Garden Chrysanthemum Cell Membrane Thermostability and Flowering Heat Delay Differences Among U.S. and South Korean Germplasm

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Abstract. Global warming has created increased nighttime temperatures both in field and greenhouse production of chrysanthemums during flower bud initiation (FBI) and development, causing heat delay or complete cessation of flowering. Integration of breeding and selection for heat delay insensitivity (HDI) has become imperative for greenhouse (cut, potted types) and must be accomplished on a genotypic basis, similar to winterhardiness. This is a breeding objective in the joint garden chrysanthemum breeding project between the Chungnam Provincial Agricultural Research and Extension Services and the University of Minnesota. The objectives of this research were to test 10 genotypes (cultivars, seedlings) from both breeding programs when grown in low-temperature (LT) and high-temperature (HT) short-day (SD) and long-day (LD) conditions (four environments: LTSD, LTLD, HTSD, and HTLD); determine the extent of heat delay and HDI for visible bud date (VBD), flowering, and other phenotypic traits; evaluate relative injury (RI) and cell membrane thermostability (CMT), and to select future parents with lowered RI values, higher CMT, shorter heat-induced flowering delay, and/or HDI. ‘Magic Ball’ and ‘Minnwhite’ had the shortest plant height in HTLD and HTSD, whereas ‘Geumbangul’ had stability for height in all treatments. Lowest long day leaf numbers (LDLN) occurred under LTSD in seven genotypes. However, both ‘Geumbangul’ and ‘Magic Ball’ had complete stability for LDLN across all environments. Sigmoid curves for RI% and temperature were found for all genotypes and environments with $R^2 = 0.79$ – 0.89 . Only ‘Mellow Moon’ had stability or equal VBDs in HTSD, LTSD, and LTLD conditions. This is the first-ever report of stability for VBD across inductive and noninductive HT/LT treatments. Only ‘Centerpiece’ flowered in all environments and also had 0 day of heat for VBD in LT and 1 day of heat delay in HT, as well as three others (Mn. Sel’n. 01-210-43, ‘Autumn Fire’, and ‘Geumbangul’). Few had linear regressions with positive slopes for heat-induced VBD or flowering delay regressed with RI%; most had no slope ($R^2 \approx 0.0$) for all treatments (‘Centerpiece’, Mn. Sel’n. 01-210-43), whereas others were negative (‘Mammoth™ Dark Bronze Daisy’, Flw LTLD–LTSD). Surprisingly, one linear regression had a slope of $R^2 = 1.0$ (‘Geumbangul’, Flw LTLD–LTSD). These responses are all novel in chrysanthemums. Selecting the best parents in both breeding programs to maximize stability of all traits across these four environments with minimal crossing and selection across generations could be accomplished by stacking parental traits. A crossing scheme involving just three parents is proposed to incorporate stability for all traits in just a few generations.

Chrysanthemums (Asteraceae) remain popular indoor (cut flowers, potted flowering plants; collectively “greenhouse” types) and outdoor (winter-hardy and nonhardy garden plants; collectively “garden”) ornamental

flowering crops in the United States and the rest of the world. Certain Korean and Chinese species are also grown to make chrysanthemum tea (Xiwang et al., 2008). Greenhouse types, *Chrysanthemum* × *grandiflorum*

Tzvelv. (= *Dendranthema* × *grandiflora* Tzvelv.; = *Chrysanthemum* × *morifolium* Ramatuelle), are ranked ninth in value among all cut flower and foliage crops with \$11.497 M (w), whereas flower potted chrysanthemums rank seventh among potted foliage and flowering products at \$21.767 M (w) in 2014 (USDA NASS, 2015). Garden types, *C. × grandiflorum* and *Chrysanthemum × hybridum* Anderson, are the no. 1 herbaceous perennial in the top 15 U.S. producing states with a wholesale farm gate value of \$117.54 M in 2014 (USDA NASS, 2015). The combined value of all U.S. grown chrysanthemums totals \$150.804 M (w) (USDA NASS, 2015).

Both greenhouse and garden types of chrysanthemums have been domesticated with a wide range of flower colors, patterns, and forms (Anderson, 2006). Both types can be programmed to flower year-round by manipulating the photoperiod although less control occurs when garden types are grown outside under naturally occurring photoperiods to flower in the fall season (Anderson, 2006; Dole and Wilkins, 2005). Typical plant habits of garden chrysanthemums are upright (cut flower types), similar to greenhouse cut flowers, and unique phenotypes of cushion (spherical shape completely covered with flowers obscuring most foliage) or groundcover types (Anderson, 2006; Langevin, 1992; Widmer, 1980). Groundcover or wave habits have been created recently (Anderson and Ascher, 2004a; Xiwang et al., 2008). The cushion habit was developed by the University of Minnesota as the ‘Minn’ series and now has the majority of the market share (Anderson, 2004, 2006; Anderson and Gesick, 2003, 2004; Kim and Anderson, 2006; Widmer, 1978).

Since most greenhouse and garden chrysanthemums are facultative SD plants for FBI, whereas qualitative (obligate) SD plants for flowering or flower bud development (FBD) (Anderson and Ascher, 2001; Cathey, 1954, 1957; Cockshull and Kofranek, 1994; Langton, 1977), HTs can cause heat delay which prevents or delays either FBI and/or FBD in greenhouse production under black out or black cloth (used to induce SD conditions) (Anderson and Ascher, 2001; Chen et al., 2001). There are exceptions to photoperiodic response and HDI to this species trend, such as day neutral (DN) garden genotypes that flower under HTs regardless of photoperiod (Anderson et al., 1989). Heat delay can occur when the night temperatures are ≥ 22 °C or more precisely at 26–32 °C (Karlsson et al., 1989; Whealy et al., 1987), under either black cloth or in the field during the summer (de Jong, 1978). Heat delay-sensitive (HDS) cultivars also have flowering delay at suboptimal temperatures for the species (≤ 10 °C; de Jong, 1978). Thus, HDI garden chrysanthemums will also undergo FBI and FBD in cool growing conditions. Chrysanthemum production year-round, particularly in the subtropics, predicates division of cultivars into two groups: heat tolerant or HDI (summer and fall seasons) and HDS (grown in winter and spring) (Wang et al., 2008). The HDI

Table 1. Mean plant height (cm), plant width (cm), number of leaves (LDLN), internode length (cm), and number of nodes on lateral branches (subtending the apical meristem) for 10 garden chrysanthemum genotypes grown under four temperature/photoperiod combination treatments.

