# EFFECTS OF DROUGHT AND/OR HIGH TEMPERATURE STRESS ON WILD WHEAT RELATIVES (*AEGILOPS* SPECIES) AND SYNTHETIC WHEATS

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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# Abstract

High temperature (HT) and drought are detrimental to crop productivity, but there is limited variability for these traits among wheat (*Triticum aestivum* L.) cultivars. Five *Aegilops* species were screened to identify HT (52 accessions) and drought (31 accessions) tolerant species/accessions and ascertaining traits associated with tolerance. Four synthetic wheats were studied to quantify independent and combined effects of HT and drought. *Aegilops* species were grown at 25/19°C day/night and 18 h photoperiod. At anthesis, HT was imposed by transferring plants to growth chambers set at 36/30°C, whereas in another experiment, drought was imposed by withholding irrigation. Synthetic wheats were grown at 21/15°C day/night and 18 h photoperiod. At anthesis or 21 d after anthesis, plants were exposed to optimum condition (irrigation + 21/15°C), HT (irrigation + 36/30°C), drought (withhold irrigation + 21/15°C), and combined stress (withhold irrigation + 36/30°C). Stresses were imposed for 16 d. High temperature and drought stress significantly decreased chlorophyll, grain number, individual grain weight, and grain yield of *Aegilops* species (≥ 25%). Based on a decrease in grain yield, *A. speltoides* and *A. geniculata* were most tolerant (~ 61% decline), and *A. longissima* was highly susceptible to HT stress (84% decline). Similarly, *A. geniculata* had greater tolerance to drought (48% decline) as compared to other species (≥ 73% decline). Tolerance was associated with higher grains spike$^{-1}$ and/or heavier grains. Within *A. speltoides*, accession TA 2348 was most tolerant to HT with 13.5% yield decline and a heat susceptibility index (HSI) 0.23. Among *A. geniculata*, TA 2899 and TA 1819 were moderately tolerant to HT with an HSI 0.80. TA 10437 of *A. geniculata* was the most drought tolerant accession with 7% yield decline and drought susceptibility index 0.14. Irrespective of the time of stress, HT, drought, and combined stress

decreased both individual grain weight and grain yield of synthetic wheats by ≥ 37%, 26%, and 50%, respectively. These studies suggest a presence of genetic variability among *Aegilops* species that can be utilized in breeding wheat for HT and drought tolerance at anthesis; and combined stress of drought and high temperature on synthetic wheats are hypo-additive in nature.

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Major Professor
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I would like to dedicate this dissertation to my father Mr. Gopal Prasad Pradhan and mother Mrs. Durga Devi Pradhan for their never ending love and *ashirwad*. Your prayers to god have been answered.

# Chapter I -Review of Literature

## Overview

Wheat (*Triticum* spp.) is the most important food crop of the world in terms of the harvested area, trade value, and human nutrition. For the last two decades, wheat has been harvested from more than 207 million hectares of land, and there is no other crop in the earth that has been grown in such a massive scale. This area is usually about 1.4 times bigger than the harvested area of paddy rice (*Oryza sativa* L.) and that of maize (*Zea mays* L.), other two important cereal crops (FAO, 2011c). In 2008, the total trade value of wheat was 95 billion dollars, which was one and a half and three times higher than the maize and rice trading, respectively (FAO, 2011a). In addition, wheat has been providing about 19% calories, and 21% protein to the world's population (FAO, 2011b).

World population has been estimated to reach 9 billion in 2050 from 6.9 billion at present. Furthermore, global food demand has been forecasted to increase continuously for another 40 years (Godfray et al., 2010). The world demand for cereal is projected to grow by 56% (1048 million metric tons) in 2050 from the demand for the base year 2000, and 26% of this increase is expected for wheat (Hubert et al., 2010). Thus, it is imperative to increase the wheat yield.

Bread wheat (*Triticum aestivum* L., 2n = 6x = 42 and genomes AABBDD) occupies 90% of world wheat area because it has an extremely buffered genotype due to polyploid (Faris et al., 2002); and three divergent alleles may be harbored at each locus. These genetic attributes enable bread wheat to display arrays of phenological responses to wide ranges of photoperiod and temperature regimes, including vernalization (Slafer and Rawson, 1994). Thus, wheat is grown

from the tropical to temperate climates and from a few meters to more than 3600 meters above sea level such as in India (http://www.krishiworld.com/html/field_crops1.html) and Nepal (Aase et al., 2010). In addition, common wheat has special gluten proteins that made it possible to prepare different kinds of delicious foods from wheat flour, like chapatti, bread, cookies, biscuits, noodles, etc., which may be the reasons for widespread acceptance and cultivation of wheat. Although wheat has a wide range of climatic adaptability, many biotic factors (diseases, insect pests, and weeds) and abiotic factors (drought, high temperature, salinity, flooding, freezing, high irradiation, and nutrient deficiency or toxicities) limit its yield. Among the abiotic factors, high temperature and drought are the most important environmental factors limiting crop production in the world (Curtis, 2002).

In human history, a most rapid increase in the population took place in $20^{th}$ century. The population of the earth was only a billion in the beginning of $19^{th}$ century, which became three billion in 1960's, and reached six billion by the end of the last century (United Nations Population Division, 1999; Kirkham, 2005). The food demand for the exploding human population was met by the technological revolution that began in 1960's, popularly known as "Green Revolution". During the green revolution, architectures of wheat and rice plant were modified through dwarfing genes (wheat, $Rht_1$ and $Rht_2$; rice Sd-1), followed by the availability and the heavy application of chemical fertilizers, irrigation, and pesticides. The sustained increase in cereal yield has then been realized by the development of location specific high yielding varieties with resistance/tolerance to diseases and pests (Evenson, 2003). Now, in order to meet the global food-demand in 2050, another technical revolution encompassing effective disease and insect pest control and tolerance to abiotic stress for cereal crops, especially for bread wheat, will be needed.

The climatic model presented by Meehl and Tebaldi (2004) predicted that most intense, frequent and longer lasting heat waves will occur in the second half of this century, in the North America; the magnitude being higher in Midwest of USA. Recently, Intergovernmental Panel on Climate Change summarized that more land areas will be experiencing warmer and frequent episode of hot days and nights and erratic precipitation in coming years IPCC (2007). The global maximum and minimum temperature trends show that, over the last century, the global increase in daily minimum temperature was double than the increase in daily maxima (Easterling et al., 1997). The climatologists have identified the global warming as the main reason for such phenomena. Although, Kutilek and Nielsen (2010) were skeptical about the hypothesis on global warming and to the projected catastrophes, frequent drought and high temperature have been already reported in the agriculture fields causing yield losses.

Wheat is mostly grown under the rainfed conditions. In 2000, 70% of world's wheat harvested area was under rainfed condition (Portmann et al., 2010). This rainfed crop frequently suffered from drought resulting in significant yield loss and decreased revenue. Periodic drought often affected 50% and 70% of wheat areas in developing and developed country, respectively (Trethowan and Pfeiffer, 1999). Back in 1982, Boyer (1982), calculating crop insurance payments, reported about 87% yield decrease in US wheat, and pointed out that only 6% yield loss was due to biotic factors, whereas environmental factors were responsible for 94% yield loss. He further noted that drought was the main environmental constraint. FAO (2011d), in February 2011, issued an alert on drought in China stating that drought affected about 5.16 million hectares of winter wheat. In 2010, drought damaged at least 10.3 million hectares of crop land in Russia and wheat harvest was forecasted to fall to 50 million MT (FAO, 2010). In USA, there were reports on wide spread spring and summer drought in the Great Plains in recent years

causing substantial revenue loss (Lott et al., 2011). Most of the wheat-growing areas of the world, including the Great Plains of the USA experienced above-optimum temperatures at some point in their life cycle with large negative impact on yield. In developing countries, continual high temperature affects 7 million ha of wheat area; and in temperate climates terminal stress often affects about 36 million ha of wheat crop (Reynolds et al., 2001). High temperature following anthesis is called terminal stress, and continual stress is experienced when the mean daily temperature exceeds 17.5°C in the coolest month of the season (Fischer, 1991). For wheat, air temperature of about 20–25°C has been considered optimum for growth and development (Acevedo et al., 2002). Semenov and Shewry (2010) reported high temperature at the flowering period as a principal yield decreasing factor in European wheat. Wheat has been the staple food of Europe, West and Central Asia and North Africa regions during the last 8000 years (Curtis, 2002); and high temperature was the major abiotic threat in this region with stress occurring at heading and grain filling period (Abdalla et al., 2010). In Kansas, USA, high temperature at grain filling frequently caused revenue loss as a result of decrease in wheat quality and quantity (Paulsen, 1997; Lott et al., 2011). Moreover, in the field, most of the time high temperatures follow the drought, i.e., drought and high temperature occur simultaneously causing significant yield loss (Mittler, 2006; Lott et al., 2011). The combined effects of drought and high temperature on physiology, growth, water relations, and yield were significantly higher than the individual effects (Nicolas et al., 1984; Machado and Paulsen, 2001; Shah and Paulsen, 2003; Sharma and Kaur, 2009; Grigorova et al., 2011).

The adverse effect of drought and high temperature on crop can be minimized by escaping stress at the most sensitive stages of crop development such as reproductive and grain filling periods (Saini et al., 1983; Saini and Westgate, 2000). This is usually achieved by

adjusting seeding date or growing early-maturing varieties. However, as the abiotic stress is unpredictable, the best way to cope with them is to develop tolerant varieties that perform well under stress and under optimum environments (Wahid et al., 2007; Prasad et al., 2008c). For this, wild relatives (*Aegilops* species) and synthetic hexaploid wheats are the best sources of genes (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001; Yang et al., 2002; Baalbaki et al., 2006; Trethowan and Mujeeb-Kazi, 2008; Kurahashi et al., 2009). They are also proven sources of disease and insect pest resistant genes (Gill et al., 2006; Ogbonnaya et al., 2008).

Therefore, in this dissertation, I have reported experimentations on effects of drought and/or high temperature stress on *Aegilops* species and synthetic hexaploid wheats. The stress was applied at the anthesis. In case of synthetic wheat, effect of stress at the late grain filling period was also examined. The objectives were (a) to identify genotypes with tolerance to adverse effect of drought and/or high temperature at the respective growth stages (b) to ascertain physiological, growth, and yield traits associated with the tolerance, and (c) to quantify combined stress of drought and high temperature on yield and yield components of synthetic and spring wheats.

## Origin and domestication of bread wheat

The fertile-crescent, the fertile regions of Mesopotamia extended to present day Iraq, Israel, and parts of Turkey, Syria, and Iran has been considered the birth place of bread wheat (Gill and Friebe, 2002). The origin of bread wheat (*Triticum aestivum* L., 2n = 42, genomes AABBDD) occurred in two separate amphidiploidization events. Circa 380,000 years ago, hybridization between the diploid *Triticum urartu* Tumanian ex Gandilyan (2n = 14, genome $A^uA^u$) and the closest extant of *A. speltoides* Tausch (2n = 14, genome SS), followed by

spontaneous chromosome doubling produced emmer wheat: *T. turgidum subsp. dicoccoides* (2n = 28, genomes AABB) (Dvořák and Zhang, 1990; Gill et al., 2007). About 10,000 years ago, the wild emmer wheat was domesticated following spontaneous mutation in its inflorescence and transformed to *T. turgidum* subsp. *dicoccum* (AABB), a cultivated form of emmer wheat. Circa 8000 years ago, at farmers' fields in Caspian Iran, second hybridization occurred between *T. turgidum* subsp. *dicoccum* (AABB) and *A. tauschii* Coss. (2n = 14, genome DD), followed by spontaneous chromosome doubling that gave rise to bread wheat, *Triticum aestivum* subsp. *aestivum* L. (2n = 42, genomes AABBDD) (Kihara, 1944; McFadden and Sears, 1946). This wheat has non-brittle rachis (brbr), soft glume (tgtg), and free threshing (QQ) spikes, which resulted into its rapid domestication and cultivation (Gill et al., 2007).

## Botany, morphology, and growth of wheat

Wheat (*Triticum* spp.) is a monocot and belongs to tribe Triticeae of family Poaceae (previously called Gramineae). Other important crops like rice (*Oryza sativa* L.), maize (*Zea mays* L.) and bamboo also belong to this family. Wheat is an annual grass with inflorescence called spike. When wheat plant switches to reproductive phase from vegetative phase, the shoot apical meristems elongate and differentiated into inflorescence meristems, on which spikelet meristems are directly formed as lateral branches. On these spikelet meristems, floret meristems are developed that gives rise to flowers or florets (Shitsukawa et al., 2006). Thus, a wheat inflorescence (spike) consists of a main axis, rachis, on which spikelets are arranged alternately on opposite sides, and the spikelets are composed of florets joined at the axis (rachilla) as two opposite rows (Shitsukawa et al., 2009). Each floret has a pistil (female organ), three stamens (male organs) and two lodicules enclosed within lemma and palae. A hexaploid wheat spikelet

may have four to six fertile florets, and all the florets are encompassed within two small bract leaves called glumes (Shitsukawa et al., 2009).

Wheat plants consist of root and shoot systems. Root system comprises of the seminal roots and the crown roots, which arise from the lower nodes of the shoot (Kirby, 2002). The shoot comprises of a series of phytomers, each having a node, a leaf, an extended internode, and a bud in the axil of the leaf. A leaf consists of a leaf-sheath and a leaf blade (lamina), with a membranous structure, the ligule, and a pair of small hairy organs, auricles at their junction. The shoot is terminated by spike as explained above. Wheat has a tendency of tillering. A tiller has the same basic structures like that of the main stem, and it arises from the axil of the basal leaves, i.e., from the points of attachment of the coleoptiles and the basal leaves on the main shoot. Each tiller has potential to develop a spike; and number of fertile tillers (spike bearing tiller) in a plant is one of the important yield components.

Wheat plant development can be classified into three broad phases: seed germination and seedling establishment phase, vegetative phase, and reproductive phase followed by maturity and ripening. Each development phase can be further classified into distinct growth stages, and there are several methods (scales) to describe them, such as, Zadoke, Huan, and Feekes staging systems. In my experimentations, I have used Feekes staging system (Large, 1954 ). The growth stages are also usually categorized into E (from the germination to the seedling emergence stage), GS1 (Growth Stage 1: from the emergence to the double ridge stage), GS2 (Growth Stage 2: from the double ridge to the anthesis stage), and GS3 (Growth Stage 3: from the anthesis to the maturity stage) (Acevedo et al., 2002).

According to Feekes scale, wheat growth has been classified as follows:

| Feekes scale | Growth stage |
|---|---|
| 1.0 | Seed germination, emergence, and shoot formation |
| 1.1 | One leaf |
| 1.2 | Two leaves |
| 1.3 | Three leaves |
| 2.0 | Tillering initiation |
| 3.0 | Tillers formed |
| 4.0 | Beginning of erect growth, leaf sheaths lengthen |
| 5.0 | Leaf sheath strongly erect |
| 6.0 | First node visible |
| 7.0 | Second node visible |
| 8.0 | Flag leaf visible |
| 9.0 | Ligules of flag leaf visible (Flag leaf fully expanded) |
| 10.0 | Boot stage |
| 10.1 | Awns visible, head emerging through slit of flag leaf sheath |
| 10.2 | Heading ¼ completed |
| 10.3 | Heading ½ completed |
| 10.4 | Heading ¾ completed |
| 10.5 | Heading completed |
| 10.5.1 | Beginning flowering: First Anther visible |
| 10.5.2 | Flowering complete to top of spike |
| 10.5.3 | Flowering complete to base of spike |
| 10.5.4 | Kernels watery ripe |
| 11.0 | Ripening |
| 11.1 | Milky ripe |
| 11.2 | Mealy ripe |
| 11.3 | Kernel hard |
| 11.4 | Harvest ready |

Adapted from: (Miller, 1999).

## Stress, water stress (drought), and heat stress (high temperature)

According to Taiz and Zeiger (2006) "stress is usually defined as an external factor that exerts a disadvantageous influence on the plant and is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes, which are related to overall growth". The survival and growth of plants under a stress depend on both stress and plant characteristics. Stress characteristics such as severity, duration, number of exposures, and combination of stresses; and plant characteristics like organ or tissue in question, stages of development and genotype determine survival and growth or death of a given plant (Larkindale et al., 2005; Farooq et al., 2009). As mentioned earlier, stress may be biotic or abiotic in nature. Abiotic stresses are the environmental conditions or combinations that adversely affect the expression of the genetic potential of a plant for normal physiology, growth, development, and yield. Under unpredictable weather pattern, as reported in many climate change reports like in (IPCC, 2007), and especially under the rainfed conditions, development of stress tolerant varieties is the judicial way of mitigating adverse effect of abiotic stresses.

Drought has been defined and understood differently under different perspectives. National Drought Mitigation Center at the University of Nebraska, Lincoln, USA has classified drought into four categories, namely, meteorological drought, agricultural drought, hydrological drought, and socio-economic drought (Fig. 1.1). Usually for the farming purpose, and for the purpose of this dissertation, drought is understood as an agricultural drought, which is supposed to have occurred when soil water is not available in enough amounts for normal growth and development of a crop at a particular time (NDMC, 2006). As depicted in Fig. 1.1, climatic variables like high temperature, strong winds, low humidity, and high solar radiance exacerbate the drought stress.

According to Wahid (2007) “high temperature stress may be defined as the increase in air temperature well above a threshold level for a period of time sufficient to cause irreversible damage to plant organ, growth and/or development”. According to this definition, under the irrigated conditions, if anthers and/or pollen grains are damaged due to extreme high temperature plants will be considered under high temperature stress, although the green leaves might still be transpiring and cooling the leaf surface. This is because the damaged anthers/pollen grains will adversely affect the grain number and the yield.

## Impact of drought on wheat physiology

Wilting is the first visible symptom of drought, which indicates excessive transpirational water loss exceeding the rate of water absorption (Buchanan et al., 2002). However, drought affects many important physiological processes that cannot be perceived with naked eyes.

### Stomatal conductance

Stomata are tiny structures present on the outer skin layer of leaves. They consist of two guard cells and a tiny opening in between. The turgidity of guard cell regulates the opening and closing of the openings. The main function of the stomata is to regulate the exchange of gases like $CO_2$, water vapor, and $O_2$ between inner part of leaves and the atmosphere. Drought decreases stomatal conductance (Lu and Zhang, 1998). Numerous studies suggest that Abscisic Acid (ABA), is the chemical signal produced in roots in response to drought stress that ultimately leads to stomatal closing. Drought (dehydration of soil) decreases soil water potential, which reduces movement of water from soil to root. This results in a decline in cell pressure potential (loss of turgidity), increase in cell osmotic potential, and disturbances in the cell membrane structure and composition (Mullet and Whitsitt, 1996; Bray, 1997). Such an increased osmotic

potential triggers ABA production genes. In a split-root experiment, Zhang and Davies (1987) observed substantial increase in ABA concentration in half of the root system located in drying soil as compared to another half remaining in wet soil. In another study, when only a little portion of the root system was exposed to air allowing dehydration, enhanced accumulation of ABA occurred within a few hours in the exposed roots as compared to ones well covered by wet soil (Neales et al., 1989). Zhang and Davies (1989) reported a good correlation between the ABA content of roots in different parts of the soil profile and the water status of the soil surrounding the individual roots. The higher the dehydrated roots, the more was the ABA. The transports of ABA from the root to the shoot occur via the xylem sap. When transpiration was prevented from leaves by covering with tin foil, enhanced ABA concentration was not detected although roots were drying and ABA was loaded in the roots. But on removal of tin foil, ABA concentration of leaf increased dramatically suggesting that ABA moves from roots to shoots through the xylem stream (Zhang and Davies, 1987). Kriedemann et al. (1972) showed that small amount of endogenous ABA was enough for rapid closing of stomata and suggested regulatory function of this hormone. ABA has been shown to regulate ion channels and the PM-ATPase in guard cells that result into stomatal closure due to loss of potassium and anion ($Cl^-$ or $malate^{2-}$) from the cell (Taiz and Zeiger, 2006). In addition to ABA, increased xylem pH under drought also decreases stomatal conductance. Wilkinson (1999) mentioned that among other potential effects, increase in xylem pH due to drought increase ABA concentration in the apoplast, next to the stomatal guard cells, which close the stomata.

## Leaf chlorophyll

Chlorophyll and carotenoid pigments are responsible for harvesting light energy that is used in producing chemical energy such as Adenosine-5'-triphosphate, ATP and Nicotinamide adenine

dinucleotide phosphate-oxidase, NADPH (Taiz and Zeiger, 2006). There was a marked decrease in chlorophyll and carotenoid content of hexaploid and diploid wheat subjected to drought for 10 days at 50, 60 and 70 days after sowing (Chandrasekar et al., 2000). Drought increases senescence by enhancing chlorophyll degradation, nitrogen loss, and lipid peroxidation (Yang et al., 2001). Liu et al. (2006) observed marked increase in electrolyte leakage and reduction in chlorophylls (Chl) a and b in wheat cultivars subjected to water stress.

**Photosynthesis**

Drought decreases photosynthesis by lowering stomatal and mesophyll conductance (Flexas et al., 2004), or by oxidative damage of the chloroplast (Zhou et al., 2007). Severe drought impairs regeneration of ribulose bisphosphate (RuBP) and decreases activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) resulting into lower photosynthesis (Bota et al., 2004). Drought decreased photosynthesis in wheat and *Aegilops* species (Shah and Paulsen, 2003; Dulai et al., 2006; Liu et al., 2006). Chlorophyll fluorescence has been widely used as a non-destructive tool to estimate maximum quantum yield of photosystem II (PS II). Measurement is usually taken after fully expanded top most leaf is dark adapted for 1 h. The maximum quantum yield of PS II is then calculated as the ratio of variable fluorescence (Fv, a difference between maximum and minimum fluorescence) to maximum fluorescence (Fm), which decreases with stress (Maxwell and Johnson, 2000; Roháček, 2002). Effect of drought on maximum quantum yield of photosystem II (Fv/Fm) in dark adapted leaves were seldom observed in wheat (Shangguan et al., 2000; Subrahmanyam et al., 2006). PS II reaction center, its oxidizing and acceptor sides, or its antennae system was highly conserved at the drought stress (Lu and Zhang, 1998).

### Canopy temperature depression

Canopy temperature depression (CTD) has been considered a reliable tool to assess drought tolerance in crop plants. It is a difference between air temperature and leaf temperature, and the higher the CTD value more will be the stress tolerance. There was a significant association between CTD and yield of wheat bulks grown under the drought conditions (Reynolds et al., 2005). However, in the field, leaf and air temperature should be measured on a clear and sunny day and the best time to measure CTD was proposed as 0900, 1300, and 1800 h (Balota et al., 2007).

## Impact of drought on wheat growth and development

### Germination to emergence (E)

Wheat seeds are usually stored at around 12% moisture content by weight. Seed germinates when the amount of water in grain reaches at least 35–45% of dry weight (Evans et al., 1975). Thus, early drought at wheat growing season adversely affects germination and crop establishment.

### Emergence to double ridge (GS1)

Drought during GS1 decreases leaf area in wheat (Giunta et al., 1995b; Royo, 2004). Leaf expansion is the most sensitive trait to drought at this stage. When drought occurs, cells lose turgidity, cell walls shrink, which subsequently decreases turgor-dependent activities like leaf expansion and root elongation (Taiz and Zeiger, 2006). At this stage, drought also increases phyllochron in wheat (Simane et al., 1993). Tillering is another trait affected by drought at this stage (Blum et al., 1990). Thus, drought during this stage can decrease relative water content,

leaf area and biomass production (Giunta et al., 1995a; Hafid et al., 1998; Dulai et al., 2006; Liu et al., 2006).

### Double ridge to anthesis (GS2)

As leaves, stem, and roots keep on growing at this stage, drought adversely affects their growth as explained above. In wheat, up to 20% grain-weight is derived from the reallocation of stem reserves under favorable conditions (Gebbing et al., 1999). Stem reserves may account for up to 50% of grain weight under post anthesis drought stress (van Herwaarden et al., 1998). Thus, drought at pre-anthesis decreases grain yield by adversely affecting photosynthesis leading to decreased accumulation of stem reserves (water soluble carbohydrates). Ehdaie et al. (2006) showed up to 23% decrease in the main stem weight when wheat was subjected to drought stress. The main sensitive trait to drought stress at this stage is spikelet number spike$^{-1}$ (Oosterhuis and Cartwright, 1983; Moustafa et al., 1996; Sangtarash, 2010), and the premature death of more distal and basal florets also occurs at this stage under drought (Oosterhuis and Cartwright, 1983). As a result, grain number decreases drastically when drought occurs at this stage.

Again, drought stress at early stages of reproductive development (meiosis in pollen mother cells) induces pollen sterility, leading to lower grain numbers (Saini and Aspinall, 1981; Ji et al., 2010). Drought during meiosis in microspore mother cells resulted into a complete male sterility in wheat cultivar ‘Gabo’, which was due to a loss of contact between microspores and tapetum (a nutritive layer of cells that lines the inner wall of the pollen sac) and the filament degeneration (Saini et al., 1984). Lalonde et al. (1997) reported water stress induced male sterility in wheat through abnormal vacuolization of tapetal cells, disorientation of reproductive cells, and the lack of starch and intine in pollen grains. It was also observed that desiccation of

the sporogenous tissue had not yet occurred at the onset of male sterility, suggesting a decrease in water potential somewhere else in the plant (Saini and Aspinall, 1981).

Pollen grains accumulate starch that they use later on for germination and pollen tube growth (Clément et al., 1994). The high male sterility leading to a decrease in grain-set may be due to impaired starch accumulation in pollen grains. Sheoran and Saini (1996) reported that reduced starch accumulation in rice pollen grains was due to arrested activities of acid invertase and soluble starch synthase. Dorion (1996) suggested that in addition to reduced acid invertase activities, inability to convert sucrose to hexose might be another reason for reduced starch accumulation in pollen grains. Drought significantly increased reactive oxygen species in rice spikelets of drought susceptible variety N118 as compared to the resistant variety N22. N22 had enhanced antioxidant (superoxide dismutase, ascorbate, and glutathione) activities and reduced percentage of spikelet sterility (Selote and Khanna-Chopra, 2004). Water deficit at meiosis down regulates transcription of vacuolar (*Ivr5*) and cell-wall (*Ivr1*) encoding genes, resulting into reduced activities of vacuolar and cell-wall invertases of anthers long before the failure of pollen development in wheat, which decreased grain-set substantially (Koonjul et al., 2005).

### Anthesis to maturity (GS3)

Drought during the flowering stage decreases grain-set in almost all field crops. It may be due to lower fertilization caused by pollen sterility and/or ovule abortion. Nicolas et al. (1985) observed 16% more sterility in the top spikelets of the wheat cultivar ‘Warigal’ when drought was imposed at anthesis. Similarly, Sangtarash (2010) documented higher decline in the grain number when drought occurred at or immediately after anthesis. Fábián et al. (2011) reported seed abortion in winter wheat varieties when drought was applied at 5-9 d after anthesis. Seed

abortion was higher in the drought sensitive cultivar 'Cappelle Desprez' than in the tolerant cultivar 'Plainsman V'.

Drought during post anthesis decreases grain yield by decreasing individual grain weight (Ahmadi and Baker, 2001; Ji et al., 2010). The decrease in individual grain weight may be due to lower grain filling duration (Wardlaw and Willenbrink, 2000; Prasad et al., 2008b) and a decreased number of endosperm cells and starch granules per cell (Nicolas et al., 1985). Wardlaw and Willenbrink (2000) recorded 38% decline in individual grain weight of the wheat cultivar 'Lyallpur 73' subjected to drought during anthesis; and Ahmadi and Baker (2001) observed 43% decline in cultivar 'Cadenza' subjected to severe drought from 15 d after anthesis.

## Impact of drought on wheat yield

Cereals are mainly grown for grain yield. In wheat, grain yield is the function of the number of plants $ha^{-1}$, the number of fertile tillers $plant^{-1}$, the number of grains $spike^{-1}$, and individual grain weight. Factors that affect one of these components directly or indirectly will affect the grain yield. Drought adversely affects these components as described above resulting into a marked decline in grain yield. Drought decreased grain yield $spike^{-1}$ by about 70% when stress was applied during early seed development in spring wheat cultivars 'Cappelle Desperez' and 'Plainsman V' (Fábián et al., 2011). There was about 40% decline in average grain yield $ha^{-1}$, when drought stress was imposed on 30 wheat cultivars and 21 landraces from tillering to maturity by installing mobile roofs (Denčić et al., 2000). Fischer and Maurer (1978) reported a decrease in average grain yield by 37 to 86%, when durum wheats, triticales, barley and bread wheats were subjected to drought by withholding irrigation at various stages before the anthesis. The effect of drought on Chinese's wheat, as alerted by FAO in February 2011 and mentioned above was on plant stand and number of fertile tillers $plant^{-1}$, because Chinese's wheat growing

areas were facing severe early season drought. In Kansas of the USA, drought at GS2 and GS3 stages is the frequent phenomenon resulting in wheat yield loss.

## Impact of high temperature on wheat physiology

### Leaf chlorophyll

High temperature decreases leaf chlorophyll. A significant decrease in high temperature was observed when two spring wheat cultivars 'Yangmani 9' and 'Xuchou 26' were subjected to high temperatures of 32/24°C and 34/22°C at 7 d after anthesis (Zhao et al., 2007). High temperature of 30/25°C, applied at 10 d after anthesis, decreased flag leaf chlorophyll of synthetic hexaploid wheats by 11% to 38% (Yang et al., 2002). Chlorophyll is harbored in the thylakoid membranes, and loss of chlorophyll may be due to high temperature-induced electrolytic leakage of thylakoid membrane (Al-Khatib and Paulsen, 1984; Ristic et al., 2007) and/or lipid peroxidation of chloroplast membranes (Djanaguiraman et al., 2010).

### Photosynthesis

High temperature decreases photosynthesis in wheat (Fokar et al., 1998; Yang et al., 2002) (Al-Khatib and Paulsen, 1990; Reynolds et al., 2000). Increase in high nighttime temperature from 14°C to 23°C decreased leaf photosynthesis rate by about 15 μmol $m^{-2}$ $s^{-1}$ in spring wheat (Prasad et al., 2008b). Photosynthesis is a temperature-dependent process, and damages due to high temperature include a wide range of changes in structures or functions of the photosystem apparatus, including enzymes (Georgieva, 1999). Wise et al. (2004) suggested photochemical reaction in thylakoid lamellae and carbon metabolism in the stroma of chloroplast are the primary sites of damage under high temperature. Thylakoid membranes harbor all integral membrane proteins such as the reaction center, the antenna-pigment-protein complex

(carotenoid, chlorophyll a, b) and electron carrier proteins (cytochrome bf, ferredoxin) (Taiz and Zeiger, 2006). High temperature induced electrolytic leakage of thylakoid membrane, thus, resulted in significant decline in photosynthetic rate in wheat genotypes from major world regions (Al-Khatib and Paulsen, 1990). The damage in thylakoid membrane can be estimated by measuring the ratio of constant fluorescence to the peak of variable fluorescence (O/P ratio) with pulse modular fluorometer (Ristic et al., 2007). The increase in O/P ratio was observed in spring wheat grown under high nighttime temperature of 23°C (Prasad et al., 2008b). The loss in chlorophyll as a result of thylakoid membrane damage might be another reason for a decrease in photosynthesis. PS II is highly thermo-sensitive and high temperature greatly reduces its activities (Camejo et al., 2005). PS I system is usually more conserved under high temperature than the PS II system (Heckathorn et al., 1998). In wheat, high temperature and excessive light might damage different sites of PS II (Sharkova, 2001). Under high temperature, Rubisco deactivation rate normally exceeds the activase's capacity to promote activation, which results into a decreased net photosynthesis (Crafts-Brandner and Salvucci, 2000).

