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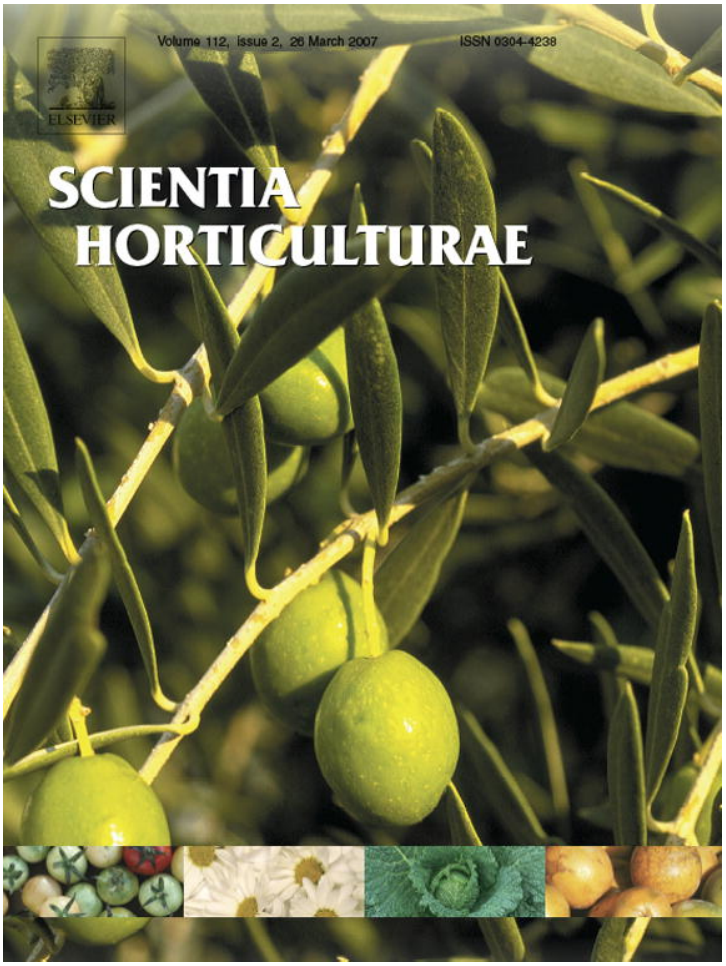

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Screening *Capsicum* species of different origins for high temperature tolerance by *in vitro* pollen germination and pollen tube length

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Abstract

Successful fruit set depends on several reproductive processes including pollen germination and tube growth processes. An experiment was conducted to determine the effects of temperature on pollen germination characteristics and to identify species/genotypic differences in *Capsicum* using the cumulative temperature response index (CTRI) concept. Pollen was collected from plants of seven genotypes from five *Capsicum* species, adapted to various parts of the world and grown outdoors in large pots. The pollen was subjected to *in vitro* temperatures ranging from 15 to 50 °C at 5 °C intervals. Pollen germination and tube lengths were recorded for all species after 24 h of incubation at the respective treatments. Species/genotypes differed significantly for *in vitro* pollen germination percentage and pollen tube length with mean values of 78% and 734 μm, respectively. The mean cardinal temperatures (T_{min}, T_{opt}, and T_{max}) averaged over genotypes, were 15.2, 30.7, and 41.8 °C for pollen germination and 12.2, 31.2, and 40.4 °C for pollen tube growth. The CTRI of each species/genotype calculated as the sum of eight relative individual stress response values, such as maximum pollen germination, maximum pollen tube length; T_{min}, T_{opt}, and T_{max} temperatures of pollen germination, and pollen tube lengths, identified species tolerance to high temperatures. *Capsicum annum* cv. Mex Serrano from Mexico was identified as tolerant, *C. chacoense* cv. 1312 and *C.* spp. cv. Cobanero from Argentina and Guatemala, respectively as intermediate and *C. frutescens* cv. Early Spring Giant from China, *C. annum* cv. Long Green from South Korea, *C.* spp. cv. NM89C130 and *C. pubescens* cv. 90002 from Guatemala as sensitive to high temperatures. The tolerant species/genotypes can be used in breeding programs to develop new genotypes that can withstand high temperature conditions both in the present climate and particularly in a future warmer climate.
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Keywords: *Capsicum*; Cardinal temperatures; Cumulative temperature response index; Pepper; Screening tool

