Plants potted for 42 d were moved into a 35 °C growth chamber for 24 h to induce heat-tolerant response. Photoperiod was set as 14 h (0800–2200 HR), providing 300 ± 50 μmol·m^{-2}·s^{-1} *PPFD* at plant height. Following the method proposed by Sharma et al. (2012) to fulfill large-scale application, recently fully expanded leaves (ca. the fourth leaf from the apex) were detached and kept in moist Ziploc bags to prevent water loss. Ziploc bags were gently pressed to exclude the excess air. Leaf samples were then transferred into an oven set at 35, 40, 45, 47.5, 50, 52.5, 55, 60, or 65 °C for 20 min. Samples were then returned to room temperature (25 °C) in the dark for 30 min. Fv/Fm measurement was taken on the adaxial surface at the first leaf lobe near the main leaf vein but excluding the vein. Each cultivar and each temperature treatment consists of four leaf samples and each sample from a different plant. Data were graphed and regressed using the following equation:

$$y = y_0 + a/(1 + e^{(-(x-x0)/-b)}),$$

where inflection point (mid-point, T_{mid}) of the S-curve is when $x = x_0$. Critical temperature (T_{crit}) was calculated following Slot et al. (2019). T_{crit} is the intersection of the maximum Fv/Fm value by regression and linear slope around the inflection point (±1.5 °C).

Another group of plants was moved from the net house to growth chambers set at 15, 20, 25, 30, 35, or 40 °C in the morning on a sunny day to clarify better heat tolerance inducing temperature. Growth chambers were set as 16 h photoperiod, providing 300 ± 50 μmol·m^{-2}·s^{-1} *PPFD* at plant height by LED lighting (LSF035–300AAD; China Electric Mfg. Co., Taipei, Taiwan). After acclimatizing for 3 d, leaf samples were collected and heat stressed at 50 °C for 20 min. Fv/Fm value was determined after being returned to room temperature for 30 min in the dark.

A third group of plants was moved from the net house to a 35 °C growth chamber with 24 h lighting (300 ± 50 μmol·m^{-2}·s^{-1} *PPFD*) to observe for gradual changes in leaf Fv/Fm value and to determine the sufficient heat tolerance inducing period. Leaf samples were collected at 0.5, 1, 2, 4, 6, 12, and 24 h after 35 °C treatment, underwent the same heat stress treatment and dark-acclimatization process, then measured for Fv/Fm value. Data were graphed and regressed using the following equation:

$$y = y_0 + a \times (1 - e^{(-bx)}).$$

The stable Fv/Fm value is calculated as 95% of the maximum y ($y' = 95\% \times$ maximum y) and time required to reach stable Fv/Fm value is then calculated using y'.

Determination of flowering heat delay. Rooted cuttings at 21 d after propagation with a sturdy root ball were planted in a plastic tunnel on 2 Jun 2021 (summer crop). Soil was amended with rice husks and ground corn stalk 3 months before planting at 5000 and 30,000 kg·ha^{-1}, respectively. Plots were set as 80 cm wide and 10 cm high, whereas rows and plant spacing were 10 cm wide. Flower net was laid out and frequently adjusted according to plant height to prevent lodging. Each cultivar consisted of at least six plants. Daylength during the experimental period was 13–14 h. Average noon time light intensity was 1400 to 1600 μmol·m^{-2}·s^{-1} *PPFD*. Air temperature was recorded. Each plant was weekly fertilized with ca. 100 mL of water-soluble 20N–8.8P–16.6K fertilizer at 1 g·L^{-1}. Pest and disease control were done as commercially suggested. Plants were observed for apical development at 2-d intervals. Lateral buds were removed when terminal bud width was ~1 cm, because these cultivars were selected as standard cut chrysanthemums. Days to visible bud (terminal buds turn into apparent flower buds and diameter is less than 3 mm) and flowering (fully expanded outermost petals) were recorded. The autumn crop was planted on 14 Sep 2021. Plant management and replication were the same as the summer crop. Daylength during the autumn experiment was 11 to 12.1 h. Average noon time light intensity was 1000 to 1200 μmol·m^{-2}·s^{-1} *PPFD*. Air temperature was also recorded. Mean day/night temperatures for summer and autumn crops were 35 to 37/21 to 26 °C and 31 to 36/15 to 21 °C, respectively. Relationship between flower heat delay (difference in days to flowering between summer and autumn crops) and Fv/Fm measurements was analyzed.

