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Authors

Godoy, J
Gizaw, S
Chao, S
et al.

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RESEARCH

Genome-wide Association Study of Agronomic Traits in a Spring-Planted North American Elite Hard Red Spring Wheat Panel

Jayfred Godoy, Shiferaw Gizaw, Shiaoman Chao, Nancy Blake, Arron Carter, Richard Cuthbert, Jorge Dubcovsky, Pierre Hucl, Ken Kephart, Curtis Pozniak, P.V. Vara Prasad, Michael Pumphrey,* and Luther Talbert

ABSTRACT

Inbred cultivars and advanced breeding lines have been subjected to numerous recombination cycles, have strong allelic selection for desired traits, and share important attributes for adaptation and agronomic performance. Genetic variation in elite gene pools captured using molecular markers is immediately useful for cultivar development. The primary goal of this study was to implement a genome-wide association study for 17 agronomic traits using elite inbred lines. A panel consisting of 237 elite hard red spring wheat (*Triticum aestivum* L.) lines from different wheat breeding institutions in North America were evaluated in 11 locations over 2 yr. A total of 19,192 polymorphic single-nucleotide polymorphism (SNP) markers from the Illumina 90K SNP array and markers linked to major genes controlling plant height, photoperiod sensitivity, and vernalization were used to assay the population. Linkage disequilibrium was observed to decay within a map distance of ~3 cM in the A and B genomes and 7 cM in the D genome. A total of 226 marker-trait associations were identified. Potentially novel associations were detected for grain yield on chromosome 2B and kernels per spike on 1B and 7D, whereas others colocalized with well-known adaptation loci for photoperiod response, vernalization, and plant height. The frequency of positive alleles for specific marker-trait associations differed among the programs, suggesting targets for introgression by the respective breeding programs.

J. Godoy, S. Gizaw, A. Carter, and M. Pumphrey, Dep. of Crop and Soil Sciences, Washington State Univ., Pullman, WA 99164; S. Chao, USDA-ARS Genotyping Lab., Biosciences Research Lab., Fargo, ND 58102; N. Blake and L. Talbert, Dep. of Plant Sciences and Plant Pathology, Montana State Univ., Bozeman, MT 59717; R. Cuthbert, Agriculture and Agri-Food Canada, Swift Current, SK S9H 3X2, Canada; J. Dubcovsky, Dep. of Plant Sciences, Univ. of California, Davis, CA 95616; P. Hucl and C. Pozniak, Crop Development Centre, Univ. of Saskatchewan, Saskatoon, SK S7N 5A8, Canada; K. Kephart, Southern Agricultural Research Center, Huntley, MT 50037; P.V.V. Prasad, Dep. of Agronomy, Kansas State Univ., Manhattan, KS 66506. Received 14 July 2017. Accepted 26 Apr. 2018. *Corresponding author (m.pumphrey@wsu.edu). Assigned to Associate Editor Jesse Poland.

Abbreviations: AM, association mapping; BLUP, best linear unbiased predictor; FWD, days to anthesis; GFD, grain-filling duration; GNY, grain yield; GWAS, genome-wide association studies; HDD, days to heading; HIN, harvest index; HRS, hard red spring; LD, linkage disequilibrium; KASP, Kompetitive Allele Specific Polymerase Chain Reaction; KPS, kernels per spike; LOESS, locally weighted polynomial regression; MAF, minor allele frequency; MAT, days to maturity; MTA, marker-trait association; PDL, peduncle length; PHT, plant height; PIC, polymorphic information content; QTL, quantitative trait locus; SDS, sodium dodecyl sulfate sedimentation; SKD, spikelets per head; SNP, single-nucleotide polymorphism; SPL, spike length; SSD, stem solidness; TCAP, Triticeae Cooperative Agricultural Project; TKW, thousand-kernel weight; TLN, tiller number; TWT, test weight; WAMI, wheat association mapping initiative; WGP, whole-grain protein concentration.

Exchange of germplasm is an integral part of successful plant breeding programs and has significant value in maintaining genetic diversity. Elite lines exchanged between programs are crossed to generate diverse progenies and breeding materials. Attributes for a particular region such as vernalization requirement, height, phenology, and maturity characteristics are dictated

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by climate, whereas end-use-quality attributes are determined by market forces. For this reason, initial crosses between adapted lines and lines from other areas do not tend to produce acceptable cultivars, as many of the genes from the introduced line are probably unfavorable in a particular environment. However, even genotypes that do not perform well have favorable genes. These are difficult to identify if the line itself has a poor phenotype due to lack of adaptation to a particular region. Recent advances in molecular biology have pointed to the possibility that breeders may be able to identify and share superior genes, instead of entire genotypes (Suslow et al., 2002).

Quantitative trait loci (QTLs) that control yield and yield components typically have small effects and low heritability and are significantly influenced by environment (Cuthbert et al., 2008). Thus, QTLs associated with these yield traits are challenging to detect in cultivar development because there is limited information on their number and location in the genome, and the contribution of each gene to the final expression of the trait (Mohan et al., 1997). Association mapping (AM) for QTL detection uses a collection of germplasm such as landraces, breeding lines, gene bank accessions, and cultivars that represent a larger population (Risch 2000). Sampling diverse individuals can significantly increase polymorphism among markers and exploit historical recombination, which leads to improved marker coverage and mapping resolution. There is likely more phenotypic diversity in an AM panel than in a biparental population, and variation for many traits can be studied using the same genotype data, making resource use very efficient (Jannink et al., 2001). Earlier AM studies were restricted to a few targeted candidate genes (Thornsberry et al., 2001) or chromosome regions due to limitations in marker availability, format, and cost. However, with the development of high-throughput genotyping platforms over the past decade, large-scale association analyses across the entire genome, known as genome-wide association studies (GWAS), became possible and were widely implemented in many species.

Wheat (*Triticum aestivum* L.) is the main source of food for roughly one-third of the world's population (Arzani and Ashraf 2017). With global population growth and cultivated production areas decreasing (Godfray et al., 2010), substantial genetic yield gains are necessary to keep up with the demand for wheat. This can be achieved by targeting genetic improvement efforts towards wheat cultivars with high yield potential, resistance to pest and diseases, and resilience to environmental instability. Genome-wide association studies in wheat have been successful in detecting QTLs for resistance to major wheat pests (Joukhadar et al., 2013), diseases (Kollers et al., 2013; Yu et al., 2011; Gurung et al., 2014; Maccaferri et al., 2015) and end-use-quality traits (Breseghello and Sorrells 2006; Reif et al., 2011), but relatively few report on QTLs for yield and yield components that are significant across environments and breeding programs. Recently, cost-efficient single-nucleotide polymorphism (SNP) platforms have been developed for wheat (Cavanagh et al., 2013; Wang et al., 2014). These platforms allow extensive analysis of linkage disequilibrium (LD) in different wheat germplasm, which is critical in designing informative and valuable association mapping panels for complex traits. In this study, we used an association mapping panel consisting of 237 elite hard red spring (HRS) wheat breeding lines and cultivars from different US and Canadian wheat breeding programs and CIMMYT to identify marker-trait associations (MTAs). We also examined the population structure, LD, and distribution of the MTAs across the breeding programs. Information from this research can be beneficial in molecular breeding and marker-assisted selection to allow breeders to efficiently use introduced germplasm.

MATERIALS AND METHODS

Genetic Materials, Genotyping, and SNP Calling

An AM panel of 250 elite HRS wheat cultivars and breeding lines was reduced to 237 genotypes after filtering for >5% missing data and duplicate genotypes indicated by an identity by state (IBS) value of one. The 237 lines represented 10 wheat breeding programs in North America (Supplemental Fig. S1). The panel represents elite diversity in each breeding program.

Fresh leaf tissues from three seedlings of each line were pooled for genomic DNA extraction following the cetyltrimethylammonium bromide (CTAB) procedure (Saghai-Maroof et al., 1984). Single-nucleotide polymorphism genotyping was implemented using the Illumina iSelect 90K SNP array at the USDA-ARS genotyping laboratory in Fargo, ND, as described in Wang et al. (2014). Monomorphic and low-quality SNPs were first discarded using GenomeStudio software 2011.1 (Illumina, 2011). The default clustering algorithm in GenomeStudio was used to select biallelic SNPs that showed distinct clusters corresponding to AA, AB, and BB genotypes. Compressed SNP allele clusters that could be discriminated by the clustering algorithm were manually curated (Cavanagh et al., 2013). Markers with a minor allele frequency (MAF) <0.05 were excluded due to lack of statistical power required to determine association with very rare alleles (Tabangin et al., 2009). A total of 19,192 SNP markers that were positioned on the wheat consensus map, developed by Wang et al. (2014), were used in the association analyses. Additional genotypic data were generated for major functional genes in wheat as described in Grogan et al. (2016). The Kompetitive Allele Specific Polymerase Chain Reaction (KASP) markers KASP-wMAS000001 and KASP-wMAS000002 were used to discriminate alleles at plant height (PHT) loci *Rht-B1* and *Rht-D1*, respectively. Alleles for photoperiod response genes were detected using KASP-Ppd-A1prodel for *Ppd-A1*, KASP-wMAS000027 and KASP-TaPpdBJ003 for *Ppd-B1*, and KASP-wMAS000024 for *Ppd-D1*. Vernalization genes were assayed using KASP-wMAS000033 and KASP-wMAS000035 for *Vrn-A1*, KASP-VrnB1_I_D, KASP-wMAS2000037 and KASP-VrnB1_C for *Vrn-B1*, and

KASP-wMAS000039 for *Vrn-D1*. Sequence-tagged sites (STS) markers UMN19, UMN25, and UMN26 were used to assay for high molecular weight glutenin genes *Glu-A1* and *Glu-D1* (Liu et al., 2008).

