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Screening ornamental pepper (Capsicum annuum L.) cultivars for temperature tolerance using pollen, physiological and seed germination parameters

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SCREENING ORNAMENTAL PEPPER (*Capsicum annuum* L.) CULTIVARS FOR TEMPERATURE TOLERANCE USING POLLEN, PHYSIOLOGICAL AND SEED GERMINATION PARAMETERS

By

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Temperature affects reproductive potential, aesthetic and commercial value of ornamental peppers. In experiment one, temperature effects were assessed in 12 ornamental cultivars using *in vitro* pollen germination and tube length, and the physiological parameters, cell membrane thermostability, chlorophyll stability index and canopy temperature depression. In experiment two, seed germination rate and maximum seed germination response to temperature were assessed. Cumulative temperature response indices (CTRI) for pollen, seed, and physiological parameters were derived and used to classify cultivars for temperature tolerance. CTRI based on pollen parameters showed significant, but poor correlation with physiological parameters. CTRI based on seed parameters showed significant correlation with CTRI-physiological parameters. It is concluded that screening using pollen parameters will be ideal for reproductive temperature tolerance while seed and physiological parameters will be suitable for screening vegetative temperature tolerance. Identified tolerant cultivars are potential

candidates for breeding programs to develop heat and cold tolerant ornamental pepper genotypes.

DEDICATION

I dedicate this dissertation to my late father Somapala Gajanayake, late mother Seetha Manathunga, wife Kanchana Weerasinghe and brother Mahesh Gajanayake. Without their love, affection, teachings, inspiration, encouragement, and sacrifices this episode of my life would have never been possible.

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CHAPTER 1

INTRODUCTION

The floriculture sector is an important segment of production agriculture in the United States. This sector plays a vital role in the US economy and is one of the fastest growing agricultural areas. The total wholesale floriculture crop value for the 15 state programs for all growers with $10,000 or more in sales is estimated at $4.10 billion for 2007. Out of the total value, the value of potted annual bedding and garden plants was estimated to be about $560 million (USDA, 2008).

Among various plants in the floricultural industry, ornamental pepper (*Capsicum annuum* L.) is widely used as a potted flowering or bedding plant. Ornamental peppers have morphologically diverse characteristics and are planted for their aesthetic value. Ornamental peppers used as potted and florist crops are popular in Europe and are gaining in popularity in the United States (Armitage and Hamilton, 1987). Most ornamental pepper cultivars produce pungent fruit. Cultivars such as ‘Chilly Chili’ and ‘Medusa’ have become popular due to their non-pungent fruit (Stummel and Bosland, 2007). Ideal cultivation conditions for ornamental peppers are similar to typical vegetable pepper production with the crop requiring high light and minimum day time temperatures between 18 to 21°C for maximum fruit set. Pepper, as a warm weather crop, requires night and soil temperatures of 14.5°C or higher to promote growth. Lower temperatures are tolerated by the pepper plants as they mature. Yellowing and dropping of leaves are caused by low temperatures, low light, and insufficient moisture or nutrients (Stummel and Bosland, 2007). Pollen fertility and fruit quality are also affected by low night temperature (Polowick and Sawhney, 1986). Young et al. (2004) found that high temperature during flowering or during pollen release and germination may affect male reproductive processes (microsporogenesis) resulting in lower fruit set and smaller fruits. Thus, both low and high temperature extremes can negatively affect reproductive and vegetative growth in pepper.

Changes projected in climate change will exacerbate the effects of several abiotic stresses on crop production. According to Taylor and Hepler (1997), the increase in earth's near surface temperature associated with carbon dioxide concentration and other greenhouse gases is an important component of global climate change and this may cause impacts on productivity of crops. This has been verified from both field and controlled-environmental studies and modeling exercises (Reddy et al., 2000). Since Industrial Revolution, global surface temperature has increased by more than 0.6°C because of anthropogenic climate-forcing agents. Sustained perturbations in climate are projected to increase global surface temperatures between 1.5 to 11°C by the year 2100 (Hansen and Sato, 2004). It has been shown that this projected variation in climate will have profound impacts on crop production (Stainforth et al., 2005). Lobell and Asner (2003) have studied the relationship between climate variation and crop production on corn and soybean grown in the United States between the years 1982 to 1998 and stated that for every 1°C increase in temperature, there was an average 17% decrease in yield.

Furthermore, extreme temperatures during the flowering period could potentially exacerbate the effects of temperature crop yield more than the general increase in temperature conditions for the long term (Hall, 1992). Therefore, there is a need to investigate the effect of changes in temperature on various crop production processes. Prior studies have reported that fruit set in many horticultural crops are sensitive to supra-optimal temperatures, namely, bell pepper (Erickson and Markhart, 2001), bean (Porch and Jahn, 2001), and tomato (Sato et al., 2002).

Compared to vegetative organs, reproductive organs of plants are much more vulnerable to temperature fluctuations. Fruit set in peppers is affected by temperature stress mostly prior to and during the early flower stage (Erickson and Markhart, 2002; Reddy and Kakani, 2007). A higher capability to survive under extreme temperature conditions during plant reproduction as well as various processes leading to fruit yield such as pollen grain development, pollen germination, pollen tube growth, fertilization, and embryo development are highly beneficial to plants. Various studies have shown that pollen germination, pollen tube growth, fertilization, embryo development, and seed production of peppers are affected by high temperature (Erickson and Markhart, 2002; Reddy and Kakani, 2007). The pollen grains are the structures which are used to transfer the male gamete with male genetic information to the female element of a flower in higher plants. According to Taylor and Hepler (1997), these small structures are developed in the anthers and once they reach maturity, they contain genetic information of the both sporophytic and gametophytic. In the process of microsporogenesis, where pollen grains are developed, two stages namely microspore mother cell meiosis and mature microspore at anthesis are more susceptible to high temperature stress (Erickson and Markhart, 2002). At the same time, these pollen grains behave as independent functional units once they release from anthers during anthesis. Aloni et al. (2001) found that any temperature exceeding 32°C, causes reduction in pollen germination and pollen tube growth, and ultimately the fruit set in peppers. Therefore, investigation of cultivar variability in ornamental pepper pollen grains to high and low temperature stress and their tolerance levels would be beneficial.

Temperature and water mainly control the rate of seed germination when aeration is not restrictive (Gummerson, 1986). Temperature alone is the major environmental factor overriding seed germination when soil moisture is not limiting (Garcia-Huidobro et al., 1982). Ornamental peppers are generally propagated through seed with an optimum temperature of 30°C. The rate of both germination and emergence is significantly reduced at temperatures ranging from 15 to 20°C (O'Sullivan and Bouw, 1984). Thermal time (Degree-day or hour), the heat unit for plant development, is a well established developmental principle for plants (Fry, 1983). This thermal time model has been successfully used to predict seed germination under non-water limiting conditions. According to the model, for most crop species, existing soil temperature determines both the percentage of seed in a sample which germinate and the rate of germination (Garcia-Huidobro et al., 1982). Using this model, cardinal temperatures (T_{opt}, T_{max}, and T_{min}) could be identified and these thermal responses from each cultivar of ornamental pepper can be used to assess the temperature tolerance.

In many studies, for a range of crops on abiotic stresses and their level of tolerance, various physiological parameters were extensively used, especially temperature. Wahid et al. (2007) emphasized that adjustments in various photosynthetic attributes under high temperature stress would be used as indicators of temperature tolerance of the plants as they show correlation with growth. Any limitation in the process of photosynthesis can limit plant growth in high temperature environments. Physiological parameters used to study temperature tolerance include net photosynthesis, chlorophyll fluorescence (Nyarko et al., 2008; Singh et al., 2008a), stomatal conductance, (Srinivasan et al., 1996), cell membrane thermostability (Balota et al., 1993; Nyarko et al., 2008; Singh et al., 2008b), and canopy temperature depression (Balota et al., 1993).

Karim et al.(2000) concluded that net photosynthesis is a reliable parameter for temperature tolerance in maize seedlings exposed to high temperature stress. It was found that moderate heat stress to plants can decrease the photosynthetic rate to near zero, even though such heat stress rarely affects photosystem II (Sharkey, 2005). Stomatal conductivity also has been used by researchers as a key parameter to screen crops and cultivars for both drought and temperature tolerance. Lu et al. (1998) found that stomatal conductance could be used as an indirect selection criterion for higher yields in irrigated pima cotton and bread wheat grown at supra-optimal temperatures. Canopy temperature depression (CTD) is the difference between air and canopy temperature. Reynolds et al. (1998) indicated that CTD is a good criterion for screening heat stress tolerance. In addition, it has been used as an indicator for overall plant stresses and environmental stresses such as moisture stress and/or heat for many crops by various researchers. Many studies on temperature tolerance made an effort to determine the correlation between various physiological and other parameters to temperature tolerance and other performances of crop plants. In such a study, Kakani et al. (2002) found that for groundnut, there exists no correlation between pollen germination and CMT under high temperature conditions. Limited research has been conducted for ornamental peppers to screen the temperature tolerance of cultivars using of pollen and seed based parameters and physiological parameters. Thus, it will be necessary to explore cultivars of ornamental peppers for temperature tolerance using multiple parameters namely pollen, seed, and physiological. Association of pollen and seed parameters with physiological parameters (CMT, chlorophyll stability, and CTD) among cultivars of ornamental pepper would be beneficial.

Central Research Question and Objectives

Is there a genetic variation for temperature tolerance in ornamental pepper cultivars and can such variation screened with (i) pollen related attributes, (ii) physiological parameters and (iii) seed related attributes? Also, is there a correlation between those parameters among cultivars for temperature tolerance?

The general objective of the study was to understand the differences in temperature tolerance among 12 different ornamental pepper cultivars. The specific objectives were to (i) quantify the responses of *in vitro* pollen germination, pollen tube growth, and seed germination of ornamental pepper cultivars to different temperatures; (ii) determine cardinal temperatures of each cultivar both for pollen germination and seed germination; (iii) classify cultivars based on their tolerance levels to temperature using cardinal temperatures and three physiological parameters, cell membrane thermostability, chlorophyll stability index and canopy temperature depression, and (iv) determine whether the observed variation among cultivars in pollen germination related parameters is correlated with seed germination and the physiological parameters.

CHAPTER II

LITERATURE REVIEW

Botanical Classification and Origin of Ornamental Pepper

Ornamental pepper (*Capsicum annuum* L.) belongs to the plant family Solanaceae, which is mainly a tropical family. The genus *Capsicum* includes 30 known pepper species. Of the 30 species, only five have been domesticated and cultivated world-wide (Bosland and Votava, 2000).

Peppers are thought to have originated from South America (Bolivia and Peru) and are endemic to tropical and subtropical climates (Purseglove, 1987). Archeological evidence of wild *Capsicum annuum* L. seed that precedes 5000 B.C. has been found at Tehuacan, Mexico. Peppers were introduced into Europe by Christopher Columbus and then were distributed throughout the world, including the tropics, sub tropics and temperate regions by the extensive trading routes of the Spanish and Portuguese (Stummel and Bosland, 2007). The *Capsicum annuum* L. is the most widely cultivated and economically important species of peppers, and is used as a vegetable pepper, sweet pepper, dried hot peppers, chili powder, paprika, and as an ornamental crop (Rubatzky and Yamaguchi, 1997).

Development of Ornamental Peppers

There exists a substantial diversity in *Capsicum* germplasm for fruit and leaf shape and size, as well as plant habit. Ornamental peppers are easy to propagate from seed, have a short cropping time, are tolerant to drought and heat and could persist throughout the season. These attributes, combined with morphological diversity of fruit and foliage, has allowed for development of distinctive ornamental cultivars (Stummel and Bosland, 2007). Furthermore, Stummel and Griesbach (2005) reported that peppers were considered more as a high value ornamental plant than as a food source, when introduced to Europe in the 15^{th} century. Since the 15^{th} century, ornamental peppers have become popular in Europe and the United States as a potted or bedding plant and florist crop (Armitage and Hamilton, 1987). In the early floriculture industry, ornamental peppers were referred to as Christmas peppers and were confined to pot plants. Ornamental peppers were popular as Christmas plants until 1960's and with the emergence and growth of the Poinsettia industry, the popularity for ornamental peppers as Christmas plants declined. However, because of their vibrant fruit and foliage colors and their sustained growth throughout the summer fall seasons, they are gaining popularity in recent years as an ideal bedding plant in many home and garden landscapes and as an ideal potted plant in the floriculture and garden industry (Stummel and Bosland, 2007).

Plant Types of Ornamental Peppers

Ornamental peppers are popular herbaceous species because of the great diversity of pod types and plant habit (Stummel and Bosland, 2007). Ornamental peppers have three main types: potted types, cut stems and bedding and garden types. Cut stems are popular in Europe and are gaining popularity in the USA as well in recent years. This type of pepper produces clusters of fruits on long stems capable for use in flower arrangements. Other ornamental peppers which are heat and drought tolerant and prostrate are grown as bedding and garden plants. Some ornamental peppers are used for both ornamental and culinary purposes (Stummel and Bosland, 2007).

Climate and Plant Culture

Ornamental peppers can be grown from sea level to an elevation of 3000 m. Generally peppers are frost sensitive and require temperatures above 20°C with a relatively long growth period to be productive. Peppers are photoperiod insensitive and can be grown with an optimum temperature range from 20 to 25°C and flowering range from 18 to 27°C (Purseglove, 1987). Generally, higher night temperatures are not conducive for fruit set, thus, temperatures above 32°C and below 15°C for extended periods during flowering often result in poor growth and low yields (Berke et al., 2003). When the night temperature is below 20°C, plant growth is improved. At the same time, flavor and color development of fruit is affected by low temperature and both plants and fruits are susceptible to chilling injury. Production conditions for ornamental bedding plant cultivation are similar to those for vegetable pepper production (Stummel and Bosland, 2007).

Ornamental pepper seed has a prolonged germination period of 10 to 14 days and an optimum germination temperature of about 30°C. For potted plant production, peppers are typically grown in 10 cm diameter (1 liter volume) pots (Ball, 1998). According to

Stummel and Bosland (2007), sowings are typically made from April until August, for Christmas sales starting from September. It takes 14 weeks from seeding to market for plants in 10 cm diameter pots when seeded in the spring or summer and 17 weeks when seeded in winter months (Harthun, 1991). Even though, ornamental peppers require high solar radition and minimum day time temperatures between, 18 and 21°C for maximum fruit set, lower temperatures are tolerated as the plants mature.

Global Temperature Rise

With globalization comes environmental changes and the results of these changes will persist into the coming decades due to increases in the earth's surface temperature. According to Meehl and Tebaldi (2004), future climate is projected to have more intense, more frequent, and longer lasting heat waves. Such climate change is a result of increased greenhouse gas concentrations in the atmosphere mainly due to anthropogenic (human) activities. Global surface temperature has increased by more than 0.6°C since the industrial revolution because of anthropogenic climate forcing agents (Hansen and Sato, 2004). Future increases in greenhouse gases are projected to increase the earth's surface temperature between 1.5 to 11°C by the year 2100 (Stainforth et al., 2005). According to Intergovernmental Panel for Climate Change (IPCC) (2007) the daily minimum temperatures are projected to increase faster (night time) compared to daily maximum temperature (daytime), leading to decreases in the diurnal temperature trend. Research predicts that climate change will have profound impacts on crop production as evidenced from studies using field and climate controlled environments and modeling exercises (Reddy et al., 2000; Doherty et al., 2003). Prior research has shown that elevated temperatures during the flowering period will have a more intense effect on crop yield than a rise in season long temperatures (Hall, 1992).

Heat and Cold Stress and their Tolerance in Crop Plants

Hall (2001) defined heat stress as the situation in which the air temperature is high enough to cause irreversible damage to plant growth and development. Wahid et al. (2007) defined heat stress as an increase in temperature beyond a threshold level for a period of time adequate to cause irreversible damage to plant growth and development. They further mentioned that any temporary increase in temperature, usually 10-15°C above ambient, is considered heat shock or heat stress to plants. Heat stress caused by high temperature is a serious threat to global crop production (Hall, 2001). Investigations have evaluated heat stress effects on plant processes.

Heat tolerance is defined as the ability of the plant to grow and produce economic yield under elevated temperatures (Willits and Peet, 1998). Two hypothesis on heat tolerance exist, one that night temperatures plays a key role whereas the other hypothesis considers that diurnal mean temperature is the key to predict the plant response to higher temperatures, with day temperature having a secondary role.

Cold acclimation of plants induced anatomical, morphological and biochemical changes that result in a lowering of the temperature at which the plant is damaged by cold (Mercado et al., 1997). In plants of temperate origin this acclimation includes the reduction of the plant growth rates and the root to shoot ratio, the modification of the leaf morphology (Huner, 1985), the increase in chlorophyll and protein in the leaves (Guy, 1990), a higher content of non-structural carbohydrates in both sink and source tissues, and the alteration of photosynthetic partitioning (Farrar, 1988). Saltveit and Morris (1990) recorded that tropical and subtropical plants suffer physiological disfunction if exposed to temperature below around 10-15C. Pepper is a chilling sensitive crop cultivated in mild winter climates and in greenhouses. Mercardo et al. (1997) found that the shoot growth, the number of leaves per plant and the individual leaf area significantly decreased when plants were cultivated under suboptimal night temperatures.

Temperature Effects on Seed Germination

Seed germination is a key stage in plant physiology affecting seedling development, survival, and population dynamics. Germination starts with water uptake by the seed and ceases with the elongation of the embryonic axis from the seed coat (Bewley, 1997). Germination events and subsequent establishment are controlled by nuclear and maternal genetics, and current and maternal environments (Baskin and Baskin, 2001). Environmental controls regulate seed dormancy release, seed germination rate and percentage, and seed deterioration and mortality processes in plants. According to Baskin and Baskin, (2001) environmental factors which regulate germination include temperature, water, and oxygen for non-dormant seeds, along with light and chemical environment for dormant seed. Temperature and water are the two main driving forces affecting rate of seed germination when aeration is not restrictive for non-dormant seeds (Gummerson, 1986). For most crop species, temperature determines germination percentage and germination rate (Garcia-Huidobro et al., 1982). Germination rate usually increases linearly with increasing temperature from a minimal or base temperature (T_{min}) up to an optimum and decreases linearly to a ceiling or maximum temperature (T_{max})

(Bradford, 1990). Cardinal temperatures, the minimum (T_{min}), the maximum (ceiling temperature, T_{max}) and optimum temperature (T_{opt}), vary in most species for seed germination (Bewley and Black, 1994). Thus, seed germination occuring in a well defined temperature range for a given cultivar and germination rate is dependent upon temperature.

According to Fry (1983), temperature is a kinetic requirement for biochemical processes for plant development. Denaturation of proteins, membrane dysfunction, and interactions with water decrease germination rate linearly with temperature at the supra-optimal temperatures (temperature between T_{opt} and T_{max}) (Bradford, 2002). Possible mechanism for the decrease of germination rate may be associated with the decrease of metabolic efficiency above the optimal temperature (Thygerson et al., 2002).

