High Temperature Tolerance in *Aegilops* Species and Its Potential Transfer to Wheat

G. P. Pradhan, P. V. V. Prasad,* A. K. Fritz, M. B. Kirkham, and B. S. Gill

ABSTRACT

High temperature (HT) stress is highly detrimental to crop productivity but there is limited variability for this trait among wheat (*Triticum aestivum* L.) cultivars. The objectives of this research were to screen wild wheats (*Aegilops* species) for tolerance to HT stress at the reproductive stage and to measure physiological and yield traits associated with the tolerance. Fifty-two accessions belonging to five *Aegilops* species were evaluated at optimum temperature (OT) (25/19°C day/night) and HT (36/30°C) with a photoperiod of 18 h. Stress was imposed at anthesis and continued for 16 d. Across species, HT decreased chlorophyll, grain number per spike, individual grain weight, and grain yield per plant by 38, 40, 56, and 70%, respectively. Based on a decrease in grain yield, *A. speltoides* Tausch and *A. geniculata* Roth were most tolerant and *A. longissima* Schweinf. & Muschl. was highly susceptible to HT stress. Tolerance was associated with higher grain number per spike and/or heavier grains. Within *A. speltoides,* accession TA 2348 was highly tolerant to HT with 13.5% decline in grain yield and a heat susceptibility index (HSI) of 0.23 whereas TA 1787 and TA 2097 were highly susceptible with >82% yield decline and HSIs > 1.4. Among *A. geniculata*, two moderately HT tolerant accessions, TA 2899 and TA 1819, were identified, with an HSI of 0.80. Our results suggest that there is genetic variability among *Aegilops* species that can be utilized in breeding wheat for HT tolerance at reproductive stage.

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Abbreviations: CTD, canopy temperature depression; GS, growth stage; HSI, heat susceptibility index; HT, high temperature; OT, optimum temperature; PAR, photosynthetically active radiation; SPAD, soil plant analysis development.

Wheat (*Triticum* spp.) is one of the most important food crops in the world in terms of the area harvested, production, and nutrition as it supplies about 19% of the calories and 21% of the protein to the world's population (FAO, 2011). Over 90% of world wheat area is planted to common or bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, genomes AABBDD). The polyploidy has a highly buffered genotype and has enormous genetic variability as each locus may harbor three divergent alleles. This genetic attribute enables bread wheat to exhibit an array of phenological responses to wide ranges of photoperiod and temperature regimes, including vernalization (Slafer and Rawson, 1994). Thus, wheat can be grown from tropical to temperate climates and from a few meters to more than 3800 m above sea level (Aase et al., 2010). Although wheat has a wide range of climatic adaptability, many biotic and abiotic factors limit its yield. High temperature stress is one of the most important environmental factors

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limiting crop production in the world. During the coming decades as a result of global warming, field crops may experience more hot days and nights (IPCC, 2007; Meehl and Tebaldi, 2004). Most of the wheat-growing areas of the world, including Great Plains of the United States, experience above-optimum temperatures at some point in their life cycle and have a negative impact on yield (Abdalla et al., 2010; Reynolds et al., 2001).

High temperature decreases crop yield by adversely affecting phenological, morphological, physiological, and biochemical traits. High temperature reduces chlorophyll and the photosynthetic capacity of leaves (Prasad et al., 2008). Thylakoid membranes are one of the most sensitive cellular structures to high temperature (HT) stress. Damaged thylakoid membranes result in loss of chlorophyll and decreased photosynthesis (Al-Khatib and Paulsen, 1984). Ristic et al. (2007) reported a strong positive correlation between HT-induced thylakoid membrane damage and chlorophyll content in 12 winter wheat cultivars. High temperature increases leaf temperature, which results in reduced canopy temperature depression (CTD), a difference between air and canopy temperature. A positive correlation between CTD and wheat grain yield has been reported and recommended as a useful trait in selecting HT-tolerant genotypes (Balota et al., 2007).

The main effect of HT stress on wheat plants is the decreased duration of all developmental stages. When spring wheat was grown in summer, a decrease in duration of growth stage (GS) 1 (emergence to double ridge), GS 2 (double ridge to anthesis), and GS 3 (anthesis to grain maturation) stages was observed (Shpiler and Blum, 1986). Prasad et al. (2008) reported a decrease in time to flowering, grain set, and physiological maturity in spring wheat when grown at high nighttime temperature. The reproductive stage has been considered the most temperature-sensitive period in wheat. High temperature at anthesis decreases the grain number per spike (Prasad et al., 2008; Yang et al., 2002) and grain size (Stone and Nicolas, 1994; Viswanathan and Khanna-Chopra, 2001), both of which have a large effect on grain yield. The decrease in grain number is mainly due to adverse effects of HT on floral organs. High temperature during meiosis reduces wheat yield due to decrease in grain set (Saini and Aspinall, 1982; Saini et al., 1983). High temperature at the grain filling stage adversely affects grain yield by decreasing individual grain size. Stone and Nicolas (1998) reported that a day of HT (40/21°C day/night) during grain filling decreased the grain size of wheat by 14% compared to the control (21/16°C day/night). Such a decrease in grain size is the consequence of shorter grain filling duration and/or grain growth rate (Gibson and Paulsen, 1999; Viswanathan and Khanna-Chopra, 2001). However, high temperature often increases grain filling rate but not enough to compensate for decreased grain filling duration (Prasad et al., 2006b). A study in wheat showed that high nighttime temperature decreased spikelet fertility, grain number, individual grain size, and grain filling duration (Prasad et al., 2008).

One way to mitigate the effect of HT stress on yield is to develop stress tolerant varieties (Wahid et al., 2007). *Aegilops* species have been considered a genetic resource for increasing the genetic potential of cultivated wheat to withstand biotic and abiotic stresses. *Aegilops* species are close relatives of bread wheat. Bread wheat acquired its D genome from *A. tauschii* Coss. (Kihara, 1944; McFadden and Sears, 1946), and *A. speltoides* Tausch. has been considered the closest extant species to B and G genomes of polyploid wheats (Dvorák and Zhang, 1990). As two of the three genomes of bread wheat came from *Aegilops*, it has been an important source for disease- and insect-resistant genes (Gill et al., 2006). Wild wheats are also sources for abiotic stress-tolerance genes. Some accessions of *A. tauschii*, *A. speltoides*, and *A. geniculata* Roth have shown the capability to withstand drought (Baalbaki et al., 2006; Zaharieva et al., 2001). A few HT stress tolerant accessions belonging to *A. geniculata*, *A. speltoides*, *A. searsii* Feldman & Kislev ex K. Hammer, and *A. longissima* Schweinf. & Muschl. also have been reported (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001). There is a lack of information on the effect of an extended period (16 d) of high day/night temperature at anthesis on growth, physiology, and yield of *Aegilops* species.

The objectives of this study were (i) to identify *Aegilops* species and accessions with tolerance to HT stress at the reproductive stage and (ii) to measure physiological, growth, and yield traits associated with high temperature tolerance.

MATERIALS AND METHODS

Plant Material

Fifty-two accessions of *Aegilops* belonging to five different species, *A. markgrafii* (Greuter) K. Hammer (syn. *A. markgrafii* L.) (9 accessions), *A. geniculata* (12 accessions), *A. longissima* (6 accessions), *A. searsii* (9 accessions), and *A. speltoides* (16 accessions), were used in this research (Table 1).

