



## Phenotypic diversity of *Aegilops cylindrica* (jointed goatgrass) accessions from the western United States under irrigated and dryland conditions

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### ARTICLE INFO

#### Article history:

Received 16 December 2011

Received in revised form 4 October 2012

Accepted 5 October 2012

#### Keywords:

Jointed goatgrass

Wheat

Gene flow

Genetically modified

Drought

Transgene escape

### ABSTRACT

*Aegilops cylindrica* Host (jointed goatgrass) is an exotic invasive weed in the western United States that causes economic loss due to competition with winter wheat (*Triticum aestivum* L.) and contamination of wheat grain. Further, because *Ae. cylindrica* is a wild relative of wheat, interspecific hybridization is a possible mechanism for the introgression of advantageous genes from wheat to the weedy species. The risk of introgression of traits conferring environmental stress tolerance, such as drought tolerance, from transgenic wheat has not been studied and is of high concern because it could increase the weed's competitiveness with wheat, as well as its ability to invade novel habitats. Genetic diversity has been reported among western U.S. *Ae. cylindrica* accessions, however, studies to determine whether genetic diversity relates to phenotypic diversity have been limited. Here, 30 accessions of *Ae. cylindrica* from the western U.S. were evaluated over two years at two Colorado locations: Haxtun in 2008–2009 and Fort Collins in 2009–2010. Days to flowering, plant height, number of tillers produced per plant, number of spikelets per spike, and number of spikelets per plant were evaluated under rain-fed and irrigated conditions. Results indicate significant ( $P<0.01$ ) variation among accessions for each of the traits for both years. Additionally, in 2009–2010 there was a significant ( $P<0.01$ ) effect of irrigation on all traits, except days to flowering. Similarly, all traits except days to flowering showed a significant effect of the interaction of accession with treatment ( $P<0.05$ ) in 2009–2010. The study shows that variation exists in traits related to competitive ability and fitness. Our data also suggest differences among accessions in sensitivity to water availability. This observation needs to be confirmed in additional environments. Implications of our results for evaluation of transgene introgression are discussed.

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### 1. Introduction

*Aegilops cylindrica* Host (jointed goatgrass) is an exotic invasive weed in the U.S. that infests winter wheat (*Triticum aestivum* L.) fields and disturbed ecosystems (Anderson, 1993). The facultative annual grass is thought to have been introduced from its center of origin in Eurasia (Mayfield, 1927). In Europe and Asia, the range of *Ae. cylindrica* is vast and encompasses the region spanning the Mediterranean to central and eastern Asia (van Slageren, 1994). *Ae. cylindrica* has been reported in 32 states in the U.S. and continues to expand its range by 50,000 acres a year (USDA-NRCS, 2010; Washington State University, 2008). *Ae. cylindrica* appears to have a high degree of phenotypic plasticity in response to moisture availability by adjusting tiller number (P. Westra, unpublished observation).

The occurrence of hybridization and gene flow between bread wheat (*T. aestivum*) and *Ae. cylindrica* has driven research to assess the risk of introgression of transgenic wheat genes into the weedy species. *Ae. cylindrica* and bread wheat are both allopolyploid species in the Triticeae tribe. Bread wheat is an allohexaploid ( $2n=6$ ABBDD=42), while *Ae. cylindrica* is an allotetraploid ( $2n=4$ CCDD=28). The two species both contain the D-genome derived from a common diploid progenitor, *Ae. tauschii* Coss. In order to evaluate the rate of gene introgression from wheat to *Ae. cylindrica*, studies have been conducted to determine the frequency of interspecies hybridization and the restoration of fertility through backcrossing (Econopouly et al., 2011; Gaines et al., 2008; Guadagnuolo et al., 2001; Hanson et al., 2005; Mallory-Smith et al., 1996; Morrison et al., 2002a, 2002b; Schoenenberger et al., 2006; Snyder et al., 2000; Stone and Peeper, 2006; Wang et al., 2001; Zemetra et al., 1998). Cytogenetic techniques have also been used to track changes in chromosome numbers and the concomitant transfer of wheat genes in hybrids and subsequent backcross generations (Cifuentes and Benavente, 2009; Mallory-Smith et al.,

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1996; Perez-Jones et al., 2006a, 2006b; Schoenenberger et al., 2005, 2006; Wang et al., 2002, 2001, 2000; Zemetra et al., 1998). Genetic analyses have been conducted as well in order to assess the introduction history of the weed into the U.S., the level of genetic diversity, and the genetic structure of the species (Gandhi et al., 2009, 2005; Naghavi et al., 2009; Okuno et al., 1998; Pester et al., 2003; Watanabe et al., 1994). However, fewer studies have been done to assess the phenotypic diversity of *Ae. cylindrica*, an important factor for determining the risk of gene introgression in wheat production areas across the western U.S. In order to assess the likelihood of introgression it is necessary to evaluate the selective advantage gaining a wheat gene will confer among *Ae. cylindrica* populations from across the western U.S.