1. Introduction

Sexual reproduction in plants is more sensitive to high temperatures than vegetative processes, and therefore plant reproductive organs will be more vulnerable to changes in short episodes of high temperatures prior to and during early flower stage. Fruit set in bell pepper (Erickson and Markhart, 2002), bean (Porch and Jahn, 2001; Gross and Kigel, 1994), corn (Herrero and Johnson, 1980), cotton (Reddy et al., 1992a,b, 1997), cowpea (Ahmed and Hall, 1993) groundnut (Prasad et al., 1999, 2003), soybean (Ferris et al., 1998), and tomato (Peet et al., 1998; Sato et al., 2002), is sensitive to high temperatures. Furthermore, plant reproductive organs will be more vulnerable to changes projected in climate such as increase in Earth's surface temperature to anywhere between 1.5 and 11 °C by 2100 due to increases projected in greenhouse gases (Stainforth et al., 2005). In addition, short episodes of extreme events including high temperatures projected to occur more frequently in the future climate (Mearns et al., 2001; Meehl and Tebaldi, 2004) will impact fruit set and yield (Reddy et al., 1992a,b). It would be advantageous for plants to exhibit greater reproductive survivability at extreme (low and high) temperatures normally encountered during plant reproduction and for processes leading to yield such as pollen grain development, pollen germination, pollen tube growth, fertilization and embryo development, and finally seed development.

High temperatures during flowering have been shown to affect pollen germination, tube growth, fertilization, flower abscission and fruit set in peppers (Wien, 1997; Han et al., 1996; Usman et al., 1999; Aloni et al., 1991, 2001; Erickson and Markhart, 2002). Low temperatures also result in reduced fruit set in peppers due to inhibition of pollen germination (Shaked

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et al., 2004). Two stages of pollen development, microspore mother cell meiosis and mature microspores at anthesis, have been reported to be highly sensitive to high temperature (Erickson and Markhart, 2002). Pollen abnormalities such as shrunk and empty pollen without a noticeable exine were observed in peppers on exposure to high temperature of 33 °C (Erickson and Markhart, 2002). Similar pollen abnormalities were observed in bean (Porch and Jahn, 2001; Gross and Kigel, 1994), snap bean (Suzuki et al., 2001) and soybean (Koti et al., 2005) when grown in high temperatures. Lower seed yield for plants grown at high temperature conditions was shown to be due to decreased pollen viability in peppers (Han et al., 1996; Erickson and Markhart, 2001, 2002; Karni and Aloni, 2002), groundnut and bean (Prasad et al., 2002, 2003).

Pollen, once released from the anthers, acts as an independent functional unit. Several recent studies have investigated *in vitro* pollen germination and pollen tube length in various crops (cotton—Burke et al., 2004; Kakani et al., 2005; groundnut—Craufurd et al., 2003, Kakani et al., 2002; *Primula*—McKee and Richards, 1998). Temperature of >32 °C is known to reduce pollen germination, pollen tube growth and fruit set in peppers (Aloni et al., 2001; Erickson and Markhart, 2002) However, to date, there have been no studies conducted to specifically document genotypic variability in peppers for *in vitro* pollen germination and pollen tube growth and tolerance to high temperatures. The objectives of this research were to (i) quantify the responses of *in vitro* pollen germination and pollen tube growth of *Capsicum* species of different origin to temperature, (ii) determine cardinal temperatures of *Capsicum* species, and (iii) develop a screening technique to identify species tolerant to high temperature.

2. Materials and methods

2.1. Plant husbandry

Seven genotypes belonging to five species of *Capsicum* originated from six countries (Table 1), were sown outdoors on 9 June 2004 in 12 L white polyvinyl chloride (PVC) pots filled with fine sand. The pots were 0.65 m tall and 0.15 m in diameter, with a small aperture at the bottom to drain excess water. There were 10 pots for each genotype, arranged in rows (40 pots per row) with an additional border row maintained on each side. The rows were oriented east to west and spaced 1 m apart. Seedlings emerged 7 days after sowing and were thinned to two per pot 1 week after emergence. All pots were irrigated using a computer-controlled drip system with half-strength Hoagland's nutrient solution (Hewitt, 1952) to maintain favorable conditions of water and nutrients.

