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SWITCHGRASS (*Panicum virgatum* L.) INTRASPECIFIC VARIATION AND TEMPERATURE TOLERANCE CLASSIFICATION USING *IN VITRO* SEED GERMINATION ASSAY

By

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An experiment was conducted to determine temperature effects on switchgrass seed germination, a native species with feedstock potential for the biofuel industry. Stratified seeds were germinated at constant temperatures, 15 to 45°C with 5°C interval. Maximum seed germination (MSG) and germination rate (GR), estimated by fitting sigmoid function to germination-time series data, varied among genotypes. Quadratic and bilinear models best described the MSG and GR responses to temperature, respectively. The mean cardinal temperatures, minimum, optimum and maximum, were 8.1, 26.6 and 45.1ºC for MSG and 11.1, 33.1 and 46.0ºC for GR, respectively, varied among genotypes. Genotypes were classified for temperature tolerance based on cumulative temperature response index: ‘Summer’ and ‘Expresso’ were identified as the most heat- and cold-tolerant genotypes, respectively. The functional algorithms and identified tolerant genotypes may be used to improve switchgrass models for field

applications and breeding programs to develop new genotypes with enhanced tolerance for niche environments.

DEDICATION

I dedicate this thesis to my father Jadoo Bance Seepaul and mother Rajpattie Seepaul for their enduring, unrelinquished and unconditional love and support throughout my life.

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"*Knowledge is in the end based on acknowledgement.*" Ludwig Wittgenstein

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CHAPTER I

INTRODUCTION

Global climate change, negative environmental consequences of unabated fossil fuel-use coupled with dwindling and unstable supply of petroleum fuels provided impetus for a renewable energy source. Global surface temperatures, influenced by both anthropogenic and natural factors, increased by 0.2°C per decade between 1950 and 1993 and are projected to increase by 2 – 4.5°C by 2100 (Meehl et al., 2007). Associated with projected temperature increases are changes in precipitation intensity and frequency, decreased seasonal and perennial snow and ice extent, and sea level rise, factors which may revolutionize global agricultural production systems in an attempt to adapt and mitigate the effects of climate change. The combined effects of elevated temperatures and reduced crop-water availability stemmed increased droughts, which may have significant impacts on global agriculture (Chiotti and Johnston, 1995) affecting yield, productivity and food security. Smit and Skinner (2002) suggested four agricultural adaptation measures to abate the effects of climate change including: (1) technological developments, (2) government programs and insurance, (3) farm production practices, and (4) farm financial management. Of agronomic importance is the development of new crop varieties that are tolerant to temperature, moisture, and other conditions associated

with climate change via breeding, selection or genetic engineering is a direct adaptive measure to abate the effects of climate change.

Coupled with climate change is the unabated and record increase in oil prices, national security implications for U.S. foreign energy dependence, which created an impetus for developing a domestic, renewable energy source in the United States (Khanna et al., 2008). Perennial grass-based energy has been purported to have significant environmental and economic benefits to society (Liebig et al., 2005), including reducing national dependence on foreign fuel, abate greenhouse gas emissions through carbon sequestration and revitalize rural economies (DOE, 2006). In 1978, switchgrass (*Panicum virgatum* L.) was identified as a model lignocellulosic biofuel feedstock by the Department of Energy's Bioenergy Feedstock Development Program (BFPD) after evaluation of yield and agronomic characteristics on 34 candidate species at the Oak Ridge National Laboratory (McLaughlin and Walsh, 1998). Switchgrass is a highly diverse species with significant genetic (Das et al., 2004) and phenotypic variation resulting from gene migration, random genetic drift, mutation, natural selection (Eberhart and Newell, 1959) combined with environmental variation due to latitude, altitude, soil type, and precipitation (Casler et al., 2007).

The adoption of a feedstock species is hinged on its ability to grow and sustain under a wide range of growing conditions and its capacity to produce high yields and quality biomass. From an agronomic perspective, the species should also be able to establish rapidly and uniformly under existing conditions to escape weed competition and late-season water unavailability (Hacisalihoglu, 2008). Establishment of warm-season

forage grasses has been limited due to slow germination and low seedling vigor (Hsu et al., 1984, Aiken and Springer, 1995), particularly in the first year after seeding, presenting a major problem in the improvement of existing pastures, or in establishing pasture land currently used for row crops (Perry and Moser, 1975). Slight or moderate successes of native grasses establishment can be attributed to dormancy and delayed germination (Robocker et al., 1953). Seeding pastures or feedstock fields require knowledge of many parameters, including optimum temperature and moisture conditions for rapid germination and establishment (Fulbright, 1988, Hanson and Johnson, 2005). Some other factors which may affect switchgrass establishment include variation in seed size and dormancy, seedling survival rate, and seedling emergence (Hanson and Johnson, 2005).

Temperature is a major environmental factor influencing seed germination capacity and rate, and seedling vigor (Hsu et al., 1984). Temperature affects the maximum seed germination and rate of germination through three distinct processes: its effect on seed deterioration (seed aging), dormancy loss, and on the germination process itself (Roberts, 1988). Extreme temperatures are the single most important factor delimiting the distribution, adaptability and yield potential of plants. High or low soil temperatures in the semi-arid tropics or temperature conditions at sowing can reduce plant populations at extreme temperatures necessitating seed temperature tolerance for adequate crop establishment. Determining temperature effects on germination using mathematical functions may be useful in evaluating germination characteristics or establishment potential among genotypes or species (Jordan and Haferkamp, 1989).

Final germination percentage and germination rate are both considered sensitive indicators of seed vigor (Larsen and Andreasen, 2004). Germination as a process may be defined by three cardinal temperatures (T_{min}, T_{max} and T_{opt}) that determines the range of temperatures over which germination can occur. Previous studies that reported the effects of temperature on switchgrass germination capacity and rate did not quantify these effects for a diverse population of switchgrass genotypes. Parrish and Fike (2005) reported that switchgrass germinates slowly when the temperature is below 15.5°C with maximum germination occurring within 3 d of imbibition at 29.5°C. Hsu et al. (1985) reported that the minimum temperature for switchgrass germination is10.3°C and optimum temperature occurring between 25 and 30°C. Minimum temperatures are critical for accurate phenological predictions because small differences in temperatures can cause large differences in germination time. Current switchgrass models that simulate switchgrass phenology use blanket minimum temperatures that range from 10 to 12°C (McLaughlin et al., 1999, Heaton et al., 2004, Kiniry et al., 2005). Limited reports are available in the literature on cardinal temperature variability among diverse switchgrass genotypes.

The interest in switchgrass as a feedstock has fostered development and selection of a wide number of genotypes, which must be screened for various abiotic stress tolerances prior to release. Current screening methods are restricted to field performance and visual evaluations which may mask a genotype's true potential or tolerance capacity due to unpredictable moisture and fluctuating temperatures in the field. Field screening for temperature tolerance is tedious, inconsistent, and seasonally limited, therefore the

need for simple, rapid, and reliable techniques to identify sources of tolerance and for evaluating a large number of breeding materials in controlled conditions (Setimela et al., 2005). Screening for abiotic stress tolerance has been achieved using biochemical and physiological parameters at the germination, emergence, vegetative, and reproductive stages. Screening genotypes prior to field testing requires a controlled environment where temperature and moisture are monitored. *In vitro* seed-based screening can provide insights into genotypic environmental adaptability and tolerance capacity prior to field evaluations. Studies related to temperature tolerance screening in switchgrass, however, are limited in general and no reported studies using seed-based parameters have been found. Seed-based parameters, in particular, germination capacity and rate have been used successfully to screen several species and genotypes for various abiotic stress factors including drought (Bouslama and Schapaugh Jr, 1984, Sadasivam et al., 2000), saline (Foolad and Lin, 1997, Misra and Dwivedi, 2004), flooding/water logging (Hou and Thseng, 1992, Sharma, 2008), chilling (Acharya et al., 1983, Tiryaki and Andrews, 2001), and heat tolerance (Emerson and Minor, 1979, Ellis et al., 1986) in other species.

The temperature tolerance capacity of different genotypes may be determined by relative ranking using single value indices, percentiles and quartiles relative to control studies and cumulative indices, grouping based on statistical separation of means (Emerson and Minor, 1979, Koti et al., 2004, Salem et al., 2007) or quantitative relationships determined by principal component analysis (Kakani et al., 2002, Kakani et al., 2005, Singh et al., 2008).

The objectives of this study were to (a) quantify the effects of temperature on seed germination capacity and rate, (b) determine the cardinal temperatures for seed germination capacity and rate, and (c) classify genotypes for temperature tolerance using cumulative temperature response index concept. The seed germination and temperature dependent functional algorithms developed from these data are a prerequisite for modeling the germination of a diverse switchgrass genotypes adapted to different climatic zones.

CHAPTER II

REVIEW OF LITERATURE

Biofuel Justification

Global climate change, negative environmental consequences of unabated fossil fuel-use coupled with dwindling and unstable supply of petroleum fuels provided impetus for a renewable energy source. Global surface temperatures, influenced by both anthropogenic and natural factors, increased by 0.2°C per decade between 1950 and 1993 and are projected to increase by 2 to 4.5°C by 2100 (Meehl et al., 2007). Associated with projected temperature increases are changes in precipitation intensity and frequency, decreased seasonal and perennial snow and ice extent, and sea level rise; factors which may revolutionize global agricultural production systems in an attempt to adapt and mitigate the effects of climate change. Karl and Trenberth (2003) contended that the incessant use of fossil fuels is the primary factor fueling the changes in atmospheric composition, and therefore continued increase in global temperature. Biofuels are "cleaner" relative to fossil fuels with significant reductions in greenhouse gas emissions, carcinogens, particulates, hydrocarbons, and sulfur (Goldemberg et al., 2008). Cellulosic ethanol has been reported to produce 94% less greenhouse gas emissions than gasoline (Schmer, 2008).

Hill et al. (2006) argues that for biofuels to be a viable alternative, it should provide a net energy gain, have environmental benefits, be economically competitive, and be producible in large quantities without reducing food supplies. Perennial grass-based energy has been purported to have significant environmental and economic benefits to society including: near-zero net emission of greenhouse gases, improved soil and water quality, and net economic returns to rural communities (Liebig et al., 2005). The efficiency of energy production for a perennial grass system such as switchgrass can exceed that for an energy-intensive annual row crop such as corn by as much as 15 times (McLaughlin and Walsh, 1998). Atmospheric CO_2 accumulation attributed to fossil fuel combustion can be abated by sequestering large quantities of carbon into the soil ecosystem through appropriate management and by replacing fossil fuels with bioenergy crops (Ma et al., 2001). Switchgrass, because of its 2.5 m deep and productive root system accounting for 72 - 84% of the total biomass when crown tissues are included (Frank et al., 2004, Liebig et al., 2005), may play a key role in soil carbon sequestration (Ma et al., 2001) because at soil depths 30 cm and deeper, carbon is less susceptible to mineralization and loss (Liebig et al., 2005). Annual biomass yields of 5 to 11 Mg ha^{-1} under moderate management practices with average estimated net energy yields of 60 GJ ha^{-1} yr^{-1} have been reported (Schmer, 2008). This is equal to about 540% more renewable than non-renewable energy consumed during the production of the crop.

Switchgrass Description

Switchgrass is a native, erect, warm-season, perennial rhizomatous C_4 grass species widely adapted in the tallgrass prairie ecosystem of the Central Great Plains of United States because of its cold, heat, and drought tolerance attributes (Cassida et al., 2005), and is widely distributed across North America including in areas with highly erodible, marginally fertile, flooded or drought stressed conditions (Casler, 2005). The botanical characteristics of the species have been described by several authors (Silveus, 1933, Hitchcock and Chase, 1971, Gould and Hamilton, 1973, Gould and Kapadia, 1975). Switchgrass adaptation across widely diverse regions and its ability to thrive in low fertility conditions are responsible for its selection as a biofuel feedstock. Switchgrass is the earliest maturing of the warm-season grasses, growing as much as 1.8 to 2.2 m high, but is typically shorter than big bluestem (*Andropogon gerardii* Vitman) or indiangrass (*Sorghastrum nutans* L.). Switchgrass typically initiates growth in late April to early May and flowers in early June and continues into early August. Seeds are dispersed in late September to October in Mississippi. More than 90% of dry matter yield for switchgrass is produced from June to August.

Uses

Switchgrass is suitable for use as an energy feedstock, either for producing ethanol, via bioconversion techniques, or electricity via co-firing with coal (Cassida et al., 2005). It was selected in 1978 as the primary herbaceous bioenergy candidate species for further research and development by the Department of Energy's Bioenergy Feedstock

Development Program (BFDP) after evaluation of yield and agronomic potential on 34 candidate species at the Oak Ridge National Laboratory (McLaughlin and Walsh, 1998). In 1992, the BFDP commenced a 10-year research program concentrated on developing dedicated herbaceous bioenergy crops that were compatible with conventional farming practices.

The selection of switchgrass was based on the following agronomic and production characteristics: broad adaptation and wide geographic distribution; high yields on marginal and erosive lands; compatibility with conventional farm practices; perenniality; high nutrient-use efficiency, hence relatively low fertilizer requirements and high biomass yield production. Its palatability and relatively high quality prior to flowering make switchgrass a productive forage used primarily for summer grazing to supplement the forage deficit caused by low cool-season grass productivity (Anderson and Matches, 1983, Vassey et al., 1985). Switchgrass has also been utilized for planting on land enrolled in the Conservation Reserve Program (Mulkey et al., 2006). Among its other uses include carbon sequestration in permanent grasslands (Ma, 1999, 2000, 2001), erosion control (Ichizen et al., 2001, 2005), riparian buffer strips (Lee, 1999), remediation of contaminated soils (Montez-Ellis et al., 2001, Chen, 2002), and habitat cover in wildlife management areas (Washburn et al., 2000, Murray and Best, 2003, Roth et al., 2005). For wildlife habitats, switchgrass is an excellent seed source for upland, nesting migratory birds and a forage source for game animals, while as a cover; it is especially beneficial in winter due to its standing canopy.

Classification

Based on ploidy levels, molecular markers, habitat preference, zone of adaptation and anatomical and physiological characteristics, switchgrass is classified as morphological types or physiological ecotypes (Cassida et al., 2005); (Table1). Two morphological ecotypes of switchgrass have evolved: lowland and upland ecotypes which are genetically and phenotypically distinct from each other. Lowland ecotypes are tall (60 to 305 cm) and erect, coarse-stemmed, glabrous and more robust, adapted to poor drainage, and found in bunches. Upland ecotypes are short (90 to150 cm), fine-stemmed, semi-decumbent, broad based, have varying amounts of pubescence on the leaf blades, and are known for good drought tolerance.

Switchgrass is highly heterozygous, self incompatible and an out-crossing species characterized by ploidy series ranging from 2n = 2x = 18 to 2n = 12x = 108 (Nielsen, 1944). Upland varieties are tetraploid, hexaploid or octaploid while lowland varieties are tetraploid only (Fike et al., 2006). Being an allogamous species, gene migration is accomplished via pollen or seed resulting in highly heterogenous and variable populations with the potential for natural selection to climatic or edaphic factors (Casler and Boe, 2003, Casler, 2005). Most cultivars are either seed increases from collections or products of a limited number of selection or breeding cycles (Casler and Boe, 2003). Photoperiod sensitivity controls the adaptation regions of switchgrass populations, such that planting more than one zone north of south of the region of adaptation will affect the vigor, survival and flowering.