To verify that leaf Fv/Fm value is valid in selecting heat-tolerant progenies in the segregating generation, reciprocal crossing of ‘Bai Tian Xing’ × ‘NCHU-001’ and ‘Huang Ching

Table 1. Critical temperature (T_{crit}) and midpoint temperature (T_{mid}) as determined by S-curve and leaf Fv/Fm values after heat stress of three chrysanthemum cultivars and one line.

Cultivars/line	T_{crit} (°C)	T_{mid} (°C)	Fv/Fm at 47.5 °C[i]	Fv/Fm at 50 °C
Bai Tian Xing	47.2	50.1	0.72 a[ii]	0.44 a
Huang Ching Chin	45.8	49.2	0.58 b	0.36 ab
Pink Pearl	46.4	49.0	0.62 ab	0.29 b
NCHU-001	47.0	49.3	0.68 ab	0.31 b

[i] 47.5 °C was the temperature that most resemble critical temperature (around 47 °C) with actual measurements.

[ii] Mean separation within columns by least significant difference test at $P < 0.05$.

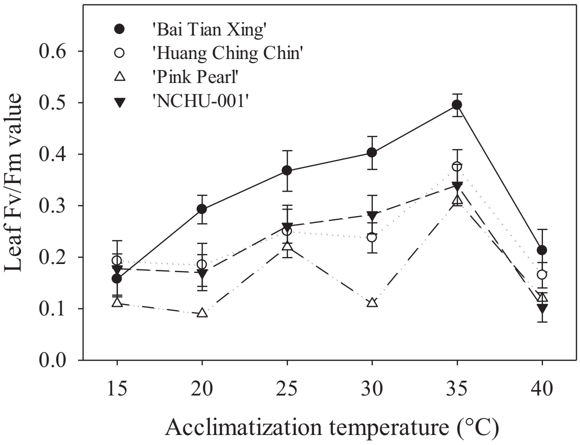

Fig. 2. Effect of acclimatization temperature on leaf Fv/Fm value of three chrysanthemum cultivars and one line. Bars indicate standard error of the mean (n = 4).

Chin' × 'Pink Pearl' were made during the summer and autumn in 2020. Pollination was done when three-fourths of the florets reached anthesis. Outer ray florets were removed from the inflorescence to expose inner tubular florets. Pollen was collected with a paint brush when anthers protruded out of the tubular corolla and then carefully brushed on the female parent. Seeds were harvested 6 weeks after pollination and sown into 200-cell plug trays (59.0 × 29.8 × 3.0 cm) containing peatmoss. Young seedlings were planted into 9-cm pots containing 3 peatmoss:1 perlite at 28 d after sowing. Fertilization was the same as previously described. Hybrid plants were heavily pruned to encourage lateral bud growth at 56 d after planting. Then cutting propagation was performed using the newly emerged lateral shoots. Cutting propagation and night break were the same as previously described. The following year, hybrid progenies were planted on 2 Jun 2021 (summer crop only). Time to flowering was recorded. Field preparation and plant management were the same as previously described. Another group of rooted cuttings were planted in pots containing 3 peatmoss: 1 perlite. At 42 d after planting, plants were given 35 °C treatment for 24 h in a growth chamber (14 h lighting, 300 ± 50 μmol·m^{-2}·s^{-1} *PPFD*). Then, leaf samples were collected, heat-stressed, and measured for Fv/Fm value after being returned to room temperature in darkness for 30 min. Fv/Fm value and days to flowering were analyzed for correlation. Among all progenies, two plants that flowered earlier and had acceptable flower appearance were selected and coded 109-W001Y and 109-W003Pi.