Genotype	Temperature, photoperiodic treatments	Plant ht (cm)	Plant width (cm)	No. of leaves (LDLN)	Internode length (cm)	No. nodes on lateral branches
Mn. Sel'n. 01-210-43	HTSD	29.3 e ^z	38.7 e ^z	43.8 d ^z	1.8 b ^z	6.7 b ^z
	HTLD	24.3 c	38.7 e	61.5 e	0.9 a	7.3 b
	LTSD	26.5 d	20.4 bc	34.8 bc	1.2 a	9.8 c
	LTLD	29.5 e	23.8 c	41.2 d	1.2 a	6.3 b
Mn. Sel'n. 01-210-77	HTSD	34.5 fg	31.5 e	49.0 e	1.1 a	13.4 d
	HTLD	32.9 e	24.8 c	51.5 e	1.1 a	9.6 c
	LTSD	33.4 f	23.3 c	38.0 c	1.2 a	13.3 d
	LTLD	36.1 g	27.0 d	35.9 c	1.3 a	10.6 c
Mn. Sel'n. 02-93-1	HTSD	23.5 c	21.0 c	30.8 ab	1.8 ab	10.5 c
	HTLD	32.0 e	19.8 bc	40.3 d	1.1 a	6.0 b
	LTSD	18.2 ab	15.8 ab	28.5 a	1.2 a	6.0 b
	LTLD	20.8 bc	18.5 b	29.2 a	2.3 b	5.3 a
'Autumn Fire'	HTSD	32.3 e	18.7 b	29.8 a	1.4 a	12.2 c
	HTLD	47.3 h	15.3 a	39.1 cd	1.3 a	5.7 a
	LTSD	32.7 e	16.1 ab	34.3 bc	1.0 a	9.3 c
	LTLD	38.0 gh	17.9 ab	33.8 b	1.3 a	6.7 b
'Centerpiece'	HTSD	34.1 fg	16.4 ab	26.9 a	1.5 a	10.9 c
	HTLD	41.2 h	14.8 a	39.0 cd	1.0 a	5.7 a
	LTSD	29.3 e	13.4 a	25.1 a	1.2 a	8.0 b
	LTLD	30.1 e	15.6 a	29.4 a	1.2 a	5.0 a
'Geumbangul'	HTSD	14.2 a	18.8 b	46.2 de	2.1 b	15.7 d
	HTLD	16.8 a	18.8 b	47.8 de	1.5 a	9.8 c
	LTSD	15.3 a	16.8 ab	41.3 d	0.7 a	10.8 c
	LTLD	14.5 a	18.7 b	43.3 d	1.0 a	12.3 c
'Magic Ball'	HTSD	16.8 a	20.3 c	62.3 e	0.8 a	19.3 d
	HTLD	19.5 b	20.1 c	62.8 e	1.0 a	13.2 d
	LTSD	16.9 a	17.8 ab	48.2 e	0.8 a	14.3 d
	LTLD	19.8 b	16.8 ab	58.0 e	2.4 b	13.5 d
'Mammoth Dark Bronze Daisy'	HTSD	24.4 c	28.1 d	38.9 c	1.9 a	9.9 c
	HTLD	37.2 gh	24.7 cd	59.8 e	1.1 a	8.7 c
	LTSD	23.0 c	19.5 c	34.8 bc	0.9 a	7.5 c
	LTLD	26.0 d	18.7 b	34.7 bc	1.0 a	8.6 c
'Mellow Moon'	HTSD	34.3 f	18.9 b	23.1 a	1.5 a	3.8 a
	HTLD	40.5 gh	15.8 a	33.3 b	1.3 a	1.7 a
	LTSD	32.6 e	15.6 a	19.7 a	1.7 a	5.7 a
	LTLD	34.6 f	14.3 a	22.3 a	1.5 a	3.3 a
'MinnWhite'	HTSD	20.0 bc	25.0 c	70.5 e	— ^y	14.5 d
	HTLD	25.9 d	16.3 ab	40.1 d	1.1 a	6.3 b
	LTSD	17.1 a	16.8 ab	32.0 b	0.7 a	7.7 b
	LTLD	16.4 a	17.2 ab	49.4 de	1.4 a	6.2 b

HTSD = high temperature short days; HTLD = high temperature long days; LTSD = low temperature short days; LTLD = low temperature long days.

^zAny two means within a column not followed by the same letter are significantly different at $P \leq 0.001$.

^yNonexistent.

phenomenon has been widely documented throughout the chrysanthemum germplasm tested to date (Anderson and Ascher, 2001; Cockshull, 1979; Karlsson et al., 1989; Wang et al., 2008; Whealy et al., 1987; Yeh and Lin, 2003).

Selection and breeding for HDI have commenced in Japanese (Shibata and Kawata, 1987), British (De Jong, 1989), American (Anderson and Ascher, 2001), Taiwanese (Wang et al., 2008), and Chinese (Xiwang et al., 2008) chrysanthemum breeding programs. An ideotype

has been proposed to select for DN and HDI garden chrysanthemums in a LD, HT environment (Anderson and Ascher, 2001) and one study tested the rigor of the ideotype (Anderson and Ascher, 2004b). In addition to screening chrysanthemums for phenotypic traits (VBD, flowering, etc.) to assess heat delay, CMT has also been used (Wang et al., 2008; Xiwang et al., 2008; Yeh and Lin, 2003). Electrolyte leakage tests measure CMT and summer- and fall-flowering types had RI values at 47–53 °C, the midpoint of the sigmoid response curves (Wang et al., 2008). Additional tests can be measured to detect HDI, e.g., proline, chlorophyll, and/or malondialdehyde concentrations, although these do not add any additional information to CMT data (Wang et al., 2008; Xiwang et al., 2008). Thus, most HDI research in chrysanthemum uses CMT as a quick, yet sensitive test (Sullivan, 1972; Wu and Wallner, 1983), when coupled with plant growth and flowering under HT, SD, and LD photoperiods.

Recent trends in global warming have created increased nighttime temperatures during FBI and FBD, causing heat delay or complete

cessation of flowering in greenhouses during spring through fall seasons and in tropical and semitropical field production of garden chrysanthemums (Yeh and Lin, 2003). Thus, HDI is now an important trait for both greenhouse (cut, potted types) as well as garden types (Liu et al., 2011) and needs to be integrated into the selection processes similar to our integration of winterhardiness screening (in northern latitudes) on a genotypic basis (Kim and Anderson, 2006). Heat tolerance or HDI screening is variable and inconsistent under field conditions (De Jong, 1989) and, thus, germplasm screening and parental or hybrid selection needs to be integrated into greenhouse or growth chamber environments (Anderson and Ascher, 2001; Yeh and Lin, 2003). A breeding objective in the joint garden chrysanthemum breeding project between the Chungnam Provincial Agricultural Research and Extension Services (South Korea) and the University of Minnesota (United States) is to select germplasm with the cushion or upright garden/landscape phenotypes that initiate and develop

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flower buds in temperatures causing heat delay in inductive conditions. We hypothesize that heat-tolerant garden chrysanthemum cultivars and seedlings exist in the breeding programs due to inadvertent selection, which would exhibit a range of expression in heat delay adaptation with lowered RI. The objectives of this research were to 1) test genotypes (cultivars, seedlings) from the University of Minnesota (United States) and Chungnam Provincial Agricultural Research and Extension Services (South Korea) when grown in LT and HT, SD and LD conditions (four environments: LTSD, LTLD, HTSD, and HTLD); 2) determine the extent of heat delay and HDI for VBD, flowering and other phenotypic traits of garden chrysanthemums; 3) evaluate RI and CMT in the germplasm; and 4) to select future parents with lowered RI values, higher CMT, shorter heat-induced flowering delay, and/or HDI.