Table 1. Ploidy level, ecotype, latitude, origin and plant hardiness zone (PHZ) of switchgrass genotypes Table

Genotype	Ploidy Level	Ecotype	Latitude	Origin	PHZ	Remarks	Reference
Alamo	T	lowland		southern TX	6	Selected for biomass	
Blackwell	H	upland	S	Blackwell, OK	5a		Riley and Vogel (1982)
Carthage	O	upland		southern IL			
Cave-in-Rock	H	lowland/ upland	S	Cave-in-Rock, IL	4b		Riley and Vogel (1982)
Dacotah	T	upland		North Dakota	4a	Early maturity, winter hardy, high stand density at northern sites, persistent	Barker et al. (1990)
Expresso		lowland		Mississippi		Selected for improved germination	
Forestburg	T	upland	N	Forestburg, SD	3b-4b	Early, maturity, excellent winter hardiness and persistence, good seed potential	Barker et al. (1988)
Kanlow	T	lowland	N	Wetumka, OK	5		
Shawnee	O	upland	S	Cave-in-Rock, IL		High forage yield and quality	Vogel et al. (1996)
Shelter	H	lowland/ upland	N	St. Mary's, WV	4		Wullschleger et al. (1996)

Table 1. (continued)

Summer	T	upland		Southern NE	4		
Sunburst	H	upland	N	South Dakota		Winter hardy, leafy, heavy-seeded, superior seedling vigor	Boe and Ross (1998); Wullschleger et al. (1996)
Trailblazer	H	upland	N	Nebraska		High forage quality, high IVDMD	Vogel et al. (1991)
Tusca		lowland		Mississippi		Selected for herbicide tolerance from Alamo	

13 Ploidy level (T = tetraploid, H = hexaploid, and O = octaploid), and latitude (S = southern and N = Northern)

Intraspecific Variation

Switchgrass is a highly diverse species with significant genetic (Das et al., 2004) and phenotypic variation (Eberhart and Newell, 1959) resulting from gene migration, random genetic drift, mutation, natural selection combined with environmental variation due to latitude, altitude, soil type and precipitation (Casler et al., 2007). Latitude of origin affects productivity, survival, and adaptation traits of switchgrass (Sanderson and Moore, 1999, Casler et al., 2004). Lowland switchgrass perform better under flooded conditions (Porter Jr, 1966), is more susceptible to drought, have a lower N requirement, and produce greater biomass yields than upland switchgrass genotypes that are more adapted to drier climates. Switchgrass morphological development is largely determined by its response to photoperiod (Vogel et al., 2002). Cassida et al. (2005) contends that optimizing biofuel production and feedstock quality requires harmonizing ecotype and morphological type to environments. Upland varieties grow faster with higher photosynthetic rates within shorter growth cycle as opposed to lowland varieties (Monti et al., 2008). Upland varieties yielded 12.6 versus 15.8 Mg ha^{-1} for lowland cultivars (Fike et al., 2006). There is genetic variation for adaptation among varieties. Upland switchgrass ecotypes tend to be adapted to mid- and northern latitudes of the USA while lowland ecotypes are more inclined to the southern region (Casler, 2005). Genetic variation for photoperiodism, cold and heat tolerance among genotypes may result in the latitudinal adaptation of switchgrass (Casler et al., 2004, 2007). Strains grown in their zone of adaptation tend to have higher relative biomass yield and survival (Casler et al., 2004, 2005, 2007). According to Cassida et al. (2004), lowland populations seem to be

limited in adaptation due to lack of cold tolerance when planted at northern locations, suggesting limited cold tolerance in lowland switchgrass germplasm. In addition, upland ecotypes exhibit higher survival, stand longevity, and sustained biomass yields at northern locations relative to lowland ecotypes. Information is needed; however, for genotype selection based on adaptability in diverse environments and higher biomass production potential (Sanderson et al., 1999).

Seed Germination

Seed germination is a complex biological process, initiating with imbibition by the quiescent dry seed and culminating with the elongation of the embryonic axis (Bewley, 1997). This process, involving complex adaptive traits and regulated by a large number of genes and environmental factors and their interactions (Koornneef et al., 2002), has been studied extensively. The visible sign of germination is the emergence of the radicle, a process that is terminated before seedling emergence. The determinant of germination varies among species since the emergence of either the coleoptile or coleorhizae can take precedence. In addition, there is a divergence in germination definitions adopted by researchers even within a single species. The lengths of both the plumule and the radicle or the ratio between these two structures are usual definitions for germination adopted in the literature.

Most seed undergo a specific sequence of events during germination with the process described as triphasic. Phase 1 is considered to be the activation stage which is typified by imbibition resulting in an increase in the seed volume. In addition to the

uptake of water, enzymes involved in endosperm digestion and other functions are activated and synthesized, respiration increases and cell elongation occurs with the radicle lengthening. Imbibition is the first key event that moves the seed from a dry, quiescent, dormant organism to the resumption of embryo growth. Phase II is considered the digestion and translocation phase where metabolic activity increases dramatically. Imbibition induces splitting of the seed coat allowing oxygen to penetrate the seed, hence respiration is hastened. Protein synthesis is initiated and stored endospermic reserves are metabolized. The synthesized enzymes that promote the loosening of cell wall are initiated for subsequent cell elongation and increase in volume. Phase III is described as the seedling emergence phase typified by the radicle rupturing the seed coat. During this phase, there is rapid cell elongation and cell division. With the emergence of the radicle, the embryo can access water and nutrients from its environment. There is a continued dramatic increase in dry weight of new seedlings and a simultaneous decrease in storage tissue dry weight. The renewed water uptake rate depends on the water potential of the soil, adaptation of the seed water potential to soil environmental conditions, and the seed-soil contact properties (Benech-Arnold and Sanchez, 2004).

Seed Size

Switchgrass seedlots are heterogeneous with respect to size, hence the seed reserves variation among seed can affect the rates of germination and emergence and growth in grasses (Aiken and Springer, 1995, Smart and Moser, 1999). However, the relationship between the seed size and seed germination is variable (Larsen and

Andreasen, 2004). (Aiken and Springer, 1995) reported that seed size affected germination and emergence of six cultivars of switchgrass nonlinearly. Higher germination and emergence for larger seed sizes were consistent among cultivars. Haynes et al. (1997) also found a similar relationship and reported that the germination of "light" seed (42.0 mg per 100 seeds) was 7 vs. 45% for "heavy" seed (91.0 mg per 100 seeds), and contends that removal of lighter seed may improve seedlot germination and establishment. Although seed size may affect early growth and development, Smart and Moser (1999) reported that 8 to10 weeks after emergence, small and large seed were at a comparable growth stage. As a result of the relationship between seed weight and early seedling vigor (Kneebone and Cremer, 1955, Glewen and Vogel, 1984), Boe and Johnson (1987) suggested that seed size should be a selection criteria for improving seedling vigor in switchgrass, since larger seed may improve the chances of successful establishment when conditions are less than ideal (Aiken and Springer, 1995).

Seed Treatments

As a result of low seed germination and seedling vigor of neoteric switchgrass seed (Jensen and Boe, 1991), seed treatments are utilized to abate these effects and to enhance seedling establishment. Seed separation based on size and density (Jensen and Boe, 1991, Aiken and Springer, 1995), scarification (Jensen and Boe, 1991), cold stratification (Haynes et al., 1997) are seed treatments that could improve seed germination. Acid scarification, resulting in the corrosion of the lemma margin in the distal region of the caryopsis, is achieved using 8 M H_2SO_4 for 5 min followed by 5.25%

NaOCl for 15 min (Haynes et al., 1997). Cold stratification was imposed on 0.2% KNO_3 (m/v) at 5°C for 14 d. A combination of seed treatments have been reported to multiplicatively increase germination, almost doubling the final emergence percentage. Aged seed; however, can be germinated without imposing seed treatment. Haynes et al. (1997) reported that the effects of seed treatment (scarification and stratification) are nullified after 32 months of dry storage. Priming is another seed germination enhancement technique utilizing solid carriers for improving seed germination. Hacisalihoglu (2008) found that priming using a synthetic calcium silicate and water at 30°C for 5 d, increased germination of Cave-in-rock, Dacotah and Kanlow by 5, 8, and 19%, respectively, compared with non-primed seed. In addition to increasing final germination rate, priming was also found to decrease mean germination time by 26 to 36% among these cultivars.

Temperature Effects on Plant Processes

Plant growth processes can be differentiated by their temperature responses (Went, 1953). Increasing temperature increases various biochemical, physiological and phenological processes such as photosynthesis, respiration, transpiration, flowering, crop maturity, metabolite storage (low temperature reduces energy use and increase carbon storage) and dormancy. Conversely, cold temperatures reduce the activity of these processes and hence all plant processes occur within an optimum range. Although increasing temperature can stimulate growth and developmental rate of plants, high temperature has been demonstrated to have deleterious irreversible effects on

reproductive capacity and economic yield. For example, in cotton (*Gossypium hirsutum* L.), a 5°C increase from 30°C resulted in 10% boll and square abscission (Reddy et al., 1992). Also, increasing temperatures can also cause an imbalance between vegetative and reproductive growth with less total biomass produced at 35.5°C than at 29.9°C (Reddy et al., 1992). No bolls were produced at higher temperature indicating that high temperature injury limited growth, in particular reproductive growth (Reddy et al., 1995). The magnitude of temperature response not only varies among species and cultivars, but also among plant processes. The effect high temperature (supra-optimal) on growth processes has been demonstrated for several species and cultivars, for example, soybean [*Glycine max* (L.) Merr.] seed growth during flowering and pod set (Egli and Wardlaw, 1980), soybean seed composition (Gibson and Mullen, 1996), corn (*Zea mays* L.) seedling emergence, tasseling and anthesis (Warrington and Kanemasu, 1983), canola (*Brassica napus* L.) pollen germination and tube growth (Singh et al., 2008), wheat (*Triticum aestivum* L.) senescence acceleration (Harding et al., 1990), big bluestem growth and development (Kakani and Reddy, 2007) and cotton growth and developmental aspects (Singh et al., 2007).

Temperature Effects on Seed Germination

Temperature plays a major role in several growth and developmental processes in plants. As a result, all process-related models use the temperature-dependent functional algorithms in developing decision support systems for management (Reddy et al., 1997, 2008). In seed germination, temperature is involved in the removal of dormancy of grass

species as well as determining the germination capacity (Roundy and Biedenbender, 1996) and the rate of germination of non-dormant seed (Madakadze et al., 2001). Response to temperature varies across and within species as well as within seedlots of the same species (Madakadze et al., 2001, Larsen and Andreasen, 2004, Hardegree et al., 2008). The position of the seed on the panicle, maternal environmental conditions, and sequentially maturing seed are some factors responsible for intra-seedlot variation. This variation within a single seedlot means a variation in thermal time requirements for individual seed germination (Ellis and Barrett, 1994). The time required for germination may be described by log normal distribution of thermal times, accumulated above a base temperature (Ellis and Barrett, 1994).

The increase in thermal response between the minimum and optimum temperature is attributed to an increase in thermal activity of molecules involved in the chemical reactions (Probert, 1992). Conversely, the decreased response at supra-optimal temperatures is attributed to molecular dysfunction caused by alterations in protein or enzyme configuration by denaturation, breaking of seed dormancy, or physiological effects of temperature on membrane components causing membrane degradation (Hsu et al., 1985, Hardegree and Van Vactor, 1999).

The response to temperature has been quantified using (1) single value indices that summarizes the germination time course with a few coefficients, or (2) method of moments, which includes total, mean and variance of time to germination, quartiles, percentiles, time to 50% germination, etc, representing the germination process and assimilate final germination (Shafi et al., 1991). Examples of single value indices include

heat tolerance index, germination rate index (GRI, the total of the daily germination percent divided by the respective accumulated number of days since placement in the temperature treatment), corrected germination rate index (CGRI, the GRI corrected for final germination by dividing by the respective final germination and multiplying by 100). The CGRI increases with increasing temperature for several warm-season grasses that were subjected to a range of temperatures (Hsu et al., 1985, Madakadze et al., 2001). The speed of germination expressed by germination rate (reciprocal of time to 50% germination) also increases with increasing temperature (Hsu et al., 1985). These authors showed that temperature is positively correlated with GRI, CGRI, and germination rate, indicating that germination is enhanced by temperature. The utility of the germination index was demonstrated by Hanson and Johnson (2005) in assessing the response of eight varieties of switchgrass to temperature. As temperature increased from 25 to 40°C, the GI decreased from 22.4 to 11.9 (unitless). Across this same temperature range germination dropped by 44% and the time required to initiate germination increased from 8.8 to 9.5 d. Using Arrhenius plots to demonstrate the effects of increasing temperature on time to reach 50% germination, Hsu et al. (1985) identified two breakpoints, one between 12 and 14°C and another between 20 and 25°C, resulting in deviations from the linear temperature response. In addition to comparing the relative speed of germination, indices can be used to screen germplasm and rank potential temperature responses of species or cultivars (Hardegree et al., 2008). Shafi et al. (1991) stated; however, that the use of indices have the following limitations: (a) insensitive, ambiguous and incomplete, (b) do not express the location; (c) rate; (d) dispersion in time and extent of germination;

(f) assume a normal distribution for the frequency of germination, and (g) represent rather than describe the germination process. Although these single value indices can be used to determine inter-seedlot differences allowing for relative ranking of seedlots, these indices may not be statistically robust or confer biologically meaningful parameters that explain intra-seedlot dynamics as it relates to the germination capacity and rate.

Growth models offer an alternative approach to using index numbers to define the germination process. Shafi et al. (1991) posited that the correct mathematical specification coupled with the appropriate statistical estimation, growth models can provide considerable information resulting in parameter estimates with meaningful and relevant biological estimates. The performance of a seedlot can be characterized by three parameters: (1) time of germination onset (lag); (2) germination speed (rate); and (3) extent or capacity (cumulative germination percentage at the end of the testing period) (El-Kassaby et al., 2008). These parameters are useful for determining the suitability of a seedlot for commercial seed production, type of seed treatment required, as well as nursery management practices for rapid and uniform germination (El-Kassaby et al., 2008). Many nonlinear asymptotic models have been proposed and utilized to describe the germination course. The models include:

(a) Logistic $y = M [1+ \exp (L – Kt)]^{-1}$, Hsu et al. (1984)
(b) Gompertz $y = M [\exp (– \exp (L – kt))]$, Brown and Mayer (1988a)
(c) Richards $y = M [1 – \exp (– K (t – L))]$ 1/(1 – c), Berry et al. (1988)
(d) Weibull $y = M[1 – \exp (-K(t – L))c)]$, Brown and Mayer (1988a)

where y = cumulative percentage germination at time t, M = asymptote (theoretical maximum for y), L = time scale (lag related) constant, K = rate of increase and c = shape parameter.

Schimpf et al. (1977) fitted the logistic function to cumulative germination data by simple linear regression and found acceptable fit for both fast and slowly germinating seed populations. Logistic curves may have limitations; however, when different curves having the same rate but their integration constants differ. Most germination curves are positively skewed and this characteristic cannot be accommodated by the symmetrical logistic curve (Nichols and Heydecker, 1968).