According to Baskin and Baskin (2001), seeds produced by most plants at high temperatures have higher germination percentages and/or rates than those produced at low temperatures. With respect to peppers, temperature has a major influence on seed germination. Peppers have a prolonged germination period and optimum germination temperature is about 30°C. The rate of germination and emergence is markedly reduced at temperatures in the range of 15-20°C (Stummel and Bosland, 2007). Kotowski (1926) found that pepper cv. Perfection Pimento, seeds germinated more profusely between 25 to 30°C than at cooler temperatures. Bierhuizen et al. (1978) found that sweet pepper seeds show less uniform germination at temperatures greater than 30°C. Maynard and Hochmuth (1997) recommend that the optimum temperature for pepper seed germination is 29°C. The inability of germination at higher than optimal temperatures is attributed to a condition called thermoinhibition. Tomato (*Lycopersicon esculentum)* seed do not germinate at higher temperatures of 35°C, but germinate when temperature is reduced to 25 or 30°C. Several studies recorded thermoinhibition occurs in other vegetables and that temperature is the most limiting factor influencing seed germination.

Generally, water and temperature stress are interrelated. Shoots of most C_3 and C_4 plants with access to ample water supply before emergence are maintained below 45°C through evaporative cooling and if water becomes limiting, this evaporative cooling is less effective and tissue temperature rises causing heat stress (Taiz and Zeiger, 2002). Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils, because wet, bare soil is typically darker and absorbs more solar radiation than drier soils (Taiz and Zeiger, 2002).Various functions of roots of the seedling, including nutrient uptake, water uptake, assimilation of metabolites and translocation, are very sensitive to temperature.

Long-term effects of heat stress on developing seeds may include delayed germination or loss of vigor leading to low rate of emergence and seedling establishment. Carter and Vavrina (2001) reported that summer greenhouse temperatures can reach 40 to 45°C for six hours or more in the southern United States. This is far above the optimum temperature (30°C) for pepper germination and shade cloth and tray-top dressings of vermiculate or perlite to cool the germination medium may not prevent inhibited or erratic germination. They further mentioned that erratic germination causes various production schedule complications and often reduces overall stand establishment. Weaich et al. (1996) reported that in maize (*Zea mays.* L), under diurnally varying temperatures, coleoptile growth was reduced at 40°C and ceased at 45°C.

Thermal Time Model

Thermal time (degree-day or hour), the heat unit for plant development, is a well established principle for plants (Fry, 1983) and can be applied to seed germination (Garcia-Huidobro et al., 1982). The thermal time model has been used successfully to predict phenological development in crops and weedy species (Alm et al., 1991) as well as seed germination under non water-limiting conditions (Garcia-Huidobro et al., 1982). The thermal time model has been used to predict optimal planting dates for corn at water potentials above – 0.5 MPa (Gupta et al., 1988).

Effects of Temperature on Growth and Development

Plants grow by accumulating material to become larger and they develop in response to internal and external stimuli, from juvenile to mature state, from vegetative to flowering, and from active to dormant. There are two main categories of temperature effects on plant growth and development. The first is the effect of temperature and temperature fluctuations on general growth and development. The second is the effect of temperature extremes on survival. However, among various abiotic factors, temperature is the key factor which determines the adaptation of plants to different climatic zones and seasons of the year. Most annual crops can be described as being adapted to either cool or warm seasons (Hall, 2001). According to Rubatzky and Yamaguchi (1997) and Hall (1990), vegetables can be loosely classified based on their adaptation and preferences to their climatic preferences as cool or warm season crops. Accordingly, pepper is categorized as a warm season crop with temperature optima between 18 and 30°C.

Different species show differences in the genetically determined range of environmentally induced photosynthetic adaptation to temperature. Photosynthetic response to high temperature can vary significantly within a species (Reynolds et al., 1990). Temperature also plays a crucial role in the determination of sowing or planting dates of a crop species based on the seed germination and survival of the seedlings. The minimum threshold temperature for seed germination differs among crop species. At the same time, the optimum temperatures depend on the stage of development of a crop plant. Kakani and Reddy, (2007) reported that the cardinal temperature for growth and development of a crop species are process dependent.

Effect of Temperature on Reproductive Parameters

Flower production, fruit set and fruit growth and development

Compared to vegetative processes, sexual reproduction is more sensitive to elevated temperatures. Increases in incidental temperature could make plant reproductive organs more susceptible to injury, especially prior to and during early flower stage (Hall and Ziska, 2000; Kakani and Reddy, 2007; Reddy et al., 1997). The undesirable impacts of high temperature stress on plant reproduction and implications on global crop production systems have been established. Previous research has been conducted on this topic for many crop species and the attention has accelerated with the predicted rise of global temperatures (IPCC, 2001).

High temperature stress (HTS) detrimentally affects reproductive processes (flowering and seed production) of many crop species (Hall, 1992). HTS causes flower and fruit abortion, which is a yield-limiting factor in many agronomic crops. Rylski and Spigelman (1982) found considerable abortion of floral buds in pepper, grown both under field and controlled environments, when day temperatures are higher than 34°C and/or night temperatures are higher than 21°C for extended periods. According to (Alsadon et al., 2006) flowering, fruit set and maturity are sensitive to heat and directly relate to final yield. Research has shown that temperature stress (Guilioni et al., 1997), low light (Aloni et al. 1991) or limited pollination (Berjano et al., 2006) causes flower abortion. Peet et al. (1998) reported that fruit set in many crops is sensitive to high temperature which is primarily due to the adverse impact on microsporogenesis.

Aloni et al. (1991) found that heat stress during flowering affects pollen germination, pollen tube growth, fertilization and embryo development and ultimately seed development in peppers. The development of pollen grains during the final period of flower bud development specifically, 16-18 days before anthesis, is inhibited by elevated temperature, leading to pollen sterility (Erickson and Markhart, 2002). Additionally, two specific stages of pollen development, microspore mother cell meiosis and mature microspores at anthesis, are highly sensitive to elevated temperature. Abnormal pollen grains such as shrunken and empty pollens without exine (outermost layer of pollen grain) develop in peppers exposed to higher temperatures. Similar abnormalities to pollen grains exposed to high temperature were found in cotton (*Gossypium hirsutum* L.) (Salem et al., 2007), soybean (*Glycine max* L. Merr.) (Koti et al., 2005) and common beans (*Phaseolus vulgaris* L.) (Suzuki et al., 2001).

According to Erickson and Markhart (2002), the reduction of pollen viability effectively reduces fruit size and fruit set under high temperature. Furthermore, they found that the injury to the pistil is minimal. However, no injuries were found to both male (staminate) and female (pistillate) organs, when flowers were exposed to higher temperature just before anthesis.

Karni and Aloni (2002) showed that the low seed yield for pepper grown under higher temperatures is caused by low pollen viability. Heat stress during flowering resulted in significant reduction in seed yield in corn (Herrero and Johnson, 1980), tomato (Peet et al., 1998), and canola (*Brassica napus* L., Young et al., 2004).

Literature confirms that environmental stresses have a significant influence on metabolic activities in anthers preceded by the structural injury in the pollen caused by desiccation (Saini, 1997). Aloni et al. (1991) found that under high temperature conditions the translocation of assimilates from the photosynthesizing leaves to the developing flower is greatly reduced compared to at normal temperatures. Therefore, pollen from plants under high temperature conditions would contain less sucrose than control plants grown under normal temperatures. However, it was found that the sucrose accumulation was increased under high temperature treatment. This was due to the reduction in sucrose utilization in the pollen grains under high temperature leading to pollen malfunctioning. It was concluded that the higher concentration of sucrose in the pollen grains extracted from plants grown under high temperature resulted from reduction in metabolism under heat stress conditions (Aloni et al., 2001).

Pollen viability and tube length

Pollen viability has been defined as having the capacity to live, grow, germinate or develop (Lincoln et al., 1998). Viable pollen grains may not germinate (either *in vitro* or *in vivo*) if the conditions are not favorable. Pollen viability has been defined differently as; pollen grains capable of germinating on the stigma (Vaughton and Ramsey, 1991), germinating *in vitro* on a artificial media (Lindgren et al., 1995), absorbing certain stains (Mione and Anderson, 1992) or effective seed set after pollination (Dafni and Firmage, 2000; Smith-Huerta and Vasek, 1984).

According to Stone (2001), among various developmental stages in crops, viability and germination of pollen is the most heat-sensitive stage. Erickson and Markhart (2002) found that in bell pepper, development of pollen grains were inhibited by high temperature during the period of final tetrad formation to tetrad dissolution, when flower buds were 16-18 d before anthesis, resulting in pollen sterility leading to poor fruit set. Fruit set was impaired in tomato (Peet et al., 1998), groundnut (*Arachis hypogaea*) (Prasad et al., 2000), and kidney bean (*Phaseolus vulgaris*) (Prasad et al., 2002) because of high temperature effect on pollen viability and germination.

Effect of Temperature on Vegetative Parameters

High temperature either in greenhouses or field conditions limits various plant physiological, biochemical, and growth processes. Membrane disruption, gas exchange (which include photosynthesis, stomatal conductance, and transpiration), and translocation are the most commonly influenced physiological processes (Singh et al., 2007).

Photosynthesis and chlorophyll fluorescence

According to Berry and Bjorkman (1980), photosynthesis is one of the most heat sensitive processes and high temperature can cause complete inhibition of the process before other symptoms of stress are detected. Long (1991) extensively studied the effect of rising temperature on photosynthesis and several other vegetative growth parameters. In the photosynthetic apparatus, photosystem II (PS II) has been identified as the most heat labile component of the electron transport chain and any inhibition of its activity may result in reduction or complete inhibition of photosynthesis (Havaux, 1993). Jifon et al. (2004) used chlorophyll fluorescence (as an indicator of membrane dependent PS II quantum efficiency) to test the heat tolerance in four genotypes of Habanero pepper. Therefore, PS II parameters could be used as a marker in screening genotype for abiotic stress tolerance.

Havaux and Tardy (1996) found that the thermal stability of the photosynthetic system differed markedly between plant species. Thus, photosynthetic rates were considered as a good marker of temperature tolerance in most agronomic crops (Reynolds et al., 2001).

Chlorophyll fluorescence is a subtle reflection of primary reactions of photosynthesis. The complex relationships between fluorescence kinetics and photosynthesis help our understanding of photosynthetic biophysical processes. According to Srinivasan et al. (1996) this technique is valuable as a non-invasive tool in many eco-physiological studies, and many researchers have used it to assess plant response to abiotic stresses (water stress, heat stress, salt stress, and chilling).

Membrane disruption and cell membrane thermostability

The stability of various cellular membranes is vital both during heat and cold (chilling and freezing) stress. Raison et al.(1982) found that at high temperature there exist an excessive fluidity of membrane lipids and it is correlated with loss of physiological function. At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of protein within the aqueous phase of membranes. High temperature therefore modifies membrane composition and structure and can cause leakage of ions. Membrane disruption also causes the inhibition of photosynthesis and respiration which are dependent on the activity of membrane associated carriers and enzymes (Taiz and Zeiger, 2002). According to Hall (1990), cell membranes may be disrupted due to temperature stress. Sharkey (2005) noted that the inhibition of photosynthesis at high temperature stress is due to increased thylakoid membrane ionic conductance and deactivation of ribulose-1, 5–bisphosphate carboxylase/oxigenase (Rubisco). Sullivan (1972) developed a test to measure heat tolerance using cellular membrane thermostability (CMT) through measuring the amount of electrolyte leakage from leaf disc submerged in demonized water after a heat treatment. This protocol was later modified to accommodate various other crops and to measure the level of heat tolerance. Bibi et al. (2003) concluded that CMT is the most sensitive technique for measuring temperature tolerance in cotton under field conditions.

Screening for Heat and Cold Tolerance

Increase of seasonal temperature in both the short and long-term could cause heat stress and affects many aspects of crop growth and development, thereby reducing the final yield. Moreover, different physiological mechanisms contribute to heat tolerance mechanisms. Identification and selection of suitable genotypes/cultivars is a primary requirement in any crop improvement program (Singh et al., 2007; Wahid et al., 2007). Due to changes projected in future climate, particularly in temperatures, the necessity of research on crop improvement for heat stress has been accelerated in recent years (Hall and Ziska, 2000; Cheikh et al., 2000; Mittler, 2007). Many scientists have developed reliable techniques to screen the available genotypes/cultivars for various ecological, physiological, morphological and reproductive characters/traits to support crop breeding programs including pepper (Reddy and Kakani et al., 2007; Salem et al., 2007; Singh et al., 2007).

Hall (1990) emphasized the importance of identifying the most sensitive stages of plant development and plant processes, responsible for the reduction of crop yield, which will facilitate the development of efficient techniques for screening germplasm for heat tolerance. Although both the male and female gametophytes are sensitive to high temperature, it is found that male gametophyte (pollen grains) show a higher vulnerability. Under extreme temperatures, pollen germination and pollen tube growth is reduced resulting in lack of fruit formation. According to Hormaza and Herrero (1996) male gametophyte selection has been widely considered an early genotype selection strategy in plant breeding programs. Reddy and Kakani (2007) studied the response of *in vitro* pollen germination and pollen tube growth of different pepper genotypes to different temperatures as a means of screening those genotypes tolerant to high and low temperatures.

Singh et al. (2008) developed a screening tool for both cold and heat tolerance in winter-grown canola cultivars using pollen related attributes, namely percentage of pollen viability, *in vitro* pollen germination and pollen tube length. They concluded that pollen-based parameters could be used to screen canola cultivars for cold and heat tolerance. Similarly, *in vitro* pollen germination and pollen tube length have been utilized as a selection tool for temperature tolerance in many other important crops such as cotton (Kakani et al., 2005; Liu et al., 2006), corn/maize (Herrero and Johnson, 1980), soybean, and groundnut (Prasad et al., 2000).

CHAPTER III

SCREENING ORNAMENTAL PEPPER CULTIVARS FOR COLD AND HEAT TOLERANCE BY *IN VITRO* POLLEN GERMINATION, POLLEN TUBE LENGTH AND PHYSIOLOGICAL PARAMETERS

Abstract

Temperature affects both reproductive potential, aesthetic and commercial value of ornamental peppers (*Capsicum annuum*). An experiment was conducted to study the influence of temperatures from10 to 45°C at 5°C intervals, in 12 cultivars of peppers on *in vitro* pollen germination (PG) and pollen tube length (PTL) on artificial pollen media. Further, three physiological parameters, cell membrane thermostability (CMT), canopy temperature depression (CTD), and chlorophyll stability index (CSI), were used to distinguish genotypic differences among the cultivars. From the modified bilinear temperature-pollen germination and tube length response functions, cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for PG and PTL, and maximum PG and PTL were estimated. Cultivars varied significantly for PG, PTL, and all three physiological parameters. Maximum PG and PTL varied from 51 to 92% and 405 to 1348 µm, respectively, with a mean of 80% and 884 µm. The mean values for cardinal temperatures were 11.8, 26.8 and 41.2°C for PG and 12.0, 28.5 and 40.9°C for PTL for T_{min}, T_{opt}, and T_{max}, respectively. Cumulative temperature response index, CTRI (unit less), of each cultivar calculated as the sum of 12 individual temperature responses derived from pollen

viability, maximum PG, maximum PTL, T_{min}, T_{opt}, and T_{max} for PG and PTL, CMT, CTD and CSI were used to classify cultivars for 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. Cell membrane thermostability and CSI showed a significant, but weak linear correlation with pollen-based parameters indicating that screening based on pollen-based parameters is an accurate measurement for reproductive heat- and cold-tolerance. The identified heat- and cold-tolerant cultivars are potential candidates in breeding programs to develop new ornamental pepper genotypes for high and low temperature environments and also selecting cultivars for a niche environment.

Introduction

Ornamental peppers (*Capsicum annuum* L.) are widely used as a potted flowering or bedding plants because of their morphologically diverse characteristics. This is one of the summer annuals grown primarily for the combination of attractive fruits and foliage. The use of potted pepper plants in florist shops gained large scale acceptance in Europe, and is gaining popularity in the United States (Armitage and Hamilton, 1987). Most ornamental pepper cultivars produce pungent fruit. However, cultivars such as 'Chilly Chili' and 'Medusa' have become popular due to their non-pungent fruit and increased concern for product safety and child safety issues (Stummel and Bosland, 2006).

Ideal cultivation conditions for ornamental peppers are similar to typical vegetable pepper production with the crop requiring high radiation and minimum day-time temperatures between 18 to 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 pepper plants as they mature. Yellowing and dropping of leaves are caused by low temperatures, low light, and insufficient moisture or nutrients (Stummel and Bosland, 2006). In addition, pollen fertility and fruit quality are also affected by low night temperature (Pollowick and Sawhhnew, 2006). Young et al. (2004) found that high temperature during flowering or during pollen release and germination affect male reproductive processes (microsporogenesis) thus resulting in lower set and smaller fruit. Both low and high temperature extremes are detrimental for both reproductive and vegetative growth of pepper. Reproduction processes have been shown to be more sensitive to temperature stress than vegetative processes in many crops including peppers (Reddy and Kakani, 2007).

With global climate change, crop production across the world will be met with challenges. According to Taylor and Hepler (1997), the increase in earth's near surface temperature associated with natural and human-induced changes in greenhouse gases is an important component of global climate change and may impact productivity of crops across the globe. This has been verified from both field and controlled-environment studies and modeling exercises (Reddy et al., 2000). Since the Industrial Revolution, global surface temperature has increased by more than 0.6°C. Because of anthropogenic climate-forcing agents (Hansen and Sato, 2004) and future increases in greenhouse gases air temperatures are projected to increase anywhere between 1.5 to 11°C by the year 2100. It has been shown that this projected variation in climate will have profound impacts on crop production (Stainforth et al., 2005). Lobell and Asner (2003) have studied the relationship between climate variation and crop production on corn (*Zea mays* L.) and soybean [*Glycine max* L. Merr.] grown in the United States between the years 1982 to 1998 and stated that for every 1°C increase in temperature, there was on average a 17% decrease in yield in both crops. At the same time, possible extreme temperatures during the flowering period will have even more severe effects on crop yield than the general increase in temperature conditions over the season in long term (Hall, 1992). Studies have reported that fruit set in many horticultural crops are sensitive to supra-optimal temperatures namely, bell pepper (*Capsicum annuum* L.) (Erickson and Markhart, 2001), bean (*Phaseolus vulgaris* L.) (Porch and Jahn, 2001), and tomato (*Solanum lycopersicum* L.) (Sato et al., 2002).

Compared to vegetative organs, reproductive organs of plants are much more vulnerable to temperature fluctuations. Fruit set in peppers has been reported to be affected by temperature stress mostly prior to and during early flower stage (Erickson and Markhart, 2002). A higher capacity to survive under extreme temperature conditions during plant reproduction as well as various processes leading to fruit yield such as pollen grain development, pollen germination, pollen tube growth, fertilization, and embryo development are highly beneficial to plants. Studies have shown that these processes in many crops including peppers are affected by high temperature (Erickson and Markhart, 2002; Reddy and Kakani, 2007). The pollen grain grains are the structures that transfer the male gamete with male genetic information to the female element of a flower in higher plants. According to Taylor and Hepler (1997), these small structures are developed in the anthers and contain genetic information of both saprophyte and gametophyte. In the process of microsporogenesis, where pollen grains are developed in two stages namely microspore mother cell meiosis and mature microspores at anthesis, is more susceptible to high temperature stress (Erickson and Markhart, 2002). Pollen grains behave as independent functional units once they are released from anthers during anthesis. Aloni et al. (2001) found that any temperature exceeding 32°C, causes reduction in pollen germination and pollen tube growth, thus leading to lower fruit set in peppers.

