Risk of Gene Introgression from Drought Tolerant Wheat to Jointed Goatgrass in the U.S. Great Plains

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Introduction

Jointed goatgrass (*Aegilops cylindrica* Host) is a wild relative of wheat (*Triticum aestivum* L.) introduced into the western U.S. from Eurasia. The species is an agricultural pest that infests wheat fields in the Western U.S. (Fig. 1) causing economic loss. Common ancestry between the two species enables interspecific hybridization, providing a mechanism for the potential introgression of advantageous wheat genes to its weedy relative (Fig. 2). Interest in the development of transgenic drought tolerant wheat cultivars has initiated concern for the potential for drought tolerance genes to introgress into jointed goatgrass.

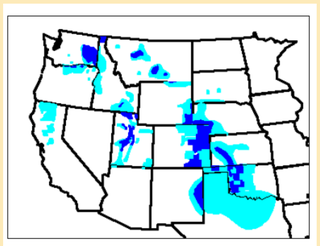


Fig. 1. U.S. distribution of jointed goatgrass (www.jointedgoatgrass.org/)

Present

Moderate to dense infestation

Fig. 2. Spikes of jointed goatgrass (left), winter wheat (right), and a wheat-goatgrass hybrid (center) collected in eastern Colorado.

Objectives

- To determine the rate of backcrossing of wheat-goatgrass F_1 hybrids to jointed goatgrass. Recurrent backcrossing to the weedy species is necessary to restore fertility and chromosome composition after hybridization with wheat.
- To assess jointed goatgrass collections from the western U.S. for phenotypic diversity under different soil moisture conditions.

Materials and Methods

Backcrossing frequency

- **Field experiments.** Trials were conducted at two Colorado locations: Fort Collins in 2007-08 and Haxtun in 2008-09. F_1 hybrid plants developed through manual crossing were transplanted in a grid pattern with 0.6 m spacing between plants. Jointed goatgrass was seeded over the entire plot. Because hybrid plants are male sterile, viable seed could only be produced through an outcrossing event with jointed goatgrass pollen.
- **Germination studies.** Spikes collected from 100 hybrid plants in 2008 and 106 hybrids in 2009 were used in germination studies. Spikes were stored at room temperature for at least 16 weeks to ensure non-dormancy (Fandrich and Mallory-Smith, 2006). They were then placed in flats with potting soil, covered with clear plastic lids, and germinated in growth chambers at 25/15 ºC with a 12-hr photoperiod .
  - Frequency of backcrossing was defined as Y/*n*, where Y is the number of seeds that germinated and *n* is the total number of florets. Since there are typically 1 to 3 viable florets per spikelet, the number of spikelets was multiplied by two to give *n* (Donald and Ogg ,1991).
  - The NlmixedProcedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC), which employs a maximum likelihood method, was used to obtain estimates of the mean and standard deviation of backcrossing, based on a complementary log-log transformation of the data. The estimates were then back-transformed to obtain median germination probabilities and upper (0.975) and lower (0.025) confidence limits.

Phenotypic diversity

- **Field experiments.** 30 jointed goatgrass accessions representing 12 western U.S. states were evaluated in Haxtun, CO in 2008-09 and Fort Collins, CO in 2009-10. The trial was arranged in 2 replications of a split plot design, with moisture treatment (rainfed or supplemented with drip irrigation) as the main plot and accession as the sub-plot. Each sub-plot consisted of two 3 m rows, with a final stand of 7 plants per row.
- **Statistical analysis.**
  - Data were collected on plant height, number of tillers/plant, and number of spikelets/spike. The first two of these traits were ln-transformed.
  - Analysis of variance was conducted with the GLM procedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC).

