

THESIS

RISK OF GENE INTROGRESSION FROM TRANSGENIC WHEAT TO JOINTED
GOATGRASS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY BETHANY F. ECONOPOULY ENTITLED RISK OF GENE INTROGRESSION FROM TRANSGENIC WHEAT TO JOINTED GOATGRASS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

RISK OF GENE INTROGRESSION FROM TRANSGENIC WHEAT TO JOINTED GOATGRASS

Transgenic technology for cultivar improvement has heightened concern for the introgression of advantageous genes from crop species into wild relatives. Interspecific gene flow has long been proposed as a mechanism for evolution in natural populations and the specific case of complexes between crop species and their wild relatives is an extension of this theory. Gene introgression from crops may lead to increased fitness of weedy relatives, enabling the new genotype to expand its range and invade new habitats, as well as increase its competitiveness with the domesticated species.

Hybridization between bread wheat (*Triticum aestivum* L.) and the related species jointed goatgrass (*Aegilops cylindrica* Host) is of concern because the latter wild species is an agricultural weed in the western U.S. The two species share the D genome derived from a common diploid progenitor, *Aegilops tauschii* Coss. Wheat is an allohexaploid ($2n = AABBDD = 42$), while jointed goatgrass is an allotetraploid ($2n = CCDD = 28$). Interspecific hybridization is known to occur where wheat and jointed goatgrass coexist, albeit at low rates due to low frequencies of outcrossing and differences in ploidy. Evaluating the risk of gene introgression from wheat to jointed goatgrass is necessary to inform both transgenic research and weed management. For example, it has been suggested that the risk of introgression is highest for genes located on the D genome of wheat. If this were the case, selecting wheat cultivars with

the gene of interest on the A or B genome would decrease the risk of gene introgression to jointed goatgrass. The goal of this study was to assess the risk of gene introgression from wheat to jointed goatgrass and what measures can be taken to minimize this risk. Three studies were conducted analyzing the genetic diversity and structure of jointed goatgrass in the native and introduced ranges, the phenotypic diversity of the species in the western U.S., and the rates of backcrossing of interspecific hybrids to jointed goatgrass. Each of these components was used in combination to assess the risk of gene introgression from wheat to jointed goatgrass.

The first chapter used a genetic analysis to evaluate the diversity and structure of jointed goatgrass in its native and introduced range. Genotyping was conducted using six microsatellite primer pairs giving rise to eight loci for three plant collections. A sample of 165 plants from 21 Eurasian countries was used to represent a large-scale sampling of the native range. In the U.S., 96 plants representing 12 western states constituted the large-scale collection for the introduced range, while a collection of 164 plants from seven eastern Colorado populations represented a fine-scale sampling. The results provide evidence for a bottleneck in the introduced range, with the U.S. representing only 41% of the total alleles amplified in the native range. Genetic diversity is present in the U.S. and is most likely the result of multiple introduction events. Genetic diversity implies that phenotypic variation may exist in jointed goatgrass in the western U.S. If variation exists in adaptive traits the benefit of gaining a wheat gene may vary across the region depending on the resulting fitness advantage. Genetic structure was not detected in either large-scale collection from the native or introduced range inferring that the agricultural movement of seed could distribute diversity across a large geographic area. However, a significant correlation ($r=0.87$; $P=0.0049$; $n=21$) was found between geographic and genetic distance among populations sampled in the fine-scale collection from eastern Colorado, providing evidence for a pattern of isolation by distance. The different findings for the two U.S.

collections suggests that gene flow is restricted as distance increases in the fine-scale collection, while in the large-scale collection gene flow occurs over large distances. Agricultural movement of seed could provide a mechanism for long distance dispersal of seed explaining the lack of genetic structure at a large-scale. Restricting human seed dispersal is important for weed management to prevent the spread of advantageous genes and genotypes across the weed's invasive range.

The second study assessed jointed goatgrass collections from the western U.S. for phenotypic diversity using a 'common garden' experiment. Few phenotypic studies have been reported for jointed goatgrass. However, evidence for genetic diversity and multiple introduction events of the species into the U.S. suggest that phenotypic variation could exist. Variation for environmental stress tolerance, such as drought tolerance, is important in order to determine how gaining a wheat gene of this type would affect fitness of different genotypes of jointed goatgrass. Thirty accessions of jointed goatgrass from the western U.S. were evaluated in four replications of a randomized complete block design under common environmental conditions in 2008-09 in Haxtun, Colorado. Plant height, the number of tillers produced per plant, the number of spikelets per spike, and the number of spikelets per plant were measured. Results indicate significant ($P < 0.001$) variation in each of the traits by accession and by replication, and show a significant ($P < 0.0001$) interaction between accession and replication. Natural variation in fitness and in the response to environment implies that the risk for gene introgression from wheat to jointed goatgrass will not be uniform across the western U.S.

The last study determined the rate of backcrossing of interspecific hybrids to jointed goatgrass. Recurrent backcrossing to the weedy species is necessary to restore fertility and chromosome composition after hybridization with wheat. Introgression occurs at the BC_2S_2 stage. Field experiments were conducted at two locations in 2007-08 and 2008-09 with jointed

goatgrass acting as the sole source of viable pollen to transplanted hybrid plants. A total of 214 BC₁ plants were produced by 100 hybrid plants collected in 2007-08 and 249 BC₁ plants were produced by 106 hybrid plants collected in 2008-09. Median backcrossing rates of 0.062% and 0.152% with ninety-five percent confidence intervals of 0.028 to 0.306% and 0.077 to 0.604%, for Fort Collins and Haxtun, respectively, were determined. Subsequent backcrossing to the BC₂ stage would make it likely that a wheat gene conferring a selective advantage would introgress into the weedy species. The results here are significant because a field study using jointed goatgrass as the sole pollen source has not previously been reported. In addition, the study was conducted in the central Great Plains, where previous field trials have been limited, but where wheat and jointed goatgrass coexist. The results suggest that backcrossing rates could vary by environment and that multiple field studies are necessary to estimate the range of backcrossing rates across the western U.S.

Overall the studies confirm the presence of genetic diversity and provide initial evidence for phenotypic diversity in jointed goatgrass from the western U.S. Taken together these data suggest that the risk for transgene introgression will be variable across this region. It was also demonstrated that wheat by jointed goatgrass hybrids are partially fertile and produce viable seed when backcrossed to jointed goatgrass in the Great Plains. Subsequent backcrossing would provide a mechanism for the stable transfer of wheat genes or genomic regions into the jointed goatgrass genome. Lastly, the evidence for long distance gene flow at a large-scale, but not at a fine-scale, illustrates that agricultural practices play an important role in dispersing jointed goatgrass seed and could facilitate the spread of introgressed transgenes across the western U.S. Management should prioritize preventing contamination of agricultural machinery, transport vehicles, and wheat grain to minimize the spread of genetic material that can lead to

the evolution of more fit genotypes, increasing both the weed's competitiveness with wheat and its ability to invade novel habitats.

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Literature Review

Relevance of assessing transgenic risk

Interspecies hybridization and gene flow have been long proposed as a mechanism for evolution in natural populations (Anderson 1949). Gene introgression between domesticated species and their wild relatives extends this evolutionary process to agricultural systems. Twelve of the world's 13 most widely cultivated crops are known to hybridize with a related wild species (Ellstrand et al. 1999). For the plant breeder, the ability to make successful crosses between crops and their wild relatives is an advantage. These wild species are sources of novel genes and genetic variability that can be exploited for crop improvement. However, gene flow between a domesticated species and a wild relative is a reason for concern. Introgression can lead to increased fitness of weedy relatives, enabling the new genotype to expand its range and invade new habitats, as well as increase its competitiveness with the domesticated species (Arnold 1992; Ellstrand et al. 1999; Snow and Palma 1997; Stewart et al. 2003). Genetic engineering in plant breeding programs has brought attention to the risks associated with interspecific gene introgression, although this phenomenon is not unique to transgenic cultivars. Nonetheless, biotechnology has increased awareness of the risk and the need to assess the risk of commercializing transgenic cultivars.

Both conventionally bred and transgenic crops have the ability to form interspecific hybrids with wild relatives, facilitating gene introgression into the wild species. Transgenic cultivars appear to pose a greater risk. Transgenic technology uses molecular techniques to insert a gene, or stack of genes, into the plant genome. Conventional breeding relies on the use of cross-pollination to transfer genes that confer a desirable phenotype into the plant. Transgenic methods can theoretically use DNA from any organism, increasing the novelty and variation of genes available for crop improvement. This is in contrast to conventional breeding, which only has the existing genetic material in sexually compatible plants available. Transgenic technology has increased the novelty of genes available for use in crop improvement and consequently the novelty of genes that can introgress into wild relatives (Raybould and Gray 1993; Snow and Palma 1997). Herbicide resistance is an example of a novel trait that has been transgenically introduced into domesticated species with the potential for introgression into wild relatives.

Single chromosomal blocks confer the improved phenotype in transgenic cultivars (Stewart et al. 2003). With conventional crops, trait improvements are more likely the result of multiple genetic changes spread across the genome. This is especially true for complex traits under polygenic control. The transfer of a single genomic region containing the target gene from a transgenic cultivar, rather than multiple regions from a conventionally bred cultivar, is a more likely event and could confer an advantageous trait in the wild relative.

The combination of the increase in novelty of target genes and the ability to confer advantageous traits with only a single change to the genome makes it necessary to evaluate the risk of transgene introgression. Global transgenic crops have increased 80-fold since 1996 with the total area cultivated up to 134 million hectares in 25 countries (James 2009), emphasizing the need to evaluate the safety of these cultivars.

Interspecific gene flow is associated with the evolution of increased weediness in wild relatives of 7 of the world's 13 most important crops (Ellstrand et al. 1999). Examples include sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wild beet and sea beat (*Beta vulgaris* ssp. *maritima*), sunflower (*Helianthus annuus*) and wild *Helianthus* species, and sorghum (*Sorghum bicolor* L.) and johnsongrass (*Sorghum halepense*), *Sorghum alnum*, and *Sorghum propinquum* (Ellstrand et al. 1999). The ability for domesticated species to hybridize with wild relatives suggests the potential for gene introgression to occur from the crop to the weed. Movement of beneficial genes could lead to increased weediness in the wild species resulting in increased competition in agricultural settings and/or range expansion into new habitats with both economic and biodiversity implications. For example, transgenic technology has been used in the development of herbicide-resistant cultivars, contributing 62% of the total world wide area under transgenic cultivation (James 2009). Introgression of the transgene into weedy relatives leads to the evolution of an herbicide-resistant weed, making that herbicide ineffective and the transgenic cultivar no longer beneficial for management of that weed. The detrimental impact to agriculture of crop-to-weed gene flow in that case is clear. However, introgression of genes conferring beneficial traits in agricultural settings is specific to site or management practice. Of greater concern, is the introgression of genes conferring tolerance to environmental stress, such as salt or drought tolerance, which could enhance the weed's performance in both agricultural and natural habitats (Hancock 2003; Stewart et al. 2003).

Wheat has been reported to hybridize with wild *Aegilops* species and wild durum wheat subspecies in the Middle East, Africa, Europe, Asia, and North America (Ellstrand et al. 1999; Kihara 1963; van Slageren 1994). Bread (*Triticum aestivum* L.) and durum (*T. turgidum* L.) wheats have been shown to hybridize with 12 related *Aegilops* species, forming 21 crop-weed complexes (Ellstrand et al. 1999; Kihara 1963; van Slageren 1994). Hybridization between bread

wheat and the related wild species, jointed goatgrass (*Aegilops cylindrica* Host), is of greatest concern because this wild species is a weed of North America, Europe, and Asia. In North America, it is one of winter wheat's greatest pests, causing losses of \$145 million annually (Anderson 1993; Ogg 1993; Stewart et al. 2003). In the United States, the invasive species has been reported in 32 states and continues to expand its range by 50,000 acres a year (USDA, NRCS 2010; Washington State University 2008). Hybridization occurs in areas where the two species co-exist. Recurrent backcrossing to jointed goatgrass after hybridization increases fertility and restores the genome and serves as a mechanism for introgression of advantageous genes from wheat.

Development of transgenic wheat for improved cultivars is a major research priority for public and private breeding programs. Bayer CropScience recently teamed with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) with a pledge to work toward GM-wheat just days after Monsanto's announcement of its WestBred acquisition and return to investing in biotech wheat research (Fox 2009). The United States Department of Agriculture (USDA) spends \$40 million annually in biotech wheat research supporting groups such as the Agricultural Research Service (ARS) and university programs (Fox 2009). In 2008, China's government spent more money on transgenic research in wheat than for any other biotech crop and is expected to release transgenic wheat commercially in the next five years (James 2009). The Chinese Academy of Agricultural Sciences (CAAS) and Henan Agricultural University are the most notable institutes in China devoted to this cause. Research programs at India's Maharashtra Hybrid Seed Company and the Indian Agricultural Research Institutes, and Australia's CSIRO, Victorian Department of Primary Industries, and La Trobe University are also world leaders in transgenic wheat development (Fox 2009). The increase in biotech research is the result of growing acceptance and the need to increase wheat productivity. While maize (*Zea*

mays L.) yields have been able to keep pace with food demand due to large investments in biotech research, wheat production has fallen short of closing the yield gap. Whereas in 2004, Monsanto's transgenic Roundup Ready wheat was withdrawn from commercialization due to lack of grower support in the U.S., seventy-five percent of wheat growers in 2009 approved of biotech wheat (James 2009). In addition, nine wheat organizations from the U.S., Canada, and Australia agreed to make commercialization of transgenic wheat a priority.

Improving wheat production under environmental stress is necessary to increase yields and increase food security. For example, drought stress is a major factor limiting optimal wheat yields. Biotechnology is an asset for developing improved cultivars for drought tolerance. Ted Crosbie of Monsanto declared that development of drought tolerance in wheat could only be solved by biotechnology (Fox 2009). Transgenic drought tolerant maize is expected to be released commercially in 2012 (James 2009). Developed by Monsanto, the drought tolerance trait is conferred by inserting a single foreign allele from *Bacillus subtilis*, *CspB*, responsible for the production of a cold shock protein, and resulting in yield advantages in dryland environments (Castiglioni et al. 2008). With Monsanto's renewed interest in wheat, it is likely this gene, as well as others, will be investigated for use to develop transgenic drought tolerant wheat. However, Monsanto is far from being the only research group interested in improving drought tolerance in wheat. Each of the world's leading groups in biotech wheat research is investigating drought tolerance. The leader, La Trobe University in Australia, has already begun field-testing transgenic drought tolerant lines and is expected to be the first to release a cultivar of this type (Fox 2009).

Although the predicted commercial release of a transgenic drought tolerant wheat cultivar is still, at best, 8 to 10 years away, assessing the risks associated with a crop of this type is important now to avoid these risks in the future (Hancock 2003; Fox 2009). For a cultivar that

could have a large impact on wheat production and food security, any delay in its availability for use in production would be a disservice. However, the potential environmental impact of introducing a cultivar with tolerance to an environmental stress is of high risk in terms of transgene introgression to a related weedy species (Hancock 2003). Out of five transgene categories, Hancock (2003) ranks those conferring environmental tolerance among those having highest risk over those that are selectively neutral or negative in the natural environment. Monsanto's demonstrated success in improving drought tolerance, typically considered a complex trait under polygenic control, with a single gene supports the hypothesis that transgenic cultivars are of higher risk for gene introgression than their conventional counterparts. For these reasons, the high risk of introgression of genes conferring tolerance to environmental stress, combined with the ability for transgenic technology to develop cultivars with only a single genomic change, assessing the risk of introgression for transgenic drought tolerant wheat is necessary. In addition, the likelihood of its development and release makes it timely in order to ensure its safety and approval.

Jointed goatgrass impact

Jointed goatgrass is an exotic weed in the U.S. that impacts both agricultural and disturbed natural ecosystems. Herbarium records report the presence of jointed goatgrass back to 1870 in Delaware and it is speculated that introduction was via contaminated winter wheat grain from Turkey (Mayfield 1927). As an invasive weed, jointed goatgrass causes economic loss to the agricultural sector. In the western U.S., jointed goatgrass has become one of the biggest pests of winter wheat cultivation (Anderson 1993). Economic loss is caused by decreases in wheat yield from competition and by contamination of wheat grain by jointed goatgrass seed (Donald and Ogg 1991). Further, containment efforts have been unsuccessful and jointed

goatgrass seed continues to spread by movement of contaminated combines, wheat seed, and grain trucks (Donald and Ogg 1991). Its invasive range in the U.S. contains almost five million acres and has been reported in 32 western states (Ogg 1993; plants.usda.gov). The most recent publication that evaluates the impact of jointed goatgrass decreased winter wheat yield by 21% in field experiments infested at 170 plants/m² at agricultural experiment stations in Oklahoma (Fast 2009). This interference from jointed goatgrass led directly to a loss in wheat grain price due to yield decreases, dockage, and grade. Because of the detrimental impact and uncontrolled spread of jointed goatgrass, continued research is necessary to understand the evolution of this invasive species to improve management. Although the agricultural impact of jointed goatgrass is now evident, its potential to invade and affect natural ecosystems is unclear.

Genetic diversity in jointed goatgrass

Many genetic studies have been published attempting to resolve the questions surrounding the introduction history and genetic structure of jointed goatgrass in the U.S. These studies are useful for detecting genetic diversity and patterns of gene flow within the species. This information can be used to determine how seed and pollen is dispersed and how this could lead to the spread of an introgressed gene from transgenic wheat.

Okuno *et al.* (1998) found a greater diversity of jointed goatgrass in Central Asia accessions than in those of North Caucasia. A total of 112 accessions of *Aegilops* species (*Ae. tauschii* Coss., *Ae. crassa* Boiss., *Ae. cylindrica* Host, *Ae. biuncialis* Vis., and *Ae. triuncialis* L.) were evaluated. Diversity was assessed by the percentage of polymorphic fragments in each region. In Central Asia, 89.2% of the total fragments were polymorphic, while in North Caucasia that number was only 30.2%. Naghavi *et al.* (2009) confirmed the presence of genetic diversity in the native range. The study analyzed 13 sites in Iran with 21 D-genome microsatellites and found

Nei's gene diversity at 0.469 with an average of 8.6 bands per primer. The studies are conclusive for the existence of genetic diversity for jointed goatgrass in its native range and that Central Asia harbors the richest diversity for the species.

The presence of genetic diversity in the native range does not mean that genetic diversity exists in the introduced range. Additional studies must be done to assess the genetic diversity of jointed goatgrass in the U.S. The usefulness of this is two-fold. First, understanding the genetic composition of jointed goatgrass in the U.S. can be used as a predictor of phenotypic diversity. Second, a comparative analysis between the introduced and native ranges can be used to re-create the introduction history of the species into the U.S. The introduction history can reveal information such as the number of introductions, origins of introductions, and secondary sources of genetic diversity. The distribution of genetic diversity will show how genetic material is spread across its range. This information will be useful to determine whether risk of introgression will be uniform across the U.S. and whether an introgression event will be localized or will spread among populations.

Molecular marker analysis performed by Pester et al. (2003) suggests limited variation in both the native and introduced ranges. The study analyzed 58 accessions representing eight western U.S. states and 13 Eurasian countries with 30 random amplification of polymorphic DNA (RAPD) markers, and 16 accessions representing three U.S. states and 13 Eurasian countries with amplified fragment length polymorphism (AFLP) markers. Only 3% of the bands produced by RAPDs were polymorphic among the U.S. accessions. This number increased to 6.7% when both U.S. and Eurasian accessions were included. Similarly, only 5% of the bands produced by AFLPs were polymorphic for both U.S. and Eurasian accessions. Cluster analysis using both the AFLP and RAPD data detected differences in introduced accessions by state, with U.S. accessions dispersed throughout the native accessions. This suggests that either multiple genotypes were

introduced into the U.S. or that evolutionary divergence occurred after the introduction of jointed goatgrass. Using a larger sample set will allow a more thorough study of the differences and distribution of genotypes.

Looking more closely at the evolutionary history of jointed goatgrass, Gandhi et al. (2005) evaluated 20 chloroplast and 19 nuclear microsatellite markers on jointed goatgrass and its progenitors, *Ae. tauschii* and *Ae. caudata*. The analysis illustrated a reduction in genetic diversity from the progenitors to jointed goatgrass implying that only a limited number of hybridization events led to the formation of the allotetraploid. This suggests that as a species, jointed goatgrass may have little genetic diversity because of an initial founder event during its formation. However, jointed goatgrass individuals were found with chloroplast DNA derived from both parental species, indicating the possibility for multiple polyploidy events with either species serving as the female parent. Thirty-six accessions of jointed goatgrass were analyzed in total, with four accessions from four U.S. states and the remaining 32 accessions representing the native range. A neighbor-joining tree derived from chloroplast and nuclear microsatellites showed the U.S. accessions of jointed goatgrass interspersed among Eurasian samples of jointed goatgrass, supporting the presence of multiple genotypes in the U.S. The authors preliminarily concluded that the formation of jointed goatgrass most likely occurred in the center of the Fertile Crescent where the most allelic similarities were found between the diploid ancestor, *Ae. tauschii* spp. *tauschii*, and jointed goatgrass.

Additional data provided by Gandhi et al. (2006) revealed information on the genetic structure and introduction history of jointed goatgrass in the U.S. The authors concluded an overall low genetic diversity in jointed goatgrass based upon chloroplast and nuclear microsatellite work on 173 accessions of jointed goatgrass from 18 countries, including 11 U.S. states. They also concluded that a single region of the native range in Eurasia could not have

contributed to all the diversity found in U.S. accessions of jointed goatgrass. Even though only low levels of genetic diversity are present within the U.S., enough significant differences exist that the introduction of only a single genotype is not plausible. Additionally, within the U.S., Gandhi found that accessions from the Great Plains region had a higher level of diversity than any other U.S. region. This implies that this area could have been the initial site of introduction with expansion occurring via its spread to other U.S. regions. By using polymorphic microsatellite markers on a more extensive sample of Eurasian and U.S. populations a more definitive conclusion surrounding the introduction of jointed goatgrass into the U.S. can be made.

