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Effect of Heat Stress Duration on Growth, Flowering and Electrolyte Leakage in Four Cultivars of *Calendula officinalis*

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Abstract

In the floriculture industry, the need for heat tolerant bedding plant cultivars is increasing because of the rising temperature around the world. A pot experiment was carried out to examine the impacts of four heat stress durations (0, 7, 14 and 21 days) on growth and ornamental traits to determine the relative heat tolerance of four cultivars of calendula (*Calendula officinalis*). Growth and development were quantified by measuring plant height, total leaf area, shoot and root fresh weight, shoot and root dry weight, time to flowering, flower number, average flower size, and flower longevity. Plant height, leaf area, and shoot and root growth at 35-42°C were significantly lower than those at normal temperatures (20-30°C). Time to flowering increased with temperature. Flower number, size and longevity were reduced by heat stress, so that among all durations, plants exposed to heat stress for 21d had the least mean. Longer heat stress reduced the plant height, leaf area, shoot and root growth, SPAD value, flower diameter and flower longevity of all cultivars., However, the range percentage reduction in growth and flowering parameters were different among cultivars. Experiment to determine the membrane damage showed an increase in percent electrolyte leakage with exposure of plants to higher temperatures. The studied cultivars differed in their sensitivity to heat stress. The results indicated that better cell membrane stability, higher shoot and root growth, and later flowering led to greater heat tolerance in 'Indian Prince' compared to other cultivars.

Keywords: Bedding plants, High temperature, Morphology, Thermotolerance.

INTRODUCTION

Temperature is one of the primary environmental factors that greatly affects the growth and development of plants (Koh, 2002). Heat stress in plants, as defined by Hall (2001), results from exposure to high enough air temperatures for a sufficient time so as to cause irreversible damage to plant growth and development. Heat stress is one of the most typical kinds of challenges which influence bedding plants growth and development in greenhouse production and landscape, during late spring and summer in most parts of the world (Natarajan, 2005).

In general, some morphological effects due to high temperature stress included the reduced plant height, root growth, shoot growth, leaf area, flower diameter, flower number, and flower size (Kertring, 1984; Lee and Erick, 2007; Niu and Rodriguez, 2006; Natarajan and Kuehny, 2008; Ortiz and Cardemil, 2001). Plants vary in their sensitivity to heat stress, and the degree of damage depends on their genotype, environmental conditions for optimum growth, and intensity, duration and rate of high temperatures (Kertring, 1984; Larkindale *et al.*, 2005; Wahid *et al.*, 2007). Cell membrane thermostability (CMT) test is a rapid laboratory technique used to evaluate variation in thermotolerance across some genotypes (Yeh and Hsu, 2004). Lee *et al.* (1990) stated that flower production, flower size, and plant growth may be useful measurements for assessing heat tolerance of floriculture crops.

Bedding plants production is one of the most important parts of the floriculture. Many bedding plants species encounter heat stress during summer months even though they are well-watered. The growth of cool-season bedding plants is often limited by high temperatures of summer months in warm climates, resulting in ceased flowering under exposure to high temperature (Natarajan, 2005). Despite the economic importance of these plants, relatively little has been done to investigate the effects on bedding plants during heat stress (Adams *et al*., 1997). Therefore, understanding the responses of bedding plant cultivars to high temperatures is important to identify heat-tolerant cultivars for the selection of traits used in breeding programs, leading to improvement in summer performance of cool-season bedding plants (Prasad *et al.*, 1999).

Calendula (*Calendula officinalis*) belonging to the family Asteraceae is an important annual herbaceous species widely used as cool-season bedding plant in landscaping and home gardens in Iran. This species prefers cooler temperatures, and its flowering and marketable quality are lost in warmer temperatures (Warner and Erwin, 2005; Braithwaite and Drost, 2009). So far, a few heat-tolerant *Calendula officinalis* cultivars have been introduced. Furthermore, little assessment has been done on major morphological and physiological characteristics associated with heat tolerance/sensitivity of popular bedding plants such as calendula. The objective of this research was to assess the changes in morphological characteristics and cell membrane stability induced by different durations of heat stress in four calendula cultivars.