Genetic diversity has been detected with random amplified polymorphic DNA (RAPD) markers and nuclear microsatellite markers in the native range of Eurasia (Naghavi et al., 2009; Okuno et al., 1998). Studies using  $\alpha$ -amylase isozymes, RAPD, and amplified fragment length polymorphism (AFLP) markers concluded there was limited, or no, genetic diversity present in U.S. *Ae. cylindrica* collections (Pester et al., 2003; Watanabe et al., 1994). Conversely, studies using microsatellite markers have found the presence of multiple genotypes among populations collected in the western U.S. Diversity estimates from microsatellite data support the hypothesis of multiple *Ae. cylindrica* introductions into the U.S., but show evidence of genetic bottlenecking compared to the diversity found across the native range (Gandhi et al., 2009, 2005). However, diversity at microsatellites, repeat regions typically considered to be selectively neutral (Awadalla and Ritland, 1997; Schlotterer and Wiehe, 1999; Tachida and Iizuka, 1992; Li et al., 2002), does not imply that phenotypic diversity exists. The combination of the detection of multiple introductions and genetic diversity with the ability of the weed to invade across a vast geographic range demonstrates that an evaluation of phenotypic variation in *Ae. cylindrica* populations across the western U.S. is warranted. If *Ae. cylindrica* introduced into the U.S. originated from multiple eco-geographical regions in the native range, genotypes adapted to different environments may exist in the U.S. Previous phenotypic trials have suggested that trait diversity exists in populations of *Ae. cylindrica*. For example, in three years of common garden studies conducted in Bulgaria, variation was found in 13 of 14 traits, including date of heading, glume length, and number of fertile spikelets per spike, in *Ae. cylindrica* populations collected from four diverse regions in Bulgaria (Zaharieva et al., 2003). In addition, eco-geographic variation in that study was found for vegetative growth traits: populations from regions of high autumn and winter rainfall were shown to have greater plant height and leaf width and length than those from low rainfall areas. In the U.S. Gealy (1988) reported few consistent differences in two years of common garden studies evaluating *Ae. cylindrica* from nine western U.S. states. Nevertheless, in the first year, statistically significant variation was found among accessions for plant height and awn length. In year two of the study, variation was found for number of tillers per plant, awn length, and leaf dry weight. Greenhouse studies found variation for gas exchange rates between plants from Colorado and Nebraska (Gealy, 1988). Phenotypic variation has also been reported for seed germination in three populations from Oregon (Fandrich and Mallory-Smith, 2005) and in vernalization requirements for five populations from Oregon and Washington (Fandrich et al., 2008). In the western U.S., the high incidence of *Ae. cylindrica* infestations in dryland wheat has drawn concern for the risk of introgression of traits conferring drought tolerance from transgenic wheat cultivars to the weed. Transgenic technology has made it possible to make improvements in cultivars for complex traits by inserting only a single gene or stack of genes to a single location of the genome. Monsanto's development of drought tolerant maize (*Zea mays* L.) demonstrates how

transgenic technology can be used to alter expression of complex traits with a single gene. A transformation event inserting a single gene, *cspB* from *Bacillus subtilis*, into maize plants conferred yield advantages over non-transformed plants under water deficit in dryland environments (Castiglioni et al., 2008). World leaders in biotech wheat research, such as Monsanto, Bayer CropScience and the Commonwealth Scientific and Industrial Research Organization (CSIRO), the Chinese Academy of Agricultural Sciences, and Henan Agricultural University, are investigating transgenic drought tolerant wheat (Fox, 2009). La Trobe University in Australia has already begun field-testing transgenic drought tolerant lines and is expected to be the first to commercialize varieties (Fox, 2009). An evaluation of transgene introgression of drought tolerance is necessary in order to minimize economic and environmental risks associated with introgression from wheat to *Ae. cylindrica*. Phenotypic variation in *Ae. cylindrica* across the western U.S. could imply that the evolutionary consequences of gene introgression from wheat would vary. The risk of introgression of wheat genes into *Ae. cylindrica* populations is dependent on the selective advantage of gaining those genes. For traits beneficial in both agricultural settings as well as in natural ecosystems, such as tolerance to environmental stress, natural variation for the trait could already exist in *Ae. cylindrica* populations, implying that the selective advantage for gaining wheat genes conferring these traits could vary as well. This in turn would make the risk of gene introgression variable across populations. Additionally, unlike purely agricultural traits such as herbicide tolerance, introgression of traits conferring tolerance to environmental stress could enable habitat extension by increasing fitness in more diverse environments where these traits are beneficial (Stewart et al., 2003) with potentially negative economic and environmental consequences. Variation in flowering time among *Ae. cylindrica* populations could also affect the risk of gene introgression. Flowering time between wheat and the *Ae. cylindrica* must overlap in order for hybridization and gene flow to occur. Variation in flowering time among *Ae. cylindrica* populations implies that the opportunity for hybridization and gene flow with wheat is not uniform due to asynchronous flowering times.

The goal of this study was to evaluate *Ae. cylindrica* accessions from the western U.S. for diversity of traits with the potential to be differentially affected by introgression of wheat genes conferring environmental stress tolerance for the purpose of providing data for risk assessments. Thirty accessions of *Ae. cylindrica* from 12 western U.S. states were evaluated in a field trial conducted over two years at two Colorado locations. Two moisture treatments were used to determine variation in phenotypic response and sensitivity to water availability.