Cardinal Temperatures

Thermal response of seed germination is similar to thermal response patterns of other physiological and developmental processes in plants (Probert, 1992). For any given process or developmental event, there is a minimum or base temperature below which the growth or developmental rate is zero, a sub-optimal range over which growth or developmental rate increases with temperature, a supraoptimal range over which the growth or developmental rate decreases with temperature, and a maximum or ceiling temperature threshold above which the developmental rate is zero (Probert, 1992). The temperatures or range at which each of these events occurs are defined as cardinal temperatures. Garcia-Huidobro et al. (1982) pointed out that for a complete description of thermal response, five cardinal temperatures are to be identified including the base, maximum and optimum temperatures and the limits for the optimum range. Roundy and

Biedenbender (1996) stated that the germination rate increases linearly with increasing sub-optimal temperatures and this relationship has led to the use of accumulated thermal units above the minimum temperature to predict germination with time. Based on the findings of Ellis and Barrett (1994) that instantaneous germination rate is independent of thermal history. Hardegree and Van Vactor (1999) asserted that models developed from temperature experiments can be used to predict the germination behavior under variable temperature conditions. Cardinal temperatures have important agronomic and management implications and generating genotype-specific cardinal temperatures may be useful for determining optimum sowing dates and potential regions of adaptation. Parrish and Fike (2005) reported that switchgrass germinated slowly when the temperature was below 15.5°C and maximum germination occurred within 3 d of imbibition at 29.5°C. Hsu et al. (1985) found that switchgrass reached maximum germination between 20 and 30°C for non-dormant seed and estimated 10.3°C as the minimum temperature for switchgrass germination. These authors reported optimum temperatures for seedling development were between 25 and 30°C. Evaluating switchgrass crowns for cold tolerance using artificial freeze tests, Hope and McElroy (1990) determined an LT50 (lethal temperature at which 50% of plants are killed) of -4.0°C prior to the onset of cold hardening.

Modeling seed germination using thermal units require accurate determination of the base temperature (Madakadze et al., 2001). Ellis et al. (1986) found no differences in base temperatures for rate of germination between six widely differing chickpea (*Cicer arietinum* L.) genotypes and concluded that base temperature may be a species-specific

characteristic, unaffected by genetical or physiological differences in quality resulting from ageing. Therefore, differences in germination rate to temperatures below the optimum are due to differences in thermal time requirements. Minimum temperature estimation can be determined by regressing the rate of germination (Y) against temperature (X) and by estimating when Y = 0 and solving for X after finding a linear relationship between rate of germination and temperature. The T_{min} can also be determined by nonlinear regression using the equation $1/t = k(T-T_b)$ where $1/t$ represents the rate of germination, T as germination temperature, T_b as the minimum temperature at which $1/t$ equals zero and k is a constant (Garcia-Huidobro et al., 1982). The T_{min} can also be estimated from the linear function between temperature and the reciprocal of time to 50% germination. Using this method, Hsu et al. (1985) estimated base temperatures for Blackwell (9.6°C) and Cave-in-rock (10.9°C). Madakadze et al. (2001) estimated minimum temperature ranges of 5.5 to 10.9°C, 7.3 to 8.7°C, 7.5 to 9.6°C and 4.5 to 7.9°C for switchgrass, big bluestem, indiangrass and prairie sandreed [*Calamovilfa longifolia* (Hook.) Scribn.], respectively. Covell et al. (1986) showed that T_{min} for germination rate is constant within populations but varies across species such as chickpea, lentil (*Lens culinaris* Medik.), soybean and cowpea [*Vigna unguiculata* (L.) Walpers ssp. *unguiculata*)], while optimum and maximum temperatures vary both within populations and across species.

The selection of T_{min} estimation method will also affect the accuracy of the prediction. Madakadze et al. (2001) found that non-linear estimation of T_{min} were 14 to 29% higher than those from linear estimation for switchgrass. Small differences in T_{min}

may mean large differences in germination time. Hsu et al. (1985) contends that species or cultivars germinating over a relatively wide range of temperatures might be easier to get established in the field than those with a highly specific temperature requirement. Among the native warm-season grasses (switchgrass, big bluestem and indiangrass), Hsu et al. (1985) found that switchgrass tended to be more temperature-specific in its temperature adaptability range.

Crop Modeling

Crop simulation models are developed for various purposes including prediction of plant growth and development, yield forecasting, hypothesis testing, and decision support, achieved through the synthesis of plant genetics, physiology, and environment interactions (Vandendriessche and Van Ittersum, 1995). Mathematical modeling is a powerful approach for understanding the complexity of biological systems (Meng et al., 2004) permitting the development and testing of models based on functional algorithms between crop growth and the environment. Modeling crop growth and development is based on quantifying environmental factors effect on several discrete phenological and physiological processes of a given species from sowing to maturity (Reddy et al., 1997, Reddy, 2008). Robust and mechanistic-field tested models will be of great value for on-farm resource management and policy decisions (Reddy et al., 2002).

A switchgrass simulation model can be a component of a biofuel decision support system (Grassini et al., 2009). One published model with validated utility to adequately simulate switchgrass yield potential in diverse environments is the ALMANAC model

(Kiniry et al., 1996, 2005, McLaughlin et al., 2006), a physiologically-based crop production model quantifying plant-environment interactions that influence crop productivity and resource utilization. This model has been parameterized based on Alamo switchgrass studies conducted in diverse locations in Texas. The model simulates growth and development of switchgrass after seedling establishment, neglecting the influence of field conditions on germination and emergence while assuming near-perfect plant densities. Since germination is a critical stage in the life cycle of plants controlling population dynamics, its inclusion in simulation modeling can enhance the decision support systems and for tactical and operational farm-level decisions.

Modeling Germination Response to Temperature

The ideal description of germination should be complete, concise, unambiguous, amenable to statistical analysis, and easy to understand. As early as 1926 (Kotowski, 1926) and up to recent models, germination thermal responses have been distilled into single value indices that attempt to describe the germination process; however, their efficacy in describing the germination process have been questionable. Single value indices cannot combine three independent aspects of germination (lag, speed and extent) into a single ambiguous value. Brown and Mayer (1988b) assessed the validity of several single value indices (Kotowski's coefficient of velocity, Maguire's speed of germination, Czabator's germination value, Diavanshir and Pourbiek's germination value, Timson's cumulative germination, Lehle and Putnam's Richards function index, Smith and Millett's sprouting index, and Tucker and Wright's regression index) and found all of the indices

with the exception of the Timson's cumulative germination method being unable to simulate field-level germination data. As a result, no single value index was recommended because of their inherent ambiguity and failure to adequately summarize the germination process. However, alternatives to the use of single value indices include the use maximum seed germination, the use of two or more statistics, or fitting a curve to the data. Fitting curves to germination data better describe the germination time course via curve coefficients while preserving essential information on the initiation of germination, the rate and its extent. Several methods of curve fitting procedures have been proposed to describe the germination process. Brown and Mayer (1988b) fitted the Weibull, Morgan-Mercer-Flodin, Richards, Mitseherlich, Gompertz, and logistic functions to a wide range of cumulative germinations of non-dormant seed and found that the Weibull function consistently provided the best fit with its four parameters revealing the maximum germination, germination rate, the lag in the onset of germination and the shape of the cumulative distribution. The effect of a specific environmental factor on germination is typified by an S-shaped germination curve, relating the cumulative germination percentage to time. Cumulative germination curves are typically Sigmoid, which can be quantified by the standardized normal distribution (Janssen, 1973) or by the logistic curve procedure as suggested by (Hsu et al., 1984).

Effect of temperature on germination rate and maximum seed germination percentage

The extent, uniformity, and rapidity of germination are desirable attributes of any seedlot. These can be modified by existing environmental conditions. Under natural conditions, environmental cues moderate dormancy and germination to ensure survival and distribution of a species. The germination rate and final germination percentage are the two important seedlot descriptive and quantification parameters that are affected differently by temperature, and the quantification of these responses is imperative to modeling using thermal parameters. Garcia-Huidobro et al. (1982) reported that rate of germination has a sharply defined optimum while the highest values of maximum germination were achieved over a range of temperatures. Schimpf et al. (1977) reported that the rate of germination and final germination percentage are positively correlated with the germination rate appearing to be more sensitive to temperature during germination than maximum seed germination percentage in *Setaria lutescens* and *Amaranthus retroflexus* (Schimpf et al., 1977). Defined as the reciprocal of time taken for half the population to germinate, the germination rate response to constant sub- and supra-optimal temperature is generally bilinear for several crops including pearl millet (*Pennisetum typhoides* S. & H.) (Garcia-Huidobro et al., 1982), chickpea, lentil, soybean, cowpea (Covell et al., 1986), carrot (*Daucus carota* L.) (Hegarty, 1973), and 31 vegetable species (Bierhuizen and Wagenvoort, 1974).

Rate of germination increases linearly with temperature from a base temperature to a sharply defined optimum, beyond which the rate decreases linearly and reaches zero at a maximum temperature (Garcia-Huidobro et al., 1982, Ellis et al., 1986). The

linearity between germination rate and temperature over a defined range, for example, between the minimum temperature and the optimum temperature means that the thermal time required for germination is a constant and can therefore be used to compare germination in different species, climates and locations. This relationship between rate and temperature is observed in many other physiological and phenological processes including rate of pollen germination and tube length growth (Kakani and Reddy, 2007), early growth of radicle and plumule (Arndt, 1945, Blacklow, 1972) and several growth and phenological events (Reddy et al., 1997).

Covell et al. (1986) and Ellis et al. (1987) suggest that the thermal time approach can be modified to provide equations that describe the variation in germination time within a seed population at sub-optimal temperatures, Eq. [1], and another which describes variation at supra-optimal temperatures, Eq. [2].

$$1/t(G) = [T-Tb]/([probit(G)- K]\sigma) \quad [1]$$

where t/(G) is the time taken for cumulative germination to reach the percentile *G* at temperature *T, T_b* is the base temperature (at which temperature *t(G)* = ∞), K is a constant and σ is the standard deviation of the distribution of thermal times for germination within the seed population.

$$1/t(G) = (([Ks- probit(G)\ ]\sigma)- T)/(\Theta_2) \quad [2]$$

Where K_s is a constant, σ is the standard deviation of the distribution of the ceiling temperature within the population [T_c(G), at which temperature t(G) = ∞], and Θ_2 is the thermal time for germination at supra-optimal temperatures. Covell et al. (1986) found that the base temperature (T_b) does not vary for different fractions within a seed

population and the thermal time over the sub-optimal range varies within each seed population.

Temperature Tolerance Screening

Temperature tolerance is a multigenic trait, hence, emphasis needs to be on relevant approaches to assess genetic variability in basal and acquired tolerance. This is a major aspect of crop improvement programs. Crop species differ in their sensitivity to high and low temperatures, which can be attributed to differential expression of stress-response genes. Temperature tolerance can be achieved by screening genotypes at either low or high or both high and low temperatures (Potaczek and Kozik, 2000). Porch (2006) pointed out that long-term goal of temperature tolerance breeding program should be the development of germplasm with improved field-level tolerance under variable temperature conditions. Any parameter that changes with temperature can be used to screen genotypes; however, Srinivasan et al. (1996) contended that screening for temperature tolerance should meet the following requirements: (a) performed with a suitable physiological parameter sensitive enough to respond to induced temperature and also have the capacity to stratify genetic differences at early stages; (b) rapid, precise and reproducible detection of selected parameter changes under variable field conditions; and (c) allow performance of large number of measurements with many breeding lines and cultivars. Temperature variation responses can be used as a method of analysis for screening seed populations based on the assumptions that there is (a) positive and negative linear relationship between rate of germination and temperature at sub- and supra-optimal temperatures, respectively; (b) no variation of minimum temperature

within one seed population, but a normal distribution of thermal times at sub-optimal temperatures; and (c) within a seed population no variation in thermal time, but a normal distribution of maximum temperatures at supra-optimal temperatures (Ellis et al., 1987). Germination and seedling establishment of switchgrass can be sensitive to early season cold temperatures, hence identifying genotype-specific minimum temperatures can aid in sowing date decisions or development of genotypes for regions where early season chilling stress is common.

Being a warm-season species that switchgrass should have a high level of inherent heat tolerance. However, upland ecotypes may be limited in their southern adaptation by reduced heat tolerance or inability to capitalize on the extended growing season compared with lowland ecotypes (Casler et al., 2004). Final germination percentage and germination rate are both considered sensitive indicators of seed vigor (Larsen and Andreasen, 2004). Generally, maximum seed germination percentage and rate of germination increases with increasing temperature (Madakadze et al., 2001).

The selection of superior genotypes from populations has been aided by stress indices based on physiological parameters associated with a desired trait. Some indices reported to screen genotypes include geometric mean, stress tolerance index, stress susceptibility index (Porch, 2006). Heat tolerance screening can be achieved using both vegetative and reproductive physiological and biochemical parameters, including chlorophyll fluorescence induction parameters [fluorescence origin (F_o), maximum fluorescence yield (F_m), variable fluorescence (F_v) and their ratios], and cell membrane thermostability (Singh et al., 2007). These traits have been used to successfully to screen

for heat tolerance among common bean (*Phaseolus vulgaris* L.) genotypes (Petkova et al., 2007), and legume species [chickpea, groundnut (*Arachis hypogaea* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], and soybean] (Srinivasan et al., 1996). Narrow leaves, small plants, dense tillers, profuse root growth, and high root-to-shoot ratio could be used to select heat tolerant cultivars. Basu and Minhas (1991) and Nagarajan and Minhas (1995) reported that vegetative parameters such as internode elongation can be useful selection criteria to screen potato (*Solanum tuberosum* L.) genotypes. Biochemical parameters including canopy net photosynthetic rate (Pn), single-leaf Pn, and RuBP carboxylase (Rubisco) have been used to screen creeping bentgrass (*Agrostis stolonifera* L.) genotypes (Basu and Minhas, 1991).

Temperature Tolerance Screening Tools

Field studies are confounded with a large number of co-varying variables including unpredictable moisture and fluctuating temperatures, which can mask a genotype's true germination potential. Germination under controlled temperatures is a relatively simple and inexpensive technique to screen large numbers of genotypes. De La Soujeole (1984) suggested that chilling tolerance in sorghum should be evaluated at germination, emergence, and seedling growth stages, contending that these three processes are independently sensitive to cold tolerance. Tiryaki and David (2001) found that germination rate better separates thermal genotypic response than maximum seed germination and early seedling growth rate.

Screening switchgrass genotypes for adaptability has been restricted to screening nurseries, field performance, and visual evaluation based on survival, which is time and resource consuming. It is difficult to separate heat stress, water stress, and biotic factors from germination potential because of uncontrollable interactions that may exist. Therefore, screening genotypes prior to field testing requires a controlled environment where temperature and moisture can be monitored. Hence, a simple, rapid and reliable screening method is required to screen large number of genotypes for temperature tolerance in controlled conditions (Setimela et al., 2005). However, studies dealing with temperature tolerance screening in switchgrass are limited in general and none using seed-based parameters have been found in the literature. Seed-based parameters, in particular, germination capacity and rate, have been used successfully to screen several other species and genotypes for various abiotic stress factors including drought, saline, flooding/water logging, chilling, and heat tolerance (Table 2).

Table 2. Abiotic tolerance screening using *in vitro* seed germination assay in several crop species.