## Canopy temperature depression

Leaf cooling is one of the major functions of transpiration. Usually, drought induces stomatal closer resulting in high leaf temperature. However, under the irrigated condition also, if the relative humidity is very high and there is high solar radiation, transpiration may be checked and leaf temperature increases. This usually occurs in tropical and greenhouse conditions. Amani et al. (1996) reported highly significant correlation between CTD and the yield of 24 spring wheat cultivars planted for two years under hot climate in Mexico, and also showed a positive correlation between CTD and stomatal conductance. High temperature decreased CTD and there

was a close relation between CTD and yield of recurrent inbred lines (RILs) and 60 advanced lines of wheat grown at several international nurseries (Reynolds et al., 1998).

## Impacts of high temperature on wheat growth and development

High temperature initially increases the growth rate of wheat in all development phases, but the growth rate declines when stress intensity and duration become higher. High temperature, however, decreases the duration of each growth period that adversely affects crop performance and yield. When 20 spring wheat cultivars were sown in summer, 12°C higher average temperature from the emergence to anthesis significantly decreased duration of all the development stages, GS1, GS2 and GS3; and duration of GS2 was identified as most sensitive to high temperature (Shpiler and Blum, 1986; Wollenweber et al., 2003). Prasad et al. (2008b) reported a decrease in time to flowering, grain set, and physiological maturity in spring wheat when grown at high nighttime temperature. Wheat is usually sown in the fall season in Asia, Europe and Great Plains of USA. Therefore, the crop does not experience high temperature during germination to emergence stage (E). However, in tropical regions, wheat may be exposed to high temperature stress at all the development stages.

### Germination to emergence (E)

High air temperature along with scorching sunshine may increase soil temperature 10° to 15°C more than the air temperature. In such condition, seedlings may die and number of plants $ha^{-1}$ will be affected (Acevedo et al., 2002). Plant population below 100 $m^{-2}$ has been considered yield limiting situation in wheat (Acevedo et al., 1991).

### Emergence to double ridge (GS1)

Acevedo et al. (1991) showed that an increase in mean seasonal temperature from 12°C to 20°C at this stage decreased GS1 duration by 33 d, plant height by 25 cm, and leaf area index by 2.3 units. Even decrease in leaf number and fertile tiller numbers plant$^{-1}$ in wheat were observed under high temperature stress at this stage (Midmore et al., 1984). An increase in phyllochron with increasing temperature might be the reason for the decreased leaf number (Cao and Moss, 1994). High temperature of 35°C significantly decreased seedling shoot length and shoot dry weight of eight wheat varieties (Tripathi et al., 2009).

### Double ridge to anthesis (GS2)

GS2 is highly sensitive to stress including high temperature. Acevedo et al. (1991) showed that increase in seasonal average temperature from 12°C to 21°C at this stage decreased duration of GS2 by 25 d, fertile tiller number m$^{-2}$ by 54, and the grain number m$^{-2}$ by 36. A further increase in mean seasonal temperature to 24°C had exacerbated the effect on all of above mentioned traits. High temperature at anthesis decreased the grain number spike$^{-1}$, and the decrease in the grain number was mainly due to adverse effects of high temperature on floral organs (Yang et al., 2002; Prasad et al., 2008b). Seed-set in wheat was dramatically decreased when a high temperature of 30°C was applied for three days at the onset of meiosis in the anthers. The reduction in grain-set was due to both abnormal ovary developments, such as the absence of an embryo sac and reduced nucellus development; shriveled pollen with abnormal cytoplasm, poor pollen dehiscence and pollen tube formation (Saini and Aspinall, 1982; Saini et al., 1983). High temperature caused low grain set in several other crop species due to low pollen production and viability. High day and night time temperature at flowering stage decreased pollen production, pollen reception, and increased floret sterility in rice (Prasad et al., 2006b; Mohammed and

Tarpley, 2009). In sorghum and maize, high temperature decreased pollen production, viability, pollen longetivity, and pollen shedding resulting into reduced grain-set (Schoper et al., 1986; Prasad et al., 2006a; Prasad et al., 2011). In barley, *Hordeum vulgare*, high temperature at spike differentiation stage resulted into pollen with normal exine but reduced or no cytoplasm (Sakata et al., 2000). At premeiotic stage, high temperature produced short anthers but without pollen grains. At meiosis, high temperature resulted into pollen that had exine and were also swollen but had little starch accumulation. All these resulted into sterile seeds (Sakata et al., 2000).

### Anthesis to maturity (GS3)

High temperature stress during GS3 induces leaf senescence that decreases availability of current assimilates to growing grain and also starch synthesis and deposition, which ultimately decrease individual grain weight (grain size). Again, high temperature at this stage also decreases grain filling duration, which outweighs the increase in grain filling rate (Prasad et al., 2006a; Prasad et al., 2006c; Prasad et al., 2008a). Decrease in grain weight under high temperature at GS3 has been well documented (Gibson and Paulsen, 1999; Khanna-Chopra and Viswanathan, 1999). Yang et al. (2002) reported about a 50% decline in average grain weight of 30 synthetic hexaploid wheats subjected to high temperature of 10°C higher than the ambient (20/15°C) at 10 d after anthesis.

## Impact of high temperature on wheat yield

The adverse effect of high temperature on physiological, morphological, growth, and yield traits, as mentioned above, ultimately lead to yield penalty. Significant decrease in wheat yield (up to 70%) under high temperature has been well documented (Fokar et al., 1998; Gibson and Paulsen, 1999; Khanna-Chopra and Viswanathan, 1999; Prasad et al., 2008b).

## Combine impact of drought and high temperature on wheat

High temperature and drought stress often occur during the grain filling period of wheat crop development stage causing severe yield loss in most of the wheat growing areas of the world, including Great Plains of the USA (Boyer, 1982; Altenbach et al., 2003; Lott et al., 2011). These two abiotic stresses often occur simultaneously in dry land wheat areas, such as Mid Western Region of USA, causing higher yield loss (Lott et al., 2011). The combined effects of drought and high temperature on plant performance cannot be directly extrapolated from the response of plant to each of the different stresses applied individually (Mittler, 2006). In tobacco leaves, under the combined effect of drought and high temperature, transcripts which were usually expressed under drought, like dehydrin and glycolate oxidase; and others usually expressed under heat shock, like ascorbate peroxidase, were highly suppressed; and rather expressions of different transcripts like oxidase glutathione peroxidase were observed (Rizhsky, 2002). In another study, different patterns of defense response of plants were observed in *Arabidopsis* subjected to combined drought and heat stress. Osmoprotectant proline, which is toxic to cells and produced during drought, was replaced by sucrose when plants were subjected to a combined drought and high temperature stress (Rizhsky, 2004). In another study, Xu and Zhou (2006) indicated a drastic reduction in the PS II function and weakened nitrogen anabolism in *Leymus chinensis* (Trin.) Tzvelev subjected to combined stress of drought and high temperature. Despite the above mentioned importance of combined effects of drought and high temperature, only few have studied the effects of these two abiotic stresses together on the grain number, grain-set, and yield of cereal crops. (Rizhsky, 2002; Mittler, 2006). Nicolas et al.(1984) observed a higher decline in wheat yield when high temperature and drought stress were applied simultaneously at an early and late period of grain development stage (cell division) as compared

to ones under either of single stress. Similar additive interaction between high temperature and drought stress was reported by Shah and Paulsen (2003) for individual grain weight and grain yield of spring wheat (cv. Len) subjected to a combination of high temperature (35/30°C) and drought stress at 7 d after anthesis. On contrary, the interaction effect of high temperature and drought on grain dry weight was not additive when a chronic heat stress (27/22°C) and drought was simultaneously applied at anthesis on spring wheat (cv. Len) (Wardlaw, 2002).

## Genetic variability in wheat, wild wheat relatives (*Aegilops* species), and synthetic hexaploid wheats

As mentioned earlier, bread wheat (*Triticum aestivum* L.) acquired its D genome from *A. tauschii* (Kihara, 1944; McFadden and Sears, 1946), and *A. speltoides* has been considered the closest extant species to B and G genomes of polyploid wheats (Dvořák and Zhang, 1990). Thus, two of the three genomes of bread wheat came from *Aegilops*, and this genus *Aegilops* species have been an important source for disease- and insect-resistant genes (Friebe et al., 1991; Gill et al., 2006). Because of the recent origin and polyploidy bottleneck, the bread wheat and its land races have narrow genetic variability (Trethowan and Mujeeb-Kazi, 2008).

In nature, thousands of *A. speltoides* Tausch. (genome BB) and *A. tauschii* Coss. (genome DD) accessions are growing in the wild form. The Wheat Genetic and Genomic Resources Center alone has about 100 and more than 500 accessions of *A. speltoides* Tausch. and *A. tauschii* Coss., respectively, in its gene bank. Along with other species of *Aegilops*, such as *A. caudata*, *A. geniculata*, *A. longissima*, *A. searsii* etc.; the center has thousands of *Aegilops* accessions (http://www.k-state.edu/wgrc/Germplasm/aegilops.html). These wild wheat relatives have shown tolerance to diseases and insect pests. A summary of disease- and insect-resistant genes transferred from wild species to cultivated wheats was reported by Gill et al (2006). Wild

wheats are also sources for abiotic stress-tolerance genes. Cakmak et al. (1999) have demonstrated *A. tauschii* as an invaluable source for tolerance to zinc deficiency. Similarly, some accessions of *A. tauschii, A. speltoides,* and *A. geniculata* have shown the capability to withstand drought (Zaharieva et al., 2001; Baalbaki et al., 2006). A few high temperature stress tolerant accessions belonging to *A. geniculata, A. speltoides, A. searsii, A. longissima* also have been reported (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001). However, more screening of wild genotypes is essential if we are to exploit them in breeding programs. Furthermore, reports on screening of *Aegilops* at anthesis with an extended period of drought or high temperature stress are not available.

As wild relatives have shown tolerance to abiotic and biotic stresses, different cultivars of durum wheat (*Triticum turgidum* L., genomes AABB) have been hybridized with several *A. tauschii* accessions in vitro to increase genetic variability in wheat; and the plants, thus produced, are termed synthetic wheats (Mujeeb-kazi, 2003; Gill et al., 2006). Synthetic hexaploid wheat genotypes (2n = 42, genomes AABBDD), so produced, have been studied for drought, high temperature, and disease tolerance (Yang et al., 2002; Trethowan and Mujeeb-Kazi, 2008; Kurahashi et al., 2009; Yang et al., 2009). However, performance of synthetic hexaploid wheats under combined effects of drought and high temperature at flowering and at late grain filling stages have not been yet studied.

## Dissertation hypotheses

- *Aegilops* species / accessions vary in their response to drought and/or high temperature stress at anthesis, and they have yield trait(s) useful for increasing abiotic tolerance of bread wheat.

- Response of synthetic hexaploid wheats to the combined effects of drought and high temperature at anthesis, and at late grain filling stages are additive.

## Dissertation objectives

The broader objectives of this dissertation were:

- to screen *Aegilops* species / accessions for high temperature tolerance during reproductive stages of crop development (Chapter II).
- to screen *Aegilops* species / accessions for drought tolerance during reproductive stages of crop development (Chapter III).
- to understand the interaction effects of high temperature and drought stress on physiology, growth and yield of synthetic and spring wheats during reproductive stages of crop development (Chapter IV and V).

The specific objectives of each Chapter were:

- Chapter II: (a) identifying *Aegilops* species / accessions with tolerance to an extended period of high temperature stress at the anthesis, and (b) ascertaining physiological, growth, and yield traits associated with tolerance.
- Chapter III: (a) identifying *Aegilops* species with tolerance to an extended period of drought at the anthesis, and (b) to identify physiological, growth, and yield traits associated with the tolerance.
- Chapter IV: (a) quantifying independent and combined effects of drought and high temperature on synthetic and spring wheats at the anthesis, and (b) determining if responses varied among synthetic and spring wheats.

- Chapter V: (a) quantifying independent and combined effects of drought and high temperature on synthetic hexaploid and spring wheat genotypes at the late grain filling period, and (b) determining if responses varied among synthetic and spring wheats.

In addition to the specific objectives, the research will help to:

- improve the knowledge of genetic and physiological basis of tolerance to drought and high temperature stress in wheat.
- develop screening tools for identifying drought and high temperature tolerance under controlled / field conditions.
- provide diverse high temperature and/or drought tolerant genetic material to breeders for use in breeding programs.

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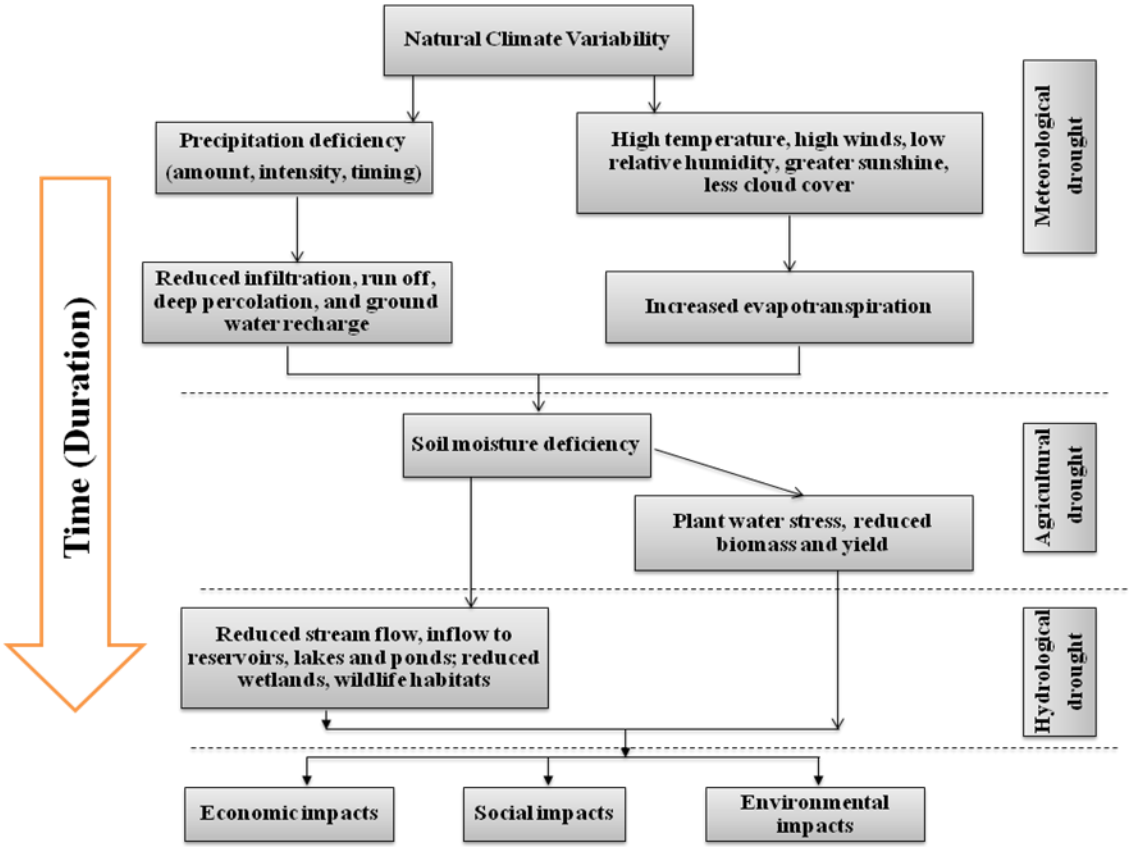
## Figures and Tables

**Figure 1.1.** Schematic diagram illustrating different kinds of drought. Adapted from the National Drought Mitigation Center (NDMC), University of Nebraska, Lincoln, Nebraska, USA. http://www.drought.unl.edu/whatis/concept.htm. With due permission from the director, NDMC.



# Chapter II -High temperature tolerance in *Aegilops* species and its potential transfer to wheat

## Abstract

High temperature stress is highly detrimental to crop productivity but there is limited variability for this trait among wheat cultivars and land races. The objectives of this research were to explore *Aegilops* species for tolerance to high temperature stress at the reproductive stage and to understand physiological, and yield traits associated with the tolerance. Fifty-two accessions belonging to five *Aegilops* species were evaluated at optimum temperature (25/19°C day/night) and high temperature (36/30°C). Stress was imposed at anthesis and continued for 16 d. Across species, high temperature decreased chlorophyll, grain number spike$^{-1}$, individual grain weight, and grain yield plant$^{-1}$ by 38%, 40%, 56%, and 70%, respectively. Among the species, *A. speltoides* and *A. geniculata* had greater tolerance to high temperature for yield (58–61% decline from optimum temperature); and *A. longissima* was highly susceptible (84% decline). Tolerance was associated with greater grain number spike$^{-1}$ and/or individual grain weight. Within *A. speltoides*, accession TA 2348 was highly tolerant to high temperature with 13.5% decline in grain yield and a heat susceptibility index (HSI) 0.23. The highly susceptible accessions were TA 1787 and TA 2097 with > 82% yield decline and HSIs > 1.4. Among *A. geniculata*, two moderately high temperature tolerant accessions TA 2899 and TA 1819 were identified, with an HSI of 0.80. The results suggest that there is genetic variability among *Aegilops* species that can be utilized for improving high temperature tolerance in wheat during reproductive stages of crop development.

# Introduction

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) because 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 a range of phenological responses to wide ranges of photoperiod and temperature regimes, including vernalization (Slafer and Rawson, 1994). Thus, wheat can been grown from tropical to temperate climates and from a few meters to more than 3800 meters above sea level. Although wheat has a wide range of climatic adaptability, many biotic, diseases and insect pests; and abiotic factors limit its yield. Among those factors, high temperature stress is one of the most important environmental factors 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 (Meehl and Tebaldi, 2004; IPCC, 2007). Most of the wheat-growing areas of the world, including Great Plains of the USA, experience above-optimum temperatures at some point in their life cycle and have a large negative impact on yield.

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., 2008b). Thylakoid membranes are one of the most sensitive cellular structures to high temperature stress (Ristic et al., 1992). 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 high

temperature-induced thylakoid membrane damage and chlorophyll content in 12 winter wheat cultivars. High temperature increases leaf temperature, which may result in reduced canopy temperature depression (CTD), the 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 high temperature-tolerant genotypes (Balota et al., 2007).

At the whole-plant level, the main effect of high temperature stress on wheat is the decreased duration of all developmental stages. When spring wheat was grown in summer, a decrease in duration of GS1 (emergence to double ridge), GS2 (double ridge to anthesis), and GS3 (anthesis to grain maturation) stages was observed (Shpiler and Blum, 1986). Prasad et al. (2008b) reported a decrease in time to flowering, grain set, and physiological maturity in spring wheat when grown at high nighttime temperature. Shpiler and Blum (1986) and Wollenweber et al. (2003) reported that GS2 was most susceptible to high temperature; this is the period when numbers of spikelet spike$^{-1}$ are determined. The reproductive stage has been considered the most temperature-sensitive period in wheat. High temperature at anthesis decreases the grain number spike$^{-1}$ (Yang et al., 2002; Prasad et al., 2008b) 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 high temperature 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 high temperature (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., 2006a; Prasad et al., 2006b; Prasad et al., 2008a). A study in wheat showed that high nighttime temperature decreased spikelet fertility, grain number, individual grain size, and grain filling duration (Prasad et al., 2008b).

One way to mitigate the effect of high temperature stress on yield is to develop stress tolerant varieties (Wahid et al., 2007). Wild wheat genotypes (*Aegilops* species) have been considered a genetic resource for increasing the genetic potential of cultivated wheat to withstand biotic as well as abiotic stresses. *Aegilops* species are close relatives of bread wheat. Bread wheat (*Triticum aestivum* L.) acquired its D genome from *A. tauschii* (Kihara, 1944; McFadden and Sears, 1946), and *A. speltoides* has been considered the closest extant species to B and G genomes of polyploid wheats (Dvořák and Zhang, 1990). As two of the three genomes of bread wheat came from *Aegilops*, this genus, *Aegilops* species, have been an important source for disease- and insect-resistant genes (Friebe et al., 1991; Gill et al., 2006). A summary of disease- and insect-resistant genes transferred from wild species to cultivated wheats was reported by Gill et. al (2006). Wild wheats are also sources for abiotic stress-tolerance genes. Cakmak et al. (1999) have demonstrated *A. tauschii* as an invaluable source for tolerance to zinc deficiency. Similarly, some accessions of *A. tauschii, A. speltoides*, and *A. geniculata* have shown the capability to withstand drought (Zaharieva et al., 2001; Baalbaki et al., 2006); a few high temperature stress tolerant accessions belonging to *A. geniculata*, *A. speltoides*, *A. searsii*, *A. longissima* also have been reported (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001). However, more screening of wild genotypes is essential if we are to exploit them in breeding programs. Further, no reports have been made on

the effect of an extended period (16 d) of high day/night temperature at anthesis on growth, physiology, and yield. Therefore, this study was conducted under controlled environmental conditions with the objectives of (a) identifying *Aegilops* species / accessions with tolerance to an extended period of high temperature stress at the reproductive stage, and (b) ascertaining physiological, growth, and yield traits associated with tolerance.

## Materials and methods

### Plant material

Fifty two accessions of *Aegilops* belonging to five different species, *Aegilops caudata* L. (9), *Aegilops geniculata* Roth (12), *Aegilops longissima* Schweinf. & Muschl. (6), *Aegilops searsii* Feldman & Kislev ex K. Hammer (9) and *Aegilops speltoides* Tausch (16) were used in this research (Table 2.1).

### Experimental and treatment conditions

This research was conducted in the Spring of 2008 at the controlled environmental facility of the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, Kansas, USA. Seeds of each accession were sown in 4-cm-deep trays containing commercial Sun Grow Metro Mix 300 potting soil (Hummert International, Topeka, Kansas). The seedlings were raised in a growth chamber (Conviron Model E15, Winnipeg, 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 and with an 8 h photoperiod. Following vernalization, three seedlings of each accession were transplanted into six 1.6-L plastic pots of dimensions 14 cm (height) × 50 cm (top perimeter) × 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, USA), 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* 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 $pot^{-1}$. Marathon, 1% G (a.i.: Imidacloprid,1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) was applied at this time to avoid infestation of sucking 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'* flowering. In all growth chambers, the canopy level photosynthetically active radiation (PAR) of 400 $\mu mol^{-1}$ $m^{-2}$ $s^{-1}$ was provided by cool white fluorescent lamps (Philips Lighting Co., Somerset, NJ, USA). 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 regime (25/19°C day/night) to one of three growth chambers maintained at high temperature of 36/30°C day/night, 18 h photoperiod, and 85% humidity. The duration of high temperature stress was 16 d; plants were then returned to their original growth chamber. 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.

## Data collection

### Leaf chlorophyll and leaf temperature

Leaf chlorophyll and leaf temperature were measured every other day from the start of treatment for 16 d. A self-calibrating chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL, USA) was used to measure chlorophyll from a fully expanded flag leaf on a tagged main stem. Each time, data were taken thrice from the middle portion of the leaf and the reading was averaged. Prior to taking SPAD meter readings, images of flag leaves were captured with a FLIR BCAM SD thermal imaging camera (FLIR Systems Inc., Wilsonville, OR, USA). To determine flag leaf temperature, these images were processed with QuickReport 1.2 software (FLIR, 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, USA) from the air temperature, collected with Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA, USA).

### 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 plant$^{-1}$ consists of both fertile (with spikes) and non-fertile (without spike) tillers. Vegetative biomass plant$^{-1}$ 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. Aboveground biomass plant$^{-1}$ includes vegetative biomass and dried spike weight plant$^{-1}$.

### 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 spike$^{-1}$ 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 number of grains spike$^{-1}$ and grain weight spike$^{-1}$. Individual grain weight was then calculated by dividing grain weight spike$^{-1}$ by number of grains spike$^{-1}$. Grain yield plant$^{-1}$ was estimated by multiplying grain weight spike$^{-1}$ by spike number plant$^{-1}$ (fertile tiller number plant$^{-1}$).

### Heat susceptibility index (HSI)

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

$$HSI = (1 - Y/Yp) / D$$

where, $Y$ = average grain yield plant$^{-1}$ of an accession at high temperature of 36/30°C; $Yp$ = average grain yield plant$^{-1}$ of the same accessions at optimum temperature of 25/19°C; D = stress intensity = $1 - X/Xp$ ; $X$ = mean $Y$ of all accessions, and $Xp$ = mean $Yp$ of all accessions.

## Statistical analyses

The statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). 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 (T) randomly assigned to main plots and accessions (A) to sub-plots.

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 (S) and accessions within species effect (A/S). 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 ten days of data were used because there was little variations 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 (Potvin and Tardif, 1988); therefore, chambers should be monitored and checked for uniformity. Before starting the 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 $\mu mol^{-1}$ $m^{-2}$ $s^{-1}$. 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 $chamber^{-1}$ 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 plant$^{-1}$ was 3.5 ± 0.1, spike number plant$^{-1}$ was 2.6 ± 0.1, and aboveground 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 minutes with the Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA, USA) (data not presented).

The environmental quality of the current experiment also was measured. Air temperature was set at optimum temperature (25/19°C day/night) in three growth chambers and at high temperature (36/30°C day/night) in three more chambers as shown in supplementary Fig. 2.1. The previous growth data and the Supplementary Fig. 2.1 suggest that growth chambers used in this study were uniform.

## Results

The F-values for growth, physiological, and yield traits obtained with SAS PROC MIXED are presented in Supplementary Table 2.1. Only the species and the accessions within species effect were significant for all traits ($P < 0.001$). The effects of temperature and temperature × species were significant unless indicated otherwise. Effects of temperature × accessions within species were non-significant for most of the traits unless mentioned specifically.

### Flag leaf chlorophyll

High temperature decreased leaf chlorophyll (SPAD value) by 38% when averaged across all species over the first 10 d of readings (Fig. 2.1). Species differed in their response to high temperature for leaf chlorophyll ($P < 0.001$). As a consequence of high temperature, leaf chlorophyll declined by 18% in *A. speltoides* and 36% or more for all the other species (Fig. 2.2).

The amount of chlorophyll in flag leaves as a function of time is shown in Fig. 2.3. At optimum temperature (25/19°C), 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. 2.3A). High temperature (36/30°C) decreased flag leaf chlorophyll over time irrespective of species (Fig. 2.3B). The rate of decrease was highest for *A. searsii* (slope = – 4.52) followed by *A. longissima* (slope = – 4.35). The effect of high temperature on flag leaf chlorophyll was the lowest on *A. speltoides* as indicated by the lowest slope of – 2.70.

## Flag leaf temperature depression

At optimum temperature (25/19°C), a decrease in flag leaf temperature depression was observed at 6 d after treatment in *A. caudata* and *A. speltoides*, and from 8 d after treatment in other species (Fig. 2.4A). High temperature decreased the flag leaf temperature depression of all species at 2 d after treatment (Fig. 2.4B). 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 = more than – 0.63). Even at 10 d after high temperature stress, *A. geniculata* had a higher flag leaf temperature depression (7.1°C) followed by *A. speltoides* (about 5.6°C). *Aegilops caudata* had the lowest flag leaf temperature depression at 10 d after the induction of high temperature stress (3.2°C) followed by *A. longissima* (4.3°C), indicating that these species could not keep their leaf temperature as cool as by other species.

## Plant height, tiller number, and biomass

Temperature and temperature × species had no significant effect on plant height, number of tillers plant$^{-1}$, and number of fertile tillers plant$^{-1}$ (Supplementary Table 2.1); however, significant differences occurred among species for all above traits ($P < 0.001$). Plant height

ranged from about 54 cm for *A. geniculata* to 106 cm for *A. speltoides* (Table 2.2). Number of tillers plant$^{-1}$ varied from 17 for *A. longissima* to 40 for *A. caudata*. *Aegilops longissima* and *A. searsii* had a minimum number of fertile tillers (about 13) plant$^{-1}$, but other species produced ≥ 18 fertile tillers plant$^{-1}$. High temperature stress affected vegetative biomass and aboveground biomass by 9 and 23%, respectively (Fig. 2.1). Species were significantly different for these traits; however, temperature × species had no significant effect on these traits. Among species, *A. speltoides* had highest vegetative biomass (about 10 g plant$^{-1}$) and aboveground biomass (about 15 g plant$^{-1}$). *Aegilops geniculata* and *A. searsii* had the lowest vegetative and aboveground biomass (4 and 7 g plant$^{-1}$, respectively; Table 2.3).

## 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 about 34 mm. Similarly, *A. longissima* had the highest spikelet number spike$^{-1}$ (16.7), and *A. geniculata* had the lowest (3.4 spike$^{-1}$; Table 2.2). High temperature decreased spike weight plant$^{-1}$ of all species. The species and accessions within species differed for this trait ($P < 0.001$), but temperature × species interaction was non-significant ($P > 0.05$; Supplementary Table 2.1). High temperature decreased spike weight by 42% when averaged across all species (Fig. 2.1). Among species, *A. longissima* and *A. speltoides* had heavier spikes (about 5 g plant$^{-1}$) than the other species (about 3 g plant$^{-1}$; Table 2.3).

## Grain number, grain weight, and individual grain weight

Effects of temperature, species, and temperature × species were evident for the number of grains spike$^{-1}$, grain weight spike$^{-1}$, and individual grain weight ($P < 0.001$; Supplementary Table 2.1).

High temperature decreased grain number spike$^{-1}$ by 40%, grain weight spike$^{-1}$ by 70% and individual grain weight by 56% when averaged across all species (Fig. 2.1). At optimum temperature, *A. geniculata* had the fewest number of grains (about 5) and *A. longissima* had the highest (about 25; Fig. 2.5A); however, decline in number of grains spike$^{-1}$ due to high temperature 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. caudata* ($P = 0.15$) and *A. geniculata* ($P = 0.82$).

At optimum temperature, *A. longissima* had the highest grain weight spike$^{-1}$ (0.21 g), and *A. caudata* and *A. geniculata* had the lowest (about 0.08 g). High temperature decreased grain weight spike$^{-1}$ in all species. *Aegilops longissima* had the maximum decrease in grain weight (84%), and *A. geniculata* and *A. speltoides* had the lowest decrease, about 58% (Fig. 2.5B). Similarly, *Aegilops* species differed for individual grain weight in both temperature regimes (Fig. 2.5C). At optimum temperature, *A. geniculata's* grain was the heaviest (13 mg) followed by *A. longissima*; and *A. searsii* had the lightest grain (4.9 mg). At high temperature, *A. longissima* had the highest decline in individual grain weight (76%) followed by *A. caudata* (64%). The lowest decline in individual grain weight was observed in *A. speltoides* (36%).

## Grain yield

The effects of temperature, species, and temperature × species on grain yield plant$^{-1}$ were highly significant ($P < 0.001$, Supplementary Table 2.1). High temperature decreased grain yield plant$^{-1}$ by 70% when averaged across all species (Fig. 2.1). At optimum temperature, *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 about 1.5 g of grain plant$^{-1}$. At high temperature, *A. longissima* had the highest decline in grain yield (84%) followed by *A. searsii* (70%) and *A. caudata* (72%). The lowest

decrease in grain yield was observed in *A. speltoides* (58%) and *A. geniculata* (61%). Among the species at high temperature, *A. speltoides* had the highest grain yield (0.98 g plant$^{-1}$) followed by *A. geniculata* (0.66 g plant$^{-1}$). The grain yield plant$^{-1}$ of all other species was about 0.45 g (Fig. 2.5D).

