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Response of *Aegilops* species to drought stress during reproductive stages of development

Gautam P. Pradhan[A], *P. V. Vara Prasad*[A,C], *Allan K. Fritz*[A], *Mary B. Kirkham*[A] *and Bikram S. Gill*[B]

[A]Department of Agronomy, Kansas State University, 2004 Throckmorton Plant Sciences Center, Manhattan, KS 66506, USA.
[B]Department of Plant Pathology, Kansas State University, 4024 Throckmorton Plant Sciences Center, Manhattan, KS 66506, USA.
[C]Corresponding author. Email: vara@ksu.edu

Abstract. Drought stress is an important abiotic factor limiting wheat yield. Thirty-one accessions of *Aegilops* species belonging to five species were screened to identify species/accessions tolerant to drought stress and to measure traits associated with the 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 stress was imposed by withholding water for 16 days. Controls were continuously irrigated. Drought stress decreased chlorophyll content, grain number, individual grain weight and grain yield by 31, 25, 68 and 76% compared with the control. *Aegilops geniculata* Roth had greater tolerance to drought stress for yield (48% decline from control) compared with other species (>73% decline from control). The tolerance was associated with higher grain number spike^{-1} and heavier grains. *A. geniculata*, GenBank accession number TA 10437, was highly tolerant to drought stress with <20% yield decline and a drought stress susceptibility index (DSI) <0.5, whereas TA 1802, TA 2061, TA 1814, TA 1819 were identified as moderately tolerant to drought stress (20–40% yield decline and DSI < 1.0). Our results suggest a presence of genetic variability among *Aegilops* species that can be utilised in breeding wheat for tolerance to drought stress at reproductive stages.

Additional keywords: abiotic stress, chlorophyll content, drought susceptibility index, grain yield, grain number, individual grain weight, *Triticum aestivum*, wild wheat.

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Introduction

Wheat (*Triticum aestivum* L.) provides 19% of world's food energy and 21% of protein intake (FAO 2011). Owing 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 constraints including high temperature and water deficit (drought stress) (Chaves *et al*. 2003; Flexas *et al*. 2004; Prasad *et al*. 2008*a*, 2008*b*).

Drought stress adversely affects physiology, morphology, growth and yield traits of wheat (Saini and Westgate 1999; Barnabás *et al*. 2008; Prasad *et al*. 2008*b*). Drought stress increases senescence by enhancing chlorophyll degradation, nitrogen loss and lipid peroxidation (Yang *et al*. 2001). Drought stress 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 stress 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 (water soluble carbohydrates) under favourable 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 stress at pre-anthesis decreases grain yield by adversely affecting photosynthesis leading to decreased accumulation of stem reserves. Ehdaie *et al*. (2006) showed up to 23% decrease in main stem weight when wheat crop was subjected to drought stress. Drought stress 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 decreased starch accumulation in anthers and pollen grains (Saini *et al*. 1984; Lalonde *et al*. 1997; Ji *et al*. 2010). A short durations of drought stress at the meiotic stage may cause pollen and ovary abortion, leading to decreased seed set (Saini and Aspinall 1981; Saini and Westgate 2000; Ji *et al*. 2010). Drought stress during post-anthesis decreases grain yield by decreasing individual grain weight (Ahmadi and Baker 2001; Ji *et al*. 2010). A decrease in individual grain weight under drought stress in wheat is due to decreased grain filling duration rather than grain filling rate (Wardlaw and Willenbrink 2000). Recent studies indicate that drought decreases both grain filling period and grain filling rate in wheat (Ehdaie *et al*. 2008). In addition, post-anthesis drought stress decreases 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 *Aegilops 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 stress tolerance. Gill *et al*. (2006) summarised disease- and insect- resistant genes identified in many *Aegilops* species such as *A. tauschii*, *A. speltoides* and *Aegilops geniculata* Roth. Screening of wild genotypes for drought stress tolerance at the reproductive stage may be useful to exploit genetic variability. There are a few reports on *Aegilops* species with drought stress 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*. 2008*b*). Reports on screening of *Aegilops* species for drought stress 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 stress at reproductive stage; and (b) to measure 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, *Aegilops markgrafii* Greuter K. Hammer (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 (see Table S1 available as Supplementary Material to this paper).

