Screening Ornamental Pepper Cultivars for Temperature Tolerance Using Pollen and Physiological Parameters

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Abstract. Temperature affects reproductive potential, aesthetic, and commercial value of ornamental peppers (*Capsicum annuum* L.). Limited information is available on cultivar tolerance to temperature stress. An experiment was conducted using pollen and physiological parameters to assess high and low temperature tolerance in ornamental peppers. In vitro pollen germination (PG) and pollen tube length (PTL) of 12 morphologically diverse ornamental pepper cultivars were measured at a range of temperatures, 10 to 45 °C with 5 °C increments. Cell membrane thermostability (CMT), chlorophyll stability index (CSI), canopy temperature depression (CTD), and pollen viability (PV) were measured during flowering. From the modified bilinear temperature–PG and PTL response functions, cardinal temperatures (T_{min} , T_{opt} , and T_{max}) for PG and PTL and maximum PG (PG_{max}) and PTL (PTL_{max}) were estimated. Cultivars varied significantly for PG, PTL, cardinal temperatures for PG and PTL, and all three physiological parameters. Cumulative temperature response index (CTRI) of each cultivar, calculated as the sum of 12 individual temperature responses derived from PV, PG_{max} , PTL_{max} , T_{min} , T_{opt} , and T_{max} for PG and PTL, CMT, CTD, and CSI were used to distinguish differences among the cultivars and classify for high (heat) and low (cold) temperature tolerance. Based on CTRI–heat, cultivars were classified as heat-sensitive ('Black Pearl', 'Red Missile', and 'Salsa Yellow'), intermediate ('Calico', 'Purple Flash', 'Sangria', and 'Variegata'), and heat-tolerant ('Chilly Chili', 'Medusa', 'Thai Hot', 'Explosive Ember', and 'Treasures Red'). Similarly, cultivars were classified for cold tolerance as cold-sensitive, moderately cold-sensitive, moderately cold-tolerant, and cold-tolerant based on CTRI–cold. 'Red Missile' and 'Salsa Yellow' were classified as cold-tolerant. Cultivar screening using pollen parameters will be ideal for reproductive temperature tolerance, whereas physiological parameters will be suitable for screening vegetative temperature tolerance. The identified heat- and cold-tolerant cultivars are potential candidates in breeding programs to develop new ornamental and vegetable pepper genotypes for high and low temperature tolerance.

Ornamental peppers (*Capsicum annuum* L.) are widely used as potted flowering or bedding plants for their morphologically diverse characteristics. Ideal cultivation conditions for ornamental peppers are similar to typical vegetable pepper production with the crop requiring high radiation and minimum

daytime temperatures between 18 and 21 °C for maximum fruit set. Pepper, being a warm-season crop, requires night and soil temperatures of 14.5 °C or higher to promote growth. Lower temperatures are tolerated by ornamental pepper plants as they mature (Stummel and Bosland, 2007). Yellowing and leaf drop are caused by low temperatures and light and insufficient moisture or nutrients (Stummel and Bosland, 2007). In addition, pollen vitality and fruit quality are also affected by low night temperature (Polowick and Sawhney, 1986). As described by Young et al. (2004), high temperature during flowering or during pollen release affects male reproductive processes (microsporogenesis) resulting in lower fruit set and smaller fruit. Both low and high temperature extremes are detrimental, especially for reproductive development of pepper plants (Polowick and Sawhney, 1986).

With global climate change, crop production across the world will be met with great

challenges. According to Intergovernmental Panel on Climate Change (IPCC) (2007), the predicted mean increases in earth's surface temperatures of 1.4 to 5.8 °C by 2100 and other changes such as variable precipitation patterns and frequent occurrence of extreme climatic events associated with natural and human-induced changes in greenhouse gases may impact productivity of crops across the globe. It has been shown that this projected variation in climate will have profound impacts on crop production (Stainforth et al., 2005). Furthermore, low night temperatures have also been reported to cause negative impacts on reproductive developmental processes leading to reduction in marketable yield of vegetable peppers (Polowick and Sawhney, 1986; Pressman et al., 2006; Shaked et al., 2004).

High temperatures including short episodes of extreme events during the plant reproductive period have been shown to cause extensive damage to grain and fruit yield in many crops (Ahmed and Hall, 1993; Erickson and Markhart, 2002; Ferris et al., 1998; Gross and Kigel, 1994; Herrero and Johnson, 1980; Peet et al., 1998; Porch and Jahn, 2001; Prasad et al., 1999, 2003; Reddy et al., 1997; Sato et al., 2002; Taylor and Hepler, 1997). Because pollen is short-lived after release and acts as an independent functional unit, in vitro responses of pollen germination and tube length response characteristics such as maximum pollen germination and pollen tube length and cardinal temperatures (minimum, optimum, and maximum) of these two processes have been used in many studies to assess genetic variability among crops (Kakani et al., 2002, 2005; Salem et al., 2007; Singh et al., 2008) including vegetable peppers (Aloni et al., 2001; Reddy and Kakani, 2007). Also, Salem et al. (2007) have demonstrated the relationship between in vitro pollen germination and tube length responses to temperature and classification of genotypes based on these parameters and whole plant thermotolerance when plants were subjected to high temperatures for a long period of time. Therefore, pollen parameters could be good indicators in determining reproductive tolerance to high and low temperatures.