More recently Gandhi et al. (2009) published new findings on the genetic diversity and structure of jointed goatgrass. The authors analyzed 76 U.S. and 97 Eurasian accessions with 24 nuclear and 20 chloroplast microsatellite markers. The results show that the highest levels of nuclear diversity for the species is in Eastern Turkey and Transcaucasia (Georgia, Armenia, Azerbaijan, and Daghestan), with the highest level of chloroplast diversity in Northwestern Iran. Two plastome types, D-type and C-type, were present, supporting the results from 2005. In a nuclear neighbor-joining tree, accessions of each plastome type clustered together illustrating the similarity of these plastome types at the nuclear level. The finding of two plastome types supports multiple hybridization events in the formation of jointed goatgrass. Interestingly, the more uncommon C-type plastome was found at a larger percentage in the U.S., with 24%, than in the native range, with only 3%. This suggests either that the introduction of jointed goatgrass included a disproportional amount of C-type plastome accessions from the native range or that selection pressure in the new habitat favored this plastome type. Additionally, the finding infers that genetic diversity exists in the U.S., giving potential for phenotypic diversity among genotypes.

Gandhi et al. (2009) also provide new clues into the genetic structure of jointed goatgrass, which had not been reported in any previous studies. Using an analysis of molecular variation (AMOVA) on the entire dataset of both the introduced and native ranges, the authors found that 84% of the genetic diversity is contained within regions while only 16% can be contributed to among region variation. Regions were arbitrarily formed based on geographic origin of accessions. The U.S. was divided into three regions while the native range was divided into seven regions. This indicates that movement of genotypes between these regions is occurring, creating heterogeneity.

The introduced and native accessions were further analyzed by Gandhi et al. (2009) using the program Structure (Pritchard et al. 2000). The program detected three clusters, K, for the entire distribution of the species. The greatest membership of Eurasian accessions was to K=1 and K=3, while in the U.S. membership was greatest for K=2 and K=3. Again, these differences in genetic composition of the introduced range and the native range are suggestive of patterns of introduction and founder events or selection of better suited genotypes in the introduced range. The evidence provides further support for genetic diversity of jointed goatgrass in the U.S. A nuclear neighbor-joining tree supports the Structure clusters. However, the tree suggests five groups of U.S. accessions, with three clustering together with minimal interspersions of Eurasian accessions. The remaining two groups are separated by the other three, and from each other, by native accessions. The neighbor-joining tree does not provide evidence of grouping by geographic regions within ranges, supporting the results of the AMOVA.

Previous genetic analyses have provided insights into the diversity and structure of jointed goatgrass in its native and introduced ranges. It is apparent that there is a decreased level of diversity in the introduced range, but there is evidence that multiple genotypes and plastome types exist in the U.S. The structure of this genetic diversity is unclear. Analyses of the

introduced and native range separately would reveal whether patterns of genetic structure are the same in the U.S. as in Eurasia. This will provide information on how the diversity is distributed across the U.S. and if different management techniques to control and prevent gene escape to the weed would be necessary. Sampling of jointed goatgrass at the population level is needed to further investigate if genetic structure exists at a smaller scale than by the geographic regions used by Gandhi et al. (2009). Last, it is necessary to couple genetic diversity with phenotypic expression. Only if genetic diversity results in differences in trait expression will it lead to variation in the risk associated with gene escape from a transgenic drought tolerant wheat.

Phenotypic diversity in jointed goatgrass

Common garden studies have been carried out on jointed goatgrass populations collected across Bulgaria (Zaharieva et al. 2003). Results indicated phenotypic variation for 11 of the 12 morphological traits evaluated. Correlation between collection site and phenotype was modest, but significant eco-geographic correlation was found for leaf width and length, as well as plant height. Additionally, greater vegetative growth observed by plant height, leaf length, and leaf width were found for populations originating from high rainfall versus low rainfall areas. Collections of nine *Aegilops* species by Zaharieva et al. (2004) across Bulgaria revealed further ecological information about jointed goatgrass in its native range. Their study concluded that jointed goatgrass, along with Lorent's goatgrass (*Ae. biuncialis*) and barbed goatgrass (*Ae. triuncialis*), had the widest distribution across Bulgaria with the largest population sizes. Jointed goatgrass was not present in the most extreme southerly region of Bulgaria where the climate is characterized by warm winters and dry summers, but was most frequently found north where winter and autumn rainfall is low and summer rainfall is high. The results suggest that jointed

goatgrass has the ability to invade ecologically diverse environments, but is best adapted to high rainfall climates with a tolerance to low temperatures.

Several studies have also been conducted for jointed goatgrass in the western U.S. Gealy (1998) concluded that 11 accessions from nine U.S. states were not different overall for germination, growth, CO₂ fixation, and water use. However, differences among accessions over two years in common garden studies were found for awn length, plant height, and dry matter. A comparison between accessions from Colorado and Nebraska for gas exchange revealed significant differences, with Colorado having higher rates of gas exchange, but Nebraska accessions outperforming in growth nevertheless (Gealy 1998). Fandrich et al. (2006) also found variation in jointed goatgrass populations in the western U.S. Germination studies conducted on six populations from Oregon and Washington revealed differences in the minimum number of vernalization days required to produce germinable seed. The authors concluded that polymorphisms for vernalization genes must exist and that these polymorphisms are environment specific.

Evaluating the phenotypic diversity and invasive potential of jointed goatgrass in the western U.S. is important for assessing the risk for introgression of drought tolerance into these populations. Diversity will imply that risk may vary among genotypes and therefore management may need to be adjusted on a case-by-case basis. Different levels of drought tolerance would suggest that the increase in frequency of a drought tolerant gene in a population will differ depending on the selection pressure for the trait. Genotypes with low tolerance to drought will experience a higher selection pressure for drought tolerance and introgression will be more likely to occur. These populations may need to be managed more intensely because they will become more invasive in low rainfall areas if drought tolerance is acquired, both in competition with wheat and in spreading its range into other ecosystems.

Hybridization between wheat and jointed goatgrass

Jointed goatgrass owes its success as an agricultural weed in part to its shared ancestry with wheat. Jointed goatgrass is an allotetraploid ($2n = CCDD = 28$) and shares the D genome with hexaploid wheat ($2n = AABBDD = 42$). The D genome of both species was derived from the diploid progenitor *Aegilops tauschii* Coss. This common ancestry gives way to the crop-weed complex between wheat and jointed goatgrass. The two related species share physical attributes, such as spikelet arrangement on the spike, seedhead height, and seed cross sectional area, as well as similarity in vernalization response, germination, and development phenology (Donald and Ogg 1991). Phenotypic similarity is to the advantage of the weed. For example, similarity in seedhead height with wheat enables it to be harvested alongside wheat, facilitating its spread and planting, and thus its reproductive success.

The genetic relationship between wheat and jointed goatgrass also gives the two species the ability to hybridize, creating a mechanism by which gene flow and co-evolution can occur. It has been shown that hybrids between winter wheat and jointed goatgrass form naturally under field conditions. Guadagnuolo et al. (2001) reported a range of hybridization rates between 1 and 7%, Morrison et al. (2002a) reported between 0.3 and 8%, Stone and Peeper (2004) reported rates of 0.074 and 0.078%, and Gaines et al. (2008) reported a rate between 0.1 and 1.6%. Conclusions from these studies suggest that rates of hybridization vary depending upon the wheat and jointed goatgrass genotype and/or the environment of infestation. Genetic factors, such as flowering time, and environmental factors, such as temperature, wind, and density of plants, will influence the rate at which hybridization occurs. Interspecific hybridization is the first step required for gene flow to occur and the frequency at which this occurs is important for determining the occurrence of gene introgression. These rates, between 0.074 and 8%, indicate that in wheat fields infested with jointed goatgrass

hybridization will occur. Whether advantageous genes will move into jointed goatgrass populations, however, is dependent not only on the ability for the initial hybridization event to occur, but on the success of advanced backcross generations and the retention of foreign genetic material.

F₁ hybrids between winter wheat and jointed goatgrass have been shown to be male sterile and therefore self infertile (Mallory-Smith et al. 1996; Zemetra et al. 1998). However, a very low female fertility exists and this serves as a mechanism for backcrossing to occur on the hybrid plant. Recurrent pollination by jointed goatgrass on hybrids to form advanced backcross plants, provides a bridge by which fertility can be restored and gene flow can occur from the wheat genome to that of jointed goatgrass. Collections of hybrid spikes from infested areas revealed seed production at rates of 1% (Morrison et al. 2002b) and 1.6% (Morrison et al. 2002a). Zemetra et al. (1998) reported 2% female fertility of the F₁ hybrid in greenhouse studies using both wheat and jointed goatgrass as the pollen donor, similar to their rate found in the field of 2.2%. Snyder et al. (2000) reported a rate of 2.3% and 3.8% over two years of field studies and Stone and Peeper (2004) report rates at 0.42%, 0.97%, and 1.10% over 3 years, again with both wheat and jointed goatgrass serving as the pollen donor. Greenhouse studies have reported various rates of female fertility, using jointed goatgrass pollen, on the hybrid plants with rates found at 1.3% (Mallory-Smith et al. 1996), 0.87% (Wang et al. 2001), and from 0.03% to 0.6% with a mean of 0.2% (Schoenenberger et al. 2006).

Overall these reports show a fertility rate of F₁ hybrids between 0.03% and 3.8%. In order to assess the risk of transgene escape, a conclusive study is needed to assess the rate of backcross seed production on hybrid plants under natural conditions in the field, with a large number of hybrid plants for statistical power, and with only jointed goatgrass serving as the pollen donor. Field studies to date have been done under mixed populations of jointed

goatgrass and wheat with rates of seed production on the hybrid being a result of pollination by either wheat or jointed goatgrass. Similarly, rates determined from collection material in wheat production areas could also be the result of pollination by wheat. Since gene introgression into jointed goatgrass populations will only occur by recurrent backcrosses to jointed goatgrass, it is imperative to understand the rate at which this phenomenon occurs. Greenhouse studies fall short in assessing this because crosses are performed artificially, sometimes with the aid of gibberellic acid or embryo rescue techniques (Zemetra et al. 1998), and with a small number of hybrid plants. Therefore, these results may overestimate the fertility rate under natural conditions.

After the first backcross by jointed goatgrass to the F_1 hybrid a second backcross is required for self-fertility to be restored. Zemetra et al. (1998) concluded that only two crosses are required after the hybrid formation for partial self-fertility to be restored. Wang et al. (2001) demonstrated self-fertility in the BC_1 generation at a rate of 0.06%. Additionally, Schoenenberger et al. (2006) reported self-fertility in two BC_1 plants at rates of 0.16 and 5.21%. BC_1 plants have been shown to have 5.1% (Zemetra et al. 1998), and 4.4% (Wang et al. 2001) female fertility. Morrison et al. (2002) state that self-fertility begins to be restored as early as the BC_2 generation. BC_2 plants jump quickly to 37.4% and 20.9% (Zemetra et al. 1998) and 18% and 6.9% (Wang et al. 2001) female and self-fertility. Selfing of the BC_2 plant produces jointed goatgrass plants with restored genomes. Preliminary data from Wang et al. (2001) showed selfed progeny of BC_2S_1 plants with 28 or 29 chromosomes, Schoenenberger et al. (2006) showed BC_1S_1 plants with 28 to 31 chromosomes, and Perez-Jones et al. (2006a, 2006b) showed BC_2S_2 plants with 28 chromosomes. It is at this stage, where the fertility and genome are restored, that introgression of foreign DNA is stable.

Gene introgression from wheat into jointed goatgrass has been shown to occur experimentally. Wheat-specific RAPD fragments were used by Schoenenberger et al. (2005) to illustrate that DNA from any of the wheat genomes can introgress into BC₁ plants. Resistance to strawbreaker foot rot, caused by *Pseudocercospora herpotrichoides* (Fron) Deighton, was shown to introgress into the genome of jointed goatgrass from the wheat cultivar 'Madsen' when hybrids were formed and reproduced to the BC₂S₂ generation (Perez-Jones et al. 2006a). Additionally, herbicide tolerance was shown to introgress into the BC₂S₂ generation after crossing with imidazolinone-resistant winter wheat (Perez-Jones et al. 2006b). The *Imi1*, conferring imidazolinone resistance, and *Pch1*, conferring resistance to strawbreaker foot rot, genes are located on the D genome of wheat. Transfer of the genes can occur via recombination of homologous chromosomes of wheat and jointed goatgrass. However, these studies were conducted using hand crosses in a greenhouse and whether introgression occurs under field conditions through natural pollination and seed production is unclear (Ellstrand et al. 1999). Herbicide resistance has been identified in hybrid plants produced in field experiments, confirming the ability for gene flow to occur between wheat and jointed goatgrass (Gaines et al. 2008; Hanson et al. 2005). In an experimental plot, Seefeldt et al. (1998) found two herbicide resistant hybrids that produced BC₁ plants also carrying the herbicide resistance. The presence of foreign genes at these initial stages does not mean that introgression will occur. Introgression of a wheat gene into jointed goatgrass has not been shown in either collections or field experiments. Field studies that follow the progression of gene transfer to the BC₂S₂ stage will provide evidence that introgression occurs under natural conditions.

Objectives and Goals

The overall objective of this study is to assess the risk of introgression from wheat to jointed goatgrass in the western U.S. with the goal of evaluating the risk of commercialization of a transgenic drought tolerant wheat cultivar. The analysis will be based upon the barriers of interspecific hybridization and recurrent backcrossing to jointed goatgrass, the ability for the gene to spread by seed and pollen movement, and the effect of the transgene on the fitness of the weed, all factors vital for risk assessment (Ellstrand et al. 1999; Raybould and Gray 1993; Snow and Palma 1997; Stewart et al. 2003). Genetic diversity and gene flow within the weed species will be analyzed by genotyping three jointed goatgrass collections spanning the native and introduced range using microsatellite markers. A phenotypic study will be conducted to investigate the fitness of 30 western accessions of jointed goatgrass under wet and dry treatments. Last, a rate of backcrossing to jointed goatgrass on wheat by jointed goatgrass hybrids will be determined by conducting field trials in two locations in Colorado.

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CHAPTER 1

Variation in genetic structure of jointed goatgrass collections reveals agriculture impact on gene flow

Abstract

Jointed goatgrass (*Aegilops cylindrica* Host) is an exotic weed introduced into the western U.S. from Eurasia. The occurrence of interspecies hybridization between jointed goatgrass and wheat (*Triticum aestivum* L.) provides a mechanism for the introgression of advantageous wheat genes into the weedy species. This can act as a means for the accumulation of novel alleles, loci, and larger chromosomal segments, increasing the species' competitiveness and ability to expand its range. Genotyping was conducted using six microsatellite markers for three collections of jointed goatgrass. A sample of 165 plants from 21 Eurasian countries was used to represent a large-scale sampling of the native range. In the U.S., 96 plants representing 12 western U.S. states constituted a large-scale collection, while a collection of 164 plants from seven populations in eastern Colorado represented a small-scale sampling for the introduced range. The results provide evidence for a bottleneck in the introduced range with the U.S. representing only 41% of the total alleles present in the native range. However, genetic diversity is present in the U.S. and is most likely the result of multiple introduction events. Diversity implies phenotypic variation could exist in jointed goatgrass in the western U.S., making the risk for introgression of wheat genes variable across the region. Genetic structure was not detected in either large-scale collection from the native or introduced range, suggesting that the

agricultural movement of seed could distribute diversity across large geographical areas. A significant correlation ($r=0.87$; $P=0.0049$; $n=21$) was found between geographic and genetic distance among populations sampled in the small-scale collection, providing evidence for a pattern of isolation by distance suggesting natural mechanisms of gene flow could be restricted by geographic distance. The results indicate that restricting agricultural movement of jointed goatgrass seed would limit gene flow at a large-scale preventing the spread of advantageous genes and genotypes across the weed's invasive range. Unrestricted gene flow would increase the risk of transgene introgression by facilitating the spread of the gene.

Abbreviations: Analysis of molecular variation (AMOVA), Heterozygosity (H_E), polymerase chain reaction (PCR), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), unweighted pair group method with arithmetic mean (UPGMA).

Jointed goatgrass (*Aegilops cylindrica* Host) is a problematic agricultural weed in the western U.S. introduced from Eurasia. It is hypothesized that this invasive grass was introduced from Turkey as a contaminant of winter wheat grain (*Triticum aestivum* L.) towards the end of the 19th century (Mayfield 1927). Lack of containment through the movement of contaminated combines, grain trucks, and wheat seed has allowed the weed to increase its range by 50,000 acres a year across the U.S. (Donald and Ogg 1991; Washington State University 2008; Ogg 1993). The closely related weed primarily infests wheat fields, where it is one of wheat's biggest pests, resembling wheat at the seed and seedling stages. It is also found in disturbed ecosystems, such as along roadsides (Anderson 1993). Jointed goatgrass is estimated to cause losses of \$145 million annually due to decreases in wheat yield from competition and through the contamination of wheat grain (Donald and Ogg 1991; Ogg 1993). Recently, Fast et al. (2009) showed a 21% yield reduction in wheat grown at experimental stations in Oklahoma due to competition with jointed goatgrass. The interference by jointed goatgrass caused a decrease in wheat grain price due to loss in grain yield, dockage, and grade. The expanding area of infestation and detrimental economic impact of this invasive weed demands that research advancing the management of this species continues.

Wheat and jointed goatgrass are both allopolyploid species in the Triticeae tribe. Wheat is an allohexaploid ($2n = AABBDD = 42$), while jointed goatgrass is an allotetraploid ($2n = CCDD = 28$). The two species both contain the D-genome derived from a common diploid progenitor, *Aegilops tauschii* Coss, allowing hybridization to occur albeit at low rates due to low frequencies of outcrossing and differences in ploidy levels. Hybridization between the two species has been shown to occur at rates between 0.074 and 8% (Gaines et al. 2008; Hanson et al. 2005; Guadagnuolo et al. 2001; Morrison et al. 2002; Stone and Peeper 2006). These hybrids are self-incompatible and have very low rates of female fertility, estimated between 0.02 and 2.2%

based on manual pollination in the greenhouse (Mallory-Smith et al. 1996; Schoenenberger et al. 2006; Wang et al. 2001; Zemetra et al. 1998) and between 0.42 and 3.8% in field trials (Morrison et al. 2002; Snyder et al. 2000; Stone and Peeper 2004). Hybrid plants have 35 chromosomes with genomes from both parents (ABCDD) making for unequal chromosome pairing of the A, B, and C genomes during meiosis (Mallory-Smith et al. 1996). Recurrent backcrossing to either a wheat or jointed goatgrass pollen donor will restore the normal genome and fertility of the plant. Introgression is not achieved until genes are introduced into the genome of stable progeny. For jointed goatgrass both self-fertility and a normal genome ($2n = CCDD = 28$) are restored at the BC_2S_2 stage (Mallory-Smith et al. 1996; Morrison et al. 2002; Perez-Jones et al. 2006a, b; Schoenenberger et al. 2006; Wang et al. 2001; Zemetra et al. 1998). It is not until this stage that introgression is achieved.

Common ancestry and crop mimicry has given rise to common physical attributes in jointed goatgrass and domesticated wheat, such as seedhead height, similarity in vernalization response, germination, and development phenology (Donald and Ogg 1991). Phenotypic similarity is to the advantage of the weed. For example, similarity in seedhead height with wheat enables it to be harvested alongside the crop, facilitating its spread and planting, and thus its reproductive success. Gene flow from wheat to jointed goatgrass appears to be advantageous because it provides one mechanism for crop mimicry to evolve in the weed species.

The potential release of new wheat cultivars has raised concern about the ability for novel traits to move into populations of jointed goatgrass that could increase its competitiveness and ability to invade new environments. Both fungal and herbicide resistances have been shown experimentally to introgress into the genome of jointed goatgrass following hybridization and backcrossing (Perez-Jones et al. 2006a,b). In order to assess the risk of

introgression under natural field conditions an understanding of gene flow, both between wheat and jointed goatgrass and among different populations of the weed, is necessary.

Several studies of genetic diversity have been used as a method to determine the introduction history of jointed goatgrass into the U.S. (Gandhi et al. 2005, 2009; Pester et al. 2003). Genetic diversity suggests the possibility of multiple introduction events and that the risk of introgression from wheat to jointed goatgrass could be variable in the U.S. A single introduction would imply uniformity of risk under comparable environments. If variation is present, differences in the rates of initial hybridization between wheat and jointed goatgrass, as well as in the rates that recurrent backcrossing to jointed goatgrass occurs, would affect the likelihood of gene introgression. In addition, variation in the selective advantage for a given wheat gene in different jointed goatgrass populations would require that risk assessment be done on a case-by-case basis.

Analysis of genetic diversity organization is also an important tool for determining how variation in the species is distributed and at what distances gene flow occurs. Gene flow in jointed goatgrass occurs by the movement of seed followed by outcrossing. The distance that seed is dispersed will determine how genetic diversity is structured. Variation in the likelihood of introgression determined by the genotypic level may equate to small-scale or large-scale variation depending on the genetic structure. Once a gene has introgressed from wheat into a population of jointed goatgrass, gene flow within the species will enable the gene to spread where a selective advantage exists. Patterns of seed dispersal and pollen-mediated gene flow will determine whether introgression will be an isolated event or whether advantageous genes will move across the range of jointed goatgrass. For example, a pattern of isolation by distance would suggest that gene movement between populations is restricted and, therefore, the spread of advantageous traits would be limited. Introgression in this case would be an isolated

event. In contrast, lack of population structure would imply that advantageous traits would move quickly among populations where selection pressure exists, spreading introgressed genes across areas infested with jointed goatgrass. The distance at which gene flow occurs will be informative for determining the range over which introgressed genes can spread across populations. Thus, understanding the genetic diversity and structure of jointed goatgrass in the western U.S. will provide a foundation for evaluating the risk for gene introgression.