Abiotic stress	Species	References
Drought tolerance	*Glycine max* (soybean)	Sapra and Anaele (1991); Kpoghomou et al. (1990); Bouslama and Schapaugh (1984)
	18 legumes	Grzesiak et al. (1996)
	Triticum aestivum (wheat)	Rauf et al. (2007); Blum et al. (1980); Ashraf and Abu-Shakra (1978)
	Zea mays (corn)	Williams et al. (1967)
	Ricinus communis (castor bean)	Manjula et al. (2003)
	Lens culinaris (lentil)	Mohammad and Haghnazari (2007)
	Trifolium repens (white clover)	Sharma (1973)
	Lolium perenne (perennial ryegrass)	
	Danthonia caespitosa (wallabygrass)	
	Atriplex vesicaria (bladder saltbush)	
	A. nummularia (bluegree saltbush)	
	Oryza sativa (rice)	Sadasivam et al. (2000)
Salt tolerance	*Lycopersicon esculentum* (tomato)	Foolad and Lin (1997); Jones (1986)
	Cucumis melo (melon)	Akinci (1997)
	Vigna unguiculata (cowpea)	Murillo-Amador et al. (2000)

Table 2. (continued)

	Phaseolus aureus (green gram)	Misra and Dwivedi (2004)
		Bayuelo-Jiménez et al. (2002)
Water logging / flooding tolerance	*Glycine max* (soybean)	Hou and Thseng (1992)
	Triticum aestivum (wheat)	Sharma (2008)
Chilling tolerance	*Triticum aestivum* (wheat)	Ashraf and Abu-Shakra (1978)
	Brassica napus (Canola)	Acharya et al. (1983)
	Sorghum bicolor (sorghum)	Tiryaki and David (2001)
	Hibiscus cannabinus (kenaf)	Angelini et al. (1998)
	Phaseolus vulgaris (common bean)	Zaiter et al. (1994)
	Lycopersicon esculentum (tomato)	Potaczek and Kozik (2000); Scott and Jones (1982)
	Oriza sativa (rice)	Cruz and Milach (2004)
	Linum usitatissimum (flax)	Saeidi (2008)
Heat tolerance	*Glycine max* (soybean)	Sapra and Anaele (1991); Emerson and Minor (1979)
	Cicer arietinum (chickpea)	Ellis et al. (1986)

Genotype Classification Methods

Techniques for genotype classification ranged from simple to statistically rigorous procedures including single value indices (Brown and Mayer, 1988a), percentiles and quartiles relative to control studies, cumulative indices and principal component analysis (PCA). Emerson and Minor (1979) classified soybean genotypes for high temperature tolerance using a confidence interval about the mean germination. Similar classification approaches have been used by Kakani and Reddy (2007) and Salem et al. (2007) to classify pepper (*Capsicum annuum* L.) and soybean genotypes, respectively, using pollen-based parameters and a temperature response index (TRI). The TRI relates the value of a genotype to the maximum or minimum value of all genotypes. The summation of individual TRI results in a cumulative TRI that is then separated by standard deviation based on the number of classes of interest. Cumulative TRI has been used to screen genotypic variability under multiple environmental conditions in soybean (Koti et al., 2004).

Genotypic classification can also be achieved by PCA, which is a multivariate technique that examines the relationships among a large number of quantitative traits. Kakani et al. (2002) and Singh et al. (2008) demonstrated the utility of this method by classifying peanut, cotton and canola genotypes based on eigen vectors and eigen values. The TRI method uses all traits of interest that may potentially contribute to a given stress condition tolerance or sensitivity, and each trait will have an equal contribution. The PCA analysis, on the other hand, will take into an account only one to three traits that have the maximum contribution in separating the genotypes.

CHAPTER III

SWITCHGRASS (*Panicum virgatum* L.) INTRASPECIFIC VARIATION AND TEMPERATURE TOLERANCE CLASSIFICATION USING *IN VITRO* SEED GERMINATION ASSAY

MATERIALS AND METHODS

Seed Material

Seeds of 14 switchgrass genotypes (representative of northern and southern, upland and lowland ecotypes) were evaluated in this experiment. For nine cultivars, seeds were collected from the plants grown during the 2006-2007 growing season at Mississippi State University, Mississippi State, MS, USA (33°28′N, 88°47′W). Seeds of 'Blackwell', 'Carthage', 'Cave-in-Rock', 'Shawnee' and 'Shelter' were obtained from the Ernst Seed Company (Meadville, PA). Air-dried seed were stored in vials at room temperature during the course of the study. Seed were homogenously mixed and 100 seeds per experimental unit for germination testing were counted by an electronic seed counter (Model 850-2; The Old Mill Company, Savage, MD).

Seed Quality Characteristics

Seed viability was determined by longitudinal dissection to reveal the embryo after 24 h imbibition using 0.1% (1g L^{-1}) triphenyl tetrazolium chloride stain. Partially or completely red or pink embryo was considered viable (ISTA, 1985). Approximately, 1 g of seed replicated thrice, was grounded, homogenized, and sieved (40 mesh) and analyzed for nitrogen (N) and carbon (C) concentration with an automated CHN combustion analyzer (Perkin Elmer 2400; Perkin Elmer Corp., Norwalk, CT, Software: Eager 300 ver. 1.01) at the USDA-ARS Laboratory, Mississippi State, MS. Individual seed C and N content were determined by multiplying the C and N concentration by the dry weight of individual seed (C and N concentration × seed mass).

Germination Testing

Germination tests were performed from March to July 2009. Moistened seeds were stratified at 5^{o}C for 14 d according to Association of Official Seed Analysts (ASOA) rules with no humidity control. Preliminary studies at low temperature (< 20^{o}C) indicated that fungal infection can affect germination, prompting the use of Captan {cis-N-[(trichloromethyl)thio-4-cyclohexene-1,2-dicarboximide]} at 0.55 g ai kg^{-1} seed as a drench prior to germination testing. Each genotype was replicated four times in a completely randomized design with 100 seed per replicate placed on a moistened single layer Whatman No. 1 filter paper (Whatman, Atlanta, GA) in a covered 90-cm sterilized disposable plastic Petri dish to minimize moisture loss. Petri dishes were vertically stacked at constant set temperature, 10 to 45^{o}C with 5°C interval, and a constant light

during an eight 8h light period with a photon flux density of 35 ± 2.6 μmol m^2 s^{-1}, provided by cool white fluorescent lamps and 16 h dark for all genotypes and temperatures in a germination chamber (Fisher Scientific, Suwanee, GA). Petri dishes were monitored daily and watered when necessary with distilled water.

Replicates for each genotype were completely randomized within the germination chamber for each temperature. Germinated seed were counted, recorded and discarded every 6 h. Counts were discontinued if no seeds germinated for five consecutive days. To minimize the potential of small temperature changes within the chambers, the Petri dishes were rearranged every 6 h (Larsen and Bibby, 2004). A seed was considered germinated when the coleoptile or coleorhizae was at least 2 mm long.

Curve Fitting Procedure and Data Analysis

Temperature and germination time-course data were fitted with a 3-parameter sigmoidal function (Eq. 1) using Sigma Plot 11 (Systat Software Inc., 2006). 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.

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

where G is the total germination percentage, G_{max} is the maximum cumulative seed germination percentage, x_{50} is the time to 50% maximum seed germination and G_{rate} is the slope of the curve.

Maximum seed germination and rate of development at each temperature were analyzed using linear and nonlinear regression techniques to quantify developmental responses to temperature (Kakani et al., 2002). Quadratic, cubic and higher order polynomials and modified bilinear equations were fitted to the data to determine the best fit model. Based on the highest coefficient of determination (r^2) value and the root mean square error (RMSE), the best curve fitting model was obtained. Maximum seed germination was modeled using a quadratic function ($r^2 = 0.88$, RMSE = 5.2) while GR was modeled by a modified bilinear function ($r^2 = 0.95$, RMSE = 1.00). Quadratic and modified bilinear equations estimates for each replicate within each genotype were estimated using PROC NLIN of SAS (SAS Institute Inc., 2004) with a modified Newton Gauss iterative method. For the quadratic model (Eq. [2]), the three cardinal temperatures (T_{min}, T_{opt} and T_{max}), were estimated using Eq. [3] to [5].

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

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

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

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

where MSG is the maximum seed germination, T_{opt}, T_{min}, and T_{max} is the optimum, minimum and maximum temperature, respectively. T is treatment temperature at which MSG were determined, and a, b, and c are genotype specific constants generated using PROC GLM in SAS (SAS Institute Inc., 2004). For the modified bilinear model [6], T_{opt} was generated using SAS (SAS Institute Inc., 2004) while T_{min} and T_{max} were estimated using Eq. [7] and [8].

$$GR = a + b_1 (T - T_{opt}) + b_2 \times ABS (T_{opt} - T) \quad [6]$$

$$T_{min} = [a + (b_2 - b_1) \times T_{opt}] / b_1 - b2 \quad [7]$$

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

where GR is germination rate, T_{opt}, T_{min}, and T_{max} is the optimum, minimum and maximum temperature, respectively. T is the treatment temperature and a, b_1 and b_2 are genotype-specific constants generated using PROC NLIN in SAS (SAS Institute Inc., 2004).

Cumulative Temperature Response Index (CTRI)

Switchgrass genotypes were classified as cold or heat tolerant groups based on the summation of seed individual temperature response index (ITRI) following the protocol used by Salem et al. (2007) for pollen germination response to temperature. Heat CTRI was calculated as the MSG and GR values for each of the cardinal temperatures (T_{min}, T_{opt} and T_{max}) of a specific genotype, divided by the maximum value observed among all genotypes (Eq. [9]) while cold CTRI was determined by dividing the minimum values among all genotypes by the value of a specific genotype (Eq. [10]). Genotypes were classified based on CTRI of all parameters as cold-tolerant (> minimum CTRI + 4 standard deviations [SD]), moderately cold-tolerant (> minimum CTRI + 3 SD), moderately cold-sensitive (> minimum CTRI + 2 SD), and cold-sensitive (> minimum CTRI + 1 SD).

$$ITRI = P_t / P_h$$

$$\text{Heat CTRI} = \left(\frac{\text{MSG } T_{min_t}}{\text{MSG } T_{min_h}} + \frac{\text{MSG } T_{opt_t}}{\text{MSG } T_{opt_h}} + \frac{\text{MSG } T_{max_t}}{\text{MSG } T_{max_h}} + \frac{\text{GR } T_{min_t}}{\text{GR } T_{min_h}} + \frac{\text{GR } T_{opt_t}}{\text{GR } T_{opt_h}} + \frac{\text{GR } T_{max_t}}{\text{GR } T_{max_h}} \right) \quad [9]$$

$$\text{Cold CTRI} = \left(\frac{\text{MSG } T_{min_h}}{\text{MSG } T_{min_t}} + \frac{\text{MSG } T_{opt_h}}{\text{MSG } T_{opt_t}} + \frac{\text{MSG } T_{max_h}}{\text{MSG } T_{max_t}} + \frac{\text{GR } T_{min_h}}{\text{GR } T_{min_t}} + \frac{\text{GR } T_{opt_h}}{\text{GR } T_{opt_t}} + \frac{\text{GR } T_{max_h}}{\text{GR } T_{max_t}} \right) \quad [10]$$

All cumulative germination data were arcsine transformed prior to analysis and back transformed for reporting. Replicated values of cardinal temperatures (T_{min}, T_{opt} and T_{max}), temperature adaptability range (TAR, = $T_{max} - T_{min}$) and MSG were analyzed using the one-way ANOVA procedure (PROC GLM) in SAS (SAS Institute Inc., 2004) to determine the effect of temperature treatment on MSG and GR and their respective cardinal temperatures (T_{min}, $T_{opt,}$ and T_{max}). Means were separated using Fishers protected least significant differences (LSD) at $P = 0.05$. Germination parameters (MSG and GR) were treated as dependent variables while temperature and time to germination as independent variables. Regression of test parameters was done using Sigma Plot 11.0 (Systat Software Inc., 2006). Genotypes were classified as lowland (Alamo, Expresso, Kanlow and Tusca) or upland (Blackwell, Carthage, Cave-in-Rock, Dacotah, Forestburg, Shawnee, Shelter, Summer, Sunburst and Trailblazer) to determine the ecotypic response to temperature.

RESULTS

Seed Quality Characteristics

Seed viability, seed weight, seed C and N content, and C:N ratio differed among genotypes ($P < 0.05$). Seed viability ranged from 73 (Kanlow) to 96% (Tusca) with a mean of 89% (Table 3). Individual seed weight ranged from 0.7 (Kanlow) to 1.89 mg seed^{-1} (Trailblazer) with a mean of 1.39 mg seed^{-1} (Table 3). Carbon content, on the other hand, ranged from 296 (Kanlow) to 823 µg seed^{-1} (Trailblazer) with a mean of 594 µg seed^{-1}, while N content ranged from 16 (Kanlow) to 47 µg seed^{-1} (Sunburst). Ecotypic classification of the genotypes reveals that seed C and N content and seed weight differ between upland and lowland genotypes ($P < 0.05$) (Table 4).

Table 3. Seed viability, weight, carbon (C) and nitrogen (N) content, and C:N ratio of 14 switchgrass genotypes

Genotype	Seed viability	Seed weight	C	N	C:N
	——% ——	—mg seed^{-1} —	——————µg seed^{-1} ——————		
Alamo	85.50 ± 1.66	0.94 ± 0.01	395.47 ± 1.19	23.86 ± 0.37	16.58 ± 0.27
Blackwell	93.25 ± 1.93	1.83 ± 0.03	769.43 ± 2.83	43.30 ± 0.33	17.77 ± 0.08
Carthage	81.50 ± 2.50	0.98 ± 0.01	426.25 ± 0.11	26.60 ± 0.46	16.04 ± 0.27
Cave-in-Rock	91.75 ± 1.31	1.82 ± 0.03	747.58 ± 1.44	37.68 ± 1.42	19.90 ± 0.79
Dacotah	93.75 ± 2.06	1.30 ± 0.04	578.88 ± 0.86	35.69 ± 1.55	16.28 ± 0.70
Expresso	93.25 ± 1.80	1.06 ± 0.01	453.80 ± 1.19	34.41 ± 0.90	13.21 ± 0.38
Forestburg	94.50 ± 1.55	1.54 ± 0.02	670.93 ± 1.34	40.23 ± 0.68	16.69 ± 0.31
Kanlow	73.25 ± 4.37	0.70 ± 0.00	296.12 ± 0.77	15.85 ± 0.52	18.72 ± 0.68
Shawnee	85.75 ± 4.40	1.75 ± 0.02	726.57 ± 1.94	38.35 ± 0.50	18.95 ± 0.26
Shelter	84.75 ± 2.81	1.64 ± 0.01	693.65 ± 3.42	40.46 ± 0.71	17.15 ± 0.22
Summer	89.50 ± 0.87	1.06 ± 0.03	454.33 ± 0.90	29.62 ± 0.18	15.34 ± 0.12
Sunburst	94.50 ± 0.96	1.75 ± 0.01	758.16 ± 1.70	47.16 ± 0.92	16.09 ± 0.32
Trailblazer	95.50 ± 0.96	1.89 ± 0.04	823.94 ± 3.92	45.71 ± 1.47	18.06 ± 0.51
Tusca	96.50 ± 0.65	1.22 ± 0.02	516.35 ± 0.78	38.38 ± 0.57	13.46 ± 0.22
Mean	89.52	1.39	593.68	35.52	16.73
LSD	2.21*	0.16*	5.54*	2.52*	1.23*

*Significant at $P = 0.05$ probability level.

Table 4. Variation of seed quality characteristics between upland (Blackwell, Carthage, Cave-in-Rock, Dacotah, Forestburg, Shawnee, Shelter, Summer, Sunburst and Trailblazer) and lowland (Alamo, Expresso, Kanlow and Tusca) ecotypes of switchgrass

Parameter	Ecotype				LSD
	Upland	CV (%)	Lowland	CV (%)	
C	664.97 a	20	415.44 b	23	228.23
N	38.48 a	17	28.13b	36	9.8
C:N	17.23 a	8	15.49 a	17	3.27
Seed Viability	90.48 a	5	87.13 a	12	12.07
Seed Weight	1.56 a	21	0.98 b	22	0.55

Means within columns followed by the same letter are not significantly different (P = 0.05). CV (%) represents the variability of the ecotype means of a particular parameter.

Germination Time Courses

The 3-parameter sigmoidal curve fitted the cumulative germination time course ($r^2 = 0.98$) of genotypes response to temperature efficiently, illustrating how the genotypes differed in their germination characteristics (Fig. 1). For clarity, only data and fitted lines for four genotypes, each representative of northern and southern upland (Cave-in-Rock and Shelter) and lowland (Alamo and Kanlow) genotypes are presented. There was no germination at 10 or at 45°C.