Several biophysical and physiological parameters have been used extensively to investigate temperature stress in a range of crops (Singh et al., 2007). Among them, CMT has been used to measure high temperature tolerance of crops because stress adaptation can be associated with cell viability and cell membrane integrity (Gusta and Chen, 1987). Canopy temperature depression (CTD), the difference between air and canopy/foilage temperature, has also been used to study temperature tolerance in certain crops such as wheat and rice. Reynolds et al. (2001) indicated that CTD is a good criterion for screening heat stress tolerance in wheat under field conditions. CSI is another physiological parameter that gained importance to study drought and high temperature tolerance in plants (Sairam et al., 2008). The CSI indicates how well chlorophyll performs under stress conditions (Kumari et al., 2004) and variability can be exploited to differentiate

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cultivar responses to temperature and drought stresses (Mohan et al., 2000).

To our knowledge, there are no reports on screening the responses of ornamental pepper cultivars under a wide range of temperatures, particularly using pollen and physiological parameters. The objectives of the current study were to 1) quantify the responses of in vitro PG and PTL of ornamental pepper cultivars to a range of temperatures; 2) determine cultivar-specific cardinal temperatures for both PG and PTL response parameters; 3) quantify the cultivar stability to heat treatments using three physiological parameters: CMT, CSI, and CTD; 4) classify cultivars based on their level of tolerance to high and low temperatures using pollen- and physiological-based (CMT, CSI, and CTD) parameters; and 5) determine whether the observed variation among cultivars in pollen germination responses to temperature are related to physiological traits.

Materials and Methods

Twelve phenologically and morphologically diverse (Stummel and Bosland, 2007; Whipker et al., 1999) commercially available cultivars of ornamental peppers ('Black Pearl', 'Calico', 'Chilly Chili', 'Explosive Ember', 'Medusa', 'Purple Flash', 'Red Missile', 'Salsa Yellow', 'Sangria', 'Thai Hot', 'Treasures Red', and 'Variegata') were grown from seed in 1-L plastic pots (15.3 cm diameter, 10 cm height) in a greenhouse at the R. R. Foil Plant Science Research Center, Mississippi State University, Mississippi State (lat. 33°28' N, long. 88°47' W) with day/night temperatures of 25/22 °C. Thirty plants of each cultivar were arranged randomly in rows oriented east to west and spaced 1 m apart and were hand-irrigated and fertilized with a constant liquid feed of Peter's 20-10-20 (N, P₂O₅ and K₂O) (Peters Peat Lite; Scotts, Marysville, OH) at 200 mg N/L as needed. The photosynthetically active radiation measured with a line quantum sensor (LI-191 Line quantum sensor; LI-COR, Inc., Lincoln, NE) on several clear days at noon time was over 1150 μmol·m⁻²·s⁻¹.

Physiological measurements. The leaf CMT in ornamental pepper cultivars was assessed according to the procedure described by Martineau et al. (1979). Two sets (control and treatment) of 10 leaf discs, each disc with 1.3 cm², cut from five fully expanded third or fourth leaves from the top of the stem axis from each cultivar during the midflowering period were placed in test tubes with 10 mL of deionized water. The leaf discs were thoroughly rinsed three times with deionized water to remove electrolytes both adhering to the leaf surface and leaching from the cut surfaces of the leaf discs. After final rinsing, all the test tubes with leaf discs were filled with 10 mL of deionized water and capped with aluminium foil to prevent evaporation of water. One set of test tubes was incubated for 20 min at 55 °C in a temperature-controlled water bath, whereas the other set was left at room temperature of ≈25 °C. After incubation, the sets of test tubes were brought to 25 °C and initial measurement of conductance of the control (CEC1) and the

treatment (TEC1) was measured by an electrical conductivity meter (Corning Checkmate II; Corning Inc., Corning, NY) at room temperature. Tubes were then autoclaved at 0.1 MPa for 12 min to kill tissues completely, releasing all the electrolytes. Tubes were then cooled to 25 °C and final conductance was measured (CEC2 and TEC2). The CMT was estimated using the following Eq. [1]:

$$\text{CMT}(\%) = [1 - (\text{TEC1}/\text{TEC2})] / [1 - (\text{CEC1}/\text{CEC2})] \times 100 \quad [1]$$

Chlorophyll stability index (CSI) was assessed according to the procedure described by Murty and Majumdar (1962). Two sets of leaf samples were collected from five fully expanded leaves for each cultivar during the midflowering period. Five leaf discs, each with 2.0 cm², from each sample were collected randomly and placed in vials containing 4 mL of dimethyl sulphoxide for chlorophyll (Chl) extraction. The sample vials were incubated at room temperature in the dark for 24 h to allow complete extraction of Chl pigments. Absorbance of the extract was measured using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA) at 470, 648, and 662 nm to calculate concentrations of Chl a and Chl b (Chapple et al., 1992). Total leaf Chl was estimated by summing Chl a with Chl b values (Lichtenthaler, 1987). Another set of leaf discs, each with 2.0 cm², was collected similarly from each cultivar and incubated at 56 °C in a temperature-controlled water bath for 1 h. The set of tubes was brought to 25 °C and the Chl content was measured from the heat-treated samples as described previously. The CSI was estimated as the ratio of Chl content in heated leaf (56 °C) to that in fresh leaf expressed as a percentage using Eq. [2]:

$$\text{CSI}(\%) = [(\text{Total Chl content in heat treated sample}) / (\text{Total Chl content in control})] \times 100 \quad [2]$$