Experimental and treatment conditions

This experiment was conducted at the facility of the crop physiology laboratory of the Department of Agronomy, Kansas State University, Manhattan, KS, USA. Seeds of *Aegilops* accessions were sown in 4-cm deep trays, containing commercial Sun Grow Metro Mix 300 potting soil (Hummert International, Topeka, KS, 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 vernalised for 42 days at 4°C with an 8 h photoperiod. Following vernalisation, seedlings of each accession were transplanted into six 1.6-L square 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 fertiliser (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 different 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. Seeds of four wheat cultivars were sown on the day the *Aegilops* accessions were transplanted. 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 (active ingredient Imidacloprid,1-((6-Chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine) was applied to avoid infestation of sucking insect pests. Within a chamber, pots were randomly shifted every 7 days to avoid any positional effect. At 45 days 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. Throughout the experiment, 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 ~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 stress treatment. Drought stress was imposed by withholding water for 16 days. The second pot was continuously irrigated and served as a control. At the end of the treatment period, the plants were re-watered and kept at well watered conditions until physiological maturity.

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.

Leaf chlorophyll content

Leaf chlorophyll content was measured every other day for 14 days from the start of the treatment. A self-calibrating chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL, USA) was used to measure chlorophyll content 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} consisted of both fertile (with spikes) and non-fertile (without spike) tillers. Vegetative biomass plant^{-1} was the weight of the oven-dried (65°C for 10 days) plant material without spikes and roots. 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, yield and harvest index

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 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. Harvest index was estimated as a ratio of grain yield and above ground biomass.

Drought susceptibility index (DSI)

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

$$\mathrm{DSI} = (1 - Y/Y\mathrm{p})/D, \tag{1}$$

where, Y is the average grain yield of an accession at drought stress (g plant^{-1}); Yp is the average grain yield of the same accessions at control (g plant^{-1}); D is stress intensity, calculated by 1 – X/Xp where X is the mean Y of all accessions (g plant^{-1}) and Xp is the mean Yp of all accessions (g plant^{-1}). Higher DSI values are associated with lower drought tolerance.

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 (three different chambers). Class variables consisted of block (replications), 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. 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). Accession 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 analysed as an independent species rather than classifying them as *T. aestivum*'s.

Results

The *F*-values for growth, physiological and yield traits obtained with SAS PROC MIXED are presented in Table S2. There were significant effects of species and accessions within species for all traits at $P < 0.001$. Effects of drought, drought × species and drought × accessions within species were found for leaf chlorophyll content, grain number, individual grain weight and grain yield.

Leaf chlorophyll content

Drought stress decreased leaf chlorophyll content (SPAD units) by 31% as compared with control, when averaged across all species and over the first 14 days readings. Differential responses of species to drought stress for leaf chlorophyll retention was evident at $P < 0.001$ (Table S2). In drought stress, *Aegilops markgrafii* and 'Halberd' had highest decrease in leaf chlorophyll content (> 50%), whereas 'Dharwar Dry' and 'Sitta' had the lowest decrease (6%). Most of the other species had ~33% decline in leaf chlorophyll content (Fig. 1).

Plant height, tiller number and biomass

Drought and drought × species had no effects on plant height, tiller number, number of fertile tillers and biomass; however, differences occurred among species for these attributes ($P < 0.001$) (Table S2). Plant height varied from ~50 cm for *A. geniculata* to 95 cm for *A. speltoides. A. speltoides* had the highest total number of tillers (39) and fertile tillers (25) plant^{-1}, whereas cultivars belonging to *Triticum* species had the minimum number of tillers (~6) and fertile tillers (~5) plant^{-1} (Table 1). *A. speltoides* had the highest vegetative biomass (~13 g plant^{-1}), whereas *A. geniculata* and cultivar 'Sitta' had the lowest vegetative biomass (~4.5 g plant^{-1}) (Table 1).