Physiological parameters have been used extensively to investigate temperature stress in a range of crops (Singh et al., 2007). Cell membrane thermostability (CMT) has been used to measure high temperature tolerance of crops because stress adaptation can be associated with viability of cells (Gusta and Chen, 1987). Membrane thermostability usually is determined by measuring conductivity of electrolytes that leak from leaves subjected to high temperature (Saadalla et al., 1990). Therefore, CMT has been widely used to study temperature tolerance by various researchers in crops including wheat

(*Triticum aestivum*) (Balota et al., 1993), cabbage (*Brassica oleracea* L.) (Nyarko et al., 2008), cowpea (*Vigna unguiculata* L. Walp.) (Balota et al., 1993; Nyarko et al., 2008; Singh et al., 2008a).

Canopy temperature depression (CTD), the difference between air and canopy/foliage temperature (Balota et al., 1993), has also been used to study temperature tolerance in certain crops such as wheat. Reynolds et al. (1998) indicated that CTD is a good criterion for screening heat stress tolerance in wheat. In addition, it has been used as an indicator for environmental stresses such as moisture and heat (Balota et al., 2007) and drought (Feng et al., 2009).

Chlorophyll stability index (CSI), the loss in the amount of Chlorophyll of a heated sample in relation to its unheated sample is another physiological parameter that gained importance in studying drought and high temperature tolerance in plants (Sairam et al., 2008). This was used by Sivasubramaniam (1992) as a method of determining drought hardiness in *Acacia* species. Sairam et al. (2008) have used CSI to evaluate both drought and temperature stress in wheat in relation to increased antioxidant activity.

Many studies on temperature tolerance, predict the correlation between various physiological and reproductive parameters to determine temperature tolerance in plants. Kakani et al. (2002) found that for groundnut, there exists no correlation between pollen germination and CMT under high temperature conditions.

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 ability to sustain with high metabolic and physiological activity under stressful environments like high and low temperatures is an important tolerance trait for incorporation into newer ornamental pepper cultivars. It is hypothesized that there exists variability in temperature tolerance characteristics among ornamental pepper cultivars and both pollen-based (reproductive) and physiological-based (vegetative) parameters can be used as screening tools and assume that these parameters contribute equally to temperature tolerance.

The objectives of this study were to (i) quantify the responses of *in vitro* pollen germination (PG) and pollen tube growth (PTL) of ornamental pepper cultivars to a range of temperature, (ii) determine cultivar-specific cardinal temperatures for both PG and PTL based on pollen parameters, (iii) quantify the cultivar stability to heat treatments using three physiological parameters; CMT (cell membrane thermostability), CSI (chlorophyll stability index), and CTD (canopy temperature depression), (iv) classify cultivars based on their level of tolerance to high and low temperatures using pollen-based cardinal temperatures and physiological-based (CMT, CSI and CTD) parameters, and (v) determine whether the observed variation among cultivars in pollen germination responses to temperature are related to physiological parameters.

Materials and Methods

Plant Husbandry

Twelve commercially available cultivars of ornamental peppers were used for this study (Table 3.1). Seeds of these cultivars were established in cell trays within germination chambers on 2 June 2008. Seedlings emerged between 10 and 15 days after sowing and were thinned to one per cell. Three weeks after establishment, seedlings were transplanted to 1 L plastic pots (15.3 cm dia., 10 cm h.) These pots were filled with commercial growing medium, Pro Mix BX W/Mycorise (Premier Horticulture Inc., Quakertown, Penn.). Thirty healthy plants of each cultivar were arranged in rows oriented east to west and spaced 1 m apart inside a greenhouse at R. R. Foil Plant Science Research Center, Mississippi State University, Mississippi State (33° 28´N, 88° 47´W) with day/night temperatures of 25/22°C. Plants were hand-irrigated and fertilized with constant liquid feed of Peter's 20-10-20 (N, P_2O_5 and K_2O) (Peters Peat Lite, Scotts, Marysville, Ohio) at 200 mg $N{\cdot}L^{-1}$ as needed.

Physiological Measurements

Canopy temperature depression (CTD)

Canopy temperature depression is the difference between the air temperature (Ta) and foliage temperature (Tc) and it is positive when the canopy is cooler than the air. Canopy temperature depression measurements were made on 12 pepper cultivars grown in the greenhouse three weeks after transplanting. Leaf temperatures of five fully expanded leaves from each cultivar and the respective air temperature was measured between 12.00 and 13.00 h (cloudless, bright days) using a handheld infrared thermometer (Model OS533E-OMEGASCOPE, OMEGA Engineering, Inc. Stamford, CT, USA). The apparatus was held 1 m above the foliage and forty measurements were taken from randomly selected plants of each cultivar once in two days for two weeks. Cell membrane thermostability was calculated following equation [Eq. 1]), where CTD is canopy temperature depression, and Ta and Tc refers to air and canopy temperature of the target.

$$CTD = Ta - Tc \qquad [1]$$

Cell membrane thermostability (CMT)

The leaf cell membrane thermostability (CMT) in ornamental pepper cultivars was assessed according to the procedure described by Martineau et al. (1979) with minor changes. A sample for assay consists of a paired set, namely control (C) and treatment (T) set of 10 leaf disks each 1.3 cm^2. The disks were cut from five fully expanded 3^{rd} or 4^{th} leaves from the top of the stem axis from each cultivar. Samples were replicated three times each. Prior to assay, the paired set of leaf disks were placed in two separate test tubes and washed thoroughly with four exchanges of de-ionized water, 10 mL each time, to remove electrolytes adhering to the cut surface of the leaf discs. After the final wash, both sets of test tubes were filled with 10 mL de-ionized water and sealed with aluminum foil to avoid evaporation. The T set of the test tubes were incubated for 20 minutes at 50°C in a temperature controlled water bath, while the C set of test tubes remained at room temperature (approximately 20°C). Both sets of test tubes were then incubated at 4°C (kept in a refrigerator) for 24 h. Initial conductance reading of both sets (CEC 1 and TEC 1) was made using an electrical conductivity meter after bringing test tubes to room temperature. Tubes were then sealed again with aluminum foil and autoclaved at 120°C and 0.15 MPa for 20 min. to completely kill the leaf tissue. Autoclaved tubes were cooled to room temperature, content mixed thoroughly and final conductance (CEC 2 and TEC 2) measurements were recorded. The CMT was calculated using the following equation

[Eq. 2]), where, TEC and CEC are a measure of conductance in treated and control test tubes, respectively at initial (CEC 1 and TEC 1) and final (CEC 2 and TEC 2) conductance measurements.

$$\text{CMT (\%)} = \frac{1-(\text{TEC1}/\text{TEC2})}{1-(\text{CEC1}/\text{CEC2})} \times 100 \qquad [2]$$

Chlorophyll stability index (CSI)

Two sets of leaf samples were collected from five recently fully expanded leaves for each cultivar. Five leaf discs of 2.0 cm^2 from each sample were collected and placed in a vial with 4 mL of dimethyl sulphoxide for chlorophyll (Chl) extraction. Three replicate leaves from three different plants were sampled from each cultivar. The sample vials were incubated at room temperature in the dark for 24 h to allow complete extraction of chlorophyll pigments into the solution. Absorbance of the extract was measured using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA) at 470, 648 and 662 nm to calculate concentrations of Chl a and Chl b (Chappelle et al., 1992). Total leaf chlorophyll was estimated by summing Chl a and Chl b values (Lichtenthaler, 1987).

Another set of leaf discs of 2.0 cm^2 were collected from five recently fully expanded leaves using another leaf sample and incubated for 20 minutes at 46°C in a temperature controlled water bath. Using the same protocol, the chlorophyll content was measured in these treated samples. The CSI was calculated by the method of Murty and Majumdar (1962) using the following equation [Eq 3].

$$\text{CSI (\%)} = \frac{\text{Total chlorophyl content (heated)}}{\text{Total chlorophyl content (control)}} \times 100 \qquad [3]$$

Reproductive Measurements

Preparation of pollen growth media

The pollen medium for highest pollen germination for 12 cultivars used was identified at 24°C temperature through modification of the medium previously used for bell pepper by Karni and Aloni (2002) and later modified by Reddy and Kakani (2007) for vegetable peppers. This initial medium consists of 100 g sucrose ($C_{12}H_{22}O_{11}$), 500 mg of calcium nitrate [$Ca\ (NO_3)_2.4H_2O$], 120 mg of Magnesium Sulphate ($MgSO_4$), 100 mg of Potassium Nitrate (KNO_3), and 120 mg of Boric Acid (H_3BO_3) dissolved in 1000 ml of de-ionized water 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 Petri dishes for each cultivar in each temperature treatment and allowed to cool for 15 minutes for agar solidification.

Pollen collection, culture, temperature treatments and viability

Twenty to thirty flowers at anthesis were randomly collected from each cultivar between 09:00 and 10:00 h. Pollen grains were collected in a Petri dish by tapping the flowers. Pollen grains were distributed uniformly onto the solidified and modified germination medium using a tiny, clean bristle paint brush. Petri dishes with medium were kept in the incubator set at treatment temperatures for half an hour prior to pollen distribution. The Petri dishes were then covered and incubated (Precision Instruments, New York, USA) at respective temperature treatments from 10 to 45°C at 5°C intervals. Each Petri dish per cultivar and temperature treatment was considered as a replicate.

Pollen viability was tested using 2.5% concentration of 2, 3, 5-triphenyl tetrazolium chloride (TTC) in de-ionized water. Pollen grains were dusted gently using the bristle paint brush on a microscopic glass slide containing a drop of staining solution according to Aslam et al. (1964). Then the slides were kept in the dark at room temperature for three hours and the numbers the total as well as TTC stained pollen grains were counted at five microscopic fields having more than 100 pollen grains per field using Nikon SMZ 800 microscope (Nikon Instruments, Kanagawa, Japan). Average pollen viability was calculated as a percentage of viable pollen grains to total pollen grains.

Pollen germination and pollen tube length

After 24 h of incubation, the Petri dishes were removed from the incubators, one at a time, and a thin layer of pollen fixing solution comprising 3% glacial acetic acid, 5% formaldehyde, 20% glycerin and 72% water (Feng et al., 2000) was sprayed evenly and the Petri dishes were immediately stored in a refrigerator until measurements of PG and PTL were recorded. Total as well as numbers pollen grains germinated were counted using a Nikon SMZ 800 microscope with a magnification of 6.3 x. Ten fields per replication were counted for percent pollen germination. When counting the pollen grains, 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 and expressed as a percentage. The pollen tube lengths of 30 pollen grains selected randomly from each Petri dish was measured with an ocular micrometer fitted to the eye piece of the microscope after 24 h of incubation.

Curve Fitting Protocol, Cardinal Temperature Determination and Analysis

The recorded PG and PTL measurements after 24 h were analyzed by linear and non-linear regression models commonly employed 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 ANOVA procedure in SAS (SAS Institute Inc., 2004). 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 PTLs were modified bilinear models. Accordingly, the cardinal temperatures (T_{min}, T_{opt}, and T_{max}) were calculated from the fitted equations for all the cultivars. The non-linear regression procedure PROC NLIN (SAS Institute Inc., Cary, NC) 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 (equation [4]) (Kakani et al., 2002), where T is actual treatment temperature and a, b1, and b_2 are equation constants. T_{min} and T_{max} were calculated using equations ([Eq. 5] and [Eq. 6]).

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

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

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

Cumulative Temperature Response Index (CTRI)

Initially, individual temperature response index (ISRI) of each parameter for heat tolerance was calculated as the value of a cultivar (P_t) divided by maximum value (P_h) observed over all the cultivars ([Eq. 7]) while for ISRI for cold tolerance was determined by dividing the minimum value (P_l) observed over all the cultivars by the value of a cultivar (P_t) ([Eq. 7]) as described by Reddy and Kakani (2007). Then, cumulative temperature response index (CTRI) for each cultivar ([Eq. 9] and [Eq.10]) was calculated as the sum of twelve ISR's derived from maximum pollen viability (PV), maximum pollen germination (PG), maximum pollen tube length (PTL), 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 [> minimum CTRI + 2.5 standard deviation (stdev)], intermediate (> minimum CTRI +2.5 stdev and < minimum CTRI + 1.5 stdev), and sensitive (> minimum CTRI to < minimum CTRI + 1.5 stdev). Similarly, Cultivars were classified based on cold CTRI of all parameters, as cold tolerant [> minimum CTRI + 3.0 standard deviation (stdev)], moderately cold tolerant [< minimum CTRI + 3.0 standard deviation-[> minimum CTRI + 2.0 standard deviation], moderately cold sensitive (> minimum CTRI +2.0 stdev and < minimum CTRI + 1.0 stdev), and cold sensitive (< minimum CTRI to, minimum CTRI + 1.0 stdev).

$$\mathrm{ISR} = \mathrm{P_t}/\mathrm{P_h} \qquad [7]$$

$$\mathrm{ISR} = \mathrm{P_l}/\mathrm{P_t} \qquad [8]$$

$$\text{Heat CTRI} = \left(\frac{\mathrm{PV\%_t}}{\mathrm{PV\%_h}} + \frac{\mathrm{PG\%_t}}{\mathrm{PG\%_h}} + \frac{\mathrm{PTL_t}}{\mathrm{PTL_h}} + \frac{\mathrm{PG_{min_t}}}{\mathrm{PG_{min_h}}} + \frac{\mathrm{PG_{opt_t}}}{\mathrm{PG_{opt_h}}} + \frac{\mathrm{PG_{max_t}}}{\mathrm{PG_{max_h}}} + \frac{\mathrm{PTL_{min_t}}}{\mathrm{PTL_{min_h}}} + \frac{\mathrm{PTL_{opt_t}}}{\mathrm{PTL_{opt_h}}} + \frac{\mathrm{PTL_{max_t}}}{\mathrm{PTL_{max_h}}} + \frac{\mathrm{CMT_t}}{\mathrm{CMT_h}} + \frac{\mathrm{CTD_t}}{\mathrm{CTD_h}} + \left(\frac{\mathrm{CSI_t}}{\mathrm{CSI_h}}\right)^{-1} \right) \qquad [9]$$

$$\text{Cold CTRI} = \left(\frac{\mathrm{PV\%_l}}{\mathrm{PV\%_t}} + \frac{\mathrm{PG\%_l}}{\mathrm{PG\%_t}} + \frac{\mathrm{PTL_l}}{\mathrm{PTL_t}} + \frac{\mathrm{PG_{min_l}}}{\mathrm{PG_{min_t}}} + \frac{\mathrm{PG_{opt_l}}}{\mathrm{PG_{opt_t}}} + \frac{\mathrm{PG_{max_l}}}{\mathrm{PG_{max_h}}} + \frac{\mathrm{PTL_{min_l}}}{\mathrm{PTL_{min_t}}} + \frac{\mathrm{PTL_{opt_l}}}{\mathrm{PTL_{opt_t}}} + \frac{\mathrm{PTL_{max_l}}}{\mathrm{PTL_{max_t}}} + \frac{\mathrm{CMT_l}}{\mathrm{CMT_t}} + \frac{\mathrm{CSI_l}}{\mathrm{CSI_t}} + \left(\frac{\mathrm{CTD_l}}{\mathrm{CTD_t}}\right)^{-1} \right) \qquad [10]$$

Results and Discussion

Greenhouse Environmental Conditions

All pepper cultivars were grown under ideal growing condition inside a greenhouse with optimum water and nutrient conditions with a mean temperature of 26 ± 3°C and on most days photosynthetically active radiation, measured with line quantum sensor (Instrument model no., LiCOR Inc., Lincoln, NE) exceeding over 1160 µmol m^{-2} s^{-1}. The morphological characteristics of 12 ornamental pepper cultivars used in the study are summarized in Table 3.1.

Table 3.1 Morphological characteristics of ornamental pepper cultivars used in the study

Cultivar	Crop maturation (d)	Plant attributes		Leaf color	Fruit		
		Plant habit [Z]	Width (cm)		Color	Shape and size [Y]	Heat [X] rating
‘Black Pearl’	90	Medium, bushy, upright and well branched	30-41	Greenish to glossy black	Shiny black to dark red	Rounded Small	Hot
‘Calico’	80	Short/compact and rounded plants. Uniform plants	36-41	Strongly variegated (purple, green and cream)	Glossy black	Round and pointed Small	Hot
‘Chilly Chili’	90	Short/Compact	33-35	Greenish	Greenish yellow to orange to dark red	Tapered Small to medium	Not heat mild
‘Explosive Ember’	85	Short to medium and compact	30-38	Dark purple to green	Deep purple to red	Tapered and oblong Small	Very hot and edible
‘Medusa’	85	Dwarf/compact and well branched	10-15	Dark green	Ivory to yellow to orange to bright red at maturity	Narrow, twisted and cone shape Medium	Not heat mild
‘Purple Flash’	85	Medium/compact and mounded. Uniform plants.	48-53	Black with flashes of bright purple	Glossy black fruit	Round Small	Hot

Table 3.1 Continued. Morphological characteristics of ornamental pepper cultivars used in the study

Cultivar	Crop maturation (d)	Plant attributes		Leaf color	Fruit		
		Plant habit [Z]	Width (cm)		Color	Shape and size [Y]	Heat [X] rating
'Red Missile'	80	Short/compact and mounded upright	15-20	Dark green	Cream/yellow to orange to bright red	Tapered Small	Hot
'Salsa Yellow'	75	Short/compact	40-45	Small dark green	Green to yellow	Tapered and upright Small	Hot
'Sangria'	80	Short, mounded and upright	41-46	Green	Bright purple to orange to red	Little longer conical Medium	Mild and non pungent
'Thai Hot'	85	Medium/ compact and mounded	30-40	Green	Green to orange to red	Slender and short Small	Very hot and edible
'Treasures Red'	60	Short/compact	20-25	Dark green and tiny leaves	White to yellow to orange to red	Short and conical Small	Mild and edible
'Variegata'	72	Tall	40-45	Variegated (white, green and purple)	Deep purple to bright red	Tapered Small	Hot

[Z] Dwarf=15-20 cm, Short=20-30 cm, Medium=30-45 cm, Tall =45–90 cm
[Y] Small = Less than 4 cm, Medium = between 4–7, Long = above 7 cm
[X] Very hot = above 25,000 of scoville rating, Hot = between 10,000-25,000 scoville rating, Mild = below 10,000 scoville rating.

Pollen Viability

Pollen viability varied significantly among 12 cultivars of pepper. Percentage pollen viability (PV %) ranged from 56% in 'Salsa Yellow' to 91% in 'Thai Hot', with an average of 80% (Table 3.2). Similar variability was observed in canola cultivars growing under field conditions (Singh et al., 2007).

Pollen Germination Responses to Temperature Treatments

The maximum PG percentage at optimum temperature varied significantly among pepper cultivars, 51% in 'Salsa Yellow' to 91% in 'Thai Hot', with a mean of 77.5% (Table 3.2). The PG_{max} found in the study is comparable with PG_{max} values reported for vegetable pepper species (59 to 95% Reddy and Kakani, 2007) and 68% for green-house-grown bell pepper var. California Wonder (Kafizadeh et al., 2008). Similar studies in other plant species found varying PG_{max} values such as 44% in cotton (Kakani et al., 2005), 56% in groundnut (Kakani et al., 2002), 54% in corn (Geetha et al., 2004), 80% in soybean (Salem, 2007), and 75% in tomatoes (Weaver, 1989).