Previous studies have been conducted analyzing the genetic diversity of jointed goatgrass in both its native range in Eurasia and its introduced range in the U.S. Genetic diversity has been detected with nuclear microsatellite and random amplification of polymorphic DNA (RAPD) markers in the native range of Eurasia (Naghavi et al. 2009; Okuno et al. 1998). Studies using RAPD markers and amplified fragment length polymorphism (AFLP) markers and α -amylase isozymes concluded limited, if any, genetic diversity is present in the U.S. (Pester et al. 2003; Watanabe et al. 1994). However, neighbor-joining trees created from analyses with chloroplast and nuclear microsatellite markers showed U.S. accessions interspersed among Eurasian accessions (Gandhi et al. 2005). This implies the introduction of multiple genotypes into the U.S. Further work by Gandhi et al. (2009) using a larger sample size has confirmed the hypothesis that multiple introductions have been made into the U.S., if only representing a subset of the diversity present in the native range.

Research investigating the genetic structure of jointed goatgrass across its range has been limited. Gandhi et al. (2009) found that the majority of diversity for the species is contained within, rather than among, geographic regions in both the introduced and native ranges. No clear relationship was found between genotype and geographic location. The scope of the study was limited to a large-scale analysis. As an agricultural weed, it is not surprising that the movement of seed within the introduced and native ranges has prevented genetic isolation.

However, for a predominately selfing species, investigating the genetic structure at a smaller geographic scale will provide information on natural mechanisms of gene flow via pollen and seed dispersal. The distance at which gene flow occurs within and among populations is informative for determining how an introgressed gene can spread. Hybridization between jointed goatgrass plants through cross-pollination followed by dispersal of seed is necessary. Both natural and human mediated mechanisms of gene flow should be taken into account to determine how an introgressed gene can spread. To do this, genetic structure needs to be analyzed at multiple scales of sampling in order to detect patterns of gene flow across large geographic areas, as well as at a fine-scale within and among populations.

Here, the genetic diversity and structure of jointed goatgrass, in both the native and introduced range, were analyzed to evaluate the risk of introgression from wheat to populations of jointed goatgrass across its invasive expanse in the western U.S. Eight nuclear microsatellite loci were used to analyze a large-scale collection of 96 U.S. plants and 195 Eurasian plants of jointed goatgrass. In addition, 164 plants collected in a wheat-growing region near Sterling, Colorado, serving as a small-scale collection of jointed goatgrass, were analyzed to investigate population level diversity and structure. The following questions were addressed: (i) How does the genetic diversity of jointed goatgrass in the introduced range of the western U.S. compare to the diversity in the native range of Eurasia? and (ii) How is genetic diversity structured in the native and introduced ranges at multiple geographic scales?

Materials & Methods

Plant Material

The large-scale U.S. collection was composed of 96 jointed goatgrass plants from 51 accessions sampled from 12 western U.S. states. Two plants were included for most locations and a single plant was used for six locations. These statewide collections of U.S. accessions were obtained from field collections held by academic institutions (Appendix 1, Table A1.1). The native range collection consisted of 195 individual plants from 21 Eurasian countries ranging as far west as France and as far east as Turkmenistan. Eurasian accessions span the native range of jointed goatgrass and were obtained from the USDA National Small Grains collections and the Institute of Plant Genetics and Crops Plant Research Gatersleben, Germany (Appendix 1, Tables A1.2 and A1.3). Accessions from both the large-scale U.S. collection and Eurasian collection were grouped together into three regions based on geographic proximity to one another (Table 1.1).

A small-scale, population level collection was made near Sterling, CO. Seed was collected from seven populations over a range of 7.8 km (Table 1.2). A population was defined by a cluster of jointed goatgrass plants residing in isolation from other jointed goatgrass plants. Four populations (Sterl-2, -3, -4, and -5), all within 1 km, were collected in a depression in the landscape where water drains after heavy rain. Three additional populations at incrementally further distances (up to 7.8 km) were sampled. These contain two populations bordering wheat fields (Sterl-1 and Sterl-7) and one population found alongside a road (Sterl-6). Seed was collected from 30 individual plants for each population. Plant tissue from 164 plants that germinated from the total 210 seeds was used for DNA extraction.

DNA extraction and polymerase chain reaction

Approximately 150 mg of leaf tissue from each accession was collected from greenhouse grown seedlings at the two to three leaf stage for DNA extraction. Leaf tissue was lyophilized and then ground for 6-10 minutes in a Skandex Shaker SO100 (Fast & Fluid Management, Sassenheim, The Netherlands) with three 1.9 mm ball bearings in 2 ml Eppendorf tubes. DNA was extracted from ground tissue according to the protocol of the ChargeSwitch gDNA plant kit (Invitrogen Corporation, Carlsbad, CA).

Six microsatellite primer pairs (Table 1.3) that amplified eight polymorphic loci in the large-scale U.S. collection were chosen from a preliminary screening of 145 primer pairs available from the GrainGenes database (<http://wheat.pw.usda.gov>). PCR reactions for primers gwm190, gwm301, and gwm383 (Roder et al. 1998; Gandhi et al. 2005) were carried out in a 15 μ l volume containing 10X Taq reaction buffer with 7.5 unit 20mM Tris-HCl, 10mM $(\text{NH}_4)_2\text{SO}_4$, 10 mM KCl, 2mM MgSO_4 , and 0.1% Triton X-100 (New England Biolabs, Ipswich, MA), 0.125 mM dNTP, 0.34 μ M of the reverse primer, 0.085 μ M of the forward primer, a M13 fluorescent tail, and 1.5 unit Taq DNA polymerase. PCR conditions consisted of an initial denaturation at 94° for 5 min followed by 35 cycles of 94° for 30 sec, annealing at either 55° or 60° (see Table 2.2) for 59 sec, 72° for 20 sec, and a final extension at 72° for 10 min.

The forward primer for each of cfd48, cfd72, and cfd79 (Guyomarc'h et al. 2002) were fluorescently tagged and PCR reactions were carried out in 25 μ l containing 12.5 unit 10X Taq reaction buffer, 0.3125 mM dNTPs, 0.5 μ M of each primer, and 1.5 unit of Taq DNA polymerase. PCR conditions consisted of an initial denaturation at 94° for 5 min followed by five cycles of 94° at 30 sec, an annealing temperature at 65°, decreasing by 1° at each cycle, for 30 sec, 72° for 30 sec followed by 25 cycles of 94° for 30 sec, an annealing temperature of 60° for 30 sec, 72° for 30 sec, and a final extensions at 72° for 10 min.

Amplified products were separated on an ABI 3130x/ sequencer (Applied Biosystems, Foster City, CA). Fragments were sized and scored according to number of base pairs using GeneMapper v 4.0 and ABI GeneScan 500 LIZ size standard.

Statistics

All statistical analyses used allele size data except for analysis with NtSysPC ver. 2.1 (Applied Biostatistics Inc.; Rohlf 2000), which required the data to be transformed into a presence/absence matrix. Eight polymorphic loci for the large-scale U.S. and Eurasian collections and six polymorphic loci for the small-scale collection were used in all analysis with the exception of gene diversity analysis using Powermarker v 3.25 (Liu and Muse 2005) in which all eight loci were used for all collections. Gene diversity values were calculated using Powermarker software. Mean values of gene diversity, or unbiased expected heterozygosity (H_E) (Nei 1978), across all eight loci are reported. The frequency of heterozygosity was calculated as the number of plants heterozygous for at least one locus divided by the number of total plants in that collection. Cluster analysis was done using NTSysPC software ver. 2.1 using the Dice-Coefficient similarity index and Unweighted Pair Group Method with the Arithmetic Mean (UPGMA) distance calculation. Analysis of molecular variance (AMOVA) tables were generated using Arlequin ver. 3.11 (Excoffier 2005) and corresponding p-values are based on 1023 permutations.

Genetic structure was analyzed for both U.S. collections using the software program Structure ver. 2.3 (Pritchard et al. 2000; Falush et al. 2003; Falush et al. 2007; Hubisz et al. 2009). Allele size data for large and small-scale U.S. collections were used. Since jointed goatgrass is a selfing species the model was run under an assumption of no admixture. Simulations were run using a burn-in length of 100,000 and Markov Chain Monte Carlo (MCMC) length of 50,000 with

five iterations at each K . Values of K from 1 to 25 for large-scale and 1 to 15 for small-scale were analyzed. Structure Harvester ver. 0.3 was used to determine the optimum number of clusters, K , in the U.S. dataset. The method is based upon the second order rate of change in the log probability between each K analyzed by Structure with the modal value at the optimal K (Evanno et al. 2005; Earl 2009). CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007) was then used to align the cluster membership coefficients for each replication of K . Output from CLUMPP was used in Distruct ver. 1.1 (Rosenberg 2004) to create the corresponding figures.

A Mantel test (Mantel, 1967) for the Sterling collection was run using Genalex ver. 6.3 (Peakall and Smouse 2006; Sherwin et al. 2006) to test for isolation by distance between populations. Matrix of genetic distance according to F_{st} , calculated in Arlequin ver 3.11, was correlated with a matrix of geographic distance in km.

Results

Genetic Diversity

Eight polymorphic loci were amplified in the large-scale U.S. and Eurasian collections of jointed goatgrass with the six microsatellite primer pairs used in this study. A total of 88 alleles were amplified in these two collections, 85 in the Eurasian collection and 35 in the U.S. collection. Of the total alleles amplified, 60% of these alleles were unique to Eurasia, while only 3% were exclusive to the U.S. In the native range, 15.4% of plants were heterozygous for at least one locus, while in the U.S. large collection this number was reduced to only 7.3%. The average gene diversity was found to be higher in the native range ($H_E=0.5423$) than in the introduced range ($H_E=0.4136$) (Table 1.1). Gene diversity values were also determined for all regions within both of these ranges. In Eurasia, the highest value of gene diversity was found in Central Asia ($H_E=0.6147$), while the lowest value was found in South Caucasus with $H_E=0.5045$. In the U.S., the greatest diversity was found in the Great Plains ($H_E=0.4026$), while the Intermountain region had the lowest diversity ($H_E=0.2881$).

In the small-scale U.S. collection from Sterling, CO, only six of the eight loci were polymorphic and only 26% of the total alleles from both ranges were amplified. Only 0.6% of plants were heterozygous for at least one locus. The average gene diversity was 0.1676, lower than both the large-scale U.S. and Eurasian collections (Table 1.2). The four geographically closest populations from this collection, Sterl-2, 3, 4, and 7, showed very low levels of diversity with $H_E=0.0981$, 0.0391, 0.0586, and 0.0709, respectively. Populations Sterl-1 and Sterl-6, the two collections at the outermost sites of the collection range, showed the greatest levels of diversity with $H_E=0.1914$ and 0.2739, respectively.

Genetic Structure

Eurasia and large-scale U.S. collections

The genetic diversity of each collection in this study was analyzed to determine how genetic diversity is organized in jointed goatgrass. An analysis of molecular variance (AMOVA) was run to compare the large-scale U.S. and Eurasian collections (Table 1.4). The results show that the majority of the total genetic diversity in these two ranges is explained by diversity present within the ranges, 85%, with only 11% of diversity explained by differences between the two ranges ($P < 0.001$). The native range contains the most alleles, with 97% of the total number of amplified alleles present there compared to only 40% in the introduced range. In addition, the native range contains 60% unique alleles.

Further analyses were done within each collection. In the Eurasian collection, variation was partitioned by region and by country. An AMOVA shows that the greatest amount of variation, 86%, is explained within countries, the smallest geographic scale used for this range (Table 1.5). Only 3% of the total Eurasian diversity is explained by variation resulting from comparing regions, and only 11% by comparing countries within these regions.

The large-scale U.S. collection was analyzed by region and by state. A similar pattern to the Eurasian structure was revealed: the largest amount of variation, 70%, is contained within U.S. states, again the smallest geographic scale used for the collection (Table 1.6). Comparing regions within the U.S. explained the smallest amount, 3%, of variation while comparing states within regions explained 26% of the variation.

The genetic structure of U.S. accessions of jointed goatgrass was further evaluated using the software Structure. When ancestry memberships for each cluster, K , from Structure were analyzed using Structure Harvester, $K=3$ was the optimum for the large-scale U.S. collection

(Figure 1.1, Table A1.4). Overall K1 has the highest membership, with 42% of individuals belonging to this cluster. The remaining individuals are equally distributed between clusters K2 and K3, with 30% and 29% membership. Each of these clusters is present in each of the three U.S. regions, although in different proportions. For regions 1 (Great Plains) and 2 (Pacific Northwest), individuals belong to K1 at the greatest proportions, 43% and 74%. For region 3 (Intermountain Region), the greatest proportion of individuals (47%) belong to K3. The presence of each cluster across the U.S. regions supports the AMOVA results that the majority of the diversity in the U.S. is partitioned within, rather than among, these regions.

A distance analysis was run for the large-scale U.S. and Eurasian collection to determine the similarity between accessions from the introduced range of jointed goatgrass with those of the native range. Cluster analysis from the UPGMA shows that geographic regions of the U.S. do not necessarily cluster together. Instead, U.S. accessions are dispersed throughout both the introduced and native ranges and cluster with accessions from both other U.S. regions, as well as accessions from the native range (Figure 1.2). Eleven clusters can be drawn with Dice-Coefficients less than or equal to 0.50. U.S. individuals are grouped across four of these clusters, while Eurasian accessions can be found in all but one cluster. The distribution of U.S. accessions shows no consistency in geographic origin confirming the results from the AMOVA and Structure analyses.

Small-scale U.S. collection

For the small-scale U.S. collection an AMOVA was used to look at variation among and between populations of jointed goatgrass. The results show the majority of variation, 70%, attributed to within population diversity with 30% explained by among population variation (Table 1.7).

An analysis using Structure was also performed for the small-scale U.S. collection. $K=2$ was optimal for this collection (Figure 1.3, Table A1.5). Overall, a very high percentage, 86%, of individuals belong to the S1 cluster. Three populations sampled within the smallest distance for the collection, Sterl-2, -3, and -4, only contain individuals with ancestry membership in the S1 cluster. Sterl-1 has five individuals and Sterl-5 and Sterl-7 each have one individual belonging to the S2 cluster. Population Sterl-6 is the only population that contains a majority, 67%, of individuals belonging to the S2 cluster. Again, the two populations representing the furthest points in the collection show a clear distinction from the remaining populations.

Cluster analysis from the UPGMA was also performed on the small-scale U.S. collection of jointed goatgrass (Figure 1.4). Three clusters are formed with Dice-Coefficients less than or equal to 0.50. The first smallest cluster contains only six individual plants with five of these plants originating from Sterl-1 and one from Sterl-6. The second cluster contains plants from Sterl-5, -6, and -7. The third and largest cluster contains plants from every Sterling population. Sterl-2, -3, and -4 again only show membership to one group. The cluster diagram from the UPGMA shows the same differentiation of individuals as the Structure results, with the addition of one extra cluster.

A Mantel test was used to test for a correlation between genetic and geographic distance at the population level. Significant evidence ($r=0.87$; $p=0.0049$; $n=21$) supports a pattern of isolation by distance (Figure 1.5) confirming that the populations collected within the smallest range, Sterl-2, -3, and -4, are most similar and populations become increasingly differentiated as the distance increases.

Discussion

Genetic Diversity

The analysis of genetic diversity in this study confirms previous studies that show jointed goatgrass in the western U.S. has an overall decreased level of diversity than in the native range (Gandhi et al. 2005, 2009; Pester et al. 2003). Gene diversity, as measured by expected heterozygosity, of 0.5423 was found in Eurasia while in the U.S. large-scale collection gene diversity was reduced to 0.4136. In addition, only 40% of the total alleles amplified in the both ranges are present in the U.S. This reduction in diversity is also visible in the UPGMA between the large-scale U.S. and Eurasian accessions; seven of the 11 total clusters only contain Eurasian samples, illustrating the absence of these genotypes in the U.S. The results support the hypothesis that the introduced range of jointed goatgrass contains only a subset of the total diversity found in Eurasia.

However, populations of jointed goatgrass across the western U.S. are not genetically uniform. The cluster diagram showed U.S. accessions from the large-scale collection dispersed across four clusters among Eurasian accessions. This confirms the results of Gandhi et al. (2009) that multiple genotypes exist in the U.S. and that a single introduction event is unlikely. Grouping of U.S. accessions into a single cluster would have suggested genotypic homogeneity and clustering with one group of Eurasian accessions would have implied a single introduction event. Further, 3 % of the total alleles were unique to the U.S. This finding could be the result of insufficient sampling in the native range or detection of new alleles that have arisen since introduction and population expansion. If the latter is the case, jointed goatgrass could regain diversity that was lost during initial founder events.

The population level sampling from Sterling, CO revealed an even greater reduction in diversity from the native range. Only 26% of the total alleles, and 66% of the total U.S. alleles, were amplified in the Sterling populations. Gene diversity was only 0.1676 overall. This suggests that at a small-scale genetic isolation exists preventing genetic heterogeneity because of the lack of successful seed dispersal and cross-pollination.

Genetic structure

Eurasia and large-scale U.S.

AMOVA revealed that in both the large-scale U.S. and Eurasian collections genetic diversity is explained by variation contained within these ranges, with only 15% of diversity due to variation between the native and introduced ranges, similar to Gandhi et al. (2009). Analyzing additional geographic areas shows that within these ranges the majority of genetic diversity continues to be found within each geographic area analyzed. Diversity was explained within regions and countries for Eurasia and within regions and states for the U.S. The Structure analysis identified three distinct groups based on allelic frequencies of the datasets. These three clusters were represented across all regions, confirming the AMOVA results that genetic diversity is found within regions rather than among regions. In the cluster analysis there is also no clear grouping of accessions according to geographic origin, further supporting a lack of genetic structure at a large-scale. The absence of genetic structure at the large-scale can be attributed to the agricultural movement of jointed goatgrass seed. Determining the introduction history, including the origin of U.S. genotypes, is confounded by the wide distribution of genetic diversity in the geographic areas analyzed. A single introduction event from one area in Eurasia could have resulted in the introduction of multiple genotypes into the U.S. Movement of jointed

goatgrass seed could disperse this diversity across the introduced range preventing the isolation of independent introduction events.

Small-scale U.S.

The AMOVA showed that the majority of genetic diversity in the Sterling collection is explained within populations rather than among populations. Analysis using Structure showed that genetic structure exists at the population level. Two clusters are assumed with 86% of all the individuals belonging to the predominant cluster. All plants, with the exception of one individual, analyzed from the four populations closest together, Sterl-2, -3, -4, and -5, all belong to the dominant cluster. Not until the distance between populations is increased does greater membership to the second cluster appear. This result confirms the results found in the gene diversity calculations that showed a near absence of diversity in three of the four closest populations, with increasing levels of diversity increasing for populations sampled at a greater distance from these central groups. The cluster diagram revealed a similar pattern for genetic structure. Only three clusters are formed with Dice-Coefficients greater than or equal to 0.50. The majority of the plants group together in just one main cluster. Again, all individuals from Sterl-2, -3, -4, and all but one for Sterl-5, were assigned to one cluster. The Mantel test gave significant support to the hypothesis that a pattern of isolation by distance is present at the small-scale level analyzed here. This implies that at the small-scale, gene flow is limited. This contrasts with the results of the large-scale analysis where genetic diversity is found at all geographic areas. Whereas at the large-scale agricultural movement may increase the distance at which gene flow occurs, at a small-scale gene flow seems to be limited to only natural mechanisms of gene flow. Movement of pollen and seed is restricted with an increase in geographic distance without the aid of human dispersal of seed.

Conclusion

The presence of genetic diversity in the introduced range of jointed goatgrass raises concerns that differences in phenotypic traits could exist. Multiple introductions could have brought genotypes into the U.S. originating from jointed goatgrass populations adapted to different environments in the native range. Field trials using common garden studies would be valuable to test for variation at the level of trait expression. This information could be used to evaluate how different populations would benefit from a gene introgression event from wheat. Variation in the fitness advantage of gaining a wheat gene would mean that the risk of introgression would vary by jointed goatgrass genotype. Also, phenotypic variation in traits such as flowering time could result in different rates of interspecific hybridization and the ability for gene flow to occur between wheat and jointed goatgrass. If risk is different among populations, preventing gene introgression would prove difficult due to the lack of genetic structure at a large-scale. Variation in risk may not be based upon geographic location, as results in genetic diversity show here, making management of the weed on a case-by-case basis implausible.

Genetic heterogeneity across regions in the large-scale Eurasian and U.S. collections suggests that there is gene flow across and within these areas. This should not come as much of a surprise, for even though the species is predominantly selfing, as an agricultural weed, seed can hitchhike alongside wheat, whether contaminating farm machinery, grain trucks, or wheat seed. For example, transport of custom harvesters from the southern U.S. states through the central Great Plains has been attributed to the distribution of jointed goatgrass across the western U.S. (Washington State University 2008). The finding of genetic diversity at multiple levels of geographic scale stresses the importance of containing jointed goatgrass in order to prevent the spread of genotypes, which may prove more competitive in different environments.

Increasing diversity can increase the ability for adaptation and selection to occur. In addition, movement can bring together different genotypes, which have the potential to hybridize and produce novel genotypes. This lack of control raises concern for the introgression of wheat genes, for the event would not be isolated unless movement of jointed goatgrass seed ceased. Movement of the weedy seed will move the genetic material and any advantageous genes, including those originating from wheat, to new areas. The management of gene flow between transgenic wheat and jointed goatgrass will not be possible without the containment of invasive seed.