Spike length and spikelet number

There were no effects of drought and drought × species on spike length and number of spikelet spike^{-1}, but species differed significantly for these traits. *A. longissima* had the longest spike (22 cm) followed by *A. speltoides* and *A. searsii* (~16 cm). The shortest spike was observed in *A. geniculata* (~4 cm) (Table 1). 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 1).

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 ($P < 0.001$) (Table S2). Drought stress decreased grain number spike^{-1} by 25% and individual grain weight by 68%, when averaged across all species. Drought stress decreased grain number spike^{-1} of *A. searsii* by 100% as compared with control, followed by *A. longissima* (69%). A minimum decrease in grain number due to drought stress was observed in 'Dharwar Dry' (0% decline) followed by 'Sitta' and 'Pavon 76' (≤7% decline) (Fig. 2*a*). Effect of drought stress on individual grain weight was the lowest in *A. geniculata* (34% decline as compared with

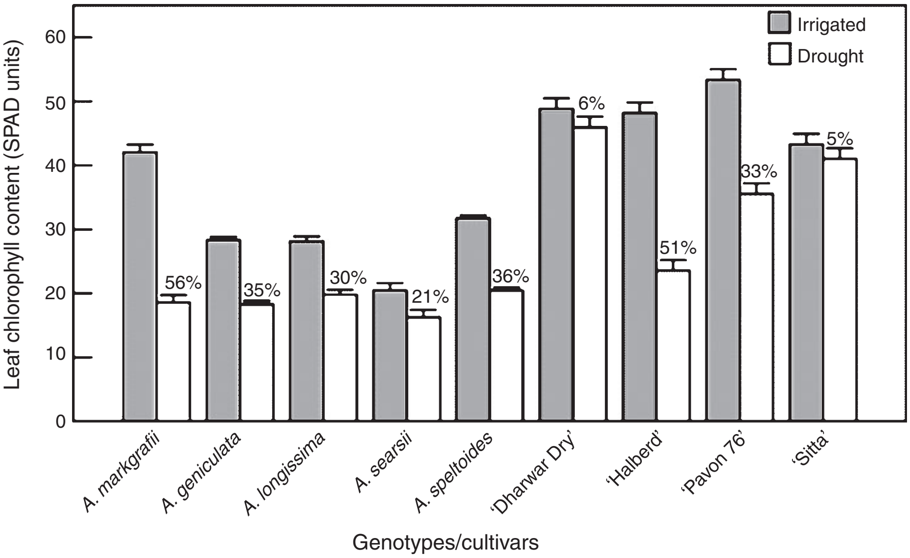

Fig. 1. Effect of drought stress on leaf chlorophyll content (SPAD units) of five *Aegilops* species and four bread wheat cultivars. Interaction effect of drought × species was significant at $P < 0.001$. Percentage decline due to drought stress as compared with control is indicated. Vertical lines on bars indicate s.e.

Table 1. Mean growth and morphological parameters of five *Aegilops* species and four bread wheat cultivars

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

Species/ cultivar	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. markgrafii	64de	34b	21.3b	5.4bc	7.8e	8f
A. geniculata	50f	30b	16.6c	4.6c	3.9f	3g
A. longissima	83b	22c	14.2d	7.7b	21.8a	16d
A. searsii	61e	30b	16.7c	6.1bc	16.3b	13e
A. speltoides	95a	39a	24.9a	12.6a	16.3b	14e
'Dharwar Dry'	72c	6d	4.5e	6.5bc	12.2c	23b
'Halberd'	69cd	5d	4.7e	5.5bc	10.7cd	21b
'Pavon 76'	72cd	7d	5.5e	8.1b	11.3cd	26a
'Sitta'	59e	6d	4.5e	4.3c	8.4de	18c

control) and the highest in *A. searsii* (100% decline). The rest of the species/cultivars had ≥63% decline in individual grain weight due to drought stress (Fig. 2*b*).