## 2. Materials and methods

### 2.1. Field design

Thirty accessions of *Ae. cylindrica* collected at various localities across 12 western U.S. states (Table 1) were grown at the Agricultural Research, Development, and Education Center (ARDEC) (40.653°N, 104.995°W, elevation 1555 m) in Fort Collins, CO, at the western edge of the U.S. Great Plains, during 2007–2008 to increase seed for the common garden experiments. The accessions were subsequently evaluated under wet and dry treatments in two years of study. In 2008–2009 the study was conducted in Haxtun, CO (40.532°N, 102.607°W, elevation 1231 m), a wheat growing region in the northeast quadrant of the state. The second year study, 2009–2010, was conducted at ARDEC.

The trial was arranged in a split-plot design with two replications. Moisture treatments, rain-fed or supplemented with

**Table 1**

Accessions of *Ae. cylindrica* from the western U.S. used in this study.<sup>a</sup> ID is the number given to the collection by Colorado State University researchers. Latitude and Longitude information is not available for the collections.

Entry	ID <sup>a</sup>	County/city	State
1	<i>Ae. cyl</i> 16	Fort Collins	Colorado
2	<i>Ae. cyl</i> 18	Kiowa County	Colorado
3	<i>Ae. cyl</i> 19	Phillips County	Colorado
4	<i>Ae. cyl</i> 21	Platner	Colorado
5	<i>Ae. cyl</i> 14	Bingham County	Idaho
6	<i>Ae. cyl</i> 43	Franklin County	Idaho
7	<i>Ae. cyl</i> 15	Twin Falls County	Idaho
8	<i>Ae. cyl</i> 50	Bunker Hill	Kansas
9	<i>Ae. cyl</i> 49	La Crosse	Kansas
10	<i>Ae. cyl</i> 25	Logan County	Kansas
11	<i>Ae. cyl</i> 40	Broadview	Montana
12	<i>Ae. cyl</i> 8	Chadron	Nebraska
13	<i>Ae. cyl</i> 1	Deuel County	Nebraska
14	<i>Ae. cyl</i> 2	Garden County	Nebraska
15	<i>Ae. cyl</i> 7	Scotts Bluff County	Nebraska
16	<i>Ae. cyl</i> 55	Clovis	New Mexico
17	<i>Ae. cyl</i> 42	Oklahoma County	Oklahom
18	<i>Ae. cyl</i> 28	Ione	Oregon
19	<i>Ae. cyl</i> 54	Sherman County	Oregon
20	<i>Ae. cyl</i> 51	Fall River County	South Dakota
21	<i>Ae. cyl</i> 29	Box Elder County	Utah
22	<i>Ae. cyl</i> 32	Cache County	Utah
23	<i>Ae. cyl</i> 48	Wasatch County	Utah
24	<i>Ae. cyl</i> 47	Weber	Utah
25	<i>Ae. cyl</i> 45	Asotin	Washington
26	<i>Ae. cyl</i> 46	Garfield County	Washington
27	<i>Ae. cyl</i> 22	Pullman	Washington
28	<i>Ae. cyl</i> 34	Ritzville	Washington
29	<i>Ae. cyl</i> 35	Archer West	Wyoming
30	<i>Ae. cyl</i> 37	Pine Bluffs	Wyoming

irrigation, were the main plots. Subplots were 30 accessions of *Ae. cylindrica*. Irrigation was supplied using Chapin drip tape<sup>1</sup> with a flow rate of  $0.062 \text{ L min}^{-1} \text{ m}^{-1}$  at the Haxtun location and Netafim Techline<sup>2</sup> CV with a flow rate of  $0.083 \text{ L min}^{-1} \text{ m}^{-1}$  at the Fort Collins location. In September of 2008 and 2009, two 3 m rows at 0.3 m spacing were planted at a rate of 20 seeds per row for each subplot. In the spring of 2009, seedlings were thinned to 10 plants per row. Drip line was installed to run down the middle of each plot to ensure watering of both rows of plants. A 1.5 m wide wheat border was planted around each replication. Weeds were controlled manually as necessary.

## 2.2. Data collection

Data were collected on a per plant basis for 5–10 plants from each subplot, with two plants in each row serving as border plants. In order to evaluate whether differences may exist in competitiveness, fitness, and the potential to hybridize with wheat, data were collected to evaluate the following traits: days to flowering, plant height, number of tillers per plant, mean number of spikelets per spike and total number of propagules per plant. Days to flowering was recorded for 10 plants per subplot in the 2009–2010 study in Fort Collins as the number of days from January 1 to date of heading (when the first spike was fully visible above the flag leaf). Plant height was measured in the field for five plants per subplot from ground level to the tip of the main tiller. Twenty intact spikes were collected from each of 10 plants per subplot. Spikelets were then counted for each of 10 random spikes per plant to give a mean number of spikelets produced per spike. Five whole plants from

<sup>1</sup> Chapin drip tape, Jain Irrigation, Inc., 2851 E. Florence Ave., Fresno, CA 93721, USA.