MATERIALS AND METHODS

Plant material and treatments application

This work was conducted in agricultural research glasshouse, Ferdowsi University of Mashhad. Seeds of calendula 'Candyman', 'Zen', 'Indian Prince' and 'Pacific Beauty' were germinated into plastic trays containing cocopeat. Healthy, uniform size seedlings (45-day old) were transplanted into 14 cm diameter plastic pots filled with mixture of 60% loam soil, 30% sand and 10% vermicompost. The calendula plants were grown in the glasshouse at a day/night temperature regime of 27ºC/18ºC with a 14 h photoperiod and a photosynthetically active radiation of 400-600 μmol m^{-2} s^{-1} provided from natural solar radiation. Fifty days after transplanting, plants were divided into two groups: one group was exposed to optimum temperature and the other was exposed to higher temperature in two separate mini plastic greenhouses. A set of eight pots per cultivar were transferred from the glasshouse at 27/18ºC (day /night) to one mini plastic greenhouse set at 20-30ºC (control treatment) and 24 plants of each cultivar were subjected to 42/35ºC at another plastic greenhouses (stress treatment) where they were kept for 7, 14 and 21 days. High temperature conditions were provided by two electric fan heaters equipped with thermostat. The photoperiod in both two mini plastic greenhouses was 14 h, the PPFD was 200-400 µmol m^{-2} s^{-1} at the top of the plant canopy provided by natural solar radiation, and supplemented by fluorescent and incandescent lamps, and the relative humidity was 75-80%. Pots in plastic greenhouses were randomly placed and rotated frequently to minimize positional effects. After the stress period, plants were returned to the glasshouse with conditions described above and kept there until final harvest. The heat stress temperature set to 35-42ºC because it commonly occurs in warm climatic regions during mid-summer.

Measurements and data collection

All plants were destructively harvested after flowering and separated into shoot and root. Total leaf area per plant was determined using Delta-T device (Delta-T, LTD, Cambridge, UK). Dry weights of separated shoots and roots were recorded on four randomly selected plants per treatment after oven drying at 75 °C for 72 h. Plant height, shoot and root fresh weight, time to flowering, flower number, average flower size, and flower longevity were also measured. Leaf greenness or chlorophyll reading values (SPAD value) were determined by three measurements per plant using a portable SPAD-502 chlorophyll meter (Konica, Minolta, Tokyo).

Electrolyte leakage (EL) of leaves was measured as per Jiang and Huang (2001)'s procedure with minor modifications. Each sample for assay consisted of ten leaf discs (10-mm diameter) cut from a group of fully expanded youngest leaves with a cork borer. Leaf discs were rinsed three times with distilled water and were then placed in glass vials containing 20 mL of distilled deionized water. The vials were kept at room temperature for 24h to allow diffusion of electrolytes from the leaf disks, and their initial solution conductivity (C1) was measured. Then, the vials were capped with foil, autoclaved at 121°C for 15 min to completely kill the leaf tissue, cooled to 25°C, and the final conductivity (C2) was measured. The relative electrolyte leakage was calculated as (C1/ C2) × 100.

Experimental design and statistical analysis

A factorial experiment based on a completely randomized design was conducted on four cultivars of *Calendula officinalis*, including 'Candyman' , 'Indian Prince', 'Zen' and 'Pacific Beauty' combined with four durations (0, 7, 14 and 21 days) of exposure to the high temperatures regime with eight replications. Analysis of variance (ANOVA) for all the variables was carried out using the JMP8 software package. Differences in means were tested by Least Significant Difference (LSD) test at $P < 0.05$ level.

RESULTS

Plant growth and development

Analysis of variance showed significant effects of high temperature duration on the plant height, shoot fresh and dry weight, root fresh and dry weight and leaf area of the examined calendula cultivars (Table 1). Means comparison for the data of four studied cultivars (Table 2) indicated that different genotypes of calendula exhibited significant differences ($P < 0.01$) in growth characteristics and that the highest values were recorded in 'Indian Prince' cultivar.

The effect of heat stress was significantly observed as the reduction in growth traits (Table 3). According to results, 21 days of high temperature produced the shortest plants (7.33 cm) with the lowest total leaf area (114.31 cm^2) as compared to control temperature. Shoot fresh and dry weight was also significantly greater in control temperature as compared to high temperature, though increasing duration from 7 to 21 d, did not significantly affect shoot fresh weight. In addition, root fresh and dry weight was significantly decreased by 56% and 58%, respectively as duration was increased from 0 to 21 d.