Grain yield and harvest index

There were significant effects of drought, species and drought × species on five *Aegilops* species and four bread wheat cultivars for grain yield plant^{-1} and harvest index ($P < 0.001$) (Table S2). Drought stress decreased grain yield plant^{-1} by 76% when averaged across all species. Effect of drought stress on grain yield plant^{-1} was the lowest in *A. geniculata*, 48% decline as compared with control; and the highest in *A. searsii*, which could not produce a grain. Other species had ≥73% decline in grain yield due to drought stress (Fig. 3*a*). The responses of *Aegilops* species to drought stress for harvest index were similar to grain yield (Fig. 3*b*).

Accessions within species variability in A. geniculata

Among five *Aegilops* species and four bread wheat cultivars, *A. geniculata* was highly tolerant to drought stress for grain yield plant^{-1}. Thus, accessions belonging to this species were further analysed for variability and data are presented in Table 2. 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). Drought stress decreased grain number spike^{-1} of TA 1808 by 86% followed by TA 10009 (45%). TA 2899, TA 10024 and TA 10437 had the lowest decrease in grain number spike^{-1} due to drought stress (≤5% decline). Effect of drought stress on individual grain weight was the highest in TA 1813 and TA 1808 (64–69% decline as compared with control) and the lowest in TA 10437 and TA 1802 (<3% decline). Drought stress decreased grain

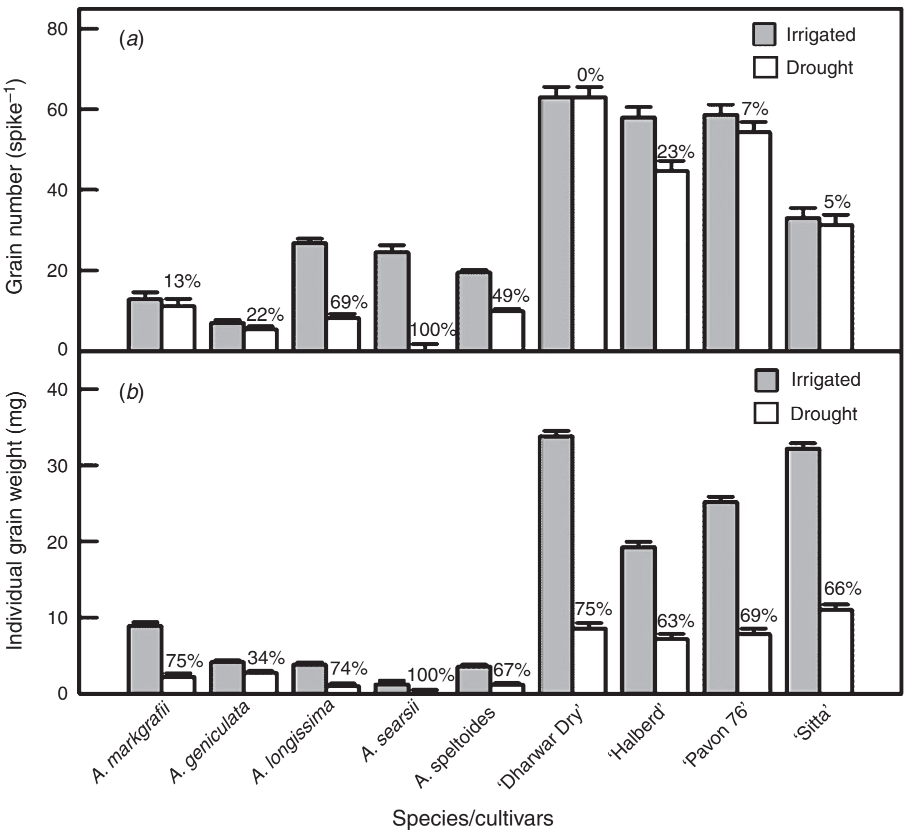

Fig. 2. Effect of drought stress on (*a*) grain number spike^{-1} and (*b*) individual grain weight of five *Aegilops* species and four bread wheat cultivars. Interaction effect of drought × species was significant at $P < 0.001$. Percentage decline due to drought stress as compared with control is indicated. Vertical lines on bars indicate s.e.