Experimental and Treatment Conditions

This research was conducted at the facility of the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, KS. Seeds of each accession were sown in 4-cm deep trays containing commercial Sun Grow Metro Mix 300 potting soil (Hummert International, Topeka, KS). The seedlings were raised in a growth chamber (Conviron Model E15, Winnipeg, MB, Canada) maintained at 20/15°C day/night temperature, 12 h of photoperiod, and 65% humidity. After 14 d, seedlings were vernalized for 42 d at 4°C, 8 h photoperiod, and photosynthetically active radiation (PAR) of 100 μmol m^{-2} s^{-1}. Following vernalization, three seedlings of each accession were transplanted into six 1.6-L plastic pots of dimensions 14 cm (height) by 50 cm (top perimeter) by 36 cm (bottom perimeter) filled with a mixture of soil and Metro Mix 300 at a ratio of 1:2 and 4 g of Osmocote Plus (Scotts, Marysville, OH), a slow-release fertilizer. The pots were placed randomly in three growth chambers designated as three replications. Environmental conditions in growth chambers were optimal for *Aegilops* species tillering: 20/15°C day/night temperature, 12 h photoperiod, and 85% humidity. Each growth chamber held two pots of each accession. After seedlings were established, one seedling from each pot was removed, leaving two seedlings per pot. At this time, Marathon 1% G (granules) {a.i.: Imidacloprid,1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine} was applied to avoid infestation of insect pests. At 45 d after transplanting, chamber conditions were changed to 25/19°C day/night, 18 h photoperiod, and 85% humidity, conditions favorable for *Aegilops* species' flowering. In all growth chambers, the canopy level PAR of 400 μmol m^{-2} s^{-1} was provided by cool white fluorescent lamps (Philips Lighting Co., Somerset, NJ). Plants in each growth chamber were randomly moved every 7 d to avoid positional effects within the chamber.

With the onset of anthesis at Feekes growth stage 10.5.1, one pot of each accession was moved from the optimum temperature (OT) regime (25/19°C day/night) to one of three growth chambers maintained at HT of 36/30°C day/night, 18 h photoperiod, and 85% humidity. The duration of HT stress was 16 d; plants were then returned to their original growth chamber (25/19°C day/night). To avoid water stress, all pots were kept in trays containing about 2 cm deep water from sowing to maturity.

At heading, one plant in each pot was randomly selected and the main stem was tagged. In addition, four other spike-bearing tillers of the same plant were tagged for growth, physiological, and yield traits. Data were collected from tagged plants.

Leaf Chlorophyll and Flag Leaf Temperature

Leaf chlorophyll and leaf temperature were measured every other day from the start of treatment for 16 d. A self-calibrating soil plant analysis development (SPAD) chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL) was used to measure chlorophyll from a fully expanded flag leaf on a tagged main stem. Each time, data were taken three times from the middle portion of the leaf and the reading was averaged. Before taking SPAD meter readings, images of flag leaves were captured with a FLIR BCAM SD (forward-looking infrared building camera, secure digital) thermal imaging camera (FLIR Systems Inc., Wilsonville, OR). To determine flag leaf temperature, these images were processed with QuickReport 1.2 software (FLIR Systems Inc., 2009). Flag leaf temperature depression was then estimated by subtracting the flag leaf temperature (measured with a BCAM SD infrared camera, FLIR Systems Inc., Wilsonville, OR) from the air temperature, collected with Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA).

Plant Height, Tiller Number, and Biomass

At maturity, plant height was measured from plant base to the tip of main stem spike excluding awns. Tiller number per plant consists of both fertile (with spikes) and nonfertile (without spike) tillers. Vegetative biomass per plant was the weight of oven dried (65°C for 10 d) plant material without spikes. The spikes were dried in an incubator (at 40°C) until they attained a constant weight. Above-ground biomass per plant includes vegetative biomass and dried spike weight per plant.

Table 1. Accession number, species, and country of origin of *Aegilops* species used for identifying high temperature tolerant lines at the reproductive stage†.

Accessions	Genus	Species (new)	Species (old)	Subspecies	Country of Origin
TA 1906	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 1908	*Aegilops*	*markgrafii*	*markgrafii*		Germany
TA 1909	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2085	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2091	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2093	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2095	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2096	*Aegilops*	*markgrafii*	*markgrafii*		Turkey
TA 2170	*Aegilops*	*markgrafii*	*markgrafii*		Syria
TA 1800	*Aegilops*	*geniculata*	*ovata*		Turkey
TA 1802	*Aegilops*	*geniculata*	*ovata*		Turkey
TA 1808	*Aegilops*	*geniculata*	*ovata*		Turkey
TA 1813	*Aegilops*	*geniculata*	*ovata*		Italy
TA 1814	*Aegilops*	*geniculata*	*ovata*		Romania
TA 1819	*Aegilops*	*geniculata*	*ovata*	*vulgaris*	Japan
TA 2061	*Aegilops*	*geniculata*	*ovata*		Morocco
TA 2787	*Aegilops*	*geniculata*	*ovata*		Croatia
TA 2899	*Aegilops*	*geniculata*	*ovata*		Israel
TA 10009	*Aegilops*	*geniculata*	*ovata*		Morocco
TA 10024	*Aegilops*	*geniculata*	*ovata*		Morocco
TA 10437	*Aegilops*	*geniculata*	*ovata*		Unknown
TA 1910	*Aegilops*	*longissima*	*longissimum*		Israel
TA 1912	*Aegilops*	*longissima*	*longissimum*		Israel
TA 1913	*Aegilops*	*longissima*	*longissimum*		Turkey
TA 1917	*Aegilops*	*longissima*	*longissimum*	*typical*	Israel
TA 1921	*Aegilops*	*longissima*	*longissimum*	*nova*	Jordan
TA 1924	*Aegilops*	*longissima*	*longissimum*		Canada
TA 1837	*Aegilops*	*searsii*	*searsii*		Jordan
TA 1925	*Aegilops*	*searsii*	*searsii*		Jordan
TA 1926	*Aegilops*	*searsii*	*searsii*		Israel
TA 2343	*Aegilops*	*searsii*	*searsii*		Syria
TA 2350	*Aegilops*	*searsii*	*searsii*		Jordan
TA 2351	*Aegilops*	*searsii*	*searsii*		Jordan
TA 2353	*Aegilops*	*searsii*	*searsii*		Jordan
TA 2355	*Aegilops*	*searsii*	*searsii*		Israel
TA 2669	*Aegilops*	*searsii*	*searsii*		Jordan
TA 1772	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Turkey
TA 1776	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Turkey
TA 1783	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Israel
TA 1787	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Turkey
TA 1789	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Iraq
TA 1790	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Iraq
TA 1793	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Syria
TA 1796	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Iraq
TA 1905	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Italy
TA 2097	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Turkey
TA 2120	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Turkey
TA 2149	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Turkey
TA 2342	*Aegilops*	*speltoides*	*speltoides*	*ligustica*	Israel
TA 2348	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Israel
TA 2362	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Turkey
TA 2780	*Aegilops*	*speltoides*	*speltoides*	*speltoides*	Israel

† Source: Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, KS.

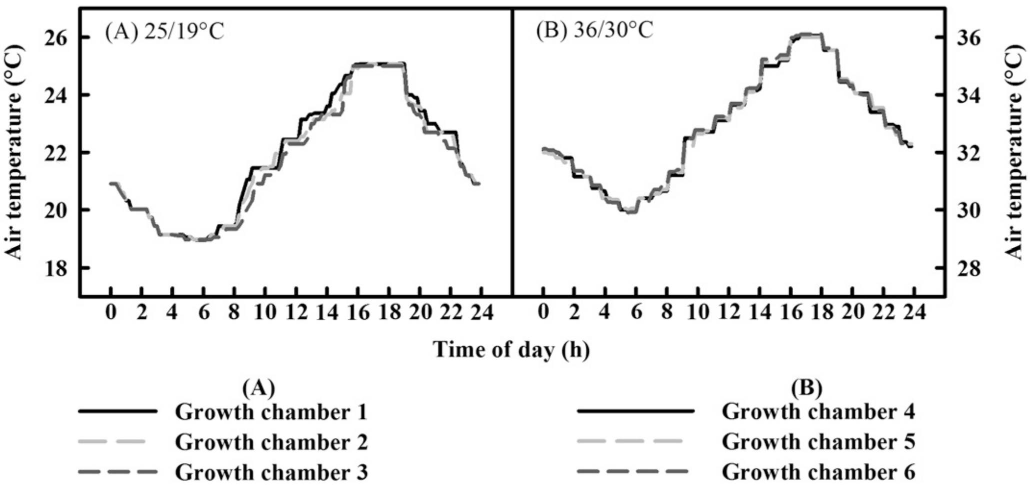

Figure 1. The targeted and measured growth chambers' air temperature at (A) optimum temperature of 25/19°C and (B) high temperature of 36/30°C. Each datum or a line is the average of four data sets collected during the flowering period.