**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 high temperature for grain yield plant$^{-1}$. Thus, accessions belonging to these two species were further analyzed and data are presented in Table 2.4 (*A. speltoides* accessions) and Table 2.5 (*A. geniculata* accessions).

Effects of temperature, accession, and temperature × accession were significant for the number of grains spike$^{-1}$, individual grain weight, and grain yield plant$^{-1}$ of *A. speltoides* accessions ($P \leq 0.005$, Table 2.4). Accession TA 2348 had the lowest decline in grain number spike$^{-1}$ (10% from optimum temperature), individual grain weight (4% from optimum temperature), and grain yield plant$^{-1}$ (14% from optimum temperature) under high temperature conditions. The maximum decline in grain number spike$^{-1}$ was observed in TA 1787 and TA 2120 (about 71% from optimum temperature). The maximum decline in individual grain weight was observed in TA 2097 (about 62% from optimum temperature), which also had the maximum decline in grain yield plant$^{-1}$ (86%).

The heat susceptibility index (HSI) calculated for *A. speltoides* is also shown in Table 2.4. Accessions were classified as highly tolerant (HSI ≤ 0.5), moderately tolerant (0.5 < HSI ≤ 1.0), or susceptible (HSI > 1.0) to high temperature stress (Viswanathan and Khanna-Chopra, 2001). Analysis of *A. speltoides* accessions for HSI showed that TA 2348 was a highly high temperature stress tolerant accession with an HSI of 0.23 (Table 2.4). 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 spike$^{-1}$ of *A. geniculata* accessions ($P > 0.05$), but accessions were different for this trait ($P = 0.004$; Table 2.5). Among accessions, TA 2787 had a lower number of grains (about 3 spike$^{-1}$) compared to others (about 6 spike$^{-1}$). Effects of temperature and accession were evident for individual grain weight and grain yield spike$^{-1}$ of *A. geniculata* accessions ($P \leq 0.003$), but temperature × accession effect was not observed for these traits ($P = 0.29$ and 4.72, Table 2.5). 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 (such as TA 10009 and TA 1800) and another with a range of 8.35 to 13.28 mg (such as TA 1808 and TA 10437). For grain yield plant$^{-1}$, 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$^{-1}$).

*Aegilops geniculata* accessions were either moderately high temperature tolerant, with $0.5 < HSI \leq 1.0$ (TA 2899, TA 1819, TA 1802, TA 1814 and TA 2061), or high temperature susceptible, with HSI > 1.0 (TA 1800, TA 10437, TA 1813, TA 1808, TA 10024, and TA 10009). *Aegilops geniculata* accessions with high levels of high temperature 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 may be a promising source of resistance to terminal heat stress (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999; Zaharieva et al., 2001). The high temperature stress (36/30°C day/night) at the reproductive stage of crop development revealed differences in high temperature responses among the accessions of the five *Aegilops* species tested. The *Aegilops* species differed in their responses to high temperature stress for physiological, yield, and yield parameters.

Among the physiological parameters, high temperature decreased relative chlorophyll (SPAD value) of all species. Chlorophyll is harbored in the thylakoid membranes, and loss of chlorophyll may be due to 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). The differential rate of decreases in chlorophyll (SPAD value) at high temperature across time (slope = – 2.70 to – 4.52; Fig. 2.3) and different magnitude of decreases in average chlorophyll across species (Fig. 2.2) showed the presence of genetic variability in *Aegilops* species for chlorophyll. The negative slope of regression lines in all species under high temperature shows an inverse relationship between duration of high temperature and leaf chlorophyll. The genetic variability in chlorophyll content of genotypes exposed to high temperature 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 carbohydrate and protein in grain comes from current assimilate and up to 20% is relocated from stem reserves

(Gebbing et al., 1999). In addition, a linear correlation was observed between loss of chlorophyll and heat stability of thylakoid membranes in winter wheat subjected to high temperature stress (Ristic et al., 2007). Thus, the amount and duration of chlorophyll retention in leaves might be crucial in realizing higher yield under high temperature stress. In this study, effect of high temperature on leaf chlorophyll was the lowest in *A. speltoides* and it had the minimum decrease in grain yield at high temperature. On the other hand, *A. longissima* had the highest decrease in leaf chlorophyll and grain yield at high temperature. This showed that leaf chlorophyll is highly valuable trait and can also be utilized in screening genotypes for high temperature stress tolerance under controlled environmental conditions.

Canopy temperature depression (CTD) has been widely used in evaluating heat stress tolerance of wheat germplasms at field conditions (Amani et al., 1996; Reynolds et al., 1998). A higher CTD value indicating cooler leaf surface as compared to air temperature is warranted. There was a significantly higher positive correlation (r = 0.91) between CTD and flag leaf temperature depression (Ayeneh et al., 2002). 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. caudata* (Fig. 2.4B); and as expected, *A. speltoides* and *A. geniculata* were the highest grain yielder at high temperature. This showed that flag leaf temperature depression may be used in evaluating germplasms for high temperature tolerance at control environmental conditions too. However, further studies are warranted before making a sound conclusion. Because, in this study, there was no facility to regulate the relative humidity of the chamber and thus vapor pressure deficit was not monitored. Vapor pressure deficit plays significant role in transpiration and thus the cooling of leaves surfaces.

High temperature decreased grain number spike$^{-1}$ in all species, resulting in yield loss. As high temperature was imposed at the Feekes 10.5.1 stage, when the first anthers already had appeared from the middle spikelet of the spike on the main tiller, the decrease in grain number spike$^{-1}$ was not due to decrease in spikelet number spike$^{-1}$ (Table 2.2), but may be due to a negative effect of high temperature on factors leading to grain set (lower pollen or ovule viability or pollen tube growth and low fertilization). High temperature caused low grain set in several crop species due to low pollen production and viability: wheat (*Triticum aestivum* L.) (Saini et al., 1983); rice (*Oryza sativa* L.) (Prasad et al., 2006b); and sorghum (*Sorghum bicolor* [L.] Moench) (Prasad et al., 2011) were all affected. In this study, genetic variability was observed for decrease in grain number spike$^{-1}$ ranging from non- significant decrease in *A. caudata* and *A. geniculata* to a highly significant decline of 62% in *A. longissima* (Fig. 2.5A). This result suggests potential for improving wheat cultivars for higher grain number spike$^{-1}$ at high temperature (Ehdaie and Waines, 1992; Khanna-Chopra and Viswanathan, 1999).

In addition to grain number spike$^{-1}$, individual grain weight (seed size) has been considered to be the most important yield component under high temperature stress at the reproductive stage (Gibson and Paulsen, 1999; Khanna-Chopra and Viswanathan, 1999). In this study, high temperature caused individual grain weight to decline by about 56% when averaged across the species. Yang et al. (2002) reported about a 50% decline in average grain weight of 30 synthetic hexaploid wheats subjected to high temperature of 10°C higher than the ambient (20/15°C) at 10 d after anthesis. *Aegilops* species responses to high temperature stress for individual grain weight differed; the decrease in grain weight ranged from 36% for *A. speltoides* to 76% for *A. longissima*. Genotypic differences for decrease in grain weight due to high temperature also 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%) exposed to > 36°C from anthesis.

In this study, because of the difficulty of threshing the wild relatives, grain yield plant$^{-1}$ was estimated by multiplying grain weight spike$^{-1}$ obtained from five tagged spikes by spike number plant$^{-1}$ (fertile tiller number plant$^{-1}$). High temperature decreased grain yield plant$^{-1}$ by 70% when averaged across the species. This decrease in yield is consistent 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 about 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 high temperature, the tolerance in *A. speltoides* was due to relative maintenance of both grain number spike$^{-1}$ and the individual grain weight. In *A. geniculata*, tolerance was primarily due to maintenance of grain number spike$^{-1}$. Previous studies conducted at field by delaying seeding/transplanting days, also suggested greater high temperature 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 high temperature for grain number spike$^{-1}$ and individual grain weight, TA 2348 (*A. speltoides*) had the lowest decrease in yield with high temperature 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 high temperature tolerant wheat genotypes. Accession TA 2348 originated in Israel (Table 2.1), a dry and hot area, and therefore, this

genotype is adapted to high temperature. Similarly, TA 2342 and TA 2780 (*A. speltoides*), which followed TA 2348 with an HSI of 0.65, are identified as moderately tolerant to high temperature; they, too, were of Israeli origin. This suggests that the place of origin can play an important role in an accession's high temperature tolerance.

*Aegilops speltoides* is a putative B genome donor of wheat and it should be feasible to introgress high temperature tolerance from this species into wheat by direct crosses and backcrosses (Chen et al., 1994; Gill et al., 2008). Similarly, *A. geniculata* is also easily hybridized with wheat. Surprisingly, there is considerable homoeologous 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).

In conclusion, this study revealed genetic variability among wild wheat species and accessions within species for high temperature tolerance. It illustrated that *A. speltoides* was the most tolerant species and that greater grain number spike$^{-1}$ and/or individual grain weight were main yield components associated with high temperature tolerance. Among *A. speltoides*, accessions TA 2348, TA 2342, and TA 2780 were identified as high temperature tolerant. Three accessions of *A. geniculata* (TA 2899, TA 1819, and TA1814) were also identified as moderately high temperature tolerant on the basis of yield and HSI. The high temperature tolerant accessions identified in this study can be used in breeding for high temperature tolerance of cultivated wheat as discussed above.

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## Figures and Tables

**Figure 2.1.** Effect of high temperature (36/30°C) stress on physiology, growth, yield, and yield components of *Aegilops* species. Percent decline in the respective parameters from optimum temperature (25/19°C) is indicated. Data are averaged across species.

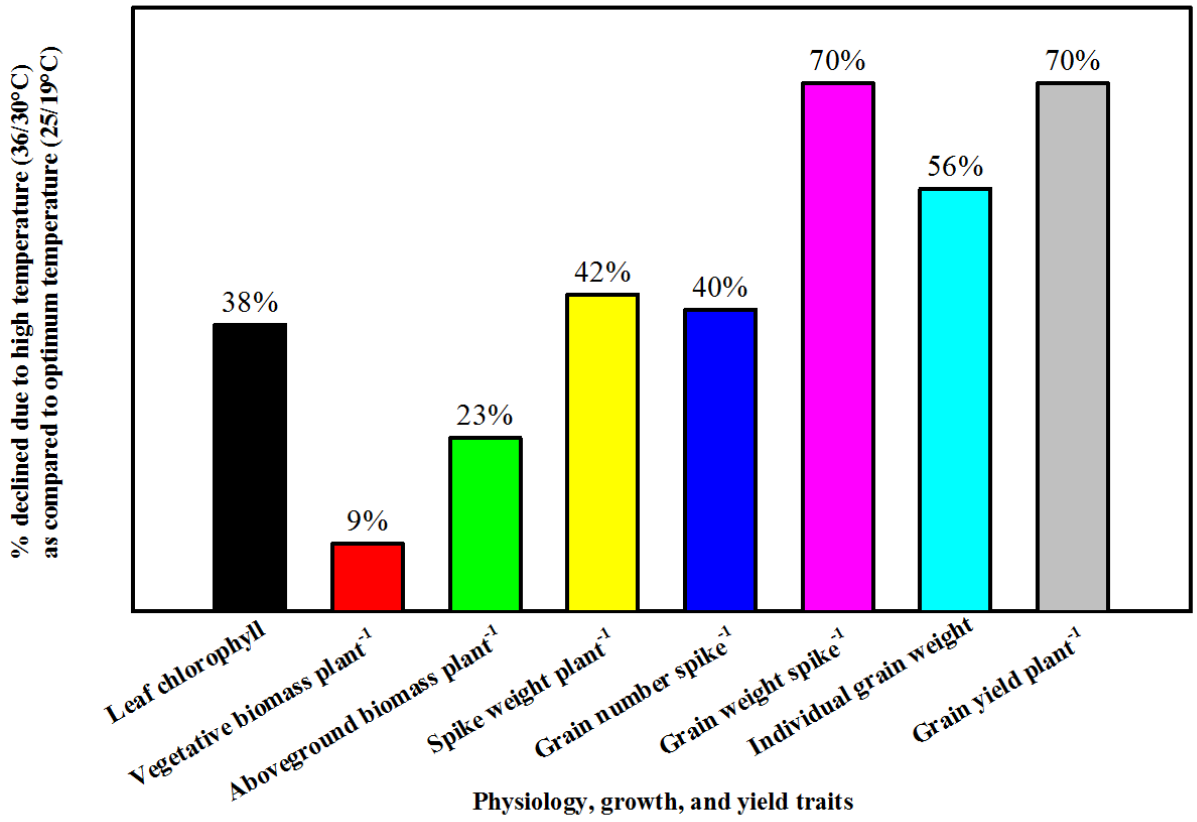


**Figure 2.2.** Effect of high temperature (36/30°C) stress on flag leaf chlorophyll content (SPAD value) of five *Aegilops* species. Percent decline in SPAD value due to high temperature as compared to optimum temperature (25/19°C) is indicated. Vertical lines on top of bars indicate standard error of means.

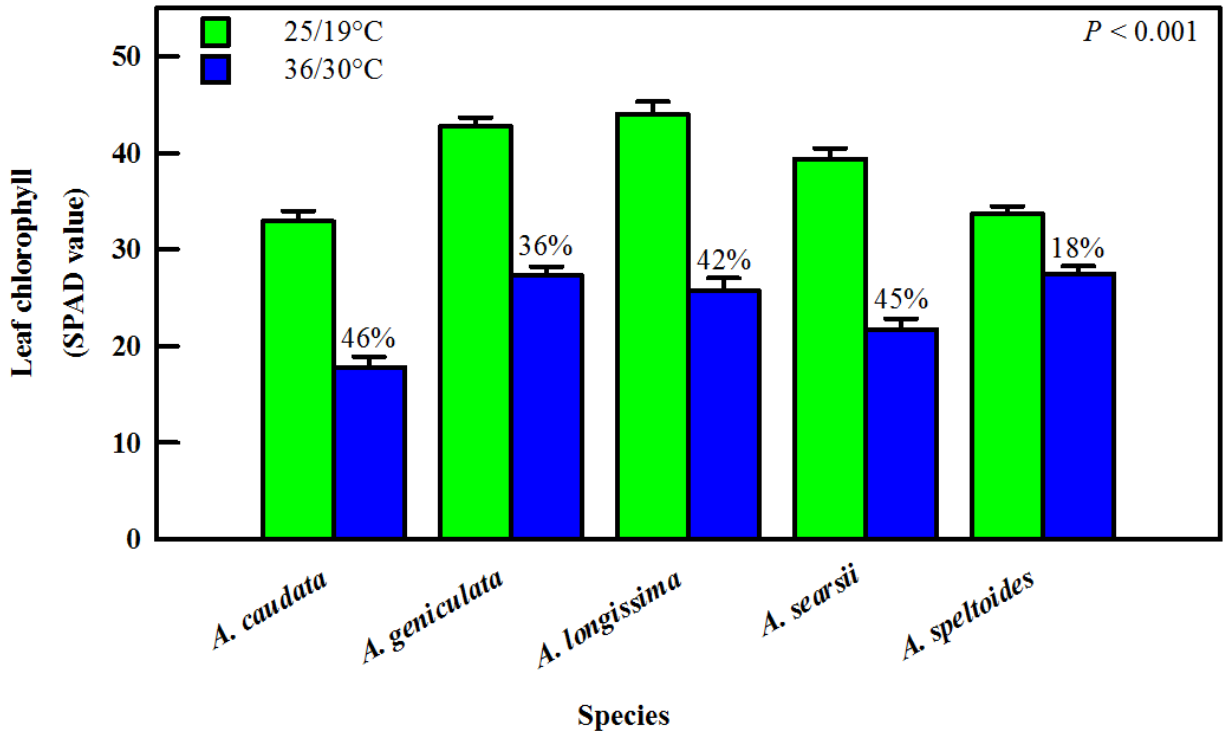


**Figure 2.3.** Flag leaf chlorophyll of five *Aegilops* species presented as a function of days after anthesis. Number of observations (n) = 6. Vertical lines on symbols indicate standard error of means. (A) Optimum temperature (25/19°C): *A. caudata*, y = – 1.68x + 41.34, $r^2$ = 0.99; *A. geniculata*, 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; *A. speltoides*, y = – 0.95x + 38.44, $r^2$ = 0.99. (B) High temperature (36/30°C): *A. caudata*, 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; *A. speltoides*, y = – 2.70x + 41.00, $r^2$ = 0.94.

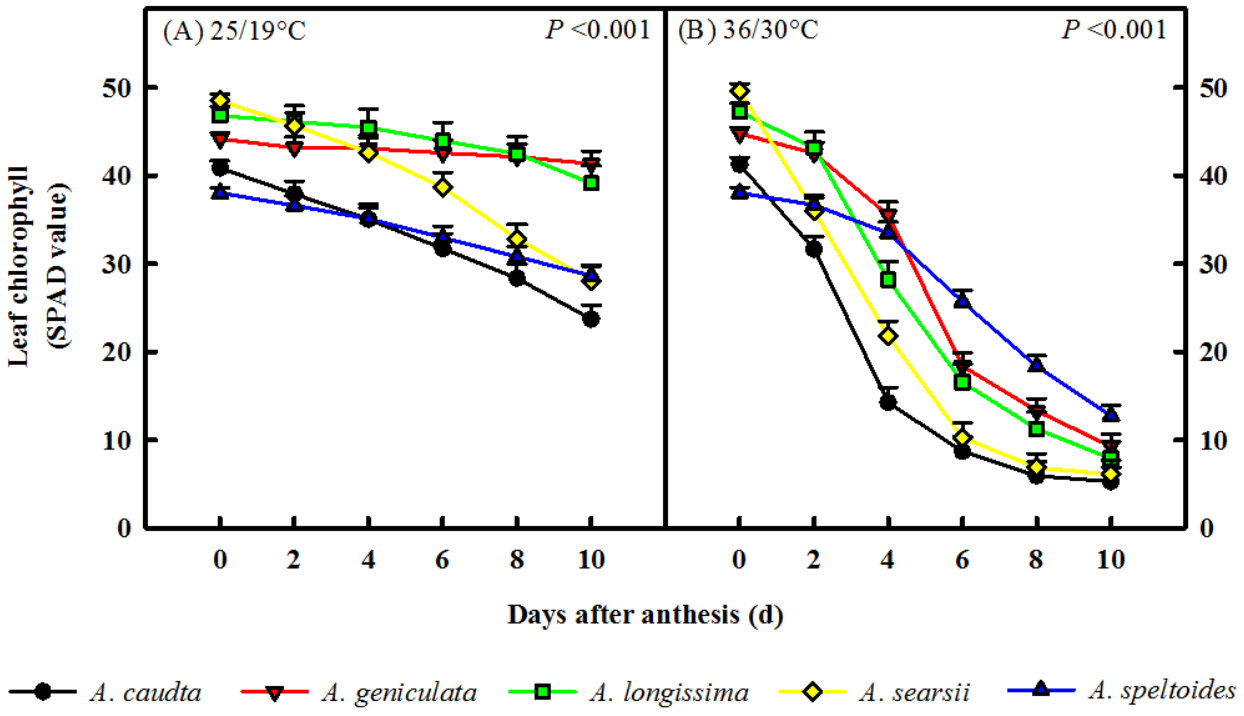


**Figure 2.4.** Flag leaf temperature depression of five *Aegilops* species presented as a function of days after anthesis. Number of observations (n) = 5. Vertical lines on symbols indicate standard error of means. (A) Optimum temperature (25/19°C): *A. caudata*, y = – 0.28x + 1.43, $r^2$ = 0.98; *A. 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; *A. speltoides*, y = – 0.23x + 1.84, $r^2$ = 0.94. (B) High temperature (36/30°C): *A. caudata*, 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, *A. speltoides*, y = – 0.67x + 12.08, $r^2$ = 0.99.

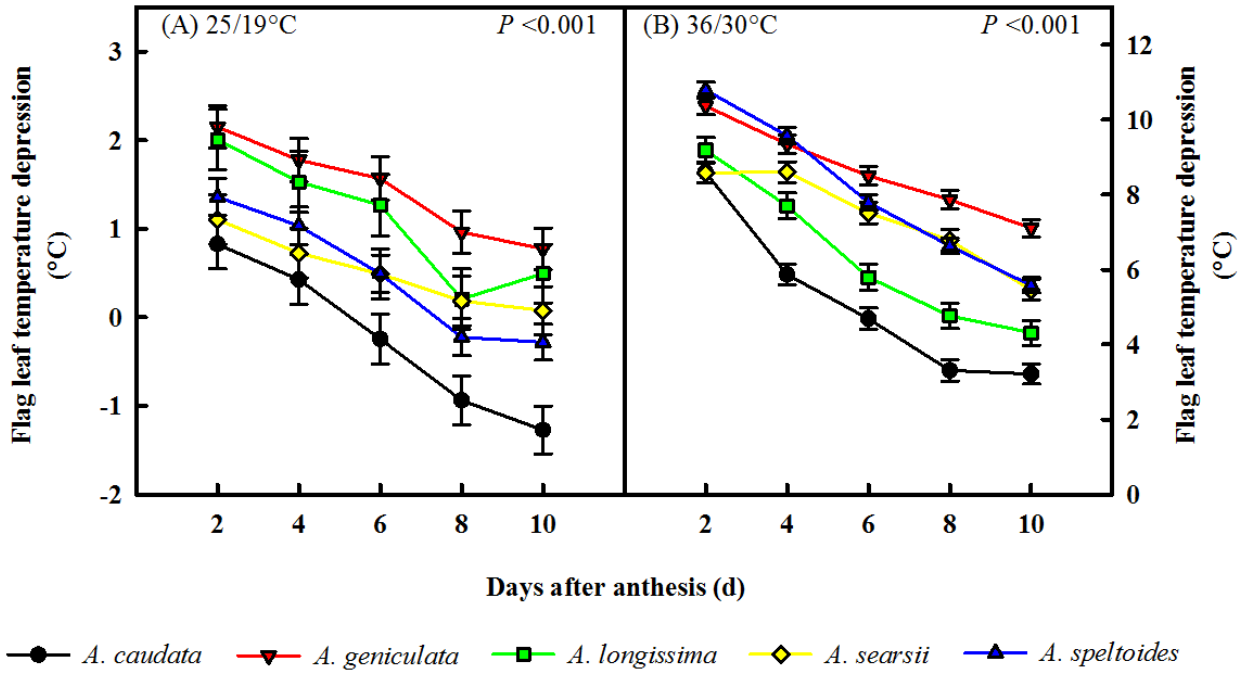
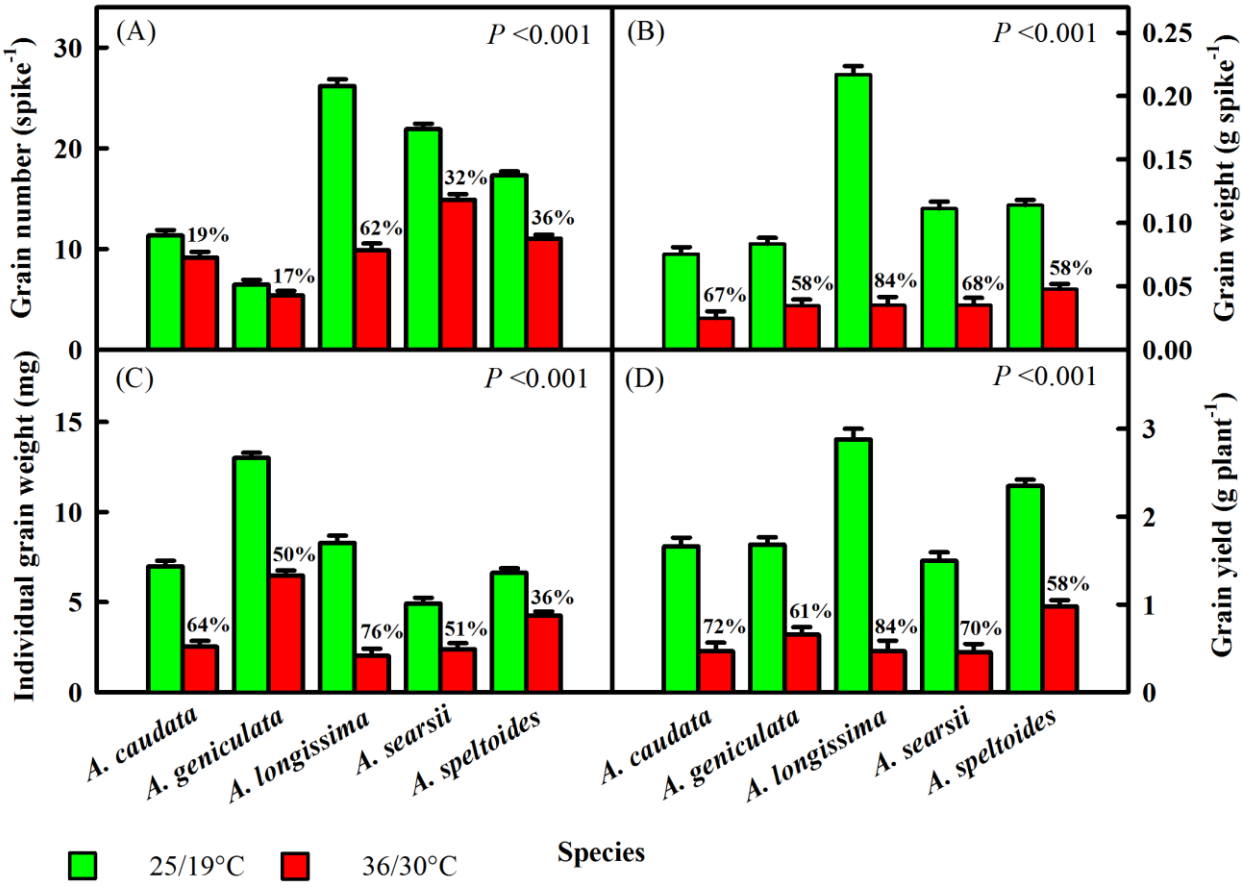


**Figure 2.5.** Effect of high temperature on (A) grain number spike$^{-1}$, (B) grain weight spike$^{-1}$, (C) individual grain weight, and (D) grain yield plant$^{-1}$ of five *Aegilops* species. Percent decline in each trait due to high temperature as compared to optimum temperature (25/19°C) is indicated. Vertical lines on top of bars indicate standard error of means.



**Supplementary Fig. 2.1.** The targeted and measured growth chambers' air temperature at (A) optimum temperature (25/19°C), and (B) high temperature (36/30°C). Each datum or a line is the average of four data sets collected during the flowering period.

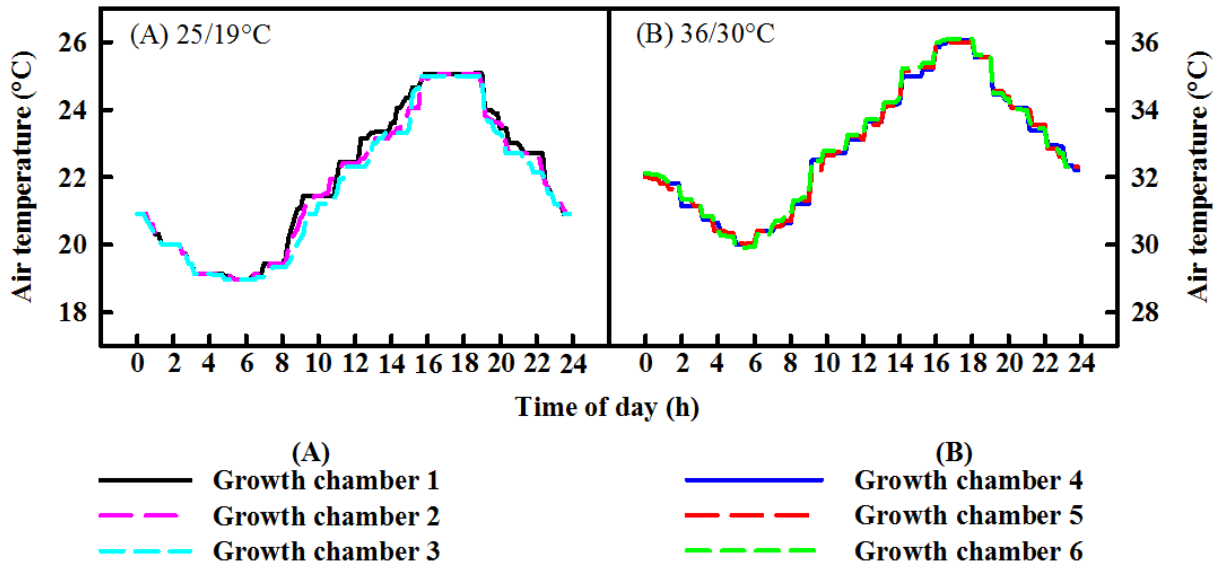


**Table 2.1.** Accession number, species, and country of origin of *Aegilops* species used for identifying high temperature tolerant genotypes at the reproductive stage.

| Accessions[†] | Genus | Species (New) | Species (Old) | Sub-species | Country of Origin |
|---|---|---|---|---|---|
| TA 1906 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 1908 | *Aegilops* | *caudata* | *markgrafii* | | Germany |
| TA 1909 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2085 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2091 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2093 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2095 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2096 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2170 | *Aegilops* | *caudata* | *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* | *typica* | 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, Kansas, USA.

**Table 2.2.** 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 (plant$^{-1}$) | Fertile tiller number (plant$^{-1}$) | Spike length (mm) | Spikelet number (spike$^{-1}$) |
|---|---|---|---|---|---|
| *A. caudata* | 71.0$^{c}$ | 40.1$^{a}$ | 21.1$^{a}$ | 88.2$^{d}$ | 7.9$^{d}$ |
| *A. geniculata* | 53.8$^{e}$ | 35.3$^{b}$ | 18.8$^{b}$ | 34.4$^{e}$ | 3.4$^{e}$ |
| *A. longissima* | 96.3$^{b}$ | 16.9$^{e}$ | 13.3$^{c}$ | 220.7$^{a}$ | 16.7$^{a}$ |
| *A. searsii* | 62.7$^{d}$ | 22.4$^{d}$ | 12.9$^{c}$ | 174.7$^{b}$ | 15.4$^{b}$ |
| *A. speltoides* | 106.1$^{a}$ | 29.9$^{c}$ | 20.1$^{ab}$ | 140.1$^{c}$ | 14.5$^{c}$ |

Tukey-Kramer grouping (Little et al., 2006) of the *Aegilops* species using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$.