Isolation by distance at the population level implies that gene flow is limited at this scale. It is at this small range that pollen-mediated gene flow, in addition to seed dispersal, can occur. The results suggest that gene flow becomes increasingly rare as geographic distance increases. In this study the populations closest together, within 1 km, were collected in a draw where water could carry seed. Homogeneity within these populations is suggestive of gene flow within and between the populations. However, at increasing distances, and away from the draw, differentiation was found among the populations, which could be the result of lack of seed and pollen movement at these distances. Understanding the history of the populations collected would be valuable to determine the patterns of gene diversity in this collection.

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Tables

Table 1.1. Gene diversity of jointed goatgrass from the large-scale U.S. and Eurasian collections by range and region.

Location	Region Information	Gene diversity (H_E)	n
U.S. region 1 (Great Plains)	CO, KS, NE, OK, NM, WY, SD	0.4026	63
U.S. region 2 (Pacific Northwest)	WA, OR	0.3842	14
U.S. region 3 (Intermountain)	UT, ID, MT	0.2881	19
U.S. total		0.4136	96
Eurasia region 1 (Central Europe)	France, Germany, Czech Republic/Slovakia, Hungary, Italy, Serbia, Romania, Bulgaria, Macedonia	0.5202	53
Eurasia region 2 (South Caucasus)	Ukraine, Turkey, Armenia, Georgia, Azerbaijan	0.5045	115
Eurasia region 3 (Central Asia)	Iran, Afghanistan, Kazakhstan, Tajikistan, Turkmenistan, Uzbekistan, Former Soviet Union	0.6147	27
Eurasia total		0.5423	195
Total		0.5273	291

Table 1.2. Gene diversity of jointed goatgrass collections from Sterling, CO.

Population	Collection Information	Gene Diversity (H_E)	n
Sterl- 1	40°30'4.80"N, 102°58'12.72"W	0.1914	25
Sterl- 2	40°31'19.80"N, 102°59'21.36"W	0.0981	26
Sterl- 3	40°31'20.12"N, 102°59'20.46"W	0.0391	24
Sterl- 4	40°31'20.04"N, 102°59'25.50"W	0.0586	23
Sterl- 5	40°31'18.06"N, 102°59'30.35"W	0.1016	25
Sterl- 6	40°34'9.66"N, 102°59'28.32"W	0.2739	24
Sterl- 7	40°31'59.10"N, 102°59'25.98"W	0.0709	17
Total		0.1676	164

Table 1.3. Microsatellite markers and corresponding annealing temperatures used in this study. Gwm markers are from Roder et al. 1998 and Gandhi et al. 2005 and cfd markers are from Guyomarç'h et al. 2002.

Marker	Forward Primer	Reverse Primer	Tm (°C)
cfd48	ATGGTTGATGGTGGGTGTTT	ATGTATCGATGAAGGGCCAA	65
cfd72	CTCCTTGAATCTCACCGAA	ATGTATCGATGAAGGGCCAA	65
cfd79	TCTGGTTCTTGGGAGGAAGA	CATCCAACAATTTGCCCAT	65
gwm190	GTG CTT GCT GAG CTA TGA GTC	GTGCCACGTGGTACCTTTG	60
gwm301	GAG GAG TAA GAC ACA TGC CC	CAG ATGCTCTTCTCTGCTGG	55
gwm383	ACG CCA GTT GAT CCG TAA AC	GAC ATCAATAACCGTGGATGG	60

Table 1.4. AMOVA comparing the native and introduced ranges using large-scale collections from Eurasia and the U.S.

Source of variation	df	Percentage of variation	p
Among ranges	1	10.88576	<0.001
Within ranges	282	85.20881	<0.001

Table 1.5. AMOVA for Eurasian accessions of jointed goatgrass.

Source of variation	df	Percentage of variation	p
Among regions	2	2.93811	<0.001
Among countries within regions	18	11.16416	<0.005
Within countries	351	85.89773	<0.001

Table 1.6. AMOVA for the large-scale U.S. accessions of jointed goatgrass.

Source of Variation	df	Percentage of variation	p
Among regions	2	4.21940	<0.05
Among states within regions	9	25.86541	<0.001
Within States	190	69.91520	<0.001

Table 1.7. AMOVA for collections of jointed goatgrass from Sterling, CO.

Source of variation	df	Percentage of variation	p
Among populations	6	29.13560	<0.001
Among individuals within populations	156	69.97507	<0.001
Within individuals	163	0.00627	<0.001

Figures

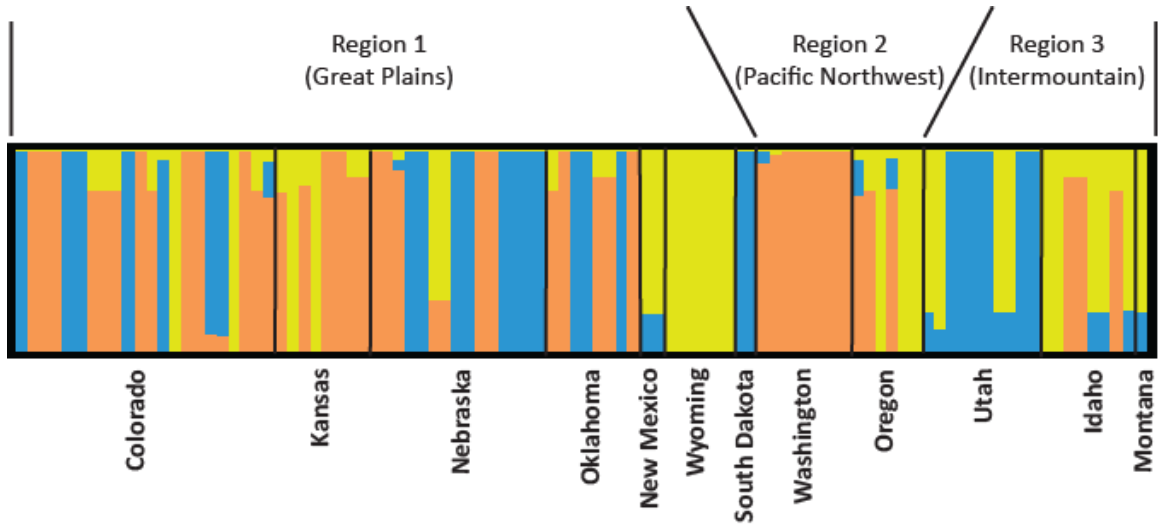


Figure 1.1. Structure results from the large-scale U.S. collection of jointed goatgrass using $K=3$, without admixture. Orange depicts K1, blue K2, and yellow K3 for the corresponding ancestry table (Table A1.4).

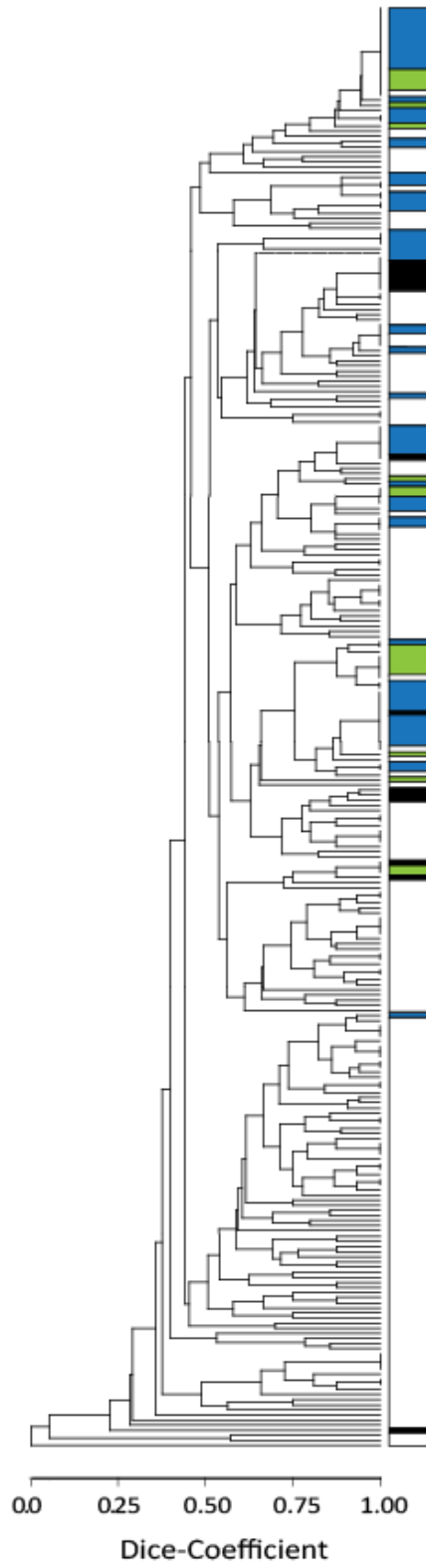


Figure 1.2. Cluster diagram based on UPGMA of the large-scale U.S. and Eurasian collections of jointed goatgrass. White indicates Eurasian samples while colored blocks indicate one of three U.S. regions: blue for region 1, black for region 2, and green for region 3.



Figure 1.3. Structure results for the small-scale Sterling collection of jointed goatgrass using $K=2$, without admixture. Purple depicts S1 and Red S2 for the corresponding ancestry table (Table A1.5). Populations are arranged by collection location.

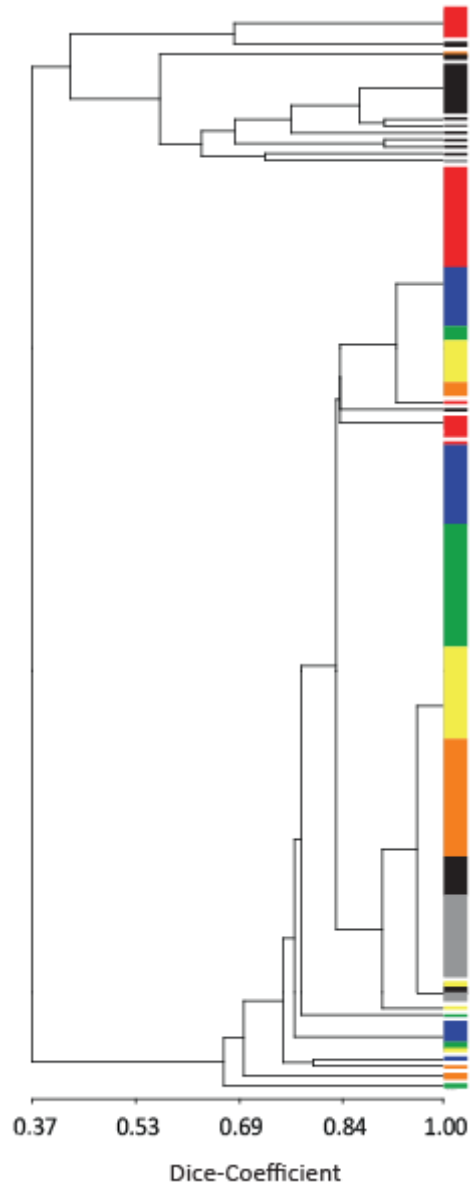


Figure 1.4. Cluster diagram based on UPGMA of 164 individual plants from the small-scale U.S. collection from Sterling, CO. Each color represents a different Sterling population: Sterl-1=red, Sterl-2=blue, Sterl-3=green, Sterl-4=yellow, Sterl-5=orange, Sterl-6=black, and Sterl-7=gray.

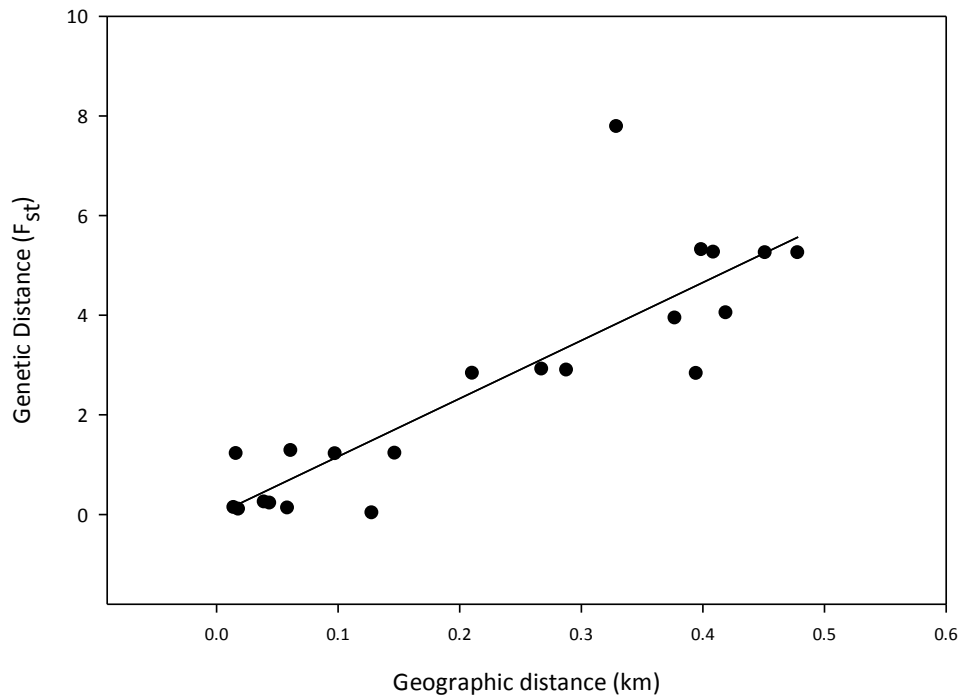


Figure 1.5. Scatter plot of results from a Mantel test for the small-scale Sterling collection correlating matrices for genetic distance (F_{st}) and geographic distance (km) between populations ($r=0.87$; $P=0.0049$; $n=21$).

Chapter 2

Phenotypic diversity in jointed goatgrass from the western U.S.

Abstract

Jointed goatgrass (*Aegilops cylindrica* Host) is an exotic weed in the western United States. The species is an agricultural pest and causes economic loss due to competition with winter wheat (*Triticum aestivum* L.) and contamination of wheat grain. Further, as a wild relative of wheat, interspecific hybridization serves as a mechanism for the introgression of advantageous genes from wheat to jointed goatgrass. The risk of introgression of ecological traits 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 studies have suggested multiple introductions of the species and the presence of genetic diversity in the western U.S. However, phenotypic studies to determine whether findings of genetic diversity relate to variation at the level of trait expression have been limited. Natural variation for environmental stress tolerance, such as drought tolerance, is important in order to determine how gaining a wheat gene of this type will affect fitness of different genotypes of jointed goatgrass. Thirty accessions of jointed goatgrass from the western U.S. were evaluated in four replications of a complete block design in Haxtun, Colorado. Plant height, the number of tillers produced per plant, the number of spikelets per spike, and the number of spikelets per plant were measured. Results indicate significant ($P < 0.001$) variation in each of the traits by

accession and by replication, and show a significant ($P < 0.0001$) interaction between accession and replication. Natural variation in fitness and in the response to environmental stress implies that the risk for gene introgression of an environmental trait from wheat to jointed goatgrass will not be uniform across the western U.S. Cultivation of drought tolerant transgenic wheat will be a greater risk for populations of jointed goatgrass in which gaining drought tolerance would confer a selective advantage. Future studies will need to be conducted under wet and dry treatments in order to evaluate whether natural variation in response to drought, in particular, exists in jointed goatgrass.

Abbreviations: Random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP).

Jointed goatgrass (*Aegilops cylindrica* Host) is an exotic weed in the U.S., where it infests winter wheat (*Triticum aestivum* L.) fields and disturbed ecosystems (Anderson 1993). The annual grass is thought to have been introduced from its center of origin in Eurasia (Mayfield 1927). The range of jointed goatgrass is vast and encompasses the region spanning the Mediterranean to central and eastern Asia (van Slageren 1994). In the U.S. the invasive species has been reported in 32 states and continues to expand its range by 50,000 acres a year (USDA, NRCS 2010; Washington State University 2008).

The occurrence of hybridization and gene flow between wheat and jointed goatgrass has driven research to assess the risk of introgression of wheat genes into the weedy species. Jointed goatgrass and wheat are both allopolyploid species in the Triticeae tribe. Wheat is an allohexaploid ($2n = AABBDD = 42$), while jointed goatgrass is an allotetraploid ($2n = CCDD = 28$). The two species both contain the D-genome derived from a common diploid progenitor, *Aegilops tauschii* Coss. In order to evaluate the rate of gene introgression from wheat to jointed goatgrass, studies have been conducted to determine the frequency of interspecies hybridization and backcrossing (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 2004; Wang et al. 2001; Zemetra et al. 1998). Cytogenetic techniques have also been used to track changes in chromosome numbers and the transfer of wheat genes in hybrids and subsequent backcross generations (Cifuentes and Benavente 2009; Mallory-Smith et al. 1996; Perez-Jones et al. 2002a,b; Schoenberger et al. 2005, 2006; Wang et al. 2000, 2001, 2002; 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. 2005, 2009; Naghavi et al. 2009; Okuno et al. 1998; Pester et al. 2003; Watanabe et al. 1994;). However, little has been done to assess the phenotypic

diversity of jointed goatgrass, an important factor for determining the risk of gene introgression in wheat production areas across the western U.S.

Genetic diversity has been detected with random amplification of polymorphic DNA (RAPD) markers and nuclear microsatellite markers in the native range of Eurasia (Naghavi et al. 2009; Okuno et al. 1998). Studies using α -amylase isozymes, RAPD, and amplified fragment length polymorphism (AFLP) markers concluded limited, if no, genetic diversity present in the U.S. (Pester et al. 2003; Watanabe et al. 1994). Conversely, evidence from microsatellite markers supports the presence of multiple genotypes in the western U.S., albeit evidence supporting a genetic bottleneck, most likely the result of multiple introduction events (Gandhi et al. 2005, 2009). Diversity at microsatellites, repeat regions typically considered to be neutral (Awadalla & Ritland 1997; Schlötterer & Wiehe 1999; Tachida & Iizuka 1992; You-Chun Li et al. 2008), does not imply that phenotypic diversity exists. However, You-Chun Li et al. (2008) caution against the assumption of microsatellites as exclusively neutral, supported by evidence for nonrandom distribution in coding and noncoding areas of the genome, in addition to, involvement in chromatin organization and regulation of DNA metabolic processes and gene activity. The combination of the detection of genetic diversity at microsatellites with the ability of the weed to invade across a vast geographic range demonstrates that an evaluation of phenotypic variation in jointed goatgrass populations across the western U.S. is warranted.

Previous phenotypic trials have suggested that trait diversity exists in introduced populations of jointed goatgrass. If multiple introductions have occurred, populations originating from various eco-geographical regions could be a source for phenotypic diversity in the U.S. In three years of field trials 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 jointed goatgrass populations collected from four diverse regions (Zaharieva et al. 2003). In addition,

eco-geographic variation was found for vegetative growth traits: populations from regions of high autumn and winter rainfall were shown to have higher 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 jointed goatgrass from nine western U.S. states. Nevertheless, in the first year, significant variation was found among accessions for plant height and awn length. In year two of the study variation was found for tillers per plant, awn length, and leaf dry weight. Greenhouse studies revealed 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 and in vernalization requirements for five populations from Oregon and Washington (Fandrich and Mallory-Smith 2005; Fandrich et al. 2008).

Phenotypic variation in jointed goatgrass across the western U.S. could imply that the evolutionary consequences of gene introgression from wheat could vary. For example, flowering time between wheat and the invasive species must overlap in order for hybridization to occur. Variation in flowering time among jointed goatgrass populations could mean that the ability for hybridization and gene flow to occur is not uniform. The risk of introgression of wheat genes into jointed goatgrass populations is also dependent on the selective advantage of gaining those genes. For non-agricultural traits, such as tolerance to environmental stress, natural variation could exist in jointed goatgrass populations, implying that the selective advantage for the introgressed wheat genes would vary as well. Introgression of traits conferring tolerance to environmental stress can increase fitness and enable habitat extension, unlike for purely agricultural traits such as herbicide tolerance, which are selectively neutral in the natural environment (Stewart et al. 2003).

In the western U.S., infestation of dryland wheat production with jointed goatgrass has drawn concern for the introgression of drought tolerance from improved 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 confer complex traits. A transformation event inserting a single allele, *CspB*, in maize plants conferred yield advantages over non-transformed plants under drought stress in dryland environments (Castiglioni et al. 2008). With Monsanto's renewed interest in wheat, it is likely that this gene, among others, will be investigated for use in developing a transgenic drought tolerant wheat cultivar. Additionally, other world leaders in biotech wheat research, such as Bayer CropScience and CSIRO, the Chinese Academy of Agricultural Sciences, and Henan Agricultural University, are investigating drought tolerance (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 jointed goatgrass.

Under selection pressure, introgression of drought tolerance from a transgenic wheat cultivar would only require the transfer of one chromosomal unit. For complex traits, transgenics pose a greater risk for introgression than their conventional counterparts where the transfer of multiple chromosomal units is required for the trait to be expressed. If transfer of a drought tolerance gene increases the fitness of jointed goatgrass populations, it would be expected that introgression could occur. If the trait already exists in jointed goatgrass, transfer of a drought tolerant gene would be unlikely to have a significant impact on fitness (Hancock 2003). However, in populations where these genes would be under positive selection, the risk

for introgression will be high (Stewart et al. 2003). For an environmental trait, introgression could have both economic and environmental consequences.

In order to assess the risk of gene introgression from wheat to jointed goatgrass a phenotypic evaluation for the weedy species is necessary. Here, 30 accessions of jointed goatgrass from 12 western U.S. states were evaluated in a field trial conducted in Haxtun, Colorado. Two moisture treatments were used to determine variation in phenotypic response to drought stress. The results will be used to evaluate whether gaining a drought tolerant gene from wheat would be advantageous to populations of jointed goatgrass, leading to the introgression of these genes and a more competitive genotype.