Materials and Methods

Genotypes. Ten named cultivars or unnamed, numbered selections from two garden

chrysanthemum breeding programs in the United States and South Korea were selected for use in this study. These included five named cultivars *C. ×grandiflorum* Autumn Fire (upright plant habit, cut flower type; Widmer and Ascher, 1976), Centerpiece (upright plant habit, cut flower type; Widmer et al., 1981), Mellow Moon (upright plant habit, cut flower type; Widmer et al., 1982) Minnwhite (short-statured cushion plant habit; Widmer and Phillips, 1967); *C. ×hybridum* Mammoth™ Dark Bronze Daisy (shrub cushion plant habit; Anderson et al., 2015), and three unnamed, numbered shrub cushion plant habit selections of *C. ×hybridum* Mn. Sel'n. 01-210-43, Mn. Sel'n. 01-210-77, and Mn. Sel'n. 02-93-1, all from the University of Minnesota flower breeding program. Two named cultivars, *C. ×grandiflorum* Geumbangul and Magic Ball (short-statured cushion plant habit; Yesan Chrysanthemum Experiment Station, 2015), were from the Chungcheongnam-do Agricultural Research and Extension Services Chrysanthemum Breeding Program. The genotypes represented a range in breeding phenotypes from upright,

cut types to cushion plant habits with all flower color classes (white, lavender/purple, red, bronze, and yellow) and several flower forms (daisy, decorative, and incurved). Before this experiment, all genotypes were established to have stable phenotypic traits and were true to type when clonally propagated (*cf.* Anderson and Ascher, 2008).

Stock plant cultural conditions. All stock plants were grown as clonal ramets from the original ortet of each genotype potted into 1620-cm³ containers filled with Sunshine no. 8/LC8 Professional Growing Mix (Sun Gro Horticulture, Bellevue, WA) and greenhouse forced to prompt vegetative shoot growth (LDs, 0800–1600 HR supplied by 400-W high-pressure sodium lamps + 2200- to 0200-HR night interruption at a minimum set point of 150 μmol·m⁻²·s⁻¹; 18.5 ± 0.1/22.0 ± 0.2 °C day/night). To maintain a vegetative state in the stock plants, weekly sprays of 500 mg·L⁻¹ ethephon (Florel) + 5 mg·L⁻¹ gibberellic acid were applied (Strefeler et al., 1996; Stuart et al., 1988).

Cuttings. Thirty vegetative tip cuttings (1–2 cm length) of each genotype were taken,

Table 2. Mean number of days to visible bud date, flower coloration, flowering, and total number of flowers under four temperature/photoperiod combination treatments of 10 garden chrysanthemum genotypes.

Genotype	Temperature, photoperiodic treatments	No. of d to visible bud date	No. of d to flower coloration	No. of d to flowering	Total no. of flowers
Mn. Sel'n. 01-210-43	HTSD	22.0 ab ^z	37.0 b ^z	42.0 a ^z	36.0 cd ^z
	HTLD	23.7 bc	— ^y	— ^y	— ^y
	LTSD	18.7 a	30.7 a	42.7 a	17.3 ab
	LTLD	21.0 a	35.7 b	46.7 ab	17.0 ab
Mn. Sel'n. 01-210-77	HTSD	21.3 a	41.7 e	42.3 a	44.7 de
	HTLD	24.7 bc	41.0 e	— ^y	— ^y
	LTSD	19.0 a	34.0 b	46.0 ab	30.7 cd
	LTLD	21.3 a	37.3 b	48.7 b	29.0 bcd
Mn. Sel'n. 02-93-1	HTSD	22.0 ab	40.7 d	43.0 a	27.7 bcd
	HTLD	24.3 bc	41.0 de	— ^y	— ^y
	LTSD	19.3 a	35.3 b	45.0 ab	15.0 ab
	LTLD	22.0 ab	40.0 d	50.0 c	11.3 a
'Autumn Fire'	HTSD	20.7 a	40.0 d	47.3 ab	32.3 cd
	HTLD	23.3 bc	— ^y	— ^y	— ^y
	LTSD	17.7 a	35.7 b	49.3 b	16.7 ab
	LTLD	21.7 a	40.3 d	48.0 b	18.0 ab
'Centerpiece'	HTSD	23.7 bc	40.7 d	46.3 ab	23.7 bc
	HTLD	23.7 bc	49.0 f	59.0 d	1.0 a
	LTSD	23.7 bc	38.7 c	45.7 ab	14.0 ab
	LTLD	23.7 bc	46.3 ef	57.0 d	18.3 ab
'Geumbangul'	HTSD	26.0 cd	45.3 ef	52.7 c	81.7 g
	HTLD	26.7 cd	— ^y	— ^y	— ^y
	LTSD	25.0 cd	42.7 e	49.7 bc	39.3 de
	LTLD	25.3 cd	55.3 g	71.3 e	26.7 bc
'Magic Ball'	HTSD	21.7 a	42.7 e	56.0 d	78.3 g
	HTLD	26.0 cd	— ^y	— ^y	— ^y
	LTSD	19.3 a	36.0 b	46.3 ab	46.0 ef
	LTLD	24.7 bc	62.7 g	70.7 e	42.0 de
'Mammoth Dark Bronze Daisy'	HTSD	21.3 a	41.7 de	48.7 b	44.7 de
	HTLD	25.0 cd	— ^y	— ^y	— ^y
	LTSD	20.0 a	36.3 b	47.7 b	13.0 ab
	LTLD	23.3 bc	45.0 ef	62.0 de	25.3 bc
'Mellow Moon'	HTSD	21.3 a	38.7 c	45.0 ab	8.0 a
	HTLD	39.3 e	— ^y	— ^y	— ^y
	LTSD	15.3 a	36.3 b	46.3 ab	7.7 a
	LTLD	20.5 a	42.5 e	66.0 e	19.3 ab
'Minnwhite'	HTSD	24.0 bc	44.0 ef	51.0 c	66.0 g
	HTLD	30.0 de	— ^y	— ^y	— ^y
	LTSD	24.0 bc	43.3 e	54.7 cd	26.0 bcd
	LTLD	29.7 de	53.0 g	63.3 e	26.7 bcd

HTSD = high temperature short days; HTLD = high temperature long days; LTSD = low temperature short days; LTLD = low temperature long days.