Spike Length and Spikelet Number

At maturity, spike length was measured from the base to the tip of the spike excluding awns from five tagged spikes (one main stem and four side tillers). The spikelet number per spike was counted from the same five spikes.

Grain Number, Grain Weight, Individual Grain Weight, and Yield

At harvest, five tagged spikes were hand threshed after drying. Grains from these spikes were counted and weighed to determine grain number per spike and grain weight per spike. Individual grain weight was then calculated by dividing grain weight per spike by grain number per spike. Grain yield per plant was estimated by multiplying grain weight per spike by spike number per plant (fertile tiller number per plant).

Heat Susceptibility Index

Heat susceptibility index (HSI) for grain yield was calculated by using the formula of Fischer and Maurer (1978):

$$\mathrm{HSI} = (1 - Y/Yp)/D,$$

where Y is the average grain yield per plant of an accession at HT of 36/30°C, Yp is the average grain yield per plant of the same accessions at OT of 25/19°C, and D is the stress intensity, equal to $1 - X/Xp$, in which X is the mean Y of all accessions and Xp is the mean Yp of all accessions.

Statistical Analyses

The statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., 2003). The PROC MIXED procedures were used with the NOBOUND option to avoid zero value of block and/or block × temperature variances (Littell et al., 2006). The experimental design was a split plot with temperature randomly assigned to main plots and accessions to subplots. There were three replications. Class variables consisted of block, temperature, species, and accessions. Block and block × temperature were treated as random effects and all other variables as fixed effects. The Tukey-Kramer adjustment was used to separate the treatment means, as this test is conservative in all cases including multiple comparisons of means with unequal sample sizes (Hayter, 1984). To assess the differences among species for growth, physiological, and yield traits, accessions effects were partitioned into species effect and accessions within species effect ($A|S$). The accessions within species effects were further partitioned into five sources of variation, one for each species. For the time series data, repeated measure analyses within PROC MIXED were conducted with REPEATED statement and TYPE = CS, a covariance structure of compound-symmetry type. For flag leaf chlorophyll and flag leaf temperature depression, only the first 10 d of data were used because there was little variation after that point. Regression analyses on time series data were conducted on average of accessions using PROC REG procedure of SAS (Littell et al., 2006).

Quality Control of Growth Chamber

One of the constraints of using controlled environmental conditions for research is the variability among and within chambers; therefore, chambers should be monitored and checked for uniformity. Before starting our experiment, the spring wheat cultivar 'Pavon' was grown in eight different chambers set at 20/15°C day/night temperature, 85% humidity, 12 h photoperiod, and PAR of 400 μmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. Plants in all chambers received identical crop management practices from seeding to final harvest. Plants were randomly moved every 7 d within the chamber. At flowering, data on growth traits from six randomly selected plants per chamber were collected and statistically analyzed to compare chamber effects for each trait. Statistical analysis showed no significant difference among the chambers for growth traits; average plant height was 64.0 ± 0.9 cm, tiller number per plant was 3.5 ± 0.1, spike number per plant was 2.6 ± 0.1, and above-ground biomass was 3.7 ± 0.2 g plant^{-1}. This implies that the growth chambers used have uniform environmental conditions, which also was supported by temperature data collected at an interval of 10 min with the Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA) (data not presented).

In this experiment, the same growth chambers mentioned above were used and the growth chambers' environments were constantly checked. Air temperature was set at 25/19°C day/night (OT) in three growth chambers and at 36/30°C day/night (HT) in three more chambers as shown in Fig. 1. The photoperiod of the growth chambers was set at 18 h. The previous growth data and the Fig. 1 suggest that growth chambers used in this study were uniform.

Table 2. Degrees of freedom (df) and *F*-values for physiological, growth, and yield components of five *Aegilops* species.

Effects	Temperature (T)	Species (S)	T × S	$A \mid S$[†]	T × $A \mid S$	$A \mid S_1$	$A \mid S_2$	$A \mid S_3$	$A \mid S_4$	$A \mid S_5$
df	1	4	4	47	47	8	11	5	8	15
Traits				*F*-values						
Leaf chlorophyll (SPAD[‡] value)	531.61***	30.50***	16.22***	5.02***	2.02***	11.28***	1.59 NS[§]	5.45***	3.64***	7.29***
Flag leaf temperature depression, °C	2968.42***	77.65***	18.30***	7.82***	5.28***	6.28***	5.9***	3.63**	1.48 NS	4.59***
Plant height, cm	4.98 NS	569.86***	0.53 NS	11.61***	0.81 NS	7.41***	5.25***	18.09***	4.5***	6.03***
Tiller number per plant	5.00 NS	340.32***	1.22 NS	18.56***	0.63 NS	23.23***	4.82***	11.36***	15.25***	6.62***
Fertile tiller number per plant	6.56 NS	79.87***	0.83 NS	15.99***	0.29 NS	41.12***	11.63***	10.04***	8.68***	6.14***
Spike length, mm	0.28 NS	2944.62***	0.31 NS	35.24***	1.27 NS	26.98***	24.73***	8.48***	21.31***	22.05***
Spikelet number per spike	0.00 NS	820.97***	0.32 NS	5.24***	1.06 NS	17.3***	1.95 NS	16.63***	3.91**	3.72***
Vegetative biomass, g plant^{-1}	27.57***	82.29***	0.73 NS	4.54***	1.09 NS	7.49***	7.51***	12.2***	2.83*	2.6***
Spike weight, g plant^{-1}	295.71***	45.92***	1.68 NS	7.22***	1.09 NS	14.05***	18.66***	4.21**	4.85***	5.21***
Above-ground biomass, g plant^{-1}	160.99***	109.06***	1.19 NS	7.18***	1.02 NS	12.92***	16.09***	10.85***	6.74***	4.09***
Grain number per spike	676.60***	183.42***	44.24***	11.60***	3.01***	4.48**	3.05**	8.95***	4.25**	164.2***
Grain weight, g spike^{-1}	724.18***	49.52***	41.81***	6.22***	2.18***	3.16**	5.03***	4.28**	2.09 NS	61.81***
Individual grain weight, mg	4530.44***	129.28***	22.76***	5.79***	2.31***	3.82**	6.03***	12.82***	0.9 NS	6.58***
Grain yield, g plant^{-1}	2732.42***	25.29***	12.63***	9.41***	2.47***	4.32**	8.49***	4.15**	3.33**	24.95***

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†] $A \mid S$ = accessions within species, $A \mid S_1$ = accessions within *A. markgrafii*, $A \mid S_2$ = accessions within *A. geniculata*, $A \mid S_3$ = accessions within *A. longissima*, $A \mid S_4$ = accessions within *A. searsii*, $A \mid S_5$ = accessions within *A. speltoides*.

[‡] SPAD, soil plant analysis development.

[§] NS, nonsignificant at the 0.05 probability level.

RESULTS

The *F*-values for growth, physiological, and yield traits obtained with SAS PROC MIXED (SAS Institute Inc., 2003) are presented in Table 2. There were significant effects of temperature, species, and accessions within species ($A \mid S$) for most of the traits measured in this study. The interaction between temperature and species was highly significant for several physiological and yield traits (Table 2). The significance of interaction between temperature and species suggests that the effect of species on measured traits has been modified by temperature and vice versa.