Materials and Methods

Thirty accessions of jointed goatgrass (Table 2.1) representing 12 western U.S. states were evaluated under wet and dry treatments in Haxtun, CO, a wheat growing region in the northeast quadrant of the state, in 2008-09. Temperature and precipitation data was retrieved from the Colorado Agricultural Meteorological Network (Colorado State University 2010).

The trial was arranged in a split plot design with two replications. Moisture treatment, rainfed or supplemented with irrigation, served as the main plot. Irrigation was supplied using Chapin drip tape¹ with a flow rate of 0.5 gpm/100 ft. Subplots were entries, 30 jointed goatgrass accessions and three checks: 'Above' wheat (Haley et al. 2003), 'Hatcher' wheat (Haley et al. 2005), and an 'Above' wheat x jointed goatgrass hybrid. Jointed goatgrass seed was obtained from field collections held by academic institutions and was increased in field plots at the Agricultural Research, Development, and Education Center in Fort Collins, CO. In the fall of 2008, 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 seven 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.

Data was collected on a per plant basis for 5 to 10 plants from each subplot, with two plants in each row serving as border plants. 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. Ten whole plants from each subplot were harvested at maturity and the number of tillers was counted. Total number of

spikelets produced per plant was estimated by multiplying the number of tillers by the mean number of spikelets per spike for each plant.

All data was analyzed per plant by accession. Accessions *Ae. cyl* 14 and 19 were not used for either spikelet analysis due to spike shattering. In addition, *Ae. cyl* 25 was not used in the analysis of total spikelets per plant. Variation in each trait was evaluated using an analysis of variance (ANOVA) from SAS software (SAS Institute Inc. 2008). Plant height, tiller number, and total spikelets per plant were ln-transformed to meet ANOVA assumptions. Spikelet number per spike required no transformation. Two models were used. The first used the Mixed procedure and analyzed the data as a modified split plot design with treatment as a fixed effect and replication, accession, and their interactions treated as random effects. The second analysis used the GLM procedure and excluded treatment from the model. Accession, replication, and their interaction were treated as random effects. Data is reported as untransformed least squares means and standard errors from the GLM procedure. Correlations between each of the traits were conducted using the CORR procedure in SAS and least squares means by accession for each trait.

The reaction norm for each accession for each of the measured traits was graphed based upon the mean value of the genotype in each replication. Each of the replications was given an environmental value according to the mean of all the genotypes for each of the traits as in Falconer (1990). Significant differences between replications was determined using Tukey's studentized range in SAS using the ln-transformed data for plant height, tiller number, and total spikelets per plant and untransformed data for mean spikelet per spike. Data by replication is reported as untransformed means and standard errors. Values were then plotted for each genotype in low productivity and high productivity replications. The regression line is the environmental sensitivity of that accession.

Results

The wet and dry treatments were confounded by above average precipitation in Haxtun, CO for the 2008-2009 season. The period from February through July received 85.6 mm, and the month of June alone received 32.5 mm of precipitation (Figure 2.1). An ANOVA revealed no significant, or only marginally significant, effect of treatment on plant height, tiller number, or spikelet number ($p=0.2472, 0.0440, 0.5460$). Since real differences in water availability between the two treatments could not be determined, treatment was removed from the statistical model and analyzed as four replications of a complete block design.

Significant variation was found among accessions for mean plant height, mean tiller number, mean spikelet number per spike, and mean spikelet per plant (Table 2.2, Figure 2.2). The overall mean for plant height was 66.1 cm. The accession from Phillips County, CO (*Ae. cyl* 19) had the highest mean plant height (69.0 cm), while the accession from Broadview, MT (*Ae. cyl* 40) had the lowest mean plant height (58.7 cm). Overall mean tiller number per plant across all accessions was 155.1 cm. The greatest mean tiller number was 180.7 for Deuel County, NE (*Ae. cyl* 1) and the smallest was 120.3 for Fall River County, SD (*Ae. cyl* 51). The overall mean spikelet number per spike was 8.4. The maximum mean spikelet number was 9.3 for the accession from Platner, CO (*Ae. cyl* 21) with the minimum mean of 7.8 for the accession from Broadview, MT (*Ae. cyl* 40). The greatest spikelet production per plant was 1677.3 for Deuel County, NE (*Ae. cyl* 1) and the smallest was 944.7 for Franklin County, ID (*Ae. cyl* 43). The overall mean for all accessions was 1327.1 spikelets per plant. Significant correlation between traits was found only for mean plant height and mean spikelet number ($R=0.6158, p=0.0005, n=28$) (Figure 2.3).

Significant variation was also detected for each of the traits among repetitions ($p < 0.0001$) (Table 2.2). Based upon Tukey's studentized range the highest and lowest means by repetition were significantly different for each of the traits (Table 2.3). Repetition three was the high environment for plant height with a mean of 65.1 cm while repetition two was the low environment with a mean of 63.6 cm. For mean tiller number the high environment was repetition one, with 167.95, and the low environment was repetition four with 141.00. For mean spikelet the high was repetition three and the low was repetition four with mean values of 8.5283 and 8.2346. Last, for mean spikelet per plant the high was repetition one, with 1481.3, and the low was repetition four with 1143.3. An ANOVA supports an effect of accession x replication ($p < 0.0001$) for each of the traits (Table 2.2.). Reaction norms for all accessions for each trait were plotted against the low and high productivity environments to illustrate the variation in environmental sensitivity of the accessions (Figure 2.4).

Discussion

Previous field and greenhouse studies have reported minimal phenotypic variation in jointed goatgrass collections from the Great Plains (Gealy 1988). Germination and vernalization studies suggest that phenotypic variation exists for those traits (Fandrich and Mallory Smith 2005, 2008). It has also been reported that phenotypic variation exists in collections from Bulgaria (Zaharieva et al. 2003). If multiple introductions of the species have occurred in the U.S., as genetic studies have indicated (Gandhi et al. 2005, 2009), it is possible that phenotypic variation exists in the western U.S. The results of this study provide evidence for phenotypic diversity in 30 accessions of jointed goatgrass.

The traits evaluated in this study were plant height, mean number of tillers per plant, mean number of spikelets per spike, and mean number of spikelets per plant. 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. Variation in these traits among jointed goatgrass accessions suggests that their level of invasiveness under a common environment is not uniform and indicates genetic differences underlying these traits. The range of variation was 10.3 cm, 60.4 tillers per plant, and 1.5 spikelets per spike, and 732.6 spikelets per plant between the largest and smallest genotypes for each trait mean. The distribution of traits by individual plant illustrates a greater range seen over the course of the study (Appendix 3, Table A2.1). Plants that show higher growth rates, such as plant height, may be of a higher risk in agricultural settings and 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 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 and range expansion.

Significant variation was found for environmental sensitivity of accessions for each of the measured traits. Although variation between experimental replications is traditionally interpreted as experimental error, under field experiments this noise can be attributed to environmental variation (Sultan 2000). Here each replication was used as a representative of a range of microenvironments in order to analyze environmental sensitivity among the 30 accessions of jointed goatgrass. A significant difference in trait means was found between these microenvironments, as well as a significant interaction between accession and environment. The ideal invader performs well under a range of environments; it is both tolerant and plastic (Baker 1974). Plasticity allows a single genotype to tolerate variation in environmental conditions of a single habitat and to spread its range across a heterogeneous area. In this study it was demonstrated that jointed goatgrass populations across the western U.S. are variable in environmental sensitivity. This implies that the fitness response to gaining an environmental trait will vary by genotype. Genotypes with the highest sensitivity, which perform poorly in low environments, but well in high environments, will gain tolerance and lose environmental sensitivity if introgression of a wheat gene of this type occurs. This scenario is of highest risk for gene introgression for it produces a genotype that is invasive under both low and high environments.

Natural and human-mediated seed dispersal within the U.S. can lead to range expansion by invasive genotypes and/or lead to hybridization between different genotypes of jointed

goatgrass through cross-pollination. 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 to remove. Similarly, introducing an invasive genotype to a novel habitat will cause ecological disturbance through the competition with native plants for resources, leading to their decline. Interspecific hybridization acts in the same way as intraspecific hybridization, serving as a mechanism for gene flow and the introgression of advantageous genes. However, the novelty of genes available from a wheat cultivar increases the risk of introgression. Management should focus on preventing the spread of jointed goatgrass seed and cultivation of wheat cultivars with environmental stress tolerance near high risk populations. Controlling jointed goatgrass within and near wheat fields would also reduce the formation of interspecific hybrid plants.

One of the objectives of this study was to evaluate the response of jointed goatgrass populations to drought stress in order to specifically assess the risk of transgenic drought tolerant wheat. Drought tolerance is an environmental trait that could vary in jointed goatgrass and the finding of phenotypic diversity and environmental sensitivity in this field experiment suggests that natural variation in response to the stress could exist. Transgenic technology has increased the risk of introgression of genes conferring environmental traits to wild relatives and therefore risk assessment is necessary to ensure the safety of its release. Gaining an advantageous gene for environmental tolerance could lead to the development of more competitive weedy populations with detrimental economic and ecological impacts. An increase in fitness and decrease in environmental sensitivity could allow invasion into novel habitats and increased competitiveness with wheat. A replication of this study with significant wet and dry

treatments is necessary to determine the fitness of accessions under drought stress. This information will be useful to identify how gaining a drought tolerance gene will alter the fitness of populations of jointed goatgrass across the western U.S. and how a wheat cultivar of this type should be managed. In addition, this study re-emphasizes the importance of minimizing human mediated gene flow in order to isolate different jointed goatgrass genotypes to prevent hybridization and the creation of novel genotypes.

Sources of Materials

¹Chapin drip tape, Jain Irrigation, Inc., 2851 E. Florence Ave., Fresno, CA 93721.

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Tables

Table 2.1. Accessions of jointed goatgrass used in a 2008-09 field trial at Haxtun, CO. Latitude and Longitude information is based on the center of the county or city of collection.

ID	County/City	State	Latitude (N)	Longitude (W)
<i>Ae. cyl</i> 1	Deuel County	NE	41.140175	102.300581
<i>Ae. cyl</i> 2	Garden County	NE	41.736972	102.254792
<i>Ae. cyl</i> 7	Scotts Bluff County	NE	41.123886	100.765422
<i>Ae. cyl</i> 8	Chadron	NE	42.829419	102.999908
<i>Ae. cyl</i> 14	Bingham County	ID	43.211231	112.362414
<i>Ae. cyl</i> 15	Twin Falls County	ID	42.335253	114.675892
<i>Ae. cyl</i> 16	Fort Collins	CO	40.585261	105.084422
<i>Ae. cyl</i> 18	Kiowa County	CO	38.376967	102.713511
<i>Ae. cyl</i> 19	Phillips County	CO	40.537133	102.346386
<i>Ae. cyl</i> 21	Platner	CO	40.155261	103.067439
<i>Ae. cyl</i> 22	Pullman	WA	46.731275	117.179617
<i>Ae. cyl</i> 25	Logan County	KS	38.950478	101.161736
<i>Ae. cyl</i> 28	Ione	OR	45.501242	119.82475
<i>Ae. cyl</i> 29	Box Elder County	UT	41.538008	113.191803
<i>Ae. cyl</i> 32	Cache County	UT	41.756003	111.761467
<i>Ae. cyl</i> 34	Ritzville	WA	47.127372	118.379975
<i>Ae. cyl</i> 35	Archer West	WY	41.155719	104.666961
<i>Ae. cyl</i> 37	Pine Bluffs	WY	41.181925	104.069117
<i>Ae. cyl</i> 40	Broadview	MT	46.097731	108.877097
<i>Ae. cyl</i> 42	Oklahoma County	OK	35.603833	97.351656
<i>Ae. cyl</i> 43	Franklin County	ID	42.210033	111.761467
<i>Ae. cyl</i> 45	Asotin	WA	46.339325	117.048211
<i>Ae. cyl</i> 46	Garfield County	WA	46.518564	117.527661
<i>Ae. cyl</i> 47	Weber	UT	41.260264	111.95225
<i>Ae. cyl</i> 48	Wasatch County	UT	40.362942	110.998353
<i>Ae. cyl</i> 49	La Crosse	KS	38.531403	99.308714
<i>Ae. cyl</i> 50	Bunker Hill	KS	38.875844	98.703967
<i>Ae. cyl</i> 51	Fall River County	SD	43.224028	103.451178
<i>Ae. cyl</i> 54	Sherman County	OR	45.4122	120.753042
<i>Ae. cyl</i> 55	Clovis	NM	34.4048	103.205228

Table 2.2. ANOVA tables for (A) plant height, (B) number of tillers, (C) number of spikelets per spike, and (D) number of spikelets per plant.

A	Source	DF	Type III SS	Mean square	F value	Pr>F
	Rep	3	0.0615	0.0205	3.79	0.0105
	Acc	29	1.1436	0.0394	7.29	<0.0001
	Rep*Acc	87	1.4689	0.0169	3.12	<0.0001

B	Source	DF	Type III SS	Mean square	F value	Pr>F
	Rep	3	5.4213	1.8071	12.25	<0.0001
	Acc	29	12.5055	0.4312	2.92	<0.0001
	Rep*Acc	87	45.5807	0.5239	3.55	<0.0001

C	Source	DF	Type III SS	Mean square	F value	Pr>F
	Rep	3	7.6790	2.5597	10.17	<0.0001
	Acc	27	69.3169	2.5673	10.20	<0.0001
	Rep*Acc	81	68.5565	0.8464	3.36	<0.0001

D	Source	DF	Type III SS	Mean square	F value	Pr>F
	Rep	3	5.5474	1.8491	13.66	<0.0001
	Acc	26	12.3357	0.4744	3.50	<0.0001
	Rep*Acc	78	35.9082	0.4604	3.40	<0.0001

Table 2.3. Least squares means over all accessions by repetition for each trait. Means sharing letters did not differ significantly in Tukey studentized range comparisons.

Repetition	1	2	3	4
Mean height	63.833AB	63.587B	65.135A	63.794B
Standard error	0.3593	0.3593	0.3608	0.3583
Mean tiller number per plant	167.95A	153.58B	156.82AB	141.00C
Standard error	3.1120	3.0774	3.1010	3.5240
Mean number of spikelets per spike	8.4738AB	8.3178BC	8.5282A	8.2346C
Standard error	0.0420	0.0423	0.0413	0.0441
Mean number of spikelets per plant	1481.31A	1326.57A	1346.19A	1143.26B
Standard error	40.0102	39.5061	40.8213	41.0858

Figures

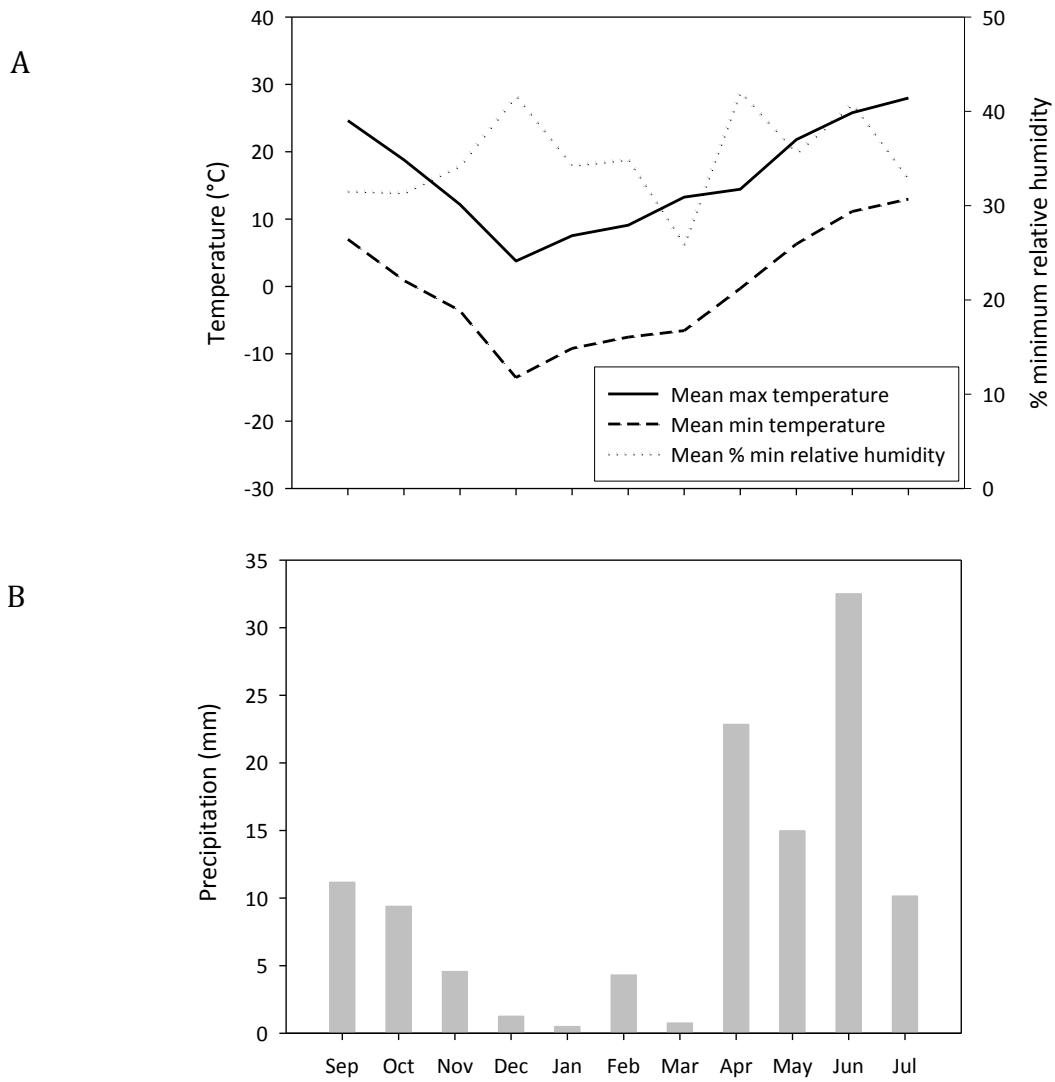


Fig. 2.1A,B. Mean monthly conditions for Haxtun, CO for time of planting in September 2008 until harvest in July 2009. (A) Mean maximum and minimum temperature (°C) and mean minimum relative humidity (%). (B) Total monthly precipitation (mm) for Haxtun, CO.

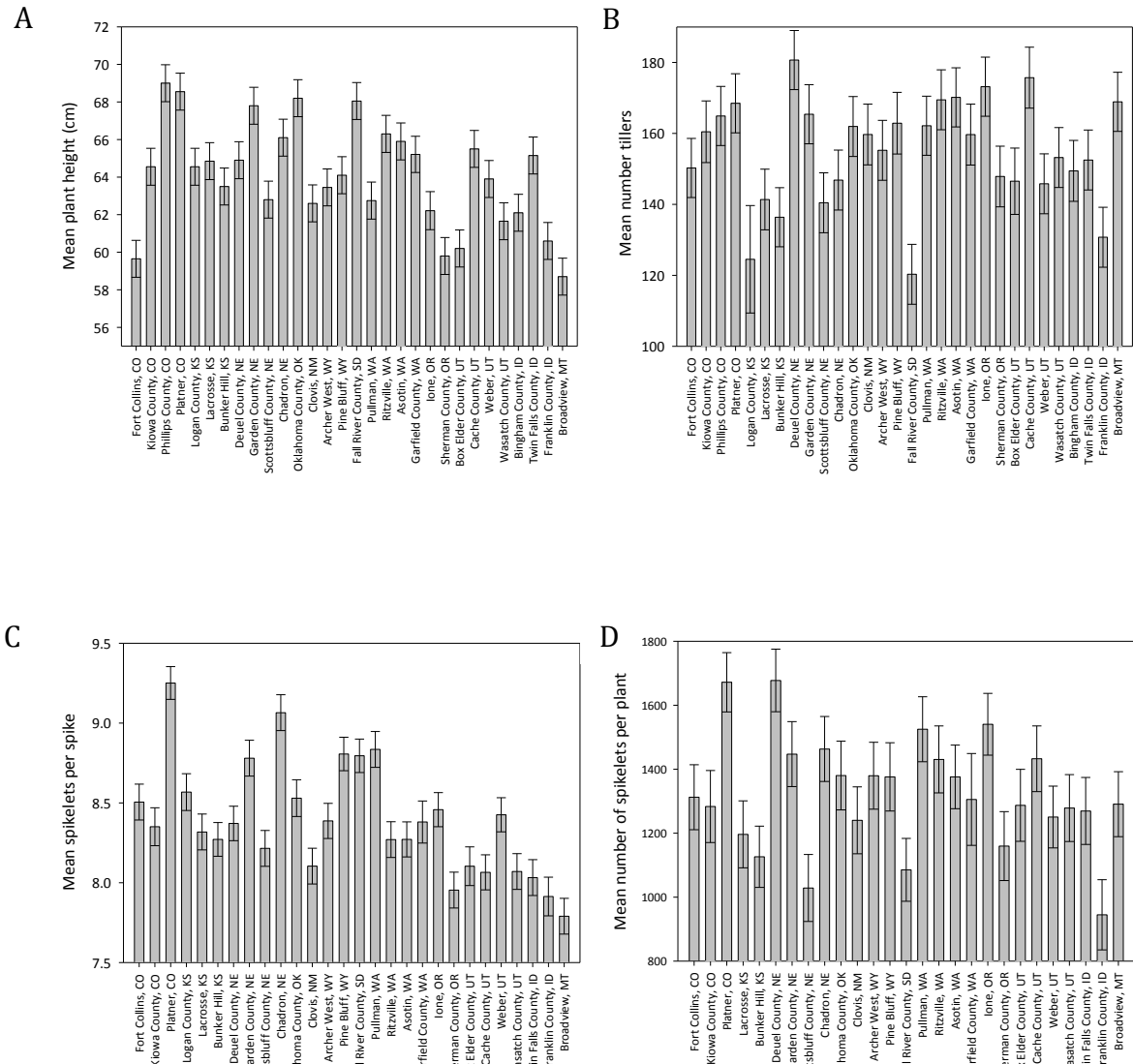


Fig 2.2A-D. Least square means \pm 1SE for each of four traits evaluated on a field trial of 30 jointed goatgrass accessions. (A) Mean height (cm) is based upon five plants per accession, (B) mean number of tillers is based upon five plants per accession, (C) mean number of spikelets per spike is based upon ten spikes from five plants per accession, and (D) mean number of spikes per plant is based upon five plants per accession.