In all cultivars, temperature above and below PG_{opt} caused a linear reduction in the percentage of PG. Therefore, the modified bilinear equation with a well-defined optimum, provided the best fit for the PG responses to temperature (Fig. 3.1A). 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 in 'Purple Flash' to 12.17°C in 'Variegata' with the mean value of 11.80°C. Significant differences were also observed both for T_{opt} and T_{max} with the means of 26.85

Table 3.2. Pollen viability (PV), maximum pollen germination percentage (PG_{max}), modified bilinear equation constants (a, b_1, b_2), regression coefficients (R^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for PG of 12 ornamental pepper cultivars.

Cultivar	PV (%)	PG_{max} (%)	Equation constants			R^2	Cardinal temperatures (°C)		
			a	b_1	b_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	-0.427	-5.78	0.90	11.84	27.65	41.29
'Chilly Chili'	90.59	90.20	90.20	-0.645	-6.21	0.90	11.86	28.08	41.24
'Explosive Ember'	88.59	87.70	87.70	-0.626	-6.02	0.92	11.87	28.12	41.31
'Medusa'	89.21	92.44	92.44	-0.822	-6.37	0.92	11.92	28.58	41.43
'Purple Flash'	78.16	79.62	79.62	-0.436	-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	-0.823	-6.19	0.92	11.88	28.6	41.40
'Treasures Red'	89.88	91.39	91.39	-0.680	-6.26	0.92	11.74	28.09	41.24
'Variegata'	77.19	78.25	78.25	-0.508	-5.49	0.92	12.17	27.86	40.90
Mean	79.98	79.00	-	-	-	0.92	11.80	26.84	41.19
LSD	4.79***	2.37***	-	-	-		0.50***	0.51***	0.66***

*** significant at the 0.001 probability level. The values without an asterisk are not significant at the 0.05 probability level; the numbers without asterisks are not significant.

Table 3.3. 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 ($T_{min,}$ $T_{opt,}$ T_{max}) (ºC) of both PG, and PTL, cell membrane thermostability (CMT, %), canopy temperature depression (CTD, ºC) and chlorophyll stability index (CSI, %) of 12 ornamental pepper cultivars.

	PV	PG_{max}	PGT_{min}	PGT_{opt}	PGT_{max}	PTL_{max}	$PTLT_{min}$	$PTLT_{opt}$	$PTLT_{ma}$	CMT	CSI
PG_{max}	0.98***										
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

*, *** significant at the 0.05 and 0.001 probability levels, respectively. The values without an asterisk are not significant at the 0.05 probability level; the numbers without asterisks are not significant.

and 41.20°C, respectively. According to Kakani and Reddy (2007), the cardinal temperatures related to growth and development of a crop species vary depending on the process similar to our findings of variability in cardinal temperatures of PG and PTL in pepper cultivars.

Pollen Tube Length Responses to Temperature Treatments

Analogous to PG, ornamental pepper cultivars significant, but varied PTL at different temperature treatments (Table 3.4). The variability for PTL in response to temperature treatments of three cultivars is illustrated in Fig. 3.2B. Similar to PG, the modified bilinear model best described the response of PTL to temperature treatments of all cultivars (R^2 = 0.94 and least root mean square deviation). Comparable to this study, *in vitro* PG and PTL to temperature have also been reported and quantified using regression models in other crop species such as canola (*Brassica napus* L.) (Singh et al., 2008b), 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 maximum PTL ranged from 478 in 'Black Pearl' to 1348 µm in 'Thai Hot' with a mean of 883 µm (Table 3.4). The mean value of PTL reported in this study was comparable to previous study using the same protocol on vegetable pepper species (737 µm - Reddy and Kakani, 2007; 550 µm -Kafizadeh et al.,2008). Similarly, the range of the PTL observed on an artificial pollen germination media in other studies for several other crops are similar to the observed results such as 410 µm - 1400 µm in cotton (Kakani et al., 2005), 198 to 357 in apricot (*Prunus armeniaca*), 209 to 335 in sweet cherry (*Prunus avium* L.), 174 µm to 188 µm in sour cherry (*Prunus cerasus* L.)

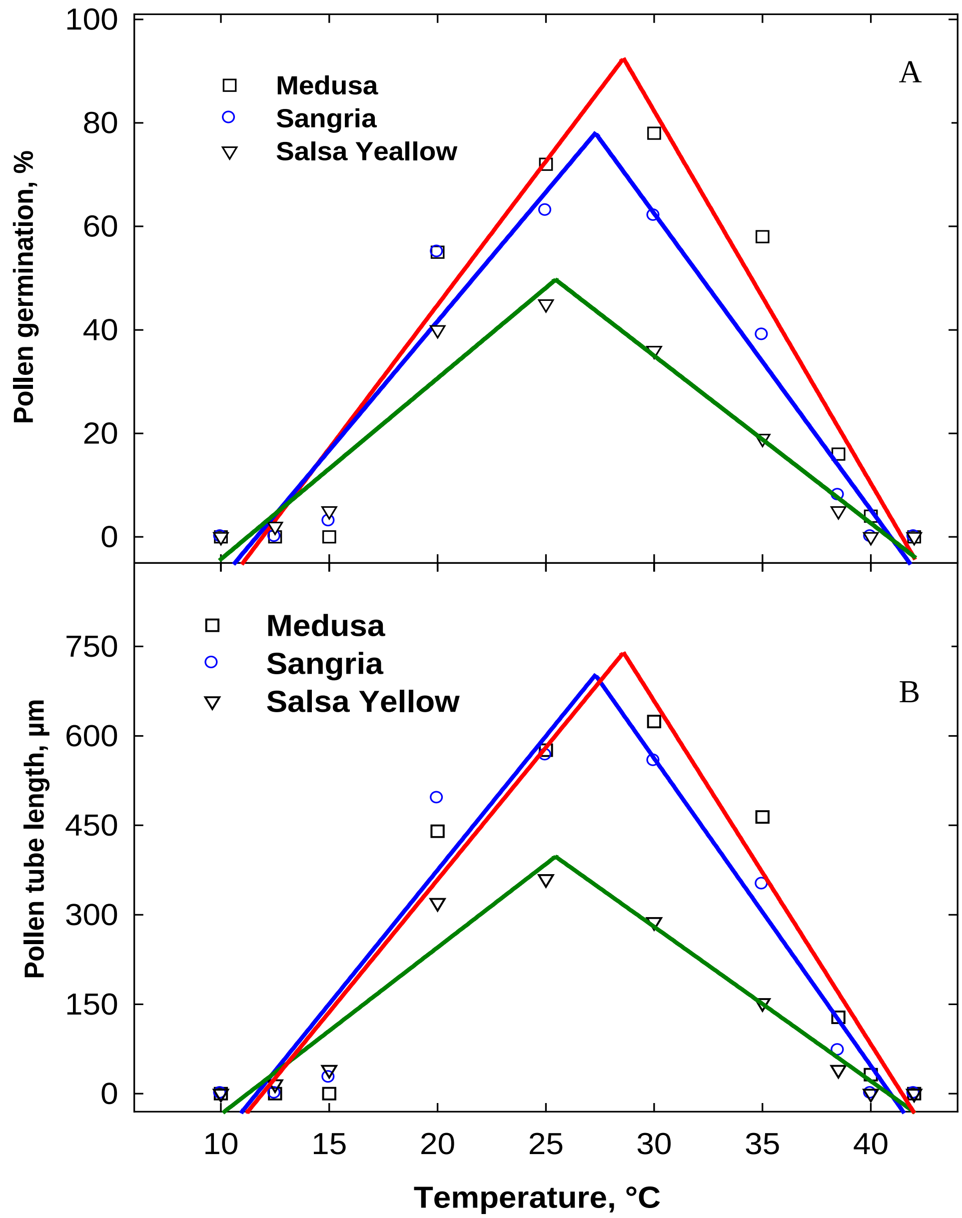

Figure 3.1 *In vitro* pollen germination (A) and pollen tube length (B) in responses to temperature (symbols) and their fitted lines derived from the modified bilinear equations, respectively, of three pepper cultivars (Medusa, Sangria and Salsa Yellow). The symbols are observed germinations percentages 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 three pepper cultivars are presented.

Table 3.4. Maximum pollen tube length (PTL_{max}), modified bilinear equation constants (a, b_1, b_2), regression coefficients (R^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for PTL of 12 ornamental pepper cultivars

Cultivar	PTL_{max} (µm)	Equation constants a	b_1	b_2	R^2	Cardinal temperatures (°C) T_{min}	T_{opt}	T_{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	1172.7	-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	884	-	-	-	0.92	12.00	28.52	40.94
LSD	3.06***	-	-	-		0.82**	1.38***	1.47

, * significant at the 0.01 and 0.001 and probability levels, respectively. The values without an asterisk are not significant at the 0.05 probability level; the numbers without asterisks are not significant.

(Bolat and Pirlak, 199), and 450 µm -1450 µm in groundnut (Kakani et al., 2002). The cardinal temperature for PTL differed significantly among cultivars (Table 3.4). The mean values of T_{max}, T_{opt}, and T_{min} were 40.9, 28.5, and 12.0°C, respectively. The optimum temperature value ranged from 23.8 in ‛Salsa Yellow to 33.5°C in ‛Sangria'.

The observed differences of the pollen germination and pollen tube lengths observed in this study are due to inherent differences among cultivars and their ability to respond to temperature. Therefore, changes in the ambient temperature conditions during pollen grain release will impact pollen germination and subsequent pollen tube growth, and fertilization process among different pepper cultivars. These differences in tolerance mechanisms will have an impact on number of fruit set and quality of the fruit and thus affecting aesthetic and/or marketable value of ornamental pepper cultivars.

Significant correlation was observed between maximum PG and maximum PTL (Table 3.3). Among the cardinal temperatures for PTL, T_{max} exhibited no significant variation, T_{min} and T_{opt} had a significant variation among the ornamental pepper cultivars (Table 3.3). The PTL T_{min} and PTL T_{max} also varied from 10.95 to 12.26°C and 39.43 to 41.62°C, respectively. PTL_{opt} was correlated with PG_{max}. Pollen germination and pollen tube growth are two parameters which prove the capability of pollen grains to perform their function of delivering sperm cells to the embryo.

Physiological Parameters and their Correlation with Pollen-based Parameters

Cell membrane thermostability

Ornamental pepper cultivars differed significantly for CMT (Table 3.5), with minimum value of 44% in 'Calico' and maximum value of 80% in 'Treasures Red'

(Table 3.5). Kuo et al. (1993) found a range of CMT values for sweet potato (*Ipomoea batatas L.*) for three growing seasons 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. Nyarko et al. (2008) found no significant variation of CMT for ten lines of cabbage (*Brassica oleracea* L.) However, Martineau et al. (1979) found large plant-to-plant variation in CMT measurements in four soybean cultivars. Among pollen parameters, there exists a positive correlation only with PG $T_{opt.}$ No significant correlation was recorded with other cardinal temperatures of PG or PTL (Table 3.4).

Table 3.5. Cell membrane thermostability (unit less), canopy temperature depression (unit less) and chlorophyll stability index measured between 50 to 70 days of planting of 12 ornamental pepper cultivars in response to temperature.

Cultivar	CMT	CSI	CTD
‘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	59.50	71.20	-3.01
LSD	9.23^{***}	5.44^{***}	0.61^{**}

, * significant at the 0.01 and 0.05 probability levels, respectively. The values without an asterisk are not significant at the 0.05 probability level.

Chlorophyll stability index

Significant cultivar differences for CSI were recorded among ornamental pepper cultivars and ranged from 61.3 in ‘Red missile’ to 86.6 in ‘Thai Hot’ with a mean of 71.2 (Table 3.5). The CSI indicates a plant’s tolerance to environmental stresses. The higher the CSI, the least amount of stress impact on chlorophyll content of the plants. A higher CSI helps plants withstand stress through better availability of chlorophyll. This leads to higher rates of photosynthesis, more dry matter production, and higher productivity (Mohan et al., 2000). Among pollen parameters, a positive correlation to CSI was found only with PTL_{max}. No significant correlation was recorded with any other pollen variables (Table 3.3). In addition to temperature stress, CSI has been used to evaluate the drought resistance of plants: Barley (*Hordeum vulgare* L.) (Anjum et al., 2003), Pine (*Pinus* spp.) (Kaloyereas, 1958) , salt resistance in Rice (*Oryza sativa* L.) (Mohan et al., 2000), Cowpea (Hussein et al., 2007) and pH stress in wheat (EI-Khawas, 2004). Positive correlation (r=0.45) was recorded with CMT (Figure 3.2).

Canopy temperature depression

The CTD differed significantly among ornamental pepper cultivars. The mean CTD recorded was -3.01, with the maximum and minimum CTD of ‘Medusa’ - 3.74 and -1.96 ‘Sangria’ (Table 3.5). Among pollen parameters, there was a significant positive correlation only with PG $T_{opt.}$ No significant correlation was recorded with other cardinal temperatures of PG and PTL (Table 3.3). No correlation was recorded with CSI but poor correlation found with CMT (Figure 3.2).

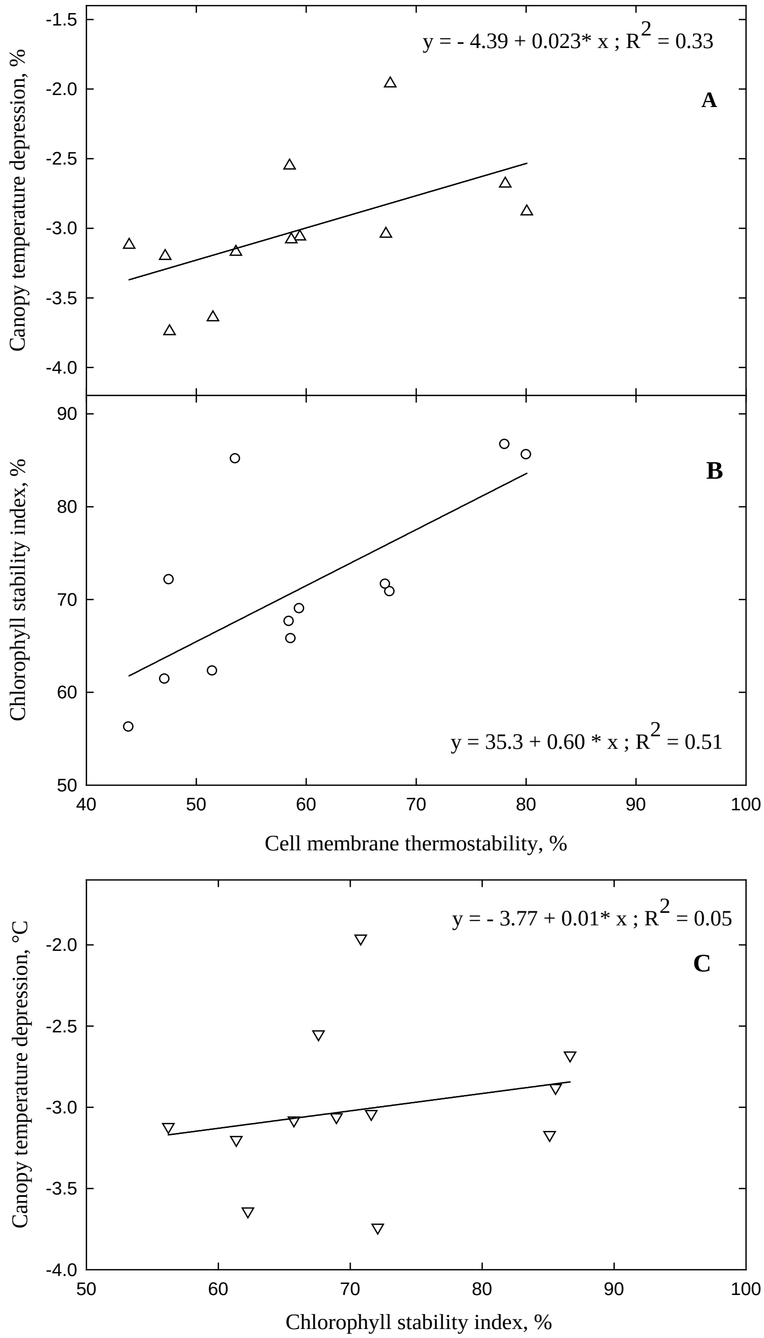

Figure 3.2 Relationships between (A) cell membrane thermostability and canopy temperature depression, (B) cell membrane thermostability and chlorophyll stability index and (C) chlorophyll stability index and canopy temperature depression of 12 ornamental pepper cultivars

Table 3.6. Pearson correlation matrix showing the relationship among CTRI (heat) and CTRI (cold) based on pollen-based parameters (PV, cardinal temperatures of MPG and PTL) and three physiological parameters (CMT, CTD and CSI) of 12 ornamental pepper cultivars.

	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 the 0.05 and 0.001 probability levels, respectively. The values

Correlation between Pollen Parameters and Physiological Parameters

Percentage of PV showed a significant positive correlation (*r*=0.98) with percentage of PG $_{max}$, PTL $_{max}$ (*r*=0.79) and PGT $_{max}$ (*r*=0.57). A reasonably positive correlation was recorded between PV and PG related parameters indicating the consistency of the evaluated procedure for the *in vitro* PG (Table 3.3). A similar trend was reported for canola in a study investigating cold and heat tolerance of pollen under field conditions (Singh et al., 2008b).

The CTRI (heat) and CTRI (cold) were derived using pollen based parameters (excluding three physiological parameters) to test the relationship between pollen based (reproductive) parameters and physiological parameters (vegetative). Significant positive correlation was observed among CTRI (heat) and two physiological parameters (CMT and CSI) (Table. 3.6). 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., 2002) also found no correlation between CMT and pollen based parameters. However, no significant correlation was found with CTD of any of the CTRIs. Moreover, a positive correlation was observed among CTRI (cold) and two physiological parameters (CMT, and CSI).

Classification of Ornamental Pepper Cultivars based on Cumulative Temperature Response Index (CTRI)

The cumulative temperature response index (CTRI) values for each ornamental pepper cultivar derived by summing individual temperature response indices for all the pollen based parameters and physiological parameters, varied significantly among pepper cultivars (Tables 3.7 and 3.8). This CTRI based technique using pollen parameters and physiological parameters, identifies cultivar variability for high and low temperatures (heat CTRI and cold CTRI).