Phenotyping and Statistical Analyses

The mapping population was evaluated in 2012 and 2013 across a total of 11 environments in the United States and Canada (Table 1, Supplemental Table S1). A description of each environment is presented in Table 1. Optimal fertilizer, weed, and pest management were implemented to avoid external factors that limit yield potential. Phenotypic measurements taken at each plot included days to heading (HDD, d), days to anthesis (FWD, d), grain-filling duration (GFD, d), days to maturity (MAT, d), PHT (cm), peduncle length (PDL, cm), tiller number (TLN, number of tillers), stem solidness (SSD, rating scale 1–5, where 1 indicates a completely hollow lumen and 5 indicates a lumen completely filled with pith; DePauw and Read, 1982), harvest index (HIN), spike length (SPL, cm), kernels per spike (KPS, number of kernels), spikelets per head (SKD, number of spikelets), thousand-kernel weight (TKW, g), test weight (TWT, g L^{-1}), sodium dodecyl sulfate sedimentation (SDS), whole-grain protein concentration (WGP, %), and grain yield (GNY, kg ha^{-1}). Full description of each trait and how they were measured can be accessed in the T3/Wheat website (https://triticeaetoolbox.org/wheat/traits.php). Plots were arranged in an augmented design with single replications for each line and six check cultivars (AC Barrie, Berkut, Thatcher, Hollis, Choteau, and Clear White) replicated across five blocks. For each location, the mixed linear model procedure using the PROC MIXED procedure in SAS 9.3 (SAS Institute, 2000) was used to obtain best linear unbiased predictors (BLUPs) considering check cultivars as fixed and genotypes and blocks as random effects. The significance of statistical differences between genotypes was tested for each trait using the mixed linear model procedure. Then, combined data analysis across environments was implemented also using PROC MIXED to obtain the BLUPs and variance components for all traits considering factors (i.e., environments and genotypes) in the model as random. The mixed linear model was $\mathbf{y} = X + \mathbf{Z}\upsilon + \mathbf{e}$, where $\mathbf{y}$ is the vector of observations for an individual quality trait, X is the intercept, υ is the vector of random effects, $\mathbf{Z}$ is the design matrix for the random effects, and $\mathbf{e}$ is the vector of residuals. Relationships between phenotypic traits were obtained using Pearson's (r) correlation statistics and principal component analyses in JMP Genomics 6.0 (SAS Institute, 2000). A repeatability estimate (r^2) was calculated across environments following the formula described in Sukumaran et al. (2015):

$$r^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_{G\times E}}{l} + \frac{\sigma^2_E}{rl}}$$

where r^2 is the repeatability estimate, σ^2_G is the genetic variance, $\sigma^2_{G\times E}$ is the genotype × environment variance, σ^2_E is the residual variance, r is the number of replications, and l is the number of environments.

Marker Polymorphism and Linkage Disequilibrium

Allelic information for SNP markers among accessions including allele frequency, gene diversity, and polymorphic information content (PIC) were estimated using PowerMarker version 3.25 software (Liu and Muse 2005). Linkage disequilibrium among mapped markers was analyzed using JMP Genomics 6.0 (SAS Institute, 2000) by computing the squared allele-frequency correlations (r^2) according to Weir (1996). Pairwise marker combinations with significant r^2 ($p < 0.01$) were included in the LD analysis. The unlinked r^2 and syntenic r^2 were estimated separately for unlinked loci (distance > 50 cM) on the same chromosome. A critical r^2 value beyond which LD is due to physical linkage was determined by taking the 95th percentile of the square-root-transformed r^2 data of unlinked markers (Breseghello and Sorrells 2006). Locally weighted polynomial regression (LOESS)-based curves were then fitted on scatter plots of r^2 values plotted against the genetic distance (cM) using the lowess function (smoother setting = 0.075) implemented in RStudio 1.1.383 (RStudio Team, 2016). The intersection of the LOESS curve fit and critical value of r^2 was considered as the estimate of the extent of LD in the chromosome.

Table 1. Weather data including average temperature, total precipitation, and amount of water supplied by irrigation during the growing season of the Triticeae Cooperative Agricultural Project elite hard red spring wheat panel in 2012 and 2013.

				Irrigated	Rainfed
Year	Location	Coordinates	Avg. temperature during the growing season	Amount of irrigation applied	Total precipitation during the growing season
			°C	mm	
2012	Bozeman, MT	45.676° N, 111.157° W	15.3	0	148.0
	Huntley, MT	45.928° N, 108.246° W	17.6	0	94.0
	Manhattan, KS	39.137° N, 96.640° W	21.9	300	245.2
	Saskatoon, SK	52.117° N, 106.65° W	16.1	0	376.8
	Davis, CA	38.526° N, 121.773° W	11.9	300	255.3
	Othello, WA	45.928° N, 108.246° W	17.1	600	65.3
2013	Bozeman, MT	45.676° N, 111.157° W	14.2	0	191.0
	Huntley, MT	45.928° N, 108.246° W	15.9	0	248.4
	Saskatoon, SK	52.117° N, 106.65° W	15.9	0	196.4
	Swift Current, SK	20.291° N, 107.707° W	16.1	0	319.8
	Othello, WA	45.928° N, 108.246° W	17.5	600	70.6

Population Structure Analysis

Seven hundred and forty-nine SNP markers spaced ~3 to 4 cM apart were sampled from all chromosomes to analyze population structure. The Bayesian model-based clustering program STRUCTURE version 2.3 (Pritchard et al., 2000) was used to estimate the number of subpopulations (K) given an admixture model and correlated frequencies. Simulations were run for 10,000 burn-in iterations, followed by 100,000 Markov chain Monte Carlo replicates. The number of K was set from 2 to 9, with 10 independent runs made for each K. The results from structure were visualized using Structure Harvester (Earl and vonHoldt 2012), and the K number of subpopulations was determined using the ad-hoc criterion described in Evanno et al. (2005). Principal component analysis was also conducted to further analyze the dispersion of the subpopulations in a three-dimensional space using JMP Genomics 6.0 (SAS Institute, 2000). Pairwise genetic distance (Nei 1972) was calculated among genotypes and breeding institutions. Genetic distance, denoted by fixation index, within and among the inferred groups and the entire population was measured by analysis of molecular variance implemented in PowerMarker version 3.25 (Liu and Muse 2005). A neighbor-joining tree based on the unweighted pair-group method with arithmetic average was also created using PowerMarker 3.25 (Liu and Muse 2005) and visualized in MEGA 5.2 (Tamura et al., 2011).

Marker-Trait Association Analysis

A total of 19,192 SNP markers were used to detect MTAs. Genome-wide association analysis on the 17 agronomic traits was performed using the FarmCPU method (Liu et al., 2016), which was implemented in GAPIT2 (Tang et al., 2016). To control for population structure, principal components were fitted as covariates and the corresponding quantile-quantile plots were compared to select the best model. Marker-trait association analysis was conducted separately for each environment and on the combined data using the BLUPs for each trait. Significant associations in the combined analysis were tested using the Bonferroni multiple test correction method at a Type I error of 5%, which is equivalent to $P = 2.61 \times 10^{-6}$ (0.05/19,192 SNPs). The proportion of phenotypic variation explained (R^2) by the SNP was calculated using a multiple linear regression model with all significant SNPs fitted as independent variable with fixed effect. Marker-trait associations with P <0.01 in two or more environments after Bonferroni correction in at least one environment were also reported as "multienvironment" MTAs.

RESULTS

Agronomic Trait Means and Correlations

Analysis of variance showed significant differences ($p < 0.05$) among genotypes for most traits in all environments and for the combined data analysis across environments. This is consistent with the wide range of values of each trait (Table 2). For traits like PHT and SPL, the maximum values are almost twice the minimum. For SSD and SDS, the maximum values were almost triple the minimum values. Traits such as TLN, HIN, SKD, KPS, TKW, WGP, and GNY had a narrower range of values than other traits. Values for HDD, FWD, MAT, and GFD values ranged from 12, 10, 9, and 4 d, respectively. On average, the panel reached heading stage in 66 d and matured 31 d later. Mean GNY across all trials was 3754 kg ha^{-1}, and the highest yield was 4198 kg ha^{-1}.