Determination of photosynthetic parameters. Plants of the four parental cultivars and two selected lines were moved into separate growth chambers at 42 d after potting, when plants had 10 to 12 unfolded leaves. Two day/night temperatures were set: 25/20 and 35/30 °C. Photoperiod was 16 h (0600–2200 HR) provided by LED lighting with 300 ± 50 μmol·m^{-2}·s^{-1} *PPFD* at plant level. Photosynthetic parameters were measured at 7 d after temperature treatments using a portable photosynthesis system (LI-6400; LICOR, Lincoln, NE, USA). The fourth apical unfolded leaf was taken for measurement. Measurements were made between 0800 and 1200 HR. Leaf temperature was set at 25 ± 2 °C. Air flow was set at 500 μmol·s^{-1}. Water vapor within the system were absorbed with silica gel desiccant (Sorbead® Orange Chameleon®; Catalyst-BASF, Iselin, NJ, USA) and humidified with ceramic humidicant (Stuttgarter Masse ceramic substrate; Pall, Port Washington, NY, USA) to maintain the relative humidity within leaf chamber at 60% ± 3%. A CO_2 cartridge was installed to maintain CO_2 concentration in air at 400 μmol·mol^{-1}. Area of measuring within the leaf chamber was 6 cm^2. Light provided with blue and red LED gave light intensities of 0, 5, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 (and 1100 for those under 25/20 °C) μmol·m^{-2}·s^{-1} *PPFD*. Measuring started from the highest light intensity and each leaf sample was balanced for at least 15 min to achieve stable photosynthetic performance. The measuring period at each light intensity was 5 min and data were taken at 50-s intervals. Linear regression between net photosynthesis and light intensity at 0–100 μmol·m^{-2}·s^{-1} *PPFD* was made using the equation: $y = y_0 + ax$, where dark respiration (Rd) is y_0. Exponential regression was made between net photosynthesis and light intensity at all light intensities range using the equation: $y = y_0 + a \times (1 - e^{(-bx)})$, where e is Euler's number. Maximum net photosynthesis was calculated based on this equation and adjusted to the 95% level to acquire estimated light-saturated rates of photosynthesis (A_{sat}). Then the respective light intensity to achieve such net photosynthetic rate, light saturation point (LSP), was calculated. Each single leaf is a replicate and each photosynthetic measurement contained three replicates.

All experiments used completely randomized design. Data were analyzed using analysis of variance procedure by *t* test or least significant difference at $P < 0.05$. The relationship between heat stress temperature and the associated Fv/Fm value of the cultivars was determined with regression analysis using Sigma Plot 10.0 programming (Inpixon, Palo Alto, CA, USA).

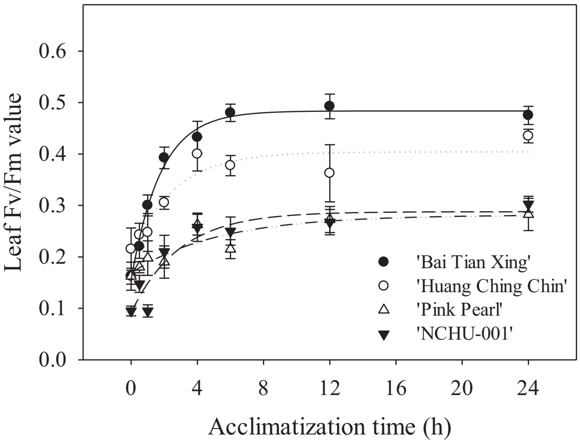

Fig. 3. Effect of acclimatization time on leaf Fv/Fm value of three chrysanthemum cultivars and one line. Bars indicate standard error of the mean (n = 4). Equations:
'Bai Tian Xing': $y = 0.155 + 0.328 \times (1 - e^{(-0.564 \times x)})$, $R^2 = 0.89$, $P < 0.001$
'Huang Ching Chin': $y = 0.205 + 0.200 \times (1 - e^{(-0.391 \times x)})$, $R^2 = 0.59$, $P < 0.001$
'Pink Pearl': $y = 0.169 + 0.113 \times (1 - e^{(-0.187 \times x)})$, $R^2 = 0.42$, $P < 0.001$
'NCHU-001': $y = 0.091 + 0.196 \times (1 - e^{(-0.344 \times x)})$, $R^2 = 0.71$, $P < 0.001$

Results

Heat-tolerant response in chrysanthemum. Results showed that response of leaf Fv/Fm value toward heat stress temperature was sigmoidal (Fig. 1). Chrysanthemum 'Bai Tian Xing' had the highest T_{crit} and T_{mid} among four tested cultivars (Table 1) and 'NCHU-001' had the second high T_{crit} and T_{mid}. 'Huang Ching Chin' and 'Pink Pearl' had the lowest values. Average T_{crit} and T_{mid} were around 47 °C and 50 °C, respectively (Table 1). Although leaf Fv/Fm value at 47.5 °C ranged between 0.58 and 0.72 among cultivars, 50 °C stress gave more distinguished difference (0.29–0.44) among cultivars based on statistical results.