Maximum Seed Germination Response to Temperature

Among the linear and nonlinear regression models tested, the quadratic function best described the response of MSG to temperature ($r^2 = 0.93$, RMSE = 5.2). For clarity, only data and fitted lines for four genotypes, each representative of northern and southern upland (Cave-in-Rock and Shelter) and lowland (Alamo and Kanlow) genotypes are presented (Fig. 2).

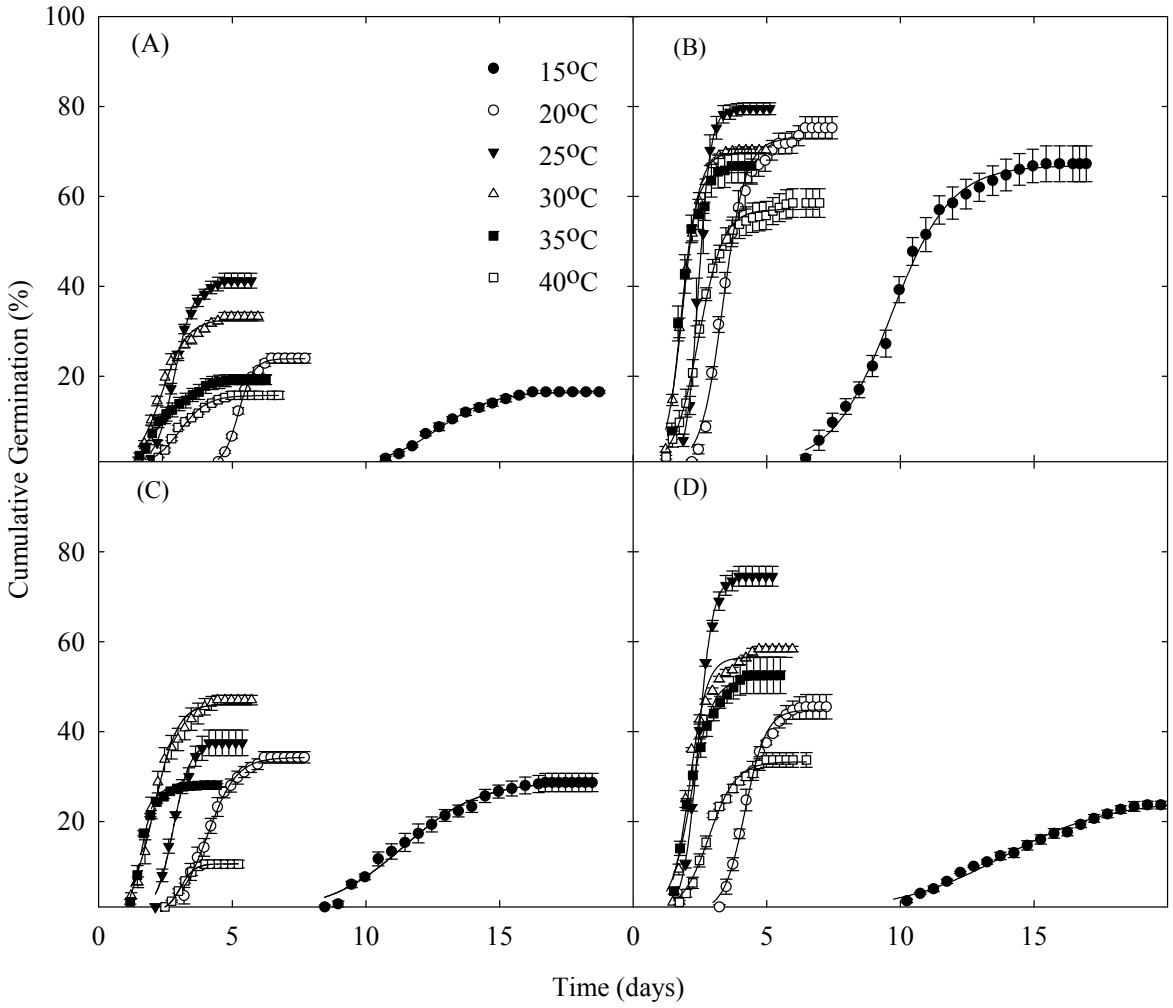

Figure 1. Germination time courses for seeds of (A) Alamo, (B) Cave-in-Rock, (C) Kanlow and (D) Shelter switchgrass germinated at a range of temperature (15 - 40ºC). The symbols indicate the observed cumulative germination data and the lines indicate the germination time courses fitted using a three-parameter sigmoidal function. Data are means and ± SE of four replications.

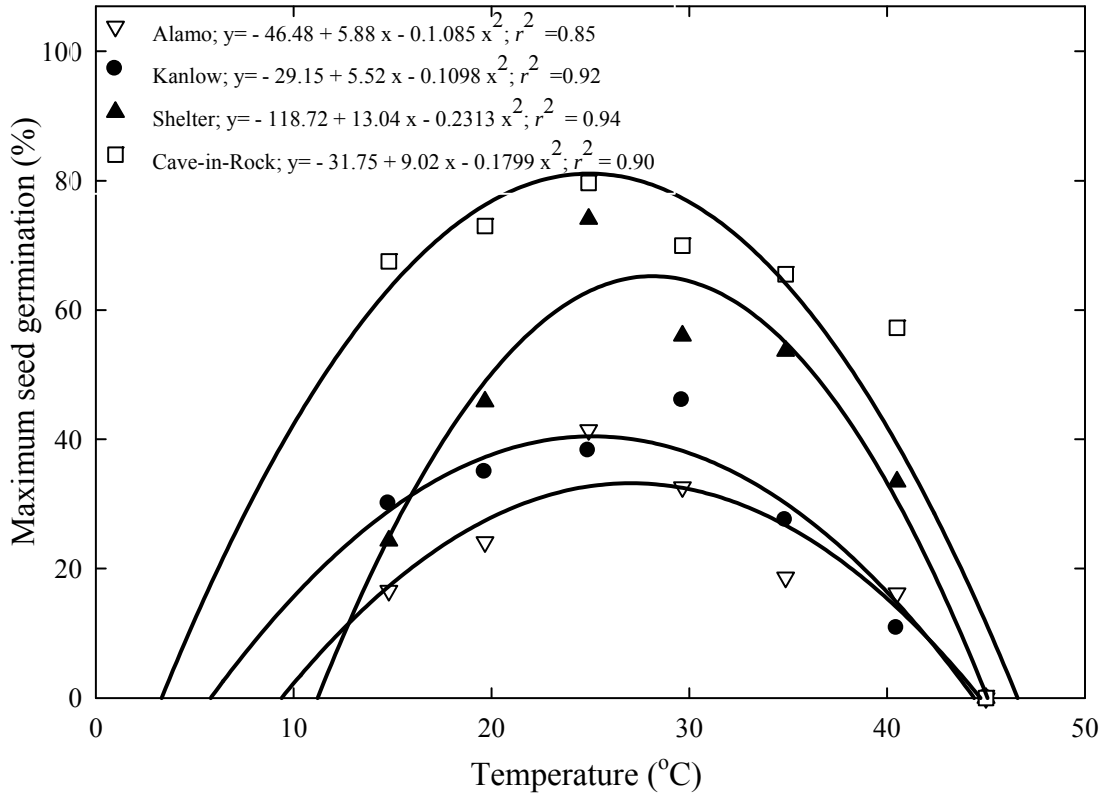

Figure 2. Influence of temperature on maximum seed germination and along with the fitted quadratic equations of four switchgrass genotypes (Alamo, Kanlow, Shelter, Cave-in-Rock). The symbols are recorded germination percentages and the curves are fitted lines using quadratic functions.

Maximum seed germination varied ($P < 0.001$) among genotypes with a mean of 73% and ranged from 41 (Alamo) to 93% (Expresso). Cardinal temperatures (T_{min}, $T_{opt,}$ and T_{max}) for MSG also differed among the genotypes ($P < 0.001$). The T_{min} values ranged from 3.69 (Expresso) to 12.83°C (Summer) with a mean of 8.08°C. The T_{opt} was 26.58^{o}C; however, there was variation among the genotypes ($P < 0.001$). Summer recorded the highest T_{opt} (28.56°C) while Tusca showed the lowest (24.04°C). The T_{max} ranged from 41.81(Tusca) to 47.07°C (Expresso) with a mean of 45.07°C (Table 5). The TAR for MSG ranged from 43.38 (Expresso) to 31.37°C (Summer) with a mean of 37°C for all genotypes.

Grouping genotypes based on upland and lowland ecotype revealed no differences ($P > 0.05$) for MSG, TAR, T_{min} and T_{max}; however, T_{opt} for MSG was different ($P = 0.0471$, LSD = 1.53) with means of 27.02 and 25.47°C for upland and lowland ecotypes, respectively. Maximum seed germination for both upland and lowland ecotypes were also varied (>10%) (Table 6). Cardinal temperature variation was small between ecotypes (<4%). Maximum seed germination T_{min} was more variable than T_{opt} and T_{max} for both upland and lowland ecotypes (Fig. 4 and Table 6). On average, MSG cardinal temperatures were 10 and 6% more variable than germination rate cardinal temperatures for upland and lowland ecotypes, respectively (Fig. 4 and Table 6).

Table 5. Maximum seed germination percentage (MSG), temperature adaptability range (TAR), quadratic equation constants (a, b, c), regression coefficients (r^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for maximum seed germination (MSG) of 14 switchgrass genotypes in response to temperature.

Genotype	MSG (%)	TAR (°C)	Equation constants			r^2	Cardinal temperatures (°C)		
			a	b	c		T_{min}	T_{opt}	T_{max}
Alamo	40.97 ± 1.56	34.94 ± 0.14	-46.48	5.88	-0.1085	0.85	9.61 ± 0.19	27.08 ± 0.12	44.55 ± 0.05
Blackwell	83.23 ± 2.16	36.01 ± 0.25	-119.03	15.28	-0.2798	0.98	9.33 ± 0.32	27.34 ± 0.20	45.34 ± 0.10
Carthage	55.09 ± 1.39	35.51 ± 0.44	-80.43	9.68	-0.1733	0.93	10.2 ± 0.35	27.95 ± 0.13	45.71 ± 0.11
Cave-in-Rock	79.48 ± 1.38	40.53 ± 1.03	-31.75	9.02	-0.1799	0.90	5.62 ± 0.96	25.88 ± 0.45	46.14 ± 0.10
Dacotah	85.68 ± 3.36	34.25 ± 0.39	-124.87	15.25	-0.2786	0.97	10.4 ± 0.41	27.52 ± 0.22	44.64 ± 0.09
Expresso	93.07 ± 0.55	43.38 ± 0.62	-41.99	11.12	-0.2176	0.79	3.69 ± 0.48	25.38 ± 0.18	47.07 ± 0.16
Forestburg	80.76 ± 2.72	37.26 ± 0.23	-72.49	11.38	-0.2172	0.95	7.68 ± 0.13	26.31 ± 0.10	44.95 ± 0.17
Kanlow	53.05 ± 6.74	37.95 ± 1.09	-29.15	5.52	-0.1098	0.92	6.40 ± 0.97	25.37 ± 0.43	44.34 ± 0.15
Shawnee	50.31 ± 1.85	35.41 ± 0.26	-74.79	9.25	-0.1675	0.98	9.90 ± 0.26	27.60 ± 0.14	45.31 ± 0.05
Shelter	74.27 ± 2.39	33.47 ± 0.20	-118.72	13.04	-0.2313	0.94	11.46 ± 0.21	28.19 ± 0.12	44.92 ± 0.08
Summer	67.52 ± 1.32	31.47 ± 0.27	-151.20	14.61	-0.2525	0.95	12.83 ± 0.11	28.56 ± 0.09	44.30 ± 0.21
Sunburst	86.95 ± 0.21	40.65 ± 1.75	-60.75	11.39	-0.2213	0.98	5.49 ± 1.07	25.81 ± 0.38	46.14 ± 0.82
Trailblazer	87.46 ± 1.98	41.78 ± 0.94	-42.23	10.63	-0.2114	0.94	4.19 ± 0.84	25.08 ± 0.37	45.97 ± 0.14
Tusca	89.56 ± 0.78	35.54 ± 1.33	-76.87	12.88	-0.2430	0.90	6.27 ± 0.82	24.04 ± 0.48	41.81 ± 0.82
Mean	73.39	37.01	-	-	-	0.93	8.08	26.58	45.09
LSD	12.66*	4.09*	-	-	-		3.09*	1.43*	1.70*

*Significant at $P = 0.05$ probability level.

Table 6. Variation of maximum seed germination (MSG) and germination rate (GR) minimum (T_{min}), optimum (T_{opt}), maximum (T_{max}) and temperature adaptability range (TAR) between upland (Blackwell, Carthage, Cave-in-Rock, Dacotah, Forestburg, Shawnee, Shelter, Summer, Sunburst and Trailblazer) and lowland (Alamo, Expresso, Kanlow and Tusca) ecotypes of switchgrass.

Parameter	Ecotype				LSD
	Upland	CV (%)	Lowland	CV (%)	
MSG	75.07 a	18	69.16 a	38	31.46
MSG T_{min}	8.71 a	37	6.49 a	33	4.96
MSG T_{opt}	27.02 a	5	25.47 b	4	1.53
MSG T_{max}	45.34 a	5	44.44 a	1	2.18
MSG TAR	36.63 a	9	37.95 a	10	6.35
GR T_{min}	11.30 a	12	10.72 a	12	2.46
GR T_{opt}	32.37 a	4	34.98 b	7	2.57
GR T_{max}	46.26 a	1	45.38 a	2	1.83
GR TAR	34.96 a	3	34.66 a	3	1.65

Means within columns followed by the same letter are not significantly different ($P = 0.05$). CV (%) represents the variability of the ecotype means of a particular parameter.

Germination Rate Response to Temperature

The modified bilinear equation best described the relationship between GR and temperature (r^2 = 0.95, RMSE = 1.0) among the linear and non-linear models tested. Cardinal temperatures (T_{min}, T_{opt} and T_{max}) were different among genotypes ($P < 0.05$) (Table 5). For clarity, only data and predictor lines of four genotypes representing four ecotypic groups are presented in Fig. 3. The T_{min} ranged from 9.09 (Dacotah) to 12.92°C (Shelter) with a mean of 11.13°C. A mean of 33.12°C was estimated for T_{opt} which ranged from 29.55 (Shelter) to 35.73°C (Tusca). Maximum T_{max} was recorded in Shelter (48.15°C, while the minimum Tmax (45.0°C) was observed in Kanlow. The TAR ranged from 32.92 (Blackwell) to 36.18°C (Dacotah) with a mean of 34.88°C (Table 7). Ecotypic classification of genotypes indicate that TAR, T_{min}, and T_{max} did not differ but T_{opt} was different ($P < 0.05$) with a mean of 32.37 and 34.98°C for upland and lowland ecotypes, respectively (P = 0.0477; LSD = 2.57). Cardinal temperatures variation was small between ecotypes (< 4%) with germination rate T_{min} being more variable than T_{opt} and T_{max} for both upland and lowland ecotypes (Fig. 4 and Table 6).