Although most of the trait variation was attributed to differences in genotype and environment, significant genotype × environment interaction was also observed. Repeatability estimates ranged from 0.28 for HIN to 0.96 for PHT. More complex traits such as GNY, HIN, and TLN had lower repeatability estimates than PHT, DTH, and WGP. Biplots showing the phenotypic correlations between traits were plotted (Fig. 1). Traits with strong positive correlations include HDD and FWD, GFD and TWT, PHT and WGP, and GNY and KPS. On the other hand, GNY was negatively correlated with WGP, PHT, SDS, and PDL. Significant Pearson coefficients showed the magnitude of the correlations seen in the biplots (Supplemental Table S2). Traits FWD and HDD had the highest correlation at 0.92, and KPS (0.52) and HIN (0.49) had the highest positive correlation with GNY, followed by GFD (0.27), MAT (0.24), and TKW (0.22). Other traits such as WGP (−0.65), PHT (−0.52), PDL (−0.27), and SDS (−0.20) were negatively correlated with GNY.

Polymorphism, Gene Diversity, and Marker Density

The B genome had the highest number of polymorphic markers (9803) and the most dense SNP coverage (1 SNP per 0.12 cM), followed by the A genome with 7593 SNPs at a density of 1 SNP per 0.16 cM (Supplemental Table S3). The D genome was the least covered with 1796 polymorphic markers and an average spacing of 1.0 cM. Among all chromosomes, 1B and 6B had the most dense marker coverage at 0.09 cM. The number of polymorphic SNP markers on each chromosome ranged from 81 (4D) to 1837 (1B). Average PIC values between A and B genomes were similar, whereas the D genome had the smallest PIC value. Among different chromosomes, average PIC values ranged from 0.25 to 0.31.

Population Structure and Linkage Disequilibrium

The maximum peak of ΔK when plotted against the number of subpopulations (2–9) occurred at $K = 2$ (Evanno et al., 2005; Supplemental Fig. S2A). Population differentiation (fixation index = 0.13) values between the two groups were significant ($p < 0.001$), suggesting moderate population structure (Hartl and Clark, 1997). The 237 accessions were then assigned into two subgroups according to the membership coefficients in the STRUCTURE analysis (Supplemental Fig. S2B). The subdivisions corresponded to the regional origin of the breeding programs contributing the accessions. The first subgroup,

Table 2. Descriptive statistics, variance parameters, and repeatability estimated for yield and yield components from 237 lines of the elite hard red spring wheat panel grown in 2012 and 2013 based on means over 11 environments.

Trait	Trait abbreviation	Descriptive statistics			Variance parameters†			
		Mean	Min.	Max.	σ^2_G	$\sigma^2_{G\times E}$	σ^2_E	r^2
Days to heading (d)	HDD	66.30	61.93	73.48	4.32	1.46	1.00	0.92
Days to anthesis (d)	FWD	61.20	57.79	67.93	2.93	0.00	0.99	0.92
Days to maturity (d)	MAT	97.20	93.64	102.80	2.39	0.99	1.15	0.85
Grain-filling duration (d)	GFD	36.57	34.58	38.37	0.71	0.89	1.00	0.60
Plant height (cm)	PHT	84.47	59.76	107.16	72.32	30.90	1.00	0.96
Peduncle length (cm)	PDL	22.41	18.16	27.63	4.80	14.25	0.94	0.49
Tiller number (no. of tillers)	TLN	422.02	395.15	455.37	366.24	2,559.83	1.00	0.42
Stem solidness (rating 1–5‡)	SSD	8.51	6.72	17.67	3.99	0.59	2.02	0.82
Harvest index	HIN	0.38	0.35	0.40	0.00	0.00	0.00	0.28
Spike length (cm)	SPL	9.30	7.38	11.46	0.77	0.00	0.90	0.72
Spikelets per head (no. of spikelets)	SKD	16.65	15.00	18.32	0.65	1.19	0.99	0.47
Kernels per spike (no. of kernels)	KPS	33.29	28.31	43.74	7.52	19.44	0.98	0.72
1000-kernel weight (g)	TGW	33.84	28.65	40.03	5.19	6.79	1.01	0.86
Test weight (g L^{-1})	TWT	779.06	739.38	809.93	169.03	181.70	1.05	0.87
Sodium dodecyl sulfate sedimentation	SDS	60.52	28.82	78.59	108.09	40.75	1.00	0.89
Whole-grain protein (%)	WGP	14.19	11.85	16.23	0.64	0.00	0.53	0.92
Grain yield (kg ha^{-1})	GNY	3,753.92	3,163.52	4,198.00	56,394.00	464,065.00	0.96	0.57

† σ^2_G, genetic variance; $\sigma^2_{G\times E}$, genotype × environment variance; σ^2_E, residual variance.

‡ Stem solidness rating 1 to 5, where 1 indicates a completely hollow lumen and 5 indicates a lumen completely filled with pith. Ratings were conducted on five stems and were summed up to get the final rating score.

QI, was composed mostly of accessions from the Northern Plains of the United States, including Canada. The second subgroup, QII, consisted mostly of accessions from the western United States and CIMMYT. A majority (127) of the accessions belonged to QII, whereas 110 accessions comprised QI. Principal component analysis also provided evidence of population structure. The first two principal components accounted for 12.5% of the total variation of the panel. The first principal component separated the panel into two major subgroups, similar to the results from STRUCTURE (Fig. 2A). The two major clusters were also identified using genetic-distance-based

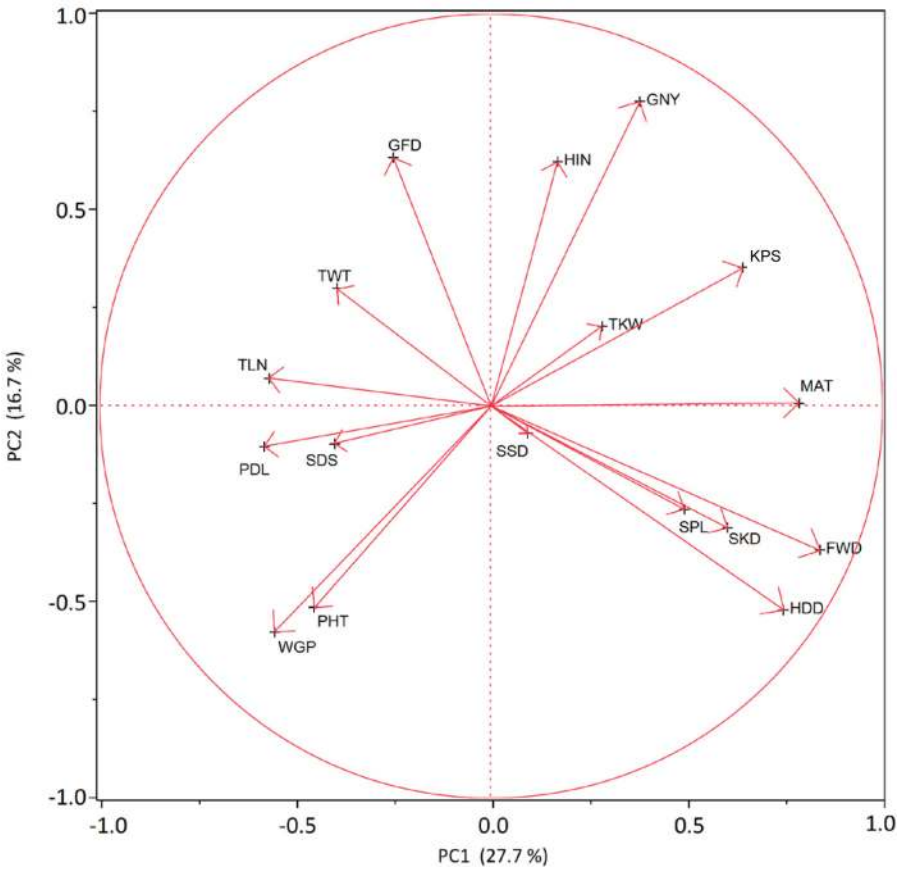

Fig. 1. Biplot of the phenotypic correlation matrix for the 17 morphoagronomic traits studied in the elite spring wheat panel grown in 2012 to 2013. PC1 and PC2 refer to Principal Components 1 and 2. FWD, days to anthesis; GFD, grain-filling duration; GNY, grain yield; HDD, days to heading; HIN, harvest index; KPS, kernels per spike; MAT, days to maturity; PDL, peduncle length; PHT, plant height; SDS, sodium dodecyl sulfate sedimentation; SKD, spikelets per head; SPL, spike length; SSD, stem solidness; TKW, thousand-kernel weight; TLN, tiller number; TWT, test weight; WGP, whole-grain protein concentration.