Further test of acclimatization temperature showed that as temperature rose from 15 to 35 °C, leaf Fv/Fm value after heat stress at

Table 2. Time to stable Fv/Fm value and respective leaf Fv/Fm value after acclimatization at 35 °C in three chrysanthemum cultivars and one line.

Cultivars/line	Time required to reach stable Fv/Fm (h)	Fv/Fm value at stable after induction
Bai Tian Xing	4.6	0.46
Huang Ching Chin	5.9	0.39
Pink Pearl	11.1	0.27
NCHU-001	7.6	0.27

50 °C gradually increased and 35 °C was more effective than 15 to 25 °C treatments (Fig. 2). It should be noticed that 'Bai Tian Xing', being as a known heat-tolerant commercial cultivar in Taiwan, showed rapid response toward acclimatization temperature of 20 °C and had higher leaf Fv/Fm value than the other cultivars after 20 to 35 °C treatments. Nevertheless, 40 °C was not a suitable acclimatization temperature for chrysanthemum or duration of 3 d might be too long and caused heat injury (Fig. 2).

Results showed that, by placing plants at 35 °C and sampled at hourly intervals, all cultivars reached a stable Fv/Fm response within 12 h (Fig. 3, Table 2), and cultivar differences became more significant as time after treatments increased from 0.5 to 6 h. Similar to the preceding results, 'Bai Tian Xing' tended to reach a stable state earlier and had higher Fv/Fm value than other cultivars, whereas 'Pink Pearl' and 'NCHU-001' took longer and reached rather lower Fv/Fm values (Table 2).

Relationships between flowering heat delay and leaf Fv/Fm value. Except for 'NCHU-001', there were no differences between summer and autumn crops in terms of time to visible bud (Table 3). However, a major difference was observed in time to flowering among all tested cultivars, for summer crops required significantly more days to flowering (Table 3). In the summer, 'Bai Tian Xing' flowered earlier than other cultivars, whereas 'Bai Tian Xing' and 'NCHU-001' flowered earlier than the other two cultivars in the autumn. All tested cultivars showed flowering heat delay that ranged from 13.8 to 39.8 d, whereas 'Bai Tian Xing' and 'NCHU-001' were the tolerant and sensitive cultivars, respectively.

By regressing the delay in days to flowering (Table 3) against leaf Fv/Fm value after heat stress (mean data from Tables 1 and 2) of the four tested cultivars, it showed that there is a negative linear relationship ($R^2 = 0.93$) between them (Fig. 4), where cultivars with higher Fv/Fm value were less delayed in time to flowering by summer conditions.

We further analyzed this relationship by using hybrid progenies from reciprocal crossing of 'Bai Tian Xing' × 'NCHU-001' and 'Huang Ching Chin' × 'Pink Pearl'. Results showed that there were weak negative linear relationships ($R^2 = 0.70–0.87$) between days to flowering and leaf Fv/Fm value after heat stress in these four populations (Fig. 5), and some progenies from 'Bai Tian Xing' crossed with 'NCHU-001' had the highest leaf Fv/Fm value and were the earliest to reach flowering. Two progenies that flowered earliest and with acceptable flowering performances were selected as line 109-W001Y and 109-W003Pi.

Photosynthetic parameters of chrysanthemum cultivars and lines in response to temperature. Results showed that, except Huang Ching Chin, all cultivars/lines had lower light-saturated net photosynthetic rate (A_{sat}) at 35/30 °C, compared with 25/20 °C. Pink Pearl had the lowest A_{sat} at 35/30 °C among all tested cultivars/lines (Table 4). Cultivars Huang Ching Chin and Pink Pearl did not show lower LSP at higher temperatures. However, both cultivars had lower LSP at 25/20 °C in comparison with other cultivars/lines. Bai Tian Xing, 109-W001Y, and 109-W003Pi had lower dark respiration rate (Rd) at 35/30 °C, whereas the other three cultivars did not show significant difference. Line 109-W001Y had the lowest Rd and 'Pink Pearl' had the highest Rd at 35/30 °C.