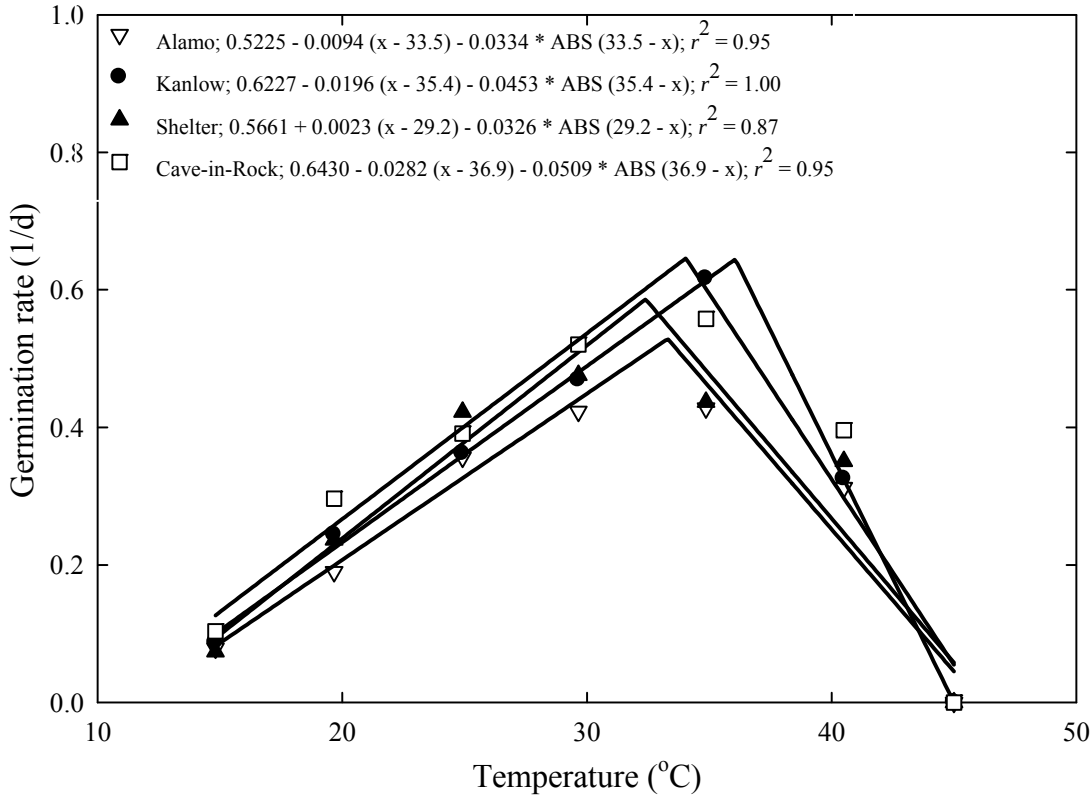

Figure 3. Effect of temperature on germination rate along with the fitted modified bilinear fitted lines and equations of four switchgrass genotypes (Alamo, Kanlow, Shelter and Cave-in-Rock). The symbols are the derived rate of development and the lines are predicted values by the fitted modified bilinear equations.

Table 7. Temperature adaptability range (TAR), modified bilinear equation constants (a, b, c), regression coefficients (r^2), and cardinal temperatures (T_{min}, T_{opt}, T_{max}) for germination rate of 14 switchgrass genotypes in response to temperature.

Genotype	TAR (°C)	Equation Constants			r^2	Cardinal temperatures (°C)		
		a	b	c		T_{min}	T_{opt}	T_{max}
Alamo	34.29 ± 0.86	0.5255	-0.0094	-0.0334	0.95	11.96 ± 0.60	33.02 ± 1.40	46.25 ± 0.78
Blackwell	32.92 ± 0.26	0.6791	-0.0142	-0.0459	1.00	12.14 ± 0.21	33.91 ± 0.08	45.06 ± 0.07
Carthage	34.06 ± 0.47	0.5945	0.0010	-0.0349	0.87	12.83 ± 0.22	30.45 ± 1.00	46.89 ± 0.64
Cave-in-Rock	35.11 ± 0.57	0.6430	-0.0282	-0.0509	0.98	10.16 ± 0.60	34.43 ± 0.88	45.27 ± 0.37
Dacotah	36.18 ± 0.30	0.6469	-0.0266	-0.0496	0.97	9.09 ± 0.49	35.34 ± 0.88	45.27 ± 0.26
Expresso	35.77 ± 0.53	0.7545	-0.0290	-0.0566	0.98	9.33 ± 0.63	35.50 ± 0.83	45.09 ± 0.10
Forestburg	35.17 ± 0.44	0.5884	-0.0121	-0.0374	0.98	10.18 ± 0.52	34.03 ± 0.78	45.35 ± 0.12
Kanlow	35.06 ± 0.84	0.6227	-0.0196	-0.0453	1.00	9.94 ± 0.84	35.65 ± 0.26	45.00 ± 0.00
Shawnee	35.01 ± 0.45	0.5940	0.0024	-0.0338	0.82	12.54 ± 0.26	30.56 ± 1.05	47.55 ± 0.71
Shelter	35.23 ± 0.15	0.5661	0.0023	-0.0326	0.87	12.92 ± 0.08	29.55 ± 0.07	48.15 ± 0.10
Summer	35.02 ± 0.36	0.4765	0.0009	-0.0270	0.86	12.06 ± 0.49	30.77 ± 1.36	47.08 ± 0.68
Sunburst	35.35 ± 0.34	0.6072	-0.0008	-0.0343	0.89	11.21 ± 0.23	30.48 ± 1.04	46.55 ± 0.50
Trailblazer	35.59 ± 0.18	0.7006	-0.0273	-0.0524	0.97	9.86 ± 0.27	34.21 ± 0.39	45.44 ± 0.15
Tusca	33.52 ± 0.39	0.6361	-0.0089	-0.0384	0.90	11.65 ± 0.34	35.73 ± 1.02	45.16 ± 0.22
Mean	34.88	-	-	-	0.93	11.13	33.12	46.01
LSD	2.47*	-	-	-	-	2.32*	4.49*	2.17*

*Significant at $P = 0.05$ probability level.

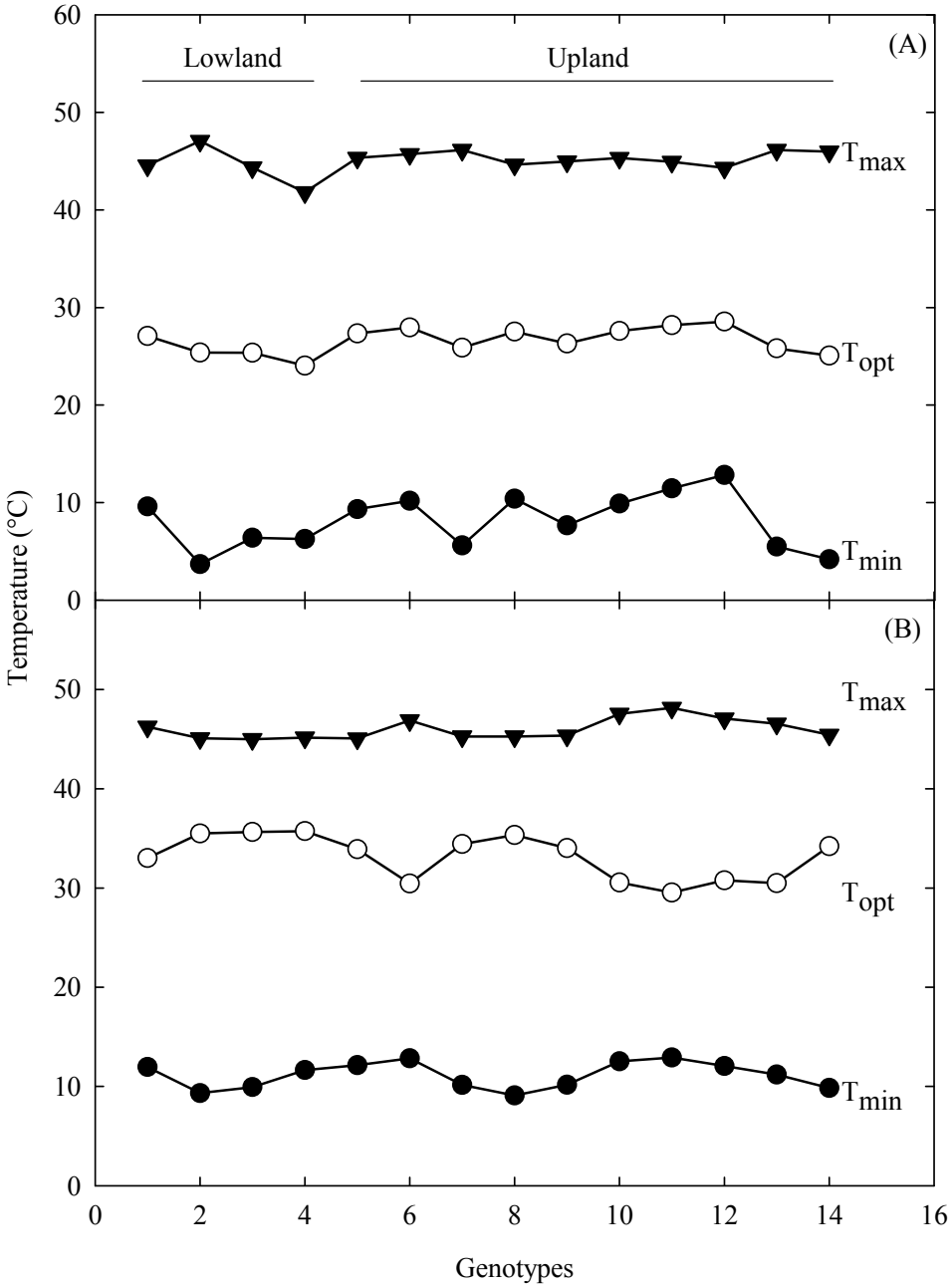

Figure 4. Ecotypic cardinal temperature variation for (A) maximum seed germination and (B) germination rate for 14 switchgrass genotypes.

Genotype Classification Using Cumulative Temperature Response Index (CTRI)

Six parameters (T_{min}, T_{opt}, and T_{max} for both MSG and GR) were used for both heat- and cold-tolerance classification based on CTRI. Each parameter contributed differently based on its relation to the minimum or maximum value for that parameter across the genotypes. Using 1 standard deviation permitted the classification of Heat-CTRI values (which ranged from 4.83 to 6.05) into three groups [heat-sensitive (4.83 – 5.43); intermediate (5.44 – 5.74), and heat-tolerant (5.73 – 6.05)]. Summer was identified as the most heat-tolerant genotype while Cave-in-Rock, Dacotah, Expresso, Forestburg, Kanlow, Sunburst, Trailblazer and Tusca as heat-sensitive genotypes (Table 8).

Using the same parameters, the genotypes were similarly classified for cold-tolerance (Table 9). Cold-CTRI values, which ranged from 4.74 to 6.21, allowed to group genotypes into four groups [cold sensitive (4.74 – 5.03); moderately cold sensitive (5.04 – 5.32), moderately cold tolerant (5.33 – 5.62), and cold tolerant (5.63 – 6.21)]. Expresso had the highest cold-CTRI (5.64), and therefore considered as most cold-tolerant genotype, while Summer had the highest heat-CTRI (5.78) and was classified as cold-susceptible genotype (Table 9).

Table 8. Classification of switchgrass genotypes into heat-tolerance groups based on cumulative temperature response index (CTRI; unitless) along with individual scores in parenthesis.

Genotype classification based on CTRI		
Heat-sensitive (CTRI = 4.83 - 5.43)	Intermediate (CTRI = 5.44 -5.74)	Heat-tolerant (CTRI = 5.75 - 6.05)
Expresso (4.83)	Alamo (5.45)	Summer (5.78)
Trailblazer (4.85)	Blackwell (5.47)	
Sunburst (5.0)	Shawnee (5.51)	
Cave-in-Rock (5.01)	Carthage (5.56)	
Kanlow (5.03)	Shelter (5.59)	
Tusca (5.06)		
Forestburg (5.16)		
Dacotah (5.36)		

Table 9. Classification of switchgrass genotypes into cold-tolerance groups based on cumulative temperature response index (CTRI; unitless) along with individual scores in parenthesis.

Genotype classification based on CTRI			
Cold-sensitive (CTRI = 4.74 - 5.03)	Moderately cold-sensitive (CTRI = 5.04 - 5.32)	Moderately cold-tolerant (CTRI = 5.33 -5.62)	Cold-tolerant (CTRI = 5.63 - 6.21)
Shelter (4.74)	Forestburg, (5.08)	Trailblazer (5.52)	Expresso (5.64)
Summer (4.74)	Tusca (5.19)		
Carthage (4.78)	Kanlow (5.21)		
Shawnee (4.8)	Cave-in-Rock (5.24)		
Blackwell (4.82)	Sunburst (5.26)		
Alamo (4.84)			

Parameter Relationships

No relationship was found between maximum seed germination T_{min} and T_{max} and T_{opt} and T_{max} ($P > 0.05$) (Fig. 5A and 5B); however, a positive linear relationship existed between T_{min} and T_{opt} ($r^2 = 0.81$, $P < 0.0001$) (Fig. 5A). As T_{min} increased among the genotypes, T_{max} generally increased ($r^2 = 0.56$, $P < 0.0021$) (Fig. 6A). An inverse relationship was found between T_{min} and T_{opt} ($r^2 = 0.58$, $P < 0.0014$) (Fig. 6A) as well as T_{opt} and T_{max} ($r^2 = 0.88$, $P < 0.0001$) (Fig. 6B). The relationship between MSG and GR cardinal temperatures varied but a weak positive relationship was found between MSG and GR T_{min} ($r^2 = 0.39$, $P = 0.0163$) (Fig. 6A), while a weak negative relationship was found between MSG and GR T_{opt} ($r^2 = 0.46$, $P = 0.0071$) (Fig. 6B).

Seed quality parameters (C and N content and seed weight) did not affect cardinal temperatures of both MSG and GR ($P > 0.05$; Figs. 6 A to F). However, MSG was correlated with seed C ($r^2 = 0.29$, $P = 0.0469$) and seed N ($r^2 = 0.57$, $P = 0.0018$) content and seed weight ($r^2 = 0.26$, $P = 0.0623$); (Figs. 7A, 7B, and 7C).

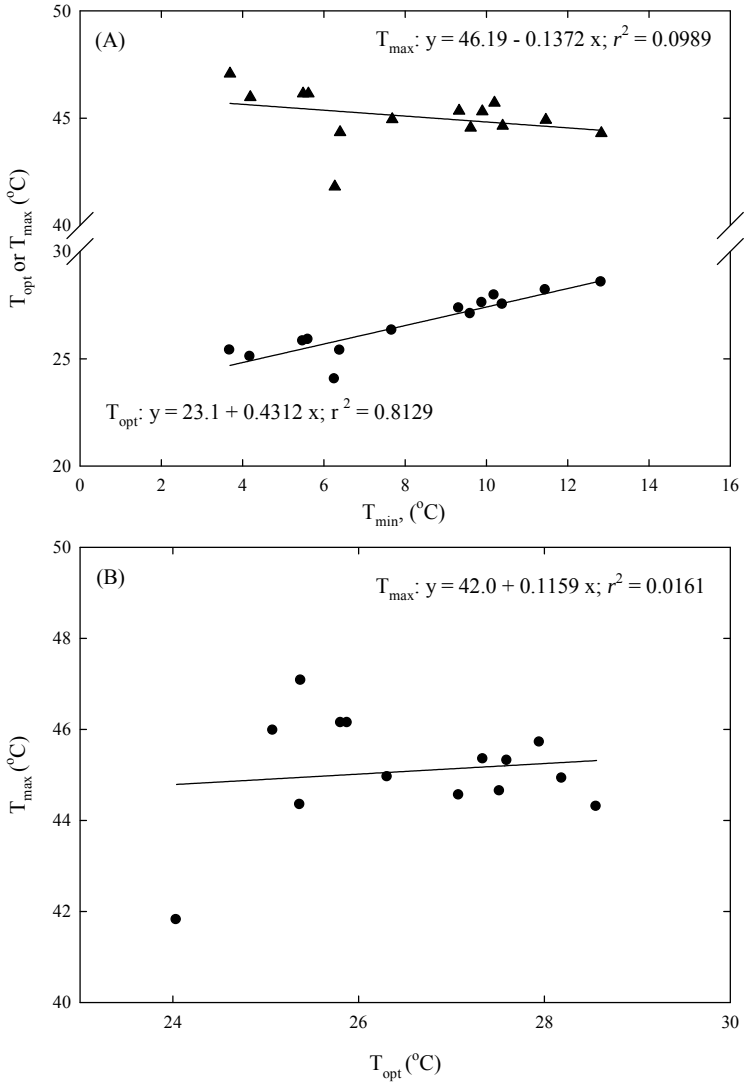

Figure 5. Relationship between (A) minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures, and (B) T_{opt} and T_{max} for maximum seed germination of 14 switchgrass genotypes.

