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Quantitative Trait Loci Mapping for Yield and Spike Characteristics in a Recombinant Inbred Line Population of Wheat

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Abstract

This study seeks to validate quantitative trait loci (QTL) for yield components in winter wheat with single nucleotide polymorphisms (SNP) identified from a Genome Wide Association Study (GWAS). Two QTL were targeted: a region on chromosome 6B of the wheat genome that is associated with kernel weight (Kw), grain width (Gw), and kernel length (Kl) and a region on chromosome 7A that is associated with the number of spikelets per spike (SPS). Wheat feeds 4.5 billion people 20% of their daily protein and food calories, making it a priority for marker-assisted selection in plant breeding programs. For this study, a recombinant inbred line (RIL) mapping population was generated by crossing the parental lines, Platte, a Great Plains winter wheat cultivar, and CO940610, an experimental line. These two lines have distinct phenotypes for Kw, Gw, Kl, and SPS, and are genetically polymorphic in the targeted genomic regions. The 224 progeny lines were grown in field experiments as two replicates in Fort Collins, CO in 2017 and Kw, Gw, Kl, and the number of SPS were measured. A three-primer KASP assay was developed for the peak SNP on chromosome 6B and a restriction enzyme assay was used to genotype the SNP on chromosome 7A. Among this segregating population, the association between these SNPs and several different yield component traits were validated. The markers developed in this project can be used by breeders to select for varieties carrying the high-value allele in each association. The ultimate goal is to clone the responsible genes and incorporate them into wheat breeding programs to aid in the development of higher yielding cultivars.

Key Words: Wheat Genetics, Quantitative Inbred Line Mapping, Recombinant Inbred Line, Food Security

1. Introduction

Common wheat (*Triticum aestivum L.)* is the most widely grown crop in the world, providing 20% of the daily protein and food calories for 4.5 billion people[1]. With the rising population, global crop production needs to double by 2050[2]. To meet the surging food demand, yields in staple crops such as corn, soybean, rice, and wheat need a 2.4% per year increase in yield[2]. The current projection of yearly increase in wheat production is 0.9%, the lowest of the cereal crops[2]. Current wheat yield having the largest deficit in meeting the required 2.4% annual increase yet being one of the largest suppliers of the world's calories, makes higher-yielding wheat an important component to reaching food security. Therefore, the understanding and eventual incorporation of genetic variants responsible for higher yields into wheat breeding programs worldwide is not only a goal but also a necessity.

Grain yield is a polygenic and highly complex trait with both environmental and genetic influences at all stages of a plant's life cycle[3]. Yield is usually analyzed as three contributing components: number of spikes per surface area, grain number per spike, and kernel weight[3]. The latter can be further dissected into the individual components of grain length, width, area, and grain filling characteristics[3]. The yield components included in this study are kernel weight (Kw), grain width (Gw), kernel length (Kl) and spikelets per spike (SPS). These grain quality traits were selected for

analysis because of their considerable influence on overall yield. These yield components vary in terms of their heritability and can be positively or negatively correlated with yield or with each other[3].

Despite the importance of these traits for agriculture, little is known of the genetic determinants underlying phenotypic variation. The discovery and validation of quantitative trait loci (QTL) can provide insight into the genetic mechanisms that govern yield characteristics. It is also necessary before employing traits of interest in marker-assisted selection. Several previous studies have identified QTLs for grain yield in wheat, although in each case they were mapped to relatively large genomic regions[3,5,6,7]. Using a CO940610/Platte doubled haploid (DH) population (n=185), El-Feki et al. (2013 and 2015), identified QTL for grain characteristics on chromosomes 1B, 6B, and 7B. The 6B QTL included variation in grain volume weight (hereafter test weight, Tw), and Kw. The doubled haploid technique results in a diverse set of homozygous lines but has a limited rate of recombination because of the single meiotic division during gamete formation[4]. Consequently, it can only be used for the QTL additive effect, and not dominant genetic effects[4]. Dominant genetic effects are an important source of variation for quantitative traits, and thus improve QTL mapping accuracy[4]. With a recombinant inbred line (RIL) population the dominant genetic effects can be observed.

Dao et al. 2017 validated the 6B QTL using a RIL population developed from the same parents (CO940610/Platte) using single seed descent to the F_6 generation. They tested the effects of the combination of alleles at three loci (*Bx7-MAR*, *Xwmc182a*, and *Xwmc182b*) near the QTL for Tw, Kw and Gw[5]. Their study revealed 12 marker-trait associations (MTA) with four simple sequence repeats (SSR) markers (*Xbarc136*, *Xwmc397*, *Xbarc198*, and *Xwmc182a)* on 6B; There were four MTA mapped to all four markers on 6B for the QTL for Tw, two MTA mapped to two SSR markers (*Xbarc198* and *Xwmc182a*) for the QTL for Kw, and 6 MTA associated with all four markers for the QTL for Gw[5]. CO940610 conferred the higher value allele at all loci[5]. It was concluded for the traits highlighted that the QTL were not robust across environments, but the regions around markers *Xwmc182a* on 6B and *Xwmc182b* on 7B should be further investigated for improving grain quality traits[5].