**Table 2.3.** Effect of high temperature stress on spike weight, vegetative biomass, and aboveground biomass of five *Aegilops* species. Individual datum is the mean of three replications.

| Species | Spike weight (g plant$^{-1}$) | | | Vegetative biomass (g plant$^{-1}$) | | | Aboveground biomass (g plant$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | OT[†] | HT | Mean | OT | HT | Mean | OT | HT | Mean |
| *A. caudata* | 4.5 | 2.4 | 3.5$^{b}$ | 6.2 | 5.1 | 5.7$^{c}$ | 10.8 | 7.5 | 9.1$^{c}$ |
| *A. geniculata* | 4.4 | 2.1 | 3.3$^{b}$ | 5.0 | 4.1 | 4.5$^{d}$ | 9.4 | 6.2 | 7.8$^{cd}$ |
| *A. longissima* | 6.4 | 3.5 | 5.0$^{a}$ | 7.4 | 7.3 | 7.3$^{b}$ | 13.8 | 10.7 | 12.2$^{b}$ |
| *A. searsii* | 3.7 | 2.3 | 3.0$^{b}$ | 3.7 | 3.9 | 3.8$^{d}$ | 7.4 | 6.1 | 6.8$^{d}$ |
| *A. speltoides* | 6.5 | 4.5 | 5.5$^{a}$ | 10.0 | 9.5 | 9.7$^{a}$ | 16.5 | 13.8 | 15.3$^{a}$ |
| Mean | 5.1$^{A}$ | 3.0$^{B}$ | | 6.5$^{C}$ | 5.9$^{D}$ | | 11.6$^{E}$ | 8.9$^{F}$ | |
| *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$^{NS}$ | |

[†]OT = optimum temperature (25/19°C); HT = high temperature (36/30°C). Tukey-Kramer grouping (Little et al., 2006) of the *Aegilops* species using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$. $^{NS}$ Nonsignificant. *** Significant at $P < 0.001$.

**Table 2.4.** Effect of high temperature stress on yield and yield components of *A. speltoides* accessions. Grain number and individual grain weight are the mean of five spikes × three replications. Yield is the mean of three replications.

| Accession # | Grain number (spike$^{-1}$) | | | Individual grain weight (mg) | | | Grain yield (g plant$^{-1}$) | | | HSI |
|---|---|---|---|---|---|---|---|---|---|---|
| | OT[†] | HT | % decline from OT | OT | HT | % decline from OT | OT | HT | % decline from OT | |
| 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): | | | $0.001^{**}$ | | | $0.005^{**}$ | | | $0.004^{**}$ | |
| Accession (A): | | | $< 0.001^{***}$ | | | $0.001^{***}$ | | | $< 0.001^{***}$ | |
| T × A: | | | $0.001^{***}$ | | | $0.004^{**}$ | | | $< 0.001^{***}$ | |

[†]OT = optimum temperature (25/19°C); HT = high temperature (36/30°C); and HSI = heat susceptibility index. $^{**}$, $^{***}$ significant at $P < 0.01$, and $< 0.001$, respectively.

**Table 2.5.** Effect of high temperature stress on yield and yield components of *A. geniculata* accessions. Grain number and individual grain weight are the mean of five spikes × three replications. Yield is the mean of three replications.

| Accession # | Grain number (spike$^{-1}$) | | | Individual grain weight (mg) | | | Grain yield (g plant$^{-1}$) | | | HSI |
|---|---|---|---|---|---|---|---|---|---|---|
| | OT† | HT | Mean | OT | HT | Mean | OT | HT | Mean | |
| TA 2899 | 7.33 | 7.00 | 7.17$^{a}$ | 15.32 | 8.91 | 12.11$^{a}$ | 2.76 | 1.45 | 2.11$^{a}$ | 0.80 |
| TA 1819 | 7.00 | 6.33 | 6.67$^{a}$ | 13.70 | 9.72 | 11.71$^{a}$ | 1.90 | 1.00 | 1.45$^{a}$ | 0.80 |
| TA 1802 | 6.67 | 6.67 | 6.67$^{a}$ | 10.33 | 6.21 | 8.27$^{b}$ | 0.94 | 0.48 | 0.71$^{bc}$ | 0.83 |
| TA 1814 | 6.33 | 6.00 | 6.17$^{a}$ | 16.41 | 7.93 | 12.17$^{a}$ | 2.19 | 0.92 | 1.55$^{a}$ | 0.98 |
| TA 2061 | 6.67 | 5.67 | 6.17$^{a}$ | 9.10 | 4.43 | 6.77$^{b}$ | 1.58 | 0.66 | 1.12$^{bc}$ | 0.98 |
| TA 1800 | 7.00 | 5.33 | 6.17$^{a}$ | 10.89 | 5.67 | 8.28$^{b}$ | 1.30 | 0.50 | 0.90$^{bc}$ | 1.04 |
| TA 10437 | 6.00 | 4.33 | 5.17$^{a}$ | 16.49 | 10.07 | 13.28$^{a}$ | 1.88 | 0.71 | 1.30$^{a}$ | 1.05 |
| TA 1813 | 6.33 | 5.67 | 6.00$^{a}$ | 18.74 | 7.34 | 13.04$^{a}$ | 2.56 | 0.90 | 1.73$^{a}$ | 1.10 |
| TA 1808 | 7.00 | 4.33 | 5.67$^{a}$ | 10.51 | 6.20 | 8.35$^{a}$ | 1.81 | 0.60 | 1.20$^{b}$ | 1.12 |
| TA 10024 | 7.67 | 6.00 | 6.83$^{a}$ | 11.05 | 4.41 | 7.73$^{b}$ | 1.60 | 0.41 | 1.01$^{bc}$ | 1.25 |
| TA 10009 | 5.67 | 5.67 | 5.67$^{a}$ | 10.34 | 2.64 | 6.49$^{b}$ | 1.09 | 0.22 | 0.66$^{bc}$ | 1.34 |
| TA 2787 | 4.00 | 1.50 | 2.75$^{b}$ | 13.17 | 4.17 | 8.67$^{a}$ | 0.58 | 0.04 | 0.31$^{c}$ | 1.56 |
| Mean | 6.47 | 5.38 | | 13.00 | 6.47 | | 1.68 | 0.66 | | |
| *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$^{NS}$ | |

†OT = optimum temperature (25/19°C); HT = high temperature (36/30°C); and HSI = heat susceptibility index. Tukey-Kramer grouping (Little et al., 2006) of the *Aegilops* species using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$. $^{NS}$ Non-significant. $^{**}$, $^{***}$ Significant at $P < 0.01$ and 0.001, respectively.

**Supplementary Tabel 2.1.** Degrees of freedom (df) and F-values for physiological, growth, and yield components of five *Aegilops* species.

| Effects | Temperature (T) | Species (S) | T x S | A/S† | T x *A/S* | $A/S_1$ | $A/S_2$ | $A/S_3$ | $A/S_4$ | $A/S_5$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 (plant$^{-1}$) | $5.00^{NS}$ | $340.32^{***}$ | $1.22^{NS}$ | $18.56^{***}$ | $0.63^{NS}$ | $23.23^{***}$ | $4.82^{***}$ | $11.36^{***}$ | $15.25^{***}$ | $6.62^{***}$ |
| Fertile tiller number (plant$^{-1}$) | $6.56^{NS}$ | $79.87^{***}$ | $0.83^{NS}$ | $15.99^{***}$ | $0.29^{NS}$ | $41.12^{***}$ | $11.63^{***}$ | $10.04^{***}$ | $8.68^{***}$ | $6.14^{***}$ |
| 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 (spike$^{-1}$) | $0.00^{NS}$ | $820.97^{***}$ | $0.32^{NS}$ | $5.24^{***}$ | $1.06^{NS}$ | $17.3^{***}$ | $1.95^{NS}$ | $16.63^{***}$ | $3.91^{**}$ | $3.72^{***}$ |
| 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$^{-1}$) | $295.71^{***}$ | $45.92^{***}$ | $1.68^{NS}$ | $7.22^{***}$ | $1.09^{NS}$ | $14.05^{***}$ | $18.66^{***}$ | $4.21^{**}$ | $4.85^{***}$ | $5.21^{***}$ |
| Aboveground 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 (spike$^{-1}$) | $676.60^{***}$ | $183.42^{***}$ | $44.24^{***}$ | $11.60^{***}$ | $3.01^{***}$ | $4.48^{**}$ | $3.05^{**}$ | $8.95^{***}$ | $4.25^{**}$ | $164.2^{***}$ |
| 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$^{-1}$) | $2732.42^{***}$ | $25.29^{***}$ | $12.63^{***}$ | $9.41^{***}$ | $2.47^{***}$ | $4.32^{**}$ | $8.49^{***}$ | $4.15^{**}$ | $3.33^{**}$ | $24.95^{***}$ |

†A/S = accessions within species, $A/S_1$ = accessions within *A. caudata*, $A/S_2$ = accessions within *A. geniculata*, $A/S_3$ = accessions within *A. longissima*, $A/S_4$ =accessions within *A. searsii*, $A/S_5$ = Accessions within *A. speltoides*.

$^{NS}$ Non-significant. $^{*}$, $^{**}$, $^{***}$ Significant at $P < 0.05$, $< 0.01$, and $< 0.001$ respectively.

# Chapter III -Response of *Aegilops* species to drought stress during reproductive stages of development


## Abstract

Drought is an important abiotic factor limiting productivity of wheat. Thirty-one accessions belonging to five *Aegilops* species (wild wheats) were screened to identify species/accessions tolerant to an extended period of drought at the reproductive stage, and to identify physiological, growth and yield traits associated with tolerance. Plants were grown at full irrigation, 25/19°C day/night temperature and an 18 h photoperiod. At anthesis (Feekes 10.5.1), drought was imposed by withholding water for 16 d. Controls were continuously irrigated. Across species, drought significantly decreased leaf chlorophyll by 31%, grain number spike$^{-1}$ by 25%, individual grain weight by 68%, and grain yield plant$^{-1}$ by 76%. *Aegilops geniculata* Roth had greater tolerance to drought for yield (48% decline from control) as compared to other species (> 73% decline from control). The tolerance was associated with greater grain number spike$^{-1}$ and/or heavier grains. *Aegilops* geniculata accessions, TA 10437 and TA 1802 were most tolerant to drought with 7 to 24% yield decline, and drought susceptibility index (DSI) ≤ 0.5; whereas susceptible accessions had > 56% yield decline and a DSI > 1.0. The results suggest a presence of genetic variability among *Aegilops* species that can be utilized in breeding wheat for drought tolerance at reproductive stages.

## Introduction

Wheat (*Triticum aestivum* L.) provides 19% of world's food energy and 21% of protein intake (FAO, 2011). Due to rapidly increasing population and changing dietary patterns, the demand for wheat by 2050 is expected to increase by 31% over the 683 million tons consumed in 2008 (Dixon et al., 2009; FAO, 2011) At present the productivity of wheat is limited due to several environmental stresses including high temperature and drought (Flexas et al., 2004; Prasad et al., 2008a; Prasad et al., 2008b).

Water stress (drought) is the most important environmental factor limiting crop growth and yield (Boyer, 1982; Jones and Corlett, 1992; Chaves et al., 2003). Drought adversely affects physiology, morphology, growth, and yield traits of wheat (Saini and Westgate, 2000; Barnabás et al., 2008; Prasad et al., 2008b). Drought increases senescence by enhancing chlorophyll degradation, nitrogen loss, and lipid peroxidation (Yang et al., 2001). Drought decreases photosynthesis by lowering stomatal and mesophyll conductance (Flexas et al., 2004), or by oxidative damage of the chloroplast (Zhou et al., 2007). Severe drought impairs regeneration of ribulose bisphosphate, and decreases activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) resulting in lower photosynthesis (Bota et al., 2004). In wheat, up to 20% grain weight is derived from the reallocation of stem reserves under favorable conditions (Gebbing et al., 1999). Stem reserve may account for up to 50% of grain weight under post anthesis drought stress (van Herwaarden et al., 1998). Thus, drought at pre-anthesis decreases grain yield by adversely affecting photosynthesis leading to decreased accumulation of stem reserves (water soluble carbohydrates). Ehdaie et al. (2006) showed up to 23% decrease in main stem weight when wheat crop was subjected to drought stress. Drought during vegetative

stages can decrease relative water content, leaf area and biomass production (Giunta et al., 1995; Hafid et al., 1998; Dulai et al., 2006; Liu et al., 2006).

Drought stress at early stages of reproductive development (meiosis in pollen mother cells) induces pollen sterility, leading to lower grain numbers (Saini and Aspinall, 1981; Ji et al., 2010). Pollen abortion occurs as a result of loss of contact between young pollen grains and the tapetum, degeneration of anther filament, and/or decreased starch accumulation in anthers and pollen grains (Saini et al., 1984; Lalonde et al., 1997; Ji et al., 2010). Short durations of drought stress at the meiotic stage may cause pollen and ovary abortion, leading to decreased grain-set (Saini and Aspinall, 1981; Saini and Westgate, 2000; Ji et al., 2010). Drought during post anthesis decreases grain yield by decreasing individual grain weight (Ahmadi and Baker, 2001; Ji et al., 2010). Decrease in individual grain weight under drought in wheat is due to decreased grain filling duration rather than grain filling rate (Wardlaw and Willenbrink, 2000). In addition, post anthesis drought stress decreases the number of endosperm cells, and number of starch granules per cell in wheat grains (Nicolas et al., 1985).

*Aegilops* species are close relatives of hexaploid wheat (AABBDD). *Aegilops tauschii* Coss. is the donor of the D genome to hexaploid wheat (Kihara, 1944; McFadden and Sears, 1946), and *A. speltoides* Tausch has been considered the closest extant species to the B and G genomes of polyploid wheats (Dvořák and Zhang, 1990). *Aegilops* species are valuable gene pools for biotic and abiotic tolerance. Screening of wild genotypes for drought tolerance at the reproductive stage is essential to exploit genetic variability. Gill et al. (2006) summarized disease- and insect- resistant genes identified in many *Aegilops* species such as *A. tauschii*, *A. speltoides*, and *A. geniculata*. There are a few reports on *Aegilops* species with drought tolerance (Molnár et al., 2004; Baalbaki et al., 2006; Dulai et al., 2006; Rampino et al., 2006). However,

those reports were based on drought stress imposed at the seedling stage. Cereals are most sensitive to high temperature and drought stress at the reproductive stage (Prasad et al., 2008b). Reports on screening of *Aegilops* species for drought tolerance at the reproductive stage are limited. Therefore, this study was conducted under controlled environmental conditions with the following objectives: (a) to identify *Aegilops* species with tolerance to an extended period of drought at the reproductive stage, and (b) to identify physiological, growth, and yield traits associated with the tolerance.

## Materials and methods

### Plant material

Seeds of 31 accessions of *Aegilops* belonging to five different species, *A. caudata* L. (2), *Aegilops geniculata* Roth (10), *Aegilops longissima* Schweinf. & Muschl. (5), *Aegilops searsii* Feldman & Kislev ex K. Hammer (2), and *Aegilops speltoides* Tausch (12); and four spring wheat cultivars, ‘‘Dharwar Dry’’, ‘Sitta’, ‘Halberd’ and ‘Pavon 76’ as standard checks were used in this experiment (Table 3.1).

### Experimental and treatment conditions

This experiment was conducted in the Fall of 2008 at the controlled environmental facility of the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, Kansas, USA. Seeds of *Aegilops* accessions were sown in 4-cm-deep trays, containing commercial Sun Grow Metro Mix 300 potting soil (Hummert International, Topeka, Kansas, USA). The seedlings were raised in a growth chamber (Conviron Model E15, Winnipeg, MB, Canada) maintained at 20/15°C day/night temperature, 12 h photoperiod, and 65% humidity. Fourteen day old seedlings were vernalized for 42 d at 4°C with an 8 h photoperiod.

Following vernalization, seedlings of each accession were transplanted into six 1.6 L squared shaped plastic pots of dimensions 14 cm (height) × 50 cm (top perimeter) × 36 cm (bottom perimeter) containing a mixture of soil and sand at a ratio of 4:1, and 4 g of controlled release fertilizer (Osmocote Plus, $N:P_2O_5:K_2O$=15:9:12; Scotts, Marysville, OH, USA). Each pot had three seedlings and pots were placed randomly in three growth chambers designated as three replications. Growth chambers were maintained at 20/15°C day/night temperature, 12 h photoperiod, and 85% humidity, conditions optimum for *Aegilops'* tillering. On the same day, three seeds of *Triticum* species were also sown in pots. Each growth chamber held two pots of each accession / cultivar.

Once seedlings were established, one seedling from each pot was removed leaving two seedlings $pot^{-1}$. At this time, Marathon 1% G (a. i.: Imidacloprid,1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) was applied to avoid infestation of sucking insect pests. Pots in each growth chamber were randomly shifted every 7 d within the chamber to avoid any positional effect. At 45 d after transplanting, growth chamber conditions were changed to 25/19°C day/night temperature, 18 h photoperiod, 85% humidity, providing an environment suitable for *Aegilops'* flowering. The canopy level photosynthetic photon flux density in growth chambers was 400 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps (Phillips Lighting Co., Somerset, NJ, USA). The pots were kept in trays containing about 2 cm deep water to avoid any drought stress.

With onset of anthesis (Feekes 10.5.1) one pot of each accession within each growth chamber was randomly assigned to the drought treatment. Drought was imposed by withholding water for 16 d. The second pot was continuously irrigated and served as a control.

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

## Data collection

### Leaf chlorophyll

Leaf chlorophyll was measured every alternate day for 14 d from the start of the treatment. A self-calibrating chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL, USA) was used to measure chlorophyll on the fully expanded flag leaf of the tagged main stem. Each time, data were taken thrice from the middle portion of the leaf and the readings were averaged.

### Plant height, tiller number, and biomass

At maturity, plant height was measured from the plant base to the tip of the main stem spike excluding awns. Tiller number plant$^{-1}$ consisted of both fertile (with spikes) and non-fertile (without spike) tillers. Vegetative biomass plant$^{-1}$ was the weight of oven dried (65°C for 10 d) plant material without spikes. Aboveground biomass plant$^{-1}$ included vegetative biomass and dried spike weight plant$^{-1}$. The spikes were dried in an incubator (at 40°C) until they attained a constant weight.

### Spike length and spikelet number

At maturity, spike length was determined from tagged spikes by measuring from the base to the tip of the spike, excluding awns. Numbers of spikelets spike$^{-1}$ were counted from the same spikes.

### Grain number, individual grain weight, and yield

At harvest, tagged spikes were hand threshed after drying. Grains from these spikes were counted and weighed to determine number of grains spike$^{-1}$ and grain weight spike$^{-1}$. Individual grain weight was calculated by dividing grain weight spike$^{-1}$ by number of grains spike$^{-1}$. Grain yield plant$^{-1}$ of *Aegilops* species was estimated by multiplying grain weight spike$^{-1}$ by spike number plant$^{-1}$ (fertile tiller number plant$^{-1}$). The yield of *Triticum* species was determined by harvesting grains from all the spikes of the tagged plant.

### Drought susceptibility index (DSI)

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

$$DSI = (1 - Y/Yp) / D$$

where, $Y$ = average grain yield of an accession at drought (g plant$^{-1}$); $Yp$ = average grain yield of the same accessions at irrigated condition (g plant$^{-1}$); D = stress intensity = $1 - X/Xp$ ; $X$ = mean $Y$ of all accessions (g plant$^{-1}$), and $Xp$ = mean $Yp$ of all accessions (g plant$^{-1}$).

## Statistical analyses

Statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The PROC MIXED procedure was used with the NOBOUND option to avoid a zero value of block and/or block × drought variances (Littell et al., 2006). The experimental design was a split-plot with drought (D) randomly assigned to main plots and accessions (A) to sub-plots. There were three replications (3 different chambers). Class variables consisted of block, drought, species, and accessions. Block and block × drought were treated as random effects and all other variables as fixed effects. The LSMEANS with option Tukey-Kramer adjustment was used to compare the

treatment means (Littell et al., 2006). The Tukey-Kramer adjustment was used because this test is conservative in all cases including multiple comparisons of means with unequal sample sizes (Hayter, 1984). To assess the differences among *Aegilops* species for growth, physiological, and yield traits, accession effects were partitioned into species effect (S) and accessions within species effect (A/S). Accessions within species effects were further partitioned into five sources of variation, one for each species. The hexaploid bread wheat cultivars were used as a standard check in this study; therefore, each of them was analyzed as an independent species rather than classifying them as *Triticum aestivum's*.

## Results

The F-values for growth, physiological, and yield traits obtained with SAS PROC MIXED are presented in Supplementary Table 3.1. There were significant effects of species (S) and accessions within species (A/S) for all traits ($P < 0.001$). Significant effects of drought, drought × species, and drought × accession within species were found for leaf chlorophyll, grain number, spike weight, individual grain weight and grain yield (Supplementary Table 3.1).

### Leaf chlorophyll

Drought decreased leaf chlorophyll by 31% as compared to control,when averaged across all species and over the first 14 d readings. Differential responses of species to drought for leaf chlorophyll retention was evident at $P < 0.001$ (Supplementary Table 1). At drought, *A. caudata* and 'Halberd' had highest decrease in leaf chlorophyll (> 50% ), whereas 'Dharwar Dry' and 'Sitta' had the lowest decrease (6%). Most of the other species had about 33% decline in leaf chlorophyll. (Fig. 3.1).

## Plant height, tiller number, and biomass

Drought and drought × species had no significant effects on plant height, tiller number, number of fertile tillers plant$^{-1}$ and biomass; however, significant differences occurred among species for these attributes ($P < 0.001$; Supplementary Table 3.1). Plant height varied from about 50 cm for *A. geniculata* to 95 cm for *A. speltoides*. *Aegilops speltoides* had the highest number of tillers (39) and fertile tillers (25) plant$^{-1}$, whereas cultivars belonging to *Triticum* species had the minimum number of tillers (about 6) and fertile tillers (about 5 plant$^{-1}$; Table 3.2). *Aegilops speltoides* had the highest vegetative biomass (about 13 g plant$^{-1}$), whereas *A. geniculata* and cultivar 'Sitta' had the lowest biomass (about 4.5 g plant$^{-1}$). Other species produced about 6 g of vegetative biomass plant$^{-1}$ (Table 3.2). Drought decreased aboveground biomass by 19% when averaged across all species.

## Spike length, spikelet number, and spike weight

Drought and drought × species had no effect on spike length and number of spikelet spike$^{-1}$, but species were significantly different for these traits. *Aegilops longissima* had the longest spike (22 cm) followed by *A. speltoides* and *A. searsii* (about 16 cm). The shortest spike was observed in *A. geniculata* (about 4 cm; Table 3.2). Cultivar 'Pavon 76' had the highest number of spikelets spike$^{-1}$ (26) followed by 'Dharwar Dry' (23) and 'Halberd' (21). The lowest number of spikelets spike$^{-1}$ was observed in *A. geniculata* (3; Table 3.2). Drought decreased spike weight plant$^{-1}$ by about 41% when averaged across all species.

## Grain number and individual grain weight

There were significant effect of drought, species, and drought × species on five *Aegilops* species and four bread wheat cultivars for grain number spike$^{-1}$ and individual grain weight ($P < 0.001$)

(Supplementary Table 1). Drought decreased grain number spike$^{-1}$ by 25% and individual grain weight by 68%, when averaged across all species. Drought decreased grain number spike$^{-1}$ of *A. searsii* by 100% as compared to control, followed by *A. longissima* (69%). A minimum decrease in grain number due to drought was observed in 'Dharwar Dry' (0% decline) followed by 'Sitta' and 'Pavon 76' (≤ 7% decline) (Fig. 3.2). Effect of drought on idividual grain weight was the lowest in *A. geniculata* (34% decline as compared to control), and the highest in *A. searsii* (100%). The rest of the species/cultivars had ≥ 63% decline in individual grain weight due to drought (Fig. 3.3A).

## Grain yield

There were significant effects of drought, species and drought × species on five *Aegilops* species and four bread wheat cultivars for grain yield plant$^{-1}$ ($P < 0.001$; Supplementary Table 3.1). Drought decreased grain yield plant$^{-1}$ by 76% when averaged across all species. Effect of drought on grain yield plant$^{-1}$ was the lowest in *A. geniculata*, 48% decline as compared to control; and the highest in *A. searsii*, which could not produce a grain. Other species had ≥ 73% decline in grain yield due to drought (Fig 3.3B).

## Accessions within species variability in *Aegilops geniculata*

Among the five *Aegilops* species and four common bread wheat cultivars, *A. geniculata* was highly tolerant to drought for grain yield plant$^{-1}$. Thus, accessions belonging to this species were further analyzed for variability and data are presented in Table 3.3.

There were significant effects of accession and drought × accession on grain number spike$^{-1}$, individual grain weight and grain yield plant$^{-1}$ of *A. geniculata* accessions ($P \leq 0.005$; Table 3.3). Drought decreased grain number spike$^{-1}$ of accession TA 1808 by 86% followed by

TA 10009 (45%). Accessions TA 2899, TA 10024 and TA 10437 had the lowest decrease in grain number spike$^{-1}$ due to drought (≤ 5% decline). Effect of drought on individual grain weight was the highest in TA 1813 and TA 1808 (64–69% decline as compared to control), and the lowest in TA 10437 and TA 1802 (< 3% decline). Drought decreased grain yield plant$^{-1}$ of TA 1808 by 89% followed by TA 1813 (74%). TA 10437 had the lowest decrease in grain yield plant$^{-1}$ due to drought (6%), followed by TA 1802 (24%) (Table 3.3).

The drought susceptibility indices (DSI) calculated for *A. geniculata* accessions are shown in Table 3.3. Accessions were classified as highly drought tolerant (DSI ≤ 0.5), moderately drought tolerant (DSI > 0.5 to 1.0), or drought susceptible (DSI > 1.0). Accessions TA 10437 and TA 1802 were highly drought tolerant with a DSI of ≤ 0.05. The moderately drought tolerant accessions were TA 2061, TA 1814, TA 1819, TA 10024 and TA 2899, with DSIs ranging from 0.65 to 1.0. TA 1808 and TA 1813 were the examples of drought-susceptible accessions.

## Discussion

Water stress (drought) at anthesis is one of the most detrimental factors that decreases wheat yield by decreasing grain number spike$^{-1}$ and individual grain weight (Saini and Aspinall, 1981; Nicolas et al., 1985; Saini and Westgate, 2000; Ahmadi and Baker, 2001; Ji et al., 2010). Development of stress tolerant varieties is one of the promising ways to sustain/increase wheat yield under drought stress. *Aegilops* species contributed two of the three genomes to bread wheat and therefore, *Aegilops* species should be considered as an important genetic resource for increasing the genetic potential of cultivated wheat to withstand biotic as well as abiotic stresses. However, there are limited reports indicating presence of drought tolerant *Aegilops* species/accessions. These reports were mostly based on work on drought imposed at seedling

stage (Zaharieva et al., 2001; Molnár et al., 2004; Baalbaki et al., 2006; Dulai et al., 2006; Rampino et al., 2006). In my knowledge, this is the first study where *Aegilops* species are explored for reproductive drought tolerance by imposing drought at anthesis. This study showed presence of variability among *Aegilops* species/accession for physiological, yield and components of yield traits.

Among the species, drought decreased the leaf chlorophyll (SPAD value) in all species. Drought triggers rapid relocation of carbohydrates and nitrogen from leaves and stems to grains in cereals to complete and ensure maturation of grain. This causes senescence of leaves and thus the decrease in chlorophyll content (Yang et al., 2001). In addition, drought also damages membranes and degrades chlorophyll (Zhang and Kirkham, 1994). In this study, there was genotypic variation for decline in leaf chlorophyll under drought ranging from about 6% in 'Sitta' and 'Dharwar Dry' to > 50% in 'Halberd' and *A. caudata*. A previous study found no change in chlorophyll content in hexaploid wheats 'Excalibur' and 'RAC875', but a decline of about 25% in 'Kukri' (Izanloo et al., 2008).

Drought decreased grain number spike$^{-1}$ of all species (Fig. 3.2). The decrease in grain number was not due to the effect of drought on spikelet number spike$^{-1}$ (Table 3.2); rather it may be due to lower fertilization caused by pollen sterility and/or ovule abortion. The most sensitive stage to drought stress in wheat was identified as pollen mother cell at meiosis and tetrad break up (Saini and Aspinall, 1981). At this stage, drought disintegrates the contact between microspore and tapetum and degenerates the filament which induces pollen sterility (Saini et al., 1984). Drought also causes abnormal vacuolization of tapetal cells and disorientation of reproductive cells resulting in male sterility (Lalonde et al., 1997). In this study, drought was imposed when first anthers had just appeared from the middle spikelet of the main stem spike

(Feekes 10.5.1 stage). At this time, although fertilization was completed in these spikelets, the reproductive cells at basal and top spikelets of the main stem spike and that of other tagged spikes may have been at a stage that is susceptible to drought. Consequently, drought might have induced pollen/ovule sterility or interfered with fertilization in these spikelets resulting in decreased grain number spike$^{-1}$. Nicolas et al. (1985) observed 16% more sterility in the top spikelets of the wheat cultivar 'Warigal', when drought was imposed at anthesis. Fábián et al. (2011) reported embryo abortion in winter wheat varieties when drought was imposed at 5-9 d after anthesis. Embryo abortion was higher in the drought sensitive cultivar 'Cappelle Desprez' than in the tolerant cultivar 'Plainsman V'. This may also be the reason for decrease in grain number spike$^{-1}$ of all the species and accessions in this study (Fig. 3.2 and Table 3.3). In this study, there was a wide range of genetic variability for decrease in grain number spike$^{-1}$ under drought (0 -100%). Therefore, this trait may be utilized in improving wheat cultivars for high grain number spike$^{-1}$ under drought stress at the reproductive stage.

In addition to grain number spike$^{-1}$, individual grain weight is the most important yield component in cereals (Saini and Westgate, 2000; Prasad et al., 2008b). Drought decreased individual grain weight by 68% (Fig. 3.3A), which is higher than the findings of Wardlaw and Willenbrink (2000) in the wheat cultivar 'Lyallpur 73' (38% decline) subjected to drought during anthesis and that of Ahmadi and Baker (2001) in cultivar 'Cadenza' (43% decline) subjected to severe drought from 15 d after anthesis. The decrease in individual grain weight may be due to lower grain filling duration (Wardlaw and Willenbrink, 2000; Prasad et al., 2008a) and a decreased number of endosperm cells and starch granules per cell (Nicolas et al., 1985). In this study, decrease in individual grain weight due to drought varied from 34% in *A. geniculata* to > 63% in rest of the species.

Drought decreased grain yield plant$^{-1}$ by 76% when averaged across all species. Decrease in yield was about 75% in all cultivars used as standard checks; including the drought tolerant cultivar 'Dharwar Dry'. *Aegilops geniculata* had remarkably lower decline in grain yield (48%) compared to the standard checks (*Triticum aestivum*) and other wild species (76-100% decline). Although *A. caudata* and cultivars 'Dharwar Dry', 'Pavon 76', and 'Sitta' showed a smaller effect of drought on grain numbers compared to *A. geniculata,* all of them had at least 25% more yield decline than *A. geniculata*. Thus, in these species individual grain weight might be the determining factor for the decrease in yield. However, in other species, both grain number spike$^{-1}$ and individual grain weight might have played a role in yield formation. The tolerance of *A. geniculata* for grain yield under drought stress was also reported by (Baalbaki et al., 2006).