Canopy temperature depression measurements were made between 50 and 60 d after

transplanting, where leaf temperatures of five fully expanded leaves from each cultivar and the respective air temperature was measured between 1200 and 1300 HR (cloudless, bright days) using a handheld infrared thermometer (Model OS533E-OMEGASCOPE; OMEGA Engineering, Inc., Stamford, CT). Canopy temperature depression was estimated using Eq. [3], in which Ta and Tc refer to air and canopy temperature of the target leaf, respectively:

$$\text{CTD} = T_a - T_c \quad [3]$$

Pollen measurements. Twenty to 30 flowers at anthesis were randomly collected from each cultivar between 0900 and 1000 HR during the flowering period, 50 to 70 d after sowing. Pollen grains were collected in a petri dish by gently tapping the flowers. Pollen grains were distributed uniformly onto the solidified and modified germination medium using a tiny, clean bristle paint brush. The pollen medium for the highest pollen germination was identified through slight modification of the medium previously used for vegetable pepper by Reddy and Kakani (2007) with pH adjusted to 7.5. To this liquid medium, 10 g of agar was added and slowly heated on a hot plate. After the agar was completely dissolved, 10 mL of germination medium was poured into three replicate petri dishes for each cultivar in each temperature treatment and allowed to cool for 15 min for agar solidification. Petri dishes with medium were kept in the incubator set at treatment temperatures for 30 min before pollen distribution. The petri dishes were then covered and incubated in an incubator (Precision Instruments, New York, NY) at respective temperature treatments from 10 to 45 °C at 5 °C increments. Each petri dish per cultivar and temperature treatment was considered as a replicate.

Pollen germination and PTL were tested after 24 h of incubation. Total pollen grains and number of pollen grains germinated were counted using a Nikon SMZ 800 microscope (Nikon Alphaphot YS microscope; Nikon Instrument, Kangava, Japan) with a magnification

Table 1. Pollen viability (PV), maximum pollen germination percentage (PG_{max}), modified bilinear equation constants (a, b₁, and b₂), regression coefficient (R²), and cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for pollen germination of 12 ornamental pepper cultivars.

Cultivar	PV (%)	PG _{max} (%)	Equation constants				Cardinal temp (°C)		
			a	b ₁	b ₂	R ²	T _{min}	T _{opt}	T _{max}
Black Pearl	76.63	73.44	73.44	0.5123	-5.13	0.90	11.80	24.81	40.70
Calico	84.92	84.76	84.76	-0.4270	-5.78	0.90	11.84	27.65	41.29
Chilly Chili	90.59	90.20	90.20	-0.6450	-6.21	0.90	11.86	28.08	41.24
Explosive Ember	88.59	87.70	87.70	-0.6260	-6.02	0.92	11.87	28.12	41.31
Medusa	89.21	92.44	92.44	-0.8220	-6.37	0.92	11.92	28.58	41.43
Purple Flash	78.16	79.62	79.62	-0.4360	-5.35	0.91	11.36	27.55	41.30
Red Missile	60.90	56.05	56.05	0.5899	-3.93	0.93	11.73	24.11	40.86
Salsa Yellow	55.99	50.57	50.57	0.5807	-3.52	0.93	11.51	23.84	41.03
Sangria	76.87	73.79	73.79	0.6528	-5.03	0.88	11.83	24.79	41.61
Thai Hot	90.80	89.83	89.83	-0.8230	-6.19	0.92	11.88	28.60	41.40
Treasures Red	89.88	91.39	91.39	-0.6800	-6.26	0.92	11.74	28.09	41.24
Variegata	77.19	78.25	78.25	-0.5080	-5.49	0.92	12.17	27.86	40.90
Mean	79.98	79.00	—	—	—	0.91	11.79	26.84	41.19
LSD	4.79***	2.37***	—	—	—	—	0.50***	0.51***	0.66***

***Significant at P α 0.001.

LSD = least significant difference.

of 6.3×. Ten fields per replication were counted for percent pollen germination. When counting the pollen grains, a pollen grain was considered germinated when its tube length equaled the diameter of the pollen grain (Luza et al., 1987). Percentage pollen germination was calculated by counting the total number of pollen grains germinated in the microscope field of view and divided by the total number of pollen grains per field of view. The pollen tube lengths of 30 pollen grains selected randomly from each petri dish were measured with an ocular micrometer fitted to the eye piece of the microscope after 24 h of incubation.