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 stress (6%), followed by TA 1802 (24%).

The drought stress susceptibility indices calculated for *A. geniculata* accessions are presented in Table 2. Accessions TA 10437 and TA 1802 were highly drought stress tolerant with a DSI of ≤ 0.5. The moderately drought stress tolerant accessions were TA 2061, TA 1814, TA 1819, TA 10024 and TA 2899, with DSIs ranging from 0.65 to 1.0. The drought stress-susceptible accessions had DSIs > 1.0 (e.g. TA 1808 and TA 1813). In contrast, considering the % decline in grain yield due to drought, the accessions could be arbitrarily classified into four classes; highly tolerant (0–20% decline, only TA10437), moderately tolerant (20–40% decline, TA 1802, TA 2061, TA 1814, TA 1819), moderately susceptible (40–60% decline, TA 10024, TA2899, TA 10009) and susceptible (>60% decline, TA 1808, TA 1813).

Discussion

Water deficit (drought stress) 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 1999; Ahmadi and Baker 2001; Ji *et al*. 2010). Development of stress tolerant varieties is one of the promising ways to sustain or increase wheat yield under drought stress. *Aegilops* species contributed two of the three genomes to bread wheat and therefore, *Aegilops* species are 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 stress tolerant *Aegilops* species/accessions. These reports were mostly based on work on drought stress 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 our knowledge, this is the first study where *Aegilops* species are explored for reproductive drought stress tolerance by imposing drought stress at anthesis. This study showed presence of variability among *Aegilops* species/accession for physiological, yield and components of yield traits.

Aegilops accessions had different times to anthesis (61 to 97 days after transplanting) and physiological maturity. However, the timing of stress was similar and imposed at anthesis. Among the species, drought stress decreased the leaf chlorophyll content (SPAD units) in all species. Drought stress 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).