Flag Leaf Chlorophyll

High temperature stress (36/30°C) decreased leaf chlorophyll (SPAD value) by 38% as compared to OT (25/19°C) when averaged across all species over the first 10 d of readings (Fig. 2). Species differed in their response to HT for leaf chlorophyll at $p < 0.001$ (Table 2). As a consequence of HT, leaf chlorophyll declined by 18% in *A. speltoides* and 36% or more for all the other species (Fig. 3). The amount of chlorophyll in flag leaves as a function of days after anthesis is shown in Fig. 4. At OT, flag leaf chlorophyll did not decrease significantly over time for *A. geniculata* ($p > 0.05$, slope = – 0.25), but a decrease was observed in *A. searsii* ($p < 0.001$, slope = – 2.07) (Fig. 4A). High temperature decreased flag leaf chlorophyll over time irrespective of species (Fig. 4B). The rate of decrease was highest for *A. searsii* (slope = – 4.52) followed by *A. longissima* (slope = – 4.35). The effect of HT on flag leaf chlorophyll was the lowest on *A. speltoides* as indicated by the lowest slope of –2.70.

Flag Leaf Temperature Depression

At OT, a decrease in flag leaf temperature depression was observed at 6 d after treatment in *A. markgrafii* and *A. speltoides* and from 8 d after treatment in other species (Fig. 5A). High temperature decreased the flag leaf temperature depression of all species at 4 d after treatment (Fig. 5B). The rate of decrease in flag leaf temperature depression was lower in *A. geniculata* and *A. searsii* (slope = –0.40) than the other species (slope ≥ –0.63). Even at 10 d after HT stress, *A. geniculata* had a higher flag leaf temperature depression (7.1°C) than the other species followed by *A. speltoides* (approximately 5.6°C). *Aegilops markgrafii* had the lowest flag leaf temperature depression at 10 d after the induction of HT stress (3.2°C) followed by *A. longissima* (4.3°C), indicating that these species could not keep their leaf temperature as cool as the other species.

Plant Height, Tiller Number, and Biomass

Temperature and temperature × species had no significant effect on plant height, number of tillers per plant, and number of fertile tillers per plant; however, significant differences occurred among species for all the above traits at $p < 0.001$ (Table 2). Plant height ranged from approximately 54 cm for *A. geniculata* to 106 cm for *A. speltoides* (Table 3). Number of tillers per plant varied from 17 for *A. longissima* to 40 for *A. markgrafii*. *Aegilops longissima* and *A. searsii* had a minimum number of fertile tillers (approximately 13) per plant, but other species produced >18 fertile tillers per plant (Table 3). High temperature stress affected vegetative biomass and above-ground biomass by 9 and 23%, respectively (Fig. 2). Species were significantly different for these traits; however, temperature × species had no significant effect on

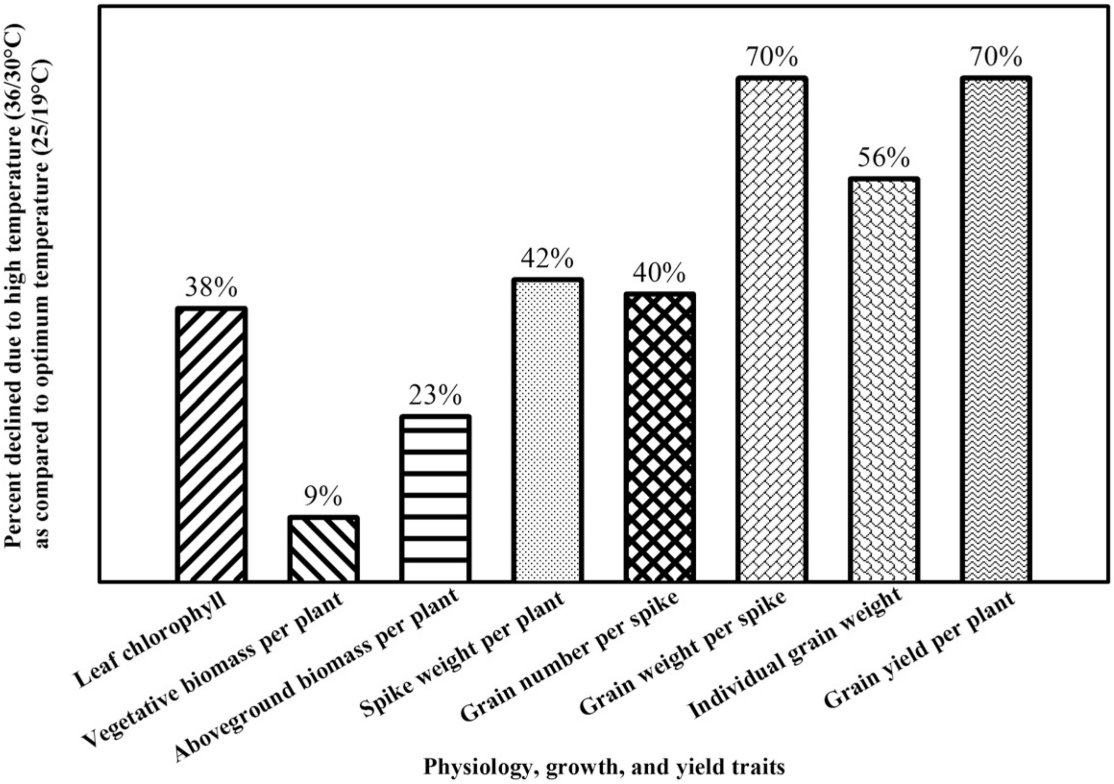

Figure 2. Effect of high temperature stress (36/30°C) on physiology, growth, yield, and yield components of *Aegilops* species. Percent decline due to high temperature as compared to optimum temperature (25/19°C) is indicated. Data are averaged across species.

these traits. *Aegilops speltoides* had the highest vegetative biomass (approximately 10 g plant^{-1}) and above-ground biomass (approximately 15 g plant^{-1}). *Aegilops geniculata* and *A. searsii* had the lowest vegetative and above-ground biomass (approximately 4 and 7 g plant^{-1}, respectively) (Table 4).

Spike Length, Spikelet Number, and Spike Weight

Temperature and temperature × species had no effect on spike length and spikelet number, but species were significantly different in these traits. The spike length of *A. longissima* was more than 200 mm whereas that of *A. geniculata* was approximately 34 mm. Similarly, *A. longissima* had the highest spikelet number per spike (16.7) and *A. geniculata* had the lowest (3.4) (Table 3). High temperature decreased spike weight per plant of all species. The species and accessions within species differed for this trait ($p < 0.001$), but temperature × species interaction was nonsignificant at $p > 0.05$ (Table 2). High temperature decreased spike weight by 42% when averaged across all species (Fig. 2). Among species, *A. longissima* and *A. speltoides* had heavier spikes (approximately 5 g plant^{-1}) than the other species (approximately 3 g plant^{-1}) (Table 4).

Grain Number, Grain Weight, and Individual Grain Weight

Effects of temperature, species, and temperature × species were evident for the number of grains per spike, grain weight per spike, and individual grain weight at $p < 0.001$ (Table 2). High temperature decreased grain number per spike by 40%, grain weight per spike by 70%, and individual grain weight by 56% when averaged across all species (Fig. 2). At OT, *A. geniculata* had the fewest number of grains (approximately 5) and *A. longissima* had the highest (approximately 25) (Fig. 6A); however, decline in number of grains per spike due to HT was highest in *A. longissima* (62%) followed by *A. speltoides* (36%) and *A. searsii* (32%; $p < 0.01$). High temperature had no effect on the grain number of *A. markgrafii* ($p = 0.15$) and *A. geniculata* ($p = 0.82$).

At OT, *A. longissima* had the highest grain weight per spike (0.21 g) and *A. markgrafii* and *A. geniculata* had the lowest (approximately 0.08 g). High temperature decreased grain weight per spike in all species. *Aegilops longissima* had the maximum decrease in grain weight (84%), and *A. geniculata* and *A. speltoides* had the lowest decrease (approximately 58%) (Fig. 6B). Similarly, *Aegilops* species differed for individual grain weight in both temperature regimes. At OT, *A. geniculata*'s grain was the heaviest (13 mg) followed by *A. longissima* (8.3 mg), and *A. searsii* had the lightest grain (4.9 mg) (Fig. 6C). At HT, *A. longissima* had the highest decline in individual grain weight (76%) followed by *A. markgrafii* (64%). The lowest decline in individual grain weight was observed in *A. speltoides* (36%) (Fig. 6C).