For pollen viability, pollen grains were collected as described for the PG procedure from 30 plants in each cultivar between 900 and 1000 HR. Pollen viability was tested using 2.5% concentration of 2, 3, 5-triphenyl tetrazolium chloride (TTC) stain in deionized water as described by Aslam et al. (1964). A drop of TTC solution was added to the dispersed pollen on the slide. Tetrazolium chloride stains the viable pollen with a reddish purple color as a result of the formation of insoluble red formazan. Thirty min after staining, the counts of the total number of pollen grains and the number of stained pollen grains were made in two microscopic fields of 2.4 mm² containing more than 100 pollen grains from each microscopic field in all replications.

Curve-fitting procedures and cardinal temperature estimations. The recorded PG and PTL measurements after 24 h were analyzed by linear and non-linear regression models commonly used to quantify pollen growth and developmental responses to temperature treatments (Kakani et al., 2002). Quadratic and or bilinear equations were applied to data to determine the best-fit models for PG and PTL processes. The mean values of all replications and of all variables were analyzed using the one-way analysis of variance procedure in SAS (SAS Institute, Inc., Cary, NC). By comparing the amount of variation of two models accounted for by *R*² and root mean square deviation (RMSD) for observed and fitted values, the best model was selected. The highest *R*² and lowest RMSD for both PG and PTL responses to temperature were best described by modified bilinear models. Accordingly, the cardinal temperatures (*T*_{min}, *T*_{opt}, and *T*_{max}) were estimated from the fitted equations for all the cultivars. The non-linear regression procedure PROC NLIN (SAS Institute, Inc.) was used to estimate the parameters of the modified bilinear equation. For the modified bilinear equation, *T*_{opt} was generated by fitting the bilinear model (Eq. [4]) (Kakani et al., 2002), in which *T* is actual treatment temperature and *a*, *b*₁, and *b*₂ are equation constants. *T*_{min} and *T*_{max} were calculated using the following equations (Eq. [5] and Eq. [6]):

$$\text{Mean PG or mean PTL} = a + b_1(T - T_{\text{opt}}) + b_2 \times \text{ABS}(T_{\text{opt}} - T) \quad [4]$$

$$T_{\text{min}} = \frac{-a + (b_1 - b_2) \times T_{\text{opt}}}{b_1 - b_2} \quad [5]$$

$$T_{\text{max}} = \frac{-a + (b_1 + b_2) \times T_{\text{opt}}}{b_1 + b_2} \quad [6]$$

Stress-response indices. Initially, individual stress response index (ISRI) for temperature of each parameter for heat tolerance was calculated as the value of a cultivar (*P*_i) divided by maximum value (*P*_h) observed overall the cultivars (Eq. [7]), whereas for

ISRI for cold tolerance was determined by dividing the minimum value (*P*_i) observed overall the cultivars by the value of a cultivar (*P*_i) (Eq. [8]) as described by Reddy and Kakani (2007). Then, CTRI for each cultivar (Eq. [9] and Eq. [10]) was calculated as the sum of 12 ISRIs derived from maximum PV, maximum PG (*PG*_{max}), maximum PTL (*PTL*_{max}), *T*_{min}, *T*_{opt}, *T*_{max} temperatures of both PGs, and PTLs, CMTs, CTDs, and CSIs. Cultivars were classified into three categories of heat tolerance based on CTRI values of 12 parameters as tolerant [greater than (minimum CTRI + 2.5 sd)], intermediate [(between

Fig. 1. In vitro pollen germination (A) and pollen tube length (B) in responses to temperature (symbols) and their fitted lines derived from the modified bilinear equations, respectively, of three pepper cultivars (Medusa, Sangria, and Salsa Yellow). The symbols are observed germination percentages and pollen tube lengths after 24 h and solid lines are the predicted values by the respective fitted equations. For clarity, data and regression lines for only three pepper cultivars are presented.

Table 2. Maximum pollen tube length (*PTL*_{max}), modified bilinear equation constants (*a*, *b*₁, and *b*₂), regression coefficient (*R*²), and cardinal temperatures (*T*_{min}, *T*_{opt}, and *T*_{max}) for pollen tube lengths of 12 ornamental pepper cultivars.

Cultivar	<i>PTL</i> _{max} (μm)	Equation constants			<i>R</i> ²	Cardinal temp (°C)		
		<i>a</i>	<i>b</i> ₁	<i>b</i> ₂		<i>T</i> _{min}	<i>T</i> _{opt}	<i>T</i> _{max}
Black Pearl	478	477.83	-2.61	-32.36	0.92	11.50	27.56	41.22
Calico	740	739.61	-6.58	-50.96	0.92	11.91	28.58	41.43
Chilly Chili	1173	1172.70	-8.38	-80.69	0.91	11.86	28.08	41.25
Explosive Ember	878	1052.93	-8.76	-84.31	0.92	14.18	28.12	39.43
Medusa	957	956.79	-40.94	-86.18	0.93	12.37	33.52	41.05
Purple Flash	782	782.10	-4.92	-54.78	0.92	12.16	27.85	40.95
Red Missile	684	684.40	-8.35	-47.46	0.96	10.95	28.45	40.71
Salsa Yellow	405	404.61	4.64	-28.17	0.93	11.50	23.83	41.03
Sangria	819	818.92	-35.12	-73.62	0.94	12.26	33.53	41.06
Thai Hot	1348	1347.54	-12.35	-92.93	0.92	11.88	28.60	41.40
Treasures Red	1280	1279.61	-9.52	-87.77	0.92	11.74	28.09	41.24
Variegata	1058	1057.70	0.34	-73.76	0.92	11.78	26.05	40.46
Mean	883	—	—	—	0.93	12.00	28.52	40.94
LSD	3.06***	—	—	—	—	0.82**	1.38***	1.47 NS

^zNS, **, ***Non-significant or significant at *P* α 0.01 and 0.001, respectively.
LSD = least significant difference.