Table 1
List of *Capsicum* species along with accession number maintained by the United States Department of Agriculture (USDA, ARS, SRPIS, University of Georgia, Plant Genetic Resources Conservation Unit, Griffin, GA, USA) and their country of origin used in the present study

Genus, species and genotype	Accession number	Country of origin
Capsicum annum cv. Mex Serrano	PI 380521	Mexico
C. annum cv. Long Green	PI 508433	South Korea
Capsicum chacoense cv. 1312	PI 260426	Argentina
Capsicum frutescens cv. Early Spring Giant	PI 419039	China
Capsicum pubescens cv. 90002	PI 593619	Guatemala
Capsicum spp. cv. NM89C130	PI 555613	Mexico
Capsicum spp. cv. Cobanero	PI 555627	Guatemala

2.2. Measurements

2.2.1. Pollen germination and pollen tube lengths

Forty to 50 flowers were randomly collected from plants in each genotype between 09:00 and 10:00 h. Maximum pollen germination was recorded during this time as determined by a preliminary time-series observations (data not shown). Pollen was distributed uniformly onto the modified germination medium of Karni and Aloni (2002). The medium consisted of 100 g sucrose ($C_{12}H_{22}O_{11}$), 500 mg calcium nitrate [$Ca(NO_3)_2{\cdot}4H_2O$], 120 mg magnesium sulphate ($MgSO_4$), 100 mg potassium nitrate (KNO_3) and 120 mg boric acid (H_3BO_3) dissolved in 1000 ml of deionized water. To this liquid medium, 10 g agar was added and slowly heated on a hot plate. After the agar was completely dissolved, 10 ml of the germinating medium was poured into three Petri dishes for each genotype in each treatment and allowed to cool for about 15 min to let the agar solidify. The plates were then covered and incubated (Precision Instruments, New York, USA) at pre-determined temperature treatments of 15–50 °C at 5 °C intervals. Each plate per genotype and treatment was considered as a replicate.

Incubators were maintained at treatment temperature and no differences were recorded between the measured cabinet and media temperatures (Kakani et al., 2005). Therefore, the average temperature of the growth cabinet during pollen germination was used in the analysis. Pollen grains were counted for pollen germination (10 fields in each Petri dish) after 24 h of incubation using a Nikon SMZ 800 microscope (Nikon Instruments, Kanagawa, Japan) with a magnification of 6.3×. A pollen grain was considered germinated when its tube length equaled the grain diameter (Luza et al., 1987). Percentage pollen germination was calculated by counting the total number of pollen grains and the number of pollen germinated in a microscope field. The pollen tube lengths were measured with an ocular micrometer fitted to the eyepiece of the microscope. A total of 100 pollen tubes were measured for each treatment.

2.2.2. Cardinal temperatures

The maximum pollen germination and pollen tube lengths recorded after 24 h were analyzed by linear and non-linear regression models commonly used to quantify pollen parameter responses to temperatures (Kakani et al., 2002). The fit of each regression equation for the response of pollen germination and pollen tube lengths to temperatures was compared for the amount of variation accounted for by R^2 and root mean square deviation (RMSD) for observed and fitted values. A modified bilinear model best described the responses of both pollen

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germination and tube length to temperature with the highest R^2 and lowest RMSD values. Accordingly, the cardinal temperatures were calculated from the fitted equations for all the species. The non-linear regression procedure PROC NLIN (SAS Institute, 1999) was used to estimate the parameters of the modified bilinear model [Eq. (1)] where T is the actual treatment temperature, and a, b_1, and b_2 are species-specific constants generated by SAS. The T_{opt} was generated by fitting the bilinear model using SAS, and T_{max}, and T_{min} were determined by Eqs. (2) and (3) (Kakani et al., 2002) as shown below:

Pollen germination (%) and pollen tube

$$\text{length} = a + b_1(T - T_{opt}) + b_2 \times \text{ABS}(T_{opt} - T) \quad (1)$$

$$T_{min} = \frac{a + (b_2 - b_1) \times T_{opt}}{b_1 - b_2} \quad (2)$$

$$T_{max} = \frac{a - (b_2 + b_1) \times T_{opt}}{b_1 + b_2} \quad (3)$$

2.2.3. Cumulative temperature response index (CTRI)

Individual stress response index (ISR) of each parameter was calculated as the value of a genotype divided by maximum value observed over all the genotypes. The cumulative temperature response index [CTRI; unit less; Eq. (4)] of each genotype was calculated as the sum of eight ISR's derived from maximum pollen germination (PG), maximum pollen tube length (PTL), T_{min}, T_{opt} and T_{max} temperatures of pollen germination, and pollen tube lengths. Species/genotypes were classified based on CTRI values of all the treatments as tolerant [>minimum CTRI + 3 standard deviations (S.D.)], intermediate (>minimum CTRI + 2 S.D. and <minimum CTRI + 3 S.D.), and sensitive (>minimum CTRI + 1 S.D. to <minimum CTRI + 2 S.D.)