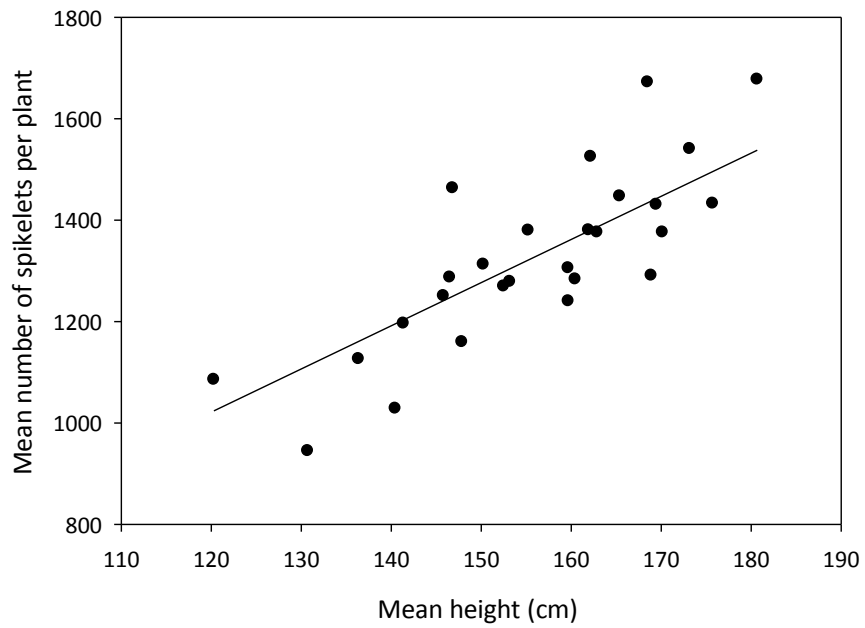


Fig 2.3. Correlation between mean spikelet number and mean plant height as least squares means by jointed goatgrass accession ($R=0.6158$, $p=0.0005$, $n=28$).

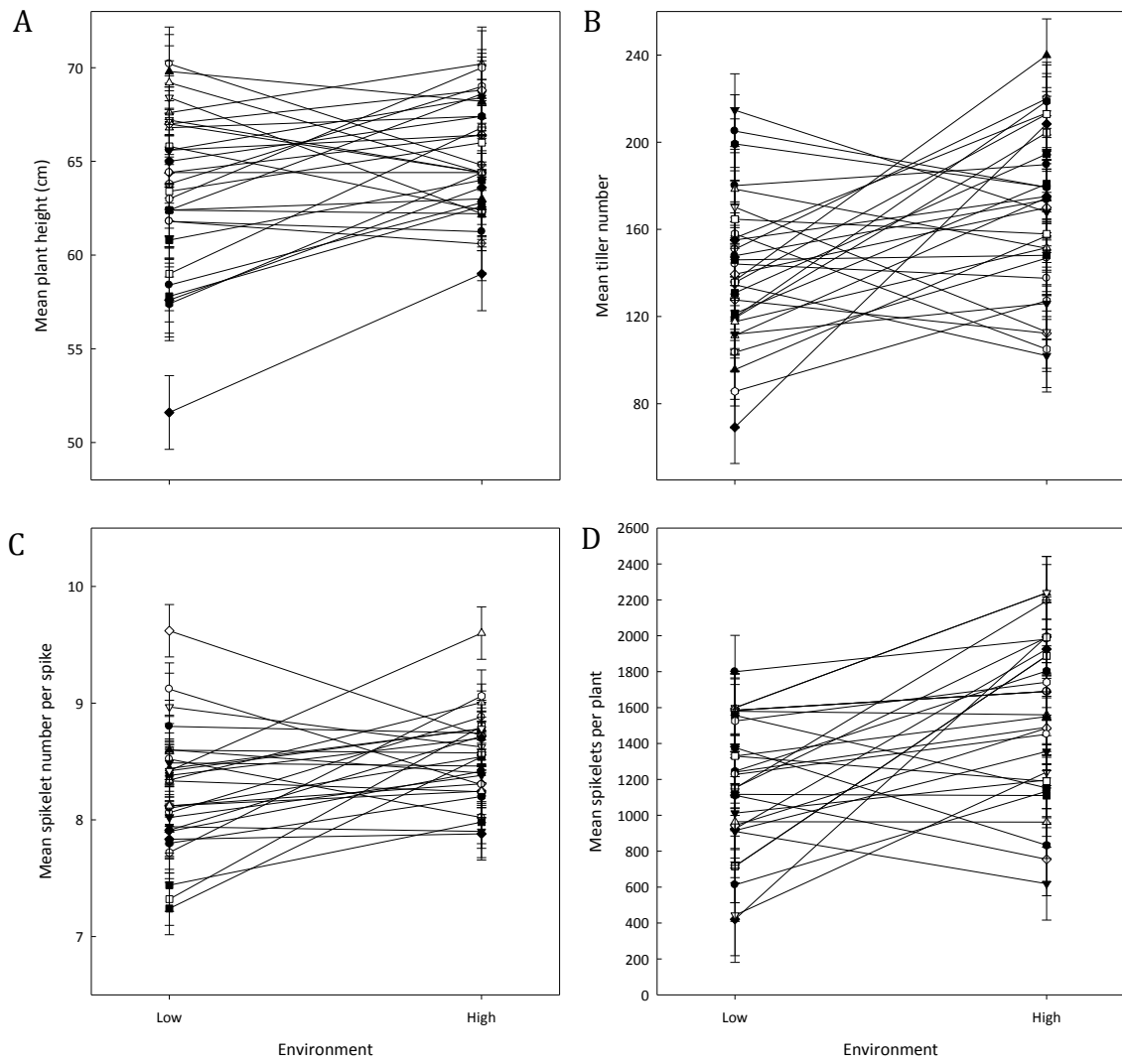


Fig 2.4A-D. Reaction norms for each of 30 accessions of jointed goatgrass in the low and high environments of each measured trait: (A) Plant height, (B) mean number of tillers per plant, (C) mean number of spikelets per spike, and (D) mean number spikelets per plant.

CHAPTER 3

Backcrossing provides an avenue for gene introgression from wheat to jointed goatgrass in the Great Plains

Abstract

Jointed goatgrass (*Aegilops cylindrica* Host) is an exotic species introduced into the western U.S. from Eurasia. The weed is an agricultural pest infesting wheat (*Triticum aestivum* L.) fields and causing economic loss. Common ancestry between the two species enables interspecific hybridization, thus providing a mechanism for the potential introgression of advantageous wheat genes to its weedy relative. This can act as a means for the accumulation of novel genes, increasing the wild species' competitiveness with wheat and its ability to invade novel habitats. Interest in the development of transgenic wheat cultivars has increased the concern for interspecific gene flow. Recurrent backcrossing to the weedy species after hybridization is a necessary step for introgression in order to restore fertility and chromosome composition. Field experiments were conducted at two locations in Colorado in 2007-08 and 2008-09, with jointed goatgrass acting as the sole source of viable pollen to transplanted hybrid plants. Backcrossing rates were determined by conducting germination studies on spikes collected from a total of 206 hybrid plants. Ninety-five percent confidence intervals estimate the rate of backcrossing at 0.028 to 0.306% and 0.077 to 0.604% in the two locations. The results demonstrate that

recurrent backcrossing to jointed goatgrass can occur, despite low rates of hybrid fertility. Subsequent backcrossing would make it likely that a wheat gene conferring a selective advantage will introgress into the weedy population. The results are significant because a field study using jointed goatgrass as the sole pollen source has not previously been reported. In addition, the study was conducted in the central Great Plains, where previous field trials have been limited, but where wheat and jointed goatgrass coexist. The results show that backcrossing rates vary by year suggesting environment could affect the likelihood for gene introgression. For the Great Plains, it is likely that future transgenic cultivars will be released and determining proper management of these crops to minimize hybridization and the potential for gene introgression is important prior to commercialization.

Abbreviations: Complementary log-log (CLL), penalized quasi-likelihood (PQL), generalized linear mixed model (GLMM).

Jointed goatgrass (*Aegilops cylindrica* Host) is an agricultural weed in the western U.S. It is presumed that this invasive grass was introduced from Eurasia as a contaminant of winter wheat (*Triticum aestivum* L.) grain towards the end of the 19th century (Mayfield 1927). Lack of containment through the movement of jointed goatgrass seed in contaminated combines, grain trucks, and wheat seed has allowed the weed to increase its range by 50,000 acres a year across the U.S. (Washington State University 2008; Donald and Ogg 1991; Ogg 1993). Its success as a pest to wheat cultivation can be in part attributed to its shared ancestry with wheat, which has resulted in similarities in physical attributes, such as spikelet arrangement on the spike, seedhead height, and seed cross sectional area, as well as, similarity in vernalization response, germination, and development phenology (Donald and Ogg 1991). It has been shown that these species have the ability to hybridize providing a mechanism for gene introgression from wheat to its wild relative (Jones-Perez et al. 2006a,b).

Interest in the development of transgenic winter wheat cultivars has increased concern for the introgression of advantageous genes from wheat into populations of jointed goatgrass. Transgenic technology makes use of single genes or blocks of genes that are inserted into the genome of interest as one unit. Movement of just one chromosomal segment containing the transgene into the jointed goatgrass genome could lead to expression of the transgenic trait. Under positive selection and with recurrent backcrossing the gene could introgress into populations of the weed (Stewart et al. 2003). Some agricultural traits, such as herbicide and disease resistance, are typically conferred by a single gene, whether using conventional or transgenic methods. Complex, quantitative traits, such as tolerance to environmental stress, are controlled by many genes, each having a small effect. Transgenic technology has provided a tool for conferring complex traits with only a single genomic region. Because only a single chromosomal unit would need to be transferred to a wild relative for the trait to be expressed,

transgenic cultivars pose a greater risk for gene introgression. Introgression can lead to increased fitness of weedy relatives, enabling the new genotype to expand its range and invade new habitats, as well as increase its competitiveness with the domesticated species (Arnold 1992; Ellstrand et al. 1999; Snow and Palma 1997; Stewart et al. 2003).

In the western U.S., infestation of dryland wheat production with jointed goatgrass has drawn concern for the introgression of drought tolerance from improved wheat cultivars to the weed. Monsanto's development of drought-tolerant maize (*Zea mays* L.) demonstrates how transgenic technology can be used to confer complex traits. A transformation event inserting a single gene responsible for expression of a cold shock protein, *CspB*, in maize plants conferred yield advantages over non-transformed plants under drought stress in dryland environments (Castiglioni et al. 2008). With Monsanto's renewed interest in wheat, it is likely that this gene, among others, will be investigated for use in developing a transgenic drought tolerant wheat cultivar. Additionally, other world leaders in biotech wheat research, such as Bayer CropScience and CSIRO, the Chinese Academy of Agricultural Sciences, and Henan Agricultural University, are investigating drought tolerance (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 the frequency and impact of introgression of wheat genes is necessary in order to minimize economic and environmental risks associated with commercialization of a cultivar of this type.

Hybridization is the first requirement for gene flow to take place between wheat and jointed goatgrass. In order for interspecific hybridization to occur, the two species must be growing within close enough proximity for cross-pollination to occur and the flowering times of both species must overlap (Raybould and Gray 1993; Ellstrand et al. 1999). Even with both of these criteria met, for two highly selfing species rates of interspecific hybridization will be low

due to their breeding systems. Successful cross-pollination is also restricted by differences in ploidy. Wheat and jointed goatgrass both contain the D genome, derived from the common progenitor *Aegilops tauschii* Coss. and are allopolyploid species in the Triticeae tribe. However, wheat is an allohexaploid ($2n = AABBDD = 42$), while jointed goatgrass is an allotetraploid ($2n = CCDD = 28$). Despite these limitations, production of F_1 hybrid plants has been shown to occur at rates of 0.074 to 8% (Gaines et al. 2008; Guadagnuolo et al. 2001; Hanson et al. 2005; Morrison et al. 2002a; Stone and Peeper 2006). Hybridization, although necessary for gene transfer between the species, does not necessarily result in gene introgression from wheat to jointed goatgrass. Introgression is dependent on fixation of the gene in a stable genome, which occurs only after recurrent backcrossing to the wild species and persistence of the new genotype (Ellstrand et al. 1999; Raybould and Gray 1993; Snow and Palma 1997; Stewart et al. 2003). Therefore, in order to assess the risk of introgression, the rates at which repeated backcrosses occur through pollination by jointed goatgrass must be quantified.

Lack of homologous chromosome pairing between the wheat and jointed goatgrass genomes results in low fertility of hybrid plants (Mallory-Smith et al. 1996). Hybrids have been shown to be self-infertile (Guadagnuolo et al. 2001; Mallory-Smith et al. 1996; Wang et al. 2001; Zemetra et al. 1998) with low rates of female fertility when crossed with either wheat or jointed goatgrass pollen. Mean rates of female fertility of hybrid plants have been estimated between 0.02 and 2.2% based on manual pollination in the greenhouse (Mallory-Smith et al. 1996; Schoenenberger et al. 2006; Wang et al. 2001; Zemetra et al. 1998). However, rates found by hand pollination do not simulate natural conditions in the plants' habitats and exclude factors such as pollen vectors, natural seed production, and environmental variability (Ellstrand et al. 1999). Field trials have found between 0.42 and 3.8% mean backcrossing rates with an unspecified pollen donor, either wheat or jointed goatgrass (Morrison et al. 2002; Snyder et al.

2000; Stone and Peeper 2004). A rate of backcrossing to jointed goatgrass alone has not been determined under field conditions. Since this is the direction of recurrent backcrossing required for gene introgression from wheat into jointed goatgrass, a field estimate of this backcross is necessary. In addition, a field study has not been conducted for hybrid seed production in the central Great Plains, a region both with extensive dryland wheat production and infested by this weedy species. The rate will be informative for understanding how frequently hybrids produce viable seeds under natural field conditions when pollinated by jointed goatgrass, providing a mechanism for introgression of wheat genes into the genome of the related weed.

Here two years of field trials were conducted in Colorado as a component of risk assessment of gene introgression from wheat to jointed goatgrass. The objective was to determine the backcrossing rate of F_1 hybrids to jointed goatgrass in two field environments. The research questions were (i) at what frequency do hybrid plants produce BC_1 seed, through a pollination event by jointed goatgrass and (ii) does the rate of backcrossing vary by year?

Materials and Methods

Two years of field trials were conducted to estimate the rate at which wheat by jointed goatgrass hybrids backcross to jointed goatgrass. In 2007-08, the trial was carried out at the CSU Agricultural Research, Development, and Education Center in Fort Collins, at the eastern edge of the Rocky Mountains. The study was repeated in 2008-09 in Haxtun, CO, a wheat growing region 215 km east of Fort Collins. Minimum and maximum temperature, relative humidity, and precipitation amounts were obtained from the Colorado Agricultural Meteorological Network (Colorado State University 2009) for each location.

Hybrid seed was produced in the greenhouse in 2007 using the wheat cultivar 'Above' (Haley et al. 2003) as the female parent and jointed goatgrass collected in Platner, CO as the male parent. The resulting seed was used in both years of study. Seeds were planted in 5 cm square pots containing potting soil¹ and germinated in the greenhouse. Seedlings at the three to four leaf stage were hardened off outside for one week prior to transplanting.

On September 19, 2007 and September 24, 2008 hybrid plants were transplanted into the field in a 16 by 14 grid pattern with 0.6 m spacing between plants. Jointed goatgrass from Paoli, CO was seeded over the entire plot, both within and between rows. Since hybrid plants are self-infertile due to male sterility, viable seed could only be produced through an outcrossing event with jointed goatgrass (Guadagnuolo et al. 2001; Mallory-Smith et al. 1996; Zemetra et al. 1998; Wang 2001).

Hybrid spikes were collected at maturity over the last two weeks of July of 2008 and 2009. Spikes from each hybrid were clipped, bagged, and labeled to keep a record of the plant identity. Bags were hung in a drying room for two weeks. Before germination studies began,

spikes were stored at room temperature for at least 16 weeks for post-ripening in order to ensure non-dormancy of seeds (Fandrich and Mallory-Smith 2006)

Germination studies were conducted for spikes harvested from 100 hybrid plants in 2008 and 106 hybrid plants from 2009. The number of spikelets produced by each plant was counted. Intact spikes and any shattered spikelets from each hybrid were planted in 27 by 53 cm flats containing potting soil and covered with clear plastic lids. Flats were placed in growth chambers set to 25/15°C with a 12-hour photoperiod determined by Fandrich and Mallory-Smith (2005) to be optimal conditions for the maximum germination of jointed goatgrass. Flats were watered every two days to ensure soil and spikes remained moist for a total of 14 days. At the end of the 14-day incubation period the number of seeds that germinated was recorded for each hybrid plant, and seedlings were transplanted into 5 cm square pots.

The frequency of backcrossing (BC₁) was defined as

$$BC_1 = \frac{Y}{n}$$

where Y was the number of seeds that germinated and n was the total number of florets. Since there are typically one to three viable florets per spikelet, the total number of spikelets was multiplied by two to give n (Donald and Ogg 1991).

Rarefaction curves were used to determine a sufficient sample size for estimating the mean frequency of backcrossing from the germination data in each year. Each observation, or floret, was scored as either producing a BC₁ plant (1) or not (0). The observations were randomized and the distribution of the cumulative average of the germination frequency was plotted against the number of florets. This was repeated three times and each curve was plotted against the actual observed mean frequency to check for convergence of the data.

Germination events can be reasonably modeled as a binomial, however it is expected that the probability of a germination event will vary from plant to plant. The distribution for the

frequency of germination by plant was highly skewed. A model with a mean value and a random effect due to this plant-by-plant variation was necessary to fit the data. The mean was fixed while the plant effect was treated as random with a normal distribution. A generalized linear mixed model (GLMM) was used in order to include both fixed and random effects. GLMM was used with a complementary log-log (CLL) link as a first step to fit a model to the data.

The CLL link is a non-linear transformation in the unit [0,1] scale used for extreme value distributions (Agresti 2002). Since the data represents a probability of success, defined by a germination event, with an asymmetrical distribution, this transformation was appropriate. The CLL transformation is defined by

$$\pi(x) = 1 - \exp [-\exp (\alpha + \beta x)]$$

where $\pi(x)$ is the probability of germination, α is the mean and βx is the random plant effect.

Proc Glimmix (SAS Institute Inc. 2008), which uses a penalized quasi-likelihood (PQL) method to fit a GLMM to the data, was used under a binomial with the CLL link to get preliminary estimates of the mean and standard deviation. These estimates were then used as starting values in the Nlmixed procedure in SAS. Nlmixed uses the maximum likelihood method to fit the model rather than the PQL method. The Nlmixed procedure was then compared to a modified model that included a zero inflated parameter (ρ), the probability of getting a zero, to test for an excess of zeros in the data. Results estimated ρ to approach zero, implying that the Nlmixed model fits the observed data without the need to add the extra parameter—that is, the data did not have an excess of zeros. To confirm this, the data set was simulated under the original Nlmixed procedure, without a zero inflated parameter, using SAS functions RANNOR and RANBIN. A constant n was used with the estimated parameters from Proc Glimmix. The simulations approximated the true data and confirmed the Nlmixed procedure as an appropriate model (Figure 3.1). Means and standard deviations were found under the CLL

transformation for both years of field studies. These estimates were then back-transformed to give medians and upper (0.975) and lower (0.025) limits, representing the range of backcrossing that can be expected after correcting for plant by plant variability. Reported mean frequencies are derived from the raw data.

Results

Environmental variation was observed between the two field locations at Fort Collins and Haxtun, CO (Figure 3.2). In the 2007-08 growing season, from planting in September 2007 through harvest in July 2008, Fort Collins received a total of 193 mm of rain. Haxtun received less total precipitation with a total of 113 mm over the same months in the 2008-09 season. Field sites also differed in relative humidity, with Haxtun having higher mean minimum relative humidity for all months except December. Flowering began the last week of May and continued through June at both locations. During these months the mean minimum relative humidity was 18.7% for Fort Collins and 38.1% for Haxtun. Haxtun had similar mean maximum temperature, but higher mean minimum temperature than Fort Collins (23.8/8.7°C and 24.1/5.7°C) during the months of flowering. Haxtun received 15 mm and 36 mm of precipitation during the months of May and June, respectively, while Fort Collins received 10 mm and 0 mm during the same months.

To test for the production of viable BC₁ seed due to pollination by jointed goatgrass, all the spikes collected from 100 hybrid plants from Fort Collins in 2008 and 106 hybrid plants from Haxtun in 2009 were used for germination studies. The average number of florets produced per hybrid plant analyzed was 2,489 and 1,202 for the Fort Collins and Haxtun sites, respectively. A total of 214 BC₁ plants from the Fort Collins collection germinated from 248,856 florets giving a mean observed frequency of germination of 0.086%. For the Haxtun collection, 249 BC₁ plants germinated from 127,428 florets resulting in a mean observed frequency of germination of 0.195%. Rarefaction curves show convergence of germination frequency for random sampling of florets to the overall mean illustrating that the sample size used in each year was sufficient (Figure 3.3).