(minimum CTRI + 2.5 SD), and greater than (minimum CTRI + 1.5 SD)] and sensitive [between (minimum CTRI and minimum CTRI + 1.5 SD)]. Similarly, cultivars were classified based on cold CTRI of all parameters as cold-tolerant (greater than minimum CTRI + 3.0 SD), moderately cold-tolerant (minimum CTRI + 3.0 SD to greater than minimum CTRI + 2.0 SD), moderately cold-sensitive (minimum CTRI + 2.0 SD and greater than minimum CTRI + 1.0 SD), and cold-sensitive (minimum CTRI to minimum CTRI + 1.0 SD).

$$\text{ISRI (Heat)} = P_t/P_h \quad [7]$$

$$\text{ISRI (Cold)} = P_l/P_t \quad [8]$$

Heat CTRI

$$= \left(\frac{PV\%_{ot} + PG\%_{ot} + PTL_t + PG_{min_t} + PG_{opt_t} + PG_{max_t}}{PV\%_{oh} + PG\%_{oh} + PTL_h + PG_{min_h} + PG_{opt_h} + PG_{max_h}} + \frac{PG_{max_t} + PTL_{min_t} + PTL_{opt_t} + PTL_{max_t}}{PG_{max_h} + PTL_{min_h} + PTL_{opt_h} + PTL_{max_h}} + \frac{CMT_t + CTD_t}{CMT_h + CTD_h} + \left(\frac{CSI_t}{CSI_h} \right)^{-1} \right) \quad [9]$$

Cold CTRI

$$= \left(\frac{PV\%_{ot} + PG\%_{ot} + PTL_t + PG_{min_t} + PG_{opt_t} + PG_{max_t}}{PV\%_{oh} + PG\%_{oh} + PTL_h + PG_{min_h} + PG_{opt_h} + PG_{max_h}} + \frac{PG_{max_t} + PTL_{min_t} + PTL_{opt_t} + PTL_{max_t}}{PG_{max_h} + PTL_{min_h} + PTL_{opt_h} + PTL_{max_h}} + \frac{CMT_t + CSI_t}{CMT_h + CSI_h} + \left(\frac{CTD_t}{CTD_h} \right)^{-1} \right) \quad [10]$$

Results and Discussion

Developing an easy and efficient screening method is one of the impediments for identifying cultivar tolerance to abiotic stress tolerance for improved field performance under high and low temperature conditions. This is the first study to investigate pollen and physiological parameters in morphologically and phenologically diverse ornamental pepper cultivars (Stummel and Bosland, 2007; Whipker et al., 1999) to temperature tolerance. In this study, cultivars differed significantly for both pollen and physiological traits leading to identification of temperature-tolerant cultivars.

Pollen viability among 12 cultivars of ornamental pepper ranged from 56% in 'Salsa Yellow' to 91% in both 'Thai Hot' and 'Chilly Chili' with an average of 80% (Table 1). The PG_{max} percentage at optimum temperature varied among the cultivars from 51% ('Salsa Yellow') to 91% ('Treasures Red') with a mean of 79%. The PG_{max} found in this study is comparable to PG_{max} values reported by Reddy and Kakani (2007) for several vegetable pepper species (59% to 95%) and by Kafizadeh et al. (2008) from the greenhouse-grown bell pepper cultivar, California Wonder (68%). In all cultivars, temperatures above and below

PG_{opt} caused a linear reduction in the percentage of in vitro PG (Fig. 1A). Therefore, the modified bilinear equation with a well-defined optimum provided the best fit for the PG responses to temperature (Table 1; Fig. 1A; mean R² = 0.91). For clarity, only observed data points and the response functions of three different cultivars are shown in the figure. The cardinal temperatures, derived from bilinear model fit of PG, differed significantly among ornamental pepper cultivars. The magnitude of T_{min} varied from 11.36 ('Purple Flash') to 12.17 °C ('Variegata') with the mean value of 11.79 °C (Table 1). Significant differences were also observed for both T_{opt} and T_{max} with the means of 26.84 and 41.20 °C, respectively.