<sup>2</sup> Netafim Techline CV dripperline, MMXI Netafim Irrigation, Inc., Fresno, CA 93727, USA.

each subplot were harvested at maturity (July 2009 and 2010) and the number of tillers was counted. Total number of propagules produced per plant was estimated by multiplying the number of tillers by the mean number of spikelets per spike. Temperature and precipitation data were retrieved from the Colorado Agricultural Meteorological Network (Colorado State University, 2011).

## 2.3. Analysis of data

Variation in each trait was evaluated using factorial analysis of variance (ANOVA) with SAS software, version 9.3 (SAS Institute Inc., 2010). In the first year of the experiment, data from accessions 14 and 19 were not used for the traits mean spikelets per spike or mean number of propagules per plant, and data from accession 25 were not used for the trait mean number of propagules per plant, due to spike shattering. In 2008–2009, plant height, tiller number, and total propagules per plant were ln-transformed to meet ANOVA assumptions. In 2009–2010 days to flowering required ln-transformation. All other traits were consistent with a normal distribution as evaluated with the Shapiro–Wilk test for normality and g-plots of the residuals versus predicted values and therefore did not require transformation. The data were analyzed by year using Proc Mixed under a split plot design with moisture treatment, accession, and their interaction as the fixed effects and replication nested with treatment as the random effect. Fixed effects are reported under the Type 3 test and using the Kenward–Roger method for the denominator degrees of freedom (Kenward and Roger, 1997). Trait means are untransformed least squares means with standard errors. In 2008–2009 irrigation was not a significant effect for any of the traits measured and therefore least squares means are reported without the treatment effect.

For the 2009–2010 data, the reaction norm for each accession for each of the measured traits, except days to flowering, was graphed based upon the mean value of the genotype in each treatment. The regression line connecting the mean values for each treatment for the accession is the environmental sensitivity of that accession.

## 3. Results

For the 2008–2009 study, during the period from planting (September 2008) through harvest (July 2009), Haxtun received 113 mm of precipitation (Fig. 1), of which 80 mm fell during the main growing season from March 1 through harvest. The irrigated treatment received an additional 68 mm of water during the last 3 weeks of the study. Both moisture treatments in the first year correspond to a relatively wet rainfed condition in Colorado. During the September through July time period in 2009–2010, the ARDEC site received a total of 236 mm of precipitation, of which 169 mm occurred from March 1 until harvest on July 1. An additional 706 mm of water was applied to the irrigated treatment during the final 5 weeks of the study. Mean minimum and maximum temperature and relative humidity also varied among the years and sites (Fig. 1).

In the 2008–2009 study the effect of irrigation was not a significant effect for any of the traits measured (Table 2). The effect of moisture treatment was removed from the analysis and accessions were analyzed across four blocks (replications). In the following season (2009–2010) irrigation was increased in terms of both frequency of application and the total amount of water applied per irrigation. Results for 2009–2010 show that irrigation was significant ( $P < 0.01$ ) for 4 of the 5 traits accessed: height, number of spikelets per spike, number of tillers per plant, and number of propagules per plant (Tables 2 and 4). Accession was a significant effect ( $P < 0.01$ ) for all traits for both years of study (Tables 3 and 4). The interaction between accession and moisture treatment was

**Table 2**

Mean value estimates for each trait as least squares means by treatment for the Haxtun and Fort Collins field trials in 2008–2009 and 2009–2010 (DF = 2). “SE” is the standard error. Each treatment contained two replications of 30 accessions of *Ae. cylindrica* collected throughout the western U.S. Days to flowering was not evaluated in the Haxtun field study. Values sharing the same letter within a trait and location are not significantly different at alpha = 0.05.

Trait	Effect	Haxtun		Fort Collins	
		Estimate	SE	Estimate	SE
Mean days to flowering	Rain-fed	–	–	156.89 <sup>a</sup>	0.546
	Irrigated	–	–	157.89 <sup>a</sup>	0.546
Mean height (cm)	Rain-fed	64.46 <sup>a</sup>	0.49	48.05 <sup>a</sup>	0.302
	Irrigated	63.71 <sup>a</sup>	0.49	69.84 <sup>b</sup>	0.300
Mean number spikelets per spike	Rain-fed	8.38 <sup>a</sup>	0.12	9.22 <sup>a</sup>	0.017
	Irrigated	8.44 <sup>a</sup>	0.12	9.88 <sup>b</sup>	0.016
Mean number of tillers	Rain-fed	148.75 <sup>a</sup>	8.01	77.22 <sup>a</sup>	3.635
	Irrigated	162.67 <sup>a</sup>	8.00	129.31 <sup>b</sup>	3.638
Mean number of propagules	Rain-fed	1254.63 <sup>a</sup>	84.55	718.74 <sup>a</sup>	36.991
	Irrigated	1382.46 <sup>a</sup>	84.53	1277.18 <sup>b</sup>	37.018

**Table 3**

Analysis of variance results for fixed effects of the Haxtun, CO 2008–2009 field data. “acc” refers to the 30 accessions of western U.S. *Ae. cylindrica*. The analysis was conducted with Proc Mixed of SAS software (SAS Institute Inc., 2010) under Type 3 effects.