Table 3. Effects of cultivars/line and seasons on time to development and delay in flowering of chrysanthemum.

Cultivars/line	Seasons	Time to development: Visible bud	Time to development: Flowering	Delay in flowering (d)
Bai Tian Xing	Summer	30.0 Ab[i]	71.0 Ab	13.8
	Autumn	30.0 Ab	57.2 Bb	
Huang Ching Chin	Summer	34.8 Aab	91.0 Aa	22.6
	Autumn	39.0 Aa	68.4 Ba	
Pink Pearl	Summer	37.0 Aa	99.0 Aa	33.8
	Autumn	32.0 Aab	65.2 Ba	
NCHU-001	Summer	34.8 Aab	99.0 Aa	39.8
	Autumn	27.6 Bc	59.2 Bb	
Significance	Cultivar (C)	***[ii]	***	
	Season (S)	NS	***	
	C × S	**	***	

[i] Uppercase and lowercase letters indicate significant difference within cultivars and seasons by *t* test and least significant difference test at $P < 0.05$, respectively.

[ii] NS, **, *** indicate nonsignificant or significant at $P < 0.01$ or 0.001, respectively.

Discussion

Chlorophyll fluorescence parameters have been proven to be useful in detecting heat stress in chrysanthemum (Janka et al. 2013, 2015). Temperatures higher than the critical temperature (T_{crit}) tend to cause a decrease in Fv/Fm value in plants (Xu et al. 2014). Difference in heat tolerance among cultivars could be evaluated or compared using T_{crit}. A single temperature with great sensitivity in detecting genotypic difference in heat tolerance would ease labor requirements (Yeh and Lin 2003). Here, we demonstrated that T_{crit} and T_{mid} for chrysanthemum were ~47 and 50 °C, respectively (Fig. 1, Table 1). Xu et al. (2014) demonstrated that T_{crit} in grape leaf disc was also 47 °C if evaluated with the Fv/Fm value. However, by comparing Fv/Fm value at 47.5 °C, a temperature with actual measurements and near T_{crit}, there was no significant difference between cultivars. And the ranking did not resemble known heat-tolerant performance of the cultivars and line. On the other hand, mean comparison at 50 °C, temperature of T_{mid}, was significant ($P < 0.05$), and the ranking was similar to known performance. Thus, T_{mid} might be a better indicator of heat tolerance in chrysanthemum than T_{crit}.

Chrysanthemum plants with 10–12 unfolded leaves were acclimatized at 35 °C for at least 12 h to induce stable heat stress–related response (Figs. 2 and 3, Table 2). Cool-season turfgrass species acclimatized at 30 °C for 3 d leads to better performances of physiological indices than those without acclimation (Xu et al. 2006). Heat-stress–related responses could be induced by exposing food legume species to 35 °C for 24 h (Srinivasan et al. 1996). Trapero-Mozos et al. (2018) also demonstrated that commercial potato cultivars could gain thermotolerance toward 40 °C heat stress when first exposing plants to 25 °C for 48 h. Further detailed study showed that 12 h of 25 °C treatment in light (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$), instead of dark, was sufficient to induce thermotolerance in potato 'Desiree'. Nevertheless, such inductive temperature and duration had its limit, as shown in this study (Fig. 2). Over-heating or a heat-stressed duration well over the tolerance of species or cultivars would cause heat injury and subsequently lead to lower measured values of Fv/Fm.

Leaf Fv/Fm value after heat stress in response to stress temperature was sigmoidal (Fig. 1), as had been shown in electrolyte leakage measurement (Yeh and Lin 2003). Nevertheless, chlorophyll fluorescence measurements are nondestructive and require less labor than electrolyte leakage method. The average T_{mid} determined by the chlorophyll fluorescence method was ~50 °C (Table 1), which is the same temperature to distinguish heat-tolerant and intolerant cultivars by the electrolyte leakage method (Yeh and Lin 2003). Therefore, heat stress at 50 °C might be crucial in distinguishing heat tolerance in present commercially available chrysanthemum cultivars. Temperature at 49 or 50 °C has been reported to screen for heat tolerance