^zAny two means within a column not followed by the same letter are significantly different at $P \leq 0.001$.

^yNonflowering.

Fig. 1. Aerial view of plant growth and flower bud initiation (buds) and development (flowers) on example chrysanthemums grown under low temperature short days (LTSD) (top left), low temperature long days (LTLD) (top right), high temperature short days (HTSD) (bottom left), and high temperature long days (HTLD) (bottom right) temperature/photoperiod treatments: (A) Mn. Sel'n. 01-210-43 and (B) 'Magic Ball'. Scale: Bar = 15.24 cm. Photo credits: Mi-Kyoung Won.

the lower 1–3 mature leaves removed and the cut ends were dipped in 1000 ppm indole-3-butyric acid in 50% ethanol. Cuttings were placed in Oasis wedges (Smithers-Oasis, Kent, OH) under intermittent mist with a mist frequency of 10-min intervals (mist nozzles, reverse osmosis water) with a 7-s duration at 21.1 ± 0.2 °C day/night (soil) and the same LD photoperiod as the stock plants. Cuttings rooted (100%) in ≈ 1.5 weeks and were transplanted for growth in 1620-cm³ containers for 2 weeks in greenhouses (Saint Paul, MN; 44°59'17.8"N lat., -93°10'51.6" W long.) for early establishment and vegetative growth (LDs, 0800–1600 HR + 2200- to 0200-HR night interruption supplied by 400-W high-pressure sodium lamps at a minimum set point of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; $18.0 \pm 0.1/21.1 \pm 0.2$ °C day/night) and a constant liquid feed of 125 ppm N from water-soluble 20N-4.4P-16.6K (Scotts, Marysville, OH).

Plant growth treatments. Twelve plants of each genotype were all pretreated with a 2-week heat acclimation (Senthil-Kumar et al., 2003, 2007; Wang et al., 2008) in the same growth chamber (Environmental Growth Chambers, Chagrin Falls, OH), starting in week 23 (3 June 2013) and ending in week 25 (17 June 2013). Preacclimation growth chamber environmental conditions were LDs, 0800–2000 HR, supplied by fluorescent [red (R) light] and incandescent [far red (FR) + R] as light sources ($4.8 \mu\text{W}\cdot\text{cm}^{-2}$ at 630 nm and $2.3 \mu\text{W}\cdot\text{cm}^{-2}$ at 750 nm) at 27 ± 0.5 °C day/night. Light levels exceeded the $2.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ minimum required at the shoot apex (Anderson and Ascher, 2001; Mastalerz, 1977).

Fig. 2. Effects of water bath temperatures on relative injury (RI) of garden chrysanthemum leaves of 10 genotypes when grown under two temperature and two photoperiod conditions [key legend: high temperature short days (HTSD); high temperature long days (HTLD); low temperature short days (LTSD); and low temperature long days (LTLD)]: (A) Mn. Sel'n. 01-210-43, (B) Mn. Sel'n. 01-210-77, (C) Mn. Sel'n. 02-93-1, (D) 'Autumn Fire', (E) 'Centerpiece', (F) 'Geumbangul', (G) 'Magic Ball', (H) 'Mammoth Dark Bronze Daisy', (I) 'Mellow Moon', and (J) 'MinnWhite'. The finely dotted lines denote the inflection point, i.e., the midpoint of the sigmoid response curve, as calculated from the regression equation for each temperature/photoperiod combination for each cultivar. SE bars are not visible if the SE are smaller than the symbol.

Following the preacclimation period, three plants per genotype per treatment were randomized into four growth chambers to test two temperature and two photoperiod effects on CMT and flowering heat delay responses and procedures were run once: LTSD and LTLD ($20.1 \pm 0.2/15.1 \pm 0.1$ °C day/night); HTSD and HTLD ($30.4 \pm 0.8/25.3 \pm 0.3$ °C day/night). All four treatments (LTSD, LTLD, HTSD, and HTLD) received 0800 to 1600 HR at a minimum set point of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; $18.3 \pm 0.1/21.1 \pm 0.2$ °C day/night, whereas the two treatments that were long days (LTLD and HTLD) also received a 2200- to 0200-HR night interruption of $2.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ lighting using incandescent (FR + R). Temperature and photoperiod treatments commenced in week 25 (17 June 2013) and ended in week 35 (1 Sept. 2013) or for a total of 10 weeks. Plants were arranged within each growth chamber in a completely randomized design.

Phenotypic data. Repeated measures plant growth data were recorded 1 month (week 29) after the commencement of the temperature/photoperiod treatments and at the termination of the experiment (week 35) for the following phenotypic traits: plant height or stem length of terminal shoot (cm; from soilless media surface to the uppermost leaf on the terminal meristem; Anderson and Ascher, 2001), plant width (cm; widest aboveground plant growth width), LDLN (the number of leaves or nodes from the crown to the flower bud of the terminal meristem in any photoperiod; Anderson and Ascher, 2001; Langton and Cockshull, 1976), internode length (cm; plant height/no. of leaves or nodes), and number of nodes on lateral branches (subtending the apical meristem). The number of days to visible bud date (FBI), first flower coloration of ray petals (FBD), and flowering (anthesis; FBD completion) were recorded daily as they

occurred, whereas the total number of flowers was determined at the end of the experiment.

Leaf CMT. In week 29, fully expanded leaves ($n = 5/\text{apex/genotype}$) from all four treatments were harvested from the treated plants using modified protocols of Yeh and Lin (2003) and Wang et al. (2008). The assay leaf samples were five discs (1.4 ± 0.2 mg dry weight) cut from five leaves with a 6-mm-diameter cork borer and then rinsed $3\times$ in distilled, deionized water. Leaf discs were immediately placed into 8-dram glass vials with 1 mL distilled, deionized, and sterilized water at 21°C and capped to maintain turgidity. Three tubes (reps)/treatment were put into an Endocal LT-50 refrigerated water bath circulator (Thermo Scientific, Newington, NH) for 30 min; water bath temperatures varied depending on the CMT test temperature measurements: 25 (control), 30, 35, 40, 45, 50, 55, 60, 65, and 70°C . After the 30-min temperature exposure, each 8-dram vial received 16 mL of distilled, deionized, and sterilized water; tubes were then kept at $10 \pm 0.5^\circ\text{C}$ for 24 h and then the initial solution conductivity (T_1C_1) was measured. Tubes were capped, then autoclaved at 120°C , 1.2 kg cm^{-2} in a Steris, Amsco Renaissance ACLV01, Stage 3 autoclave (Steris Corp., Mentor, OH) for 15 min followed by a slow exhaust autoclave cycle, cooled to 25°C , followed by incubation for 24 h before final solution conductivity measurements (T_2C_2) were recorded. Percent RI was calculated using Yeh and Lin's (2003) formula:

$$\text{RI}(\%) = 1 - \left[\frac{1 - (T_1 - T_2)}{1 - (C_1/C_2)} \right] \times 100$$

In this equation, T denotes the treatment conductivity values and C is the control (25°C).