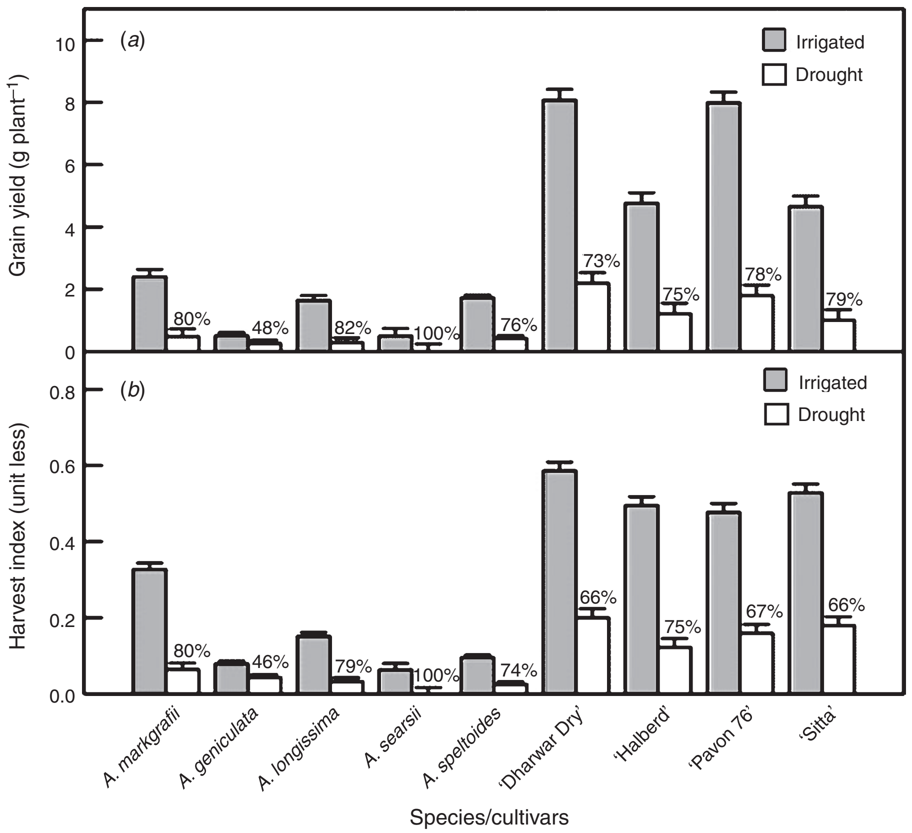

Fig. 3. Effect of drought stress on (*a*) grain yield plant^{-1} and (*b*) harvest index of five *Aegilops* species and four bread wheat cultivars. Interaction effect of drought × species was significant at $P < 0.001$. Percentage decline due to drought stress as compared with control is indicated. Vertical lines on bars indicate s.e.

Table 2. Effect of drought stress on yield and yield components of *Aegilops 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. DSI, drought susceptibility index

Accession no.	Grain number (spike^{-1})			Individual grain weight (mg)			Grain yield (g plant^{-1})			DSI
	Control	Drought stress	% Decline	Control	Drought stress	% Decline	Control	Drought stress	% Decline	
TA 10437	7.3	7.0	4.5	4.81	4.78	0.5	0.42	0.39	6.5	0.14
TA 1802	7.3	6.3	13.6	3.06	2.98	2.6	0.37	0.28	24.4	0.51
TA 2061	6.3	5.3	15.8	2.93	2.38	18.9	0.30	0.21	31.1	0.65
TA 1814	8.3	6.3	24.0	4.46	3.77	15.5	0.72	0.46	36.0	0.75
TA 1819	8.0	6.3	20.8	5.71	4.50	21.2	0.79	0.49	38.4	0.80
TA 10024	7.7	7.3	4.4	2.87	1.68	41.5	0.23	0.12	47.2	0.98
TA 2899	6.3	6.3	0.0	4.11	1.90	53.7	0.65	0.34	47.9	1.00
TA 10009	3.7	2.0	45.5	3.28	1.83	44.1	0.16	0.07	56.4	1.18
TA 1813	7.7	6.3	17.4	6.27	1.94	69.1	0.59	0.15	74.0	1.54
TA 1808	7.3	1.0	86.4	4.00	1.44	63.9	0.75	0.08	89.1	1.86
Mean	7.0	5.4	–	4.15	2.72	–	–	–	–	–

In addition, drought stress also damages membranes and degrades chlorophyll (Zhang and Kirkham 1994). In this study, there was genotypic variation for decline in leaf chlorophyll content under drought stress ranging from ~6% in 'Sitta' and 'Dharwar Dry' to > 50% in 'Halberd' and *A. markgrafii*. A previous study found no change in chlorophyll content in hexaploid wheats 'Excalibur' and 'RAC875', but a decline of ~25% in 'Kukri' (Izanloo *et al.* 2008).

Drought stress decreased grain number spike^{-1} of all species (Fig. 2*a*). The decrease in grain number was not due to the effect of drought stress on spikelet number spike^{-1} (Table 1); rather it may be due to lower fertilisation caused by pollen sterility or

Table 3. Statistical results on effect of drought stress on yield and yield components of *Aegilops geniculata* accessions

NS, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Source	*P*-values		
	Grain number (spike^{-1})	Individual grain weight (mg)	Grain yield (g plant^{-1})
Drought	0.07NS	0.04*	0.02*
Accessions	<0.001***	<0.001***	<0.001***
Drought × accession	0.001**	0.005**	<0.001***