Analogous to PG, cultivars showed varied PTL response to temperature (Table 2; Fig. 1B). Similar to PG, the modified bilinear model best described the response of PTL to temperature in all cultivars (R² = 0.93). Comparable to this study, in vitro PG and PTL response to temperature have also been reported and quantified using regression models in other crop species such as canola (*Brassica napus* L.) (Singh et al., 2008), vegetable pepper species (Reddy and Kakani, 2007), cotton (*Gossypium hirsutum* L.) (Kakani et al., 2005), and groundnut (*Arachis hypogaea* L.) (Kakani et al., 2002). The PTL_{max} values ranged from 478 ('Black Pearl') to 1348 μm ('Thai Hot') with a mean of 883 μm (Table 2). The range of the PTL observed on an artificial pollen germination media for several other crops 410 μm to 1400 μm in cotton (Kakani et al., 2005), 198 to 357 μm in apricot (*Prunus armeniaca*), 209 to 335 μm in sweet cherry (*Prunus avium* L.), 174 μm to 188 μm in sour cherry (*Prunus cerasus* L.) (Bolat and Pirlak, 1999), and 450 μm to 1450 μm in groundnut (Kakani et al., 2002) is similar to the observed results in ornamental pepper cultivars. The cardinal temperatures for PTL differed among cultivars (Table 2). The mean values of T_{max}, T_{opt}, and T_{min} were 40.9, 28.5, and 12.0 °C, respectively. The optimum temperature values ranged from 23.8 ('Salsa Yellow') to 33.5 °C ('Sangria'). A significant correlation was observed between PG_{max} and PTL_{max} (Table 3). Among the cardinal temperatures for PTL, T_{max} exhibited no variation, whereas T_{min} and T_{opt} varied among the ornamental pepper cultivars (Table 2). The PTL T_{min} and PTL

T_{max} also varied from 10.95 to 12.37 °C and 39.43 to 41.43 °C, respectively. PTL T_{opt} was correlated with PG T_{max}. Pollen germination and pollen tube growth are the two parameters that prove the capability of pollen grains to perform their function of delivering sperm cells to the embryo for effective fertilization and seed/fruit set.

Pollen viability was positively correlated (r = 0.98) with PG_{max}, PTL_{max} (r = 0.80), and PG T_{max} (r = 0.57) (Table 3). A similar trend was reported for canola in a study investigating cold and heat tolerance of pollen under field conditions (Singh et al., 2008). Recent studies indicate that pollen vitality (viability, germination, and tube lengths) plays a major role for successful seed/fruit set in many crops (Prasad et al., 1999; Salem et al., 2007; Young et al., 2004), including vegetable peppers (Aloni et al., 2001). Because sustained pollen germination and growth of pollen tubes at high and low temperatures could lead to higher fruit set, pollen-based parameters could be exploited as a tool to screen crop cultivars suitable for production in high and low temperature environments.

Several biophysiological methods have been suggested as indicators of cultivar performance under adverse conditions and as potential screening tools for cultivars to abiotic stress tolerance (Singh et al., 2007). In this study, we have evaluated three physiological parameters, CMT, CSI, and CTD, to test their ability to distinguish differences among ornamental pepper cultivars and to test their association with pollen-based parameters. Ornamental pepper cultivars differed for CMT, a measure of cell membrane damage under high temperature treatment, with a minimum value of 44% in 'Calico' and maximum value of 80% in 'Treasures Red' (Table 4). Kuo et al. (1993) found a range of CMT values for sweetpotato (*Ipomoea batatas* L.) during a 3-year study as 38%, 45%, and 32% in April, July, and November, respectively, and hot pepper as 6%, 28%, and 19% in April, July, and November, respectively, under field conditions. However, Martineau et al. (1979) found large plant-to-plant variation in CMT measurements in four soybean cultivars.

The CSI recorded across 12 cultivars ranged from 61% in 'Red Missile' to 87% in 'Thai Hot' with a mean of 71.16% (Table 4).

Table 3. Pearson correlation matrix showing the relationship among maximum pollen viability (PV, %), maximum pollen germination (PG_{max}, %), maximum pollen tube length (PTL_{max}, μm), cardinal temperatures (T_{min}, T_{opt}, and T_{max}, °C) of both pollen germination (PG, %) and pollen tube length (PTL, μm), cell membrane thermostability (CMT, %), canopy temperature depression (CTD, °C), and chlorophyll stability index (CSI, %) of 12 ornamental pepper cultivars.

	PV	PG _{max}	PGT _{min}	PGT _{opt}	PGT _{max}	PTL _{max}	PTLT _{min}	PTLT _{opt}	PTLT _{max}	CMT	CSI
PG _{max}	0.98***z										
PGT _{min}	0.19	0.13									
PGT _{opt}	0.79	0.78*	0.40								
PGT _{max}	0.57*	0.56*	-0.16	0.38							
PTL _{max}	0.80*	0.79*	0.26	0.73	0.49						
PTLT _{min}	0.50	0.47	0.09	0.44	0.50	0.31					
PTLT _{opt}	0.47	0.49	-0.01	0.10	0.67*	0.28	0.26				
PTLT _{max}	0.34	0.23	0.16	0.33	0.24	0.07	0.25	-0.04			
CMT	0.36	0.37	0.20	0.57*	0.10	0.62	-0.04	-0.14	0.09		
CSI	0.51	0.49	0.11	0.49	0.42	0.75*	0.51	0.11	0.17	0.70	
CTD	0.48	0.53	0.05	0.60*	0.32	0.51	0.07	0.22	-0.01	0.54*	0.23

z*, ***, significant at P α 0.05 and 0.001, respectively.