Data analyses. Phenotypic data for plant height, plant width, LDLN, internode length, and number of nodes on lateral branches were analyzed with univariate, repeated measures general linear model analysis of variance (ANOVA) along with mean separations using Tukey's honestly significance difference (HSD) tests at $\alpha = 0.05$ [Statistical Package for the Social Sciences (SPSS) v.22; University of Chicago, Chicago, IL]. All other phenotypic data were analyzed using a univariate ANOVA and Tukey's HSD tests.

RI values and water bath temperature treatment R^2 were determined with regression analyses for each temperature/photoperiod treatment (LTSD, LTLD, HTSD, and HTLD) using Sigma Plot (v. 8; SPSS Inc.). The inflection point or midpoint of the sigmoid response curve for each cultivar in their respective LTSD, LTLD, HTSD, and HTLD treatments were determined from the regression analyses. Degree of heat delay (Shibata and Kawata, 1987) was calculated as the difference between days to VBD (FBI) for LTLD–LTSD; HTLD–HTSD. Similarly, the degree of heat delay was also calculated for the number of days to flowering (FBD) for

Fig. 2. Continued.

LTLD–LTSD; HTLD–HTSD. Regressions were run for each degree of heat delay with their respective RIs at 50°C water bath treatment (the approximate inflection point for all genotypes; Wang et al., 2008).

Results

Phenotypic data for plant height ranged from 14.2 cm ('Geumbangul' HTSD) to 47.3 cm ('Autumn Fire' HTLD; Table 1).

Treatments, genotypes, and treatment $\times$ genotype interaction were all significant ($P \leq 0.001$) for plant height. Three short-statured, cushion plant habit chrysanthemum cultivars had the significantly shortest plant heights either under HTSD and LTSD ('Magic Ball'), LTSD and LTLD ('MinnWhite'), or all treatments, HTSD, HTLD, LTSD, and LTLD ('Geumbangul'; Table 1). Mean plant heights for upright, cut flower genotypes ('Autumn Fire', 'Centerpiece', and 'Mellow Moon') were significantly taller for all treatments than the cushion types although treatment effects demarcated different groupings of plant height. For instance, LTSD ('Mellow Moon', 'Autumn Fire', and 'Centerpiece') and LTLD ('Centerpiece') and HTSD ('Autumn Fire') were significantly taller, followed by a grouping of HTSD and LTLD ('Mellow Moon'), HTSD ('Centerpiece'), LTLD ('Autumn Fire'), and HTLD ('Mellow Moon') and, finally, the tallest of upright habits under HTLD ('Autumn Fire' and 'Centerpiece') (Table 1). Unlike field trials with these plant types (Anderson et al., 2015), all shrub cushion plant habit genotypes had plant heights within the range of the short-statured cushion plant habit cultivars. For most genotypes, the tallest plant heights occurred in the HTLD treatment, with the exceptions of the full sibs Mn. Sel'ns. 01-210-43 and 01-210-77, which were significantly taller in the HTSD treatment and 'Geumbangul', which had no difference in plant height in all four temperature/photoperiod treatments (Table 1).

Plant width varied in distribution from 13.4 cm ('Centerpiece', LTSD) to 38.7 cm (Mn. Sel'n. 01-210-43, HTSD, and HTLD; Table 1). In general, as would be expected, the widest plants were the shrub cushion plant habits, followed by the short-stature cushion cultivars and, subsequently, the upright types. As with plant height, plant width for treatments, genotypes, and their interaction was all significant, $P \leq 0.001$. At least three genotypes (Mn. Sel'ns. 01-210-77, 02-93-1, 'Autumn Fire', and 'Centerpiece') had similar mean plant widths for all treatments although they were in overlapping groups.

The number of leaves (LDLN) of the primary shoot ranged from 19.7 ('Mellow Moon', LTSD) to 70.5 ('MinnWhite', HTSD) (Table 1). Unlike plant heights and widths, LDLN was not related to plant habit since the highest number of leaves occurred in 'Minnwhite', 'Magic Ball', 'Geumbangul' (short-stature cushions) as well as 'Mammoth™ Dark Bronze Daisy' (HTLD), Mn. Sel'ns. 01-210-43 and 01-210-77 (HTSD and HTLD). Once again, the treatments, genotypes and treatment $\times$ genotype interaction were all significant, $P \leq 0.001$. Five genotypes from the University of Minnesota breeding program had the significantly lowest LDLN in the LTSD treatment, although occasionally LDLN did not differ from LTLD ('Centerpiece', Mn. Sel'ns. 01-210-77, 02-93-1, 'Mammoth™ Dark Bronze Daisy') or HTSD ('Centerpiece', 'Mellow Moon') (Table 1).

Fig. 2. Continued.

'Autumn Fire' had the lowest LDN in the HTSD treatment. Interestingly enough, both cultivars from the South Korean breeding program had no significant difference in LDN among all four treatments.

Internode lengths were significantly different, $P \leq 0.05$, for treatments, genotypes, and their interaction, ranging from 0.7 cm (LTSD: 'Geumbangul' and 'Minnwhite') to 2.4 cm (LTLD: 'Magic Ball'; Table 1). The significantly longest internodes occurred in HTSD with Mn. Sel'n. 01-210-43, Mn. Sel'n. 02-93-1, 'Geumbangul', and LTLD ('Magic Ball'). Mn. Sel'n. 01-210-77, 'Autumn Fire', 'Centerpiece', 'Mammoth™ Dark Bronze Daisy', 'Mellow Moon', and 'Minnwhite' all had statistically similar internode lengths across all four test environments (Table 1).

For the number of nodes on lateral branches, treatments and genotypes were significant, $P \leq 0.001$, whereas the treatment $\times$ genotype interaction was barely so ($P = 0.035$). A range from 3.3 ('Mellow Moon', LTLD) to 19.3 ('Magic Ball', HTSD) nodes on lateral branches was found (Table 1). The lowest frequency occurred predominantly in the upright plant habit cut flower cultivars in either all treatments (Mellow Moon) or selected ones (HTLD, LTLD: Centerpiece; HTLD: Autumn Fire). Only one other genotype (Mn. Sel'n. 02-93-1) had one treatment (LTLD) with the same number of nodes on lateral branches. Three genotypes had the same number of nodes on lateral branches for all four treatments: 'Magic Ball', 'Mammoth™ Dark Bronze Daisy', and 'Mellow Moon' although the number differed significantly among cultivars (Table 1).