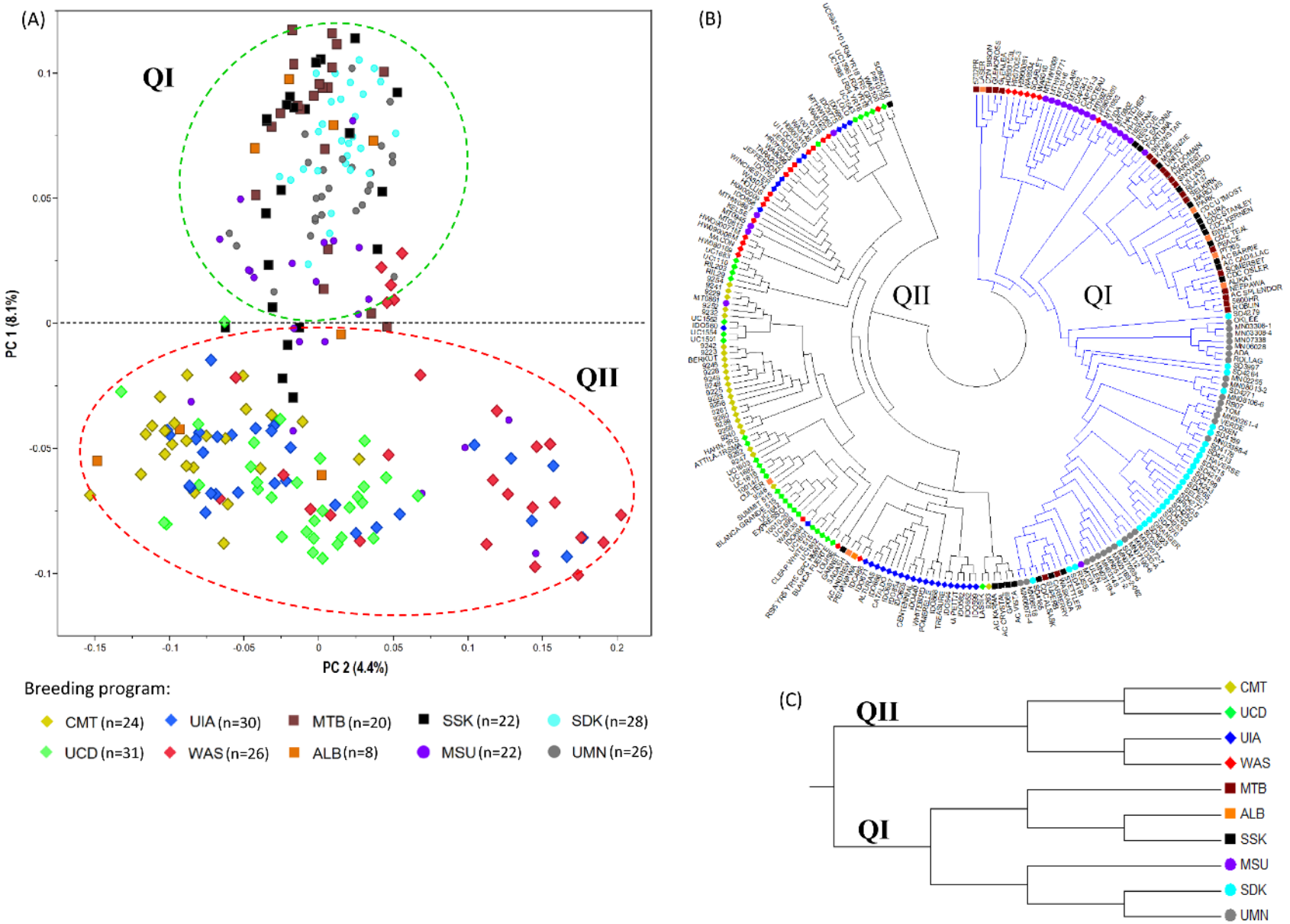

Fig. 2. Population structure analysis using (A) principal component analysis and (B) genetic distance-based cluster analysis of the elite hard red spring genotypes using 19,192 single-nucleotide polymorphism markers from all genomes. Subgroup I (QI) includes 110 genotypes from breeding programs in the Northern Plains (Montana, South Dakota, Minnesota) and Canada. Subgroup II (QII) consists of 127 genotypes from breeding programs in the western United States (Washington, Idaho, California) and CIMMYT. Cluster analysis (C) was only conducted among the 10 breeding programs that created the genotypes in the panel. PC1 and PC2 refer to Principal Components 1 and 2. ALB, University of Alberta; CMT, CIMMYT; MSU, Montana State University; MTB, Agriculture and Agri-Food Canada (Manitoba); SDK,South Dakota State University; SSK, University of Saskatchewan–Agriculture Agri-Food Canada (Saskatchewan); UCD, University of California–Davis; UIA, University of Idaho–Aberdeen; UMN, University of Minnesota; WAS, Washington State University.

cluster analysis (Fig. 2B), although it did not show perfect concordance in membership. Some genotypes in certain breeding programs were genetically much closer to one or more lines from a different program. For example, eight, seven, and three genotypes from Montana, Saskatchewan, and Alberta, respectively, grouped with genotypes in QII instead of QI. Conversely, two Washington genotypes were genetically closer to members of QI instead of QII, where most Washington genotypes clustered. Cluster analysis also showed the two major subpopulations corresponding to that identified by STRUCTURE (Fig. 2C).

Multiple regression analysis with population structure in the model showed that most traits were affected by population structure (Table 3). Traits affected by population structure included WGP (38%), followed by KPS (37.19%), PHT (29.84%), MAT (27.46%), FWD (24.85%), and TLN (23.60%). Traits GFD and SSD were the only ones whose variation was not significantly explained by population structure.

Linkage disequilibrium was measured using the r^2 of significant ($p < 0.01$) intrachromosomal marker pairs (Supplemental Table S4). A total of 11,906,433 intrachromosomal pairs were tested, of which close to 33% were significant at $p < 0.01$. The mean r^2 across the genome was 0.20. The D genome (43.52%) had the highest percentage of markers in LD, followed by the A genome (34.28%) and the B genome (31.51%). The largest proportion of markers in complete LD ($r^2 = 1$) was located on the D genome (9.7%). Chromosome 5D (47.50%) had the highest percentage of marker pairs in LD, followed by chromosome 2D (46.67%), whereas chromosome 3D (25.49%) had the fewest markers in LD. The highest proportion (15%) of markers in complete LD was on chromosome 1D. The genome-wide critical value of $r^2 > 0.36$ (95th percentile of r^2 between unlinked SNPs) was determined to be the appropriate threshold for LD due to physical linkage. The intersection of the smoothing curve with this threshold indicated that the length of LD decay was at ~3 cM genome-wide (Supplemental Fig. S3A). Linkage disequilibrium decayed at ~2 and 3 cM in the A and B genomes, respectively. The longest LD decay was seen in the D genome, which extended up to 8 cM (Supplemental Fig. S3B).

Table 3. Percentage of phenotypic variation explained (R^2) by population structure according to the combined data across environments in 2012 and 2013.

Trait	Environments†	R^2	*P* value‡
		%	
Days to heading	7	10.38	<0.0001
Days to flowering	4	24.85	<0.0001
Days to maturity	5	27.46	<0.0001
Grain-filling duration	4	0.02	0.8165
Plant height	11	29.84	<0.0001
Peduncle length	3	13.55	<0.0001
Tiller number	5	23.60	<0.0001
Stem solidness	3	0.65	0.2177
Harvest index	3	4.57	0.0009
Spike length	3	11.33	<0.0001
Spikelets per head	3	10.72	<0.0001
Kernels per spike	7	37.19	<0.0001
1000-kernel weight	9	14.43	<0.0001
Test weight	7	11.5	<0.0001
Sodium dodecyl sulfate sedimentation	3	6.82	<0.0001
Whole-grain protein	10	38.00	<0.0001
Grain yield	11	18.56	<0.0001

† The number of environments used in combined analysis.

‡ Significance threshold *P* value is 0.001.

Marker-Trait Associations

A total of 226 MTAs was identified for the 17 traits evaluated in the study, including 168 multienvironment MTAs and 58 that were detected using the combined data (BLUPs). A complete list of the MTAs for each trait is presented in Supplemental Table S5. Manhattan and quantile-quantile plots of the MTAs identified in the combined analysis are shown in Supplemental Fig. S4 and S5, respectively. Generally, the MTAs accounted for a modest amount of the phenotypic variation for each trait. Across genomes, most of the MTAs were identified on the A genome (99), followed by the B (95) and D (32) genomes. Multiple MTAs were observed in each chromosome, with the most associations located on chromosomes 4A and 5A with 25 MTAs each (Supplemental Fig. S6). Only two MTAs were found for chromosome 3D. The most MTAs per trait detected across environments and combined analysis was 35 MTAs for PHT.