The CSI indicates a plant's tolerance to environmental stresses. The higher the CSI, the lower the amount of stress impact on Chl content of the plants. A higher CSI values signifies a plant's ability to withstand stress through greater stability of chloroplast membranes leading to higher rates of photosynthesis, more dry matter production, and higher productivity (Mohan et al., 2000). Among pollen parameters, a significant positive correlation between CSI and PTL_{max} was observed. No significant correlation was recorded with any other pollen variables (Table 3). CSI was positively correlated to CMT ($r = 0.45$; Fig. 2B).

The difference between air and foliage temperature referred as CTD, a measure of a plant's ability to lower canopy temperature through transpirational cooling, differed among ornamental pepper cultivars. The mean CTD recorded was -3.01°C with the maximum and minimum CTD of -3.74°C in 'Sangria' and -1.96°C in 'Medusa', respectively (Table 4). Among pollen parameters, there was a CTD positive correlation only with PG T_{opt}. No significant correlation was recorded with other cardinal temperatures of PG and PTL (Table 3). No correlation of CTD was recorded with CSI but a poor correlation was found with CMT (Fig. 2).

The CTRI-heat and CTRI-cold were derived using pollen-based parameters excluding three physiological parameters, CTD, CMT, and CSI, to test the relationship between pollen-based (reproductive) parameters and physiological parameters (vegetative). A positive correlation was observed among CTRI-heat and two physiological parameters (CMT and CSI) (Table 5). However, no significant correlation was found with CTD with either of the CTRIs. Moreover, a positive correlation was observed among CTRI (cold) and two physiological parameters (CMT and CSI). In contrast, Salem et al. (2007) found no significant correlation between pollen-based parameters and CMT in soybean. Studies on temperature tolerance in groundnut (Kakani

et al., 2002) and cotton (Kakani et al., 2005) also found no correlation between CMT and pollen-based parameters.

Classification of ornamental pepper cultivars. The CTRI values for each ornamental pepper cultivar derived by summing individual temperature response indices for all the pollen- and physiological-based parameters varied among ornamental pepper cultivars (Tables 6 and 7). The CTRI-based technique using pollen parameters and physiological parameters identified cultivar variability for high and low temperature (heat-CTRI and cold-CTRI) tolerance in ornamental pepper cultivars. Heat CTRI varied from 8.56 'Salsa Yellow' to 11.33 'Thai Hot'. Based on

heat CTRI, five cultivars were classified as heat-tolerant and four each were as intermediate and as heat-sensitive cultivars (Table 6). Using cold CTRI, three cultivars were classified as cold-sensitive, four cultivars as moderately cold-sensitive, three cultivars as moderately cold-tolerant, and two cultivars as cold-tolerant (Table 7).

Capacity to sustain both higher metabolic and physiological activity with reproductive survivability in environments with multiple stress conditions is an imperative tolerant trait in crop plants both in present and future changing climatic conditions. These traits could be incorporated into new varieties of both vegetable and ornamental pepper

Fig. 2. Relationships between (A) cell membrane thermostability and canopy temperature depression, (B) cell membrane thermostability and chlorophyll stability index, and (C) chlorophyll stability index and canopy temperature depression of pooled data from the 12 ornamental pepper cultivars.

Table 4. Cell membrane thermostability (CMT), canopy temperature depression (CTD), and chlorophyll stability index (CSI) measured between 50 and 70 d of planting of 12 ornamental pepper cultivars.

Cultivar	CMT (%)	CSI (%)	CTD ($^{\circ}\text{C}$)
Black Pearl	51.51	62.24	-3.64
Calico	43.90	56.21	-3.12
Chilly Chili	58.48	67.59	-2.55
Explosive Ember	53.60	85.11	-3.17
Medusa	67.64	70.80	-1.96
Purple Flash	59.42	68.96	-3.06
Red Missile	47.17	61.37	-3.20
Salsa Yellow	58.64	65.73	-3.08
Sangria	47.56	72.08	-3.74
Thai Hot	78.11	86.66	-2.68
Treasures Red	80.06	85.56	-2.88
Variegata	67.25	71.60	-3.04
Mean	59.44	71.16	-3.01
LSD	9.23***	5.44***	0.61**

, *Significant at $P \alpha 0.01$ and 0.05 , respectively.
LSD = least significant difference.

Table 5. Pearson correlation matrix showing the relationship among cumulative temperature response index (CTRI) heat and CTRI cold based on pollen related parameters (pollen viability, cardinal temperatures of pollen germination, and pollen tube length) and three physiological parameters, cell membrane thermostability (CMT), canopy temperature depression (CTD), and chlorophyll stability index (CSI), of 12 ornamental pepper cultivars.