The number of days to VBD ranged from 17.7 ('Autumn Fire', LTSD) to 39.3 ('Mellow Moon', HTLD)—more than twice the number of days (Table 2). ANOVA results showed that treatments, genotypes, and treatment $\times$ genotype interaction were significant, $P \leq 0.001$, for the number of days to VBD. Although most upright plant habit, cut flower types had higher numbers of days to VBD, a few treatments had a significantly shorter time period, e.g., 'Autumn Fire' and 'Mellow Moon' (HTSD, LTSD, and LTLD treatments). Unexpectedly, 'Magic Ball', 'Mammoth™ Dark Bronze Daisy', 'Mellow Moon', and 'Minnwhite' had statistically similar VBDs under HTSD and LTSD treatments or no heat delay in the SD photoperiods. In contrast, 'Centerpiece', 'Geumbangul', and 'Minnwhite' had no difference in VBDs between HTLD and LTLD treatments. Mellow Moon was the only cultivar with equal (nonsignificantly different) VBDs in three treatments (HTSD, LTSD, and LTLD). VBD and flower color were highly significantly correlated, $r = 0.76$ ($P \leq 0.01$) as well as VBD and the number of days to flower ($r = 0.58$, $P \leq 0.01$) and VBD and the number of flowers ($r = 0.207$, $P \leq 0.05$).

The earliest genotype to reach flower coloration, Mn. Sel'n. 01-210-43 (LTSD; Fig. 1A), did so in 30.7 d, whereas the latest was 'Magic Ball' (LTLD; Fig. 1B) at 62.7 d (Table 2). Two other genotypes were almost as late as 'Magic Ball' (LTLD): 'Minnwhite'

at 53 d and 'Geumbangul' at 55.3 d—both in the LTLD treatment. No genotype had the same number of days to flower bud coloration in all treatments, although many had two or three treatments that did. The number of days to flower bud coloration, treatments, genotypes, and their interaction were significant, $P \leq 0.001$. Several treatments and genotypes did not have flower buds developing to flower coloration in the highest temperature, noninductive photoperiod treatment (HTLD): Mn. Sel'n. 01-210-43, 'Autumn Fire', 'Geumbangul', 'Magic Ball', 'Mammoth™ Dark Bronze Daisy', 'Mellow Moon', and 'Minnwhite'. Thus, Mn. Sel'n. 01-210-77, Mn. Sel'n. 02-93-1, and 'Centerpiece' had no HT delay under LDs (Table 2). In contrast, no genotype had heat delay under short days (HTSD). This trait was significantly correlated with the number of days to flower, $r = 0.839$ ($P \leq 0.01$) and the number of flowers ($r = 0.209$, $P \leq 0.05$).

A range of more than 3 weeks, i.e., 42.0 d (Mn. Sel'n. 01-210-43, HTSD) to 66.0 d ('Mellow Moon', LTLD), was found for the number of days to flowering (Table 2). For the number of days to flower, treatments, genotypes, and treatment $\times$ genotype interaction were significant, $P \leq 0.001$. However, the number of days to flowering was not significantly correlated with flower number ($r = 0.057$, $P > 0.05$). In contrast with the number of days to flower coloration where three genotypes did not exhibit heat delay in the HTLD treatment, only 'Centerpiece' actually flowered in this and all other treatments (Table 2). Thus, 'Centerpiece' has the best HT tolerance or lack of heat delay, regardless of photoperiod.

For the number of flowers, treatments, genotypes, and treatment $\times$ genotype interaction were significant, $P \leq 0.001$. A wide range in the number of flowers occurred, from 1.0 ('Centerpiece', HTLD) to 81.7 ('Geumbangul', HTSD) (Table 2). Six genotypes had statistically similar numbers of flowers in the LTSD and LTLD treatments, whereas only one ('Mellow Moon') had similar flower numbers in HTSD and LTSD (Table 2).

Most CMT ANOVA results for water bath treatments on RI of leaves at varying temperatures (30, 35, 40, 45, 50, 55, 60, 65, and 70 °C) showed treatments, genotypes, and their interactions to be significant (ranging from $P = 0.03$ to $P \leq 0.001$). Exceptions to this were a nonsignificant genotype effects at 30 °C ($P = 0.26$), 40 °C ($P = 0.13$), 45 °C ($P = 0.11$), 55 °C ($P = 0.27$), 65 °C ($P = 0.07$), and 70 °C ($P = 0.21$). A few treatment $\times$ genotype interactions were also nonsignificant at 55 °C ($P = 0.93$), 60 °C ($P = 0.14$), 65 °C ($P = 0.24$), or 70 °C ($P = 0.7$). Differences in heat tolerance of leaf tissues were found for genotypes and treatments, as measured by CMT. The effects of water bath temperatures on RI of leaves when grown under the four temperature/photoperiod treatment combinations were sigmoidal for all the 10 genotypes (Fig. 2). Rise in RI response occurred either

at 40 or 45 °C. In several instances, LTLD curve responses were at higher temperatures (50 °C), separating the sigmoidal curves in LTLD at higher temperatures, e.g., Mn. Sel'n. 01-210-43 (Fig. 2A), Mn. Sel'n. 01-210-77 (Fig. 2B), Mn. Sel'n. 02-93-1 (Fig. 2C), 'Magic Ball' (Fig. 2G), and 'Minnwhite' (Fig. 2J). In only one case, 'Geumbangul' (Fig. 2F), did this occur in HTSD. Inflection points or the midpoints of each sigmoid response curve peaked at 50% RI for all genotypes and treatments. In one genotype, 'Autumn Fire' (Fig. 2D), the inflection points all occurred at the same temperature of 48.5 °C. Two genotypes each had two inflection points for the four treatments: Mn. Sel'n. 01-210-77 (Fig. 2B) and Mn. Sel'n. 02-93-1 (Fig. 2C) had identical inflection points of 48.8 °C for HTSD, HTLD, and LTSD treatments but varied slightly for LTLD (52.0 and 51.9 °C, respectively). The remaining genotypes had three inflection points across the four treatments, ranging from 46.9 ('Mammoth™ Dark Bronze Daisy', LTSD, Fig. 2H) to 52.8 °C ('Minnwhite', LTLD, Fig. 2J). All regressions were significant, $P \leq 0.001$ and a cohesive range in R^2 values was found [from $R^2 = 0.79$ LTLD for Mn. Sel'n. 01-210-77 (Fig. 2B) and LTSD for 'Autumn Fire' (Fig. 2D) to $R^2 = 0.89$ HTSD for Mn. Sel'n. 01210-43 (Fig. 2A)].