The distribution of the frequency of BC₁ production per hybrid plant was not normal (Figure 3.4). Of the total 206 hybrid plants tested, 54 (26.2%) plants produced no BC₁ plants. Analysis using the Nlmixed model gave medians with 95% confidence intervals. For Fort Collins the median germination rate was 0.062% with a 95% confidence interval between 0.028 and 0.306%. For Haxtun the medium germination rate was 0.152% with a 95% confidence interval of 0.077 to 0.604%.

Discussion

The production of BC₁ seed on wheat by jointed goatgrass hybrids due to pollination by jointed goatgrass has previously been reported in greenhouse studies at rates from 0.02 to 2.2% based on manual pollination (Mallory-Smith et al. 1996; Schoenenberger et al. 2006; Wang et al. 2001; Zemetra et al. 1998). Field trials and collections in Oregon and Oklahoma have found between 0.42 and 3.8% mean backcrossing rates with an unspecified pollen donor, either wheat or jointed goatgrass (Morrison et al. 2002b; Snyder et al. 2000; Stone and Peeper 2004). Since F₁ hybrids are self infertile (Guadagnuolo et al. 2001; Mallory-Smith et al. 1996; Wang et al. 2001; Zemetra et al. 1998) the production of BC₁ seed can be attributed to an outcrossing event. Field studies were conducted here in order to determine the rate of backcrossing to jointed goatgrass as the sole pollen donor at two sites in Colorado. The objective was to estimate the production of BC₁ seed in the direction of the weedy parent under natural field conditions to determine the risk of introgression of wheat genes to its wild relative.

From this study it can be concluded with 95% confidence that the rate of backcrossing to hybrid plants is between 0.028 to 0.306% and from 0.077 to 0.604%, using data collected from Fort Collins and Haxtun, respectively. Evaluating the backcrossing rate using a median is more representative of the true rate than a mean due to the highly skewed data. From the reported medians it can be concluded that for Fort Collins the typical backcrossing rate for a hybrid plant was 0.062%, while for Haxtun the typical rate was 0.152%. This equates to the production of approximately two BC₁ plants per hybrid. These rates fall within the range of previous reports of backcrossing to wheat by jointed goatgrass hybrids, but represent the lower end of this spectrum. Differences are most likely a result of experimental method. In the study conducted here, jointed goatgrass provided the only source of pollen. Past field studies have

included both wheat and jointed goatgrass as sources of pollen for backcrossing. Cross-pollination also varies by the density of available pollen and the distance between the pollen source and hybrid plants. Greenhouse studies using manual pollination techniques do not rely on natural means of pollination and may not accurately estimate the rate of seed production in the field. Wheat and jointed goatgrass genotype is also likely to contribute to differences in reported backcrossing rates. In addition, variation in these rates can also be due to differences in environment. In this study, two environments were used and results indicate differences in the rate of backcrossing between the two locations. During the months of flowering, Haxtun had an overall higher mean minimum relative humidity, a higher mean minimum temperature, and received more rainfall. Pollen dispersal varies by environmental conditions, such as in day and night temperature, wind speed and direction, water availability, humidity, and light (Jarosz et al. 2003, 2005; Waines and Hedge 2003). For example, in wheat cross pollination is enhanced by drought and high temperatures (Briggs et al. 1999; Waines and Hedge 2003). Although optimal conditions for cross pollination are not known for jointed goatgrass, it has been shown that for wheat a temperature of 21°C with a minimum of 9.5°C is optimal (Porter and Gawith 1999). For this reason, in order to determine the risk of interspecific hybridization and gene introgression, hybridization and backcrossing rates should be evaluated under multiple environments.

Initial hybrids between wheat and jointed goatgrass must be fertile and produce backcross offspring in order for the possibility for introgression to exist (Stewart et al. 2003). The results presented here demonstrate that backcrossing to jointed goatgrass can occur to the BC₁ stage under field conditions. It is likely that a wheat gene conferring a selective advantage will introgress into the weedy population if subsequent backcrossing and selfing occurs. Further studies investigating the rate of subsequent backcrossing to jointed goatgrass past the BC₁ stage and under field conditions are necessary to fully assess the risk of introgression. It will also be

necessary to understand the fitness advantage that a gene will confer to populations of jointed goatgrass. For example, the risk of the transfer of wheat genes conferring environmental stress tolerance to its weedy relative has not been reported, however an estimate of this is necessary since transgenes of this type could drastically improve fitness in the wild species. The Great Plains is under extensive dryland wheat production and transgenic cultivars conferring environmental stress tolerance, such as tolerance to drought stress, would benefit production in this region. Gaines et al. (2008) showed that interspecies hybridization occurs in the Great Plains at a rate of 0.1 to 1.6%. The study reported here confirms that fertility exists in these hybrids demonstrating that introgression into populations of jointed goatgrass in the Great Plains is a potential risk. Further studies will be informative for developing proper management of transgenic wheat conferring environmental tolerances, such as drought tolerance, to minimize the impact of interspecific hybridization and introgression.

Sources of Materials

¹ Premier Pro-mix with Bx biofungicide, J.R. Johnson Supply, 2582 Long Lake Rd., Roseville, MN 55113.

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Figures

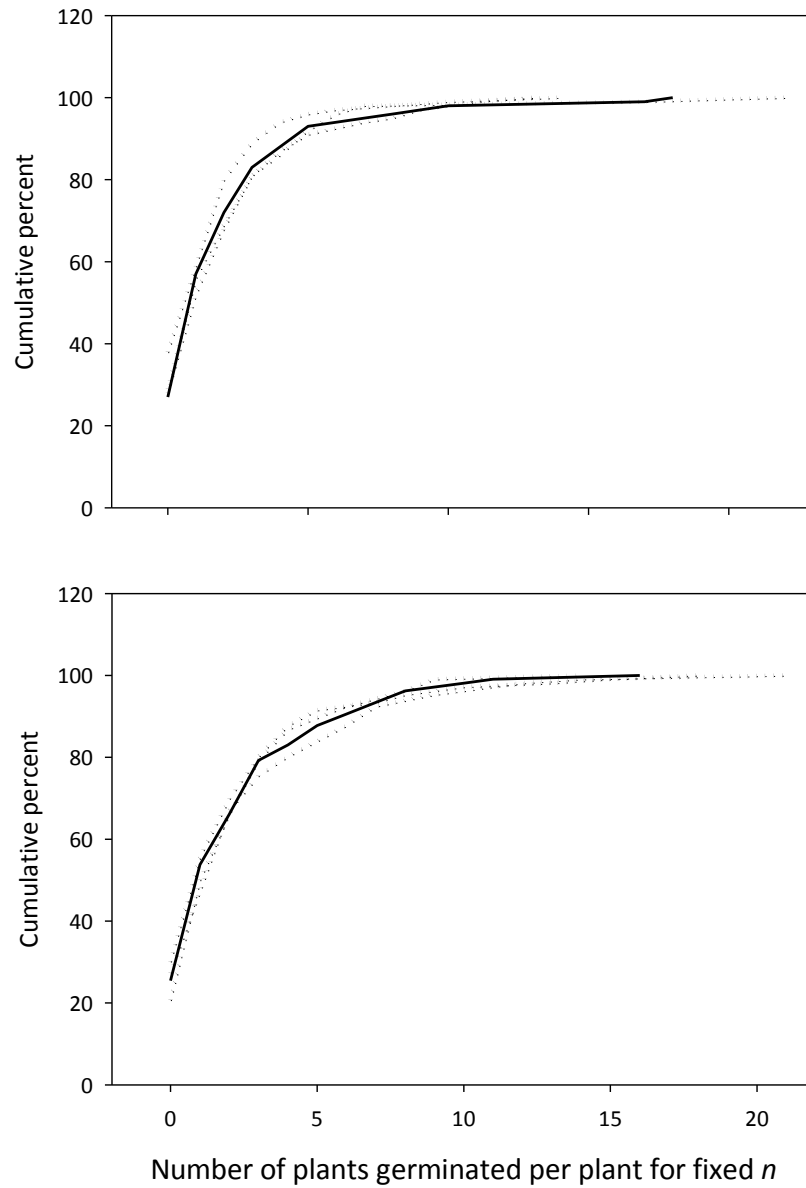


Figure 3.1. Simulations under the Nlmixed procedure in SAS (SAS Institute Inc., 2008) to test the fitness of the model to the germination data from (A) 2008 and (B) 2009. Dotted lines represent simulations and solid lines represent the real data.

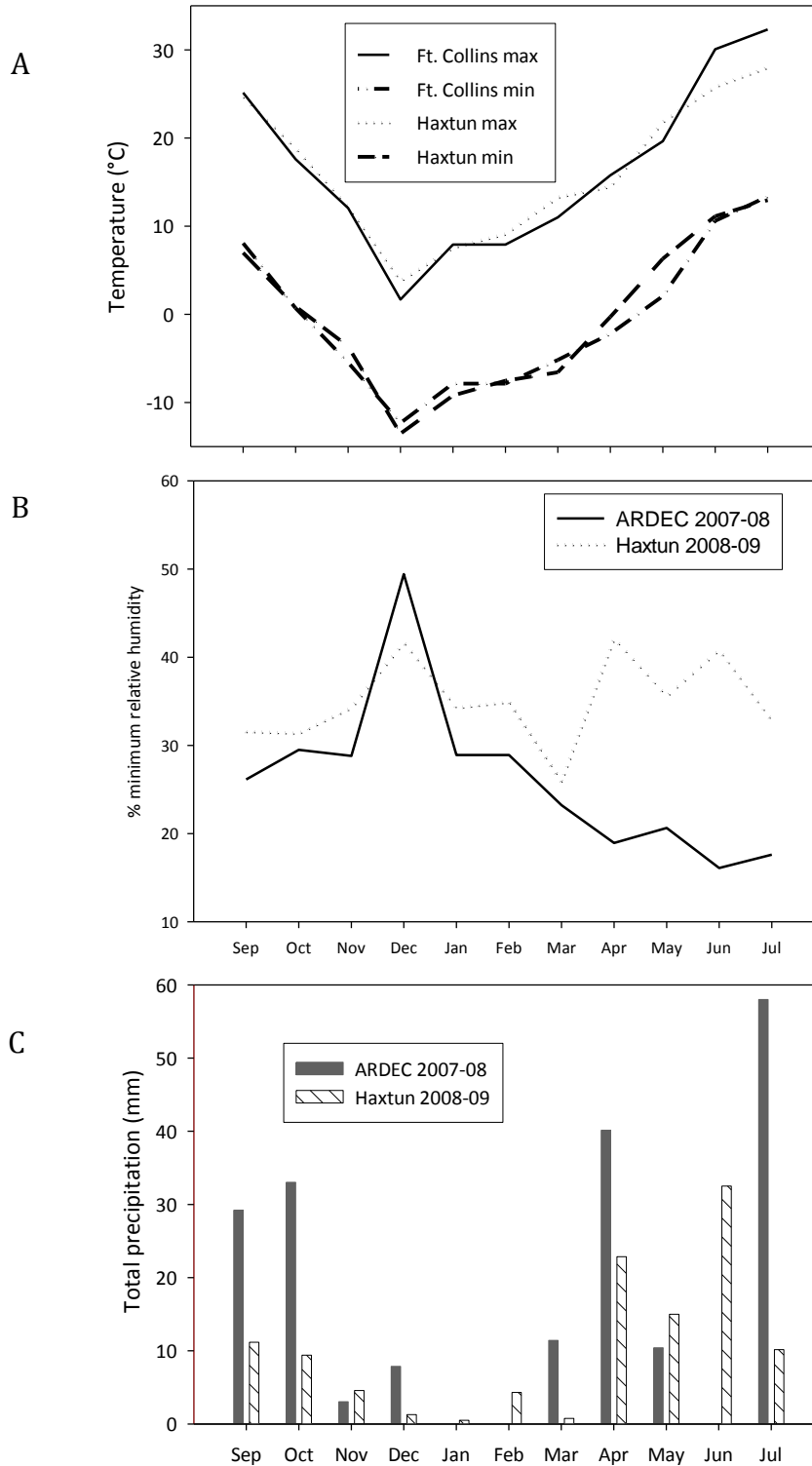


Figure 3.2. Environmental variation between two field trials as seen in (A) mean maximum and minimum temperature (°C), (B) mean minimum relative humidity (%), and (C) total monthly precipitation (mm) by month. Data for Fort Collins, CO is for September 2007 through July 2008. Data for Haxtun, CO is for September 2008 through July 2009.

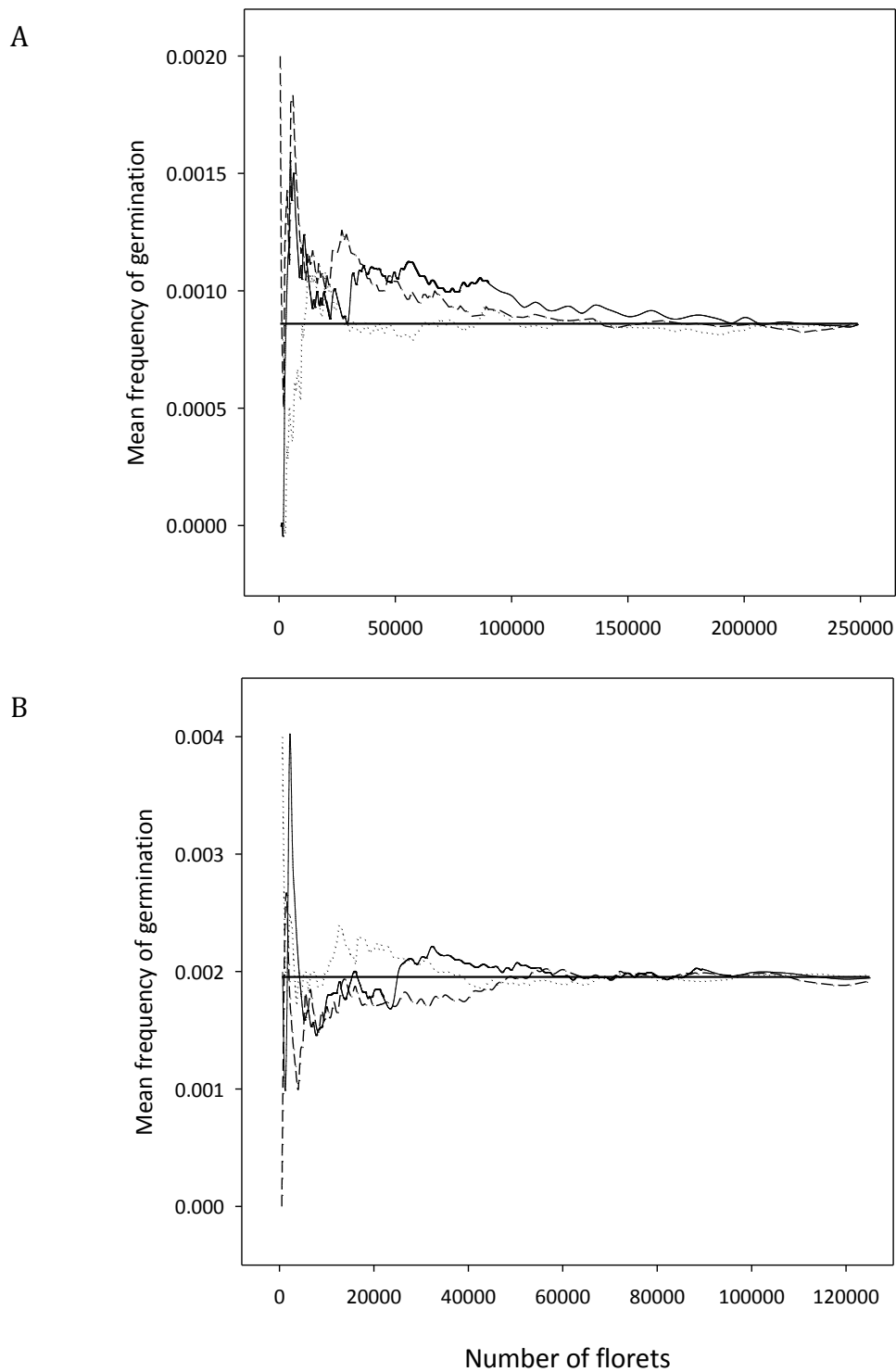


Figure 3.3. Rarefaction curves for germination studies conducted on hybrid spikes collected at (A) Fort Collins and (B) Haxtun. Three curves are pictured for each location representing random sampling of the data and the cumulative mean frequency as the sample size increases. Each random sampling converges at the overall mean, 0.00086 for Fort Collins and 0.00195 for Haxtun, represented by a solid line.

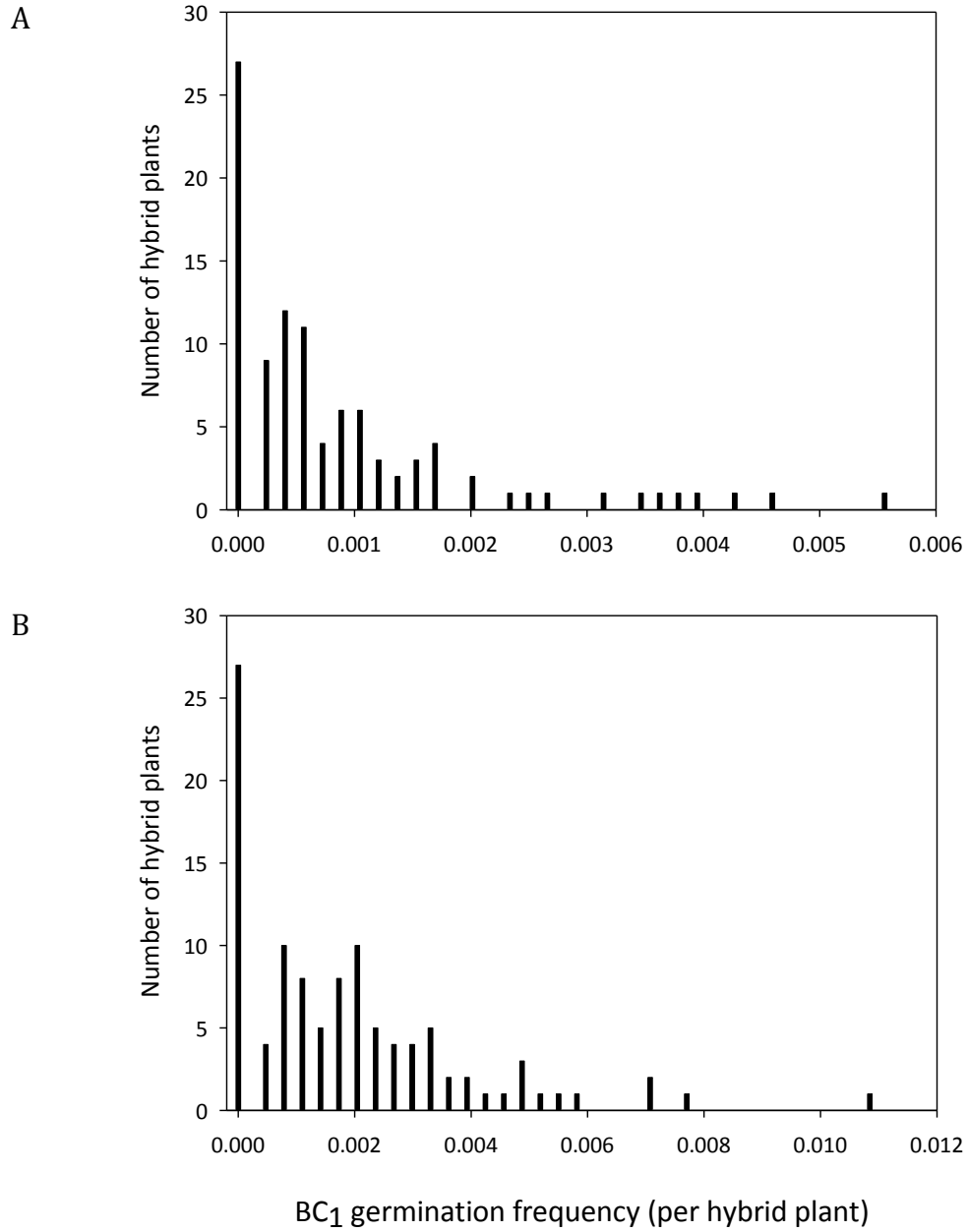


Figure 3.4. Distribution of the frequency of BC₁ plants that germinated from spikes collected from hybrid plants at (A) Fort Collins, CO 2008 and (B) Haxtun, CO 2009.

Appendix 1

Table A1.1. U.S. accessions for the large-scale analysis. Latitude and longitude information based upon the county or city center.