Trait	Pollen-based CTRI (heat)	Pollen-based CTRI (cold)	CMT	CSI
Pollen-based CTRI (cold)	-0.98***			
CMT	0.54*	-0.55*		
CSI	0.63*	-0.55*	0.70	
CTD	0.43	-0.45	0.54*	0.23

**Significant at $P \alpha 0.05$.

Table 6. Classification of ornamental pepper cultivars into heat-tolerant, intermediate, and heat-sensitive groups based on cumulative temperature stress response index (CTRI; unit less) along with individual score of CTRI values in parentheses.^z

Classification of ornamental pepper cultivars based on heat CTRI		
Heat-tolerant ^y (CTRI >10.57)	Intermediately heat-tolerant (CTRI = 9.74–0.57)	Heat-sensitive (CTRI <9.74)
Thai Hot (11.33)	Variegata (10.32)	Black Pearl (9.33)
Treasures Red (11.19)	Purple Flash (10.04)	Red Missile (9.08)
Medusa (11.18)	Sangria (9.89)	Salsa Yellow (8.56)
Explosive Ember (10.75)	Calico (9.84)	
Chilly Chili (10.73)		

^zCTRI is the sum of individual component response of nine pollen and three physiological parameters, cell membrane thermostability (CMT), canopy temperature depression (CTD), and chlorophyll stability index (CSI).

^yHeat-tolerant [CTRI = greater than (minimum CTRI + 2.5 sd)], intermediately heat-tolerant [CTRI = (greater than minimum CTRI + 1.5 sd) to (minimum CTRI + 2.5 sd)], and heat-sensitive [CTRI = (minimum CTRI) to (minimum CTRI + 1.5 sd)].

Table 7. Classification of ornamental pepper cultivars into cold-tolerant, moderately cold-tolerant, moderately cold-sensitive, and cold-sensitive groups based on cumulative stress response index (CTRI; unit less) along with individual score of CTRI values in parentheses.^z

Classification of ornamental pepper cultivars based on cold CTRI			
Cold-sensitive ^y (CTRI < 9.29)	Moderately cold-sensitive (CTRI = 9.29–10.07)	Moderately cold-tolerant (CTRI = 10.08–10.86)	Cold-tolerant (CTRI >10.86)
Medusa (8.92)	Chilly Chili (9.33)	Black Pearl (10.71)	Red Missile (10.89)
Thai Hot (8.91)	Purple Flash (9.84)	Calico (10.17)	Salsa Yellow (11.34)
Treasures Red (9.03)	Variegata (9.63)	Sangria (10.10)	
	Explosive Ember (9.33)		

^zCTRI is the sum of individual component response of nine pollen and three physiological parameters, cell membrane thermostability (CMT), canopy temperature depression (CTD), and chlorophyll stability index (CSI).

^yCold-sensitive [CTRI = (minimum CTRI) to (minimum CTRI + 1.0 sd)], moderately cold sensitive [CTRI = greater than (minimum CTRI + 1.0 sd) to (minimum CTRI + 2.0 sd)], moderately cold-tolerant [CTRI = greater than (minimum CTRI + 2.0 sd) to (minimum CTRI + 3.0 sd)], and cold-tolerant [CTRI = greater than (minimum CTRI + 3.0 sd)].

breeding programs to mitigate the negative effects of climate change on pepper production. Identification and development of cultivars that are heat- and cold-tolerant are beneficial, particularly with extreme climatic events projected to occur more frequently along with a general increase in average temperatures (IPCC, 2007; Mearns et al., 2001). Because short-term extreme temperature events cause detrimental effects to reproductive processes, particularly for pollen that are short-lived, compared with a rise in long-term average temperatures, cultivars with heat- and cold-tolerant characteristics are more important both in the present as well projected warmer and variable climate (Hall and Ziska, 2000). Because higher temperature is one of the major constraints to commercial production of vegetable peppers in the tropical and subtropical areas (Jifon et al., 2004), there exists a dire need to develop cultivars with higher heat tolerance.

In summary, *in vitro* PG and PTL of cultivars showed a typical bilinear response to temperature in ornamental pepper cultivars with diverse phenological and morphological characteristics. The cardinal temperatures for PG and PTL varied significantly among the cultivars. The narrowest range in cardinal temperatures was recorded in ‘Explosive Ember’ and the widest in ‘Purple Flash’. Among the physiological parameters, the highest CMT, CSI, and CTD were observed in cultivars Treasures Red, Thai Hot, and Medusa.

The *in vitro* pollen study in combination with physiological parameters confirms the

degree of tolerance and sensitivity of ornamental pepper cultivars to high and low temperature conditions. Based on the CTRI, ‘Thai Hot’, ‘Treasures Red’, ‘Medusa’, ‘Explosive Ember’, and ‘Chilly Chili’ were recorded as heat-tolerant cultivars, whereas ‘Red Missile’ and ‘Salsa Yellow’ were identified as cold-tolerant cultivars. The CTRI derived from pollen parameters showed a significant but weak correlation with physiological parameters. This infers that screening based on pollen parameters is an accurate approach for reproductive temperature tolerance. The identified tolerant traits can be incorporated into both vegetable and ornamental pepper breeding programs to produce cultivars tolerant to high and low temperatures. Also, the ornamental industry can target the cultivars suitable to a niche environment based on the level of heat and cold tolerance among the ornamental pepper cultivars. However, further studies are needed to test additional cultivars for heat and cold tolerance and to test the identified heat- and cold-tolerant cultivars for whole plant or field performance across a wide range of growing conditions.

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