$$\text{CTRI} = \left(\frac{\text{PG}\%_t}{\text{PG}\%_h} + \frac{\text{PTL}_t}{\text{PTL}_h} + \frac{\text{PGT}_{\text{min}_t}}{\text{PGT}_{\text{min}_h}} + \frac{\text{PG}_{\text{opt}_t}}{\text{PG}_{\text{opt}_h}} + \frac{\text{PG}_{\text{max}_t}}{\text{PG}_{\text{max}_h}} + \frac{\text{PTLT}_{\text{min}_t}}{\text{PTLT}_{\text{min}_h}} + \frac{\text{PTLT}_{\text{opt}_t}}{\text{PTLT}_{\text{opt}_h}} + \frac{\text{PTLT}_{\text{max}_t}}{\text{PTLT}_{\text{max}_h}} \right) \quad (4)$$

3. Results and discussion

The maximum percentage germination recorded at optimum temperature varied significantly among *Capsicum* species (Table 2), with 59% in *C. frutescens* (China) to 95% in *C. chacoense* (Argentina). For pollen response to temperature, use of linear and nonlinear regression models such as quadratic (Yan and Wallace, 1998), cubic or higher order polynomials (Tollenaar et al., 1979); beta distribution (Yin et al., 1995) and bilinear models (Omanga, 1994; Kakani et al., 2002, 2005) are not uncommon. Based on our results, a modified bilinear model (with $R^2 > 0.89$) fitted well to describe pollen germination in response to temperature for various *Capsicum* species (Table 2; Fig. 1A). The modified and solidified pollen germination media resulted in up to 300% higher pollen germination compared to that in the liquid media of Aloni et al. (2001) and Karni and Aloni (2002). The cardinal temperatures (T_{min}, T_{opt}, and T_{max}), derived form modified bilinear fit for pollen germination differed significantly among species (Table 2). The magnitude of T_{min} ranged from 14.8 °C (*C. annum* cv. Mex Serrano and Long Green) to 16.0 °C (*C.* spp. Cobanero), with an average of 15.2 °C. T_{opt} ranged from 29.0 °C (*C. chacoense* cv. 1312) to 32.8 °C (*C. frutescens* cv. Early Spring Giant), with an average of 30.7 °C. T_{max} ranged from 40.0 °C (*C. annum* cv. Long Green from South Korea) to 43.7 °C (*C. annum* cv. Mex Serrano from Mexico), with an average of 41.8 °C. Pollen germination percentages observed in the present study are similar to several other published studies that used artificial media: 44% in cotton (Kakani et al., 2005), 56% in groundnut (Kakani et al., 2002), 68% in pigeon pea (Jayaprakash and Sarla, 2001), and 64–76% in soybean (Gwata et al., 2003; Koti et al., 2005). Therefore, the high percentage pollen germination observed at optimum temperature and pollen germination response to temperature in the current study represents real species variability which can be used to screen *Capsicum* species and genotypes within the species for high temperature tolerance. Among the seven genotypes tested, *C. annum* cv. Mex Serrano originating from Mexico, exhibited widest temperature range (T_{max}–T_{min} = 28.9 °C), and had wider temperature adaptability while

Table 2
Maximum pollen germination percentage, modified bilinear equation constants, and cardinal temperatures for pollen germination of seven *Capsicum* accessions in response to temperature