Table 1. Means of squares of the studied traits of calendula in response to four high temperature length.

	Means of squares											
	PH	LA	SFW	SDW	RFW	RDW	TF	FD	FN	FL	SPAD	EL
C	631.20**	36152.83**	424.87**	11.11**	44.90**	1.65**	1610.23**	259.70**	6.17*	36.52**	432.06**	271.58**
D	274.08**	33893.59**	92.88**	1.05**	177.35**	4.34**	3197.49**	229.08**	31.98*	7.95**	20.74**	180.50**
C×D	37.59**	4755.24**	19.63**	0.18**	4.88**	0.21**	80.31^{ns}	21.86*	2.68^{ns}	2.31**	25.61**	64.81**
E	14.57	1025.10	3.76	0.06	1.38	0.03	121.50	10.10	1.59	0.69	4.59	16.75
CV (%)	28.26	15.78	28.39	21.91	25.13	20.39	9.77	10.02	3.68	25.84	11.98	7.70

C: Cultivar, D: duration, C×D: Cultivar ×duration, E: Error, PH: Plant height, LA: Leaf area, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, TF: Time to flowering, FD: Flower diameter, FN: Flower number, FL: Flower longevity, SPAD: SPAD unit, EL: Electrolyte leakage; *, ** indicate significance at $P < 0.05$, 0.01, respectively.

Table 2. Means comparison of the investigated growth characteristics of four studied cultivars.

Cultivar	PH	LA	SFW	SDW	RFW	RDW	TF	FD	FN	FL	SPAD	EL
Candyman	8.94^{b}	110.40^{d}	9.12^{c}	1.86^{c}	7.87^{bc}	1.09^{b}	132.78^{c}	58.25^{a}	3.50^{a}	8.16^{b}	34.49^{b}	11.51^{b}
Indian Prince	14.16^{a}	218.14^{a}	21.40^{a}	3.80^{a}	11.33^{a}	1.77^{a}	148.87^{a}	50.58^{c}	2.81^{b}	9.18^{a}	31.18^{c}	12.30^{b}
Zen	6.11^{c}	138.24^{c}	14.11^{b}	2.38^{b}	8.64^{b}	1.12^{b}	141.33^{b}	49.61^{c}	3.56^{a}	5.25^{d}	38.99^{a}	20.97^{a}
Pacific Beauty	15.76^{a}	181.99^{b}	12.75^{b}	2.37^{b}	7.71^{c}	1.18^{b}	132.90^{c}	55.08^{b}	3.97^{a}	6.67^{c}	31.27^{c}	12.79^{b}

PH: Plant height, LA: Leaf area, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, TF: Time to flowering, FD: Flower diameter, FN: Flower number, FL: Flower longevity, SPAD: SPAD unit, EL: Electrolyte leakage; the data with the same letter are not significantly different at $P < 0.05$.

Table 3. Means comparison of the investigated growth characteristics of calendula plants exposed to four high temperature durations.

Duration	PH	LA	SFW	SDW	RFW	RDW	TF	FD	FN	FL	SPAD	EL
0 days	12.83^{a}	215.20^{a}	17.88^{a}	2.96^{a}	12.89^{a}	1.93^{a}	128.62^{c}	58.60^{a}	4.81^{a}	8.53^{a}	34.98^{a}	10.34^{b}
7 days	14.09^{a}	184.52^{b}	12.57^{b}	2.59^{b}	10.33^{b}	1.49^{b}	133.37^{c}	53.65^{b}	3.47^{b}	7.00^{b}	33.36^{b}	12.35^{b}
14 days	10.45^{b}	134.73^{c}	13.76^{b}	2.49^{bc}	6.59^{c}	0.93^{c}	141.63^{b}	51.12^{c}	3.03^{bc}	6.87^{b}	34.30^{ab}	17.79^{a}
21 days	7.33^{c}	114.31^{c}	13.16^{b}	2.37^{c}	5.75^{d}	0.81^{d}	152.53^{a}	50.14^{c}	2.43^{c}	6.73^{b}	33.30^{b}	18.28^{a}

PH: Plant height, LA: Leaf area, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, TF: Time to flowering, FD: Flower diameter, FN: Flower number, FL: Flower longevity, SPAD: SPAD unit, EL: Electrolyte leakage; the data with the same letter are not significantly different at $P < 0.05$.