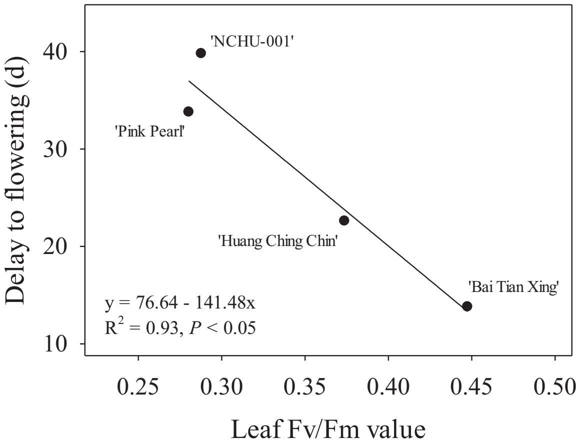

Fig. 4. Relationship between delay in flowering and leaf Fv/Fm value after heat stress in three chrysanthemum cultivars and one line. The degree of heat delay was calculated as the difference between days to flowering in plants grown in summer and autumn (Table 3).

in coffee (Marias et al. 2017) and peanut (Selvaraj et al. 2011).

The ability of a plant to rapidly acquire thermotolerance might be the key of heat tolerance and is thought to be related to the induction of molecular responses of heat shock protein–related genes (Amano et al. 2012). In chrysanthemum, Wang et al. (2008) showed that both heat-tolerant and heat-intolerant cultivars responded to 30/25 °C heat stress at the same pace. However, intolerant cultivar failed to maintain a lower malondialdehyde content at 12 d after treatment and electrolyte leakage remained high at 24 d after treatment, which is the critical period of flower bud visibility. Therefore, the rapid response and higher stable leaf Fv/Fm value in ‘Bai Tian Xing’ (Tables 1 and 2) might be critical in contributing to its heat tolerance as compared with other cultivars. As for ‘Pink Pearl’ and ‘NCHU-001’, both showed lower Fv/Fm values after 50 °C heat stress, each required more time to reach a stable Fv/Fm value, and their respective value was rather low (Tables 1 and 2). These indicated that both cultivars had weaker thermotolerance acquiring abilities, both rhythmical and their degree of response.

Time to visible bud (VB) in chrysanthemum ‘NCHU-001’ was significantly longer in the summer, whereas the other three cultivars did not differ significantly in two crop seasons (Table 3). Time to VB did not differ significantly in summer-flowering cultivars grown at 30/25 or 20/15 °C under 12.0- to 13.5-h daylengths, whereas autumn-flowering cultivars were not able to reach VB under 30/25 °C (Wang et al. 2008). This might indicate ‘NCHU-001’ had a critical daylength slightly shorter than the tested summer-flowering cultivars. All tested cultivars required more time to flowering in the summer when compared with the autumn crop (Table 3). This phenomenon has been observed in many cultivars (Wang et al. 2008) and only few cultivars had similar time to reach flowering irrespective of 30/25 or 20/15 °C (Wang et al. 2008). Chrysanthemum ‘Bai Tian Xing’ is still a qualifying summer-flowering cultivar based on time required to reach flowering, whereas the other three cultivars are doubtful to fulfill summer production.

There is a negative linear relationship between delay in flowering and leaf Fv/Fm value ($R^2 = 0.93$; Fig. 4). The delay in flowering had been shown to be positively linearly related to the relative injury of chrysanthemum leaf (Wang et al. 2008). Nevertheless, linear relationship also meant that there are no thresholds or critical value could be taken as a definite indicator for heat tolerance. We aimed to further prove this relationship between time to flowering and leaf Fv/Fm value by using hybrid progenies between tested parental cultivars (Fig. 5). However, the coefficient of determination was lower ($R^2 = 0.70$–0.87) compared with the one shown by parental cultivars ($R^2 = 0.93$). Several reasons could have caused the