Heat CTRI varies from 8.56 ‘Salsa Yellow’ to 11.33 ‘Thai Hot’. Based on heat CTRI, five cultivars, ‘Chilly Chili’, ‘Explosive Ember’, ‘Medusa’, ‘Treasures Red’, and ‘Thai Hot’ were classified as heat tolerant, four cultivars, ‘Calico’, ‘Purple Flash’, ‘Sangria’, and ‘Variegata’, classified as intermediate and another four cultivars, ‘Black Pearl’, ‘Red Missile’, and ‘Salsa Yellow’, were classified as heat sensitive (Table 3.7). Under CTRI (cold) three cultivars, ‘Medusa’, ‘Treasures Red’, and ‘Thai Hot’ were classified as cold sensitive, four cultivars, ‘Chilly Chili’, ‘Purple Flash’, ‘Variegata’, and ‘Explosive Ember’, were classified as moderately cold sensitive and three cultivars, ‘Black Pearl’, ‘Calico’, and ‘Sangria’, were classified as moderately cold tolerant and two cultivars, ‘Red Missile’ and ‘Salsa Yellow’, were classified as cold tolerant (Table 3.8). Identification and development of cultivars which are heat and cold tolerant is

Table 3.7. 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 parenthesis. CTRI is the sum of individual component response of nine pollen parameters and three physiological parameters (CMT, CTD, and CSI).

Classification of ornamental pepper cultivars based on heat CTRI [z]		
Heat-tolerant (CTRI >10.57)	Intermediate (CTRI = 9.74 -10.57)	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)		

[z] Heat tolerant [CTRI = > (minimum CTRI + 2.5 stdev)], Intermediate [CTRI = (minimum CTRI + 1.5 stdev)-(minimum CTRI + 2.5 stdev)], Heat sensitive [CTRI = (minimum CTRI)-(minimum CTRI + 1.5 stdev)

Table 3.8 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 parenthesis. CTRI is the sum of individual component response of nine pollen based parameters and three physiological parameters (CMT, CTD, and CSI).

Classification of ornamental pepper cultivars based on cold CTRI [z]			
Cold sensitive (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)		

[z] Cold sensitive [CTRI = (minimum CTRI)-(minimum CTRI + 1.0 stdev)], Moderately cold sensitive [CTRI = (minimum CTRI + 1.0 stdev) to (minimum CTRI + 2.0 stdev)], Moderately cold tolerant [CTRI = (minimum CTRI + 2.0 stdev) to (minimum CTRI + 3.0 stdev)], Cold tolerant [CTRI = > (minimum CTRI + 3.0 stdev)]

beneficial as there will be more frequent future happenings of extreme climatic events (Mearns et al., 2001). Since short term extreme temperature events cause detrimental effects to reproductive processes when compared to a rise in long term average temperatures, cultivars with such heat and cold tolerant characteristics are much more important (Hall, 1992). However, additional studies are needed to fully understand and quantify the heat and cold tolerance mechanisms in ornamental pepper cultivars.

Conclusion

The *in vitro* PG and PTL of ornamental pepper cultivars showed a typical bilinear response to temperature treatments. The cardinal temperatures for PG and PTL showed a significant difference among the cultivars of ornamental pepper. With respect to PG, T_{min}, the values ranged from 11.51 ('Salsa Yellow') to 12.17°C ('Variegata'). T_{opt} ranged from 23.84 ('Salsa Yellow') to 28.6°C ('Medusa'). T_{max} ranged from 40.70 ('Black Pearl') to 41.61°C ('Sangria'). The narrowest range in cardinal temperatures was recorded in 'Explosive Ember' and the widest in 'Purple Flash'. The highest CMT, CSI and CTD were observed in ornamental cultivars of 'Treasures Red', 'Thai Hot' and 'Medusa'. The *in vitro* pollen study in combination with physiological parameters confirms the degree of tolerance and sensitiveness 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 only with pollen parameters showed a significant correlation with physiological parameters. This infers that screening based on pollen parameters is a more accurate approach for reproductive temperature tolerance. Pollen parameters identified in the present study can be used as a screening tool for breeding programs. The identified heat and cold tolerant cultivars are potential candidates for ornamental pepper breeding programs. Also, the “Green Industry” can target the cultivars suitable to a niche environment based on the level of heat and cold tolerance among the ornamental pepper cultivars.

CHAPTER IV

SCREENING ORNAMENTAL PEPPER CULTIVARS FOR COLD AND HEAT TOLERANCE USING TEMPERATURE RESPONSE PARAMETERS OF SEED GERMINATION

Abstract

Fluctuating and extreme changes in temperature during seed germination will disrupt several physiological processes and thus early seedling establishment. An experiment was conducted to study the influence of temperatures, 10 to 45°C at 5°C interval, in 12 cultivars of ornamental peppers using *in vitro* seed germination rate (SGR) and maximum seed germination (MSG) determined from time-series seed germination response data. Quadratic and bilinear functions best described SGR- and MSG-temperature response functions. Cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for SGR and MSG estimated from these functions varied significantly for SGR (0.38 to 0.12 d^{-1}) and MSG (76 to 97%). The mean values for cardinal temperatures were 15.3, 27.6 and 42.9°C for SGR and 9.9, 24.2 and 42.5°C for MSG for T_{min}, T_{opt},, and T_{max}, respectively. Cumulative temperature response index (CTRI) of each cultivar, calculated as the sum of eight individual temperature responses derived from Temperature Adaptability Range ($TAR = T_{max} - T_{min}$), T_{min}, T_{opt}, and T_{max} for SGR and MSG, were used to classify cultivars for temperature tolerance. Cultivars were classified based on CTRI (heat) as

heat tolerant (‘Medusa’ and ‘Treasures Red’), intermediate (‘Thai Hot’, ‘Variegata’ and ‘Red Missile’), and heat sensitive (‘Purple Flash’, ‘Salsa Yellow’, ‘Black Pearl’, ‘Chilly Chili’, ‘Explosive Ember’, ‘Calico’, and ‘Sangria’). Similarly, cultivars were also classified for cold tolerance as cold sensitive (‘Medusa’, ‘Treasures Red’, ‘Thai Hot’ and ‘Variegata’), moderately cold sensitive (‘Red Missile’, ‘Purple Flash’, ‘Salsa Yellow’ and ‘Chilly Chili’), moderately cold tolerant, (‘Black Pearl’, ‘Explosive Ember’, and ‘Calico’), and cold tolerant (‘Sangria’) based on CTRI (cold). The CTRI heat and cold showed a significant linear correlation (r = 0.75 and - 0.80, respectively) with physiological-based CTRI, indicating that seed and vegetative temperature tolerance behave similarly. Therefore, screening based on seed parameters is a simple, inexpensive and reliable method for screening vegetative temperature tolerance in ornamental peppers and could be used for selecting cultivars and breeding.

Introduction

The floriculture sector in the United States is an important segment of production agriculture. Ornamental peppers (*Capsicum annuum* L.) are widely used as potted flowering or bedding plants because of their morphologically diverse foliage and fruit characteristics. The use of ornamental peppers as potted and florist crops are popular in Europe and are gaining in popularity in the United States (Armitage and Hamilton, 1987). Since ornamental peppers grow well under high radiation and set fruit under a wide range of temperatures (Stummel and Bosland, 2007), temperature effects on seed germination and early seedling establishment is important for successful plant production.

Seed germination is a complex process involving many physiological and biochemical processes contributing to embryo activation. Temperature and water mainly control the rate of seed germination when aeration is not restrictive (Gummerson, 1986). Temperature alone is the major environmental factor controlling the seed germination when soil moisture is not limiting (Garcia-Huidobro et al., 1982). Therefore, extreme and fluctuating temperatures are the single most important factors restricting the distribution, adaptability and yield potential of plants. According to Hsu et al. (1985), temperature is the major environmental factor affecting both seed germination capacity, rate and seedling vigor in many crops. Thus, high and low soil temperatures at sowing can affect plant populations leading to fewer plants which evoked research to develop seeds tolerant to extreme temperatures. Roberts (1988) reported that temperature affects both rate of seed germination and maximum seed germination through three distinct processes namely seed aging, dormancy loss, and germination. Thus, responses of seeds to temperature are of considerable importance in agriculture. Determining temperature effects on germination using mathematical functions may be useful in evaluating germination characteristics or establishment potential among genotypes or species (Jordan and Harferkamp, 1989).

Ornamental peppers are generally propagated through seeds at the optimum temperature of 30°C. The rate of both germination and emergence significantly decreases at temperatures ranging from 15 to 20°C (O'Sullivan and Bouw, 1984). Thermal time (degree-day or hour), the heat unit for plant development, is a well established developmental principle for plants (Fry, 1983). This thermal time model has been successfully used to predict seed germination under non-water limiting conditions.

According to the model, based on many crops, the existing soil temperature determines both the maximum seed germination MSG and the rate of germination (SGR) (Garcia-Huidobro et al., 1982). Using this model, cardinal temperatures (T_{opt}, T_{max}, and T_{min}) for both MSG and SGR, and maximum SGR and MSG can be identified.

Such thermal responses from each cultivar are used to identify the temperature tolerance. Carter and Vavrina (2001) reported that in the southern United States pepper seeds are sown in the fall when summer greenhouse temperatures are reaching 40 to 45°C for 6 h or more. Since this is far above the optimum temperature of pepper, it may result in inhibition or erratic germination causing complications in production scheduling and reductions in overall stand establishment. Availability of heat tolerant cultivars, in particular to seed germination, can overcome such problems. Similarly, identification of cold tolerant cultivars becomes important for cold weather conditions.

Physiological and pollen-based parameters have been widely used to determine the temperature tolerance in many crops (Singh et al., 2007). Few studies using seed-based parameters including seed germination rate and maximum germination or germination capacity, have also reported where they used to screen several crop species and genotypes for various abiotic stress factors including moisture stress (Sadasivam et al., 2000), water logging (Sharma, 2008), salinity (Misra and Dwivedi, 2004), and heat stress (Ellis et al., 1986). Seed-based *in vitro* screening can provide insights into the genotypic environmental adaptability and tolerance capacity of crops.

To date, there are studies on evaluating temperature tolerance of ornamental pepper cultivars using seed parameters except the work of Carter and Vavrina (2001).

Thus, it would be beneficial to explore cultivars of ornamental peppers for temperature tolerance using seed germination and temperature response parameters.

Among the various approaches to determine and classify temperature tolerance of different cultivars and genotypes of crop plants, Salem et al. (2007) and Koti et al. (2004) grouped genotypes by relative ranking using single value indices and cumulative indices based on statistical separation of means. Singh et al. (2008) grouped the cowpea genotypes for temperature tolerance through quantitative relationships determined by principal component analysis. In those approaches, they tried to isolate the cultivar variability with respect to heat and cold tolerance and grouped the cultivars.

The goals of this study were to understand the differences in temperature tolerance for seed germination of 12 ornamental pepper cultivars. The specific objectives of the research study were to (i) quantify the responses of seed germination rate and maximum seed germination of ornamental pepper cultivars to different temperatures, (ii) determine cardinal temperatures for both seed germination rate and maximum seed germination using parameters of the seed germination thermal model, and (iii) classify pepper cultivars for temperature tolerance using seed-based parameters.

Materials and Methods

Seed Material

Seeds of 12 ornamental pepper cultivars were obtained from Ball Horticultural Company, Chicago, Illinois. Weight of 1000 seed was recorded for each cultivar and 50 seedlots were then sealed in a polythene bags, and stored in a refrigerator (4°C) until further use.

Measuring Seed Germination with Temperature Treatments

Time-series seed germination data at various temperature treatments were carried out from February to May 2009. Fifty seeds from each cultivar were placed on 9-cm sterilized plastic Petri dishes layered with two sheets of moistened Whatman no. 2 filter paper (Whatman, Atlanta, GA). Petri dishes were placed in incubators (Fisher Scientific, Suwanee, GA) in the dark under a range of temperatures from 5 to 55°C at 5°C intervals. Four replications from each cultivar were used and the Petri dishes were covered to minimize moisture loss. The filter papers containing seeds were moistened with distilled water daily, as needed. Germinated seeds were counted, recorded, and discarded every six hours. A seed was considered germinated when the radical length was equal or longer than the diameter of the seed.

Temperature and germination time-course data were fitted with a 3-parameter sigmoidal function (Eq. 1) using Sigma Plot 11 (Systat Software Inc., Chicago, IL). This function estimated *a*, the maximum cumulative germination percentage (germination capacity); *b*, the shape and steepness of the curve; and *x0*, time to reach germination half-maximal (time to 50% of maximum germination). The rate of development was derived by the reciprocal of time to 50% of maximum seed germination.

$$Y = SG_{max}/\{1 + \exp[-(x - x_{50})/G_{rate}]\} \quad [1]$$

Curve Fitting of Germination Time Course for Seed Germination

Cumulative seed germination time-course data were analyzed by fitting a 3-paramter Sigmoid function [Eq. 1] using Sigma Plot 11 similar to procedure adopted by several others while modeling seed germination response to time (Garcia-Huidobro et al., 1982a; Shafii and Price, 2001). This function estimates (1) SGmax, the maximum cumulative seed germination percentage (germination capacity) at a given time, t; (2) the shape and steepness of the curve, G_{rate}, and (3) time to reach germination half-maximal (time to 50% of maximum germination, t_{50}).

$$Y_t = SG_{max}/\{1 + \exp[- (t - t_{50})/G_{rate}]\} \qquad [1]$$

The maximum percentage germination and the reciprocal over time to 50% germination (GR) were used for further data analysis.

Determination of Cardinal Temperatures

Both linear and nonlinear models were used to analyze maximum seed germination (MSG) and germination rate (GR) responses to temperature. The best models were determined based on the overall highest coefficient of determination (R^2) and the least root mean square error (RMSE) values using non-linear regression procedure, PROC NLIN. Based on these criteria, quadratic model best described the MSG response to temperature (Mean R^2 = 0.72, mean RMSE = 4.8), while modified bilinear function best described seed GR responses to temperature (Mean R^2 = 0.78, mean RMSE = 1.6). Quadratic and modified bilinear equation estimates for each replicate within each genotype were estimated by the non-linear regression procedure, PROC NLIN (SAS

Institute Inc., Cary, NC) by a modified Newton Gauss iterative method. For the MSG quadratic function model [Eq. 2], the three cardinal temperatures (T_{min}, T_{opt}, and T_{max}) were estimated using the following equations [3, 4, and 5].

$$MSG = a + bT - cT^2 \quad [2]$$

$$T_{opt} = -b/(2\,c) \quad [3]$$

$$T_{min} = -b + (\sqrt{b^2} - 4ac)/2c \quad [4]$$

$$T_{max} = -b - (\sqrt{b^2} - 4ac)/2c \quad [5]$$

where T is the treatment temperature at which MSG were determined for each cultivar and a, b, and c are cultivar specific constants generated by PROC GLM by SAS.

For the modified bilinear model [Eq. 6], T_{opt} was generated by SAS, while T_{min} and T_{max} were estimated by the following equations [7] and [8].

$$SGR = a + b_1\,(T - T_{opt}) + b_2 \text{ x ABS}\,(T_{opt} - T) \quad [6]$$

$$T_{min} = [a + (b_2 - b_1) \text{ x } T_{opt}]/\ b1 - b_2 \quad [7]$$

$$T_{max} = [a - (b_2 + b_1) \text{ x } T_{opt}]/\ b1 + b_2 \quad [8]$$

Where T is the treatment temperature and a, b_1, and b_2 are cultivar specific constants generated by PROC NLIN by SAS. A mean curve was fitted to each genotype for MSG to determine the parameter estimates.

Cumulative Stress Response Index (CSRI) for Seed Germination

Initially, individual temperature response index (ISRI) of each parameter for heat tolerance was calculated as the value of a cultivar (P_t) divided by maximum value (P_h) observed over all the cultivars ([Eq. 9]) while for ISRI for cold tolerance was determined by dividing the minimum value (P_l) observed over all the cultivars by the value of a cultivar (P_t) ([Eq. 10]) as described by Reddy and Kakani (2007). Heat CTRI was calculated as the value of the cultivar divided by the maximum value observed over all cultivars while cold CTRI was determined by dividing the minimum value among the genotypes by the maximum value. Then, heat and cold CTRI's for each cultivar [Eq. 11, and 12] were calculated as the sum of eight ISR's derived from cardinal temperatures (T_{min}, T_{opt}, T_{max}) and temperature adaptability range (TAR= T_{max} - T_{min}) for both MSG and SGR. Cultivars were classified based on heat CTRI of all parameters, as heat tolerant [> minimum CTRI + 2.5 standard deviation (stdev)], intermediate (> minimum CTRI +2.5 stdev and < minimum CTRI + 1.5 stdev), and heat sensitive (> minimum CTRI to, minimum CTRI + 1.5 stdev). Similarly, Cultivars were classified based on cold CTRI of all parameters, as cold tolerant [> minimum CTRI + 3.0 standard deviation (stdev)], moderately cold tolerant [< minimum CTRI + 3.0 standard deviation-[> minimum CTRI + 2.0 standard deviation], moderately cold sensitive (> minimum CTRI +2.0 stdev and < minimum CTRI + 1.0 stdev), and cold sensitive (< minimum CTRI to, minimum CTRI + 1.0 stdev).

$$\mathrm{ISRI} = \mathrm{P_t}/\mathrm{P_h} \qquad [9]$$

$$\mathrm{ISRI} = \mathrm{P_l}/\mathrm{P_t} \qquad [10]$$

$$\text{Heat CTRI} = \left(\begin{array}{l} \dfrac{\text{TAR MSG}_t}{\text{TAR MSG}_h} + \dfrac{\text{MSG T}_{\min_t}}{\text{MSG T}_{\min_h}} + \dfrac{\text{MSG T}_{\text{opt}_t}}{\text{MSG T}_{\text{opt}_h}} + \dfrac{\text{MSG T}_{\max_t}}{\text{MSG T}_{\max_h}} + \\ \dfrac{\text{TAR SGR}_t}{\text{TAR SGR}_h} + \dfrac{\text{SGR T}_{\min_t}}{\text{SGR T}_{\min_h}} + \dfrac{\text{SGR T}_{\text{opt}_t}}{\text{SGR T}_{\text{opt}_h}} + \dfrac{\text{SGR T}_{\max_t}}{\text{SGR T}_{\max_h}} \end{array} \right) \qquad [11]$$

$$\text{Cold CTRI} = \left(\frac{\text{TAR MSG}_{l}}{\text{TAR MSG}_{t}} + \frac{\text{MSG T}_{\text{min}_l}}{\text{MSG T}_{\text{min}_t}} + \frac{\text{MSG T}_{\text{opt}_l}}{\text{MSG T}_{\text{opt}_t}} + \frac{\text{MSG T}_{\text{max}_l}}{\text{MSG T}_{\text{max}_t}} \atop \frac{\text{TAR SGR}_{l}}{\text{TAR SGR}_{t}} + \frac{\text{SGR T}_{\text{min}_l}}{\text{SGR T}_{\text{min}_t}} + \frac{\text{SGR T}_{\text{opt}_l}}{\text{SGR T}_{\text{opt}_t}} + \frac{\text{SGR T}_{\text{max}_l}}{\text{SGGR T}_{\text{max}_t}} \right) \quad [12]$$

Data Analysis

Seed germination over time and the regression procedures of fitting sigmoid functions for the cumulative-time series data were estimated using SigmaPlot 10. Replicated values of cardinal temperatures (T_{min}, T_{opt}, and T_{max}) and MSG were analyzed using one-way ANOVA procedure (PROC GLM) in SAS to determine the effect of temperature treatments on MSG and SGR, and their respective cardinal temperatures. Means were separated using Fisher's protected least significant difference (LSD) at $P<0.05$. Germination parameters (MSG and SGR) were treated as dependent variables and temperature and time to germination as independent variables.