Trait	Effect <sup>a</sup>	Num DF <sup>b</sup>	Den DF <sup>c</sup>	F value	P
Mean height (cm)	acc	29	567	5.48	<0.0001
Mean number of spikelets per spike	acc	27	533	6.76	<0.0001
Mean number of tillers	acc	29	559	2.62	<0.0001
Mean number of propagules	acc	26	515	2.52	<0.0001

<sup>a</sup> “Effect” is the source of variation tested by the AMOVA.

<sup>b</sup> “Num DF” is the numerator degrees of freedom,  $k - 1$ , where  $k$  is the number of groups evaluated associated with the effect tested (number of accessions).

<sup>c</sup> “Den DF” is the denominator degrees of freedom,  $N - (k - 1)$ , where  $k$  is the number of groups (accessions and replications) and  $N$  is the number of observations (plants).

significant ( $P < 0.05$ ) for all traits except days to flowering in the second year study (Table 4).

In 2009–2010, days to flowering varied significantly by accession ( $P < 0.0001$ ), although not by treatment ( $P = 0.3269$ ) (Table 4). The mean days to flowering across all 30 accessions and two moisture levels was 157.4 days. The range of accession mean values was from June 5 (153.8 days to flower, SE = 0.72 days), observed in the rain-fed treatment, to June 16 (165 days to flower, SE = 0.76 days), in the irrigated treatment (Supplemental Table 1). The range of observed values for individual plants covered a greater time

period extending from May 29 to June 26. The flowering date does not follow a pattern of latitudinal change in the collection sites for the *Ae. cylindrica* accessions, but instead varies within and across latitude and collection site.

Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2012.10.005>.

Accession was a significant effect ( $P < 0.0001$ ) for plant height in both years of study (Tables 3 and 4); in 2009–2010 irrigation was also a significant ( $P < 0.001$ ) source of variation for this trait (Tables 2 and 4). In the first year study the mean plant height

**Table 4**

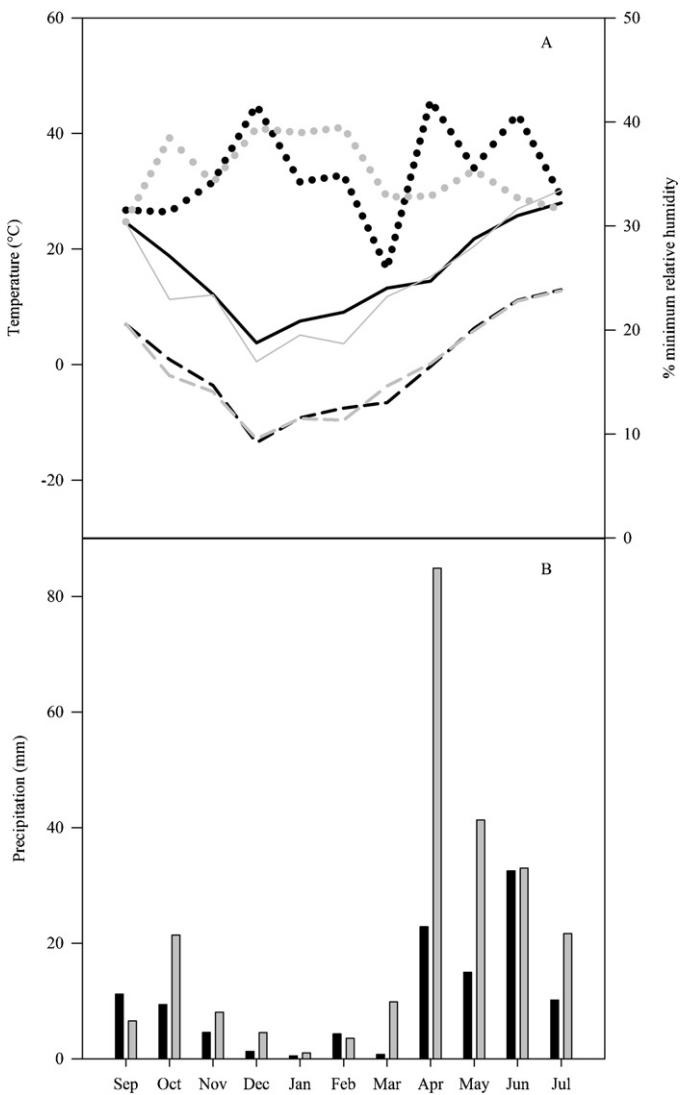
Analysis of variance results for fixed effects of the Fort Collins, CO 2009–2010 field data. “Trt” refers to the effect of irrigated or rain-fed conditions; “acc” refers to the 30 accessions of western U.S. *Ae. cylindrica*; and “trt\*acc” is the interaction between the irrigation treatment and the accession. The analysis was conducted with the Proc Mixed of SAS software (SAS Institute Inc., 2010) under Type 3 effects.