Grain Yield

The effects of temperature, species, and temperature × species on grain yield per plant were highly significant at

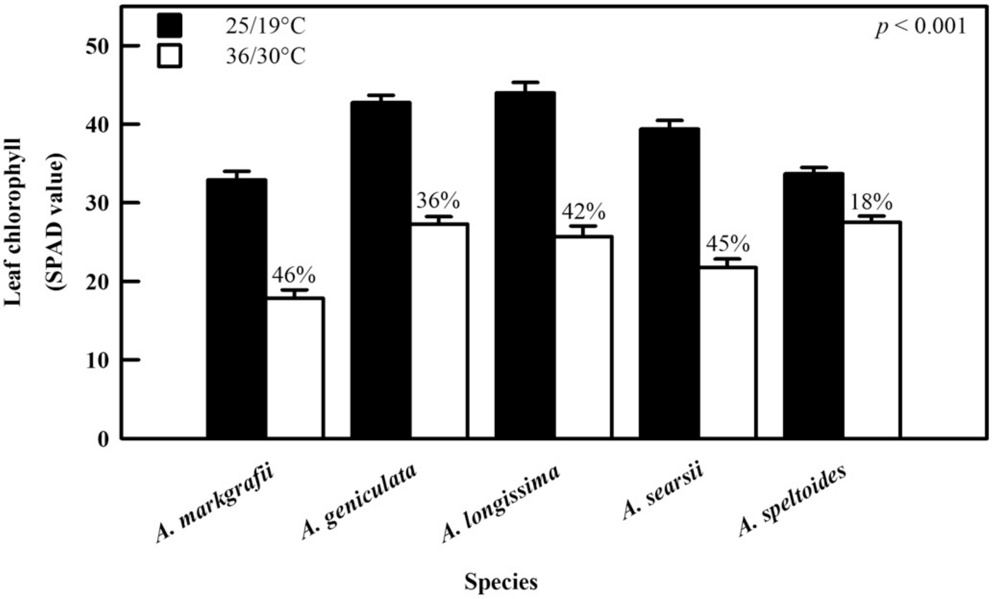

Figure 3. Effect of high temperature stress (36/30°C) on flag leaf chlorophyll content (soil plant analysis development [SPAD] value) of five *Aegilops* species. Percent decline due to high temperature as compared to optimum temperature (25/19°C) is indicated. Vertical lines on top of bars indicate standard error of means.

$p < 0.001$ (Table 2). High temperature decreased grain yield per plant by 70% when averaged across all species (Fig. 2). At OT, *A. longissima* had the highest grain yield (2.9 g plant^{-1}) followed by *A. speltoides* (2.4 g plant^{-1}). The rest of the species yielded approximately 1.5 g grain plant^{-1}. At HT, *A. longissima* had the highest decline in grain yield (84%) followed by *A. searsii* (70%) and *A. markgrafii* (72%). The lowest decrease in grain yield was observed in *A. speltoides* (58%) and *A. geniculata* (61%). Among the species at HT, *A. speltoides* had the highest grain yield (0.98 g plant^{-1}) followed by *A. geniculata* (0.66 g plant^{-1}). The grain yield per plant of all other species was approximately 0.45 g (Fig. 6D).

Accessions within Species Variability in *Aegilops speltoides* and *Aegilops geniculata*

Among the five species, *A. speltoides* and *A. geniculata* were found to be highly tolerant to HT for grain yield per plant. Therefore, accessions belonging to these two species were further analyzed and data are presented in Table 5 (*A. speltoides* accessions) and Table 6 (*A. geniculata* accessions).

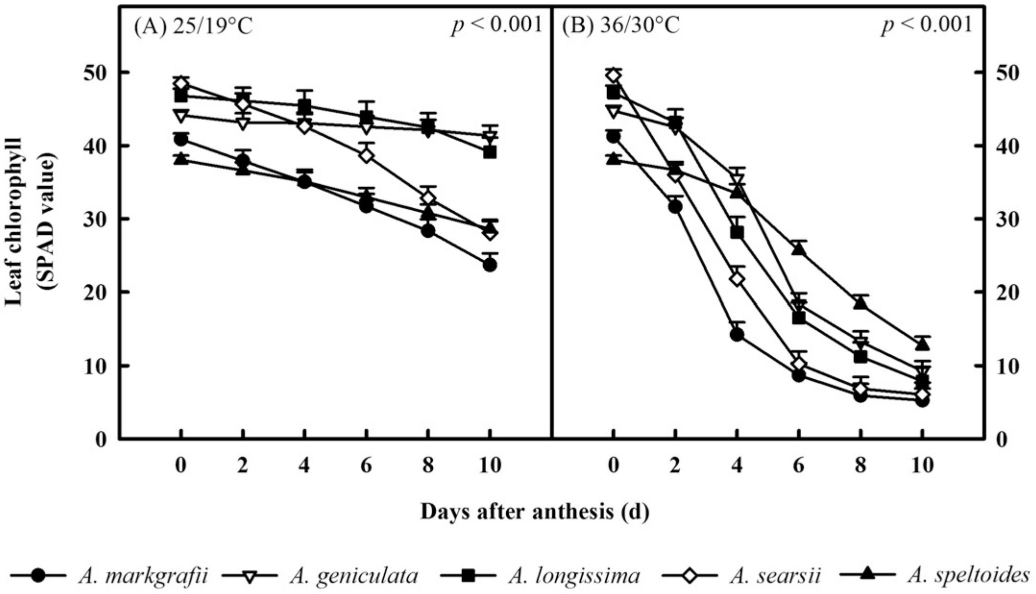

Figure 4. Flag leaf chlorophyll of five *Aegilops* species presented as a function of days after anthesis. Number of observations (n) = 6 for all species. Vertical lines on symbols indicate standard error of means. (A) Optimum temperature (25/19°C): *A. markgrafii*, $y = -1.68x + 41.34$, $r^2 = 0.99$; *A. geniculata*, $y = -0.25x + 44.00$, $r^2 = 0.95$; *A. longissima*, $y = -0.73x + 47.62$, $r^2 = 0.87$; *A. searsii*, $y = -2.07x + 49.72$, $r^2 = 0.98$; and *A. speltoides*, $y = -0.95x + 38.44$, $r^2 = 0.99$. (B) High temperature (36/30°C): *A. markgrafii*, $y = -3.75x + 36.60$, $r^2 = 0.83$; *A. geniculata*, $y = -4.03x + 47.46$, $r^2 = 0.93$; *A. longissima*, $y = -4.35x + 47.42$, $r^2 = 0.94$; *A. searsii*, $y = -4.52x + 44.37$, $r^2 = 0.88$; and *A. speltoides*, $y = -2.70x + 41.00$, $r^2 = 0.94$. SPAD, soil plant analysis development.