Heat delay for VBD in LTs (LTLD–LTSD) ranged from none (0 d) in 'Centerpiece' (Fig. 3E; all reps.) to 9 d for 'Autumn Fire' (Fig. 3D; one rep.). In contrast, heat delay for VBD in HTs (HTLD–HTSD) had a wider range of 1 d for one to two reps. in four genotypes (Mn. Sel'n. 01-210-43, Fig. 3A; 'Autumn Fire', Fig. 3D; 'Centerpiece', Fig. 3E; 'Geumbangul', Fig. 3F) to 17.5 d in 'Mellow Moon' (Fig. 3I). Flowering heat delay had a wider range in variation than VBD heat delay (LTLD–LTSD): 0 ('Autumn Fire', one rep., Fig. 3D) to 29 d ('Magic Ball', one rep., Fig. 3G). Only one genotype flowered in the HTLD treatment, so heat delay for HTLD–HTSD was 10–11 d for 'Centerpiece' (two reps., Fig. 3E). Varying linear relationships and R^2 occurred when the delay in days to VBD and flowering for the differential between photoperiod treatments in both temperatures (LTSD–LTSD and HTLD–HTSD) was regressed with RI% at 50 °C (Fig. 3). All regressions that could be performed were significant ($P \leq 0.001$); many regressions could not be done due to small numbers of reps. that either reached VBD or Flw in the LTLD–LTSD or HTLD–HTSD treatments (denoted as $R^2 = -$, Fig. 2). The slopes of many regressions were flat, nearly approximating zero (all treatments for Mn. Sel'n. 01-210-43, Fig. 3A; 'Centerpiece', Fig. 3E) in contrast with others with a positive (Flw LTLD–LTSD, 'Magic Ball', Fig. 3G) or negative (Flw LTLD–LTSD, 'Mammoth™ Dark Bronze Daisy', Fig. 3H) or vertical (Flw LTLD–LTSD, 'Geumbangul', Fig. 3F) slopes. The majority of genotypes had contrasting linear regression slopes between VBD and Flw differentials for

Fig. 3. Regression of degree of heat delay (Shibata and Kawata, 1987) for VBD and flowering (Flw) dates expressed in relation to percent relative injury (RI%) at 50 °C for each of the treatments (LTLD–LTSD; HTLD–HTSD) and delay in flowering (d) for 10 tested chrysanthemum genotypes: (A) Mn. Sel'n. 01-210-43, (B) Mn. Sel'n. 01-210-77, (C) Mn. Sel'n. 02-93-1, (D) 'Autumn Fire', (E) 'Centerpiece', (F) 'Geumbangul', (G) 'Magic Ball', (H) 'Mammoth™ Dark Bronze Daisy', (I) 'Mellow Moon', and (J) 'MinnWhite'. Key: Many regressions could not be done due to either none or one to two reps. that reached VBD or Flw (denoted as $R^2 = ---$). HTSD = high temperature short days; HTLD = high temperature long days; LTSD = low temperature short days; LTLD = low temperature long days.

both LTLD–LTSD and HTLD–HTSD, e.g., 'Geumbangul' (Fig. 3F).

Discussion

Since most main effects (genotypes, treatments) and/or their interactions were significant, there was considerable genetic variation within and among genotypes, treatments, and their interactions for all phenotypic traits measured. Similarly, there were numerous similarities and differences among genotypes from both chrysanthemum breeding programs (Tables 1 and 2; Figs. 2 and 3). For example, plant height matched expectations from field-based plant habits such that upright, cut types were the tallest ('Autumn Fire' in HTLD, Table 1), whereas the short-statured cushion habits were frequently the shortest ('Geumbangul' in HTSD, Table 1). Interestingly, 'Magic Ball' and 'Minnwhite' were the shortest only in HTLD and HTSD environments (Table 1), whereas 'Geumbangul' had the shortest plant height in all treatments (15.3 cm, LTSD; 14.5 cm, LTLD; 14.2 cm, HTSD; and 16.8 cm, HTLD; Table 1). 'Geumbangul's height matches the height for one of the DN/HDI genotypes (14.6 cm), Mn. Sel'n. 81-45-15, for the day-neutral/heat-delay insensitive ideotype (Anderson and Ascher,

2001). Mean plant height ranged from 14 to 47 cm (Table 1), which was similar to that reported in HDI progeny screening, 18–45 cm (Anderson and Ascher, 2004b). In both instances, these plant height ranges were within Anderson and Ascher's (2001) DN/HDI ideotype. It is intriguing, however, that only 1/10 or 10% of the genotypes from both chrysanthemum breeding programs had consistent stability. Thus, 'Geumbangul' would be the parent to use in breeding compact or dwarf plant habits due to its stability (no genotype $\times$ environment or $G \times E$ interaction) in height regardless of LT/HTs and SD/LD photoperiods. Given the high heritability of plant height in the DN/HDI ideotype ($h^2 = 0.66$; Anderson and Ascher, 2004b), the stability of expression in 'Geumbangul' should be found in its progenies.

Although rarely bred for, stability in plant width expression (no $G \times E$) across all four treatments did occur in full sibs Mn. Sel'ns. 01-210-43 and 01-210-77, 'Autumn Fire' and 'Centerpiece' (Table 1). To the best of our knowledge, this is the first report of such stability for width in chrysanthemums with HTs and noninductive photoperiods. Both the shrub and upright, cut flower habits would benefit with the incorporation of these parents.

The significantly lowest LDLN values occurred under LTSD conditions in seven genotypes from the University of Minnesota breeding program: 'Centerpiece', 'Mn. Sel'ns. 01-210-43, 01-210-77, 02-93-1, 'Mammoth™ Dark Bronze Daisy', 'Centerpiece', and 'Mellow Moon' (Table 1). Although these would be ideal parents to choose for breeding, both 'Geumbangul' and 'Magic Ball' from the South Korean breeding program had complete stability for LDLN across all four treatments that would be even more desirable, particularly since LDLN $h^2 = 0.79$ (Anderson and Ascher, 2001) but were closer to progeny in a 6×6 diallel (12.5–32; Anderson and Ascher, 2004b). Interestingly, none of the genotypes tested herein had low enough LDLN in any treatment that matched the DH/HDI ideotype range (LDLN = 13–20; Anderson and Ascher, 2001). Only 'Mellow Moon' approached this with LDLN = 22.3 in LTLD and LDLN = 23.1 in HTSD (Table 1).