Interaction of heat duration and cultivars affected plant growth characteristics (Table 1). In general, growth of all cultivars declined as the duration was increased from 0 to 21 days. Among the four cultivars, 'Zen' had the shortest plants under control and high temperature treatments (Fig. 1A). Plant height of 'Indian Prince' and 'Pacific Beauty' grown at high temperature for 14 d significantly reduced by ~30 and ~31% respectively, compared to control, while 'Zen' with ~10% reduction was not affected significantly by exposure to heat stress. Total leaf area of calendula decreased for all cultivars exposed to high temperature (Fig. 1B). The highest and the lowest leaf area in most durations was related to 'Indian Prince' and 'Candyman' cultivars, respectively., However, there was no significant difference between 'Candyman' and 'Zen'. Shoot fresh and dry weight of calendula cultivars decreased progressively in extended heat stress durations (Fig. 1C, D). Heat stress also reduced root fresh and dry weight of all cultivars in all durations (Fig. 1E, F).

Flower characteristics

Based on analysis of variance (Table 1), the main effect of cultivar and heat duration was statistically significant on ornamental characteristics including time to flowering, flower number, flower diameter, and flower longevity. Among the four cultivars, the greatest flower longevity, time to flowering, and the lowest flower number were related to 'Indian Prince', whereas 'Candyman' had the biggest flowers (Table 2). As it was shown in Table 3, control plants flowered the earliest (~128 days) as compared to plants exposed to heat stress. Flowering was delayed by higher duration., For example, plants exposed to high temperature for 21 d flowered after, on average