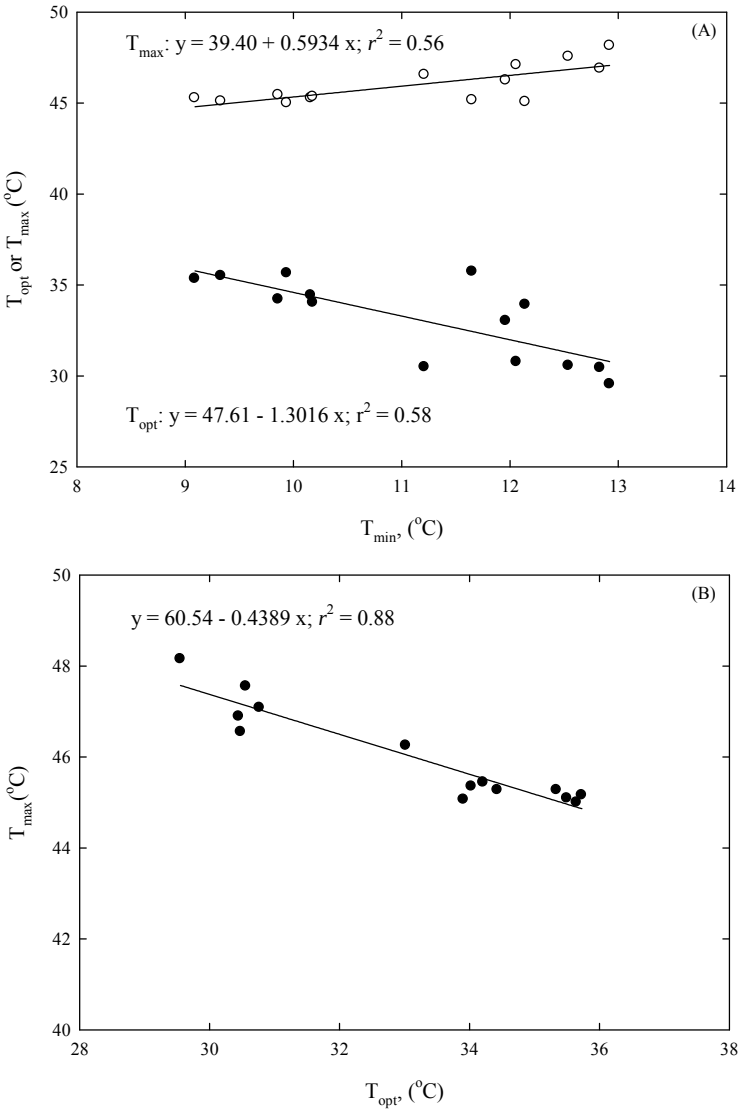

Figure 6. Relationship between (A) minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures, and (B) T_{opt} and T_{max} for germination rate of 14 switchgrass genotypes.

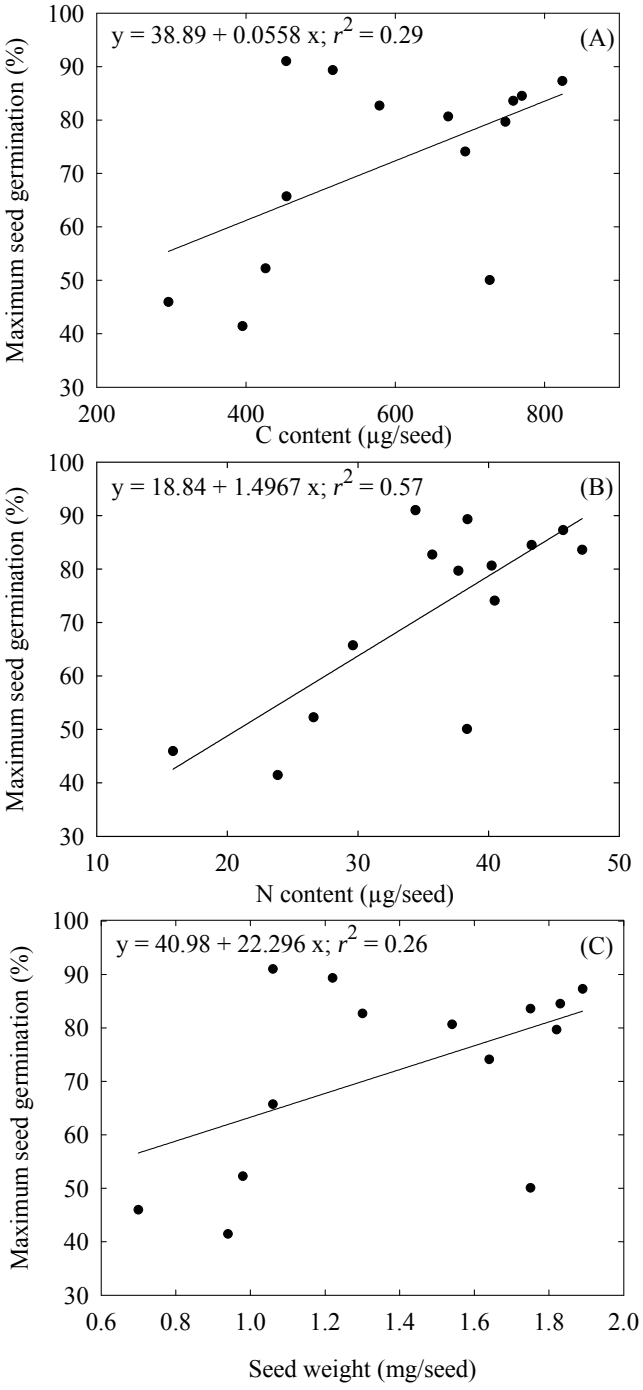

Figure 7. Relationship between seed carbon (C) content (A and B), nitrogen (N) content (C and D) and seed weight (E and F) and germination rate (A, C and E) and maximum seed germination (B, D and F) temperatures: (i) T_{max}, (ii) T_{opt} and (iii) T_{min} for 14 switchgrass genotypes.

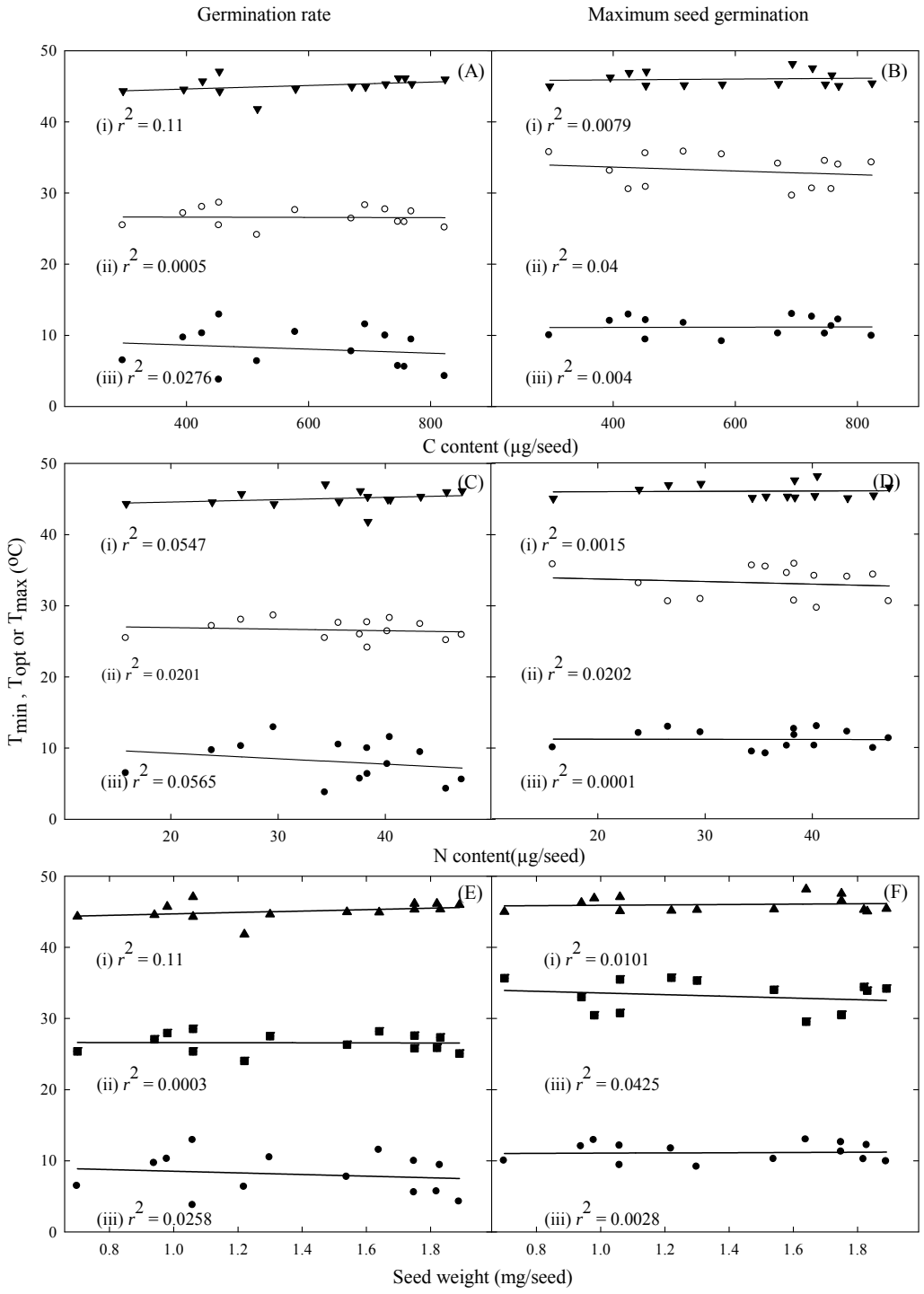

Figure 8. The relationship between maximum seed germination and seed (A) carbon (C) content, (B) nitrogen (N) content, and (C) seed weight for14 switchgrass genotypes.

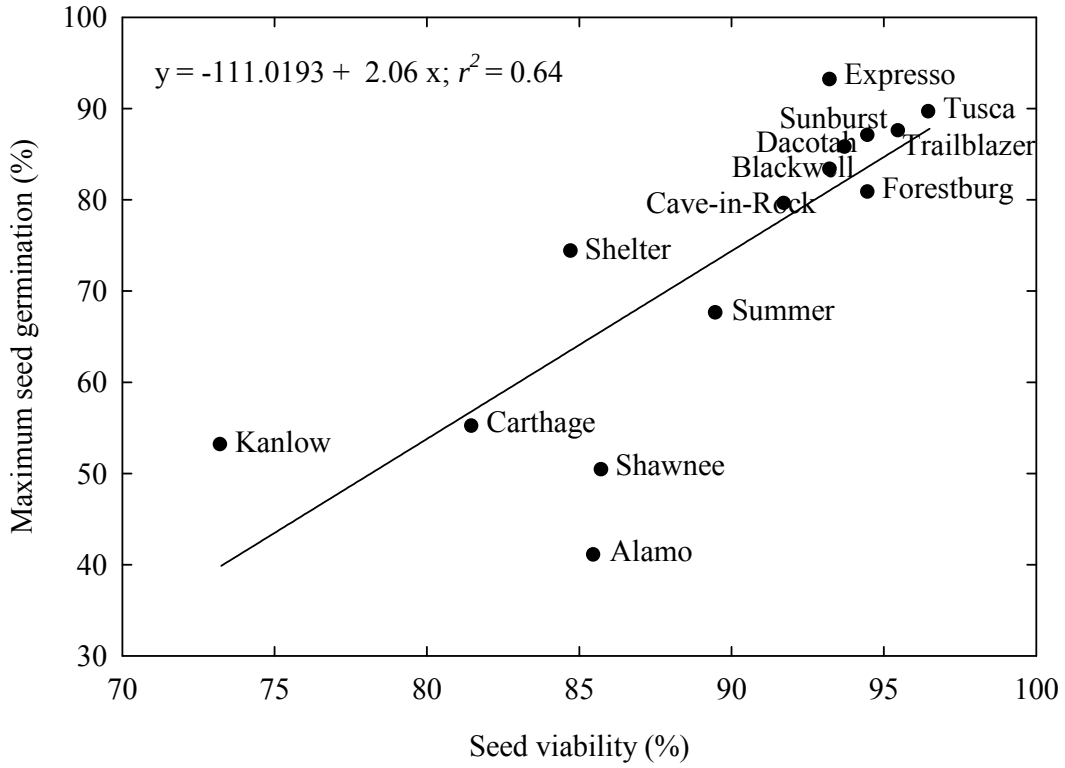

Figure 9. The relationship between seed viability and maximum seed germination for 14 switchgrass genotypes.

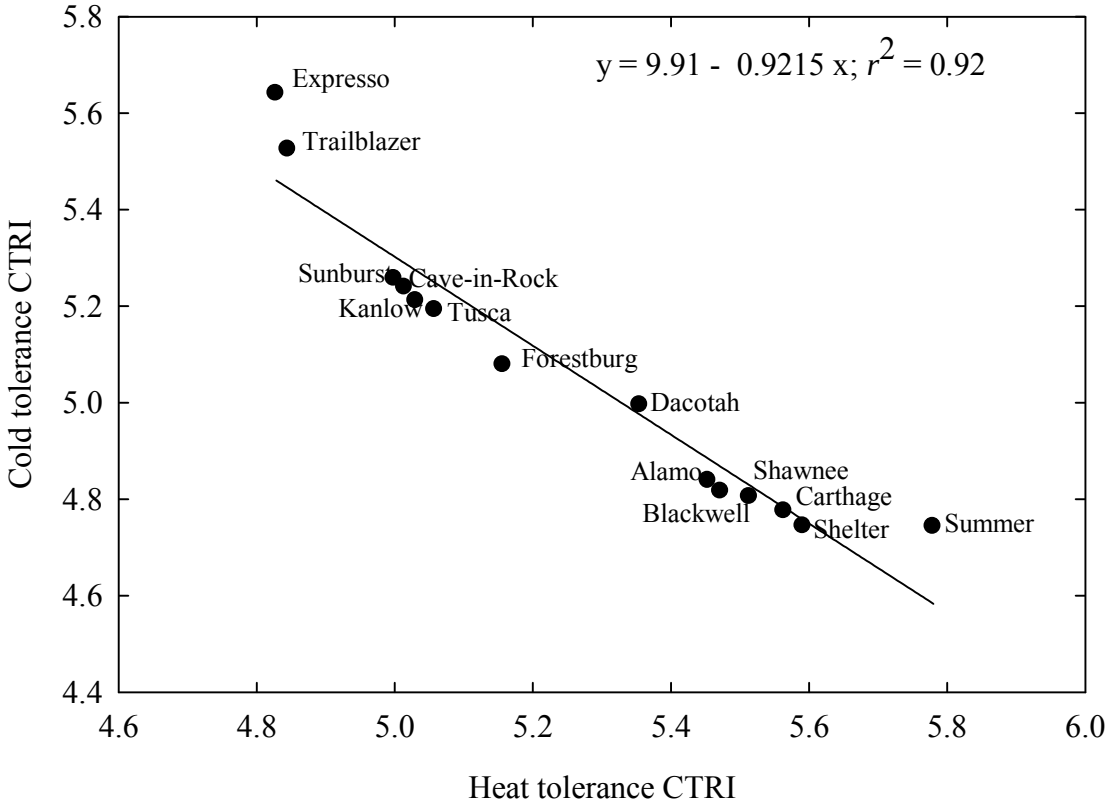

Figure 10. The relationship between heat- and cold-tolerance cumulative temperature response index (CTRI) for 14 switchgrass genotypes.