Species and genotype	Maximum pollen germination (%)	Equation constants			R^2	Cardinal temperature (°C)		
		a	b_1	b_2		T_{min}	T_{opt}	T_{max}
C. annum cv. Mex Serrano	75.8	72.9 ± 2.6	−0.56 ± 0.4	−5.12 ± 0.4	0.99	14.8	30.8	43.7
C. annum cv. Long Green	79.9	87.4 ± 6.3	−0.95 ± 0.4	−7.05 ± 0.4	0.97	14.8	29.1	40.0
C. chacoense cv. 1312	95.2	100.0 ± 2.1	−0.69 ± 0.2	−8.00 ± 0.2	0.99	14.9	29.0	40.9
C. frutescens cv. Early Spring Giant	58.7	65.7 ± 5.1	−2.70 ± 0.6	−6.39 ± 0.6	0.98	14.9	32.8	40.1
C. pubescens cv. 90002	75.9	83.4 ± 2.0	−1.30 ± 0.7	−6.24 ± 0.5	0.99	15.2	32.1	43.3
C. spp. cv. NM89C130	77.2	72.1 ± 1.3	−1.12 ± 0.1	−6.00 ± 0.1	0.91	15.9	30.7	40.8
C. spp. cv. Cobanero	81.6	76.9 ± 5.7	−0.63 ± 0.4	−5.65 ± 0.4	0.89	16.0	30.3	43.6
Mean	77.8	–	–	–	0.96	15.2	30.7	41.8
S.E.D.	5.04***	–	–	–	–	0.19***	0.55***	0.47***

–: data not analyzed statistically.
*** Significant at $P = 0.001$ level.

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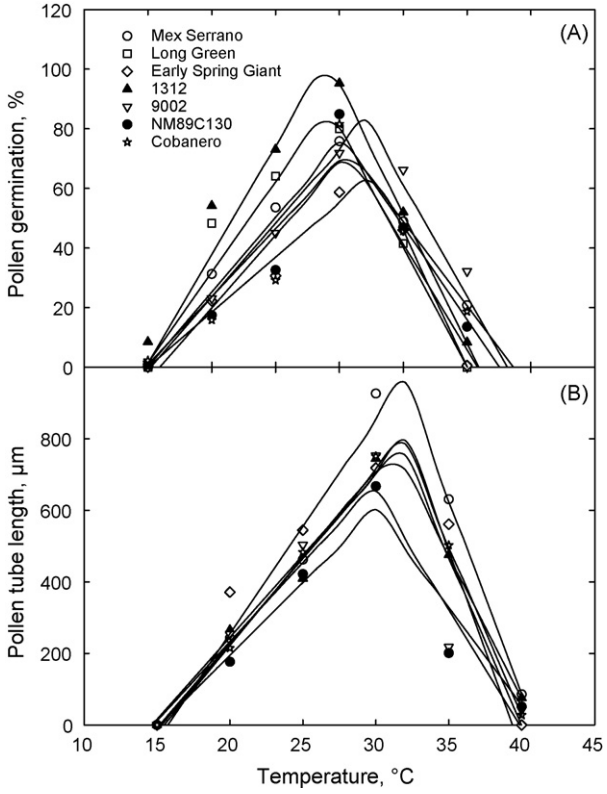

Fig. 1. Influence of temperature on (A) pollen germination percentage and (B) pollen tube length of various *Capsicum* species.

C. spp. cv. NM89C130 also originating from Mexico had the smallest temperature range (T_{max}–T_{min} = 24.9 °C) and consequently narrower temperature adaptability.

Capsicum species were also significantly different for pollen tube length in response to temperature (Fig. 1B). Similar to pollen germination response to temperature, the modified bilinear model described the response of pollen tube length to temperature in all *Capsicum* species (with $R^2 > 0.93$; Table 3). The maximum pollen tube length ranged from 655 µm (*C. pubescence* cv. Early Spring Giant) to 926 µm (*C. annum* cv. Mex Serrano from Mexico), with a mean of 737 µm (Table 3). The cardinal temperatures for pollen tube length differed significantly ($P = 0.001$) among *Capsicum* species (Fig. 1B and Table 3). Values of T_{min} ranged from 14.7 °C (*C. pubescence* cv. 90002 from Guatemala) to 15.7 °C (*C. annum* cv. Mex Serrano from Mexico), with an average of 15.2 °C. The T_{opt} ranged from 29.8 °C (*C. pubescence*) to 31.9 °C (*C. annum* cv. Mex Serrano from Mexico), with an average of 31.2 °C. Maximum temperature ranged from 39.9 °C (*C. pubescence* cv. 90002) to 41.0 °C (*C.* spp. cv. NM89C130 from Mexico), with an average of 40.4 °C. The observed differences and the ranges recorded in pollen tube lengths in the present study are similar to the observations in artificial pollen germination media for several other crops (1000–1800 µm for corn—Binelli et al., 1985; 450–1400 µm for groundnut—Kakani et al., 2002, 20–60 µm for muskmelon—Maestro and Alvarez, 1988; and 410–1400 µm for cotton—Kakani et al., 2005). Therefore, the observed differences in pollen tube lengths in the present study are reflecting the species variability in *Capsicum* species.