Results and Discussion

Seed Weight and Germination Time Course

Individual seed weights were different among cultivars, and ranged from 2.06 x 10^{-3} mg ('Black Pearl') to 5.49 x 10^{-3} mg ('Explosive Ember'), with a mean of 3.81 x 10^{-3} mg (Table 4.1). Generally, seed size or weight reflects potential food reserves for seedling growth and are considered important traits determining the successful establishment of individual plants (Zhang, 1996). In contrast, Vaughton and Ramsey (1998) reported that seed size variation within an individual plant is attributed to parental environment, fruiting position, and time from harvest to seed germination,. Therefore, it is not used to classify ornamental pepper cultivars.

For each temperature and cultivar, the time course of germination was modeled using a three parameter sigmoid function using equation [1]. The three parameter sigmoid curve fit the cumulative germination time course of cultivar seed germination response to temperature efficiently ($R^2 > 0.85$). This shows how cultivars differed in their germination characteristics with respect to different temperature treatments (Figure 4.1). No germination was observed at temperature treatments below 15°C. Most cultivars did not germinate beyond 38°C except 'Black Pearl', 'Medusa', 'Thai Hot', and 'Variegata'. Similarly, Carter and Vavrina (2001) found a significant cultivar difference in seed germination of vegetable peppers for temperatures from 20 to 30°C. However, no germination was recorded at temperatures higher than 35°C in all five cultivars they tested. This may indicate that vegetable pepper cultivar seeds are less heat tolerant than ornamental pepper cultivars. Maximum seed germination, relative rate of germination, and time to 50% of maximum germination were different across temperatures and cultivars. In contrast, Carter and Vavrina (2001) reported that temperature had little effect on the time to 50% of maximum germination. This is because of the narrow temperature range of temperature treatments they used in their study, 20 to 35°C. However, the thermal response patterns to seed germination observed in this study is consistent with thermal response patterns of a number of other seed germination studies (Shafii and Price, 2001; Tokumasu etc. 1985) and physiological processes (Probert, 2000).

Maximum Seed Germination (MSG) Response to Temperature

Out of the modified bi-linear and quadratic models tested, the bilinear function best described the response of MSG to temperature treatments based on coefficient of determination (R^2) and root mean square error (RMSE). These response curves represented how cultivars responded to different temperature treatments with respect to maximum seed germination (Figure 4.1). There exists a significant difference for MSG and their cardinal temperatures among cultivars. MSG ranged from 75.6 ('Medusa') to 96.8% ('Thai Hot') with a mean of 89.7% among cultivars. Cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for MSG differed among cultivars. Predicted T_{min} value ranged from 7.3°C in 'Black Pearl' to 15.9°C in 'Medusa' with a mean of 9.9°C. Cultivar Calico recorded highest T_{opt} value (28.0°C), while 'Thai Hot' registered the lowest T_{opt} value (21.2°C) with the mean value of 24.2°C among cultivars. T_{max} value ranged from 38.1°C ('Sangria') to 45.1°C ('Medusa') with a mean of 42.5°C (Table 4.1).

In addition to maximum germination responses to temperature, MSG was quantified for each cultivar. This parameter was not incorporated into the classification process as these responses may be applicable to one seed population because of experiment-specific conditions (Ellis et al., 1987). Thus, that parameter is limited to incorporate into the classification process due to variations in seed quality (Ellis et al., 1987), seed maturation environment (Fenner, 2008) and time from harvest to seeding (Jenson and Boe, 1991).

Seed Germination Rate (SGR) Response to Temperature

Similar to MSG, out of the two models tested, the quadratic equation best described the relationship between germination rate (1/T) and temperature treatments. Cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for SGR based on seed germination rate differed significantly among cultivars (Table 4.2). In general, germination rate increased linearly with temperature at suboptimal (T_{min} - T_{opt}) temperatures, but decreased linearly with temperature at supra optimum temperatures (T_{opt} - T_{max}). This typical thermal response pattern has been observed in several species such as pearl millet (Garcia-Huidobro et al., 1982), soybean (Covell et al., 1986), and sorghum (Benech-Arnold et al., 1990).

The germination rate response curves to temperatures demonstrated how the cultivars differed in their rate of seed germination with respect to different temperature treatments (Figure 4.2). T_{min} values ranged from 12.9°C in 'Calico' to 17.2°C ('Thai Hot') with a mean of 15.3°C. Cultivar 'Treasures Red' recorded the highest (29.5°C) T_{opt} value, while 'Explosive Ember' showed the lowest T_{opt} value with the mean value of 27.7 °C among cultivars. T_{max} values ranged from 39.0°C in 'Calico' to 46.0°C in 'Treasures

Table 4.1 Seed weight (SW), temperature adaptability range for maximum seed germination (TAR_{MSG}), maximum seed germination percentage (MSG), modified bilinear equation constants (a, b_1, b_2), regression coefficients (R^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for maximum seed germination percentage (MSG) of 12 ornamental pepper cultivars.

Cultivar	SW ($mg\ 10^{-3}$)	TAR_{MSG}	MSG (%)	Equation constants			R^2	Cardinal temperatures (°C)		
				a	b_1	b_2		T_{min}	T_{opt}	T_{max}
'Black Pearl'	2.33	33.43	89.73	122.9	-1.73	-7.74	0.71	07.30	27.75	40.73
'Calico'	3.87	31.11	91.86	130.3	-2.41	-9.02	0.65	08.33	28.04	39.44
'Chilly Chili'	2.52	34.17	83.65	120.0	2.65	-7.91	0.73	10.29	21.65	44.46
'Explosive Ember'	5.49	31.82	93.06	125.9	2.39	-8.58	0.73	10.85	22.33	42.67
'Medusa'	2.20	29.11	75.55	85.49	4.50	-8.31	0.76	15.98	22.65	45.09
'Purple Flash'	3.64	31.35	95.04	127.9	2.50	-8.87	0.71	10.84	22.09	42.19
'Red Missile'	5.06	33.36	89.60	123.9	-1.47	-7.71	0.70	07.61	27.47	40.97
'Salsa Yellow'	3.05	34.20	92.72	125.7	-0.86	-7.45	0.73	08.66	27.73	42.86
'Sangria'	5.01	27.92	91.32	125.4	1.03	-9.10	0.81	10.25	22.63	38.17
'Thai Hot'	5.21	33.36	96.76	121.7	2.95	-8.34	0.85	10.45	21.23	43.81
'Treasures Red'	2.06	34.33	88.85	120.8	2.56	-7.87	0.57	10.61	22.19	44.94
'Variegata'	5.23	35.16	80.74	114.8	2.37	-7.30	0.66	09.97	21.84	45.13
Mean	3.81	32.44	89.07				0.72	10.10	23.97	42.54
LSD	0.26***	0.49**	4.58**					0.42**	0.68*	0.49***

*,**, *** significant at the, 0.05, 0.01 and 0.001 probability levels, respectively. The values without an asterisk are not significant.

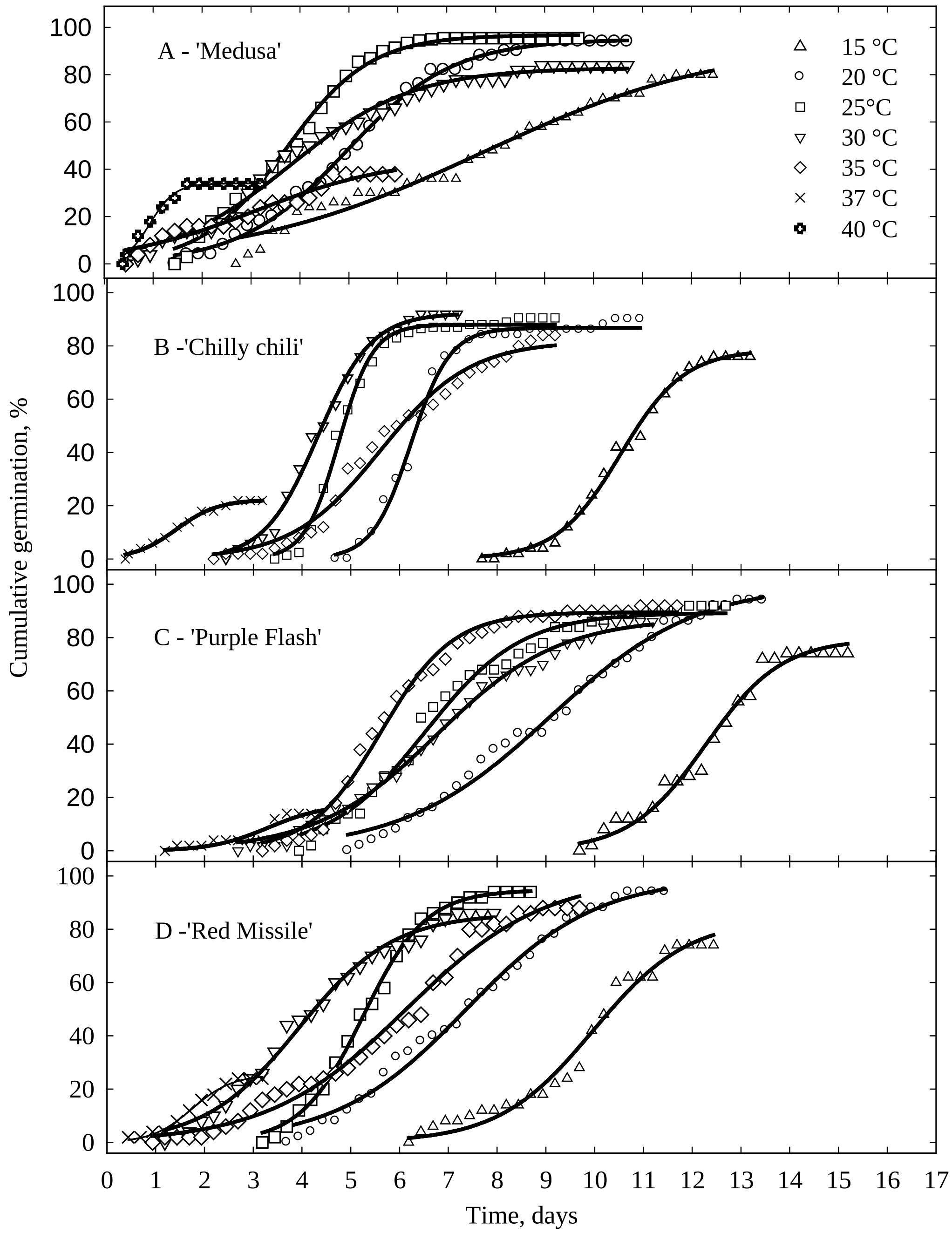

Figure 4.1. Observed (symbol) and predicted (lines) germination time course of seeds of (A) ‘Medusa’, (B) ‘Chilly Chili’, (C) ‘Purple Flash’ and (D) ‘Red Missile’ cultivars of ornamental pepper (*Capsicum annuum* L.) seeds germinated at a range of temperatures (10-45°C). Predicted lines are based on three parameter sigmoid function. For clarity, data and regression lines for four cultivars representing different rates of seed germination are presented.

Red'. This is consistent with Roberts (1988) who found that many species typically have higher optimum temperatures for SGR than for maximum seed germination percentage. According to Garcia-Huidobro et al. (1982), seeds not germinating within seven days of imbibitions usually have reduced survival due to pathogenic infection and/or insect attacks and exhaustion of seed reserves. Schimpf et al. (1977) found that SGR is more temperature sensitive than final germination percentage in yellow foxtail (*Setaria lutescens* Weigel.) and Redroot pigweed *(Amaranthus retroflexus* L.) (Schimpf et al., 1977). Ellis et al. (1987) reported that maximum seed germination is affected by seed quality. However, in commercial seeds, quality is maintained at a higher level with less variation in seed quality. Fenner (2008) further reported that seed maturation environment has a profound impact on maximum seed germination and duration of time from harvest (Boe and Ross, 1998). Therefore, the seed maturation environment and the duration time from maturation affects maximum seed germination, but not the cardinal temperatures and temperature adaptability range (T_{max} –T_{min}). This limits the use of MSG percentage as parameter in the screening cultivars for temperature tolerance. The difference found in quantative characteristics (T_{min}, T_{opt} and T_{max}) of both SGR and MSG among cultivars is attributed to genetic variability rather than seed quality.

Classification of Cultivars for Temperature Tolerance using Seed-based CTRI

Eight parameters (TAR, T_{min}, T_{opt}, and T_{max} for both MSG and SGR) were used for both heat and cold tolerance classification using seed-based CTRI. Under this protocol, each parameter contributed differently based on its relativity to the minimum and maximum value of each parameter across the cultivars. The cumulative temperature response index (CTRI) values for each ornamental pepper cultivar were derived by summing up individual temperature response indices for all seed based parameters and varied significantly among pepper cultivars (Tables 4.3 and 4.4). The CTRI based technique using seed germination parameters, identifies cultivar variability for high and low temperature tolerance among crop cultivars (heat CTRI and cold CTRI).

Heat CTRI varied from 6.5 ('Sangria') to 7.6 ('Medusa'). Based on heat CTRI, the cultivars, 'Medusa' and 'Treasures Red' were classified as heat tolerant, 'Red Missile', 'Thai Hot', and 'Variegata' as intermediate and 'Purple Flash', 'Salsa Yellow', 'Black Pearl', 'Chilly Chili', 'Explosive Ember', 'Calico', and 'Sangria' as heat sensitive to temperature (Table 4.3).

Cold CTRI also varied among cultivars from 6.6 ('Medusa') to 7.5 ('Sangria'). Based on cold CTRI, the cultivars, 'Medusa', 'Treasures Red', 'Thai Hot', and 'Variegata' were classified as cold sensitive, 'Red Missile', 'Purple Flash', 'Salsa Yellow', and 'Chilly Chili' as moderately cold sensitive and 'Calico', 'Black Pearl' and 'Explosive Ember' as moderately cold tolerant and 'Sangria' as cold tolerant (Table 4.4).

In this study, SGR and MSG were assessed as estimators of high and low temperature tolerance using 12 ornamental pepper cultivars. Cultivars were classified based on their capacity to tolerate high and low temperature conditions. A similar study was done by Tiryaki and Andrew (2001) to screen 12 genotypes of sorghum for cold tolerance in controlled *in vitro* germination studies. They found that SGR was strongly correlated with rate of emergence under field conditions. This confirms that screening using *in vitro* seed germination is a rapid and low cost approach to handle a large number of genotypes before evaluating in the field.

Table 4.2 Seed germination rate (SGR), temperature adaptability range for seed germination rate(TAR_{SGR}), quadratic equation constants (a, b_1, b_2), regression coefficient (R^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for SGR of 12 ornamental pepper cultivars.

Cultivar	SGR (day^{-1})	TAR_{SGR}	Equation constants			R^2	Cardinal temperatures (°C)		
			a	b_1	b_2		T_{min}	T_{opt}	T_{max}
'Black Pearl'	0.25	27.36	-0.573	0.060	-0.001	0.95	15.05	27.36	42.41
'Calico'	0.38	26.11	-1.124	0.115	-0.002	0.84	12.96	26.11	39.07
'Chilly Chili'	0.17	26.69	-0.397	0.043	-0.001	0.67	14.66	26.69	41.35
'Explosive Ember'	0.23	25.96	-0.582	0.062	-0.001	0.95	13.67	25.96	39.63
'Medusa'	0.12	29.37	-0.226	0.024	0.000	0.34	17.21	29.38	46.58
'Purple Flash'	0.26	27.81	-0.619	0.063	-0.001	0.67	15.1	27.81	42.91
'Red Missile'	0.16	28.25	-0.316	0.034	-0.001	0.57	16.42	28.25	44.67
'Salsa Yellow'	0.20	26.54	-0.481	0.052	-0.001	0.93	14.39	26.55	40.93
'Sangria'	0.28	26.44	-0.767	0.079	-0.002	0.86	13.61	26.43	40.05
'Thai Hot'	0.33	29.22	-0.609	0.064	-0.001	0.96	17.27	29.23	46.49
'Treasures Red'	0.33	29.54	-0.63	0.065	-0.001	0.81	17.26	29.55	46.8
'Variegata'	0.19	28.57	-0.377	0.040	-0.001	0.89	16.64	28.57	45.21
Mean	0.24	27.66				0.79	15.35	27.66	43.01
	0.018***	0.22***				0.38**	0.46**	0.68*	0.49***

*. **, *** significant at the, 0.05, 0.01, and 0.001 probability levels, respectively. The values without an asterisk are not significant.

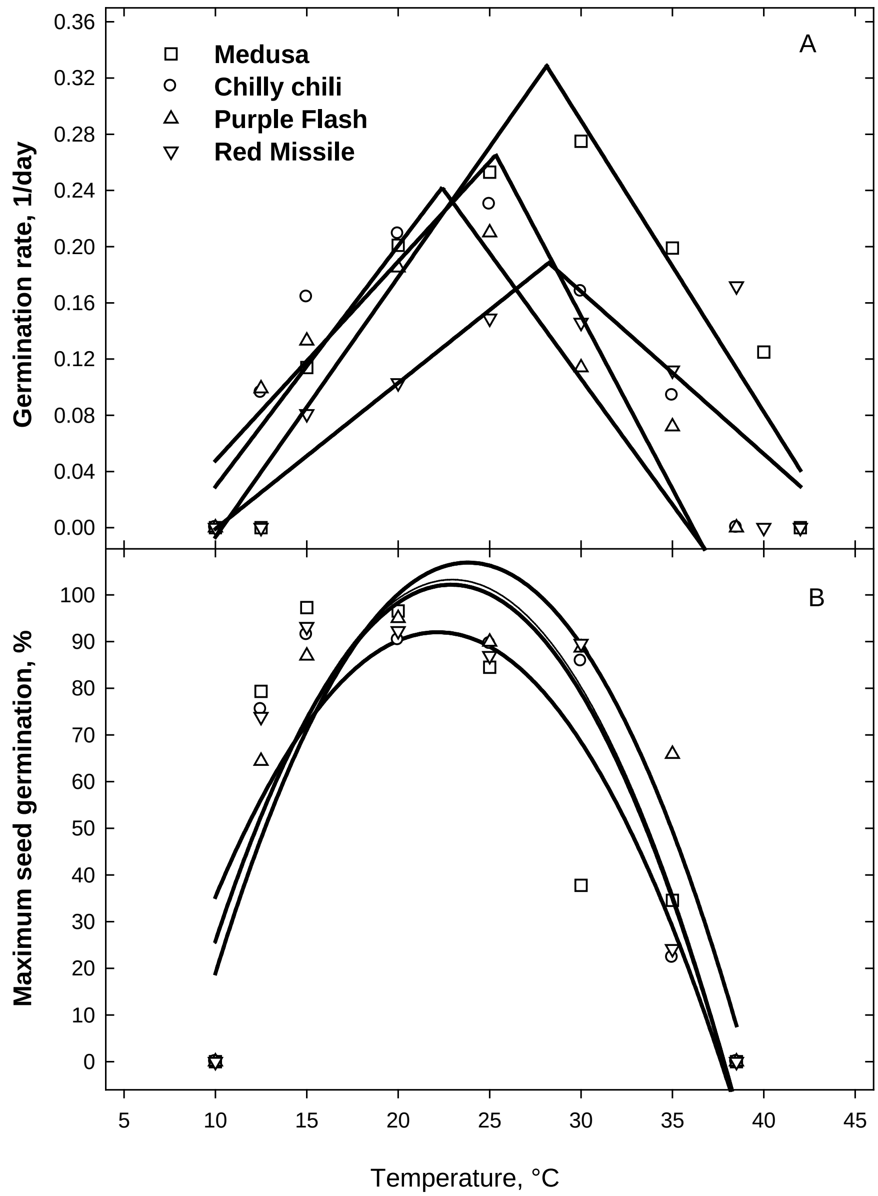

Figure 4.2 (A) Seed germination rates to temperature (symbols) and their fitted lines derived from the bilinear equation, respectively, of four ornamental pepper cultivars ('Medusa', 'Chilly Chili', 'Purple Flash' and 'Red Missile'). Predicted lines are based on bilinear function, (B) maximum seed germination responses to temperature (symbols) and their fitted lines derived from the quadratic equations, respectively, of four ornamental pepper cultivars ('Medusa', 'Chilly Chili', 'Purple Flash' and 'Red Missile'). Predicted lines are based on quadratic function. For clarity, data and regression lines for four cultivars are presented.