Trait	Effect <sup>a</sup>	Num DF <sup>b</sup>	Den DF <sup>c</sup>	F value	P
Mean days to flowering	trt	1	2	1.67	0.3269
	acc	29	1120	22.33	<0.0001
	trt*acc	29	1120	1.46	0.0529
Mean height (cm)	trt	1	2	2622.41	0.0003
	acc	29	536	4.85	<0.0001
	trt*acc	29	536	4.11	<0.0001
Mean number of spikelets per spike	trt	1	2	790.28	0.0009
	acc	29	536	4.70	<0.0001
	trt*acc	29	536	2.12	0.0007
Mean number of tillers per plant	trt	1	2	102.61	0.0096
	acc	29	531	1.89	0.0036
	trt*acc	29	531	1.63	0.0220
Mean number of propagules	trt	1	2	113.87	0.0087
	acc	29	531	1.97	0.0022
	trt*acc	29	531	1.73	0.0110

<sup>a</sup> “Effect” is the source of variation tested by the AMOVA. The effects are: treatment, accession, or the interaction of treatment and accession.

<sup>b</sup> “Num DF” is the numerator degrees of freedom,  $k - 1$ , where  $k$  is the number of groups evaluated associated with the effect tested. The groups are treatment or accession.

<sup>c</sup> “Den DF” is the denominator degrees of freedom,  $N - k$ , where  $k$  is the number of groups and  $N$  is the number of observations associated with the effect tested. The groups are treatment and accession. The observations are replication or plants.



**Fig. 1.** Weather data for Haxtun, CO September 2008 to July 2009 (black) and Fort Collins, CO September 2009–July 2010 (gray). Data is presented as (A) mean minimum temperature (°C) (dashed line), mean maximum temperature (°C) (solid line), % minimum relative humidity (dotted line), and (B) precipitation (mm) by month.

ranged from 58.7 cm to 69.0 cm ( $SE = 1.14$ ) (Supplemental Table 1). In 2009–2010, the mean height ranged from 39.11 cm ( $SE = 2.19$ ) to 57.10 cm ( $SE = 1.95$ ) in the rain-fed treatment and from 60.6 cm to 76.1 cm ( $SE = 1.95$ ) in the irrigated treatment (Supplemental Table 1).

Variation was also observed in each propagule production trait; spikelets per spike ( $P < 0.0001$ ), mean number of tillers ( $P < 0.01$ ), and propagules per plant ( $P < 0.01$ ) varied by accession in both years of study (Tables 3 and 4). Again, irrigation was also a significant source of variation in the second year study ( $P < 0.01$ ) (Tables 2 and 4). The mean spikelet number per spike ranged from 7.79 ( $SE = 0.15$ ) to 9.16 ( $SE = 0.15$ ) in the first year study (Supplemental Table 1). In the second year the range was from 8.24 ( $SE = 0.25$ ) to 9.89 spikelets ( $SE = 0.22$ ) in the rain-fed treatment and 9.15–10.65 spikelets ( $SE = 0.22$ ) in the irrigated treatment (Supplemental Table 1). For 2008–2009, the range of mean number of tillers was 117.56 ( $SE = 13.79$ ) to 197.97 ( $SE = 12.88$ ) (Supplemental Table 1). For the second year study, the mean number of tillers per plant ranged from 54.40 ( $SE = 17.56$ ) to 148.30 ( $SE = 15.72$ ) in the rain-fed treatment and 84.90–179.10 tillers ( $SE = 15.72$ ) in the irrigated treatment (Supplemental Table 1). Lastly, the range in the

mean total number of spikelets produced per plant was 992.16 ( $SE = 132.95$ ) to 1657.32 ( $SE = 122.67$ ) in 2008–2009 (Supplemental Table 1). In the second year the mean total number of spikelets ranged from 496.89 to 1431.10 ( $SE = 154.17$ ) in the rain-fed treatment and 811.79 to 1737.38 ( $SE = 154.17$ ) in the irrigated treatment (Supplemental Table 1).

Irrigation was a significant ( $P < 0.01$ ) source of variation for mean height, mean number of spikelets per spike, mean number of tillers per plant, and mean number of spikelets per spike in 2009–2010 (Tables 2 and 4). The mean height difference between treatments was 21.80 cm; the mean number of spikelets per spike differed by 0.66; the mean number of tillers per plant differed by 52.09; and the mean number of propagules per plant differed by 558.44 (Table 4, Supplemental Table 1).