ID	County/City	State	Latitude	Longitude	n
Ae. cyl 1	Deuel County	NE	41.140175	102.300581	2
Ae. cyl 2	Garden County	NE	41.736972	102.254792	2
Ae. cyl 3	Cheyenne County	NE	41.250925	103.081789	1
Ae. cyl 4	Cheyenne County	NE	41.250925	103.081789	2
Ae. cyl 5	Kimball	NE	41.235814	103.662997	2
Ae. cyl 6	Kimball	NE	41.235814	103.662997	2
Ae. cyl 8	Chadron	NE	42.829419	102.999908	2
Ae. cyl 9	Chadron	NE	42.829419	102.999908	2
Ae. cyl 10	NA	OK	NA	NA	1
Ae. cyl 11	NA	OK	NA	NA	1
Ae. cyl 12	NA	OK	NA	NA	2
Ae. cyl 13	NA	OK	NA	NA	2
Ae. cyl 14	Bingham County	ID	43.211231	112.362414	2
Ae. cyl 15	Twin Falls County	ID	42.335253	114.675892	2
Ae. cyl 16	Fort Collins	CO	40.585261	105.084422	2
Ae. cyl 17	Fort Collins	CO	40.585261	105.084422	2
Ae. cyl 18	Kiowa County	CO	38.376967	102.713511	2
Ae. cyl 19	Phillips County	CO	40.537133	102.346386	2
Ae. cyl 20	Phillips County	CO	40.537133	102.346386	2
Ae. cyl 21	Platner	CO	40.155261	103.067439	2
Ae. cyl 22	Pullman	WA	46.731275	117.179617	1
Ae. cyl 23	Lacrosse	WA	46.814044	117.881883	1
Ae. cyl 24	Kingman County	KS	37.607158	98.221297	2
Ae. cyl 25	Logan County	KS	38.950478	101.161736	2
Ae. cyl 26	Rush	CO	38.839992	104.092181	2
Ae. cyl 27	Cheyenne Wells	CO	38.821394	102.353242	2
Ae. cyl 28	Ione	OR	45.501242	119.82475	2
Ae. cyl 29	Box Elder County	UT	41.538008	113.191803	2
Ae. cyl 30	Box Elder County	UT	41.538008	113.191803	2
Ae. cyl 31	Box Elder County	UT	41.538008	113.191803	2
Ae. cyl 32	Cache County	UT	41.756003	111.761467	2
Ae. cyl 34	Ritzville	WA	47.127372	118.379975	2
Ae. cyl 35	Archer West	WY	41.155719	104.666961	2
Ae. cyl 36	Archer East	WY	41.155719	104.666961	2
Ae. cyl 37	Pine Bluffs	WY	41.181925	104.069117	2
Ae. cyl 40	Broadview	MT	46.097731	108.877097	1
Ae. cyl 41	NA	CO	NA	NA	2
Ae. cyl 42	Oklahoma County	OK	35.603833	97.351656	2
Ae. cyl 43	Franklin County	ID	42.210033	111.761467	2
Ae. cyl 44	Otis	CO	40.148872	102.962992	2
Ae. cyl 45	Asotin	WA	46.339325	117.048211	2
Ae. cyl 46	Garfield County	WA	46.518564	117.527661	2
Ae. cyl 47	Weber	UT	41.260264	111.95225	2
Ae. cyl 48	Wasatch County	UT	40.362942	110.998353	2
Ae. cyl 49	La Crosse	KS	38.531403	99.308714	2
Ae. cyl 50	Bunker Hill	KS	38.875844	98.703967	2
Ae. cyl 51	Fall River County	SD	43.224028	103.451178	2
Ae. cyl 52	NA	CO	NA	NA	2
Ae. cyl 53	Ione	OR	45.501242	119.82475	2
Ae. cyl 54	Sherman County	OR	45.4122	120.753042	2
Ae. cyl 55	Clovis	NM	34.4048	103.205228	2
Total					96

Table A1.2. Eurasian accessions of jointed goatgrass from the USDA National Small Grains Collection.

New ID	USDA ID	Collection Detail	Country	Latitude (N)	Longitude (E)	n
Ae. cyl 210	172357	Bayburt	Turkey	40.26667	40.25	1
Ae. cyl 211	172358	Erzurum	Turkey	40.05	42.18333	1
Ae. cyl 212	172681	Kars	Turkey	40.08333	42.6	1
Ae. cyl 213	172683	Kars	Turkey	38.93333	44.03333	1
Ae. cyl 216	228333	Kordestan	Iran	35.63333	47.15	1
Ae. cyl 218	263553	Van	Turkey	38.55	42.76667	1
Ae. cyl 199	298891	Faryab	Afghanistan	35.71667	64.9	1
Ae. cyl 200	298893	Faryab	Afghanistan	35.85	64.51667	1
Ae. cyl 201	314185	Tashkent	Uzbekistan	41.46667	69.55	1
Ae. cyl 202	314406	NA	Georgia	41.71667	44.78333	1
Ae. cyl 205	344778	NA	Serbia	44.01667	20.91667	1
Ae. cyl 206	349035	NA	Armenia	40.5	45	1
Ae. cyl 207	374320	NA	Serbia	43.7425	21.45639	1
Ae. cyl 208	374322	NA	Serbia	44.18889	22.46528	1
Ae. cyl 209	374332	NA	Serbia	43.9125	22.18028	1
Ae. cyl 222	374345	NA	Macedonia	41.35833	21.61667	1
Ae. cyl 223	374347	NA	Serbia	43.54167	21.70778	1
Ae. cyl 224	374348	NA	Macedonia	41.11667	20.8	1
Ae. cyl 225	374353	NA	Macedonia	42.05	21.6	1
Ae. cyl 226	374377	NA	Serbia	43.23417	21.58806	1
Ae. cyl 227	374378	NA	Serbia	42.48333	21.5	1
Ae. cyl 228	374380	NA	Serbia	42.88333	20.86667	1
Ae. cyl 229	378186	NA	Macedonia	42.13222	21.71444	1
Ae. cyl 230	378187	NA	Macedonia	42.13222	21.71444	1
Ae. cyl 231	378188	NA	Serbia	42.66667	21.16667	1
Ae. cyl 234	407639	Ankara	Turkey	39.48333	32.56667	1
Ae. cyl 235	428560	NA	Georgia	42	43.5	2
Ae. cyl 236	428561	NA	Georgia	42	43.5	1
Ae. cyl 237	486235	Van	Turkey	38.3	43.16667	1
Ae. cyl 238	486236	Hakkari	Turkey	37.3	44.56639	1
Ae. cyl 239	486237	Hakkari	Turkey	37.2	44.61667	1
Ae. cyl 240	486238	Hakkari	Turkey	37.33333	44.53333	1
Ae. cyl 241	486239	Hakkari	Turkey	37.78333	44.33333	1
Ae. cyl 242	486241	Van	Turkey	38.58333	43.93333	1
Ae. cyl 243	486242	Van	Turkey	38.83333	43.43333	1
Ae. cyl 244	486243	Van	Turkey	38.91667	43.6	1
Ae. cyl 245	486244	Agri	Turkey	39.61667	44.18333	1
Ae. cyl 186	486245	Agri	Turkey	NA	NA	1
Ae. cyl 187	486246	Kars	Turkey	NA	NA	1
Ae. cyl 189	486248	Kars	Turkey	NA	NA	1
Ae. cyl 191	486250	Erzurum	Turkey	NA	NA	1
Ae. cyl 246	542179	Balikesir	Turkey	39.35	26.75	1
Ae. cyl 248	554200	Ankara	Turkey	39.55	33.48333	1
Ae. cyl 249	554201	Malatya	Turkey	38.36667	37.7	1
Ae. cyl 250	554202	Elazig	Turkey	38.48667	39.40667	1

Ae. cyl 251	554203	Van	Turkey	38.3	43.16667	1
Ae. cyl 252	554204	Van	Turkey	38.38333	43.41667	1
Ae. cyl 253	554205	Van	Turkey	38.38333	43.41667	1
Ae. cyl 254	554207	Hakkari	Turkey	37.23333	44.65	1
Ae. cyl 255	554208	Hakkari	Turkey	37.78333	44.33333	1
Ae. cyl 256	554209	Hakkari	Turkey	37.78333	44.33333	1
Ae. cyl 257	554210	Van	Turkey	38.58333	43.55	1
Ae. cyl 258	554212	Van	Turkey	38.81667	43.41667	1
Ae. cyl 259	554213	Van	Turkey	38.91667	43.6	1
Ae. cyl 260	554214	Kars	Turkey	40.13333	43.06667	1
Ae. cyl 261	554215	Kars	Turkey	40.05	42.83333	1
Ae. cyl 262	554216	Kars	Turkey	40.11667	42.66667	1
Ae. cyl 263	554217	Ankara	Turkey	39.28333	32.26667	1
Ae. cyl 264	554218	Ankara	Turkey	39.28333	32.26667	1
Ae. cyl 266	554220	Nevsehir	Turkey	38.98333	34.53333	1
Ae. cyl 267	554221	Malatya	Turkey	38.33333	38.16667	1
Ae. cyl 268	554222	Elazig	Turkey	38.91667	40.21667	1
Ae. cyl 270	554225	Bitlis	Turkey	38.4	42.6	1
Ae. cyl 271	554226	Van	Turkey	38.41667	43.3	1
Ae. cyl 272	554227	Van	Turkey	38.5	43.36667	1
Ae. cyl 273	554228	Van	Turkey	38.46667	43.38333	1
Ae. cyl 274	554229	Hakkari	Turkey	37.13333	44.53333	1
Ae. cyl 275	554230	Hakkari	Turkey	37.13333	44.51667	1
Ae. cyl 277	554232	Van	Turkey	38.53333	43.33333	1
Ae. cyl 278	554233	Van	Turkey	38.83333	43.43333	1
Ae. cyl 279	560508	Mus	Turkey	38.81667	41.58333	1
Ae. cyl 280	560509	Siirt	Turkey	37.48333	42.55	1
Ae. cyl 281	560510	Hakkari	Turkey	37.71667	43.8	1
Ae. cyl 282	560511	Hakkari	Turkey	37.33333	44.55	1
Ae. cyl 283	560513	Hakkari	Turkey	37.88333	44.03333	1
Ae. cyl 284	560514	Van	Turkey	38.83333	43.4	1
Ae. cyl 285	560515	Van	Turkey	38.81667	43.3	1
Ae. cyl 286	560516	Van	Turkey	38.7	43.5	1
Ae. cyl 287	560517	Hakkari	Turkey	37.71667	44.6	1
Ae. cyl 288	560518	Hakkari	Turkey	37.71667	44.5	1
Ae. cyl 289	560519	Hakkari	Turkey	37.73333	44.08333	1
Ae. cyl 290	560520	Mus	Turkey	39.13333	42.53333	1
Ae. cyl 291	560521	Bitlis	Turkey	38.46667	41.83333	1
Ae. cyl 292	560522	Mus	Turkey	38.9	41.51667	1
Ae. cyl 293	560733	Hakkari	Turkey	37.48333	43.53333	1
Ae. cyl 294	560735	Hakkari	Turkey	37.48333	43.65	1
Ae. cyl 295	560736	Hakkari	Turkey	37.71667	44.01667	1
Ae. cyl 296	560738	Hakkari	Turkey	37.38333	44.45	1
Ae. cyl 297	560739	Hakkari	Turkey	37.53333	43.6	1
Ae. cyl 298	560740	Hakkari	Turkey	37.7	43.96667	1
Ae. cyl 299	568161	Tashkent	Uzbekistan	41.7	70.1	1
Ae. cyl 301	573363	Bilecik	Turkey	39.58333	30.93333	1
Ae. cyl 302	573364	Cankiri	Turkey	40.81667	32.98333	1
Ae. cyl 303	573365	Cankiri	Turkey	40.48333	33.68333	1
Ae. cyl 304	573366	Cankiri	Turkey	40.48333	33.66667	1

Ae. cyl 305	573367	Ankara	Turkey	40.03333	32.91667	1
Ae. cyl 306	573368	Ankara	Turkey	39.93333	32.93333	1
Ae. cyl 307	573369	Ankara	Turkey	39.43111	32.49556	1
Ae. cyl 308	574460	NA	Uzbekistan	41	64	1
Ae. cyl 309	574461	Naxcivan	Azerbaijan	39.25	45.5	1
Ae. cyl 310	574462	NA	Azerbaijan	40.5	47	1
Ae. cyl 312	614614	Krym	Ukraine	44.40444	33.825	1
Ae. cyl 313	614615	Krym	Ukraine	44.57028	33.94361	1
Ae. cyl 314	614616	Krym	Ukraine	44.74111	33.92028	1
Ae. cyl 315	614617	Krym	Ukraine	44.53861	33.59972	1
Ae. cyl 316	614618	Krym	Ukraine	44.51361	33.49222	2
Ae. cyl 317	614619	Krym	Ukraine	44.51167	33.83472	1
Ae. cyl 318	614620	Krym	Ukraine	44.46778	33.75889	1
Ae. cyl 319	614621	Krym	Ukraine	44.79222	34.62333	1
Ae. cyl 320	614622	Krym	Ukraine	44.91694	35.22056	1
Ae. cyl 321	614623	Krym	Ukraine	45.03889	35.2825	1
Ae. cyl 322	614624	Krym	Ukraine	44.74389	34.47611	1
Ae. cyl 323	639286	Alma-Ata	Kazakhstan	43.13889	76.6075	1
Ae. cyl 324	639293	Alma-Ata	Kazakhstan	43.27944	76.71694	1
Ae. cyl 325	639294	Alma-Ata	Kazakhstan	43.35389	76.83639	1
Ae. cyl 328	639317	Qurghonteppa	Tajikistan	38.87194	70.05972	1
Ae. cyl 330	639332	Badakhshoni Kuhi	Tajikistan	38.48889	70.98611	1
Total						119

Table A1.3. Eurasian accessions of jointed goatgrass from the Institute of Plant Genetics and Crops Plant Research Gatersleben, Germany.

New ID	IPK ID	Collection Detail	Country	n
Ae. cyl 101	AE 1024	NA	Czech Republic/Slovakia	2
Ae. cyl 105	AE 1025	NA	Czech Republic/Slovakia	2
Ae. cyl 123	AE 1035	Odessa	Ukraine	1
Ae. cyl 117	AE 1050	NA	Hungary	1
Ae. cyl 134	AE 1162	NA	Hungary	1
Ae. cyl 135	AE 1171	Tallard, Hautes Alpes	France	1
Ae. cyl 136	AE 1173	NA	USSR	1
Ae. cyl 104	AE 1347	Löss-Boden	Kazakhstan	2
Ae. cyl 151	AE 1613	NA	Germany	1
Ae. cyl 113	AE 225	NA	Azerbaijan	1
Ae. cyl 132	AE 289	Kopet-Dag	Turkmenistan	2
Ae. cyl 182	AE 293	NA	USSR	1
Ae. cyl 181	AE 298	Mamaia	Romania	1
Ae. cyl 120	AE 314	NA	Ukraine	1
Ae. cyl 168	AE 359	NA	Armenia	1
Ae. cyl 164	AE 365	Taskent	Uzbekistan	1
Ae. cyl 161	AE 368	NA	Armenia	1
Ae. cyl 153	AE 382	NA	Germany	1
Ae. cyl 138	AE 450	NA	Ukraine	1
Ae. cyl 154	AE 460	Tibilisi	Georgia	1
Ae. cyl 158	AE 466	Melnik	Bulgaria	1
Ae. cyl 160	AE 468	NA	USSR	1
Ae. cyl 133	AE 475	Erebuni	Armenia	1
Ae. cyl 119	AE 479	Remollon, Hautes Alpes	France	1
Ae. cyl 170	AE 495	NA	Hungary	1
Ae. cyl 169	AE 634	Krim	Ukraine	1
Ae. cyl 175	AE 640	Budaörs	Hungary	1
Ae. cyl 178	AE 656	Krim	Ukraine	1
Ae. cyl 183	AE 663	Tallard, Hautes Alpes	France	2
Ae. cyl 156	AE 676	Budaörs	France	1
Ae. cyl 162	AE 677	NA	Armenia	1
Ae. cyl 165	AE 694	NA	USSR	1
Ae. cyl 112	AE 70	NA	France	1
Ae. cyl 166	AE 705	Talloires	France	2
Ae. cyl 177	AE 715	NA	Kazakhstan	1
Ae. cyl 176	AE 718	Jerewan	Armenia	1
Ae. cyl 159	AE 719	Jerewan	Armenia	1
Ae. cyl 157	AE 720	Jerewan	Armenia	1
Ae. cyl 152	AE 726	Ordzhonikidze	Azerbaijan	1
Ae. cyl 109	AE 727	Maraza	Azerbaijan	1
Ae. cyl 172	AE 728	Baku	Azerbaijan	1
Ae. cyl 167	AE 745	Khorramabad	Iran	1
Ae. cyl 111	AE 77	Alma-Ata	Kazakhstan	1
Ae. cyl 116	AE 78	Alma-Ata	Kazakhstan	1
Ae. cyl 184	AE 790	Podo Iloaiei	Romania	1
Ae. cyl 185	AE 793	NA	Armenia	1

Ae. cyl 155	AE 805	Tallard, Hautes Alpes	France	1
Ae. cyl 171	AE 813	Sashegy, Budapest	Hungary	1
Ae. cyl 180	AE 836	Plovdiv	Bulgaria	1
Ae. cyl 106	AE 848	NA	Uzbekistan	2
Ae. cyl 174	AE 870	Tallard, Hautes Alpes	France	1
Ae. cyl 107	AE 887	Pavia	Italy	2
Ae. cyl 108	AE 889	Slaveni	Romania	2
Ae. cyl 130	AE 891	NA	Hungary	2
Ae. cyl 114	AE 93	Remollon, Hautes Alpes	France	2
Ae. cyl 125	AE 935	Bjurakan	Armenia	1
Ae. cyl 137	AE 936	Erevan	Armenia	1
Ae. cyl 115	AE 937	Arkatcharyntse to Vokhchaberd	Armenia	1
Ae. cyl 131	AE 958	NA	Kazakhstan	1
Ae. cyl 103	AE 987	NA	Bulgaria	1
Ae. cyl 121	AE 989	NA	Bulgaria	1
Ae. cyl 110	AE 990	NA	Bulgaria	1
Ae. cyl 163	AE 991	NA	Bulgaria	1
Ae. cyl 102	AE 998	NA	Bulgaria	2
Total				76

Table A1.4. Average ancestry coefficients for each state and region in the large-scale U.S. analysis from Structure.

Collection	K1	K2	K3	n
Colorado	0.542	0.318	0.140	22
Kansas	0.674	0	0.326	8
Nebraska	0.362	0.537	0.102	15
Oklahoma	0.570	0.375	0.055	8
New Mexico	0	0.194	0.806	2
Wyoming	0	0	1	6
South Dakota	0	0.999	0.0002	2
Region 1	0.434	0.324	0.242	63
Washington	0.991	0.007	0.002	8
Oregon	0.399	0.056	0.545	6
Region 2	0.737	0.028	0.235	14
Utah	6.667	0.559	0.441	12
Idaho	0.427	0.102	0.471	6
Montana	0	0.2	0.8	1
Region 3	0.135	0.396	0.469	19
Total	0.419	0.295	0.286	96

Table A1.5. Average ancestry coefficients for each population in the small-scale U.S. analysis from Structure. Populations are arranged by collection location.

Collection	S1	S2	n
Sterl-1	0.8	0.2	25
Sterl-2	1	0	26
Sterl-3	1	0	24
Sterl-4	1	0	23
Sterl-5	0.960	0.040	25
Sterl-7	0.941	0.059	17
Sterl-6	0.326	0.674	24
Total	0.859	0.141	164

Appendix 2

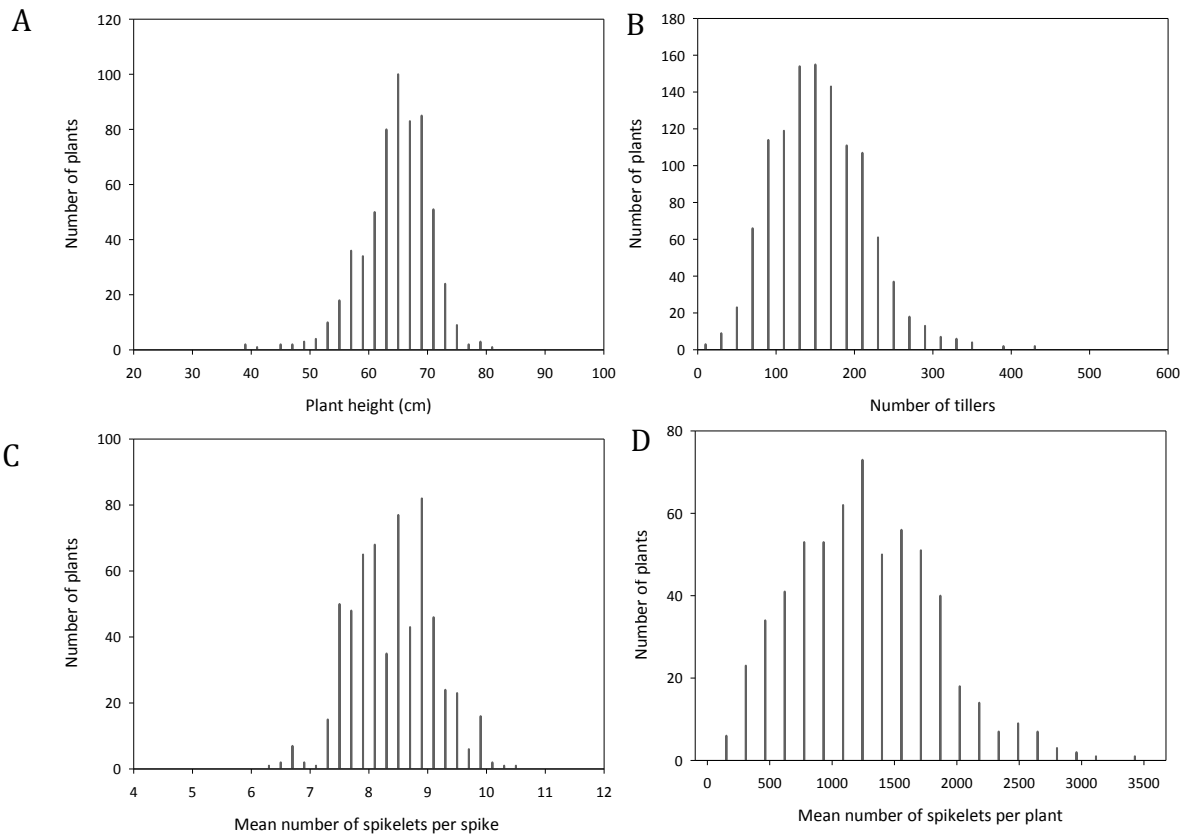


Figure A2.1A-D. Distribution of each of four traits evaluated on a field trial conducted on 30 accessions of jointed goatgrass: (A) mean plant height, (B) mean number tillers per plant, (C) mean number spikelets per spike, and (D) mean number of spikelets per plant.