Among *A. geniculata* accessions, drought stress barely affected grain number spike$^{-1}$ of TA 2899, TA 10024, and TA 10437 (Table 3.3). Thus, these may be potential sources of genes for maintaining fertility under reproductive drought stress in wheat. *Aegilops geniculata* accessions TA 10437 and TA 1802 had the minimum decline in individual grain weight; and they may be utilized in maintaining grain weight of wheat under terminal drought stress. In addition, accessions TA 10437 and TA 1802 also maintained higher yield at drought stress and had the drought susceptibility index (DSI) of $\leq 0.05$. DSI is a measure of yield stability under drought stress that can be utilized in selecting drought tolerant genotypes (Sio-Se Mardeh et al., 2006). Therefore, accessions TA 10437 and TA 1802 were classified as highly drought tolerant genotypes. In addition, accessions TA 2061, TA 1814, TA 1819 and TA 10024, with $0.5 \leq DSI \leq 1.0$ were identified as moderately tolerant to drought stress. *Aegilops geniculata* has the $U^g$ and $M^g$ genomes which are not related to the genomes of hexaploid bread wheat, *Triticum aestivum* (ABD) (Gill et al., 2006). Therefore, *A. geniculata* are categorized under tertiary gene pool and

gene transfer from this gene pool cannot be achieved by homologous combination (Qi et al., 2007). However, considerable homoeologous pairing between *A. geniculata* and wheat chromosomes had been observed (Gill et al., 2008) and several genes have been transferred from *A. geniculata* into wheat presumably as a result of spontaneous pairing (Kuraparthy et al., 2007).

In conclusion, this study revealed genetic variability among *Aegilops* species and accessions within species for drought tolerance. It identified *A. geniculata* as the most tolerant species and that greater grain number spike$^{-1}$ and/or individual grain weight were the main yield components associated with drought tolerance. Among *A. geniculata*, accessions TA 10437 and TA 1802 were identified as highly drought tolerant. The results of this study were from a controlled environment experiment. Field evaluation of accessions under drought conditions would, therefore, be useful and desirable. After field testing and evaluation, drought tolerant accessions identified in this study may be utilized in breeding for drought tolerance of cultivated wheat as discussed above.

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## Figures and Tables

**Figure 3.1.** Effect of drought stress on flag leaf chlorophyll (SPAD value) of five *Aegilops* species and four bread wheats. Interaction effect of drought × species was significant at $P < 0.001$. Percent decline due to drought as compared to control is indicated. Vertical lines on bars indicate standard error of means.

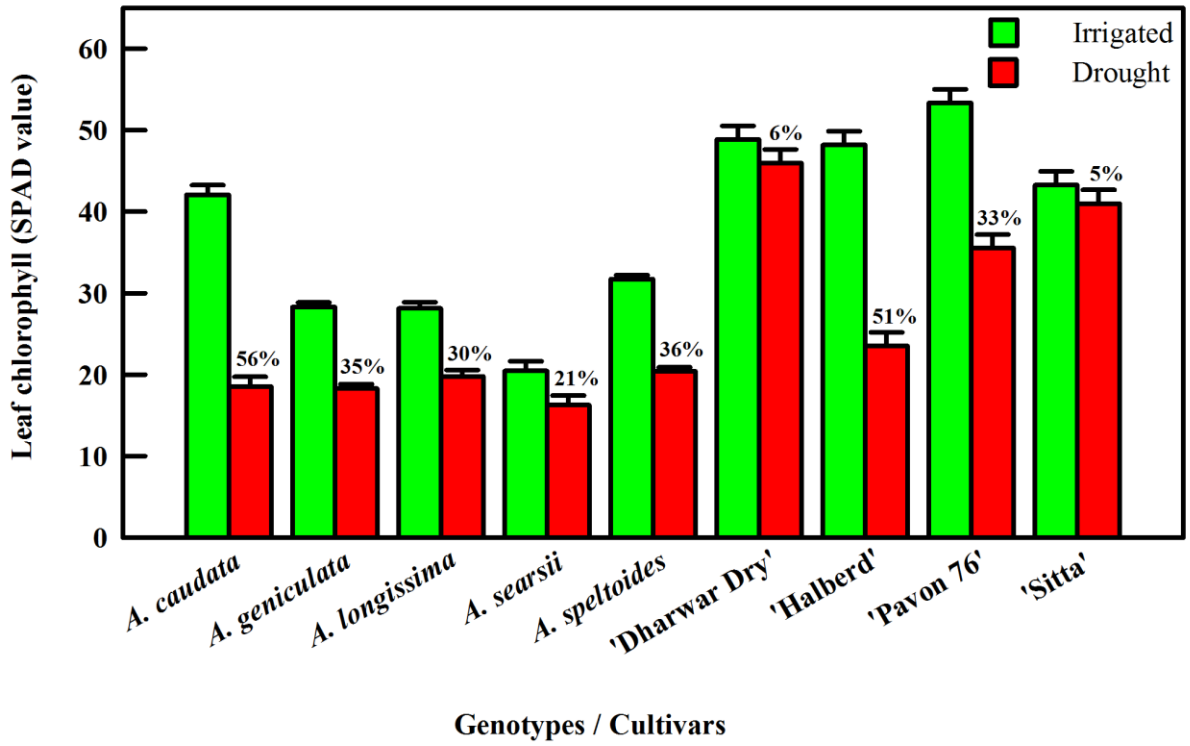

**Figure 3.2.** Effect of drought stress on grain number spike$^{-1}$ of five *Aegilops* species and four bread wheats. Interaction effect of drought × species was significant at $P < 0.001$. Percent decline due to drought as compared to control is indicated. Vertical lines on bars indicate standard error of means.

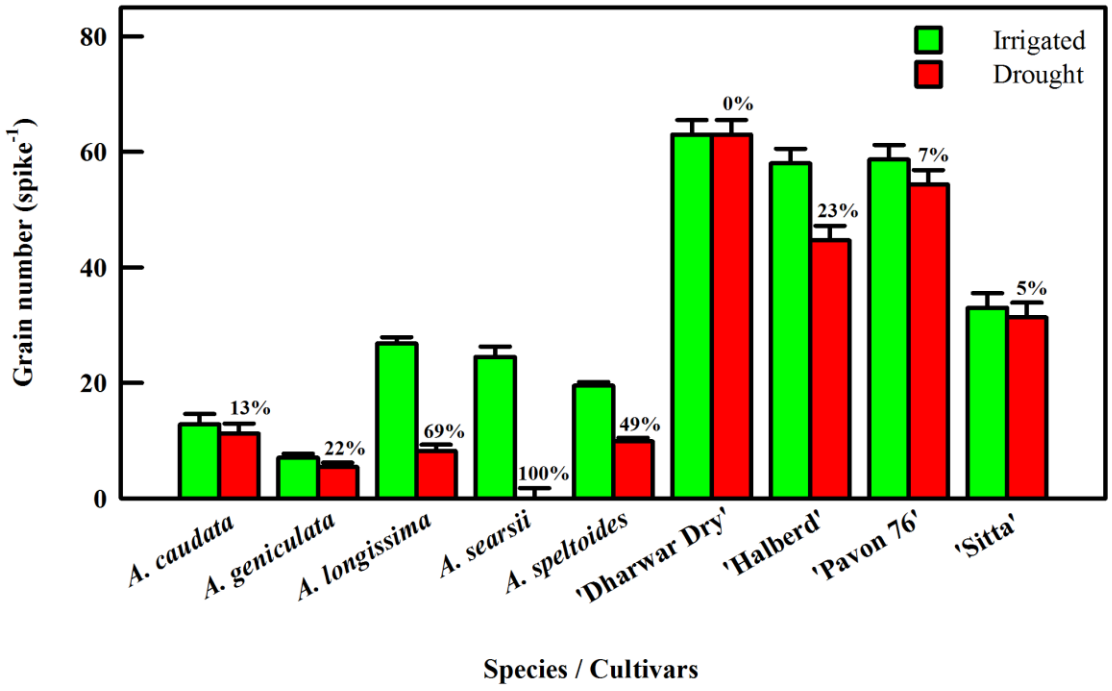

**Figure 3.3.** Effect of drought stress on (A) individual grain weight, and (B) grain yield plant$^{-1}$ of five *Aegilops* species and four bread wheats. Interaction effect of drought × species was significant at $P < 0.001$. Percent decline due to drought as compared to control is indicated. Vertical lines on bars indicate standard error of means.

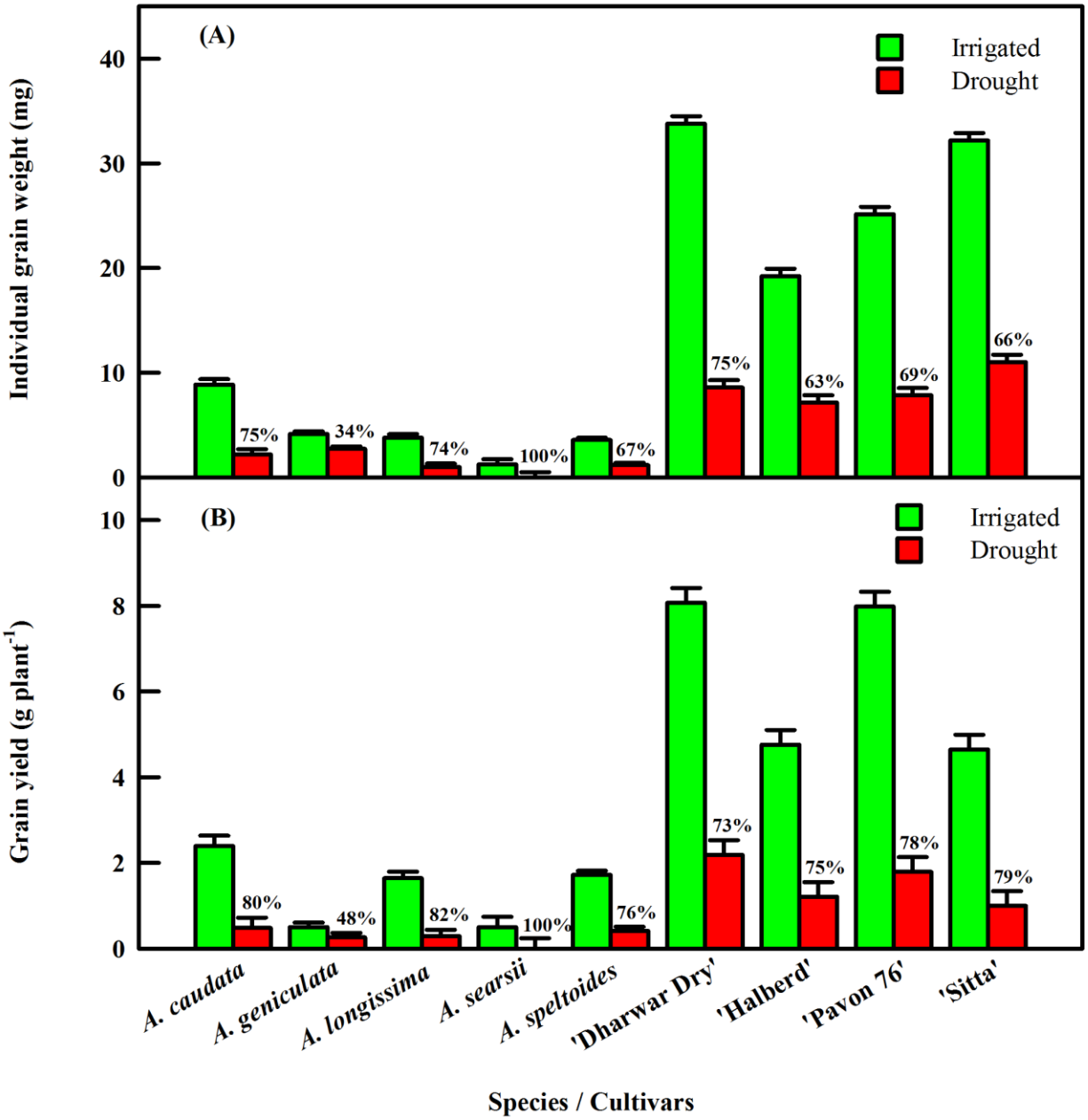

**Table 3.1.** Accession number, species, and country of origin of *Aegilops* species, and bread wheat cultivars used for identifying drought tolerant genotypes at the reproductive stage.

| Accession #[†] | *Genus* | *Species (New)* | *Species (Old)* | *Sub-species* | Country of Origin |
|---|---|---|---|---|---|
| TA 1906 | *Aegilops* | *caudata* | *markgrafii* | | Turkey |
| TA 2170 | *Aegilops* | *caudata* | *markgrafii* | | Syria |
| 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 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* | *typica* | Israel |
| TA 1921 | *Aegilops* | *longissima* | *longissimum* | *nova* | Jordan |
| TA 1837 | *Aegilops* | *searsii* | *searsii* | | Jordan |
| TA 1925 | *Aegilops* | *searsii* | *searsii* | | Jordan |
| TA 1772 | *Aegilops* | *speltoides* | *speltoides* | *ligustica* | 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 2149 | *Aegilops* | *speltoides* | *speltoides* | *speltoides* | Turkey |
| TA 2342 | *Aegilops* | *speltoides* | *speltoides* | *ligustica* | Israel |
| TA 2348 | *Aegilops* | *speltoides* | *speltoides* | *speltoides* | Israel |
| 'Dharwar Dry' | *Triticum* | *aestivum* | | | |
| 'Sitta' | *Triticum* | *aestivum* | | | |
| 'Halberd' | *Triticum* | *aestivum* | | | |
| 'Pavon 76' | *Triticum* | *aestivum* | | | |

[†]Source: Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, Kansas, USA.

**Table 3.2.** Mean growth and morphological parameters of five *Aegilops* species and four bread wheat cultivars.

| Species/Cultivars | Plant height (cm) | Tiller number (plant$^{-1}$) | Fertile tiller number (plant$^{-1}$) | Vegetative biomass (g plant$^{-1}$) | Spike length (cm) | Spikelet number (spike$^{-1}$) |
|---|---|---|---|---|---|---|
| *A. caudata* | 64$^{de}$ | 34$^{b}$ | 21.3$^{b}$ | 5.4$^{b\,c}$ | 7.8$^{e}$ | 8$^{f}$ |
| *A. geniculata* | 50$^{f}$ | 30$^{b}$ | 16.6$^{c}$ | 4.6$^{c}$ | 3.9$^{f}$ | 3$^{g}$ |
| *A. longissima* | 83$^{b}$ | 22$^{c}$ | 14.2$^{d}$ | 7.7$^{b}$ | 21.8$^{a}$ | 16$^{d}$ |
| *A. searsii* | 61$^{e}$ | 30$^{b}$ | 16.7$^{c}$ | 6.1$^{b\,c}$ | 16.3$^{b}$ | 13$^{e}$ |
| *A. speltoides* | 95$^{a}$ | 39$^{a}$ | 24.9$^{a}$ | 12.6$^{a}$ | 16.3$^{b}$ | 14$^{e}$ |
| ‘Dharwar Dry’ | 72$^{c}$ | 6$^{d}$ | 4.5$^{e}$ | 6.5$^{b\,c}$ | 12.2$^{c}$ | 23$^{b}$ |
| ‘Halberd’ | 69$^{cd}$ | 5$^{d}$ | 4.7$^{e}$ | 5.5$^{b\,c}$ | 10.7$^{cd}$ | 21$^{b}$ |
| ‘Pavon 76’ | 72$^{cd}$ | 7$^{d}$ | 5.5$^{e}$ | 8.1$^{b}$ | 11.3$^{cd}$ | 26$^{a}$ |
| ‘Sitta’ | 59$^{e}$ | 6$^{d}$ | 4.5$^{e}$ | 4.3$^{c}$ | 8.4$^{de}$ | 18$^{c}$ |

Tukey-Kramer grouping (Little et al., 2006) of the wild and bread wheats using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$.

**Table 3.3.** Effect of drought on yield and yield components of *A. geniculata* accessions. Data on grain number and individual grain weight are the mean of five spikes × three replications. Data on yield are the mean of three replications.

| Accession # | Grain number (spike$^{-1}$) | | | Individual grain weight (mg) | | | Grain yield (g plant$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Irrigated | Drought | % Decline | Irrigated | Drought | % Decline | Irrigated | Drought | % Decline | DSI$^{\dagger}$ |
| TA 10437 | 7.3 | 7.0 | 4.5 | 4.8 | 4.8 | 0.5 | 0.42 | 0.39 | 6.5 | 0.14 |
| TA 1802 | 7.3 | 6.3 | 13.6 | 3.1 | 3.0 | 2.6 | 0.37 | 0.28 | 24.4 | 0.51 |
| TA 2061 | 6.3 | 5.3 | 15.8 | 2.9 | 2.4 | 18.9 | 0.30 | 0.21 | 31.0 | 0.65 |
| TA 1814 | 8.3 | 6.3 | 24.0 | 4.5 | 3.8 | 15.5 | 0.72 | 0.46 | 36.0 | 0.75 |
| TA 1819 | 8.0 | 6.3 | 20.8 | 5.7 | 4.5 | 21.2 | 0.79 | 0.49 | 38.4 | 0.80 |
| TA 10024 | 7.7 | 7.3 | 4.4 | 2.9 | 1.7 | 41.5 | 0.23 | 0.12 | 47.2 | 0.98 |
| TA 2899 | 6.3 | 6.3 | 0.0 | 4.1 | 1.9 | 53.7 | 0.65 | 0.34 | 47.9 | 1.00 |
| TA 10009 | 3.7 | 2.0 | 45.5 | 3.3 | 1.8 | 44.1 | 0.16 | 0.07 | 56.4 | 1.18 |
| TA 1813 | 7.7 | 6.3 | 17.4 | 6.3 | 1.9 | 69.1 | 0.59 | 0.15 | 74.0 | 1.54 |
| TA 1808 | 7.3 | 1.0 | 86.4 | 4.0 | 1.4 | 63.9 | 0.75 | 0.08 | 89.1 | 1.86 |
| Mean | 7.0 | 5.4 | | 4.2 | 2.7 | | 0.50 | 0.26 | | |
| *P - values:* | | | | | | | | | | |
| Drought: | | | $0.069^{NS}$ | | | $0.035^{*}$ | | | $0.017^{*}$ | |
| Accessions: | | | $< 0.001^{***}$ | | | $< 0.001^{***}$ | | | $< 0.001^{***}$ | |
| Drought × Accession: | | | $0.001^{**}$ | | | $0.005^{**}$ | | | $< 0.001^{***}$ | |

$^{\dagger}$DSI = drought susceptibility index. $^{NS}$ nonsignificant. $^{*}$, $^{**}$, $^{***}$ significant at $P < 0.05$, $< 0.01$, and $< 0.001$ respectively.

**Supplementary Table 3.1. Degrees of freedom (df) and F-values for physiological, growth, and yield components of five *Aegilops* species, and accessions within each species.**

| Effects | Drought (D) | Species (S) | D × S | A/S[†] | D × A/S | $A/S_1$ | $A/S_2$ | $A/S_3$ | $A/S_4$ | $A/S_5$ |
|---|---|---|---|---|---|---|---|---|---|---|
| df | 1 | 8 | 8 | 26 | 26 | 1 | 9 | 4 | 1 | 11 |
| Traits | F-values | | | | | | | | | |
| Plant height (cm) | $0.00^{NS}$ | $359.30^{***}$ | $0.28^{NS}$ | $11.69^{***}$ | $0.29^{NS}$ | $1.26^{NS}$ | $24.4^{***}$ | $9.44^{***}$ | $16.15^{*}$ | $9.07^{***}$ |
| Tiller number (plant$^{-1}$) | $0.73^{NS}$ | $193.37^{***}$ | $0.15^{NS}$ | $16.15^{***}$ | $0.32^{NS}$ | $40.50^{***}$ | $4.65^{***}$ | $18.7^{***}$ | $0.60^{NS}$ | $18.3^{***}$ |
| Fertile tiller number (plant$^{-1}$) | $1.07^{NS}$ | $198.24^{***}$ | $0.20^{NS}$ | $32.35^{***}$ | $0.68^{NS}$ | $1.11^{NS}$ | $20.94^{***}$ | $4.09^{*}$ | $0.53^{NS}$ | $38.41^{***}$ |
| Spike length (mm) | $0.08^{NS}$ | $343.92^{***}$ | $0.47^{NS}$ | $17.59^{***}$ | $0.94^{NS}$ | $64.37^{***}$ | $4.89^{***}$ | $11.14^{***}$ | $8.12^{***}$ | $35.08^{***}$ |
| Spikelet number (spike$^{-1}$) | $0.49^{NS}$ | $552.57^{***}$ | $0.76^{NS}$ | $10.17^{***}$ | $0.49^{NS}$ | $48^{**}$ | $1.66^{NS}$ | $5.81^{**}$ | $24.00^{**}$ | $11.90^{***}$ |
| Vegetative biomass (g plant$^{-1}$) | $1.53^{NS}$ | $93.41^{***}$ | $0.36^{NS}$ | $9.84^{***}$ | $0.48^{NS}$ | $0.02^{NS}$ | $25.00^{***}$ | $19.42^{***}$ | $12.26^{*}$ | $8.71^{***}$ |
| Spike weight (g plant$^{-1}$) | $126.30^{***}$ | $65.17^{***}$ | $8.53^{***}$ | $8.80^{***}$ | $0.87^{NS}$ | $7.1^{NS}$ | $37.25^{***}$ | $4.32^{*}$ | $39.67^{**}$ | $14.06^{***}$ |
| Aboveground biomass (g plant$^{-1}$) | $27.55^{***}$ | $99.51^{***}$ | $1.60^{NS}$ | $10.70^{***}$ | $0.70^{NS}$ | $1.08^{NS}$ | $38.08^{***}$ | $10.60^{***}$ | $17.77^{*}$ | $12.46^{***}$ |
| Leaf chlorophyll (SPAD value) | $479.13^{***}$ | $124.54^{***}$ | $18.59^{***}$ | $22.17^{***}$ | $7.90^{***}$ | $18.05^{***}$ | $6.70^{***}$ | $4.10^{**}$ | $0.90^{NS}$ | $8.71^{***}$ |
| Grain number (spike$^{-1}$) | $289.77^{***}$ | $245.25^{***}$ | $16.97^{***}$ | $3.01^{***}$ | $2.47^{***}$ | $10.29^{*}$ | $12.11^{***}$ | $2.1^{NS}$ | $3.77^{NS}$ | $3.62^{***}$ |
| Individual grain weight (mg) | $1005.98^{***}$ | $465.08^{***}$ | $143.09^{***}$ | $5.33^{***}$ | $2.97^{***}$ | $284.17^{***}$ | $7.70^{***}$ | $2.6^{NS}$ | $0.00^{NS}$ | $9.36^{***}$ |
| Grain yield (g plant$^{-1}$) | $619.02^{***}$ | $92.55^{***}$ | $36.15^{***}$ | $3.41^{***}$ | $1.77^{*}$ | $114.63^{***}$ | $18.68^{***}$ | $2.51^{NS}$ | $0.87^{NS}$ | $10.17^{***}$ |

[†]A/S = accessions within over all species, $A/S_1$ = Accessions within *A. caudata*, $A/S_2$ = Accessions within *A. geniculata*, $A/S_3$ = Accessions within *A. longissima*, $A/S_4$ = Accessions within *A. searsii*, $A/S_5$ = Accessions within *A. speltoides*.

$^{NS}$ nonsignificant. $^{*}$, $^{**}$, $^{***}$ significant at $P < 0.05$, $< 0.01$, and $< 0.001$ respectively.

# Chapter IV -Effects of high temperature and drought at anthesis on synthetic hexaploid wheats


## Abstract

High temperature and drought often occur simultaneously at anthesis causing significant wheat yield losses. The objectives of this research were to quantify independent and combined effects of drought and high temperature on synthetic and spring wheats, and to determine if responses varied among the genotypes. Four synthetic hexaploid and two spring wheats were grown from seeding to anthesis at a full irrigation and an optimum temperature of 21/15°C. Thereafter, treatments were imposed as (a) drought stress: withhold irrigation + 21/15°C, (b) high temperature stress: irrigation + 36/30°C, (c) combined stress: withhold irrigation + 36/30°C, and (d) optimum condition: irrigation + optimum temperature. Stresses were imposed for 16 d. Combined stress decreased leaf chlorophyll, grain number, individual grain weight, and grain yield of all genotypes with higher magnitude than the decrease by high temperature or drought stress alone. ALTAR 84 / AO'S' had a minimum decrease in grain number spike$^{-1}$ (64%), individual grain weight (77%), and grain yield plant$^{-1}$ (81%) under the combined stress, and was more tolerant than others. The maximum decreases in grain number (83%), individual grain weight (94%), and grain yield (98%) was observed in genotype GAN / *A. tauschii* (WX 897). The results suggest that the interaction between drought and high temperature for individual grain weight and grain yield was hypo-additive, i.e. combined effect was lower than the sum of individual effects; and genotypes varied in their response to independent and combined stresses. I recommend for further studies under a more controlled and quantifiable drought condition.

# Introduction

Wheat (*Triticum* species) is the most important food crop of the world in terms of the harvested area and trade value. In 2008, it was grown in 222.7 million hectares of land, which was about 1.4 times bigger than the land under the maize (*Zea mays* L.) and under the paddy rice (*Oryza sativa* L.) (FAO, 2011). The trade value of wheat in the same year was about 95.2 billion dollar, which was about 1.6 times bigger than maize trading and > 50 times bigger than paddy rice trading values (FAO, 2011). Most of the wheat growing areas of the world experience environmental stresses like drought (water stress), high temperature (heat stress), cold, and salinity. Among them, drought and high temperature are two important environmental factors that adversely affect performance and yield of wheat crop (Prasad et al., 2008c; Rang et al., 2011). Recent reports show that due to global warming, there will be more frequent hot days and nights and unreliable precipitation pattern in future (Meehl and Tebaldi, 2004; IPCC, 2007). These abiotic stresses, at any time of crop development, decrease leaf chlorophyll and photosynthesis, and hasten senescence (Gibson and Paulsen, 1999; Yang et al., 2001; Altenbach et al., 2003; Dulai et al., 2006). High temperature and drought stress during vegetative stages of crop development decrease leaf area, number of tillers, plant height, and biomass (Zhong-hu and Rajaram, 1993; Hafid et al., 1998; Nouri et al., 2011).

In wheat, reproductive stages of crop development are the most vulnerable stage to high temperature and drought stress (Shpiler and Blum, 1986). High temperature at GS2 stage (double ridge to anthesis) adversely affects spikelet formation (Shpiler and Blum, 1986; Wollenweber et al., 2003). High temperature stress at meiosis decreases grain number spike$^{-1}$ by inducing ovule and pollen sterility and anther indehiscence (Saini and Aspinall, 1982; Prasad et al., 2006b; Prasad et al., 2008a; Prasad et al., 2008b). At anthesis, high temperature stress decreases the

grain number (Stone and Nicolas, 1994; Yang et al., 2002; Prasad et al., 2008b) by adversely affecting ovary development, pollen germination, and pollen tube growth (Saini et al., 1983; Prasad et al., 2011). During the grain filling period (a period from grain set to physiological maturity) high temperature decreases leaf chlorophyll content and accelerates senescence (Yang et al., 2002; Zhao et al., 2007) leading to a shorter grain filling duration with an ultimate decrease in individual grain weight and yield (Gibson and Paulsen, 1999; Altenbach et al., 2003). The increase in grain filling rate under high temperature cannot compensate the decrease in grain filling duration (Prasad et al., 2006a; Prasad et al., 2006b; Prasad et al., 2008a).

Similarly, drought stress at meiosis may reduce grain set by 35 to 50% in wheat (Saini and Aspinall, 1981; Dorion et al., 1996). At anthesis, drought stress decreases grain set in wheat by inducing pollen sterility (Saini and Westgate, 2000; Ji et al., 2010). Loss of contact between young pollen grains and tapetum, degeneration of anther filament, and/or decreased starch accumulation in anthers and pollens might be the reasons for pollen abortion (Saini et al., 1984; Lalonde et al., 1997; Ji et al., 2010). Drought at the grain filling period also decreases individual grain weight, and the decrease is often due to decrease in grain filling duration rather than decrease in grain filling rate (Saini and Westgate, 2000; Wardlaw and Willenbrink, 2000). Drought at an early stage of the grain filling period (anthesis to 14 d after anthesis) decreases the number of endosperm cells, and number of starch granules per cell, which are also the reasons for a decrease in grain size (Nicolas et al., 1985; Fábián et al., 2011).

High temperature and drought stress often occur at anthesis causing greater loss of wheat yield. These two abiotic stresses frequently occur simultaneously in dry land wheat areas, such as Mid Western Region of the USA, causing yield loss (Lott et al., 2011). The simultaneous effects of these two stress on crop performance, and yield may be quite different than the individual

stress, but there are limited studies on this aspect and need attentions (Rizhsky, 2002; Mittler, 2006). Nicolas et al.(1984) reported a higher decline in wheat yield when high temperature and drought stresses were applied simultaneously at an early and late period of grain development stage (cell division) as compared to the independent stress. Shah and Paulsen (2003) also reported the similar additive interaction between high temperature and drought stress for individual grain weight, when spring wheat (cv. Len) was subjected to a combination of high temperature (35/30°C) and drought stress at 7 d after anthesis. However, the interaction effect of high temperature and drought on grain dry weight was not additive, when a chronic heat stress (27/22°C) and drought was simultaneously applied at anthesis on spring wheat (cv. Len) (Wardlaw, 2002).

Hexaploid wheat (*Triticum aestivum* L., genome AABBDD) evolved from rare hybridization between the tetraploid wheat (*Triticum turgidum* L., AABB), and wild wheat relatives (*A. tauschii* Coss., DD) that occurred fairly recently (about 8000 years ago) at farmers' field in the West Caspian region of Iran (Gill et al., 2006). Thus this crop has a narrow genetic base. Thus, in order to increase genetic variability in wheat different cultivars of durum wheat (*Triticum turgidum* L.) have been hybridized with several *A. tauschii* accessions; and the plants, thus produced, are termed synthetic wheats (Mujeeb-kazi, 2003; Gill et al., 2006). These two species are good sources of biotic and abiotic stress tolerant genes (Molnár et al., 2005; Gill et al., 2006, refer Table III). Synthetic hexaploid wheat genotypes, thus produced, have been identified for high temperature or drought tolerance (Yang et al., 2002; Trethowan and Mujeeb-Kazi, 2008; Kurahashi et al., 2009). However, there are no reports on combined effects of high temperature and drought at reproductive stage of crop development on synthetic wheats. Therefore, this study was conducted under controlled environmental conditions with objectives

of (a) quantifying independent and combined effects of drought and high temperature on synthetic and spring wheats, and (b) determining if responses varied among the genotypes.

## Materials and methods

### Plant materials

Four synthetic hexaploid wheats, ALTAR 84 / *A. tauschii* (WX 193) [TA 4152-4], ALTAR 84 / AO'S' [TA 4049], GAN / *A. tauschii* (WX 897) [TA 4152-73], GR'S / BOY'S' [TA 4047], and two spring wheat cultivars, 'Halberd' and 'Dharwar Dry' as standard checks, were used in this experiment. Genotypes ALTAR 84 / AO'S' and GR'S / BOY'S' were selected on the basis of their relative performance for SPAD value, grain filling days, and grain yield at high temperature of 30/25°C in previous study (Yang et al., 2002). And, genotypes GAN / *A. tauschii* (WX 897) and ALTAR 84 / *A. tauschii* (WX 193) were selected on the basis of their relative yield performance at reduced field moisture condition (Villareal et al., 1998). 'Dharwar Dry' is a drought tolerant spring wheat cultivar (Kirigwi et al., 2007) and 'Halberd' is a relatively high temperature tolerant cultivar (Hays et al., 2007). Seeds of all these genetic materials were obtained from the Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Kansas State University.

