Genetic diversity and quantitative trait loci associated with seed and grain production traits of intermediate wheatgrass (*Thinopyrum intermedium*)

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Background and Objectives

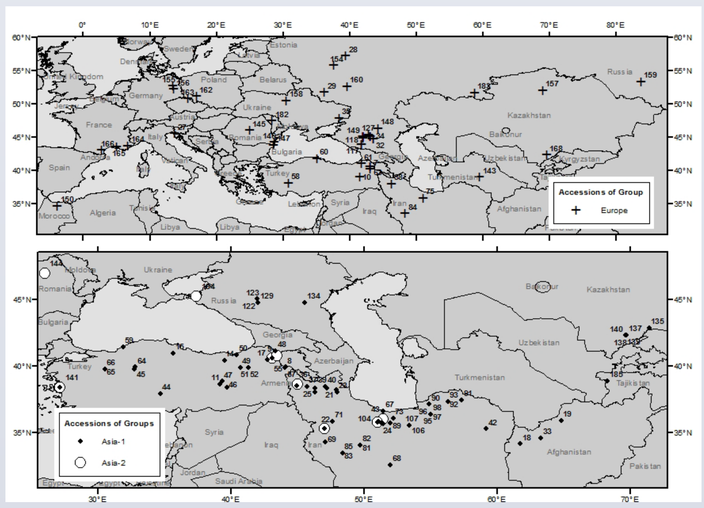
Intermediate wheatgrass (*Thinopyrum intermedium*) is a cool-season perennial grass used for soil conservation, forage production, and consumable grain products. Intermediate wheatgrass ranks among closest perennial relatives of wheat (*Triticum aestivum*) and has been used as a tertiary source of disease resistance and other agronomic traits in wheat breeding. Moreover, intermediate wheatgrass (IWG) has demonstrated potential as a low-input biomass crop. Like wheat, IWG is allohexploid (2*n*=6*x*=42) and both species have similar geographic origins, centers of diversity, and range of adaptation. However, improvement of seed size and other seed production traits is critical for the development of IWG as a multipurpose perennial-grain, forage, and biomass crop. The objectives of research described here include:

1) Evaluate genetic diversity, geographic origins, and broad-scale population structure of IWG plant materials available in the USDA National Plant Germplasm system,
2) Construct linkage maps and analyze genome organization of IWG,
3) Evaluate seed and grain production traits in a full-sib IWG family, and
4) Identify quantitative trait loci (QTLs) controlling these traits.

Genetic diversity, geographic origins, and population structure

A total of 910 plants representing 182 accessions from the USDA National Plant Germplasm System (NPGS) were genotyped with six AFLP primer pairs, which detected 643 polymophic DNA markers. Analysis of Molecular Variance (AMOVA) partitioned 31% of the total DNA variation among accessions and 69% of the variation within accessions. Bayesian cluster analysis identified three geo-genetic groups (Fig. 1).

Figure 1. Origins the Europe (52 accessions), Asia-1 (96 accesssions), and Asia-2 (10 accessions) geo-genetic IWG germplasm groups.



Linkage mapping and genome organization

A full-sib family comprised of 351 individuals was derived from a cross of two parents (M26 and M35) and three grandparents (Figure 2). Plants were sequenced and genotyped using the two-enzyme (*Pst*I and *Msp*I) approach (Poland et al. 2012) with 4 barcodes per plant, on a HiSeq 2500 (Illumina Inc., San Diego). Genotypes were called using the UNEAK pipeline (Lu et al. 2013). A total of 2,347 markers with less than 5% missing data (by marker) were mapped into 21 linkage groups (Fig. 3) using maximum likelihood mapping and CP model of JoinMap 4.1 (Kyazma B.V., Wageningen). Alignment of GBS markers from these 21 linkage groups to the seven chromosome sequences of barley (*Hordeum vulgare*), using Strudel (Bayer et. al. 2011), identified three sets of chromosomes in seven homoeologous groups (Fig. 3).

Figure 2. Pedigree of M26xM35 mapping population and inheritance of free-threshing (FT) seed trait.