Association with Known Major Genes for Morphology and Phenology

The frequency of the photoperiod-insensitive *Ppd-A1a* allele was below the cutoff value (MAF < 0.05) and therefore was not included in the association analysis. Significant MTAs were identified between the functional genes and their corresponding traits. Marker-trait association for *Ppd-D1* with HDD was localized on chromosome 2DS at 22.46 cM. *Ppd-D1* was also associated with SPL across environments and in the combined analysis (Table 4, Supplemental Table S5). *Ppd-B1* was positioned on chromosome 2BS (20.21 cM) and had significant association with SPL and TKW. Vernalization locus *Vrn-A1* was associated with multiple traits such as HDD, FWD, MAT, GFD, PHT, WGP, and GNY at the 82.7-cM region of chromosome 5A. Plant height loci *Rht-B1* and *Rht-D1* were both associated with PHT, on chromosomes 4B (76.20 cM) and 4D (80.68 cM), respectively.

Grain Yield and Yield Components

A total of 29 markers distributed across 14 chromosomes were associated with GNY (Table 4, Supplemental Table S5). Five MTAs were identified from the combined

Table 4. Significant ($p < 2.61 \times 10^{-6}$) marker-trait associations for the different phenotypic traits using the combined data across environments in 2012 and 2013.

Trait†	SNP‡	SNP ID	Chr.	Pos§	Allele¶	*P*	MAF#	Effect††	R^2	Coincident QTL or gene‡‡
HDD	IWB22274	Excalibur_c13489_867	1A	71.1	T/C	1.32×10^{-6}	0.31	0.50	0.028	
	IWB52406	Ra_c6672_1679	4A	145.2	A/G	7.58×10^{-12}	0.24	0.91	0.052	Zanke et al. (2014)
	IWA1	wsnp_AJ612027A_Ta_2_1	5A	90.5	A/G	1.64×10^{-6}	0.26	0.63	0.212	*Vrn-A1*
	IWB2746	BobWhite_c35035_317	6A	135.8	A/G	1.48×10^{-6}	0.33	−0.48	0.037	
FWD	IWA1	wsnp_AJ612027A_Ta_2_1	5A	90.5	A/G	1.37×10^{-8}	0.26	0.69	0.400	*Vrn-A1*
	IWB53851	RAC875_c14064_177	7B	167.0	T/C	4.59×10^{-7}	0.43	0.44	0.044	
GFD	IWB64353	RFL_Contig3424_931	4A	137.0	T/C	2.08×10^{-6}	0.30	−0.20	0.016	
	IWB28183	Excalibur_c63243_434	6B	60.8	T/C	6.30×10^{-8}	0.38	0.21	0.084	
	IWB55857	RAC875_c25839_225	6D	82.8	A/G	8.85×10^{-8}	0.24	0.28	0.129	
MAT	IWA5287	wsnp_Ex_rep_c66689_65010988	5A	91.3	A/G	5.31×10^{-13}	0.28	0.74	0.272	*Vrn-A1*
PHT	IWB8131	BS00038418_51	1D	82.8	A/G	2.75×10^{-7}	0.22	1.81	0.038	
	IWB27094	Excalibur_c49875_479	2B	145.1	T/C	3.21×10^{-7}	0.24	−1.43	0.030	
	IWA6906	wsnp_Ku_c3237_6024936	4A	70.0	T/C	2.75×10^{-10}	0.32	−1.69	0.023	
	IWA752	wsnp_CAP11_c356_280910	4D	69.2	A/G	5.78×10^{-7}	0.31	1.63	0.061	*Rht-D1*
	IWB4446	BobWhite_c8266_227	5A	140.6	A/C	1.82×10^{-12}	0.14	3.33	0.369	Cuthbert et al. (2008)
	IWA7774	wsnp_Ra_c2421_4647159	5B	41.9	T/C	6.22×10^{-21}	0.08	−5.07	0.166	
	IWA1721	wsnp_Ex_c13034_20630123	6B	57.0	A/G	4.16×10^{-13}	0.10	−3.22	0.027	
PDL	IWB31875	GENE-0427_442	1B	56.9	A/G	2.01×10^{-8}	0.31	−0.69	0.040	
	IWB13323	CAP12_c2677_138	4A	37.1	T/C	1.39×10^{-9}	0.38	0.81	0.051	
	IWB36033	IACX5818	5B	53.5	A/T	3.34×10^{-10}	0.37	−0.89	0.134	
	IWB4117	BobWhite_c62620_150	6A	58.5	A/G	1.05×10^{-6}	0.19	−0.86	0.002	
SSD	IWB10731	BS00074345_51	3B	144.7	A/G	5.73×10^{-35}	0.14	1.64	0.514	Varella et al. (2015)
	IWB22082	Excalibur_c12383_251	6A	56.2	T/C	3.78×10^{-8}	0.08	0.71	0.073	
SPL	IWA989	wsnp_CAP12_c812_428290	2D	19.0	A/G	4.11×10^{-13}	0.39	0.33	0.083	*Ppd-D1*
	IWB49380	Kukri_rep_c109051_116	3A	86.2	A/G	1.97×10^{-6}	0.38	−0.22	0.058	
	IWB34424	IAAV1677	3B	120.0	T/C	9.50×10^{-7}	0.35	−0.21	0.089	
	IWB52488	Ra_c700_1024	5A	62.7	A/G	6.11×10^{-9}	0.21	0.31	0.114	
SKD	IWB525	BobWhite_c1361_1187	1A	13.7	T/C	1.92×10^{-7}	0.26	−0.29	0.018	
	IWB22971	Excalibur_c1747_429	2B	26.5	A/C	1.95×10^{-7}	0.16	0.38	0.013	Kumar et al. (2007)
	IWB2081	BobWhite_c27251_77	7A	156.2	A/G	9.40×10^{-7}	0.09	−0.42	0.035	Börner et al. (2002)
KPS	IWB73713.1	Tdurum_contig8669_131	1B	154.8	A/G	6.69×10^{-7}	0.46	0.69	0.206	
	IWB6033	BS00009263_51	2B	46.8	T/C	4.19×10^{-8}	0.20	0.86	0.058	
TKW	IWB74701	tplb0041a22_935	1A	71.1	A/G	4.05×10^{-7}	0.25	0.59	0.067	
	IWA7656	wsnp_Ra_c1660_3275687	2B	20.6	T/C	8.11×10^{-8}	0.42	0.65	0.056	*PpD-B1*
	IWB8083	BS00037023_51	5B	138.7	T/C	2.27×10^{-7}	0.20	0.62	0.012	
	IWB27650	Excalibur_c56264_188	6A	80.1	A/G	8.98×10^{-11}	0.35	0.75	0.197	Sukumaran et al. (2015)
	IWB2584	BobWhite_c32883_84	7A	228.4	T/C	4.08×10^{-7}	0.40	0.53	0.047	
	IWB56648	RAC875_c31791_400	7B	140.0	A/G	6.69×10^{-8}	0.38	−0.63	0.011	Neumann et al. (2011)
TWT	IWB25538	Excalibur_c34964_326	2A	113.3	T/C	2.75×10^{-8}	0.10	−5.02	0.034	
	IWB45102	Kukri_c41117_824	7B	10.1	T/G	9.94×10^{-7}	0.38	2.84	0.134	
WGP	IWB64569	RFL_Contig4030_493	2A	18.6	A/C	3.57×10^{-8}	0.42	0.14	0.145	
	IWB31342	Excalibur_rep_c83640_791	2B	145.1	A/G	1.31×10^{-7}	0.22	−0.15	0.003	
	IWA4276	wsnp_Ex_c55777_58153636	5A	93.2	T/C	8.53×10^{-7}	0.29	−0.17	0.312	
	IWB8052	BS00036434_51	5B	100.8	T/C	5.48×10^{-9}	0.16	0.18	0.020	
SDS	IWB39039	Ku_c28007_1398	1A	21.6	A/G	3.58×10^{-9}	0.24	2.51	0.074	*Glu-A3*
	IWB48117	Kukri_c8740_75	1B	64.1	A/G	1.46×10^{-26}	0.08	−6.01	0.259	
	IWB72142	Tdurum_contig50988_500	1B	117.8	T/C	1.49×10^{-8}	0.41	−2.04	0.036	*Glu-B1*

Table 4. Continued.