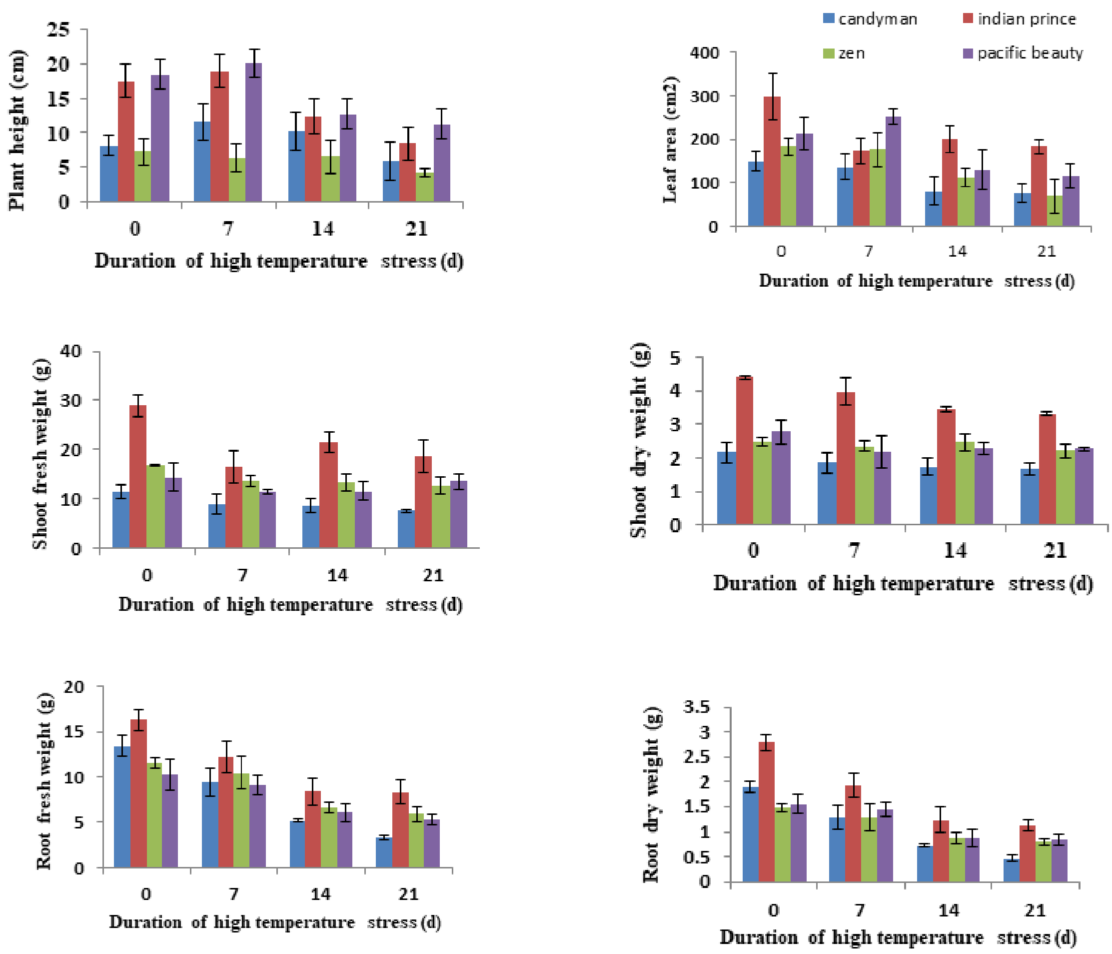

Fig.1. Effects of different durations of high temperature on measured growth indexes: a) plant height, b) leaf area, c) shoot fresh weight, d) shoot dry weight, e) root fresh weight, and f) root dry weight. Bars indicate the mean standard deviations (±SD), which are significantly different at P<0.05 (LSD test).

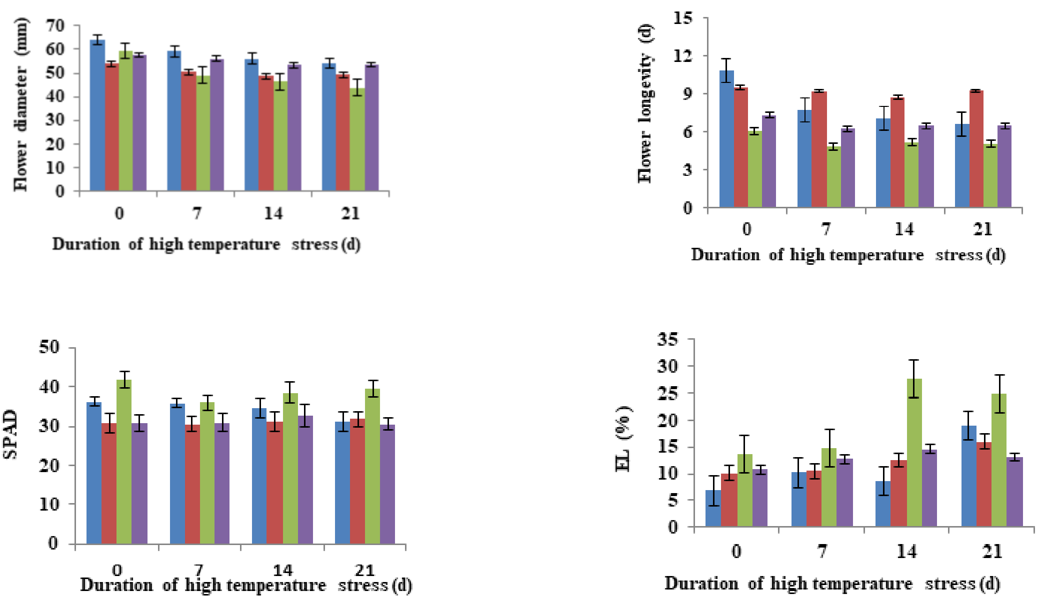

Fig. 2. Effects of different durations of high temperature on a) flower diameter, b) flower longevity, c) SPAD unit, and d) electrolyte leakage. Bars indicate the mean standard deviations (SD), which are significantly different at P < 0.05 (LSD test).

~152 days. High temperature duration also affected the flower number, size and longevity., For example, extending high temperature duration from 0 to 7, 14 and 21 d resulted in a decrease in flower number by ~28%, ~37%, and ~50%, respectively (Table 3).