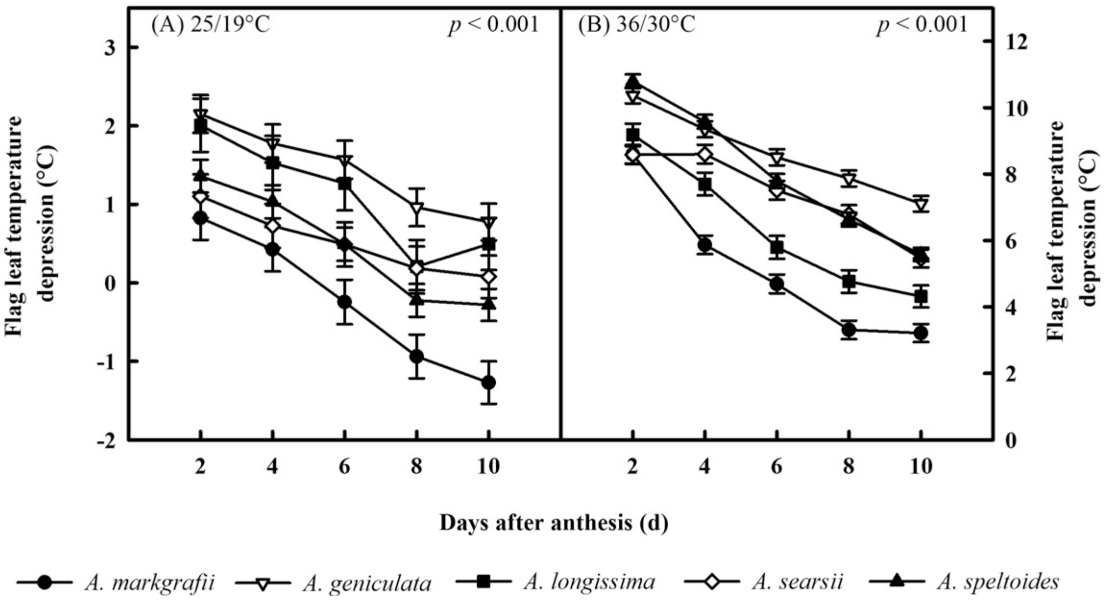

Figure 5. Flag leaf temperature depression of five *Aegilops* species presented as a function of days after anthesis. Number of observations (n) = 5 for all species. Vertical lines on symbols indicate standard error of means. (A) Optimum temperature (25/19°C): *A. markgrafii*, $y = -0.28x + 1.43$, $r^2 = 0.98$; *A. geniculata*, $y = -0.18x + 2.52$, $r^2 = 0.97$; *A. longissima*, $y = -0.22x + 2.40$, $r^2 = 0.81$; *A. searsii*, $y = -0.13x + 1.29$, $r^2 = 0.96$; and *A. speltoides*, $y = -0.23x + 1.84$, $r^2 = 0.94$. (B) High temperature (36/30°C): *A. markgrafii*, $y = -0.67x + 09.13$, $r^2 = 0.86$; *A. geniculata*, $y = -0.40x + 11.04$, $r^2 = 0.99$; *A. longissima*, $y = -0.63x + 10.15$, $r^2 = 0.94$; *A. searsii*, $y = -0.40x + 09.81$, $r^2 = 0.91$; and *A. speltoides*, $y = -0.67x + 12.08$, $r^2 = 0.99$.

Effects of temperature, accession, and temperature × accession were significant for the number of grains per spike, individual grain weight, and grain yield per plant of *A. speltoides* accessions at $p \leq 0.01$ (Table 5). Accession TA 2348 had the lowest decline in grain number per spike (10% from OT), individual grain weight (4% from OT), and grain yield per plant (14% from OT) under HT conditions. The maximum decline in grain number per spike was observed in TA 1787 and TA 2120 (approximately 71% from OT). The maximum decline in individual grain weight was observed in TA 2097 (approximately 62% from OT), which also had the maximum decline in grain yield per plant (86%) (Table 5).

The heat susceptibility indices calculated for *A. speltoides* accessions are shown in Table 5. Accessions were classified as highly tolerant (HSI ≤ 0.5), moderately tolerant (0.5 < HSI ≤ 1.0), or susceptible (HSI > 1.0) to HT stress (Viswanathan and Khanna-Chopra, 2001). Analysis of *A. speltoides* accessions for HSI showed that TA 2348 was a highly HT stress tolerant accession with an HSI of 0.23 (Table 5). The moderately tolerant accessions were TA 2342, TA 2780, TA 2362, TA 1793, TA 1789, and TA 1796, which had HSIs from 0.65 to 1.0. The most heat susceptible accessions were TA 2097 and TA 1787 (HSI ≥ 1.41) followed by others with HSI > 1.0.

Effects of temperature and temperature × accession were not evident for grain number per spike of *A. geniculata* accessions ($p > 0.05$), but accessions were different for this trait at $p = 0.004$ (Table 6). Among accessions, TA 2787 had lower number of grains (approximately 3 per spike) compared to others (approximately 6 per spike). Effects of temperature and accession were evident for individual grain weight and grain yield per spike of *A. geniculata* accessions ($p \leq 0.003$), but temperature × accession effect was not observed for these traits at $p = 0.29$ and 4.72 (Table 6). For individual grain weight, two distinct groups of *A. geniculata* accessions were observed: one with individual grain weight from 6.49 to 8.28 mg (e.g., TA 10009 and TA 1800) and another with a range of 8.35 to 13.28 mg (e.g., TA 1808 and TA 10437). For grain yield per plant, one group of *A. geniculata* had higher grain yield, from 1.3 g (e.g., TA 10437) to 2.11 g (e.g., TA 2899) plant^{-1}; other accessions had moderate grain yield, from 0.66 to 1.2 g plant^{-1}, and TA 2787 had the lowest grain yield (0.31 g plant^{-1}) (Table 6).

Aegilops geniculata accessions were either moderately HT tolerant, with 0.5 < HSI ≤ 1.0 (TA 2899, TA 1819, TA 1802, TA 1814 and TA 2061), or HT susceptible, with HSI > 1.0 (TA 1800, TA 10437, TA 1813, TA 1808, TA 10024, and TA 10009) (Table 6). *Aegilops geniculata* accessions with high levels of HT tolerance were not identified in this study.

DISCUSSION

Heat stress following anthesis, also described as terminal heat stress, is one of the most important constraints affecting wheat crop productivity. There is only limited variability within wheat for breeding for terminal heat stress (Trethowan and Mujeeb-Kazi, 2008), and wild relatives of wheat are promising source of resistance to terminal heat stress (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001).

In this study, HT stress decreased relative chlorophyll (SPAD value) of all species. Thylakoid membranes harbor

Table 3. Mean growth and morphological parameters of five *Aegilops* species. Each value is an average of three replications and two temperature regimes.

Species	Plant height (cm)	Tiller number (per plant)	Fertile tiller number (per plant)	Spike length (mm)	Spikelet number (per spike)
A. markgrafii	71.0c[†]	40.1a	21.1a	88.2d	7.9d
A. geniculata	53.8e	35.3b	18.8b	34.4e	3.4e
A. longissima	96.3b	16.9e	13.3c	220.7a	16.7a
A. searsii	62.7d	22.4d	12.9c	174.7b	15.4b
A. speltoides	106.1a	29.9c	20.1ab	140.1c	14.5c

[†] Within columns, means followed by the same letter are not significantly different at $p \le 0.05$ according to Tukey-Kramer test.

Table 4. Effect of high temperature stress on spike weight, vegetative biomass, and above-ground biomass of five *Aegilops* species. Individual datum is the mean of three replications.

	Spike weight (g plant^{-1})			Vegetative biomass (g plant^{-1})			Above-ground biomass (g plant^{-1})		
Species	OT[†]	HT	Mean	OT	HT	Mean	OT	HT	Mean
A. markgrafii	4.5	2.4	3.5b[‡]	6.2	5.1	5.7c	10.8	7.5	9.1c
A. geniculata	4.4	2.1	3.3b	5.0	4.1	4.5d	9.4	6.2	7.8cd
A. longissima	6.4	3.5	5.0a	7.4	7.3	7.3b	13.8	10.7	12.2b
A. searsii	3.7	2.3	3.0b	3.7	3.9	3.8d	7.4	6.1	6.8d
A. speltoides	6.5	4.5	5.5a	10.0	9.5	9.7a	16.5	13.8	15.3a
Mean	5.1	3.0		6.5	5.9		11.6	8.9	
p-values:									
Temperature (T)		<0.001***			<0.001***			<0.001***	
Species (S)		<0.001***			<0.001***			<0.001***	
T × S		0.157 NS[§]			0.575 NS			0.317 NS	

***Significant at the 0.001 probability level.

[†] OT, optimum temperature (25/19°C); HT, high temperature (36/30°C).