Table 4.3. Classification of 12 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 parenthesis. CTRI is the sum of individual component response of eight seed-based parameters.

Classification of ornamental pepper cultivars using seed-based heat CTRI [Z]		
Heat-tolerant (CTRI >7.34)	Intermediate (CTRI = 7.02 - 7.33)	Heat-sensitive (CTRI < 7.01)
Medusa (7.61)	Thai Hot (7.32)	Purple Flash (6.96)
Treasures Red (7.42)	Variegata (7.27)	Salsa Yellow (6.96)
	Red Missile (7.13)	Black Pearl (6.92)
		Chilly Chili (6.91)
		Explosive Ember (6.72)
		Calico (6.63)
		Sangria (6.52)

[Z] Heat tolerant [CTRI = > (minimum CTRI + 2.5 stdev)], Intermediate [CTRI = (minimum CTRI + 1.5 stdev)-(minimum CTRI + 2.5 stdev)], Heat sensitive [CTRI = (minimum CTRI)-(minimum CTRI + 1.5 stdev)

Table 4.4. Classification of ornamental pepper cultivars into cold tolerant, moderately cold tolerant, moderately cold sensitive and cold sensitive groups using seed based cumulative stress response index (CTRI; unit less), along with individual score of CTRI values in parenthesis. CTRI is the sum of individual component response of eight seed based parameters.

Classification of ornamental pepper cultivars using seed based cold CTRI [Z]			
Cold sensitive (CTRI < 6.90) [A]	Moderately cold sensitive (CTRI = 6.91 - 7.21) [B]	Moderately cold tolerant (CTRI = 7.22 - 7.53) [C]	Cold tolerant (CTRI > 7.54) [D]
Medusa (6.56)	Red Missile (7.00)	Black Pearl (7.22)	Sangria (7.54)
Treasures Red (6.65)	Purple Flash (7.07)	Explosive Ember (7.33)	
Thai Hot (6.77)	Salsa Yellow (7.13)	Calico (7.49)	
Variegata (6.80)	Chilly Chili (7.14)		

[Z] Cold sensitive [CTRI = (minimum CTRI)-(minimum CTRI + 1.0 stdev)], Moderately cold sensitive [CTRI = (minimum CTRI + 1.0 stdev) to (minimum CTRI + 2.0 stdev)], Moderately cold tolerant [CTRI = (minimum CTRI + 2.0 stdev) to (minimum CTRI + 3.0 stdev)], Cold tolerant [CTRI = > (minimum CTRI + 3.0 stdev)]

Correlation between Pollen-based CTRI and Seed-based CTRI

The relationship between seed-based CTRI (both heat and cold) and pollen (both heat and cold) and physiological-based CTRI (determined in experiment I) were tested. A positive correlation (*r*=0.78) was observed between seed-based CTRI (heat) and physiological-based CTRI, and a negative correlation (*r*=0.80) with CTRI (cold). In contrast, weak (*r*= 0.26) correlation was found between pollen- and seed-based CTRI (Table 4.5 and 4.6). The strong correlation between seed- and physiological parameters indicate that physiological- and seed-based parameters can go together, and seed-based screening for cultivar tolerance for temperature will be a simple and effective technique. In contrast, the week, correlation between seed and physiological CTRI with pollen-based CTRI indicate that reproductive and physiological mechanisms operate differently, and pollen-based screening protocols will be more ideal for reproductive temperature tolerance in ornamental pepper cultivars.

Conclusions

Seed germination response to a range of temperature treatments is quantified in 12 ornamental pepper cultivars for the first time. From the cumulative seed germination-time response curves, seed germination capacity (maximum germination) and seed germination rate were determined at each temperature for all cultivars. MSG showed a typical quadratic response to temperature treatments whereas SGR showed a bilinear response. The cardinal temperatures for MSG and SGR varied significantly among

Table 4.5. Pearson correlation matrix showing the relationship among CTRI (heat) based on pollen, seed and physiological parameters of 12 ornamental pepper cultivars.

Tolerance Parameters	Pollen based CTRI (heat)	Seed-based CTRI (heat)	Physiological parameter-based CTRI
Pollen-based CTRI (heat)	1.00		
Seed-based CTRI (heat)	0.26	1.00	
Physiological parameter-based CTRI	0.65*	0.78**	1.00

Table 4.6. Pearson correlation matrix showing the relationship among CTRI (cold) based on pollen, seed and physiological parameters of 12 ornamental pepper cultivars.

Tolerance Parameters	Pollen- based CTRI (cold)	Seed-based CTRI (cold)	Physiological parameter - based CTRI
Pollen-based CTRI (cold)	1.00		
Seed-based CTRI (cold)	0.28	1.00	
Physiological parameter-based CTRI	- 0.58*	- 0.80**	1.00

*, ** significant at the, 0.05 and 0.01 probability levels, respectively. The values without an asterisk are not significant at the 0.05 probability level; the numbers without asterisks are not significant.

ornamental pepper cultivars. Mean optimum temperatures for MSG and SGR were 24.0 and 27.7°C, respectively. The narrowest range in cardinal temperature (TAR) was recorded in 'Sangria' and the greatest in 'Variegata' indicating that the cultivar Variegata has greater survival potential than any other cultivar.

Based on CTRI, temperature tolerance variability was found among the cultivars. For heat tolerance, two out of 12 cultivars were classified as heat tolerant, three as intermediate and seven as heat sensitive. For cold tolerance, one cultivar was classified as cold tolerant, three cultivars as moderately cold-tolerant, four cultivars were moderately cold-sensitive, and four were cold sensitive. Accordingly, the cultivar Medusa was recorded as heat tolerant whereas Sangria was recorded as cold tolerant among the cultivars tested. The CTRI using seed parameters (determined in experiment 1) showed a significant linear correlation with physiological parameters indicating that seed and physiological can be used for temperature tolerance characteristics among the cultivars. Temperature tolerance classification via *in vitro* seed germination assay is a simple, reliable and inexpensive technique for screening temperature tolerance in ornamental peppers. The weak correlation observed between seed and physiological parameters (determined in experiment 1) and pollen-based parameters indicate that reproductive and physiological or vegetative tolerance operate differently to temperature, and screening based on pollen parameters will be more useful and appropriate in discriminating reproductive tolerance to temperature in ornamental peppers.

CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

Two experiments were conducted with the overall objective of understanding cultivar differences for heat and cold tolerances using pollen germination, physiological and seed germination parameters in 12 ornamental pepper cultivars with varied morpho-physiological characteristics. In experiment I, 12 ornamental pepper cultivars were screened for temperature tolerance using pollen and physiological parameters while in experiment II, seed germination cardinal temperatures and temperature adaptability range (TAR, Tmax – Tmin) were used to identify tolerance variation to temperature among the same cultivars.

Modified bilinear best described temperature-pollen germination and pollen tube length response functions. The CTRI (unit less) for each cultivar was calculated as the sum of 12 individual temperature responses derived from pollen viability, maximum PG, maximum PTL, T_{min}, T_{opt}, and T_{max} for PG and PTL, CMT, CTD and CSI, and used to classify cultivars for temperature tolerance.

Based on CTRI-heat, 'Black Pearl', 'Red Missile', and 'Salsa Yellow' were heat sensitive, 'Calico', 'Purple Flash', 'Sangria', and 'Variegata' were heat-intermediate, and 'Chilly Chili', 'Medusa', 'Thai Hot', 'Explosive Ember', and 'Treasures Red' were heat tolerant. Based on CTRI-cold, 'Medusa', 'Treasures Red', and 'Thai Hot' were cold sensitive, ‘Chilly Chili’, ‘Purple Flash’, ‘Variegata’, and ‘Explosive Ember’, were moderately cold sensitive and ‘Black Pearl’, ‘Calico’, and ‘Sangria’, were moderately cold tolerant and ‘Red Missile’ and ‘Salsa Yellow’, cold tolerant.

In experiment II, Quadratic and bilinear models best described the seed germination rate and maximum seed germination response to temperature, respectively. Seed based CTRI, of each cultivar was used to classify cultivars for temperature tolerance. Cultivars were classified based on CTRI (heat) as heat sensitive, ‘Purple Flash’, ‘Black Pearl’, ‘Salsa Yellow’, ‘Chilly Chili’, ‘Explosive Ember’, ‘Calico’, and ‘Sangria’, heat intermediate, ‘Red Missile’, ‘Thai Hot’, and ‘Variegata’ and heat tolerant, ‘Treasures Red’ and ‘Medusa’. Based on CTRI (cold) cultivars ‘Medusa’, ‘Treasures Red’, ‘Thai Hot’ and ‘Variegata’ were classified as cold sensitive, ‘Red Missile’, ‘Purple Flash’, ‘Salsa Yellow’ and ‘Chilly Chili’, as moderately cold sensitive, ‘Black Pearl’, ‘Explosive Ember’, and ‘Calico’ as moderately cold tolerant, and ‘Sangria’ as cold tolerant.

CTRI heat and cold (seed) showed a significant linear correlation (r = 0.78 and -0.80, respectively) with physiological based CTRI (heat and cold) whereas a weak (r = 0.26) correlation found between pollen- and seed-based CTRI. However, cultivars tested in the study can be summarized further based on their pollen and physiological based CTRI and seed based CTRI to gain a quick understanding of their degree of temperature tolerance with respect to both pollen and physiological and seed germination related parameters (Table 5.1). This information is helpful to growers to identify a suitable cultivar based on their desire. Ornamental pepper cultivars were highly responsive to temperature and the variability among cultivars was captured in this study. Screening based on seed germination is a simple, inexpensive and reliable measure of screening vegetative temperature tolerance in ornamental peppers. Further, screening based on pollen viability and tube growth was found to be a reliable method for testing reproductive temperature tolerance in ornamental peppers. The identified heat- and cold-tolerant cultivars are potential candidates in breeding programs to develop new pepper genotypes for high and low temperature environments and in selecting cultivars for a niche environment. However, in order to make a complete understanding of those cultivars temperature tolerant capacity, an extended study in field condition and controlled environmental condition of several aspects will be desirable.

Table 5.1. Classification of cultivars into heat and cold tolerant groups using pollen and physiological based CTRI and seed germination based CTRI values.

Cultivar	Seed germination CTRI		Pollen and physiological CTRI	
	Heat [Z]	Cold [Y]	Heat [Z]	Cold [Y]
'Black Pearl'	HS	MCT	HS	MCT
'Calico'	HS	MCT	IHT	MCT
'Chilly Chili'	HS	MCS	HT	MCS
'Explosive Ember'	HS	MCT	HT	MCS
'Medusa'	HT	CS	HT	CS
'Purple Flash'	HS	MCS	IHT	MCS
'Red Missile'	IHT	MCS	HS	CT
'Salsa Yellow'	HS	MCS	HS	CT
'Sangria'	HS	CT	IHT	MCT
'Thai Hot'	IHT	CS	HT	CS
'Treasures Red'	HT	CS	HT	CS
'Variegata'	IHT	CS	IHT	MCS

[Z] HT = Heat tolerant, IHT = Intermediate heat tolerant, HS= Heat sensitive,
[Y] CT = Cold tolerant, MCT = Moderately cold tolerant, MCS = Moderately cold sensitive, CS = Cold sensitive

REFERENCES

Alm, D.J., M.E. McGiffen, and J.D. Hesketh. 1991. Weed phenology. p. 191-218 *In* T. Hodges (ed.) Predicting crop phenology. CRC Press, Boca Raton, FL.

Aloni, B., T. Pashkar, and L. Karni. 1991. Partitioning of [14C] sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Annals of Botany 67:371-377.

Aloni, B., M. Peet, M. Pharr, and L. Karni. 2001. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum* L.) pollen in relation to its germination. Physiologia Plantarum 112:505-512.

Alsadon, A.A., M.A. Wahb-allah, and S.O. Khalil. 2006. *In vitro* evaluation of heat stress tolerance in some tomato cultivars. Journal of King Saud University 19:13-24.

Anjum, F., M.Yaseen, E. Rasool, A.Wahid, and S. Anjum. 2003. Water stress in Barley (*Hordeum vulgare*. L) II. Effect on chemical composition and chlorophyl contents. Pakistan Journal of Agricultural Sciences 40:45-49.

Armitage, A. and B. Hamilton. 1987. Ornamental peppers: A hot new crop. Greenhouse Grower 5:92-95.

Aslam, M., M. Brown, and R. Kohel. 1964. Evaluation of seven tetrazolium salts as vital pollen stains in cotton, *Gossypium hirsutum* L. Crop Science 4:508–510.

Ball, V. 1998. Capsicum. p. 417-418. *In* V. Ball (ed.). Ball red book. Ball Publishing, Batavia, IL.

Balota, M., I. Amani, M.P. Reynolds, and E. Acevedo. 1993. Evaluation of membrane thermostability and canopy temperature depression as screening traits for heat tolerance in wheat. Wheat special report No. 20. CIMMYT, Mexico, DF.

Balota, M., W.Payne, S. Evett, and M. Lazar. 2007. canopy temperature depression sampling to assess grain yield and genotypic differentiation in winter wheat. Crop Science 47:1518-1529.

Baskin, C. and J. Baskin. 2001. Seeds: Ecology, biogeography, and evolution of dormancy and germination, Academic Press, San Diego, CA.

Benech-Arnold, R.L., C. M. Ghersa, R. A. Sanchez, and P. Insausti. 1990. Temperature effects on dormancy release and germination rate in *Sorghum halepense* (L.) Pers. seeds: A quantitative analysis. Weed Research. 30:81-89.

Berjano, R., C. de Vega, M. Arista, P. Ortiz, and S. Talavera. 2006. A multi-year study of factors affecting fruit production in *Aristolochia paucinervis* (Aristolochiaceae). American Journal of Botany 93:599-606.

Berke, T., L. Black, R. Morris, N. Talekar, and J. Wang. 2003. Suggested cultural practices for sweet pepper. International cooperators guide. Available at http://www.avrdc.org/LC/pepper/swtpepper.pdf (verified November 20, 2009). AVRDC Publication No. 99-497R, Shanhua, Taiwan.

Berry, J. and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31:491-543.

Bewley, J.D. 1997. Seed germination and dormancy. The Plant Cell 9:1055-1066.

Bewley, J.D. and M. Black. 1994. Seeds physiology of development and germination. Plenum Press, New York.

Bibi, A.C., D.M. Oosterhuis, R.S. Brown, E.D. Gonias, and F.M. Bourland. 2003. The physiological response of cotton to high temperatures for germplasm screening. Summaries of Arkansas cotton research 2003. AAES Research Series 521:87-93.

Bierhuizen, J.F., W.A. Wagenvoort, and H. Nilwik. 1978. Analysis of the relationship between temperature and germination of sweet pepper. Acta Horticulturae 83:195-200.

Boe, A. and J.G. Ross 1998. Registration of 'Sunburst' switchgrass. Crop Science 38:540-540.

Bolat, I. and L. Pirlak. 1999. An investigation of pollen viability, germination and tube growth in some stone fruits. Journal of Agriculture and Forestry 23:383-388.

Bosland, P.W. and E. Votava. 2000. Peppers: Vegetable and spice capsicums, CABI Publishing, New York.

Bradford, K. 1990. A water relations analysis of seed germination rates. Plant Physiology 94:840-849.

Bradford, K. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. Weed Science 50:248-260.

Carter, A.K. and C. S. Vavrina. 2001. High temperature inhibits germination of Jalapeno and Cayenne pepper. HortScience 36:724-725.

Cheikh, N., P.W. Miller, and G. Kishore. 2000. Role of biotechnology in crop productivity in a changing environment. p. 425-436. *In* Reddy, K.R. and H.F. Hodges (ed.). Climate change and global crop productivity. CAP International, Oxon, UK.

Covell, S., R. H. Ellis, E. H. Roberts, and R. J. Summerfield. 1986. The influence of temperature on seed germination rate in grain legumes I. A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. Journal of Experimental Botany 37:705-715

Dafni, A. and D. Firmage. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. Plant Systematics and Evolution 222:113-132

Damania, A.B. 1986. Inhibition of seed germination in Lettuce at high temperature. Seed Research 14:177-184.

Doherty, R. M., L.O. Mearns, K.R. Reddy, M. Downton, and L.M. Daniel. 2003. Spatial scale effects of climate scenarios on simulated cotton production in the southeastern USA. Climatic Change 60:99-129.

EI-Khawas, S.A. 2004. Phyisiological and biological adaptation of *Triticum vulgaris* L. to pH stress by hormonal application. Pakistan Journal of Biological Sciences 7:852-860.

Ellis, R.H., S. Covell, E.H. Roberts, and R.J. Summerfield 1986. The influence of temperature on seed germination rate in grain legumes II. Intraspecific variation in chickpea (*Cicer arietinum* l.) at constant temperatures. Journal of Expermental Botany 37:1503-1515.

Ellis, R.H., T.D. Hong, and E.H. Roberts. 1987. Comparison of cumulative germination and rate of germination of dormant and aged barley seed lots at different constant temperatures. Seed Science and Technology 15:717-727.

Ellis, R.H., G. Simon., and S. Covell. 1987. The influence of temperature on seed germination rate in grain legumes III. A comparison of five faba bean genotypes at constant temperatures using a new screening method. Journal of Experimental Botany 38:1033-1043.

Erickson, A.N. and A.H. Markhart. 2001. Flower production, fruit set, and physiology of bell pepper during elevated temperature and vapor pressure deficit. Journal of American Society for Horticultural Science 126:697-702.

Erickson, A. and A. Markhart. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant, Cell and Environment 25:123-130.

Erickson, A.N and A.H. Markhart. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant, Cell and Environment 25:123-130.

Fry, K. 1983. Heat-unit calculations in cotton crop and insect models. USDA-ARS, Advances in Agricultural Technology, Fresno, Western Series 23.

Farrar, J. F. 1988. Temperature and the partitioning and translocation of carbon p. 203-235. *In* Plants and Temperature, S.P. Long and F.I. Woodward Eds. The Company of Biologists Limited, Cambridge.

Feng, B., H.Yu, Y.Hu, X.Gao, J.Gao, D.Gao, and S. Zhang. 2009. The physiological charactersitics of low canopy temperature Wheat (Triticum aestivum L.) genotypes under simulated drought condition. Acta Physiol Plant 31:1229-1235.