Interaction of cultivar and duration had a significant effect on flower longevity and flower diameter, but no significant difference was observed in time to flowering and flower number (Table 1). The results indicated a reduction in average flower size for all cultivars as duration was increased from 0 to 21 d. At control treatment, average flower size was significantly smaller in 'Indian Prince', while after 21d of heat stress 'Zen' had significantly smaller flowers than 'Indian Prince' (Fig. 2A). Heat stress resulted in the loss of flower longevity in 'Candyman' but did not affect significantly flower longevity in other cultivars. Flower longevity of 'Indian Prince' was influenced the least by high temperature as compared to other cultivars, while 'Candyman' was influenced the most (Fig. 2B).

SPAD

High temperature (35-42°C) had a significant effect on SPAD value, and the interaction of genotype and duration was significant for this trait ($P < 0.01$). With the increase of heat stress duration, the SPAD value of plants was significantly decreased, so that calendula exposed to heat stress for 21d duration had the lowest SPAD value compared to control, although the difference between temperature durations was not significant (Table 3).

Under heat stress, the SPAD value for 'Zen' and 'Candyman' leaves declined significantly with the increase in duration from 0 to 7 d and 21 d, respectively. Conversely, the SPAD values for 'Indian Prince' and 'Pacific Beauty' leaves appeared to be unaffected by heat (Fig. 2C).

Electrolyte leakage

The effect of cultivar, duration and their interaction on the electrolyte leakage was significant ($P < 0.01$) (Table 1). 'Zen' had the highest electrolyte leakage among the tested cultivars and showed a significant difference with other cultivars, but no significant difference was observed between three other cultivars (Table 2). At the challenging temperature of 35-42°C, electrolyte leakage percent of plants was increased for all cultivars, compared to control temperature. 'Zen' had the greatest mean among cultivars in all durations and it was also most affected by the increase in duration of high temperature exposure, while 'Indian Prince' was less affected (Fig. 2D).

DISCUSSION

Our results showed that four calendula cultivars studied in this experiment varied in their appearance, morphological growth pattern, and ability to tolerate high temperature stress regardless of heat duration. Differences in heat tolerance among cultivars or genotypes have been identified for several species. For example, 12 cultivars of pansy *Viola × wittrockiana* Gams. varied in growth response to heat stress based on differences in relative growth rate, leaf number, flower number, flower size and days to flower (Warner and Erwin, 2006). Two cultivars of *Salvia splendens* (Natarajan, 2005), two ecotypes of *Cercis canadensis* L. (Griffin *et al.,* 2004), 12 open-pollinated families of *Picea galuca.* (Bigras, 2000), 11 genotypes of *Lycopersicon esculentum* Mill. (Warner and Erwin, 2001), and seven cultivars of *Linum usitatissimum* L. (Gusta *et al.*, 1997) also showed differences in growth performance when exposed to high temperatures.

Data presented in this study clearly show that overall growth and development of calendula including plant height, total leaf area, root fresh weight, root dry weight, shoot fresh and shoot dry weight, flowering time, flower number, flower diameter, flower longevity, and SPAD values are largely affected by high temperature stress during greenhouse production. Growth reduction under heat stress has been already reported for a number of species (Medina and Cardemil, 1993; Gusta *et al.,* 1997; Ortiz and Cardemil, 2001; Natarajan and Kuehny, 2008).

Initial plant heights of the cultivars used in this investigation were significantly different, so that 'Pacific Beauty' and 'Indian Prince' had the highest plant height. Exposure of plants to high temperature (35-40°C) reduced the plant height in all four cultivars., However, the percent reduction in plant height was much greater for 'Indian Prince' and 'Pacific Beauty' than for two other cultivars.

As exposure duration was extended, both root and shoot dry weight decreased for all cultivars but root growth was more severely inhibited than shoot growth. The loss of root growth might be related to the use of black pots resulted in an increase in root zone temperature (Vige *et al.,* 2003). Among calendula cultivars, 'Indian Prince' had the greatest shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight under heat stress at all exposure durations and in control treatment. More extensive roots could affect water movement and nutrient uptake which increased transpirational cooling and survival capacity under high temperature (Kolb and Robberecht, 1996; Huang and Xu, 2000; Natarajan and Kuehny, 2008). Similar conclusions were made in earlier studies where survival under high temperature in heat tolerant cultivars was positively related to producing more extensive roots and shoots (Jiang and Huang, 2001; Xu and Huang, 2001; Natarajan and Kuehny, 2008).