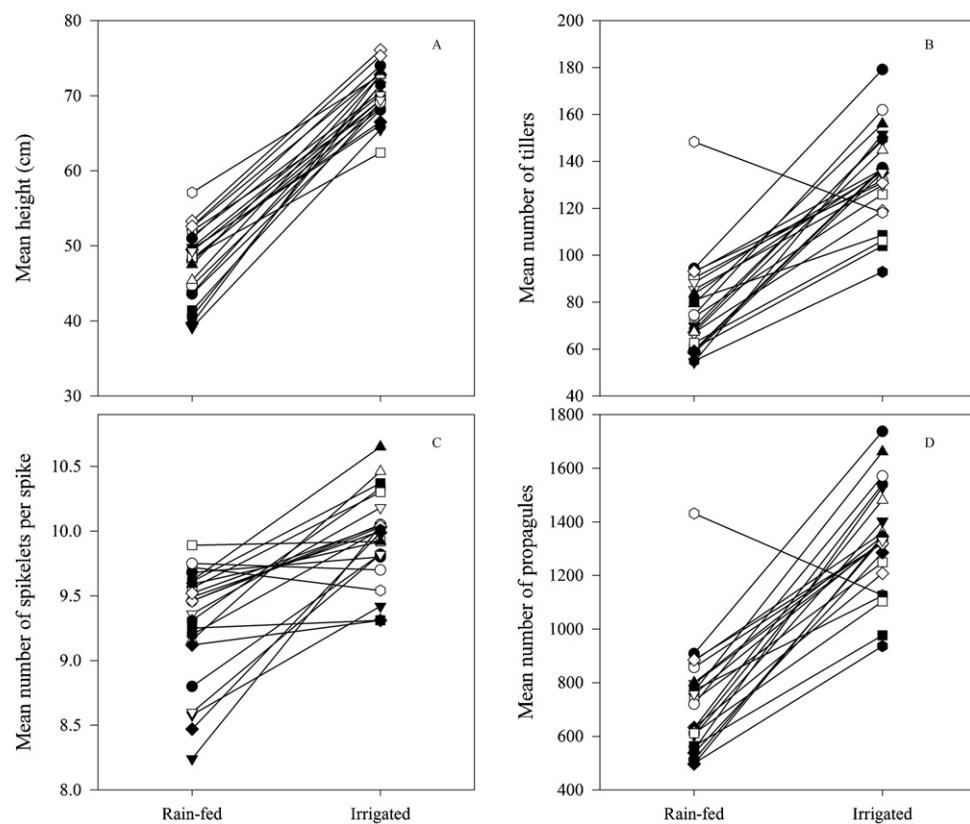
The interaction between accession and treatment was also significant ( $P < 0.05$ ) for 4 of 5 traits in 2009–2010 (Table 4). Reaction norms for all accessions for each of these traits in the 2009–2010 study, where irrigation was also a significant effect, were plotted as least squares means by treatment to illustrate the variation in sensitivity among accessions to water treatment (Fig. 2).

#### 4. Discussion

Field and greenhouse studies by Gealy (1988) found minimal phenotypic variation in *Ae. cylindrica* collections from the Great Plains. However, subsequent germination and vernalization studies have suggested that phenotypic variation exists (Fandrich and Mallory-Smith, 2005; Fandrich et al., 2008). Zaharieva et al. (2003) reported that phenotypic variation is present in collections from Bulgaria. If multiple introductions of the species have occurred in the U.S., as genetic studies have indicated (Gandhi et al., 2009, 2005), these introductions could be a source for phenotypic variation. The results of this study provide further evidence that phenotypic diversity in important traits is present in *Ae. cylindrica* populations growing in the western U.S.

The traits evaluated in this study were days to flowering, plant height, mean number of tillers per plant, mean number of spikelets per spike, and mean number of spikelets per plant. Days to flowering could impact the opportunity for both inter- and intra-specific hybridization to occur. Plant height is a measurement of growth rate and size and can be used as a predictor of competitiveness, while tiller number and spikelet number are both measurements of reproductive fitness (van Kleunen et al., 2010). van Kleunen et al. (2010) showed in a meta-analysis of 125 invasive and 196 non-invasive plant species that traits related to growth rate, size, and fitness, among others, can be used as predictors of invasiveness. Plants that show higher growth rates may have a higher risk in agricultural settings and be more problematic in terms of economic cost and extermination (Baker, 1974). However, those with higher fitness, determined by reproductive capacity, may be more evolutionarily successful even if reproductive capacity increases at the expense of plant growth and competitiveness (Baker, 1974). These latter genotypes may be less agriculturally competitive, but allocation of resources to reproduction could increase the ability for long term survival of the population as well as range expansion. However, in this study plant height was positively correlated with propagule production (data not shown) suggesting that *Ae. cylindrica* can be both a successful competitor in terms of allocating resources, and also a reproductively fit plant.

Significant variation among accessions was observed in this study for all traits evaluated. Variation in these traits suggests that the competitiveness, fitness, and ability to hybridize would vary as well. Variation under a common environment suggests that this phenotypic diversity is genetically determined. The hybridization of different *Ae. cylindrica* genotypes could lead to the introgression



**Fig. 2.** Reaction norms for each of the four traits in 2009–2010 that varied significantly both by treatment ( $P < 0.01$ ) (rain-fed or irrigated) and accession, and also had a significant interaction effect between accession and treatment ( $P < 0.05$ ): (A) mean height, (B) mean number of tillers per plant, (C) mean number of spikelets per spike, and (D) mean number of total propagules. The least squares mean of each accession in each treatment is plotted. The slope of the line connecting these two values illustrates the sensitivity of that accession to the moisture treatment. Differences in slopes of the lines shows that sensitivity of moisture treatment varies by accession.

of beneficial genes into new populations and could also provide a source of new genetic variation through recombination both of which could lead to an increase in invasiveness (for example, Novak and Mack, 1995; Kolbe et al., 2004). If containment of genetic diversity is desired, the spread of *Ae. cylindrica* seed should be minimized.