Feng, H., L.An, L.Tan, Z.Hou, and X.Wang. 2000. Effect of enhanced ultraviolet-B radiation on pollen germination and tube growth of 19 taxa *in vitro*. Environmental and Experimental Botany 43:45-53.

Fenner, M. 2008. The effects of the parent environment on seed germinability. Seed Science Research. 1:75-84.

Fry, K.E. 1983. Heat-unit calculations in cotton crop and insect models. USDA-ARS, Advances in Agricultural Technology, Fresno, Western Series 23:30-31.

Garcia-Huidobro, J., J. Monteith, and G. Squire. 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides*): Constant temperature. Journal of Experimental Botany 33:288-296.

Geetha, K., S. Vijayabaskaran, and N. Jayaraman. 2004. *In vitro* studies on pollen germination and pollen tube growth in maize. Food, Agriculture and Environment 2:205-207.

Guilioni, L., J. Wery, and F. Tardieu. 1997. Heat stress induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs. Annals of Botany 80:159-167.

Gummerson, R. 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. Journal of Experimental Botany 37:729-741.

Gupta, S., J. Swan, and E. Schneider. 1988. Planting depth and tillage interactions on corn emergence. Soil Science Society of America Journal 52:1122-1127.

Gusta, L.V. and T.H.H. Chen. 1987. Physiology of water and temperature stress, In: E. G. Heyne (Ed.), ASA, SSA, and SSSA, Madison, WI. pp. 115-149.

Hall, A. 1990. Breeding for heat tolerance: An approach based on whole-plant physiology. HortScience 25:17-19.

Hall, A.E. 1992. Breeding for heat tolerance. Plant Breeding Reviews 10:129-168.

Hall, A.E. 1992. Plant breeding reviews, volume 10, John Wiley and Sons Inc., Malden, MA.

Hall, A. 2001. Crop Responses to Environment: Considerations of crop responses to environment in plant breeding. CRC Press, Baco Ratan, FL.

Hall, A.E. and L.H. Ziska. 2000. Crop breeding strategies for the 21st century. pp 407 – 423. *In* Reddy, K.R., and H.F. Hodges (ed.). Climate change and global crop productivity. CAB International, Oxon, UK.

Hansen, J. and M. Sato. 2004. Greenhouse gas growth rates. Proceedings of the National Academy of Sciences of the United States of America 101:16109-16114.

Harthun, E. 1991. Ornamental peppers and Christmas cherry. p. 670-672. *In* Ball (ed.). Ball Red Book. Geo. J. Ball Publishing, Chicago, IL.

Havaux, M. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. Plant, Cell and Environment 16:461-461.

Havaux, M. and F. Tardy. 1996. Temperature-dependent adjustment of the thermal stability of photosystem II *in vivo*: Possible involvement of xanthophylls content. Plant, Cell Environment 19:1359-1368.

Herrero, M. and R. Johnson. 1980. High temperature stress and pollen viability of maize. Crop Science 20:796-800.

Hormaza, J. and M. Herrero. 1996. Male gametophytic selection as a plant breeding tool. Scientia Horticulturae 65:321-333.

Hussein, M., L. Balbaa, and M. Gaballah. 2007. Developing a salt tolerant cowpea using alpha tocopherol. Journal of Applied Sciences Research 3:1234-1239.

Hsu, F.H., C. J. Nelson, and A.G. Matches. 1985. Temperature effects on germination of perennial warm-season forage grasses. Crop Science 25:215-215.

Huner, N.P.A. 1985. Morphological, anatomical, and molecular consequences of growth and development at low temperature in *Secale cereale* L., cv. Puma. American Journal of Botany 72:1290-1306.

IPCC, 2007. Intergovernmental panel on climate change report: Synthesis: Summary for policy makers, IPCC, World Meteorological Organization, Switzerland.

Jifon, J., K. Crosby, and D. Leskovar. 2004. Heat stress tolerance in closely related genotypes of Habanero pepper (*Capsicum chinensis* Jacq.) p. 10. *In* 17^{th} international pepper conference, Naples, FL. Kakani, V. G. and K. R. Reddy. 2007. Temperature response of C_4 species big bluestem (*Andropogon gerardii*) is modified by growing carbon dioxide concentration. Environmental and Experimental Botany 61:281-290.

Jordan, G.L. and M. R. Haferkamp.1989. Temperature responses and calculated heat units for germination of several range grasses and shrubs. Journal of Range Management 42:41-45.

Kafizadeh, N., J. Carapetian, and K.M. Kalantari. 2008. Effects of heat stress on pollen viability and pollen tube growth in Pepper. Research Journal of Biological Sciences 3:1159-1162.

Kakani, V., P. Prasad, P. Craufurd, and T. Wheeler. 2002. Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant, Cell and Environment 25:1651-1661.

Kakani, V.G. and K.R Reddy. 2007. Temperature response of C_4 species big bluestem (*Andropogon gerardii*) is modified by growing carbon dioxide concentration. Environmental and Experimental Botany 61: 281-290.

Kakani, V.G., K.R. Reddy, S. Koti, T.P. Wallace, P.V.V. Prasad, V.R. Reddy, and D. Zhao. 2005. Differences in *in vitro* pollen germination and pollen tuber growth of cotton cultivars in response to high temperature. Annals of Botany 96:59-67.

Kaloyereas, S. 1958 A new method of determining drought resistance. Plant Physiology 33:232-233.

Karim, M., Y. Fracheboud, and P. Stamp. 2000. Effect of high temperature on seedling growth and photosynthesis of tropical maize genotypes. Journal of Agronomy and Crop Science 184:217-223.

Karni, L. and B. Aloni. 2002. Fructokinase and hexokinase from pollen grains of bell pepper (*Capsicum annuum* L.): Possible role in pollen germination under conditions of high temperature and CO_2 enrichment. Annals of Botany 90:607-610.

Koti, S., K. Reddy, V. Reddy, V. Kakani, and D. Zhao. 2005. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. Journal of Experimental Botany 56:725-736.

Kotowski, F. 1926. Temperature relations to germination of vegetable seed. Proceedings of American Society of Horticulture Science 23:176-184.

Kuo, C., H. Chen, and H. Sun. 1993. Membrane thermostability and heat tolerance of vegetable leaves. Adaptation of Food Crops to Temperature and Water Stress. Shanhua, Taiwan: Asian Veg. Res. Dev. Center:160-168.

Lincoln, R., G. Boxshall, and P. Clark. 1998. A dictionary of ecology, evolution and systematics. Cambridge University Press, Cambridge, UK.

Lindgren, D., L. Paule, S. Xihuan, R. Yazdani, U. Segerström, J. Wallin, and M. Lejdebro. 1995. Can viable pollen carry Scots pine genes over long distances? Grana 34:64-69.

Liu, Z., Y. Yuan, S. Liu, X. Yu, and L. Rao. 2006. Screening for high-temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. Journal of Integrative Plant Biology 48:706-714.

Lobell, D.B. and G.P. Asner. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. Science 299:1032.

Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant, Cell and Environment 14:729-739.

Lichtenthaler, H. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382.

Lu, Z., R. Percy, C. Qualset, and E. Zeiger. 1998. Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. Journal of Experimental Botany 49:453-460.

Luza, J., V. Polito, and S. Weinbaum. 1987. Staminate bloom date and temperature responses of pollen germination and tube growth in two walnut (*Juglans*) species. American Journal of Botany 74:1898-1903.

Martineau, J., J. Williams, and J. Specht. 1979. Temperature tolerance in soybeans. II. Evaluation of segregating populations for membrane thermostability. Crop Science 19:79-81.

Maynard, D.N. and G.J. Hochmuth. 1997. Knott's Handbook for Vegetable Growers. Wiley, New York.

Mearns, L., W. Easterling, C. Hays, and D. Marx . 2001. Comparison of agricultural impacts of climate change calculated from high and low resolution climate change scenarios: Part I. The uncertainty due to spatial scale. Climatic Change 51:131-172.

Mearns, L.O., M. Hulme, T.R. Carter, R. Leemans, M. Lal, and P. Whetton. 2001. The Scientific Basis, Cambridge University Press, UK

Meehl, G. and C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305:305:994.

Mercado, J., M. Reid , V. Valpuesta, and M. Quesada. 1997. Metabolic changes and susceptibility to chilling stress in Capsicum annuum plants grown at suboptimal temperature. Australian Journal of Plant Physiology 24:759-768.

Mione, T. and G. Anderson. 1992. Pollen ovule ratios and breeding system evolution in *Solanum* section Basarthrum (Solanaceae). American Journal of Botany 79:279-287.

Misra, N. and U.N. Dwivedi. 2004. Genotypic difference in salinity tolerance of green gram cultivars. Plant Science 166:1135-1142.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends Plant Science 11:15-19.

Mohan, M.M., S.L. Narayanan, and S.M. Ibrahim . 2000. Chlorophyl stability index (SCI): Its impact on salt tolerance in rice. Crop Management and Physiology 25:38-40.

Murty, K. and S. Majumdar. 1962. Modification of the technique for determination of chlorophyll stability index in relation to studies of drought resistance in rice. Current Science 32:470-471.

Nyarko, G., P.G. Alderson, J. Craigon, E. Murchie, and D.L. Sparkes. 2008. Comparison of cell membrane thermostability and chlorophyll fluorescence parameters for the determination of heat tolerance in ten cabbage lines. The Journal of Horticultural Science and Biotechnology 83:678-682.

O'Sullivan, J. and W. Bouw. 1984. Pepper seed treatment for low-temperature germination. Canadian Journal of Plant Science 64:387-393.

Peet, M.M., S. Sato, and R.G. Gardner. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. Plant, Cell Environment 21:225-231.

Polowick, P. and V. Sawhney. 1986. A scanning electron microscopic study on the initiation and development of floral organs of *Brassica napus* (cv. Westar). American Journal of Botany 73:254-263.

Porch, T. and M. Jahn. 2001. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. Plant, Cell and Environment 24:723-731.

Prasad, P.V.V., K.J. Boote, L.H. Allen, Jr., and J.M.G. Thomas. 2002. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.) Global Change Biology 8:710-721.

Prasad, V., V. Pagadala, P. Craufurd, R. Summerfield, and T. Wheeler. 2000. Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). Journal of Experimental Botany 51:777-784.

Probert, R. 2000. The role of temperature in the regulation in seed dormancy and germination. P. 261-292. *In.* M. Fenner (ed) Seeds: The ecology of regeneration in plant communities, 2nd ed. CABI Pub., Wallingford, UK.

Purseglove, J. 1987. Tropical crops: Dicotyledons (volumes 1 and 2 combined). Longman, Essex, UK.

Raison, J., C. Pike, and J. Berry. 1982. Growth temperature-induced alterations in the thermotropic properties of *Nerium oleander* membrane lipids. Plant Physiology 70:215-718.

Reddy, K.R., H.F. Hodges, and B.A. Kimball. 2000. Crop response to elevated carbon dioxide and interaction with temperature: Cotton P. 161-187. *In* K.R. Reddy and H.F. Hodges (eds.) Climate change and global crop productivity. CABI Publishing, New York, NY.

Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1997. Crop modeling and applications: a cotton example. Advances in Agronomy 59:225-290.

Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1997. A comparison of scenarios for the effect of global climate change on cotton growth and yield. Australian Journal of Plant Physiology 24: 707-713.

Reddy, K.R. and V.G. Kakani. 2007. Screening *Capsicum* species of different origins for high temperature tolerance by *in vitro* pollen germination and pollen tube length. Scientia Horticulturae 112:130-135.

Reynolds, M.P., R.P. Singh, A. Ibrahim, O.A.A. Ageeb, A. Larque-Saavedra, and J.S. Quick. 1998. Evaluating the physiological traits to complement empirical selection for wheat in warm environments. Euphytica 100: 85-94.

Reynolds, M., S. Nagarajan, M. Razzaque, and O. Ageeb. 2001. Heat tolerance. p. 124-135. *In* M.P. Reynolds, O. M., Monasterio, and O. McNab (eds.) Application of physiology in wheat breeding. CMMYT, Mexico.

Roberts, E.H. 1988. Temperature and seed germination, p. 109-132. *In* S. P. Long and F. I. Woodword (Eds.), Plants and temperature, Symp. Soc. Exp. Biol., Company of Biologists, Cambridge, UK.

Rubatzky, V.E. and M. Yamaguchi. 1997. World vegetables: Principles, production, and nutritive values. Chapman and Hall, New York.

Rylski, I. and M. Spigelman. 1982. Effects of different diurnal temperature combinations on fruit set of sweet pepper. Scientia Horticulturae 17:101-106.

Saadalla, M.M., J.F.Shanahan, and J.S. Quick. 1990. Heat tolerance in winter Wheat: I. Hardening and genetaic effects on membrane thermostability Crop Science 30:1243-1247.

Sadasivam, S., R.C. Babu, M. Raveendran, and J.A.J. Raja. 2000. Genetic variation in seed germination, root traits and drought recovery in rice. Indian Journal of Plant Physiology 5:73-78.

Saini, H. 1997. Effects of water stress on male gametophyte development in plants. Sexual Plant Reproduction 10:67-73.

Sairam, R.K., P.S. Deshmukh, and D.S. Shukla. 2008. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. Journal of Agronomy and Crop Science 178:171-178.

Salem, M.A., V.G. Kakani, S. Koti, and K.R. Reddy. 2007. Pollen-Based Screening of Soybean Genotypes for High Temperatures. Crop Science 47:219-231.

Sato, S., M. Peet, and J. Thomas. 2002. Determining critical pre-and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. Journal of Experimental Botany 53:1187-1195.

Schimpf, D.J., S.D. Flint, and I.G. Palmblad. 1977. Representation of germination curves with the logistic function. Annals of Botany 41:1357-1360.

Sharkey, T. 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. Plant, Cell and Environment 28:269-277.

Shafii, B. and W. Price. 2001. Estimation of cardinal temperatures in germination data analysis. Journal of Agricultural, Biological, and Environmental Statistics 6:356-366.

Sharma, S.K. 2008. Crops tolerance to waterlogging, salinity and alkalinity stresses and their Interactions and physiological mechanisms.

Singh, S., V.G. Kakani, D. Brand, B. Baldwin, and K. Reddy. 2008a. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. Journal of Agronomy and Crop Science 194:225-236.

Singh, S., G. Surabhi, W. Gao, and K. Reddy. 2008b. Assessing genotypic variability of cowpea (*Vigna unguiculata* [L.] Walp.) to current and projected ultraviolet-B radiation. Journal of Photochemistry and Photobiology, B: Biology 93:71-81.

Singh, S., G. Surabhi, W. Gao, and K. Reddy. 2008c. Assessing genotypic variability of cowpea (*Vigna unguiculata* [L.] Walp.) to current and projected ultraviolet-B radiation. Journal of Photochemistry and Photobiology, B: Biology 93:71-81.

Singh, R.P., P.V. Vara Prasad, K. Sunita, S.N. Giri, and K.R. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton: A Review. Advances in Agronomy 93:313-386.

Sivasubramaniam, K. 1992. Chlorophyll stability index methods determining drought hardiness in *Acacia* species. Nitrogen FixingTree Research Reports 10:111-112.

Smith-Huerta, N. and F. Vasek. 1984. Pollen longevity and stigma pre-emption in Clarkia. American Journal of Botany 71:1183-1191.

Srinivasan, A., H. Takeda, and T. Senboku. 1996. Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. Euphytica 88:35-45.

Stainforth, D., T. Aina, C. Christensen, M. Collins, N. Faull, D. Frame, J. Kettleborough, S. Knight, A. Martin, and J. Murphy. 2005. Uncertainty in predictions of the climate response to rising levels of greenhouse gases. Nature 433:403-406.

Stone, P. 2001. The effects of heat stress on cereal yield and quality. p. 243-291 *In* A.S. Basra, (ed.) Crop responses and adaptations to temperature stress. Food Products Press, Binghamton, NY.

Stummel, J.R and P. Bosland. 2007. Ornamental pepper *Capsicum annuum*. p. 561-599. *In* N. O. Anderson (ed.) Flower breeding and genetics: Issues, challenges and opportunities for the 21st century. Stringer, Dordrecht, The Netherlands.

Stummel, J.R. and R.J. Griesbach. 2005. *Capsicum annuum* L. 'Black Pearl'. HortScience 40:1571-1573.

Sullivan, C.Y. (ed.). 1972. Sorghum in the Seventies. Oxford and IBH Publishing Co., New Delhi, India.

Suzuki, K., H. Takeda, T. Tsukaguchi, and Y. Egawa. 2001. Ultrastructural study on degeneration of tapetum in anther of snap bean (*Phaseolus vulgaris* L.) under heat stress. Sexual Plant Reproduction 13:293-299.

Taiz, L. and E. Zeiger. 2002. Plant Physiology, Sunderland Massachusetts Sinauer Associates. Inc., Sunderland, MA.

Taylor, L. and P. Hepler. 1997. Pollen germination and tube growth. Annual Review of Plant Biology 48:461-491.

Thygerson, T., J. Harris, B. Smith, L. Hansen, R. Pendleton, and D. Booth. 2002. Metabolic response to temperature for six populations of winterfat (*Eurotia lanata*). Thermochimica Acta 394:211-217.

Tiryaki, I. and D.J. Andrews. 2001. Germination and seedling cold tolerance in sorghum: II. Parental lines and hybrids. Agronomy Journal. 93:1391-1397.

Tokumasu, S., I. Kanada, and M. Kato. 1985. Germination behavior of seeds as affected by different temperatures in some species of Brassica. Journal of Japanese Society of Horticultural Science. 54 (3):364-370.

USDA. 2008. Floriculture crops 2007 summary, United States Department of Agriculture, National agriculture statistical service. Available at http://usda.mannlib.cornell.edu/usda/current/FlorCrop/FlorCrop-04-24-2008.pdf. (verified November 17, 2008).

Vaughton, G. and M. Ramsey. 1991. Floral biology and inefficient pollen removal in *Banksia spinulosa* var. Neoanglica (Proteaceae). Australian Journal of Botany 39:167-177.

Vaughton, G and M. Ramsey. 1998. Sources and consequences of seed size variation in *Banksia marginata* (Proteaceae). Journal of Ecology 86:563-573.

Vavrina, C.S. 1994. Plastic tray inserts. Florida Vegetable Transplant Growers News 5:3-4.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: an overview. Environmental and Experimental Botany 61:199-223.

Weaich, K., K. Bristow, and A. Cass. 1996. Modeling pre-emergent maize shoot growth: II. High temperature stress conditions. Agronomy Journal 88:398-403.

Weaver, M.L. 1989. Screening tomato for high temperature tolerance through pollen viability tests. HortScience 24:493-495.

Willits, D. and M. Peet. 1998. The effect of night temperature on greenhouse grown tomato yields in warm climates. Agricultural and Forest Meteorology. 92:191-202.

Young, L., R. Wilen, and P. Bonham-Smith. 2004. High temperature stress of *Brassica napus* during flowering reduces micro-and megagametophyte fertility, induces fruit abortion, and disrupts seed production. Journal of Experimental Botany 55:485-495.

Zhang, J. H. 1996. Seed mass effect across environments in an annual dune plant. Annals of Botany 77:555-563.