Our results indicated that although flowering continued with high temperature in all cultivars, but its qualitative parameters exhibited some reductions. Calendula growing at 20-30°C had the greatest number of flowers per plant, flower diameter, and flower longevity, while they were severely reduced at 35-42°C. The reduced flower number and size under high temperature observed here is consistent with results for other species, including campanula (Niu *et al*., 2001), impatiens, snapdragon, mimulus, torenia (Warner and Erwin, 2005) and pansy (Warner and Erwin, 2006).

The data in this research showed that flowering was delayed in plants at supraoptimal temperature as compared to control. 'Indian Prince' flowered later than other cultivars allowing more time for establishment., For example 'Zen' flowered a week earlier in comparison to 'Indian Prince'. Similar results of heat tolerant and heat sensitive cultivars of chrysanthemum (*Dendranthema × grandifolia*) was reported in which the heat sensitive cultivars displayed earlier flowering when exposed to high temperature compared to heat tolerant cultivars. It was concluded that there was a positive linear correlation between the degree of heat-induced delay in chrysanthemum flowering with leaf electrolyte leakage (Yeh and Lin, 2003).

This study showed that heat stress increased cell membrane damage and decreased the chlorophyll content (SPAD value). EL could be used as stress indicators of cell membrane stability (CMS) in initial screening for heat-tolerant cultivars (Natarajan, 2005; Wahid et al., 2007; Wang and Yeh, 2008). In our experiment, membrane damage increased in all cultivars with the increase in temperature duration., However in 'Zen' the EL was greater and enhanced more severely as duration was increased than three other cultivars which suggests that the leaves of 'Zen' are less heat tolerant. After the heat stress for 7 and 14 d, there was no difference in EL response between the three other cultivars, although as duration was increased to 21 d, 'Candyman' exhibited a significantly greater CMS value which indicated that foliar tissues in 'Indian Prince' and 'Pacific Beauty' were able to maintain more cell membrane stability. Similar results indicating higher EL in more heat sensitive plants were already reported for *Glycine max* and *Prosopis chilensis* (Ortiz and Cardemil, 2001), *Festuca arundinacea* and *Lolium perenne* (Jiang and Huang, 2001), *Hedera helix* (Yeh and Hsu, 2004) and *Salvia splendens* (Natarajan, 2005).

Previous studies have indicated that some adaptable characteristic are usually found in plants that are adapted to high temperature conditions including shortened internodes, smaller and thicker leaves, higher membrane thermostability, deeper root system, and delayed flowering (Yeh and Hsu, 2004; Natarajan, 2005; Mader, 2009). Xu and Huang (2001) reported that better performance of heat tolerant cultivars was primarily due to their morphological traits.

In the present study, comparisons between different cultivars of calendula showed that 'Indian Prince' was more suitable than other tested cultivars for warm climates. This cultivar showed a lesser degree of damage as compared to other cultivars., Therefore it may be more stress tolerant cultivar. Comparing the overall plant growth, heat tolerant 'Indian Prince' had taller plants with greater leaf area and upward leaf inclination, better shoot and root growth, smaller flowers with later flowering and more cell membrane stability. Heat stress resulted in 'Indian Prince' developing more compact plants with significantly less leaf area and shoot growth as compared to control. In addition, since upward inclined leaf type reduces leaf temperature and so membrane leakage (Kalyar *et al.*, 2013), steeper leaf inclination of 'Indian Prince' could contribute to its resistance to heat stress.

In general, although 'Zen' showed some heat tolerant morphological traits such as shorter plants or compact growth and darker green foliage in control treatment, it was considered more sensitive cultivar to heat stress than other three cultivars. A higher heat sensitivity of 'Zen' could be attributed to certain morphological traits of this cultivar such as poor shoot and root growth, downward leaf inclination, and more cell membrane damage. The rapid decrease in SPAD value of 'Zen' in response of heat stress may also imply its less tolerant to heat stress.

CONCLUSION

In conclusion, growth and development of calendula cultivars was significantly affected by exposure to heat stress. Extended exposure may result in more profound differences in calendula growth and floral traits. Not all cultivars responded the same way to the high temperatures. The results indicated that 'Zen' was considered as the most heat sensitive cultivar for this study among all studied cultivars, while 'Indian Prince' enjoying more heat tolerant morphological traits would perform better in warm climates.

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