DISCUSSION

Seed germination is a complex physiological process modulated by internal and external factors and their interactions. Similar to other growth and developmental processes, temperature influences seed dormancy, germination capacity and rate, and seedling emergence. To our knowledge, this is the first study to evaluate the influence of *in vitro* temperature effects on diverse switchgrass genotypes. The resulting data provided functional algorithms for modeling and segregating genotypes for cold- and heat-tolerance based seed-based parameters. The seed germination rate and final germination percentage, two important seedlot descriptive and quantification parameters, are affected differently by temperature and the quantification of these responses are imperative to thermal modeling.

Optimal temperatures for MSG and GR differed among the genotypes with MSG optimum occurring over a range and GR having a sharply defined optimum. Seeds not germinating within 7 d of seeding usually have reduced survival potential due to the effects of pathogenic infection and insect attacks, and exhaustion of seed reserves (Garcia-Huidobro et al., 1982). Relative to MSG, GR consistently had higher T_{min}, T_{opt}, and T_{max} values, even though the temperature adaptability range (TAR, T_{min}-T_{max}) among MSG and GR cardinal temperatures was small (< 6.18^{o}C) with the exception of T_{min} of MSG, which ranged from 3.69 to 12.83^{o}C. This is consistent with Roberts (1988) findings that many species typically have higher optimum temperatures for GR than for maximum seed germination percentage. Germination rate is reported to be more temperature sensitive than final germination percentage in *Setaria lutescens* and

Amaranthus retroflexus (Schimpf et al., 1977). A higher range value is indicative of the temperature adaptability range (TAR) of a species, suggesting better survival potential to temperature variation.

Seed Quality Characteristics

The influence of temperature on MSG has been quantified, however, these responses may be applicable to one seed population because of experiment-specific conditions (Ellis et al., 1987). Maximum seed germination is reportedly affected by seed quality (Ellis et al., 1982, 1986, 1987), seed maturation environment (Orozco-Segovia et al., 1993, Sharif-Zadeh and Murdoch, 2007, Fenner, 2008) and time from harvest to seeding (Shaidaee et al., 1969, Jensen and Boe, 1991), hence limiting the utility of MSG as a screening tool. Ellis et al. (1987) contended that the same criticism can be made for GR responses to temperature; however, cardinal temperatures (T_{min} and T_{opt}) have been reported to be unchanged by temperature and therefore are a better parameters to evaluate the dispersion of responses across genotypes. Ellis et al. (1987) found no variation among T_{min} for three seedlots of onion (*Allium* cepa L.) differing in viability, suggesting that germination minimum temperature is a genotypic characteristic unaffected by seed quality.

Maximum Seed Germination

Germination is a function of accumulated thermal time, hence limiting the germination period to 28 d at suboptimal temperatures may not be reflective of the true

germination potential at these temperatures. Nevertheless, genotype responses based on MSG can be used to differentiate among genotypes. All switchgrass genotypes tested exhibited a quadratic response to temperature ($r^2 = 0.93$), similar to indiangrass (Fulbright, 1988), another native warm-season species. Mean MSG (73%) in the current study is similar to the 78% reported by Hacisalihoglu (2008), 77% by Aiken and Springer (1995) and 77% by Hanson and Johnson (Hanson and Johnson, 2005) for similar genotypes. With the exception of Expresso, which has been selected for increased precocious germination, MSG of the other two lowland genotypes (Alamo and Kanlow) were < 55%.

The linear and significant correlation between MSG and seed viability (Fig. 9) suggests that ungerminated seed are dormant even after the AOSA (1991) recommended two-week stratification. Switchgrass is a highly dormant species influenced by seed coat or embryo coverings that may impede water influx and gas exchange (respiration), contain germination inhibitors, modify light quantity and quality reaching the embryo, or act as a mechanical restraint to the emerging coleoptile or coleorhizae (Adkins et al., 2002). As seed viability increases within a seedlot, seed germination percentage should increase as well; however, the extent of the difference between viability and germination indicates the percentage of infertile or dormant seeds. Increasing MSG can be accomplished by aging (Shen et al., 1999, Shen et al., 2001), priming (Beckman et al., 1993, Hacisalihoglu, 2008), prolonged stratification (Shen et al., 2001), KNO_3 or gibberellic acid conditioning, polyethylene glycol (PEG) osmoconditioning (Madakadze et al., 2000) or mechanically scarification (Jensen and Boe, 1991).

The optimum temperature for switchgrass MSG in the current study varied between 24.04 and 28.56^{o}C among the genotypes, which is within the range of other warm-season grasses. An optimum range of 20 – 30^{o}C was reported (Roundy and Biedenbender, 1996) for Cane beardgrass [*Bothriochloa barbinodis* (Lag.) Herter], sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.], and tanglehead [*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.]. 16.5 – 27^{o}C for Indiangrass (Sabo and Forest, 1979). Minimum temperature of MSG averaged 8.08^{o}C and ranged from 3.69 to 12.83^{o}C, which is similar to T_{min} of other warm-season grasses reported by Madakadze et al.(2001), for example, 5.5 to 10.9°C for switchgrass; 7.3 to 8.7°C for big bluestem; 7.5 to 9.6°C for Indiangrass and 4.5 to 7.9^{o}C for prairie sandreed.

Germination Rate

Thermal response of seed germination is consistent with thermal response patterns of a number of other physiological processes (Probert, 2000). At suboptimal temperatures (T_{min} to T_{opt}), germination rate (reciprocal time to 50% germination) generally increases linearly with temperature, but decreases linearly with temperature at supra-optimal temperatures (T_{opt} to T_{max}). This characteristic thermal response is similar to germination rate of chickpea (Covell et al., 1986, Ellis et al., 1986), lentil and soybean (Covell et al., 1986), pearl millet (Garcia-Huidobro et al., 1982), sorghum (Benech-Arnold et al., 1990), and cool season weeds (Hardegree, 2006). A decline in germination rate with decreasing temperature is partly associated with an observed decline in the imbibition rate observed with a reduction in temperature (Lopez et al., 2000). Germination rate response to

temperature was described previously by two linear equations; the first describing the positive linear relationship between the minimum and optimum temperatures and the second describing the negative linear relationship between optimum and maximum temperature (Covell et al., 1986, Ellis et al., 1986). In this study, GR was modeled using a single modified bilinear equation, which was previously used by several studies (Kakani et al. (2002); Kakani et al. (2005); Reddy and Kakani (2007); Salem et al. (2007) and Singh et al. (2008) to quantify pollen germination and pollen tube growth responses to temperature. Analogous to pollen, seeds are considered independent functional units that are responsive to temperature changes.

Even though MSG percentage is the most important parameter determining commercial value of seedlots, GR influences the uniformity and rapidity of emergence in nurseries (El-Kassaby et al., 2008). Germination rates are most rapid at optimum temperature ranging from 29.5 to 35.6^{o}C.

The variability in quantitative characteristics of rate of germination (T_{min}, T_{opt}, and T_{max} and TAR) among the genotypes may be attributed to genetic variability rather than seed quality. Seed quality characteristics did not correlate with MSG or GR cardinal temperatures; however, N content of seeds affected MSG suggesting cardinal temperatures are insensitive to seed quality characteristics tested while MSG is affected by seedlot quality.

Cardinal Temperatures

Biological processes are typically characterized by cardinal temperatures describing the range of temperature over which a process can occur. The effect of temperature on seed germination can be expressed in terms of cardinal temperatures, that is, T_{min}, T_{opt}, and T_{max} at which germination will occur (Copeland and McDonald, 2001). Cardinal temperatures may be used to describe the range of adaptation of a species.

Though switchgrass is reported to be the most temperature specific of the warm-season grasses (Hsu et al., 1985), there exists significant intraspecific differences in cardinal temperatures that may be related to the different areas of origin or adaptation (Madakadze et al., 2001, Casler and Boe, 2003). The genotypes Cave-in-Rock, Dacotah, Forestburg, Shawnee, Shelter, Summer, Sunburst and Trailblazer are from the more cooler northern regions where average minimum temperatures range from –23.3 to –17.8°C , while Alamo, Blackwell, Expresso, Kanlow and Tusca are from the more warmer growing regions with average minimum temperatures ranging from –17.8 to 4.4°C. Cardinal temperature coefficients can be directly compared for screening germplasm (Hardegree, 2006). The cardinal temperatures derived for both MSG and GR can be used in evaluation of potential regions for introduction of switchgrass and also aid in on-farm operational practices such as appropriate sowing dates when soil temperature would be conducive to optimum germination and emergence and ultimately optimum stand establishment and crop performance. Genotypes with lower T_{min} values can be subjected to early-season sowing because of their inherent capacity to germinate in cooler temperatures. The variability of cardinal temperatures both for MSG and GR indicates

broad latitudinal adaptation across the various plant hardiness zones of the USA (Casler et al., 2004).

The cardinal temperatures derived for GR may be comparable with subsequent developmental stages of switchgrass ontogeny (morphological development). Kiniry et al. (2005) assumed a base temperature of 12°C for all growth stages of switchgrass in the ALMANAC model; however, the results in this study suggest that cardinal temperatures are genotype-specific and may be process-specific as well. Therefore, the derived cardinal temperatures in this study may be used to refine model algorithms for on-farm application and policy assessments.

Temperature Tolerance Classification

Temperature tolerance refers to the ability of an organism to cope with excessively high or low temperatures. Direct selection under field conditions is generally difficult because uncontrollable environmental factors affect the precision and repeatability of such trials. Stress tolerance is a developmentally regulated, stage-specific phenomenon; hence species may show different sensitivity to stress at different developmental stages. All stages through a plant's ontogeny are sensitive to temperature; therefore, screening for tolerance should be conducted at the most sensitive stage. Seed germination is temperature dependent and can be used to screen for temperature tolerance. *In vitro* assays are not subjected to uncontrollable biotic and abiotic stress factors marring true tolerance potential. In the field, genotypes with high minimum

temperature would experience little germination in early spring when temperatures would frequently drop below the T_{min} level.

In the current study, the successful use of CTRI, based on the summation of individual temperature response indices and then separated by standard deviation based on the number of classes of interest, confirms that seed-based parameters derived from *in vitro* seed germination assay can be used for genotype temperature tolerance classification. Genotype variability associated with temperature tolerance was demonstrated in this study. Alamo, Blackwell, Carthage, Dacotah, Shawnee, Shelter and Summer were classified as cold-sensitive while Expresso was classified as cold-tolerant. Conversely, Cave-in-Rock, Dacotah, Expresso, Forestburg, Kanlow, Sunburst, Trailblazer and Tusca were determined to be heat-sensitive and Summer as heat-tolerant. Since basal temperature tolerance is a function of genetics and acquired temperature tolerance is latitude and temperature-induced, corroborating seed-based temperature tolerance with vegetative or other reproductive responses will validate the use of seed-based parameters as a screening tool. This information is lacking in the literature with respect to screening temperature tolerance of diverse switchgrass genotypes, even though several studies link intraspecific differences in germination to geographical and ecological areas of distribution or origin (Orozco-Segovia et al., 1996). The classification method tested suggests that CTRI for heat- and cold-tolerance are inversely related ($r^2 = 0.64$, $P = 0.0006$), indicating that heat- and cold-tolerance may be unique and independent traits and may not occur simultaneously within a single genotype (Fig.

10). Variability among genotypes for heat- and cold-tolerance suggests that selection or breeding among genotypes is a viable objective.

Switchgrass adaptation to a specific ecoclimatic and edaphic region is determined by the growth rate, photoperiodism, heat tolerance, and cold or freezing tolerance of a specific genotype (Casler et al., 2007). Ecotype classification in this study did not necessarily confer the temperature tolerance characteristic of a specific ecotype. For example, Alamo, a lowland genotype, was classified as intermediately heat tolerant while Summer, an upland genotype was classified as heat tolerant using seed-based parameters. Genotype temperature tolerance is determined not only by ecotypic classification, but also latitude of origin, photoperiodism and genetics. Being photoperiod sensitive (Moser and Vogel, 1995), switchgrass morphological development is determined primarily by its response to photoperiod (Mitchell and Moser, 2000). Since ecotypic classification are more related to photoperiod responsiveness than temperature, the small or little variation observed between upland and lowland ecotypes for seed germination characteristics may be as result of ecotypic temperature insensitivity.

Since tolerance mechanisms are developmentally regulated, it is prudent to validate controlled *in vitro* seed germination assay with field performance tests. In the current study, GR and MSG were evaluated as estimators of temperature tolerance using 14 diverse genotypes. Using similar techniques, Tiryaki and Andrews (2001) screened 12 genotypes of sorghum for cold tolerance in controlled *in vitro* germination studies and found that GR was strongly correlated with rate of emergence under field conditions,

confirming that screening using parameters based on *in vitro* studies is a rapid and reliable method for handling large number of genotypes before evaluation in the field.

The current study quantified the relation between GR and temperature, highlighting genotypic differences. It is necessary in future work, therefore, to determine whether *in vitro* seed germination assay has potential in selection and screening procedures in breeding programs (Covell et al., 1986).

CHAPTER IV

SUMMARY AND CONCLUSIONS

The current study quantified the effects of temperature on seed germination rate and capacity of 14 diverse switchgrass genotypes and determined the cardinal temperatures for maximum seed germination and germination rate characteristics. Genotypic variability for maximum seed germination, germination rate, their respective cardinal temperatures, and temperature adaptability range were found to exist among the diverse switchgrass genotypes tested. Mean minimum temperatures for maximum seed germination and germination rate were 8.08 and 11.1°C, respectively, while optimum temperatures were 26.6 and 33.1°C, respectively. Using cumulative temperature response index, temperature tolerance variability was found among the genotypes. For cold sensitivity, seven of the 14 genotypes classified were as cold-sensitive (Alamo, Blackwell, Carthage, Dacotah, Shawnee, Shelter and Summer), six as moderately cold-sensitive (Cave-in-rock, Forestburg, Kanlow, Sunburst and Tusca), one each as moderately cold-tolerant (Trailblazer) and cold tolerant (Expresso). For heat sensitivity, eight of the 14 genotypes were classified as heat-sensitive (Cave-in-rock, Dacotah, Expresso, Forestburg, Kanlow, Sunburst, Trailblazer and Tusca), five as heat-intermediate (Alamo, Blackwell, Carthage, Shawnee and Shelter) and one as heat-tolerant

(Summer). Temperature tolerance classification based *in vitro* germination seed assay is therefore a simple and inexpensive technique for screening of a large number of genotypes. The inverse relationship between heat and cold tolerance cumulative temperature response index suggests that these two traits are independent and can be selected for separately.

The method used in the current study identified both heat and cold tolerant genotypes and demonstrated that variability existed among genotypes and ecotypes. The cardinal temperature estimates would be useful to improve switchgrass models for field applications. Additionally, the identified cold- and heat-tolerant genotypes can be selected for niche environments and in switchgrass breeding programs to develop new genotypes for cold and hot environments.

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