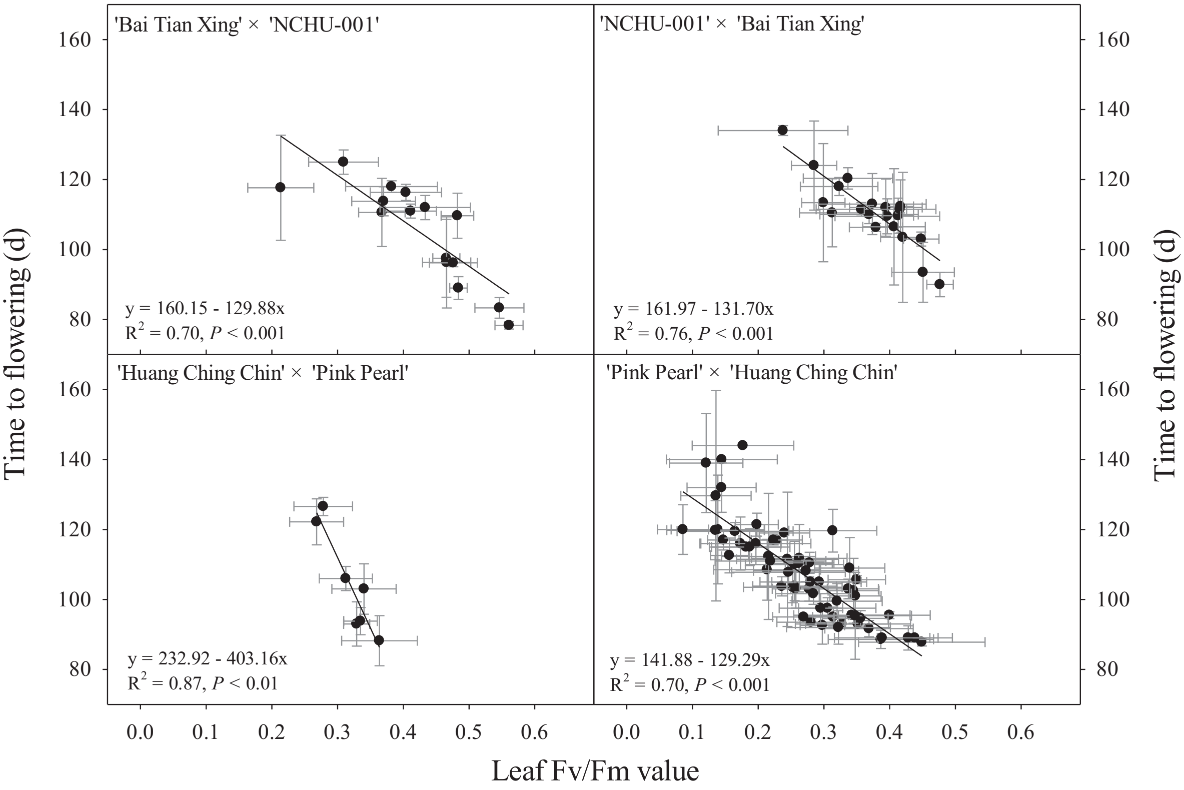

Fig. 5. Relationships between leaf Fv/Fm value after heat stress and time to flowering for summer-planted progenies in chrysanthemum. Bars indicate standard deviation of the mean (n = 6).

Table 4. Effects of cultivars/lines and temperature on light-saturated net photosynthetic rate (A_{sat}), light saturation point (LSP), and dark respiration (Rd) of chrysanthemum.

Cultivars/lines	Day/night temp. (°C)	A_{sat} ($\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2)	LSP ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Rd ($\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2)
Bai Tian Xing	25/20	15.9 Aab[i]	668.2 Aabc	1.05 Aab
	35/30	11.0 Ba	352.3 Bbc	0.33 Bab
Huang Ching Chin	25/20	11.9 Ac	525.9 Abc	0.63 Ac
	35/30	9.7 Aa	421.7 Aab	0.31 Aab
Pink Pearl	25/20	11.8 Ac	387.2 Ac	0.73 Abc
	35/30	6.8 Bb	292.8 Ac	0.55 Aa
NCHU-001	25/20	13.6 Abc	887.4 Aa	0.75 Abc
	35/30	9.8 Ba	317.2 Bbc	0.41 Aab
109-W001Y	25/20	16.3 Aa	913.5 Aa	0.76 Abc
	35/30	10.4 Ba	331.2 Bbc	0.23 Bb
109-W003Pi	25/20	16.5 Aa	792.7 Aab	1.14 Aa
	35/30	10.9 Ba	530.9 Ba	0.43 Bab
Significance	Cultivar (C)	***[ii]	**	NS
	Temp. (T)	***	***	***
	C × T	NS	**	NS

[i] Uppercase and lowercase letters indicate significant difference within cultivars/lines and temperature treatments by *t* test and least significant test at $P < 0.05$, respectively.