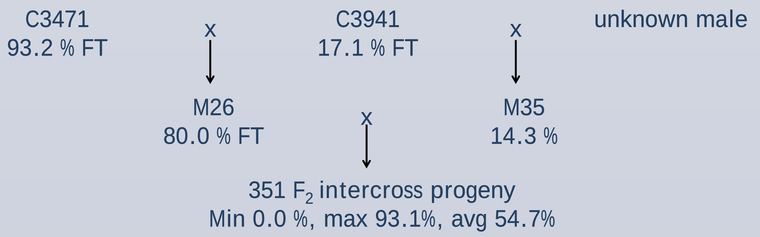
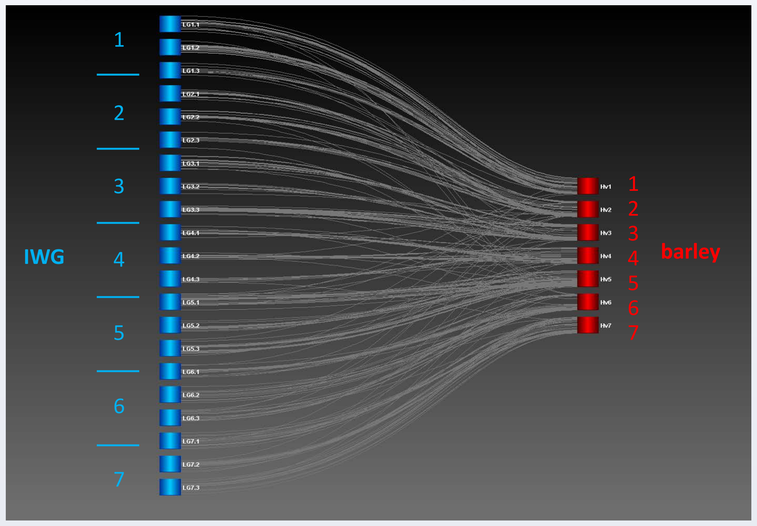


Figure 3. Alignment of 21 intermediate wheatgrass (IWG) linkage groups to the physical sequences of the seven barley chromosomes.



Trait evaluations

A total of 266 of the 351 genotyped M26xM35 F2 progeny were transplanted into clonally replicated field evaluations, on 1 m centers, with two randomized complete blocks near Salina, Kansas (November 28, 2012) and three randomized complete blocks near Logan, Utah (May 7, 2013). Spikes and seed from ten of the tallest culms were harvested, measured, and threshed using a small Vogel plot thresher and measured (Table 1, Fig. 4).

Figure 4. Sample of IWG spikes and free-threshing seed.

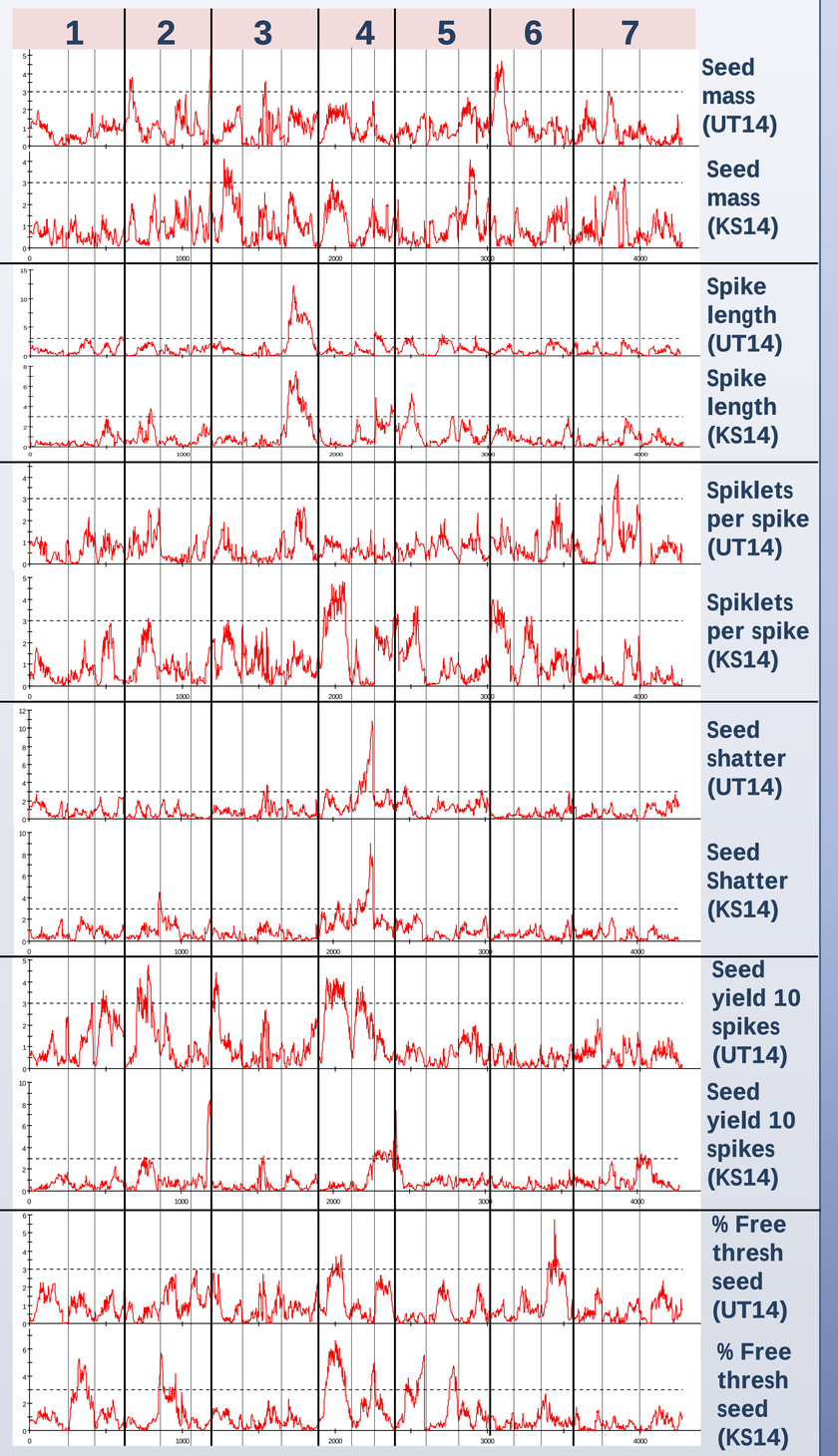
Table 1. Trait means (standard errors) for parents and F_2 progeny of the M26 x M35 mapping population.