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 stress disintegrates the contact between microspore and tapetum and degenerates the filament which induces pollen sterility (Saini *et al*. 1984). Drought stress also causes abnormal vacuolisation of tapetal cells and disorientation of reproductive cells resulting in male sterility (Lalonde *et al*. 1997). In our study, drought stress 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 fertilisation 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 stress. Consequently, drought stress might have induced pollen/ovule sterility or interfered with fertilisation 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 stress was imposed at anthesis. Fábián *et al*. (2011) reported embryo abortion in winter wheat varieties when drought stress was imposed at 5–9 days after anthesis. Embryo abortion was higher in the drought stress 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 our study (Fig. 2*a*; Table 2). There was no spike shattering in any of the accessions, thus the grain number is indicative of pollen or ovule sterility or embryo abortion. In our study, there was a wide range of genetic variability for decrease in grain number spike^{-1} under drought stress (0–100%). Therefore, this trait may be utilised 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 1999; Prasad *et al*. 2008*b*). Drought stress decreased individual grain weight by 68% (Fig. 2*b*), which is higher than the findings by Wardlaw and Willenbrink (2000) in the wheat cultivar 'Lyallpur 73' (38% decline) subjected to drought stress during anthesis and that by Ahmadi and Baker (2001) in cultivar 'Cadenza' (43% decline) subjected to severe drought stress from 15 days after anthesis. The decrease in individual grain weight may be due to lower grain filling duration (Wardlaw and Willenbrink 2000; Prasad *et al*. 2008*a*) and a decreased number of endosperm cells and starch granules per cell (Nicolas *et al*. 1985). In the present study, decrease in individual grain weight due to drought stress varied from 34% in *A. geniculata* to > 63% in rest of the species. Drought stress following anthesis can also cause loss of stem weight due to mobilisation of stem carbohydrate reserves (Wardlaw and Willenbrink 2000). In our study, the water soluble carbohydrates were not measured and requires further investigation.

Drought stress decreased grain yield plant^{-1} by 76% when averaged across all species. Decrease in yield was ~75% in all cultivars used as standard checks; including the drought stress tolerant cultivar 'Dharwar Dry'. *A. geniculata* had remarkably lower decline in grain yield (48%) compared with the standard checks (*T. aestivum*) and other *Aegilops* species (76–100% reduction). Baalbaki *et al*. (2006) reported tolerance of *A. geniculata* for grain yield under drought stress. Although *A. markgrafii* and cultivars 'Dharwar Dry', 'Pavon 76' and 'Sitta' showed a smaller effect of drought stress on grain numbers compared with *A. geniculata*, all of them had at least 25% more yield decline than *A. geniculata*. Thus, in these species, individual grain weight was the determining factor for the decrease in yield. However, in other species, both grain number spike^{-1} and individual grain weight played role in yield formation.

Among *A. geniculata* accessions, drought stress barely affected grain number spike^{-1} of TA 2899, TA 10024 and TA 10437 (Table 2). Thus, these may be potential sources of genes for maintaining fertility under reproductive drought stress in wheat. TA 10437 and TA 1802 had the minimum decline in individual grain weight; and they may be utilised in maintaining grain weight of wheat under reproductive drought stress. The accession TA 10437 had the lowest decline in grain yield components and grain yield due to drought stress (<10% decline from the control) and also had the drought stress susceptibility index (DSI) of <0.5. DSI is a measure of yield stability under drought stress that can be utilised in selecting drought stress tolerant lines (Sio-Se Mardeh *et al*. 2006); however, the stress index value shall be considered along with other traits (Yang *et al*. 2002). Therefore, accession TA 10437 was classified as highly drought stress tolerant; whereas TA 1802, TA 2061, TA 1814, TA 1819 were identified as moderately tolerant to drought stress (20–40% yield decline and DSI < 1.0). *A. geniculata* has the U^g and M^g genomes which are not related to the genomes of hexaploid bread wheat, *T. aestivum* (ABD) (Gill *et al*. 2006). Therefore, *A. geniculata* are categorised under tertiary gene pool and gene transfer from this gene pool cannot be achieved by homologous combination (Qi *et al*. 2007). However, considerable homeologous 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). So, the *A. geniculata* accessions identified in this study may be utilised in breeding program.

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

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