Trait†	SNP‡	SNP ID	Chr.	Pos§	Allele¶	*P*	MAF#	Effect††	R^2	Coincident QTL or gene‡‡
	IWB53449	RAC875_c11911_431	2D	34.2	A/G	1.95×10^{-8}	0.10	−3.59	0.075	
	IWB3924	BobWhite_c5640_282	4A	49.0	A/G	4.75×10^{-11}	0.09	−5.09	0.205	
	IWB48897	Kukri_rep_c103613_253	6B	39.1	T/C	3.68×10^{-7}	0.13	2.03	0.020	
HIN	IWB32494	GENE-1469_196	3A	90.3	A/G	4.72×10^{-7}	0.41	−0.01	0.009	
	IWB64600	RFL_Contig4167_1164	5B	135.6	T/C	3.43×10^{-8}	0.19	−0.01	0.049	Sukumaran et al. (2015)
	IWB10166	BS00067836_51	6B	66.4	T/G	4.75×10^{-9}	0.30	−0.01	0.067	
GNY	IWB56801	RAC875_c33037_578	2A	141.4	A/G	1.14×10^{-6}	0.09	−98.58	0.044	Chen et al. (2016)
	IWB54789	RAC875_c19210_348	2B	97.0	A/C	1.48×10^{-7}	0.19	−75.72	0.065	
	IWB6756	BS00021752_51	4A	35.3	A/C	6.13×10^{-7}	0.07	−110.17	0.011	Kumar et al. (2007)
	IWA4057	wsnp_Ex_c49423_54028488	5B	104.6	T/C	6.05×10^{-8}	0.25	60.72	0.086	Addison et al. (2016)
	IWB7048	BS00022372_51	6A	126.2	T/C	1.35×10^{-6}	0.07	104.84	0.029	

† HDD, days to heading; FWD, days to flowering; GFD, grain-filling duration; MAT, days to maturity; PHT, plant height; PDL, peduncle length; SSD, stem solidness; SPL, spike length; SKD, spikelets per head; KPS, kernels per spike; TKW, thousand-kernel weight; TWT, test weight; WGP, whole-grain protein; SDS, sodium dodecyl sulfate sedimentation; HIN, harvest index; GNY, grain yield.

‡ SNP, single-nucleotide polymorphism.

§ Chromosome and position based on the consensus map in Wang et al. 2014.

¶ The minor allele for each SNP marker is underlined.

Minor allele frequency.

†† Effect of allele substitution. The sign of the allelic effect estimate is with respect to the minor allele.

‡‡ Quantitative trait loci (QTL) for similar trait or gene reported in the same region.

data and 24 multienvironment MTAs. Among the MTAs in the combined analysis, *IWA4057* (5B) had the largest effect on GNY, explaining 9% of the phenotypic variation. The positive allele, associated with higher GNY, was carried by 25% of the genotypes in the panel (Table 4). Marker *IWB54789* (2B) was detected in both combined and across environment analyses. The allele for greater yield is present at a high frequency (80%) in the panel. Several MTAs were identified for the major yield component traits such as KPS, SKD, TKW, and TWT. For KPS, the strongest association in the combined data was with marker *IWB73713.1* on 1B, which explained ∼20% of the phenotypic variation, and the positive allele is present in 46% of the genotypes. Eight multienvironment MTAs for KPS were detected on chromosomes 1B, 4A, 5A, 5B, 6D, and 7D. Among these MTAs marker, *IWA2522* on 7D was responsible for 21% of the phenotypic variation in KPS. Six markers on chromosomes 1A, 2B, 5B, 6A, 7A, and 7B were associated with TKW in the combined analysis. Among them, *IWB27650* on 6A had the strongest association and largest effect on TKW (R^2 = 20%) and was also significant across environments. Nineteen multienvironment MTAs for TKW were detected on 16 chromosomes. Seven markers were associated with SPL. One marker, *IWA989*, was in LD with the gene for photoperiod response (*Ppd-D1*) on chromosome 2D. *IWA989* was significant in both combined and across environment analyses explaining between 7 and 8% of the variation in SPL. The largest effect MTA for SPL was *IWB52488* on 5A which explained as much as 11% of the phenotypic variation. Two and three MTAs were associated with TWT and SKD, respectively, in the combined analysis with R^2 between 1 and 13%. Multienvironment MTAs for TWT were also observed on chromosomes 1A, 2A, 2B, 3B, 5A, 6A, and 7B. For SKD, MTAs that were significant in more than one environment were detected on chromosomes 3A, 4A, 7A, and 7B.

Grain Protein and SDS Sedimentation

Four markers on A and B genomes of chromosomes 2 and 5 were associated with WGP in the combined analysis (Table 4). The largest effect MTA was *IWA4276* on chromosome 5A, which was responsible for up to 31% of the phenotypic variation and is near the vernalization gene (*Vrn-A1*). The allele for higher grain protein is present in 83% of the genotypes in the panel. Another marker for WGP (*IWB64569*) on 2A accounted for 15% of the variation in WGP, but only 42% of the genotypes carried the allele for higher grain protein. Marker-trait associations for SDS on the short arm of chromosomes 1A (*IWB39039*) and 1B (*IWB48117*) were significant in both combined and across-environment analyses. *IWB48117* on 1B accounted for up to 31% of the variation in SDS. The allele for higher SDS in *IWB48117* was highly represented (92%) in the

panel; however, that of *IWB39039* was present at a lower frequency (24%). Another large-effect (R^2 = 21%) MTA for SDS with identified on chromosome 4A (*IWB3924*). There were 23 and 6 multienvironment MTAs for WGP and SDS, respectively (Supplemental Table S5).

Stem Solidness and Tiller Number

IWB10731 on chromosome 3BL had the largest effect on SSD (R^2 = 51%). This marker was identified in the combined analysis and was significant in all test environments (Table 4, Supplemental Table S5). The positive allele for this SNP is carried by 14% of the genotypes. No MTA for TLN passed the significance threshold in the combined analysis; however, five multienvironment MTAs were identified.

Plant Height, Peduncle Length, and Harvest Index

Thirty-five MTAs for PHT were detected in all but five chromosomes in the D genome (2D, 3A, 5D, 6D, and 7D) (Supplemental Table S5). *IWB4446* on 5A had the largest effect on PHT (R^2 = 37%), followed by markers near known PHT genes on 4B (*IWB23338*, R^2 = 16%) and 4D (*IWA752*, R^2 = 19%). *IWB4446* and *IWA752* were significant in both combined and across-environment analyses. Marker *IWB36033* on chromosome 5B explained the highest percentage (13%) of the phenotypic variation in PDL (Table 4). Five multienvironment MTAs for PDL were detected on chromosomes 1A, 2B, 3B, 4A, and 4B. Marker-trait associations with HIN were identified on chromosomes 3A, 5B, and 6B with the alleles for higher HIN present in at least 50% of the lines. *IWB10166* (6B) had the largest effect on HIN explaining 7% of phenotypic variation.

Phenological Traits

Many MTAs detected were related to plant phenology such as HDD, FWD, GFD, and MAT. For HDD, FWD, and MAT, the strongest association was identified at 90.54 cM of chromosome 5A with markers *IWA1* and *IWB12023* (Supplemental Table S5). Marker *IWA1* is in LD with the vernalization gene *Vm-A1*. Other markers in this region, between 91 and 93 cM, were also associated with HDD, FWD, GFD, and MAT. These MTAs were significant in both the combined and across-location analyses. *IWA1* explained 21 and 40% of the variation in HDD and FWD in the combined analysis (Table 4). Other MTAs for HDD were identified on chromosomes 1A, 1B, 1D, 2B, 2D, 4A, 5A, 6A, 7A, and 7D. Most of these regions were also associated with FWD, GFD, and MAT (Supplemental Table S5).

Markers and Chromosome Regions Associated with Multiple Traits

Close linkage, pleiotropic effects, strong correlation between traits, or a combination of these factors may lead to MTAs that affect several traits. These were observed on many chromosomes. Their chromosome positions are shown with another trait-specific MTA in Supplemental Table S5. A cluster of MTAs was located between the 83 and 93 cM of chromosome 5A, colocalizing with the *Vrn-A1* gene. Marker-trait associations for HDD, FWD, GFD, MAT, and GNY were detected in this region. Similarly, MTAs for HDD and SPL were also observed in the region of *Ppd-D1* on chromosome 2D. A clustered region for yield and yield-related traits (TKW, KPS, and GNY) was detected in the 74- to 102-cM region of chromosome 1B. The QTLs for KPS, SKD, SPL, and TKW were grouped between the 20 and 46 cM of chromosome 2B. Marker-trait associations related to SDS and WGP clustered on the short arm of chromosome 1B. Grain yield shared a locus with TLN on chromosome 2AS, PHT on chromosome 5AL, and HDD, PDL, and TKW on 1AS.