### Experimental and treatment conditions

This research was conducted in May 2009, using the facility at the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, Kansas, USA. Three seeds of each synthetic and spring wheat were sown in 1.6 L squared shaped plastic pots of dimensions 14 cm (height) × 50 cm (top perimeter) × 36 cm (bottom perimeter) filled with a mixture of Metro Mix 300 potting soil (Hummert International, Topeka, Kansas, USA) and 10 g of

controlled release fertilizer (Osmocote Plus, N:$P_2O_5$:$K_2O$ = 15:9:12; Scotts, Marysville, OH, USA). Plants were grown in three growth chambers (three replications) maintained at 21/15°C (day/night temperature), 18 h photoperiod, and 85% relative humidity. At 21 d after seeding, one seedling from each pot was removed, and pesticide Marathon 1% G (a. i.: Imidacloprid,1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) was applied to avoid infestation of sucking insect pests. There were eight pots of each genotype in every growth chamber. The plants were grown at full irrigation (100% pot capacity) from sowing to anthesis (Feekes 10.5.1 stage). Miracle Gro water soluble fertilizer (N: $P_2O_5$:$K_2O$ = 24:8:16; Scotts Miracle-Gro Products, Inc., Marysville, OH, USA) was added in irrigation water (according to manufacturer instructions) once every 7 d until anthesis. Thereafter, for 16 d, one-fourth of pots (two) were exposed to drought stress: optimum temperature of 21/15°C and withhold irrigation; one-fourth to high temperature stress: high temperature of 36/30°C and irrigation; one-fourth to combined stress: high temperature and drought stress. The remaining one-fourth of plants was kept at an optimum condition: optimum temperature and irrigation. For high temperature treatments pots were moved to a chamber at 36/30°C for the duration of the stress. Similar photoperiod and relative humidity conditions were used to expose plants to drought, high temperature and combined stress treatments. After stress treatment, plants were moved back to the original chamber. The 100% pot capacity of each pot was estimated at the beginning of the experiment. Fifteen pots of Metro Mix (390 g $pot^{-1}$) were fully irrigated and allowed to drain for 48 h, and pots were weighed. The Metro Mix was then oven dried for 10 d at 65°C, and dry weight was recorded. The pot capacity was estimated as:

$$Pot\ capacity = \frac{(Wet\ Metro\ Mix\ weight - Dry\ Metro\ Mix\ Weight)}{(Dry\ Metro\ Mix\ Weight)} \times 100$$

At the beginning of treatment, two extra pots of each genotype from three chambers were harvested to include weight of plants for calculating the amount of water needed to add for attaining 100% pot capacity. In all growth chambers, the canopy level photosynthetically active radiation (PAR) of 400 $\mu mol^{-1}$ $m^{-2}$ $s^{-1}$ was provided by cool white fluorescent lamps (Philips Lighting Co., Somerset, NJ, USA). PAR was monitored every month with Fieldscout Light Sensor (Spectrum Technologies, Inc., Plainfield, IL, USA) and air temperature was monitored in every 20 minutes with Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA, USA). Every alternate day, plants in each growth chamber were randomly moved to avoid any positional effect within the chamber.

At anthesis, one plant in each pot was randomly selected, and the main stem was tagged. Physiological measurements were taken from one pot, but yield parameters were collected from tagged plants in both pots.

## Data collection

### Leaf chlorophyll

Leaf chlorophyll was measured every alternate day from the start of treatment for 16 d. Leaf chlorophyll was measured with a self-calibrating chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL, USA) from the fully expanded flag leaf of the tagged main stem. Each time, data were taken thrice from the middle portion of the leaf, and the readings were averaged.

### Plant height, tiller number, and biomass

At maturity, plant height was measured from the plant base to the tip of the main stem spike excluding awns. Tiller number plant$^{-1}$ was counted and the number of spike bearing tillers was recorded to differentiate between fertile (with spikes) and non-fertile (without spike) tillers. Vegetative biomass plant$^{-1}$ was recorded after plant material (without root and spikes) was dried in an oven (65°C) for 10 d.

### Spikelet number, grain number, and individual grain weight

At harvest, the number of spikelets spike$^{-1}$ was counted from the spikes of each tagged main stem. The spikes were dried in an incubator at 45°C for 4 d. Dried spikes were hand-threshed and grains were counted and weighed. Individual grain weight was then calculated by dividing grain weight spike$^{-1}$ by the number of grains spike$^{-1}$.

### Grain Yield

Grain yield plant$^{-1}$ was recorded by harvesting all spikes in tagged plants followed by drying, threshing and weighing of grains.

## Statistical analyses

The experimental design was a split-split-plot with temperature randomly assigned to main plots, drought to sub-plots and genotypes to sub-sub-plots. There were three replications. The statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The PROC MIXED procedures were used with block, temperature, drought, and genotypes as class variables. Block, block × drought, block × temperature, and block × drought × temperature were treated as random effects and all other variables as fixed effects (Littell et al., 2006). 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).

## Results

The *P*-values for growth, physiological, and yield traits obtained with SAS PROC MIXED are presented in Supplementary Table 4.1. The independent effects of temperature, drought, and genotypes; and interaction effects of drought × temperature, and drought × temperature × genotypes were significant for flag leaf chlorophyll, grain number, individual grain weight, and yield. Temperature, drought, and combined stress had no effect on plant height, tiller number, spikelet number, and biomass ($P > 0.05$); but genotypes were different for these traits ($P < 0.001$, Table 4.1).

### Flag leaf chlorophyll

High temperature, drought, and combined stresses significantly decreased flag leaf chlorophyll and genotypes responded differentially to stresses. At optimum condition, leaf chlorophyll ranged from 45 SPAD units for GAN / *A. tauschii* (WX 897) to about 55 SPAD units for 'Halberd' (Fig. 4.1). High temperature decreased leaf chlorophyll by 32% in ALTAR 84 / *A. tauschii* (WX 193) and GAN / *A. tauschii* (WX 897). Effect of high temperature on leaf chlorophyll was lower in ALTAR 84 / AO'S' and 'Dharwar Dry' (~ 22% decline from the optimum condition). Drought decreased leaf chlorophyll of ALTAR 84 / *A. tauschii* (WX 193) and 'Halberd' by about 14% and 'Dharwar Dry' had the lowest decline in leaf chlorophyll (4%) as a consequence of drought. Combined effect of high temperature + drought decreased leaf chlorophyll by 62% in GAN / *A. tauschii* (WX 897 followed by GR'S' / BOY'S' (54%). The combined stress had minimum effect on ALTAR 84 / AO'S' for leaf chlorophyll (37% decline).

## Plant height, tiller number, spikelet number, and biomass

Plant height ranged from 86 cm for ALTAR 84 / AO'S' to 102 cm for ALTAR 84 / *A. tauschii* (WX 193) (Table 4.1). 'Halberd' and 'Dharwar Dry' had the highest number of tillers (5.6 plant$^{-1}$), and ALTAR 84 / *A. tauschii* (WX 193) had the lowest number of tillers (3.8 plant$^{-1}$). The fertile tiller number plant$^{-1}$ ranged from 2.2 in GAN / *A. tauschii* (WX 897) to 5.4 in 'Dharwar Dry. Genotype ALTAR 84 / *A. tauschii* (WX 193) had the highest number of spikelet spike$^{-1}$ (23.6) followed by cultivar 'Dharwar Dry' (20.7); and the GAN / *A. tauschii* (WX 897) and ALTAR 84 / AO'S' had the lowest number of spikelet spike$^{-1}$ (17.2–17.6). 'Dharwar Dry' had the highest amount of vegetative biomass 7.7 g plant$^{-1}$ and all the other genotypes had about ≤ 4 g of vegetative biomass plant$^{-1}$.

## Grain number and individual grain weight

High temperature, drought, and combined stresses significantly decreased grain number spike$^{-1}$ by 47%, 16% and 70%, respctively; and genotypes behaved differentially for these stresses (Fig. 4.2). At the optimum condition, grain number ranged from about 27 spike$^{-1}$ in ALTAR 84 / AO'S' and GAN / *A. tauschii* (WX 897), to 51 spike$^{-1}$ in ALTAR 84 / *A. tauschii* (WX 193). High temperature decreased grain number spike$^{-1}$ of ALTAR 84 / *A. tauschii* (WX 193) by 62% followed by GAN / *A. tauschii* (WX 897) (58% decline over the optimum condition). Effect of high temperature stress was lowest in 'Halberd', 'Dharwar Dry' and GR'S' / BOY'S' for grain number spike$^{-1}$ (36–39% decline). Drought decreased grain number spike$^{-1}$ of GAN / *A. tauschii* (WX 897) by 28% followed by ALTAR 84 / AO'S' (25%), and 'Dharwar Dry' had the lowest decline in grain number spike$^{-1}$ (5%). Combined stress of high temperature + drought had the highest effect on GAN / *A. tauschii* (WX 897) (83% decline) for grain number spike$^{-1}$ followed by ALTAR 84 / *A. tauschii* and GR'S' / BOY'S' (72–75% decline). The minimum effect of

combined stress was on cultivar ‘Halberd’ and genotype ALTAR 84 / AO’S’ for grain number spike$^{-1}$ (62–64% decline over the optimum condition).

High temperature, drought, and combined stresses significantly decreased individual grain weight by 66%, 41% and 83%, respectively; and genotypes behaved differentially for these stresses (Fig. 4.3A). At the optimum condition, cultivar ‘Dharwar Dry’ had the highest individual grain weight (52 mg) followed by genotype GAN / *A. tauschii* (WX 897) (49 mg). Individual grain weight of other genotypes at the optimum condition was about 41 to 44 mg. High temperature decreased individual grain weight of GAN / *A. tauschii* (WX 897) by 94% followed by ALTAR 84 / *A. tauschii* (WX 193) (87% ). Effect of high temperature was the lowest in cultivars ‘Halberd and ‘Dharwar Dry’ (~ 47% decline) followed by ALTAR 84 / AO’S’ (50% decline). Drought decreased individual grain weight of ALTAR 84 / *A. tauschii* (WX 193) by 61%, followed by ‘Halberd’ and ‘Dharwar Dry’ (42–46%). All other genotypes had a decline of about 31–34% in individual grain weight under drought stress. Combined stress of high temperature + drought decreased individual grain weight by 94% in GAN / *A. tauschii* (WX 897) followed by 91% in ALTAR 84 / *A. tauschii* (WX 193) and GR’S’ / BOY’S’ (84% decline). All other genotypes had a decline of about 77% in individual grain weight over the optimum condition.

## Grain yield

High temperature, drought, and combined stress significantly decreased grain yield plant$^{-1}$ by 81%, 69% and 92%, respectively; and genotypes behaved differentially for these stresses (Fig. 4.3B). At the optimum condition, cultivar ‘Dharwar Dry’ had the highest amount of grain yield (9 g plant$^{-1}$) followed by ALTAR 84 / *A. tauschii* (WX 193) and ‘Halberd’ (~ 6 g plant$^{-1}$), and the rest of the genotypes had 3–4 g of grain yield plant$^{-1}$. High temperature stress decreased grain

yield plant$^{-1}$ of ALTAR 84 / *A. tauschii* (WX 193) and GAN / *A. tauschii* (WX 897) by about 96% followed by GR'S' / BOY'S' (89% decline over the optimum condition). ALTAR 84 / AO'S' and the 'Halberd' had the lowest decline in grain yield under high temperature stress (66–68%). Drought decreased grain yield plant$^{-1}$ of ALTAR 84 / *A. tauschii* (WX 193), 'Halberd, and 'Dharwar Dry' by 73–76%. The decline in grain yield was about 55–57% in GR'S' / BOY'S' and ALTAR 84 / AO'S'. Combined stress of high temperature + drought decreased grain yield plant$^{-1}$ by 98% in ALTAR 84 / *A. tauschii* (WX 193), GAN / *A. tauschii* (WX 897) and GR'S' / BOY'S'. 'Halberd had a decline of 94% in grain yield followed by 'Dharwar Dry' (89%). Effect of combined stress on grain yield plant$^{-1}$ was minimum in genotype ALTAR 84 / AO'S' (81% decline over the optimum condition).

## Discussion

High temperature and drought stress often occur together at reproductive stage of crop development causing significant yield losses in wheat, but their effects usually are studied individually, and limited knowledge is available on the combined effect of drought and high temperature. Synthetic hexaploid wheats have been developed by crossing *Triticum turgidum* L. with *A. tauschii* Coss. to increase genetic variability in wheat for biotic and abiotic stress tolerance (Trethowan and Mujeeb-Kazi, 2008). They have been studied separately for high temperature stress tolerance at 10 d after anthesis (Yang et al., 2002), and drought stress tolerance at seedling stage (Kurahashi et al., 2009). In this study, we have investigated the combined effects of high temperature and drought on synthetic hexaploid wheat at flowering stage of crop development. Combined effect of high temperature + drought at anthesis adversely affected grain number spike$^{-1}$ and individual grain weight of synthetic hexaploid and spring

wheats resulting into a substantial decrease in grain yield plant$^{-1}$. Synthetic hexaploid wheats differed in their response to physiological and yield components (Fig. 4.1, 4.2 and 4.3).

In this study, combined effect of high temperature + drought stress decreased leaf chlorophyll by greater magnitude than by the high temperature or drought stress alone, and genotypic variation was observed in synthetic hexaploid wheats for this trait. Wang et. al (2010) reported similar effects of these stresses in transgenic and wild type wheat seedlings. High temperature decreases leaf chlorophyll by damaging thylakoid membrane (Al-Khatib and Paulsen, 1984; Ristic et al., 2007) and/or lipid peroxidation of chloroplast membranes (Djanaguiraman et al., 2010). Drought after anthesis activates rapid reallocation of metabolites from leaves and stems to developing grain in wheat resulting in quick loss of chlorophyll and senescence (Yang et al., 2001). Under severe drought, reactive oxygen species are produced in higher level, which also accelerates leaf chlorosis (Zhang and Kirkham, 1994).

Combined effect of high temperature + drought stress decreased grain number spike$^{-1}$ of all genotypes with greater magnitude than under drought or high temperature alone, and the decrease at the high temperature was at least two times higher than at the drought stress (Fig. 4.2). Among the synthetic hexaploid genotypes, 'ALTAR 84 / AO'S' had the lowest decline in the grain number, and the decline was at par with that in Dharwar Dry' and 'Halberd'. Drought decreases the grain number in wheat by disintegrating contact between microspore and tapetum, degenerating filament and disorienting reproductive cells resulting in pollen sterility (Saini et al., 1984; Lalonde et al., 1997). High temperature decreases grain number by adversely affecting pollen production, pollen and ovule viability, pollen tube growth, and fertilization (Saini et al., 1983; Saini et al., 1984; Prasad et al., 2006b; Prasad et al., 2011). The differential magnitude of high temperature and drought's effects on the grain number in this study might be because of the

way the treatments were imposed. Drought was imposed by withholding water and Metro Mix was used as a soil medium. Thus, several days might have lapsed before plants actually experienced severe drought responsible for damaging and malfunctioning of male reproductive organ. On the other hand, plants had severe high temperature (36/30°C) stress once they were transferred to chamber with high temperature.

Individual grain weight is one of the major components of grain yield in cereal crops. High temperature, drought, and combined stress decreased individual grain weight of all genotypes (Fig. 4.3A). The effect of high temperature on individual grain weight was higher than the effect of drought. Drought decreases individual grain weight by decreasing grain filling duration rather than grain filling rate (Wardlaw and Willenbrink, 2000). High temperature also decreases individual grain weight by decreasing grain filling duration and grain filling rate (Al-Khatib and Paulsen, 1984; Gibson and Paulsen, 1999). However, there are reports indicating an increase in grain filling rate under high temperature stress, but this increase was not enough to compensate for the loss due to decreased grain filling duration (Prasad et al., 2006a; Prasad et al., 2006b; Prasad et al., 2008a). Effect of combined stress of high temperature + drought on individual grain weight was higher than the high temperature or drought stress but lower than the sum of these stresses (Fig. 4.3A) indicating that the interaction effect was hypo-additive. Both high temperature and drought stress affect individual grain weight by a common mechanism, i.e. decreasing grain filling duration, which might be the reason for hypo-additive interaction between them. Hypo-additive interaction between high temperature and drought for individual grain weight was reported (Wardlaw, 2002). The combined effect of high temperature and drought was of different magnitude among the genotypes, and ALTAR 84 / AO'S', 'Dharwar Dry', and 'Halberd' can be selected as tolerant genotypes for individual grain weight.

High temperature decreased grain yield plant$^{-1}$ by 68–96%, which was higher than the decrease under the drought stress (55–76%) (Fig. 4.3B). The decrease in the grain yield under combined stress was higher than the individual stress, high temperature or drought; but the decrease was of smaller magnitude than the sum of individual stresses. This suggests that high temperature × drought interaction effect for grain yield was hypo-additive in nature. This finding supports the hypothesis of Mittler (Mittler, 2006), presented in the form of "stress matrix", that the high temperature and drought belonged to the category of potential negative interaction". Synthetic hexaploid wheats have genotypic variability for yield under the combined stress, and combined effect of high temperature + drought for yield was lowest in ALTAR 84 / AO'S'. This synthetic hexaploid wheat genotype also had a minimal effect of combined stress on leaf chlorophyll, and effect on grain number and individual grain weight were as smaller as in "Dharwar Dry' and 'Halberd'.

In conclusion, combined effects of high temperature + drought stress at anthesis were more detrimental than the separate effect of each stress, and the interaction effect was hypo-additive. The study showed that synthetic hexaploid wheats varied in their response to combined effect of drought and high temperature stress, and genotype ALTAR 84 / AO'S' was the more tolerant. However, further investigations are needed to confirm these interaction effects in more controlled and measurable drought stress conditions, where leaf water potential and fraction of transpirable soil water are taken into consideration.

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## Figures and Tables

**Figure 4.1.** Effects of high temperature, drought, and combined stress on flag leaf chlorophyll (SPAD value) of four synthetic hexaploid wheat genotypes and two spring wheat cultivars. The interaction effects was significant at $P < 0.01$. For each genotype, a percent decline from optimum condition is indicated. Vertical lines on bars indicate standard errors of mean.

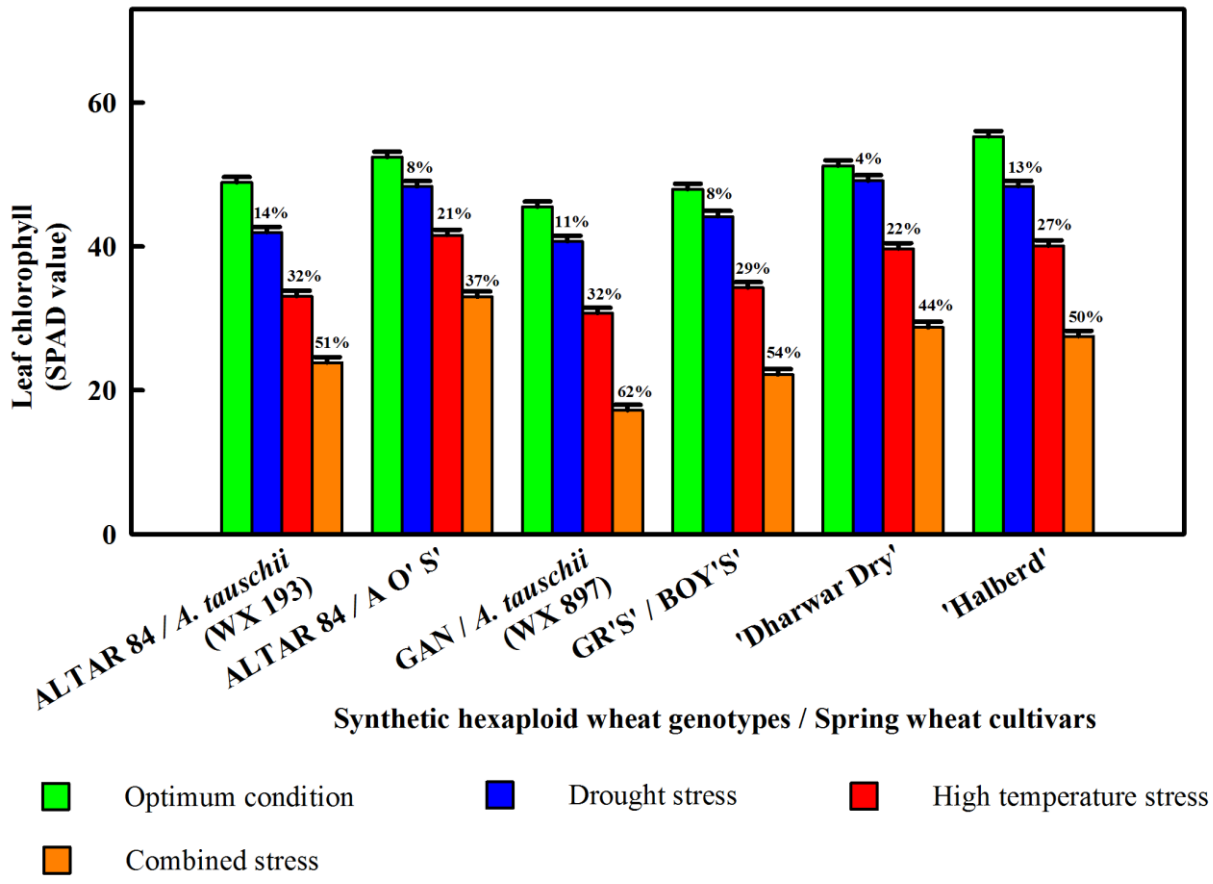

**Figure 4.2.** Effect of high temperature, drought, and combined stress on grain number spike$^{-1}$ of four synthetic hexaploid wheats and two spring wheat cultivars. The interaction effects was significant at $P < 0.001$. For each genotype, a percent decline from optimum condition is indicated. Vertical lines on bars indicate standard errors of mean.

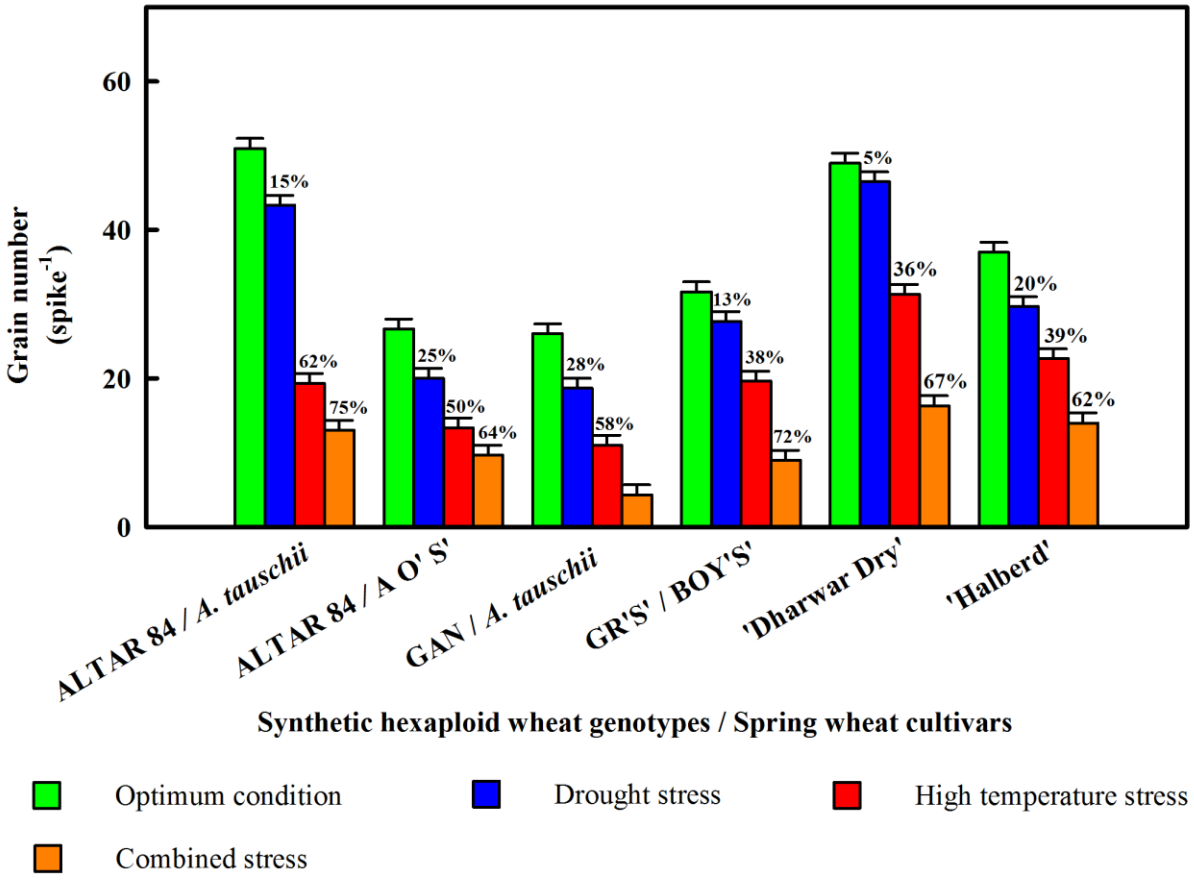

**Figure 4.3.** Effect of high temperature, drought, and combined stress on (A) individual grain weight, and (B) grain yield plant$^{-1}$ of four synthetic hexaploid wheats and two spring wheat cultivars. For both traits, the interaction effects was significant at $P < 0.001$. For each genotype, a percent decline from optimum condition is indicated. Vertical lines on bars indicate standard errors of mean.

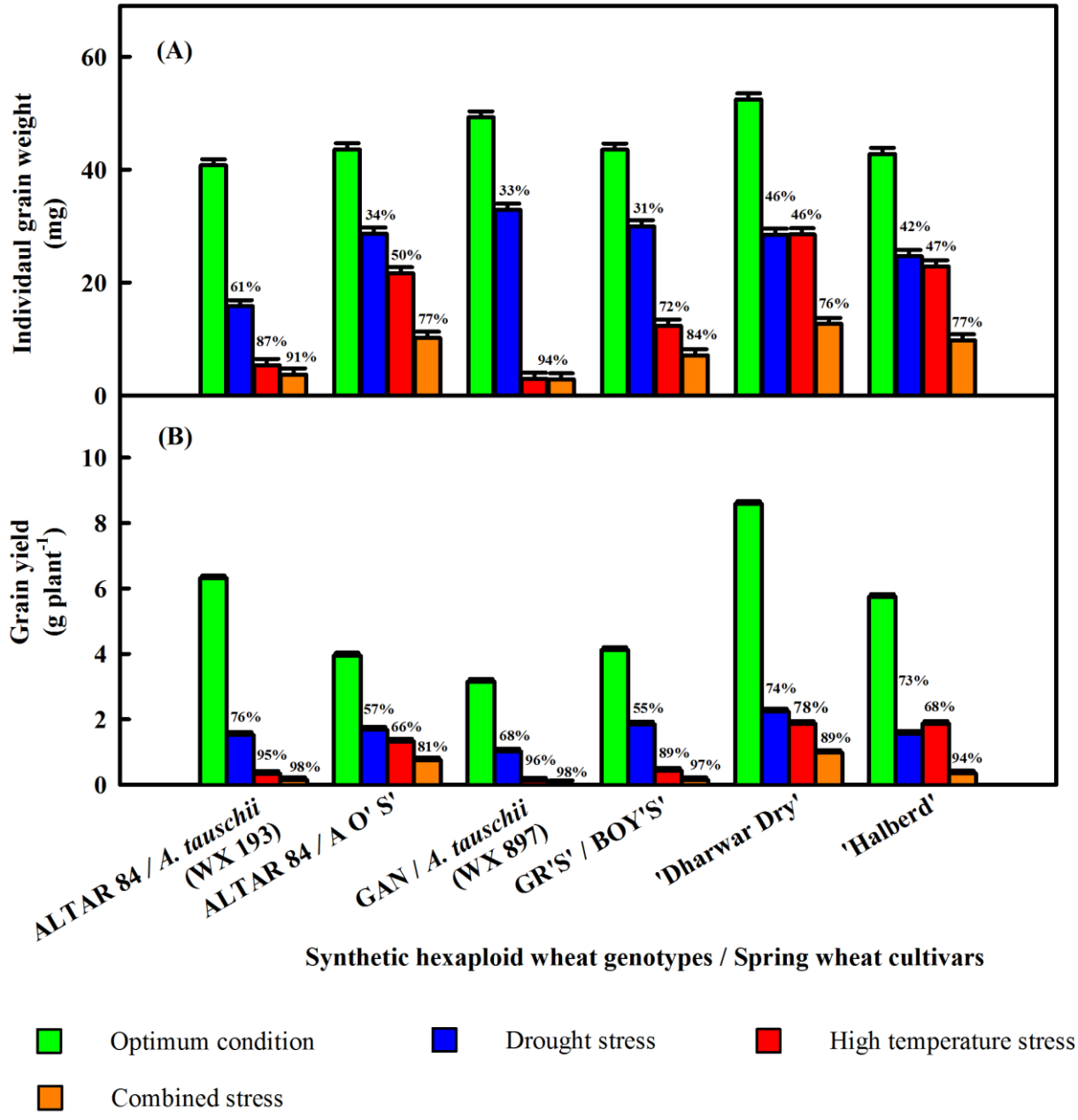

**Table 4.1.**Mean growth and morphological parameters of four synthetic hexaploid wheat and two spring wheat cultivars.

| Species | Plant height (cm) | Tiller number (plant$^{-1}$) | Fertile tiller number (plant$^{-1}$) | Spikelet number (spike$^{-1}$) | Vegetative biomass (g plant$^{-1}$) |
|---|---|---|---|---|---|
| ALTAR 84 / *A. tauschii* (WX 193) | 102$^{a}$ | 3.8$^{c}$ | 3.5$^{b}$ | 23.6$^{a}$ | 4.5$^{b}$ |
| ALTAR 84 / A O' S' | 86$^{c}$ | 5.2$^{ab}$ | 3.4$^{b}$ | 17.6$^{cd}$ | 3.4$^{b}$ |
| GAN / *A. tauschii* (WX 897) | 100$^{ab}$ | 5.2$^{ab}$ | 2.2$^{c}$ | 17.2$^{d}$ | 3.5$^{b}$ |
| GR'S' / BOY'S' | 90$^{bc}$ | 4.5$^{bc}$ | 2.8$^{bc}$ | 18.9$^{c}$ | 3.4$^{b}$ |
| 'Dharwar Dry' | 97$^{abc}$ | 5.6$^{a}$ | 5.4$^{a}$ | 24.3$^{a}$ | 7.7$^{a}$ |
| 'Halberd' | 93$^{abc}$ | 5.6$^{a}$ | 3.5$^{b}$ | 20.7$^{b}$ | 3.8$^{b}$ |

Tukey-Kramer grouping (Little et al., 2006) of the synthetic hexaploid and spring wheats using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$.