Traits	C3471	C3941	M26	M35	Range F_2	Avg. F_2
Seed mass UT14 (mg)	7.98 (0.21)	5.66 (0.21)	7.08 (0.27)	6.35 (0.21)	2.88 – 8.35 (0.44)	5.61 (0.05)
Seed mass KS14 (mg)	7.70 (0.17)	5.28 (0.17)	4.94 (0.17)	5.32 (0.17)	3.61 – 7.88 (0.40)	5.48 (0.05)
Spike length UT14 (cm)	27.8 (0.5)	27.1 (0.5)	29.4 (0.5)	25.7 (0.5)	11.5 – 35.4 (1.1)	25.5 (0.2)
Spike length KS14 (cm)	26.8 (0.9)	28.1 (0.9)	32.3 (0.9)	26.6 (0.9)	18.3 – 41.3 (2.1)	27.9 (0.3)
Spiklets per spike UT14	22.9 (0.9)	22.7 (0.9)	23.6 (1.0)	19.6 (0.9)	10.0 – 37.0 (2.1)	19.9 (0.2)
Spiklets per spike KS14	24.1 (0.7)	21.5 (0.7)	24.6 (0.7)	19.9 (0.7)	14.0 – 28.0 (1.6)	20.8 (0.2)
Seed shatter UT14	11.0 (4.6)	50.7 (5.1)	56.1 (5.5)	95.9 (4.8)	0.0 – 98.0 (8.9)	52.0 (1.8)
Seed shatter KS14	2.5 (2.9)	7.1 (2.9)	2.3 (2.9)	19.6 (2.9)	0.0 – 35.5 (6.4)	6.5 (0.5)
Seed yield 10 spikes (g) UT14	7.98 (0.21)	5.66 (0.21)	7.08 (0.27)	6.35 (0.21)	2.88 – 8.35 (0.44)	5.59 (0.05)
Seed yield 10 spikes KS14	25.7 (1.1)	3.3 (1.1)	11.8 (1.1)	5.2 (1.1)	2.6 – 27.3 (2.5)	10.1 (0.3)
% Free thresh seed UT14	95.2 (4.3)	33.4 (4.9)	89.3 (5.8)	24.0 (4.3)	21.7 – 98.1 (9.5)	78.0 (0.9)
% Free thresh seed KS14	91.1 (3.4)	0.8 (3.4)	70.6 (3.4)	4.6 (3.4)	0.0 – 81.3 (7.7)	24.8 (1.5)

QTL analysis

Trait means (Table 1) were analyzed using an interval-mapping mixture model for a cross-pollinated (CP) population type in MapQTL version 6.0 (Kyazma B.V., Wageningen). A threshold of 3.0 LOD was used as the threshold test statistic for the tentative identification of QTLs (Fig. 5). A two-way pseudo-testcross model was also used to analyze QTLs from each parent (M26 and M35) separately (results not shown).

Figure 5. Genome-wide QTL scans (LOD vs. cM) over 21 IWG linkage groups corresponding to the 7 homoeologous groups (*x*=7).



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