Without exception, all 10 tested genotypes had shorter internode lengths (0.7–2.4 cm, Table 1) than previously reported in day-neutral/heat-delay insensitive chrysanthemums (7.2–19 cm; Anderson and Ascher, 2001). Several genotypes were also found with stability of internode lengths across all

four environments: Mn. Sel'n. 01-210-77, 'Autumn Fire', 'Centerpiece', 'Mammoth™ Dark Bronze Daisy', 'Mellow Moon', and 'Minnwhite' (Table 1). At least two of these genotypes, i.e., 'Mammoth™ Dark Bronze Daisy' and 'Mellow Moon', along with 'Magic Ball' also had stability across all four environments for lateral node number—a trait that has neither been previously measured in chrysanthemum HDI studies nor included in the DN/HDI ideotype (Anderson and Ascher, 2001).

A total of four genotypes ('Magic Ball', 'Mammoth™ Dark Bronze Daisy', 'Mellow Moon', and 'Minnwhite') from both breeding programs had equal VBDs or stability in HTSD and LTSD treatments (Table 2), indicating HDI in inductive photoperiods. Only one cultivar (Mellow Moon) had stability or equal VBDs in HTSD, LTSD, and LTLD conditions (Table 2). This is the first-ever report of stability for VBD across inductive and noninductive HT/LT treatments. In contrast, no genotype had stability across all treatments for flowering, particularly since only 'Centerpiece' flowered in all environments (Table 2). 'Centerpiece' also had 0 d of heat delay (LTLD–LTSD; Fig. 3E) for VBD and 1 d of heat delay (HTLD–HTSD), as well as three other genotypes (Mn. Sel'n. 01-210-43, Fig. 3A; 'Autumn Fire', Fig. 3D; 'Geumbangul', Fig. 3F). 'Mellow Moon' had the longest heat delay of 17.5 d (HTLD–HTSD; Fig. 3I). 'Autumn Fire' had 0 d in heat delay for LTLD–LTSD (Fig. 3D). Since only 'Centerpiece' flowered in HTLD–HTSD as a DN/HDI genotype (Fig. 3E), clearly it is the best HDI source among these 10 genotypes for FBI and FBD.

Fig. 3. Continued.

Unlike previous reports with positive linear slopes between heat-induced VBD or flowering delay for chrysanthemums (Wang et al., 2008; Yeh and Lin, 2003), very few of the 10 genotypes had linear regressions with positive slopes (Fig. 3). Typical regressions had no slope ($R^2 \approx 0.0$) for all treatments in 'Centerpiece' (Fig. 3E) and Mn. Sel'n. 01-210-43 (Fig. 3A), whereas others were negative ('Mammoth™ Dark Bronze Daisy' for Flw LTLD-LTSD, Fig. 3H). Surprisingly, one linear regression had a slope of $R^2 = 1.0$ ('Geumbangul' for Flw LTLD-LTSD, Fig. 3F). These responses are all novel in chrysanthemums.

RI values of 32% to 84% in six chrysanthemum cultivars (Yeh and Lin, 2003) were similar to our findings (Fig. 2). The presence of sigmoidal curves for RI% and temperature found herein (Fig. 2) match those found in other greenhouse and garden chrysanthemum germplasm (Wang et al., 2008; Yeh and Lin, 2003), as well as fruit and vegetable horticultural (Ahrens and Ingram, 1988; Inaba and Crandall, 1988; Ingram and Buchanan, 1984; Lester, 1985) and agronomic crops (Chen et al., 1982; Ismail and Hall, 1999; Martineau et al., 1979; Sullivan, 1972). There was a very tight R^2 range ($R^2 = 0.79-0.89$, Fig. 2) and these values were similar to previous findings in chrysanthemum (Wang et al., 2008).

Only 'Autumn Fire' (Fig. 2D) had stability for CMT inflections or midpoints at 48.5 °C across all the four treatments. All other genotypes had 2–3 inflection points, depending on the test environment (Fig. 2). Significant genotypic differences for CMT were found at 50 °C, the approximate inflection or midpoints for all genotypes (Wang et al., 2008; Yeh and Lin, 2003), whereas this was not true at other test temperatures of 30, 40, 45, 55, 65, and 70 °C (Fig. 2). Thus, this study confirms Wang et al. (2008) and Yeh and Lin's (2003) postulation that 50 °C is the single, best critical test temperature for measuring CMT ratings and HDI germplasm. We propose to use the sole temperature of 50 °C for future screenings of populations for HDI expression. Implementation of such a screening program would match similar methodologies implemented in peanuts (*Arachis hypogaea* L.; Chauhan and Senboku, 1996), peppers (*Capsicum annuum* L.; Anderson et al., 1990), soybeans [*Glycine max* (L.) Merr.; Martineau et al., 1979], and winter wheat (*Triticum aestivum* L.; Saadalla et al., 1990).

An untested question remains as to whether HDI could be screened using leaves from vegetative (juvenile) chrysanthemum seedlings without the added cost and time inputs to initiate and develop flower buds. Similarly, it would be useful to test whether the HDI genotypes have over-expression of *AtDREB1A* since this gene enhances tolerance to heat stress in other chrysanthemums (Hong et al., 2009). This is a vital objective for future research to further reduce screening costs for HDI selection in large chrysanthemum populations.

In summation of the stability of trait expression across all the four environments,

not one genotype had stability for all traits in all temperature/photoperiod treatments. Instead, parental choices for future joint breeding efforts between the South Korean and U.S. chrysanthemum breeding programs would vary, depending on the trait(s) of interest. For short stature or plant height and LDLN, 'Geumbangul' would be the best choice. 'Magic Ball' had stability for LDLN as well as lateral node number. Both 'Autumn Fire' and Mn. Sel'n. 01-210-77 had two traits (plant width, internode lengths) with stability in all environments. Two genotypes had stability for three traits: 'Centerpiece' (for plant width, internode lengths, and flowering) and 'Mellow Moon' (for internode lengths, lateral node number, and VBD in HTSD, LTSD, and LTLD). Stacking of traits in parents could be done by selecting the best parents to maximize stability of all traits across these four environments with minimal crossing and selection across generations. For example, crossing 'Geumbangul' × 'Centerpiece' could concentrate stability for five traits (short plant height, plant width, LDLN, internode lengths, and DN/HDI expression). Subsequent progeny, screened for stability of these traits could be used as male parents with 'Mellow Moon' (which has to be the female parent due to double flowers or 100% ray and gynoeious florets), which has stability for internode lengths, lateral node number, and VBD in HTSD, LTSD, and LTLD conditions. Thus, all traits could potentially be incorporated in relatively few generations of crossing and selection. This crossing scheme will be tested for its effectiveness and the number of generations of selections required to concentrate favorable alleles for trait stability, given the heritability of each trait (Anderson and Ascher, 2004b).

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