**Supplementary Table 4.1.** *P*-value and significance of the effects of temperature, drought, genotype, and their interactions on physiological, growth and yield traits of four synthetic hexaploid wheat and two spring wheat cultivars.

| Effects | Temperature (T) | Drought (D) | Genotype (G) | T × D | T × D × G |
|---|---|---|---|---|---|
| Traits | | | *P*-values | | |
| Leaf chlorophyll (SPAD units) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $0.002^{**}$ |
| Plant height (cm) | $0.575^{NS}$ | $0.757^{NS}$ | $< 0.001^{***}$ | $0.519^{NS}$ | $0.144^{NS}$ |
| Tiller number (plant$^{-1}$) | $0.121^{NS}$ | $0.400^{NS}$ | $< 0.001^{***}$ | $0.079^{NS}$ | $0.646^{NS}$ |
| Fertile tiller number (plant$^{-1}$) | $0.244^{NS}$ | $0.286^{NS}$ | $< 0.001^{***}$ | $0.520^{NS}$ | $0.463^{NS}$ |
| Vegetative biomass (g plant$^{-1}$) | $0.408^{NS}$ | $0.259^{NS}$ | $< 0.001^{***}$ | $0.314^{NS}$ | $0.081^{NS}$ |
| Spikelet number (spike$^{-1}$) | $0.947^{NS}$ | $0.167^{NS}$ | $< 0.001^{***}$ | $0.914^{NS}$ | $0.598^{NS}$ |
| Grain number (spike$^{-1}$) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $0.022^{*}$ | $0.001^{**}$ |
| Individual grain weight (mg) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ |
| Grain yield (g plant$^{-1}$) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ |

$^{NS}$ nonsignificant. *, **, *** significant at $P < 0.05$, $< 0.01$ and $< 0.001$ respectively.

# Chapter V -Effect of drought and high temperature at the late grain filling period of synthetic wheat genotypes


## Abstract

Drought and high temperature often simultaneously occur in wheat growing regions of the world causing significant yield losses. The objectives of this study were to (a) quantify independent and combined effects of drought and high temperature on synthetic hexaploid and spring wheat genotypes at the late grain filling period, and (b) determine if responses to stress varied among genotypes of synthetic and spring wheat. Four synthetic hexaploid and two spring wheats were grown from seeding to 21 d after anthesis with full irrigation and optimum temperature of 21/15°C day/night. Thereafter, treatments were imposed as (a) optimum condition: irrigation + 21/15°C, (b) drought stress: withhold irrigation + 21/15°C, (c) high temperature stress: irrigation + 36/30°C, (d) combined stress: withhold irrigation + 36/30°C. Stresses were imposed for 16 d. Drought, high temperature, and combined stress decreased leaf chlorophyll, chlorophyll fluorescence (Fv/Fm), and grain yield in an increasing magnitude. Drought stress decreased individual grain weight and grain yield plant$^{-1}$ by 26%. Corresponding decreases due to high temperature were 29 and 57%. Combined stress exacerbated the effect and declined grain weight and yield by 54% and 50%. Overall, the interaction was hypo-additive, i.e., combined effect was lower than the sum of individual effects. The responses of genotypes for stresses were different. Genotype ALTAR 84 / *A. tauschii* (WX 193) produced significantly higher grain yield under all stress conditions in terms of the absolute and proportionate term as compared to other genotypes. The results suggested that, at the late grain filling period, combined effect of drought and high temperature stress were more detrimental, and synthetic wheat genotypes varied in their response to independent and combine stress.

## Introduction

Drought (water stress) and high temperature (heat stress) are two important environmental factors that adversely affect performance and grain yield of field grown crops (Prasad et al., 2008c; Rang et al., 2011). These abiotic stresses, at any time of crop development, decrease leaf chlorophyll and photosynthesis, and hasten senescence (Gibson and Paulsen, 1999; Yang et al., 2001; Altenbach et al., 2003; Dulai et al., 2006). High temperature and drought stress, during vegetative stages of crop development, decrease plant height, leaf area, number of tillers, and biomass (Zhong-hu and Rajaram, 1993; Hafid et al., 1998; Nouri et al., 2011).

Reproductive stages of crop development in cereal crops, including wheat (*Triticum aestivum* L.), are more sensitive to high temperature and drought stress than vegetative stages (Shpiler and Blum, 1986). High temperature stress at meiosis decreases grain number spike$^{-1}$ by inducing ovule and pollen sterility and anther indehiscence (Saini and Aspinall, 1982; Prasad et al., 2006; Prasad et al., 2008a; Prasad et al., 2008b). High temperature stress during anthesis decreases grain number spike$^{-1}$ (Stone and Nicolas, 1994; Yang et al., 2002; Prasad et al., 2008b) by adversely affecting ovary development, pollen germination, pollen tube growth, and seed-set (Saini et al., 1983; Prasad et al., 2011). Similarly, drought stress at anthesis decreases seed set in wheat by inducing pollen sterility (Saini and Westgate, 2000; Ji et al., 2010). Loss of contact between young pollen grains and the tapetum, degeneration of the anther filament, and/or decreased starch accumulation in anthers and pollen might be reasons for pollen abortion (Saini et al., 1984; Lalonde et al., 1997; Ji et al., 2010).

The grain filling period is also highly sensitive to high temperature and drought stress. High temperature at the grain filling stage decreases leaf chlorophyll content and accelerates senescence (Yang et al., 2002; Zhao et al., 2007) leading to a shorter grain filling duration and/or

lower grain filling rate with the ultimate decrease in individual grain weight and yield (Gibson and Paulsen, 1999; Altenbach et al., 2003). Up to 15% decrease in individual grain weight was reported by Stone and Nicolas (1998) when a wheat crop was subjected to a day of high temperature (40/21°C day/night) during the grain filling period as compared to the control (21/16°C day/night). Drought at the grain filling period also decreases individual grain weight but the decrease is often due to decrease in grain filling duration rather than decrease in grain filling rate (Saini and Westgate, 2000; Wardlaw and Willenbrink, 2000). Drought at an early stage of the grain filling period decreases the number of endosperm cells and number of starch granules per cell, which are also the reasons for a decrease in grain size (Nicolas et al., 1985; Fábián et al., 2011).

High temperature and drought stress often occur during the grain filling period of a wheat crop development stage causing severe yield loss in most of the wheat growing areas of the world, including the Great Plains of the USA (Boyer, 1982; Altenbach et al., 2003; Lott et al., 2011). Further, these two abiotic stresses frequently occur simultaneously in dry-land wheat areas, such as mid western regions of USA, causing yield loss (Lott et al., 2011). The simultaneous effects of these two stresses on crop performance, and yield may be quite different than the individual stress, but there are limited studies on this topic (Rizhsky, 2002; Mittler, 2006). Nicolas et al. (1984) observed a higher decline in wheat yield when high temperature and drought stress were applied simultaneously at the early and late period of the grain development stage (cell division) as compared to either of single stress. Similar additive interaction between high temperature and drought stress was reported by Shah and Paulsen (2003) for individual grain weight and grain yield of spring wheat (cv. Len) subjected to a combination of high temperature (35/30°C) and drought stress at 7 d after anthesis. On the contrary, the high

temperature × drought interaction effect on grain dry weight was not additive when a chronic heat stress (27/22°C) and drought was simultaneously applied at anthesis on spring wheat (cv. Len) (Wardlaw, 2002).

Wheat grain filling period can be divided into several stages, viz., 0-10, 10-20, 20-30, 30-35 d after anthesis, which in general corresponds to pre-milk stage, milking stage, soft dough stage, and hard dough stage respectively (Noda et al., 1994). Between 20-30 d after anthesis (late grain filling stage), the embryo differentiates further and becomes a fully developed miniature plant with its own reserve accumulations, mainly triacylglycerols and osmoprotectant proteins. At this period, the reserve accumulation in the endosperm shows the second peak, and endosperm will have a soft dough consistency (Noda et al., 1994). Therefore, late grain filling stage might also be a very important phase of grain development in wheat. However, there are limited reports on the combined and independent effects of high temperature and drought at late grain filling stage on wheat yield and yield traits.

Hexaploid wheat (*Triticum aestivum* L., AABBDD) evolved from rare hybridization between tetraploid wheat (*Triticum turgidum* L., AABB) and wild wheat (*A. tauschii* Coss., DD) that occurred about 8000 years ago at farmers' field in the West Caspian region of Iran (Gill et al., 2006). *Aegilops tauschii* Coss. and *Triticum turgidum* L. are good sources of biotic and abiotic stress tolerant genes (Molnár et al., 2005; Gill et al., 2006, refer Table III). Therefore, these two genotypes have been hybridized in vitro to produce synthetic wheats with stress tolerant genes (Mujeeb-kazi, 2003; Gill et al., 2006). Synthetic hexaploid wheat lines have been studied for high temperature and drought tolerance (Yang et al., 2002; Trethowan and Mujeeb-Kazi, 2008; Kurahashi et al., 2009). However, they have not been yet tested for tolerance to independent and combined effects of high temperature and drought at late grain filling stage.

Therefore, this study was conducted under controlled environmental conditions with the objectives to (a) quantify independent and combined effects of drought and high temperature on synthetic hexaploid and spring wheat genotypes at the late grain filling period, and (b) determine if responses to stress varied among genotypes of synthetic and spring wheat.

## Materials and methods

### Plant materials

Four synthetic hexaploid wheats, ALTAR 84 / *A. tauschii* (WX 193) [TA 4152-4], ALTAR 84 / AO'S' [TA 4049], GAN / *A. tauschii* (WX 897) [TA 4152-73], GR'S / BOY'S' [TA 4047], and two spring wheat cultivars, 'Halberd' and 'Dharwar Dry' as standard checks, were used in this experiment. Genotypes ALTAR 84 / AO'S' and GR'S / BOY'S' were selected on the basis of their relative performance for SPAD value, grain filling days, and grain yield at high temperature of 30/25°C in previous study (Yang et al., 2002). And, genotypes GAN / *A. tauschii* (WX 897) and ALTAR 84 / *A. tauschii* (WX 193) were selected on the basis of their relative yield performance at reduced field moisture condition (Villareal et al., 1998). 'Dharwar Dry' is a drought tolerant spring wheat cultivar (Kirigwi et al., 2007) and 'Halberd' is a relatively high temperature tolerant cultivar (Hays et al., 2007). Seeds of all these genetic materials were obtained from the Wheat Genetic and Genomic Resources Center, the Department of Plant Pathology, Kansas State University.

### Experimental and treatment conditions

This research was conducted in the summer of 2009 at the control environmental facility of the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, Kansas, USA. Three seeds of each synthetic and spring wheat were sown on June 18

2009, in 1.6 L squared shaped plastic pots of dimensions 14 cm (height) × 50 cm (top perimeter) × 36 cm (bottom perimeter) filled with a mixture of Metro Mix 300 potting soil (Hummert International, Topeka, Kansas) and 10 g of controlled release fertilizer (Osmocote Plus, $N:P_2O_5:K_2O$ = 15:9:12; Scotts, Marysville, OH, USA). Plants were grown in three growth chambers (three replications) maintained at 21/15°C (day/night temperature), 18 h photoperiod and relative humidity of 85%. About 21 d after seeding, one seedling from each pot was removed leaving two seedlings $pot^{-1}$, and at the same time Marathon 1% G (a. i.: Imidacloprid,1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) was applied to avoid infestation of sucking insect pests. There were eight pots of each entry in every growth chamber. The plants were grown at full irrigation (100% pot capacity) and optimum temperature of 21/15°C from sowing to 21 d after anthesis (Sep – Oct, 2009). Thereafter, one-fourth of pots (two) were exposed to drought stress: irrigation withhold + 21/15°C, one-fourth to high temperature stress: irrigation + 36/30°C, and one-fourth to combined stress: irrigation withhold + 36/30°C. The remaining one-fourth of plants was kept at the optimum condition: irrigation + 21/15°C. The stress period was of 16 d. For temperature treatments pots were moved to a chamber at 36/30°C for the duration of the stress. Photoperiod and relative humidity conditions similar to the control were used to expose plants to high temperature and combined stress treatments. After stress treatment, plants were moved back to the original chamber. The 100% pot capacity of each pot was estimated at the beginning of the experiment. Fifteen pots of Metro Mix (390 g $pot^{-1}$) were fully irrigated and allowed to drain for 48 h, and pots were weighed. The Metro Mix was then oven dried for 10 d at 65°C, and dry weight was recorded. The pot capacity was estimated as:

$$Pot\ capacity = \frac{(Wet\ Metro\ Mix\ weight - Dry\ Metro\ Mix\ Weight)}{(Dry\ Metro\ Mix\ Weight)} \times 100$$

At the beginning of treatments, two extra pots of each accession from three chambers were harvested to include weight of plants for calculating the amount of water needed to add for attaining pot capacity. In all growth chambers, the canopy level photosynthetically active radiation (PAR) of 400 $\mu mol^{-1}$ $m^{-2}$ $s^{-1}$ was provided by cool white fluorescent lamps (Philips Lighting Co., Somerset, NJ, USA). PAR was monitored every month with a Fieldscout Light Sensor (Spectrum Technologies, Inc., Plainfield, IL, USA) and air temperature was monitored every 20 minutes with a Stowaway Tidbit Temp Loggers (Onset Computer Corporation, Bourne, MA, USA). Every alternate day, plants in each growth chamber were randomly moved to avoid any positional effect within the chamber.

At heading, one plant in each pot was randomly selected, and the main stem was tagged. Physiological measurements were taken from one pot, but yield parameters were collected from tagged plants in both pots.

## Data collection

### Leaf chlorophyll, leaf temperature and maximum quantum yield of PS II

Leaf chlorophyll, leaf temperature, and maximum quantum yield of PS II were measured every alternate day from the start of treatment and continued for 16 d. Leaf chlorophyll was measured with a self-calibrating chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL, USA) from the fully expanded flag leaf on a tagged main stem. Each time, data were taken thrice from the middle portion of the leaf, and the readings were averaged. Before using the SPAD meter, images of flag leaves were captured with a FLIR BCAM SD thermal imaging camera (FLIR Systems Inc., Wilsonville, OR, USA). To determine flag leaf temperature, the images were processed with QuickReport 1.2 software (FLIR, 2009). After the SPAD reading,

maximum quantum yield of PS II (Fv/Fm) was recorded with a pulse modulated chlorophyll fluorometer (OS-30p, Opti-Science Inc., Hudson, NH, USA). Measurements were taken after the flag leaf was dark adapted for 1 h. The maximum quantum yield of PS II is the ratio of variable fluorescence (difference between maximum and minimum fluorescence (Fv) to maximum fluorescence (Fm), which decreases with stress (Roháček, 2002).

**Phenology**

The date of complete heading, anthesis (Feekes 10.5.1 stage), 21 d after anthesis and physiological maturity were noted on each genotype. Days to anthesis were calculated from sowing to appearance of the first anther on the tagged main stem spike. Days to physiological maturity were calculated from sowing to the day when the peduncle of the spike on the tagged main stem became completely yellow.

**Plant height, tiller number, and biomass**

At maturity, plant height was measured from the base of the plant to the tip of main stem spike excluding awns. Tiller number plant$^{-1}$ was counted and the number of spike bearing tillers was recorded to differentiate between fertile (with spikes) and non-fertile (without spike) tillers. Vegetative biomass plant$^{-1}$ was recorded after plant material without root, and spikes were dried in an oven (65°C) for 10 d.

**Spikelet number, grain number, and grain weight**

At harvest, the number of spikelets spike$^{-1}$ was counted from the spikes of each tagged main stem. The spikes were dried in an incubator at 45°C for 4 d. Dried spikes were hand threshed and grains were counted and weighed.

### Individual grain weight and yield

Individual grain weight was calculated by dividing grain weight spike$^{-1}$ by the number of grains spike$^{-1}$ obtained from tagged main stems. Grain yield plant$^{-1}$ was recorded by harvesting all spikes from tagged plants followed by drying, threshing, and weighing of grains.

### Statistical analyses

The statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). 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-split-plot with temperature randomly assigned to main plots, drought to sub-plots, and genotypes to sub-sub-plots. There were three replications. Class variables consisted of block, temperature, drought, and genotypes. Block, block × drought, block × temperature, and block × drought × 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). For the time series data, repeated measure analyses within PROC MIXED were conducted with REPEATED statement and TYPE = CS, a covariance structure. Regression analyses on time series data were conducted on averages of genotypes using PROC REG procedure of SAS (Littell et al., 2006).

## Results

The *P*-values for growth, physiological, and yield traits obtained with SAS PROC MIXED are presented in Supplementary Table 5.1. The independent effects of drought, temperature, and genotypes; and interaction effects of drought × temperature, and drought ×

temperature × genotypes were significant for physiological and yield traits, unless indicated otherwise. Genotypes were significantly different for growth traits, but effects of drought, temperature, and their interaction were non-significant (Table 5.1).

## Flag leaf chlorophyll

Drought, high temperature, and combined stress decreased flag leaf chlorophyll by 14%, 47%, and 65%, respectively, when averaged across genotypes and over the 16 d periods (Fig. 5.1A). Genotypes responded differentially to stress. At the optimum condition, cultivars 'Halberd' and 'Dharwar Dry' and genotypes GR'S' / BOY'S' and ALTAR 84 / AO'S' had maximum leaf chlorophyll (~ 51 SPAD units), and genotypes ALTAR 84 / *A. tauschii* (WX 193) and GAN / *A. tauschii* (WX 897) had flag leaf chlorophyll of about 46 SPAD units (Fig. 5.2A). Drought decreased leaf chlorophyll of ALTAR 84 / *A. tauschii* (WX 193) by 25% followed by GAN / *A. tauschii* (WX 897) and GR'S' / BOY'S' by about 17%. A minimum decrease in leaf chlorophyll due to drought was observed in ALTAR 84 / AO'S' (5% from the optimum condition) followed by cultivars 'Halberd' and 'Dharwar Dry' (~ 9-11% decline). The decrease in leaf chlorophyll due to high temperature ranged from about 32-37% in 'Dharwar Dry' and ALTAR 84 / AO'S' to 50-59% in the rest of the genotypes. The combination of drought and high temperature decreased leaf chlorophyll in the range of 60% for 'Dharwar Dry' to 71% for ALTAR 84 / AO'S'. The leaf chlorophyll as a function of time is presented in Fig. 5.3A. In plants under the optimum condition, the significant decline in leaf chlorophyll was observed 10 d after treatment and the decline was of smallest magnitude (slope = – 0.680). The average leaf chlorophyll at 16 d after treatment was still about 46 SPAD units. In other treatments, a significant decrease in chlorophyll was observed from 4 d onwards. Drought decreased leaf chlorophyll with a smaller magnitude (slope = –1.51). The leaf chlorophyll at 16 d after drought stress was 27 SPAD units.

High temperature decreased leaf chlorophyll with a magnitude almost 2.3 fold higher than that from drought (slope = –3.54), and leaf chlorophyll reached below 10 SPAD units 12 d after treatment. The combined effect of drought and high temperature on leaf chlorophyll was of greater magnitude than the individual stresses. The rate of decline in chlorophyll under combined stress was the highest (slope = –5.35); and within 8 d, leaf chlorophyll decreased to < 10.

**Maximum quantum yield of photosystem II (Fv/Fm)**

Drought, high temperature, and combined stress decreased Fv/Fm by 27%, 53%, and 74%, respectively when averaged across all genotypes and over the 16 d of measurements (Fig. 5.1B). The genotypes behaved differentially for each stress (Fig. 5.2B). Under the optimum condition, genotypes were not significantly different for Fv/Fm, and the average Fv/Fm was about 0.73 (average of 16 d readings). Drought decreased Fv/Fm by about 47% in ALTAR 84 / *A. tauschii* (WX 193) and 'Halberd', and a minimum decline due to drought was observed in ALTAR 84 / AO'S' (~ 1%) and GR'S' / BOY'S' (5%) (Fig. 5.2B). Effect of high temperature on Fv/Fm was the highest in ALTAR 84 / *A. tauschii* (WX 193) (~ 75% decline from the optimum condition) followed by GR'S' / BOY'S' (64%), and was a minimum in ALTAR 84 / AO'S' and 'Dharwar Dry' (~ 33% decline). The combination of drought and high temperature stress decreased Fv/Fm by > 70% in all genotypes. The Fv/Fm as a function of time is presented in Fig. 5.3B. Under the optimum condition, the decline in Fv/Fm was of very small magnitude (slope = –0.005), and the significant decline occurred 12 d after treatment. Drought decreased Fv/Fm from 8 d after treatment and the rate of decline was smaller than other stresses (slope = –0.055). High temperature decreased Fv/Fm from 6 d after treatment and the rate of decrease was smaller than the combined effect (slope = –0.062). The combined effect of drought and high temperature on

Fv/Fm was higher than the individual stresses and the slope of decline was – 0.098, and decline started from 4 d after treatment.

## Plant height, tiller number, and biomass

As expected, drought, temperature, drought × temperature, and drought × temperature × genotype had no significant effect on plant height, tiller number, fertile tiller number, and vegetative biomass plant$^{-1}$ (Supplementary Table 5.1). However, genotypes were different for these traits. Plant height ranged from 77 cm for GAN / *A. tauschii* (WX 897) to 98 cm for ALTAR 84 / *A. tauschii* (WX 193) (Table 5.1). Cultivar 'Halberd' had the highest number of tillers (5.8) and fertile tillers plant$^{-1}$ (4.1). The lowest tiller number was observed in ALTAR 84 / *A. tauschii* (WX 193) (3 plant$^{-1}$); and the lowest fertile tiller number was observed in GR'S' / BOY'S' (~ 2 plant$^{-1}$). Cultivar 'Halberd' produced the maximum amount of vegetative biomass (5.8 g plant$^{-1}$) followed by cultivar 'Dharwar Dry' (4.5 g plant$^{-1}$). All the synthetic hexaploid wheat genotypes produced about 3 g of vegetative biomass plant$^{-1}$.

## Spikelet number and grain number

As expected, there was no effect of drought, high temperature, drought × high temperature, and drought × high temperature × genotype on spikelet number spike$^{-1}$ and grain number spike$^{-1}$ (Supplementary Table 5.1). However, genotypes differed significantly for these traits ($P$ < 0.001). The spikelet number spike$^{-1}$ ranged from 14.6 in GAN / *A. tauschii* (WX 897) to 24.2 in ALTAR 84 / *A. tauschii* (WX 193), and the number of grain spike$^{-1}$ ranged from 21 in GAN / *A. tauschii* (WX 897) to 52 in ALTAR 84 / *A. tauschii* (WX 193) (Table 5.2).

## Individual grain weight and yield

Effects of drought, high temperature, genotypes, and drought × high temperature × genotypes on individual grain weight were significant at $P < 0.001$ and that of drought × high temperature was significant at $P < 0.05$ (Supplementary Table 5.1). Drought, high temperature, and combined stress decreased individual grain weight by 26%, 39%, and 54%, respectively, when averaged across the genotypes (Fig. 5.1C). Under the optimum condition, cultivars 'Dharwar Dry' and 'Halberd' had the highest individual grain weight (57–60 mg), and genotype ALTAR 84 / *A. tauschii* (WX 193) had the lowest individual grain weight (~ 38 mg). Individual grain weight of other genotypes under the optimum condition was about 50 mg. Drought decreased individual grain weight of 'Halberd' by 47% followed by GAN / *A. tauschii* (WX 897) (~ 36%; Fig. 5.4A). Genotype GR'S' / BOY'S' had the lowest decline in individual grain weight due to drought (~ 13%) followed by ALTAR 84 / *A. tauschii* (WX 193) and ALTAR 84 / AO'S' (~ 18%; Fig. 5.4A).

High temperature decreased individual grain weight of genotype GAN / *A. tauschii* (WX 897) by 56% followed by genotypes GR'S' / BOY'S' and ALTAR 84 / AO'S' (~ 42% decline). All other genotypes had about 33% decline in individual grain weight due to high temperature. The combined effect of drought and high temperature on individual grain weight was highest in genotype GAN / *A. tauschii* (WX 897) (66% decline) followed by genotype ALTAR 84 / AO'S' (62% decline) and cultivar 'Halberd' (59% decline). Genotype ALTAR 84 / *A. tauschii* (WX 193) had a minimum effect of combined stress on individual grain weight (38% decline; Fig. 5.4A).

Effects of drought, high temperature, and drought × high temperature on grain yield plant$^{-1}$ were significant at $P < 0.01$, and effects of genotypes and drought × high temperature ×

genotypes on grain yield plant$^{-1}$ were significant at $P < 0.001$ and $P < 0.05$, respectively (Supplementary Table 1). Drought, high temperature, and combined stress decreased grain yield plant$^{-1}$ by 26%, 37%, and 50%, respectively (Fig 5.1D). Under the optimum condition, cultivars 'Dharwar Dry' and 'Halberd' had the maximum grain yield plant$^{-1}$ (7.2 g and 6.5 g respectively) followed by genotype ALTAR 84 / *A. tauschii* (WX 193) (5.8 g plant$^{-1}$) (Fig. 5.4B). The genotype GAN / *A. tauschii* (WX 897) had the lowest grain yield under the optimum environmental conditions (3 g plant$^{-1}$). Drought decreased grain yield of cultivar 'Halberd' by 47% followed by genotype GAN / *A. tauschii* (WX 897) (35%). A minimum decline in grain yield due to drought was observed in GR'S' / BOY'S' (10%) followed by ALTAR 84 / *A. tauschii* (WX 193) (16%).

High temperature decreased grain yield plant$^{-1}$ of genotypes GAN / *A. tauschii* (WX 897) by 56% followed by ALTAR 84 / AO'S' (42%) and cultivar 'Dharwar Dry' (39%). Genotypes ALTAR 84 / *A. tauschii* (WX 193) and GR'S' / BOY'S' and cultivar 'Halberd' were least affected by high temperature (~ 32% yield decline). The combined effect of drought + high temperature on yield was the highest in genotypes GAN / *A. tauschii* (WX 897) (64% decline) followed by genotype ALTAR 84 / AO'S' and cultivar 'Halberd' (about 60% decline). The combined effect of drought + high temperature on yield was the lowest in the genotype ALTAR 84 / *A. tauschii* (WX 193) (38% decline, Fig. 5.4B).

## Discussion

Drought and high temperature frequently occur simultaneously during the late grain filling period of wheat development. Wheat grain filling stages can be divided into pre-milk stage, milking stage, soft dough stage, and hard dough stage (Noda et al., 1994). During soft dough stage (20 – 30 d after anthesis) the embryo differentiates further and becomes a fully

developed miniature plant with its own reserve accumulations, mainly triacylglycerols and osmoprotectant proteins. So, stress at this stage might results into yield penalty. However, there are limited reports on the combined and independent effects of high temperature and drought during this stage on wheat yield and yield traits.

Synthetic hexaploid wheats have been developed for increasing genetic variability in wheat for biotic and abiotic stress tolerance (Trethowan and Mujeeb-Kazi, 2008), and they have been studied for tolerance to drought (Kurahashi et al., 2009) and high temperature (Yang et al., 2002). However, to my knowledge this is the first attempt where effects of drought and/or high temperature were studied on synthetic hexaploid and spring wheats at the late grain filling period. This research showed that drought and/or high temperature at the late grain filling period decreased yield of synthetic hexaploid and spring wheats, and the decrease in yield was due to a decline in individual grain weight (Figures 5.1 and 5.4). In addition, synthetic wheat genotypes and spring wheat cultivars responded differently to stress for physiological (Fig. 5. 2) and yield traits (Fig. 5.4).

In this study, drought, high temperature, and the combination of drought and high temperature decreased leaf chlorophyll by 14%, 47%, and 65%, respectively (Fig 5.1A). Drought activates rapid relocation of carbohydrates and nitrogen from leaves and stems to grains in cereals to complete and ensure maturation of grain. This causes senescence of leaves and thus the decrease in chlorophyll content (Yang et al., 2001). Severe drought also favors the production of reactive oxygen species that damage membranes and degrades leaf chlorophyll (Zhang and Kirkham, 1994). In addition, high temperature induces electrolytic leakage from thylakoid membranes (Al-Khatib and Paulsen, 1984; Ristic et al., 2007) and/or lipid peroxidation of chloroplast membranes (Djanaguiraman et al., 2010) that decreases leaf chlorophyll. The adverse

effect of high temperature on leaf chlorophyll was three times higher than that of drought (Fig. 5.1A). The effect of high temperature on disintegrating chlorophyll was very rapid and of greater magnitude than that of drought (Fig. 5.3A), which might be the reason for increased damage under high temperature stress. Under combined stress conditions, damage of photosystem II was greater than either of drought or high temperature stress. The pattern of decline in Fv/Fm at the high temperature was similar to the pattern of decline in leaf chlorophyll under the high temperature (Fig. 5.3A, 5.3B). This suggests a close relationship between leaf chlorophyll and maximum quantum PS II yield under the high temperature condition. Ristic et al. (2007) reported similar relationship between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat subjected to high temperature stress. On the contrary, the declining pattern of Fv/Fm under drought did not match with that of leaf chlorophyll decline under the drought. This phenomenon suggests that although greenness of leaf remains intact for longer periods under drought, photosystem II might have been damaged earlier in plants subjected to drought. This observation needs to be confirmed with further studies.

In this study, drought stress decreased individual grain weight by 26% and high temperature stress decreased it by 39% when averaged across the genotypes/cultivars (Fig. 5.1). Post anthesis drought decreased grain yield by decreasing individual grain weight, and decrease in individual grain weight was mainly due to decrease in grain filling duration rather than grain filling rate (Wardlaw and Willenbrink, 2000). Drought decreased days to physiological maturity by 7 d and high temperature and combined stress decreased it by about 17 d when averaged across all genotypes (data not presented). This implies that the grain filling duration under high temperature stress and combined stress was significantly lower than under drought stress alone. This may be the reason for greater decreases in individual grain weight under high temperature

stress than the drought stress. Decreases in grain filling duration at high temperature were reported earlier (Al-Khatib and Paulsen, 1984; Prasad et al., 2008b). The interaction effect was hypo-additive (negative interaction) in nature, i.e., the combined effect (drought + high temperature) was less than the sum of the individual effect (drought or high temperature) on grain weight (Fig. 5.4A). Both drought and high temperature stress affects individual grain weight by a common mechanism, i.e. decreasing grain filling duration, which might be the reason for the hypo-additive interaction between them. This result is in contradiction to the one reported by Shah and Paulsen (2003), who saw an additive interaction between drought and high temperature when imposed about a week after anthesis. But the results are in agreement with the report of Wardlaw (2002), who reported a hypo-additive interaction between drought and high temperature for individual grain weight. The responses of synthetic wheat genotypes and spring wheat cultivars to stresses were different for individual grain weight. Under drought stress, except GAN / *A. tauschii* (897), other synthetic genotypes had higher performance for individual grain weight than check cultivar 'Dharwar Dry'. Under high temperature stress, ALTAR 84 / *A. tauschii* (WX 193) had a decline in individual grain weight equal to the decline in check variety 'Halberd' and less than the other genotypes. Under combined stress of drought and high temperature, genotype ALTAR 84 / *A. tauschii* (WX 193) had a minimum decrease in individual grain weight compared to the others (Fig. 5.4A).