Distribution of QTL Alleles among Breeding Programs

The distribution of positive alleles for the MTAs varied among the breeding programs. Positive alleles were defined as those that provided higher values for yield and its components. For traits such as PHT and HDD, alternative alleles may be preferred, depending on the environment. Figure 3 shows that the positive allele for the marker associated with SKD, *IWB2018*, was present in a high frequency in most breeding programs except Agriculture and Agri-Food Canada (Manitoba) (Panel A). All genotypes from South Dakota State University, University of Minnesota, University of California–Davis, and University of Idaho–Aberdeen carried the allele for higher number of spikelets. The frequency of the positive alleles for *IWB73713.1* (KPS) and *IWB27650* (TKW) were higher among the lines representing the western United States and CIMMYT than among those lines from the US Northern Plains and Canada (Panels B and C). At least 80% of the lines representing the western United States had higher frequencies of the positive allele for GNY (*IWB64789*). A higher frequency (at least 90%) was observed in the lines from University of Saskatchewan–Agriculture and Agri-Food Canada (Saskatchewan), South Dakota State University, and University of Minnesota (Panel D). The allelic state of all MTAs in the combined analysis for yield and component traits of the 237 genotypes in the panel is presented in Supplemental Table S6.

DISCUSSION

SNP Polymorphism and Diversity

The average PIC in this population was 0.29, which is like the PIC of 1440 improved wheat lines (breeding lines and cultivars) from the USDA National Small Grains Collection (Bonman et al., 2015). Polymorphic information content is widely used to measure informativeness (high

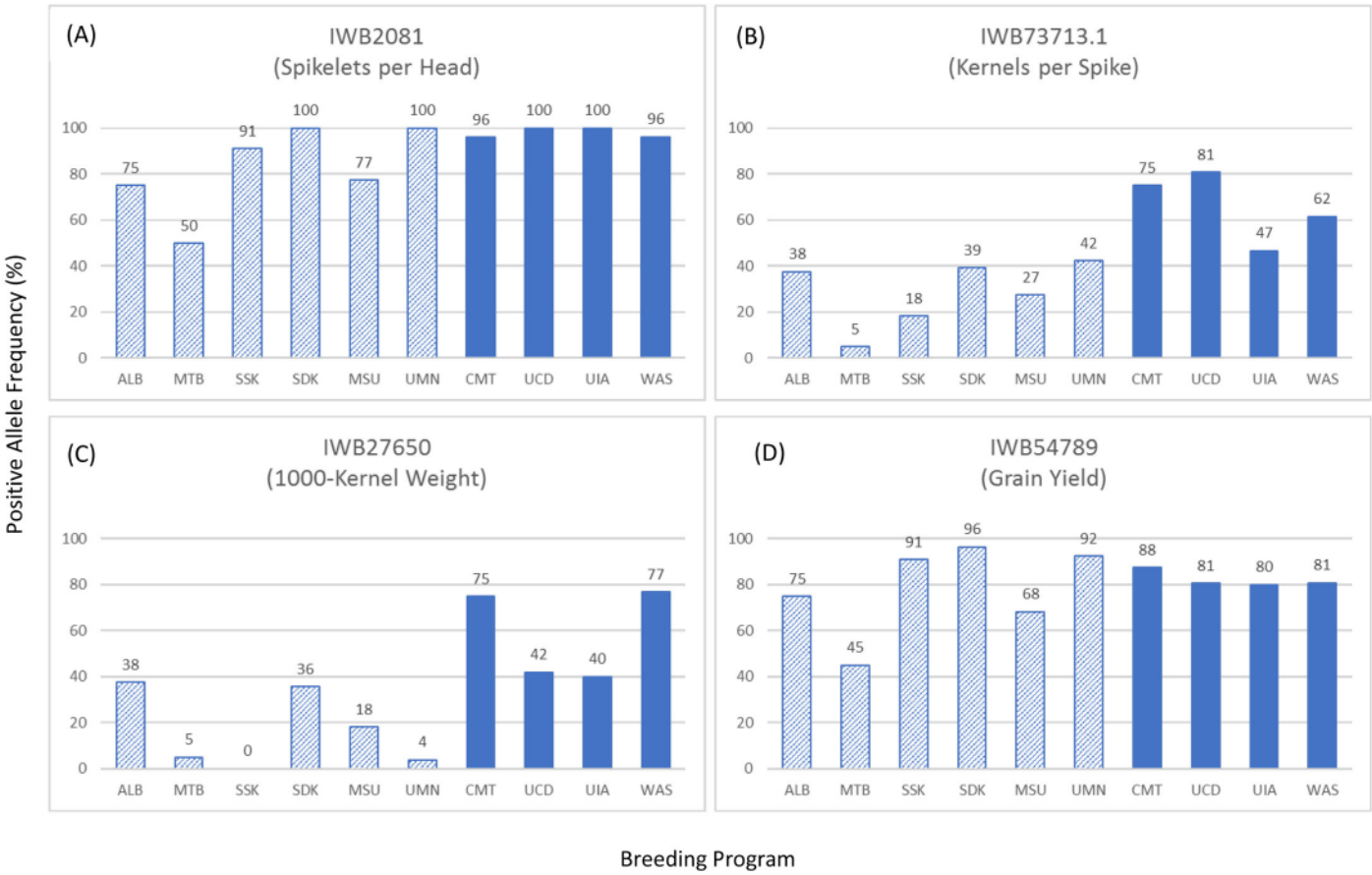

Fig. 3. Frequency of positive alleles for (A) *IWB2081*, (B) *IWB73713.1*, (C) *IWA27650*, and (D) *IWB54789* across breeding programs. Horizontal bars with a diagonal pattern correspond to genotypes in Northern Plains of the United States and Canada, whereas solid colored bars are genotypes from western United States and CIMMYT. Positive alleles are defined as those that provided higher trait values. ALB, University of Alberta; CMT, CIMMYT; MSU, Montana State University; MTB, Agriculture and Agri-Food Canada (Manitoba); SDK,South Dakota State University; SSK, University of Saskatchewan–Agriculture Agri-Food Canada (Saskatchewan); UCD, University of California–Davis; UIA, University of Idaho–Aberdeen; UMN, University of Minnesota; WAS, Washington State University.

PIC values preferred) of molecular markers (Botstein et al., 1980). Overall, the D genome had the lowest PIC values compared with genomes A and B, and this pattern was generally seen across different chromosomes. Lopes et al. (2015) reported a similar pattern in the level of PIC values on the wheat association mapping initiative (WAMI) population developed by CIMMYT. Lower PIC values were also observed in D genome from a panel of elite US spring (0.14–0.18) and winter (0.14–0.16) wheat lines (Chao et al., 2010).

Gene diversity was high and similar in genomes A and B but low in the D genome. Although higher relative to the WAMI population (0.37 vs. 0.34), gene diversity in this panel was less than the gene diversity (0.57) in a pool of elite winter wheat breeding lines from major US regional performance nurseries (Zhang et al., 2010). The amount of gene diversity present in the panel was comparable with previously established AM populations, although we expect the WAMI population to have more diversity in the D genome, which should have been enriched by including more synthetic-derived lines in their panel (Zhang et al., 2010). The high gene diversity and dense marker coverage (average density = 1 SNP per 0.43 cM) used in this panel further justified its potential to identify novel associations, especially in previously unexplored regions of the wheat genome.

Population Structure and Linkage Disequilibrium

Both model-based (STRUCTURE) and distance-based (Cluster) approaches revealed two similar subpopulations (QI and QII) that coincided with the major geographical locations of the breeding programs that developed the elite lines. Despite a distinct genetic grouping in the panel, there was also interrelatedness across subpopulations. Ascertainment bias, especially in this type of genotyping platform, could have a possible effect on the different measures of population structure. Hence, we carefully examined the pedigree of the genotypes in the panel and discussed with the wheat breeders to further validate our results. One possible reason for this mixture can be attributed to the dynamics of germplasm exchange, especially among publicly funded wheat breeding programs in North America. Wheat breeders have historically shared germplasm with other

breeding programs on a regular basis to bring new alleles that can be beneficial for trait improvement.

The variation in LD and LD decay rates across different studies reflects the complexity of the evolutionary and breeding history of wheat. The longer extent of LD in the D genome may be explained by a strong genetic bottleneck that happened after the last polyploidization event, which resulted in the origin of hexaploid wheat (Akhunov et al., 2010). After this polyploidization event, gene flow between the tetraploid and hexaploid wheat forms through fertile pentaploids increased the diversity of the A and B genomes, but not of the D genome (Dubcovsky and Dvorak 2007). We observed a high proportion of marker pairs in LD ($p < 0.01$) on chromosomes with known major wheat adaptive genes 1D (*Eps1*), 2D (*Ppd-D1*), 4B (*Rht-B1*), and 5A (*Vrn-A1*) (Supplemental Table S4). Given the current LD decay rate in our panel and the number of markers available, MTAs (except in the D genome) can be resolved to within 3 cM. Similar values of LD decay rates were reported in the WAMI population using two different marker platforms: Diversity Arrays Technology (DArT) (Edae et al., 2014) and SNP (Sukumaran et al., 2015).