Table 3
Maximum pollen tube length, modified bilinear equation constants, and cardinal temperatures for pollen tube length of seven *Capsicum* accessions in response to temperature

Species and genotype	Maximum pollen tube length (µm)	Equation constants			R^2	Cardinal temperature (°C)		
		a	b_1	b_2		T_{min}	T_{opt}	T_{max}
C. annum cv. Mex Serrano	926	969 ± 22	−2.45 ± 0.54	−8.45 ± 0.46	0.95	15.7	31.9	40.8
C. annum cv. Long Green	740	783 ± 13	−2.36 ± 0.53	−7.10 ± 0.29	0.96	15.1	31.7	40.0
C. chacoense cv. 1312	744	770 ± 20	−1.59 ± 0.08	−6.41 ± 0.10	0.93	15.3	31.3	40.9
C. frutescens cv. Early Spring Giant	706	805 ± 65	−3.32 ± 0.23	−7.91 ± 0.29	0.98	15.2	32.2	40.0
C. pubescens cv. 90002	655	767 ± 32	−1.10 ± 0.23	−5.53 ± 1.10	0.95	14.7	29.8	39.9
C. spp. cv. NM89C130	667	681 ± 33	−0.70 ± 0.16	−4.77 ± 0.31	0.94	15.2	30.0	41.0
C. spp. cv. Cobanero	718	805 ± 56	−2.28 ± 0.49	−7.22 ± 0.50	0.94	15.4	31.8	40.3
Mean	736.7	–	–	–	0.96	15.2	31.2	40.4
S.E.D.	34.6***	–	–	–	–	0.20**	0.44***	0.50***

Significant at ***$P = 0.001$ and *0.05 levels. –: data not analyzed statistically.

Table 4
Classification of *Capsicum* accessions into tolerant, intermediate, and sensitive groups based on cumulative temperature response index (CTRI) along with individual score of CTRI values in parenthesis

Tolerant (CTRI > 7.52)	Intermediate (CTRI = 7.36–7.52)	Sensitive (CTRI = 7.18–7.35)
C. annum cv. Mex Serrano (7.65)	*C. chacoense* cv. 1312 (7.51) *C.* spp. cv. Cobanero (7.52)	C. *frutescens* cv. Early Spring Giant (7.18) C. *pubescens* cv. 90002 (7.27) *C. annum* cv. Long Green (7.29) *C.* spp. cv. NM89C130 (7.31)

The CTRI is the sum of individual component responses such as maximum pollen germination, maximum pollen tube length, and cardinal temperatures for pollen germination and pollen tube length.

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The cumulative temperature response index (CTRI) derived as the sum of all reproductive parameters varied significantly among *Capsicum* species (Table 4). This technique uses all pollen parameters of interest to identify species variability to high temperature. Based on CTRI, *C. annum* cv. Mex Sarrano originating from Mexico was identified as tolerant, *C. chacoense* cv. 1312, and *C.* spp. cv. Cobanero originating from Argentina and Guatemala, respectively, were intermediate, and *C. frutescens* cv. Early Spring Giant, *C. pubescence* cv. 90002, *C. annum* cv. Long Green and *C.* spp. cv. NM89C130 originating from China, Guatemala, South Korea, and Mexico, respectively, were sensitive to high temperatures. Identification of heat tolerance and developing cultivars tolerant to high temperature are particularly important in a future global climate where extreme climatic events are forecast to be more frequent (Mearns et al., 2001). Extreme temperatures have been shown to damage reproductive processes more drastically than just a rise in season-long average temperature (Hall, 1992; Reddy et al., 1992a, 1997, 1999).

4. Conclusion

In this first study, with pepper pollen on solidified media, *in vitro* pollen germination and tube length responses of *Capsicum* species to temperature were similar and there is no clear pattern in tracing heat tolerance to the country of origin. The pollen parameters identified in the present study can be used as one of the parameter in the breeding programs to develop new genotypes for a niche environment. However, narrow range of cardinal temperatures among studied species suggests exploration of *Capsicum* germplasm for identifying species/genotypes tolerant to high temperature conditions. Pollen heat tolerance will be essential both in the present climate as well as in a projected future warmer and variable climate. In addition, the identified cardinal temperatures and response functions could be incorporated into *Capsicum* simulation models to increase prediction accuracy under current extreme and projected future climates.

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