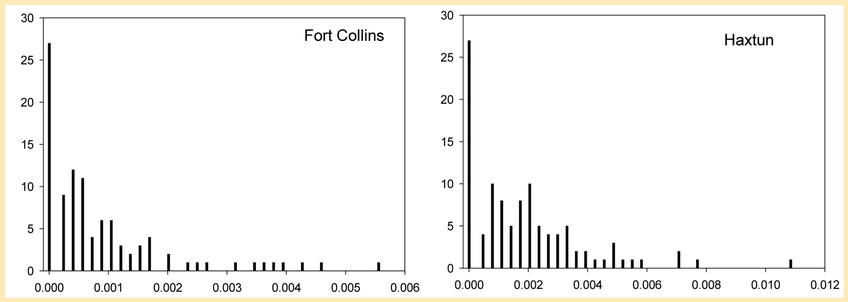
Results

Backcrossing frequency

- Flowering began the last week of May and continued through June at both locations. However, the degree of overlap in flowering times varied: in Fort Collins, hybrid plants began flowering prior to jointed goatgrass, while at Haxtun flowering of the hybrid and jointed goatgrass plants occurred concurrently.
- 214 BC_1 plants germinated from 248,856 florets from the Fort Collins collection giving a mean observed germination rate of 0.086%.
- For the Haxtun collection, 249 BC_1 plants germinated from 127,428 florets, resulting in a mean observed germination rate of 0.195%.
- Frequency distribution of BC_1 production per hybrid plant was not normal (Figure 2). Of the total 206 hybrid plants tested, 54 (26.2%) plants produced no BC_1 plants. The highest backcrossing rate observed for a single hybrid plant was 0.56% and 1.10% from Fort Collins and Haxtun, respectively.
- 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 median germination rate was 0.152% with a 95% confidence interval of 0.077 to 0.604%.

Fig. 3. Germination method for hybrid spikes (left 2/3 of flat) and control jointed goatgrass spikes (right 1/3).

Fig. 4. BC_1 germination frequency per hybrid plant at two Colorado locations (Econopouly, 2010).



- Our results indicate that in the Great Plains backcrossing occurs at a low rate, but that a field of hybrid plants could double its population size after one generation of backcrossing to the invasive species. Subsequent backcrossing would provide a mechanism for drought tolerance genes to introgress and spread to populations of the weed that are sensitive to drought stress, which as shown here, could vary across the western U.S.

Phenotypic diversity

- Data from Fort Collins 2009-10 are not completely analyzed. Therefore, only results from Haxtun 2008-09 are presented here.
- Due to unusually heavy rainfall in late spring and early summer of 2009, differences between the rainfed and irrigated treatments were small (Fig. 5). Therefore, means were calculated for all four replications rather than for each irrigation treatment.

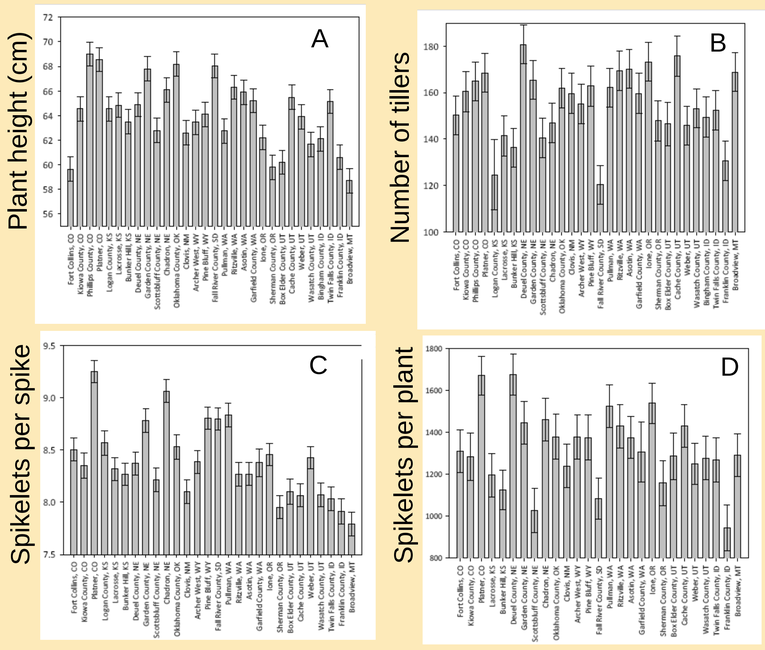
Fig. 5. Heavy rainfall in Haxtun resulted in vigorous growth and only minor differences between irrigation treatments.

Results

Phenotypic diversity (continued)

- Significant variation (P<0.001) among the 30 goatgrass accessions was observed for flowering time, plant height, tiller number, and spikelet number per spike and per plant (Fig. 6).
- This large range in values of traits related to competitiveness and reproductive fitness indicates that the risk of transgene introgression from wheat may vary with region within the U.S.
- Results from the 2009-10 season will provide information on differences in drought tolerance among the accessions.

Fig. 6. Means and standard errors for four traits evaluated for 30 jointed goatgrass accessions in Haxtun, CO 2009-10 (Econopouly, 2010).



Summary

- Frequency of backcrossing of jointed goatgrass to wheat-goatgrass hybrids had median values of 0.062% per plant at Fort Collins and 0.152% at Haxtun. Although low, these rates provide an avenue for gene introgression into goatgrass populations.
- Significant phenotypic variation was observed among 30 jointed grass accessions for traits related to competitiveness and reproductive fitness (plant height, number of tillers, spikelets per spike, and spikelets per plant).
- Together these results indicate a potential risk for introgression of wheat genes into jointed goatgrass populations.
- Ongoing work on this project is aimed at determining the patterns of wheat genome introgression into jointed goatgrass, based on cytogenetic and molecular marker analysis.

Acknowledgements

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