The flowering data collected in the second year study showed that the mean dates to heading ranged across 11 days from June 5 to June 16, with a mean date of June 8. A field study using 187 Great Plains wheat cultivars and advanced breeding lines of wheat conducted in the same year approximately 200 m from the *Ae. cylindrica* trial showed that the flowering time of the two species overlapped. The 50% heading date for the wheat was 158.9 days (June 10), with a minimum of 154 days (June 5) and a maximum of 165 days (June 16) (P. Byrne, unpublished data). These data suggest that the flowering times of the two species are synchronous and therefore provide an opportunity for cross-pollination to occur. In the future, studies that compare the flowering dates of wheat cultivars and *Ae. cylindrica* growing side-by-side in specific growing regions could be useful to determine the overlap of flowering time and will contribute to determining the risk of cross-pollination for that location.

In the 2009–2010 study, accessions were taller and produced more seeds under the irrigated treatment, as seen in differences in mean values, suggesting that water could be a limiting factor for the growth and reproductive capacity of *Ae. cylindrica*. A trait that increased the tolerance of the plant to water-limiting conditions could provide a mechanism for the plant to overcome barriers to water sensitivity, giving that plant a selective advantage. The significance of the interaction between treatment and accession shows that *Ae. cylindrica* accessions in the western U.S. responded differently to the water treatments with some accessions being more

sensitive than others. For example, on average the accession from Sherman County, OR produced 119.4 more spikelets per plant in the irrigated compared to the rain-fed treatment, while the accession from Scottsbluff County, NE produced 978.54 more spikelets in the irrigated treatment. These results from a single location must be considered preliminary. However, if confirmed, data such as these can be used to predict to what degree an accession will benefit if introduced into a more favorable environment or if it acquires genes that would allow it to overcome limiting conditions, such as water availability. Beneficial genes could introgress both from other *Ae. cylindrica* genotypes which have a higher performance in rain-fed conditions as well as from improved wheat cultivars (both transgenic and non-transgenic). Accessions with the highest sensitivity, which perform poorly in low environments, but well in high environments, could become invasive over a heterogeneous area if tolerance to this stress was gained. This scenario is of high risk for it produces a genotype that is invasive under both low and high productivity environments and therefore can invade a greater range of environments.

Natural and human-mediated seed dispersal within the U.S. can lead to range expansion by invasive genotypes and increased potential to hybridize between previously isolated genotypes of *Ae. cylindrica*. Hybridization among differentiated populations of the same species can act as a mechanism to increase invasiveness by increasing the genetic variation for selection to act upon leading to higher fitness (Ellstrand and Schierenbeck, 2006). A more-fit genotype introduced into a wheat-growing region is likely to increase competition with wheat and be more troublesome and costly to remove. Similarly, introducing an invasive genotype to a novel habitat could lead to ecological disturbance of the native habitat.

Interspecific hybridization between *Ae. cylindrica* and wheat, serves as an additional mechanism to intraspecific hybridization for gene flow and the introgression of advantageous genes into *Ae. cylindrica* populations. The novelty of genes available from a wheat cultivar could provide an additional source of genetic variation for this introduced species. Improvements in wheat cultivars could therefore provide a source of advantageous genes for *Ae. cylindrica* that may not be available otherwise due to a depleted gene pool, isolation of *Ae. cylindrica* genotypes, and reproductive barriers with other organisms. The possibility of gene transfer from wheat to *Ae. cylindrica*, as in other crop-weed complexes, is a potential agricultural and environmental risk. The results of this study suggest that a trait such as drought tolerance could benefit *Ae. cylindrica* populations by increasing their fitness and competitiveness. Controlling *Ae. cylindrica* within and near wheat fields would reduce the formation of interspecific hybrid plants and prevent the opportunity for gene flow to occur. Management focusing on preventing the spread of *Ae. cylindrica* seed would contain invasive genotypes and advantageous genes.

## 5. Conclusion

The main objective of this study was to evaluate the response of *Ae. cylindrica* populations to drought stress in order to assess the risk of gene flow from transgenic drought-tolerant wheat. Transgenic technology has raised awareness of the risk of interspecific hybridization and gene flow between crop species and their related weedy species. Introgression of genes conferring tolerance to environmental stress, such as drought, is particularly worrisome because it could be beneficial in both agricultural and natural ecosystems. An increase in fitness and decrease in environmental sensitivity of *Ae. cylindrica* could lead to increased competitiveness with wheat and invasion into novel habitats. Although not conclusive, the variation in sensitivity to water treatment among the accessions shown in this study suggests that the risk of introgression would not be uniform, but will be dependent upon the selective advantage that results from gaining the gene. Introducers of improved wheat cultivars should consider the risk of gene introgression and focus on preventing the formation of hybrid plants in and near wheat fields. Additionally, restricting the spread of *Ae. cylindrica* should continue to be a primary goal in order to prevent interspecific hybridization between different genotypes which is also a source for beneficial gene flow.

## Acknowledgements

Funding for this research was provided by the U.S. Department of Agriculture Biotechnology Risk Assessment Grants Program award 2007-33120-16481. We are thankful for the contributions of Dr. Nora Lapitan, Dr. Christopher Preston, Dr. Phillip L. Chapman, and Kelsi Grogan.

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