[‡] Within columns, means followed by the same letter are not significantly different at $p \le 0.05$ according to Tukey-Kramer test.

[§] NS, nonsignificant at the 0.05 probability level.

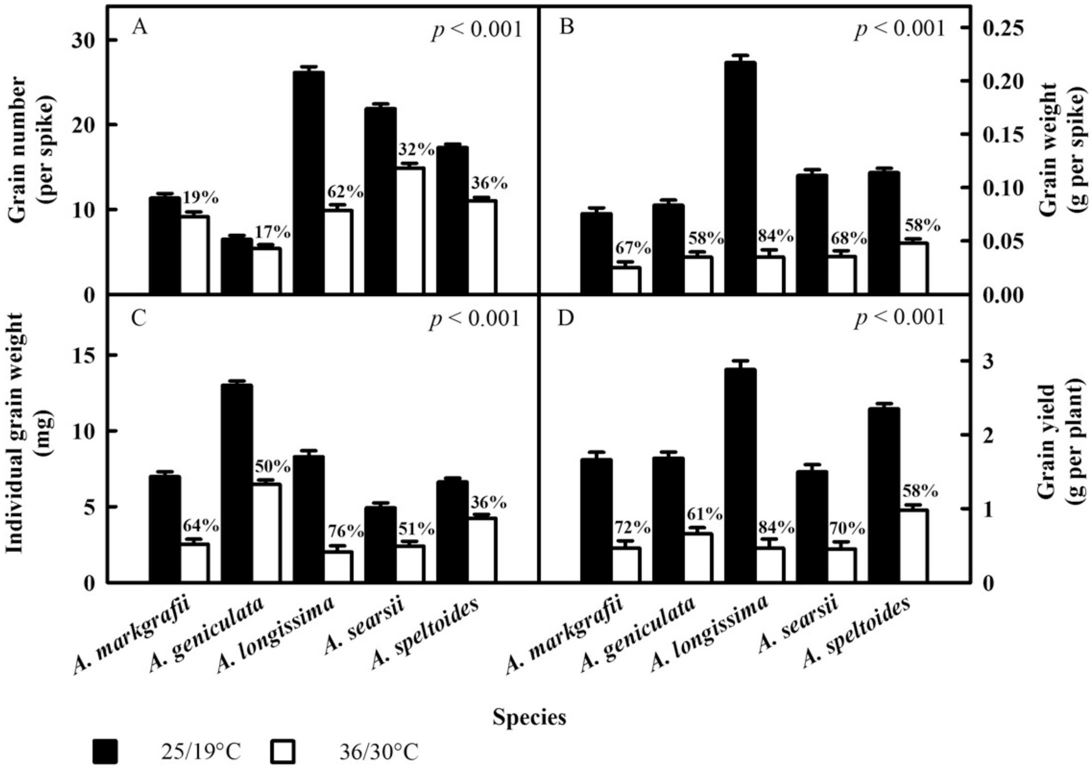

Figure 6. Effect of high temperature (36/30°C) stress on (A) grain number per spike, (B) grain weight per spike, (C) individual grain weight, and (D) grain yield per plant of five *Aegilops* species. Percent decline due to high temperature as compared to optimum temperature (25/19°C) is indicated. Vertical lines on top of bars indicate standard error of means.

Table 5. Effect of high temperature stress on yield and yield components of *Aegilops speltoides* accessions. Grain number and individual grain weight are the mean of five spikes × three replications. Yield is the mean of three replications.

Accession number	Grain number (per spike)			Individual grain weight (mg)			Grain yield (g plant^{-1})			
	OT[‡]	HT	Percent decline from OT	OT	HT	Percent decline from OT	OT	HT	Percent decline from OT	HSI[†]
TA 2348	20.0	18.0	10.0	4.5	4.3	4.0	2.2	1.9	13.5	0.23
TA 2342	11.7	9.0	22.9	3.8	2.9	24.1	1.0	0.6	38.1	0.65
TA 2780	20.0	14.3	28.3	7.9	7.2	8.7	4.5	2.8	38.1	0.65
TA 2362	21.7	15.0	30.8	7.4	6.2	15.0	2.4	1.4	41.2	0.71
TA 1793	25.3	15.0	40.8	5.1	4.6	10.2	3.4	1.7	49.2	0.84
TA 1789	17.0	9.3	45.1	5.5	4.6	16.1	1.5	0.7	52.9	0.91
TA 1796	16.0	13.3	16.7	6.1	2.8	54.4	1.8	0.8	58.4	1.00
TA 1776	11.3	9.0	20.6	10.3	4.8	52.9	1.8	0.7	60.6	1.04
TA 1905	7.7	6.0	21.7	6.3	3.5	45.0	1.0	0.4	62.1	1.07
TA 2149	12.0	9.3	22.2	6.6	3.9	40.7	1.9	0.7	64.1	1.10
TA 1790	21.0	10.0	52.4	7.1	4.5	36.3	2.3	0.7	67.6	1.16
TA 2120	5.7	1.7	70.6	8.1	5.8	28.4	0.8	0.2	71.1	1.22
TA 1772	28.7	20.0	30.2	7.7	3.3	57.5	5.6	1.5	73.0	1.25
TA 1783	20.0	13.7	31.7	7.6	3.5	54.0	2.7	0.7	73.3	1.26
TA 1787	25.0	7.3	70.7	6.1	3.6	40.5	2.7	0.5	82.0	1.41
TA 2097	14.0	5.7	59.5	5.9	2.2	62.0	2.0	0.3	86.3	1.48
Mean	17.3	11.0		6.6	4.2		2.4	1.0		
p-values:										
Temperature (T)			0.001***			0.01**			0.004**	
Accession (A)			<0.001***			0.001***			<0.001***	
T × A			<0.001***			0.004**			<0.001***	

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†] HSI, heat susceptibility index.

[‡] OT, optimum temperature (25/19°C); HT, high temperature (36/30°C).

Table 6. Effect of high temperature stress on yield and yield components of *Aegilops geniculata* accessions. Grain number and individual grain weight are the mean of five spikes × three replications. Yield is the mean of three replications.

Accession number	Grain number (per spike)			Individual grain weight (mg)			Grain yield (g plant^{-1})			
	OT[‡]	HT	Mean	OT	HT	Mean	OT	HT	Mean	HSI[†]
TA 2899	7.3	7.0	7.2a[§]	15.3	8.9	12.1a	2.8	1.5	2.1a	0.80
TA 1819	7.0	6.3	6.7a	13.7	9.7	11.7a	1.9	1.0	1.5a	0.80
TA 1802	6.7	6.7	6.7a	10.3	6.2	8.3b	0.9	0.5	0.7bc	0.83
TA 1814	6.3	6.0	6.2a	16.4	7.9	12.2a	2.2	0.9	1.6a	0.98
TA 2061	6.7	5.7	6.2a	9.1	4.4	6.8b	1.6	0.7	1.1bc	0.98
TA 1800	7.0	5.3	6.2a	10.9	5.7	8.3b	1.3	0.5	0.9bc	1.04
TA 10437	6.0	4.3	5.2a	16.5	10.1	13.3a	1.9	0.7	1.3a	1.05
TA 1813	6.3	5.7	6.0a	18.7	7.3	13.0a	2.6	0.9	1.7a	1.10
TA 1808	7.0	4.3	5.7a	10.5	6.2	8.4a	1.8	0.6	1.2b	1.12
TA 10024	7.7	6.0	6.8a	11.1	4.4	7.7b	1.6	0.4	1.0bc	1.25
TA 10009	5.7	5.7	5.7a	10.3	2.6	6.5b	1.1	0.2	0.7bc	1.34
TA 2787	4.0	1.5	2.8b	13.2	4.2	8.7a	0.6	0.0	0.3c	1.56
Mean	6.5	5.4		13.0	6.5		1.7	0.7		
p-values:										
Temperature (T)			0.073 NS[¶]			<0.001***			0.003**	
Accession (A)			0.004**			<0.001***			<0.001***	
T × A			0.896 NS			0.290 NS			0.472 NS	

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†] HSI, heat susceptibility index.