Drought stress decreased grain yield by 26%; high temperature stress decreased it by 37%; and combined stress decreased it by 50%, which is similar to the pattern observed in individual grain weight (Fig 5.4A and 5.4B). There was no effect of treatments on grain number plant$^{-1}$. This showed that, at the late grain filling period of the wheat crop, individual grain weight was the main determinant of grain yield under drought and/or high temperature stress. In

addition, interaction between drought and high temperature stress was hypo-additive (negative interaction), i.e., the effect of combined stress was higher than the individual effects but lower than their sum (Fig. 5.4B). This contrasts with the findings of Nicolas et al. (1984) who reported an additive or synergistic effect of drought and high temperature on wheat yield. The reason for the contradictory result may be due to different timing of stress and different genotypes. However, Mittler (2006), while proposing a "stress matrix", indicated that drought and high temperature belonged to the category of "potential negative interaction". Synthetic hexaploid genotypes and spring wheat cultivars responded differently to drought stress, high temperature stress, and combined stress of drought and high temperature. Under drought stress, synthetic genotypes GRS' / BOY'S' and ALTAR 84 / *A. tauschii* (WX 193) had fewer decline in yield than other genotypes including check variety 'Dharwar Dry'. In another experiment, performance of ALTAR 84 / *A. tauschii* (WX 193) under drought stress was reported poorer than 'Dharwar Dry' for yield (Villareal et al., 1998). In that field experiment drought was imposed by reducing irrigation (one-irrigation in the season), which might be the reason for contradicting performance of ALTAR 84 / *A. tauschii* (WX 193). Under high temperature stress, ALTAR 84 / *A. tauschii* (WX 193) and GR'S' / BOY'S' had about the same percent decline in grain yield as in 'Halberd' but less decline than the other genotypes (Fig. 5.4B) . Despite the differences in two experimental conditions as mentioned above, GR'S' / BOY'S' also yielded higher grain yield under high temperature stress applied at 10 d after anthesis (Yang et al., 2002). ALTAR 84 / *A. tauschii* (WX 193) yielded higher grain yield in both absolute and proportionate terms under all four treatments (Fig 5.4). Therefore, it was considered more tolerant to independent and combined effects of drought and high temperature stress.

In conclusion, the combined effect of drought and high temperature were more detrimental than the effect of individual stress, and the interaction between drought and high temperature stress was hypo-additive. This research showed that synthetic hexaploid and spring wheat cultivars had differential responses to independent and combined effect of drought and high temperature stress at the late grain filling period. Genotype ALTAR 84 / *A. tauschii* (WX 193) was the most tolerant to the combined effect of drought and high temperature at the late grain filling period of crop development. Individual grain weight was the yield component associated with tolerance at the late grain filling period. However, further studies are needed to confirm these interactions under more controlled drought stress conditions with particular attention to measuring leaf water potentials and using a fraction of transpirable soil water as a means of estimating drought stress.

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## Figures and Tables

**Figure 5.1.** Effect of drought, high temperature, and combined stress on physiology, yield, and yield components of four synthetic hexaploid wheat genotypes and two spring wheat cultivars. Data are averaged across genotypes/cultivars. For each genotype, a percent decline from the optimum condition is indicated. Vertical lines on bars indicate standard errors of mean.

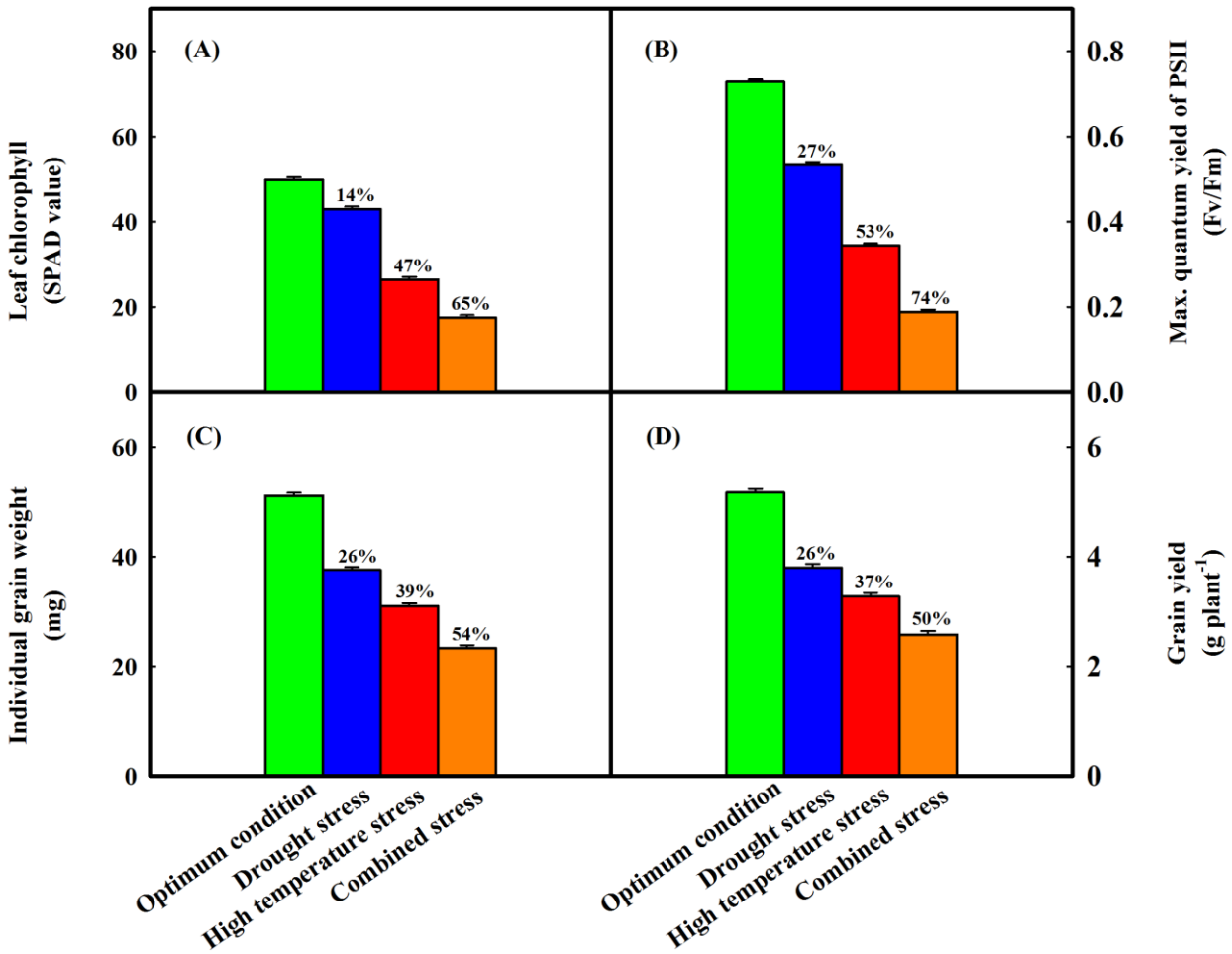


**Figure 5.2.** Effect of drought, high temperature, and combined stress on (A) flag leaf chlorophyll (SPAD value), and (B) maximum quantum yield of photosystem II (Fv/Fm) of four synthetic hexaploid wheat genotypes and two spring wheat cultivars. The interaction effects were significant at $P < 0.05$. For each genotype, a percent decline from the optimum condition is indicated. Vertical lines on bars indicate standard errors of mean.

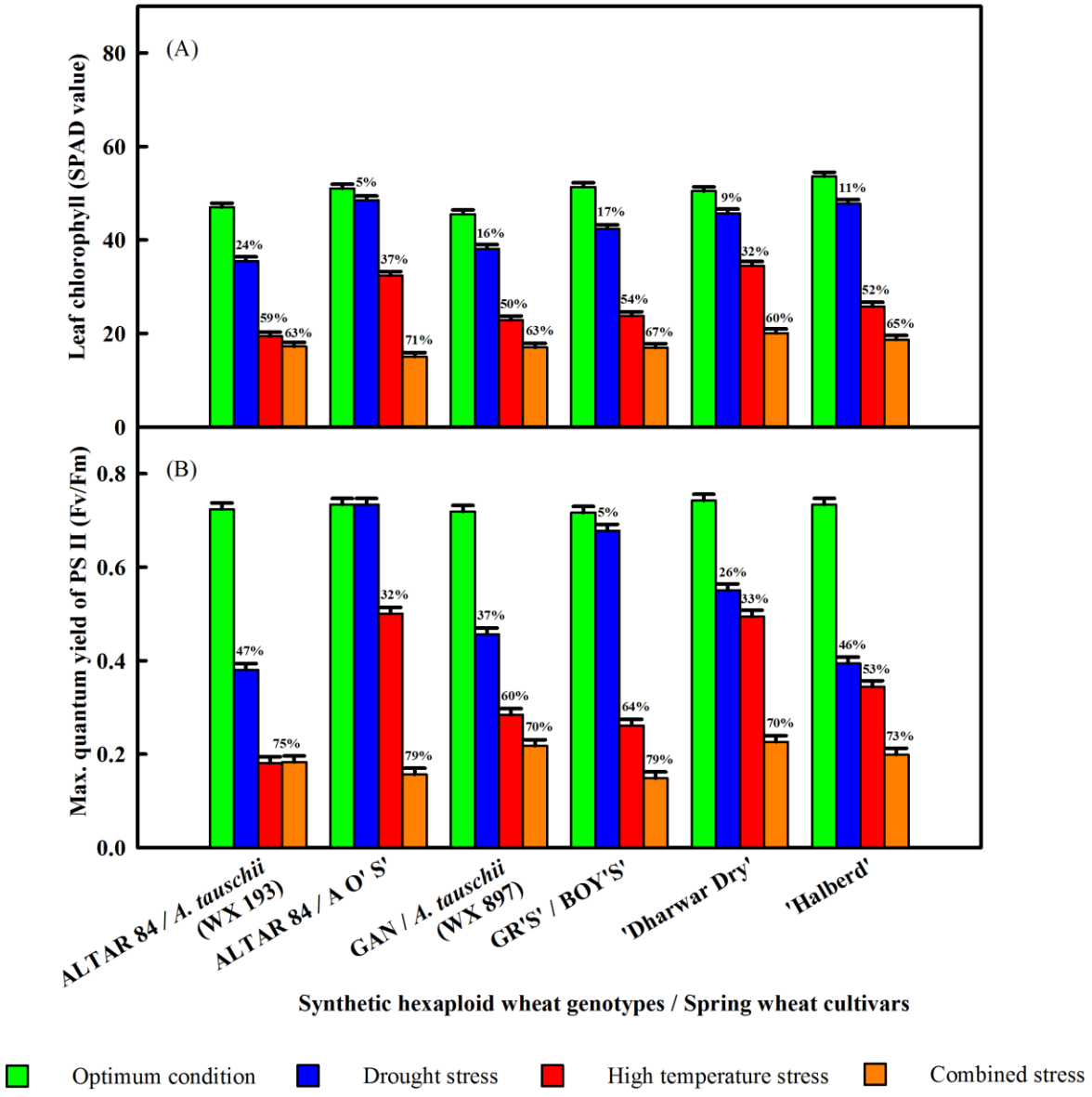


**Figure 5.3.** Physiological traits of synthetic hexaploid wheat genotypes and two spring wheat cultivars under the different stress combination presented as the function of days. Vertical lines on symbols indicate standard errors of mean. (A) Leaf chlorophyll: Optimum condition, y = – 0.680x + 56.42 , $r^2$ = 0.96, n = 5 (8 to16 d); Drought stress, y = – 1.513x + 56.22, $r^2$ = 0.83, n = 8 (0 to16 d); High temperature stress, y = – 3.539x + 57.40, $r^2$ = 0.91, n = 8 (0 to16 d); and Combined stress, y = – 5.35x + 5 4.45, $r^2$ = 0.89, n = 5 (0 to10 d). (B) Maximum quantum yield of PS II: Optimum condition, y = – 0.005x + 0.771, $r^2$ = 0.92, n = 8 (0 to16 d); Drought stress, y = – 0.055x + 1.060, $r^2$ = 0.97, n = 6 (6 to16 d); High temperature stress, y = – 0.062x + 0.904, $r^2$ = 0.94, n = 7 (4 to16 d); and Combined stress, y = – 0.098 + 0.811, $r^2$ = 0.83, n = 4 (0 to 8 d).

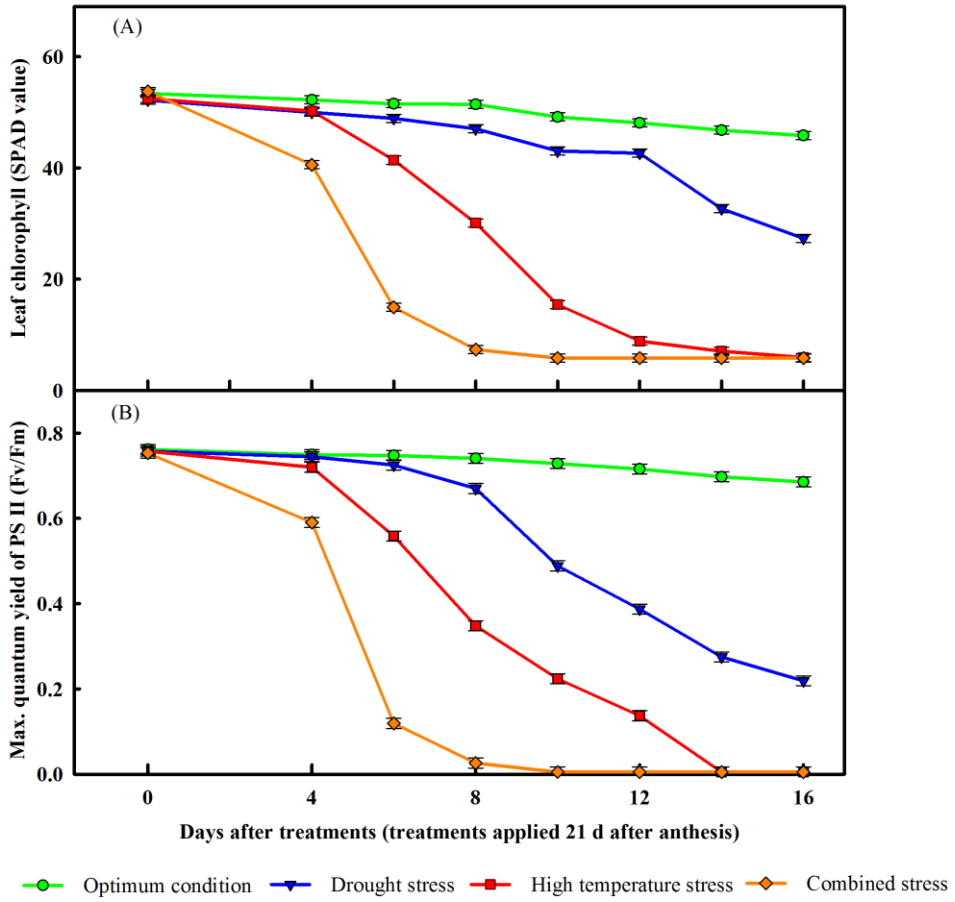


**Figure 5.4.** Effect of drought, high temperature, and combined stress on (A) individual grain weight, and (B) grain yield plant$^{-1}$ of four synthetic hexaploid wheat genotypes and two spring wheat cultivars. The interaction effects were significant at $P < 0.05$. For each genotype, a percent decline from control is indicated. Vertical lines on bars indicate standard errors of mean.

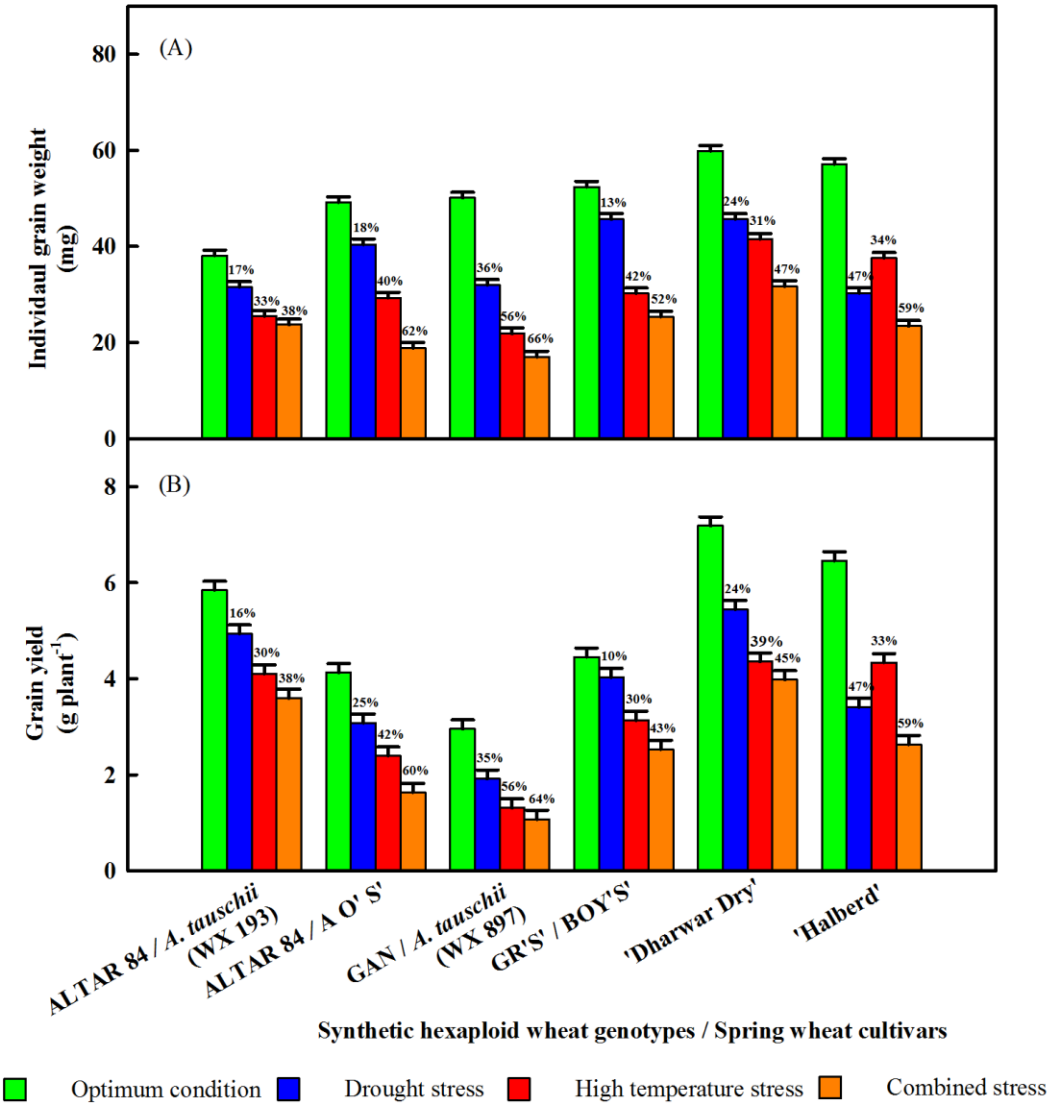


**Table 5.1.** Mean growth and morphological parameters of four synthetic hexaploid wheat genotypes and two spring wheat cultivars.

| Species | Plant height (cm) | Tiller number (plant$^{-1}$) | Fertile tiller number (plant$^{-1}$) | Vegetative biomass (g plant$^{-1}$) |
|---|---|---|---|---|
| ALTAR 84 / *A. tauschii* (WX 193) | 97.9$^{a}$ | 3.0$^{d}$ | 2.8$^{bc}$ | 3.2$^{c}$ |
| ALTAR 84 / A O' S' | 83.0$^{cd}$ | 5.1$^{ab}$ | 2.8$^{bc}$ | 3.6$^{c}$ |
| GAN / *A. tauschii* (WX 897) | 76.9$^{d}$ | 4.7$^{bc}$ | 2.4$^{cd}$ | 2.9$^{c}$ |
| GR'S' / BOY'S' | 89.7$^{bc}$ | 4.3$^{c}$ | 1.9$^{d}$ | 2.9$^{c}$ |
| 'Dharwar Dry' | 93.6$^{ab}$ | 4.5$^{bc}$ | 3.1$^{b}$ | 4.5$^{b}$ |
| 'Halberd' | 88.6$^{bc}$ | 5.8$^{a}$ | 4.1$^{a}$ | 5.8$^{a}$ |

Tukey-Kramer grouping (Little et al., 2006) of the synthetic hexaploid and spring wheats using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$.

**Table 5.2.** Mean yield parameters of four synthetic hexaploid wheat genotypes and two spring wheat cultivars.

| Species | Spikelet number (spike$^{-1}$) | Grain number (spike$^{-1}$) |
|---|---|---|
| ALTAR 84 / *A. tauschii* (WX 193) | 24.2[a] | 52.1[a] |
| ALTAR 84 / A O' S' | 17.3[d] | 27.1[e] |
| GAN / *A. tauschii* (WX 897) | 14.6[e] | 20.5[f] |
| GR'S' / BOY'S' | 19.3[c] | 30.3[d] |
| 'Dharwar Dry' | 21.3[b] | 41.5[b] |
| 'Halberd' | 19.3[c] | 38.2[c] |

Tukey-Kramer grouping (Little et al., 2006) of the synthetic hexaploid and spring wheats using least square means option in MIXED procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different at $P = 0.05$.

**Supplementary Table 5.1.** *P*-value and significance of the effects of drought, temperature, genotype, and their interactions on physiological, growth and yield traits of four synthetic hexaploid wheat genotypes and two spring wheat cultivars.

| Effects | Drought (D) | Temperature (T) | Genotype (G) | D × T | D × T × G |
|---|---|---|---|---|---|
| Traits | | | *P* - values | | |
| Leaf chlorophyll (SPAD units) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $0.020^{*}$ | $< 0.001^{***}$ |
| Maximum quantum yield of PS II (Fv/Fm) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ |
| Plant height (cm) | $0.149^{NS}$ | $0.901^{NS}$ | $< 0.001^{***}$ | $0.886^{NS}$ | $0.638^{NS}$ |
| Tiller number (plant$^{-1}$) | $0.390^{NS}$ | $0.252^{NS}$ | $< 0.001^{***}$ | $0.320^{NS}$ | $0.011^{*}$ |
| Fertile tiller number (plant$^{-1}$) | $0.804^{NS}$ | $0.968^{NS}$ | $< 0.001^{***}$ | $0.549^{NS}$ | $0.780^{NS}$ |
| Vegetative biomass (g plant$^{-1}$) | $0.818^{NS}$ | $0.853^{NS}$ | $< 0.001^{***}$ | $0.242^{NS}$ | $0.862^{NS}$ |
| Spikelet number (spike$^{-1}$) | $0.199^{NS}$ | $0.402^{NS}$ | $< 0.001^{***}$ | $0.627^{NS}$ | $0.220^{NS}$ |
| Grain number (spike$^{-1}$) | $0.234^{NS}$ | $0.079^{NS}$ | $< 0.001^{***}$ | $0.525^{NS}$ | $0.023^{*}$ |
| Individual grain weight (mg) | $< 0.001^{***}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $0.025^{*}$ | $< 0.001^{***}$ |
| Grain yield (g plant$^{-1}$) | $0.005^{**}$ | $< 0.001^{***}$ | $< 0.001^{***}$ | $0.004^{**}$ | $0.038^{*}$ |

$^{NS}$ nonsignificant. *, **, *** significant at $P < 0.05$, $< 0.01$ and $< 0.001$ respectively.

# Chapter VI -General conclusions and future directions

Four experiments were conducted under the controlled environment conditions with the objectives of (a) identifying wild wheat relatives (*Aegilops* species/accessions) tolerant to an extended period of drought, and high temperature stress at anthesis; (b) ascertaining the traits associated with tolerance; (c) quantifying the effect of drought and/or high temperature at the anthesis, and at the late grain filling period of crop development on synthetic and spring wheats; and (e) determining if responses to stress varied among genotypes of synthetic and spring wheat. The conclusions from each experiment (Chapter) were as follows:

Chapter II (Experiment 1): Screening of 52 accessions at anthesis, belonging to five different *Aegilops* species, revealed genetic variability among wild wheat species, and accessions within species for high temperature tolerance. It illustrated that *A. speltoides* was the most tolerant species and that the greater grain number spike$^{-1}$ and/or individual grain weight were main yield components associated with high temperature tolerance. Within *A. speltoides*, accessions TA 2348, TA 2342, and TA 2780 were identified as high temperature tolerant. Three accessions of *A. geniculata* (TA 2899, TA 1819, and TA1814) were identified as moderately high temperature tolerant on the basis of yield and HSI. The high temperature tolerant accessions identified in this study can be used in breeding for high temperature tolerance of cultivated wheat as discussed earlier.

Chapter III (Experiment 2): Screening of 31 accessions at anthesis, belonging to five different *Aegilops* species, revealed genetic variability among *Aegilops* species, and accessions within species for drought tolerance. It identified *A. geniculata* as the most drought tolerant species and that greater grain number spike$^{-1}$ and/or individual grain weight were the main yield components associated with drought tolerance. Within *A. geniculata*, accessions TA 10437 and

TA 1802 were identified as highly drought tolerant. The results of this study were from a controlled environment experiment. Field evaluation of accessions under drought conditions would, therefore, be useful and desirable. After field testing and evaluation, drought tolerant accessions identified in this study may be utilized in breeding for drought tolerance of cultivated wheat as discussed above.

Chapter IV (Experiment 3): Experimentation on four synthetic hexaploid wheats [ALTAR 84 / *A. tauschii* (WX 193), ALTAR 84 / AO'S', GAN / *A. tauschii* (WX 897), and GR'S / BOY'S'], and two spring wheats [‘Dharwar Dry’ and ‘Halberd’] at the anthesis showed that combined effects of high temperature + drought stress were more detrimental than the individual effect of each stress, and the interaction effect was hypo-additive, i.e. the magnitude of combined effect was less than the sum of the individual effects. The study showed that genotypes varied in their response to combined effect of drought and high temperature stress, and genotype ALTAR 84 / AO’S’ was the more tolerant. However, further investigation are needed to confirm these interaction effects in a more controlled and measurable drought stress conditions, where leaf water potential and a fraction of transpirable soil water are taken into consideration.

Chapter V (Experiment 4): Experimentation on four synthetic hexaploid wheats [ALTAR 84 / *A. tauschii* (WX 193), ALTAR 84 / AO'S', GAN / *A. tauschii* (WX 897), and GR'S / BOY'S'], and two spring wheats [‘Dharwar Dry’ and ‘Halberd’] at the late grain filling period of crop development (21 d after anthesis) showed that the combined effects of drought and high temperature stress were more detrimental than the effect of individual stress, and the interaction between the drought and the high temperature stress was hypo-additive. The study showed that genotypes varied in their response to stresses, and genotype ALTAR 84 / *A. tauschii* (WX 193) was the most tolerant to the combined effect of drought and high temperature at the late grain

filling period of crop development. Individual grain weight was the yield component associated with the tolerance at the late grain filling period. However, further studies are needed to confirm these interactions under more controlled drought stress conditions with particular attention to measuring leaf water potentials and using a fraction of transpirable soil water as a means of estimating drought stress.

## Overall outputs

(1) *A. speltoides'* accessions TA 2348, TA 2342 and TA 2780; and *A. geniculata's* accessions TA 2899, TA 1819 and TA1814 were identified as tolerant genotypes to high temperature stress.

(2) *A. geniculata's* accessions TA 10437 and TA 1802 were identified as tolerant genotypes to drought stress.

(3) Among four synthetic hexaploid wheats [ALTAR 84 / *A. tauschii* (WX 193), ALTAR 84 / AO'S', GAN / *A. tauschii* (WX 897), and GR'S / BOY'S'], genotype ALTAR 84 / *A. tauschii* (WX 193) was more tolerant to combined stress of drought and high temperature stress at the anthesis.

(4) Among four synthetic hexaploid wheats [ALTAR 84 / *A. tauschii* (WX 193), ALTAR 84 / AO'S', GAN / *A. tauschii* (WX 897), and GR'S / BOY'S'], genotype ALTAR 84 / AO'S' was more tolerant to combined stress of drought and high temperature at the late grain filling period.

## Future research

These experiments showed presence of extreme genetic variability in *Aegilops* species for drought and high temperature stress at anthesis. So, further research might be directed towards following:

(1) Crossing program to use the identified genotypes to improve drought / high temperature need to be initiated to check the suitability of gene transfer.

(2) Many more *Aegilops* accessions are available in gene bank. Screening of these potential sources for drought and high temperature stress tolerance would be useful.

(3) The results presented in this dissertation were primarily derived from effects of drought and high temperature on yield components. Therefore, accessions identified in this study shall be further investigated for biochemical and physiological basis of drought and high temperature tolerance. Research on photosynthetic rate, membrane thermo-stability, respiration, reactive oxygen species and antioxidant activity under drought and high temperature stress in *Aegilops* will enlighten us to understand their defense mechanism(s) that may be useful in studying stress sensitivity of other crops too.

(4) The reviews above showed that GS2 is also a highly sensitive stage to drought and high temperature stress. So, further research on the effects of drought and high temperature stress on reproductive processes of *Aegilops* species, such as microsporogenesis, pollen dehiscence and pollen longevity, stigma receptivity, pollen tube growth, pollen germination, and embryo development would be instrumental in understanding the mechanisms of tolerances.

(5) In these studies, drought was imposed by withholding water. Although this is an easiest method and tons of experiments have been conducted in this way, intensity of drought

in different pots could not be quantified in a given time and compared. So in future experiments, efforts shall be made to quantify the actual drought occurring at the given time of the day. Measurement like leaf water potential and fraction of transpirable soil water might aid in this endeavor.

(6) The combined effect of drought and high temperature at anthesis resulted in production of grains with maximum weight of about 10 mg (synthetic or spring wheats), which in my knowledge is not useful quantitatively and qualitatively as well. It means the combined stress (stopped irrigation + 36/30°C) was very harsh on hexaploid wheats. So, if we are to conduct future research on combined effects, the temperature setting at the higher side need to be decreased.

(7) Once the biochemical / physiological bases of tolerance or susceptible is determined, it will be useful to determine genes associated with tolerance.

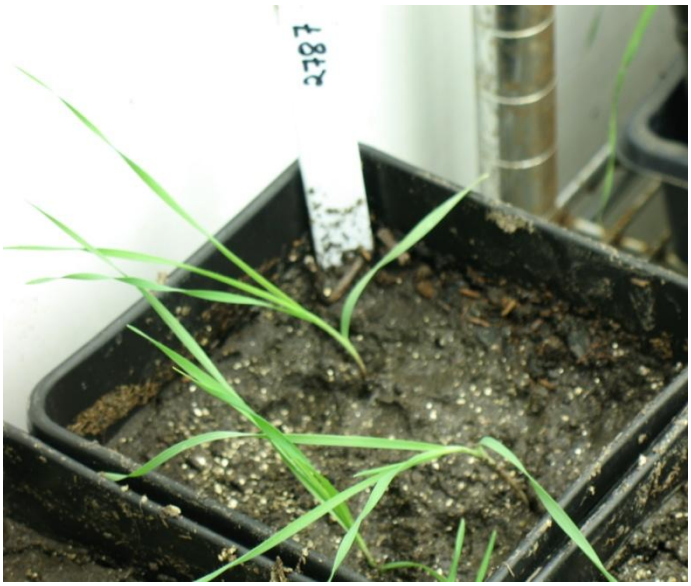
# Appendix A - **Pictures**

**Picture 1.** Growth chambers used in the experiments.

**Picture 2.** (A) *Aegilops* seedlings were raised in 4 cm tray. (B) After vernalization, they were transplanted into plastic pots (Chapter II).

(A)


(B)



**Picture 3**. Sample instruments used in collecting physiological data.

(A) SPAD Meter:
(To estimate leaf chlorophyll)

(B) FLIR BCAM SD Thermal Imaging Camera
(For recording leaf temperature)

(C) Stowaway Tidbit Temp Loggers
(For recording air temperature)


(D) Chlorophyll Fluorometer
(To estimate maximum quantum yield of the photosystem II)

**Picture 4**. Sample picture of a leaf temperature recorded with FLIR BCAM SD Thermal Imaging Camera, and edited with QuickReport 1.2 software.