[ii] NS, **, *** indicate nonsignificant or significant at $P < 0.01$ or 0.001, respectively.

weaker relationships. First, we used time to flowering in progenies rather than delay in time to flowering as done in parental cultivars. This was due to reduction in the labor required to manage lots of stock plants and field work. But progenies from commercial cultivars, mostly heterozygous, are expected to greatly divert in time required to reach VB and flowering compared with 6- to 12-d difference among parental cultivars in the autumn crop (Table 3). Therefore, the inherited heat tolerance for flowering would be masked by the flowering natures of progenies and vice versa. Second, as shown by the four cross combinations, time to flowering of progenies was probably significantly affected by the female parent. Only two progenies had mean days to flowering at ∼80 d after planting, which is from 'Bai Tian Xing' crossed with 'NCHU-001'. Progenies from the other three cross combinations had days to flowering longer than 88 d. Last, these progenies were cutting propagated for only one generation. Based on the large variation of the measured values (Fig. 5), these progenies would need more than one generation of observation. Therefore, we could suggest measuring Fv/Fm value as a tool to aid in the selection of progenies to exclude those with significant lower Fv/Fm value. But in the end, it is crucial to make crosses using parental cultivars (such as 'Bai Tian Xing') with high Fv/Fm value and rapid response.

All tested cultivars/lines showed decreased light-saturated net photosynthesis (A_{sat}) at 35/30 °C when compared with 25/20 °C, except 'Huang Ching Chin' (Table 4). High temperatures impair PSII, reduce ribulose-1,5-bisphosphate carboxylase/oxygenase activity, and lead to lower photosynthesis efficiency (Mathur et al. 2014). Although 'Bai Tian Xing' and the two selected lines showed decreased dark respiration (Rd) as temperature increased from 25/20 to 35/30 °C, Rd of the other three tested cultivars did not differ significantly (Table 4). Sucrose is a primary photosynthetic product (Wang et al. 2015). Spraying chrysanthemum 'Floral Yuuka' with 50 mM sucrose every 4 d could accelerate time to flower bud visibility by 2 weeks under night break condition at 23 °C (Sun et al. 2017). Cho and Kim (2020) demonstrated that high night temperatures not only prolonged time to VB and flowering, but also caused flower abnormality in two chrysanthemum cultivars. We could postulate that cultivars with greater balance between carbon income (photosynthesis) and expense (respiration) under high temperatures are those showing better heat tolerance. Among tested cultivars/lines, 'Pink Pearl' showed the lowest A_{sat} and highest Rd (Table 4); therefore, the significant delay in flowering (Table 3) might have been caused by the low carbon assimilation. Net photosynthetic rate is positively correlated with multiplication of photosynthetic active radiation and Fv/Fm value, as observed in four plant species (Sun and Wang 2018). We did not find significant relationships among Fv/Fm values after 50 °C heat stress and light-saturated net photosynthesis (data not shown). However, chrysanthemums with higher Fv/Fm grown in the field or greenhouses during summer production might have higher photosynthetic rate, carbon assimilation, and thus flower earlier than those with lower Fv/Fm values.

Conclusion

We propose that heat tolerance in chrysanthemum cultivars could be compared using the following method. Plants with 10 to 12 unfolded leaves should acclimatize at 35 °C (in light or 14-h photoperiod, 300 ± 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPFD*) for at least 12 h. The fourth leaf from the apex (recently fully expanded leaf) is detached and kept in a sealed moist plastic bag and heat stressed at 50 °C for 20 min, then re-acclimatized at 25 °C (room temperature) for 30 min in the dark. Plants with higher leaf Fv/Fm value are advised to be used for future breeding programs and those with lower leaf Fv/Fm value should be discarded from the heat-tolerant breeding program. Parental plants with higher Fv/Fm values after heat stress should produce progenies with better heat tolerance, whereas the heat-tolerant seed parents might contribute more to early flowering under high temperature conditions than its pollen parent.

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