[‡] OT, optimum temperature (25/19°C); HT, high temperature (36/30°C).

[§] Within columns, means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey-Kramer test.

[¶] NS, nonsignificant at the 0.05 probability level.

chlorophyll. High temperature induced electrolytic leakage from thylakoid membrane (Al-Khatib and Paulsen, 1984; Ristic et al., 2007) and/or lipid peroxidation of chloroplast membranes (Djanaguiraman et al., 2010), leading to loss of chlorophyll. The differential rate of decreases in chlorophyll (SPAD value) at HT across time (slope = –2.70 to –4.52; Fig. 4), and different magnitude of decreases in average chlorophyll across species (Fig. 3) showed the presence of genetic variability in *Aegilops* species for chlorophyll retention. The negative slope of regression lines in all species under HT showed an inverse relationship between duration of HT and leaf chlorophyll. The genetic variability in chlorophyll content of lines exposed to HT was also observed in bread wheat (Fokar et al., 1998; Ristic et al., 2007) and synthetic wheats (Yang et al., 2002). In wheat, 80% of grain carbohydrates and proteins are derived from current photosynthesis and up to 20% are relocated from the stem reserves (Gebbing et al., 1999). Thus, the amount and duration of chlorophyll retention in leaves might be crucial in realizing higher yield under HT stress. In this study, the effect of HT on leaf chlorophyll was the lowest in *A. speltoides* and had minimal effect on grain yield. On the other hand, at HT stress *A. longissima* had the greatest decrease in leaf chlorophyll and grain yield compared to the other *Aegilops* species. This showed that leaf chlorophyll is highly valuable trait and can also be utilized in screening genotypes for HT stress tolerance under controlled environmental conditions.

Canopy temperature depression has been widely used in evaluating heat stress tolerance of wheat germplasms at field conditions. A higher CTD value, indicating cooler leaf surface as compared to air temperature, is a desirable trait (Balota et al., 2007). Ayeneh et al. (2002) reported high correlation (r = 0.91) between CTD and flag leaf temperature depression. In this study, there was genotypic variation for flag leaf temperature depression. At 10 d after treatment, *A. speltoides* and *A. geniculata* had comparatively higher flag leaf temperature depression than *A. longissima* and *A. markgrafii* (Fig. 5B) and, as expected, *A. speltoides* and *A. geniculata* were the highest grain yielder at HT. This showed that flag leaf temperature depression can be used in evaluating germplasms for high temperature tolerance at control environmental conditions. However, further studies are needed before making a sound conclusion. In this study, the relative humidity of growth chambers was not monitored and thus the vapor pressure deficit was not measured. Vapor pressure deficit plays significant role in transpiration and thus the cooling of leaves surfaces.

High temperature decreased grain number per spike in all species, resulting in yield loss. In this study, HT was imposed when the first anthers had appeared from the middle spikelet of the spike on the main tiller (Feekes 10.5.1). Therefore, the decrease in grain number per spike was not due to a decrease in spikelet number per spike (Table 3). It may be due to a negative effect of HT on factors leading to grain set (e.g., lower pollen or ovule viability or pollen tube growth and low fertilization). High temperature caused low grain set due to low pollen production and viability in wheat (Saini et al., 1983), rice (*Oryza sativa* L.) (Prasad et al., 2006b), and sorghum [*Sorghum bicolor* (L.) Moench] (Prasad et al., 2006a, 2011). In our study, genetic variability was observed for a decrease in grain number per spike ranging from a nonsignificant decrease in *A. markgrafii* and *A. geniculata* to significant decline of 62% in *A. longissima* (Fig. 6A). This result suggests potential for improving wheat cultivars for higher grain number per spike at HT.

In addition to grain number per spike, individual grain weight (seed size) has been considered to be the most important yield component under HT stress at the reproductive stage (Gibson and Paulsen, 1999; Khanna-Chopra and Viswanathan, 1999). In this study, HT caused individual grain weight to decline by approximately 56% when averaged across all species. Yang et al. (2002) reported approximately 50% decline in average grain weight of 30 synthetic hexaploid wheats subjected to HT of 10°C higher than the ambient (20/15°C) from 10 d after anthesis until maturity. *Aegilops* species responses to HT stress for individual grain weight differed; the decrease ranged from 36% for *A. speltoides* to 76% for *A. longissima*. The decrease in grain weight due to HT of plants exposed to >36°C at anthesis were observed by Yang et al. (2002) in hexaploid synthetic wheats (ranging from 31 to 63%) and by Fokar et al. (1998) in five spring wheat cultivars (ranging from 39.3 to 58.3%).

In this study, as the wild relatives of wheat were difficult to thresh, data from five tagged spikes were used for estimating grain yield. High temperature decreased grain yield per plant by 70% when averaged across all species. The decrease in yield in our study is in agreement with Gibson and Paulsen (1999) and Khanna-Chopra and Viswanathan (1999), who reported yield declines of a similar magnitude in hexaploid and/or diploid and tetraploid wheats. The genotypic difference in yield reduction observed in this study (from approximately 60% for *A. speltoides* and *A. geniculata* to 84% for *A. longissima*) is consistent with those of Fokar et al. (1998) and Gibson and Paulsen (1999) in spring wheat.

Although *A. speltoides* and *A. geniculata* demonstrated at least moderate tolerance to HT, the tolerance in *A. speltoides* was due to relative maintenance of both grain number per spike and the individual grain weight. In *A. geniculata*, tolerance was primarily due to maintenance of grain number per spike. Previous studies conducted at field by delaying seeding or transplanting days also suggested greater HT tolerance of *A. speltoides* and *A. geniculata* accessions (Ehdaie and Waines, 1992; Zaharieva et al., 2001).

Analysis of accessions belonging to *A. speltoides* and *A. geniculata* revealed that, due to a higher degree of tolerance to HT for grain number per spike and individual grain weight, TA 2348 (*A. speltoides*) had the lowest decrease in yield at HT stress. This accession had an HSI of less than 0.5. The HSI also has been used by Yang et al. (2002) and Viswanathan and Khanna-Chopra (2001) to identify HT tolerant wheat genotypes. Accession TA 2348 originated in Israel (Table 1), a dry and hot area, and therefore is adapted to HT. Similarly, TA 2342 and TA 2780 (*A. speltoides*), which followed TA 2348 with an HSI of 0.65, were identified as moderately tolerant to HT; they were also of Israeli origin. This suggests that the place of origin can play an important role in selecting HT tolerant accessions.

Aegilops speltoides is a putative B genome donor of wheat and it should be feasible to introgress HT tolerance from this species into wheat by direct crosses and backcrosses (Gill et al., 2008). Similarly, *A. geniculata* is easily hybridized with wheat. Surprisingly, there is considerable homeologous pairing between *A. geniculata* and wheat chromosomes (Gill et al., 2008). Several genes have been transferred from *A. geniculata* into wheat presumably as a result of spontaneous pairing (Kuraparthy et al., 2007). Thus, the HT tolerant *A. speltoides* and *A. geniculata* species, identified in this study, can be utilized in wheat breeding programs to develop modern high yielding cultivars with HT tolerance.

In conclusion, this study revealed genetic variability among wild wheat species and accessions within species for HT tolerance. It illustrated that *A. speltoides* was the most tolerant species and grain number per spike and/or individual grain weight were main yield components associated with HT tolerance. Among *A. speltoides*, accessions TA 2348, TA 2342, and TA 2780 were identified as HT tolerant. Three accessions of *A. geniculata* (TA 2899, TA 1819, and TA1814) were also identified as moderately HT tolerant on the basis of yield and HSI. The HT tolerant accessions identified in this study can be used in breeding for HT tolerance of cultivated wheat as discussed above.

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