Marker-Trait Associations

Germplasm exchange is one basis for maintaining diversity in plant breeding programs. Evaluating lines from other programs is a challenge, in that phenotypes of introduced lines may be inferior due to lack of specific adaptation to the geographical area. However, superior genes may be present in lines that do not show superior phenotypes. Genome-wide association mapping provides an opportunity to identify the superior genes in introduced germplasm and to screen a larger number of accessions than biparental populations. However, given the extensive LD in wheat, biparental populations provide a complementary tool to validate the GWAS associations and to increase resolution by developing large and more isogenic segregating populations. The elite HRS panel, developed as part of the Triticeae Cooperative Agricultural Project (TCAP) is a sample of spring wheat breeding lines from 10 wheat breeding programs in North America assembled to represent the current genetic and phenotypic diversity of each state's breeding program. The wide phenotypic variation due to differences in the priority and target traits of each breeding program makes this panel suitable for genome-wide association analysis. In fact, all 17 agronomic traits evaluated across several locations showed a mid- to high range of differences in their phenotypic performance. The degrees of relationship and correlations seen among the traits may be taken into consideration in future improvement strategies.

A total of 226 MTAs were identified that passed relatively stringent criteria for significance, as well as expression across environments. The amount of phenotypic variation explained by each marker (R^2) ranged from 1 to 51% in the combined analysis. These values were consistent with the genetic architecture of complex traits expected to be influenced by many loci, each with a relatively small effect. Recent GWAS studies related to agronomic traits reported a similar range of R^2 values independent of the genotyping platform used and the composition of the mapping panel (Crossa et al., 2007; Yao et al., 2009; Neumann et al., 2011; Dodig et al., 2012; Edae et al., 2014; Lopes et al., 2015; Sukumaran et al., 2015; Tadesse et al., 2015; Chen et al., 2016). Marker-trait associations in a panel of elite lines may allow the identified QTLs to be effectively moved into the breeding program without the negative effects of linkage drag and adaptability of QTLs introduced from more exotic germplasm. We further compared the identified MTAs with previous studies using the latest comparative map of wheat (Maccaferri et al., 2015).

Grain yield MTAs were identified on 14 chromosomes, and half of these MTAs corresponded to chromosome positions reported in previous studies, further validating our results. The MTAs on chromosomes 1A, 1B, 3B, 4AS, and 5B were also reported in QTL mapping studies using biparental populations (Börner et al., 2002; Kumar et al., 2007; Li et al., 2015; Addison et al., 2016). This shows that both genetic strategies to identify genomic regions associated with different traits can be used to complement and/or cross-validate results. Fine mapping of a QTL region identified in GWAS is done using biparental populations from crosses of individuals that are polymorphic at the target region. The distribution of the GNY in many genomic regions and their minor effects further showed the complexity of the genetic architecture of GNY. Although increasing GNY is the primary objective of wheat programs (Green et al., 2012), other traits that contribute to yield should always be included in the breeding process. Marker *IWB54789* (2B) is a potentially new source of yield-increasing allele, especially in Canada and the Great Plains of the United States.

In this study, TKW showed significant correlation to GNY. Marker-trait associations for TKW on chromosomes 1A, 6A, and 7A are at most within 4 cM to MTAs for GNY. An MTA for TKW on chromosome 6A, *IWB27650*, which was detected across environments and in the combined analysis, was 2 cM from a TKW MTA reported in Sukumaran and Yu (2014). Given the relatively large effect ($R^2 = 20\%$) of marker *IWB27650* on TKW and stability across environments, upon further validation, this marker can potentially be used in marker-assisted selection for heavier kernels. Other MTAs for TKW on 1B, 4AS, 6D, 7A, and 7B have been reported in literature (Neumann et al., 2011; Edae et al., 2014; Zanke et al., 2014; Chen et al., 2016) (Supplemental Table S5). The trait KPS was also highly correlated with GNY. Among the 10 MTAs for KPS, markers *IWB73713.1* (1B) and *IWA2522* (7D) had the largest effects ($R^2 = 20\%$) on KPS. To our knowledge, these

two markers have not been reported elsewhere; hence they can be new sources of useful alleles for KPS. Other traits such as SKD, SPL, and TLN are known contributors to yield potential particularly in determining kernel number. Markers *IWB35184* and *IWB525* for SKD are within 2 and 4 cM from markers *IWB49380* (SPL) and *IWB61124* (GNY) on chromosomes 3A and 1A, respectively. Marker *IWB8815* for TLN colocalized with *IWB10151* for GNY on chromosome 2A. Using a combination of these various MTAs, breeding programs can design specific crosses towards the improvement of these traits.

The major QTLs for SSD, which were also related to stem sawfly (*Cephus cinctus* Norton) resistance, were also identified on chromosomes 3B and 5B (Sherman et al., 2010; Varella et al., 2015). However, the MTAs for SSD on 1D and 6A can be novel sources for horizontal resistance to stem sawfly. Several MTAs for PHT were identified aside from the region of major PHT genes (4B and 4D) in almost all chromosomes, consistent with the findings of Börner et al. (1996) and Snape et al. (1977). *Rht-D1* (4D) was significant in both combined and across environment analyses with the dwarfing allele present in 69% of the genotypes (Table 4). The importance of this height-reducing gene in breeding programs is due in part to its contribution in improving HIN and resistance to lodging in high-rainfall zones. In this panel, PHT was negatively correlated with GNY, which is in agreement with the report of Belluci et al. (2015) using Scandinavian winter wheat lines. As a result, it was not surprising that there were several MTAs for PHT near (2 cM) MTAs for GNY in chromosomes 2B, 6A, and 7A. In these MTAs, average GNY for genotypes with the dwarfing alleles was higher than for genotypes that carried the alleles for taller PHT.

Loci that were associated with multiple traits were also identified, aside from those regions governed by major developmental genes. The clustering of these MTAs is also supported by their phenotypic correlations. Chromosome 4A was a hotspot of MTAs for GNY, KPS, TKW, HDD, GFD, and PDL. Specifically, QTLs for GNY and PDL were detected in the same region at 35 to 37 cM, and another MTA for GNY and SDS was localized at 49 to 51 cM. A cluster of MTAs for GFD, HDD, KPS, PDL, TKW, and GNY was also detected at 137 to 155 cM of 4AL. A GWAS study that focused on chromosome 4A identified MTAs for six agronomic traits, including PHT, SPL, seeds per spike, spikelet density, grains per spike, and TKW (Liu et al., 2010). A region spanning ~6 cM on the distal end of the long arm of chromosome 7B harbored MTAs for TKW, SKD, and SDS. A 3-cM region on chromosome 6A also harbored MTAs for GFD, TKW, PHT, TWT, and GNY. Colocalization of loci is advantageous but can also be problematic if undesirable repulsion linkages are maintained. In this case, haplotype analysis or targeted selection of recombinants is needed to identify allelic combinations associated with the desired phenotypic performance. The relationship between these traits can be further dissected by increasing marker resolution or creating designed crosses from individuals that are polymorphic for markers in these regions. However, for alleles that are in coupling phase, these can be efficiently deployed in breeding programs using marker-assisted selection. Due to their elite background, germplasm improvement can quickly benefit from these QTLs by reducing the number of backcrosses needed if sourced from landraces or wild relatives.

Identification of MTAs from this association mapping panel will allow breeders to select parents for crossing with complementary alleles at loci affecting GNY. Germplasm exchange has the potential to target specific loci rather than just on elite lines per se. Thus, beneficial alleles may be detected in lines that do not perform at a high level in the tested environment. As an example, the positive alleles for *IWB73713.1* (KPS) and *IWB27650* (TKW) are prevalent in the genotypes from breeding programs in the western United States and can be used for germplasm enrichment in other programs (Fig. 3). Likewise, the US Northern Plains and Canadian genotypes are good sources for the *IWB54789* allele associated with higher GNY, which is found at relatively lower frequency in the western genotypes, University of Alberta, Agriculture and Agri-Food Canada (Manitoba), and Montana State University. To facilitate this approach, Supplemental Table S6 shows the allele status for all 237 lines for the MTAs with GNY and yield components.

CONCLUSION

In this study, we have shown that the TCAP elite HRS wheat mapping panel has sufficient diversity, polymorphism, and resolution to conduct meaningful whole-genome scans for complex traits. The major division in the panel coincided with the broader geographical location of the breeding programs that contributed the elite lines. We identified associations between 17 different agronomic traits and SNP markers. Some of these MTAs were validations from previously conducted QTL mapping studies, but newly discovered associations, especially for yield and yield components, can be valuable in MAS after proper validation. These results will help breeders select complementary parents for crossing to enhance the value of germplasm exchange programs.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

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