The RIL population used by Dao et al. 2017 was adopted for this study except the F_6 population was followed by another single seed descent to develop the F_7 population. The data published in Dao et al. 2017 distinguished the markers on 6B as significantly associated with Kw and Gw with almost all four markers being correlated with both traits mentioned. Hence the focus of this paper was to target the QTL region on 6B between the SSR markers *Xwmc397* and *Xwmc182a* previously used in Dao et al. 2017.

The QTL for SPS (p = 5.88E-08) on chromosome 7A was identified from a genome wide association study (GWAS) of 299 Great Plains cultivars and advanced lines, including the parental lines in this study[8]. This QTL was significant across all four tested environments, thus inspiring the hypothesis that variation in the CO940610/Platte RIL population would have marker-trait associations on 7A for SPS. When choosing the markers specifically for this QTL study, a high density 90k gene-associated SNP array for wheat was consulted[9].

The objectives of this study were to (1) validate the QTL previously identified on chromosome 6B in Dao et al. 2017, but specifically focus on the QTL responsible for Kw and/or Gw and Kl using the SNP marker (*BobWhite_c22638_135*); and (2) to identify a region on chromosome 7A that is responsible for the number of SPS using the SNP marker (*IWA5913_7A*). The combination of allelic effects for both markers was tested in the F_7 RIL population. The hypothesis was the lines with the allelic combination CO940610-Platte (C-P) at the 6B and 7A loci respectively would have the highest yield, and the lines with the combination P-C would have the lowest yield (Table 1). This hypothesis was developed with the speculation that the CO940610 allele at the SNP on 6B would contribute to high Kw, and the Platte allele at SNP 7A would contribute a larger number of SPS.

Table 1. Possible allelic combinations for 6BL and 7AL; P = Platte; C = CO940610

Allelic combinations	Selected alleles	
	BobWhite_c22638_135	IWA5913_7A
P-P	P	P
P-C	P	C
C-P	C	P
C-C	C	C

2. Materials and Methods

2.1. Plant Materials

CO940610 (GSTR 107020) is a hard white winter wheat experimental line developed by the Colorado State University (CSU) Wheat Breeding Program from the cross KS87H22/MW095[5]. CO940610 has shown excellent yield under rainfed conditions in the West-Central US Great Plains[5]. Platte (PI 596297; GSTR 10701) is a hard white winter wheat cultivar developed by HybriTech Seed International (a former division of Monsanto Co., St. Louis, MO), with the pedigree 'Tesia 79'/Chat 'S'//'Abilene'[5]. Platte and CO940610 were found to be polymorphic at both the chromosome 7A marker (*IWA5913_7A*) and the chromosome 6B marker (*BobWhite_c22638_135*). A RIL population was produced from the cross of CO940610 as the female parent and Platte as the male, followed by single seed descent to the F_7 generation.

In Dao et al. 2017 study of this RIL population, CO940610 conferred the higher value allele at SSR markers (*Xwmc397* and *Xwmc182a*) on 6B for the QTL associated with Gw, and at *Xwmc182a* for the QTL for Kw[5]. Platte conferred the higher value allele for the researched QTL associated with SPS on 7A[8]. To have a segregating population, it was established CO940610 conferred the higher value allele at the SNP (*BobWhite_c22638_135*) and Platte provided the higher value allele at the SNP (*IWA5913_7A*) in the F_7 population.

2.2. Field Trials

Replicated yield trials were conducted at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, Colorado (40.652 N, 104.996 W, elevation 1558 m) during the 2017 growing season. Entries included two sets of 224 RIL, both parents, and five check cultivars or experimental lines (Kharkof, Longhorn, OK 06318, WB Cedar, and WS-08). Seed was planted at a density of 1,700,000 seeds ha^{-1} in paired rows 0.9 m long with 23 cm spacing between rows. The trials were grown under rainfed conditions. Fertilization and weed control were typical of practices for winter wheat in the area, and no disease or insect pest control was applied. Ten heads from each plot were hand-collected for analysis and dried for at least 3 days at 40 °C.

Four grain yield-related traits (kernel weight, grain width, kernel length, spikelet per spike) were measured in both replicates of the RIL (n=224) population, using seed obtained from the 10-head samples. Seeds were scanned with a MicroTek Scanmaker 9800XL (Microtek, Santa Fe Springs, CA), and the resulting images were processed with GrainScan software, adopted from CSIRO Agriculture Flagship, to obtain estimates of grain number, width, and length. Seed was then weighed and converted to 1000 kernel weight. The spikelets per spike were hand-counted. Both filled and unfilled spikelets were counted since an empty spikelet is typically due to environmental effects rather than genetic ones.

2.3. Phenotypic Data Analysis

Variation at each marker was analyzed for association with each trait in a single-factor analysis of variance (ANOVA) using the GLM procedure of SAS software 9.3 (SAS Institute Inc., Cary, NC). Trait was the dependent variable, and marker was the independent variable. A probability level of 0.05 was used as the significance threshold, indicating the presence of a QTL in the genomic region of the marker. The percent phenotypic variance explained by the marker ($\%R^2$) was obtained by multiplying the R^2 value (coefficient of determination) provided in the SAS GLM output by 100.

2.4. Molecular Marker Genotyping

Genomic DNA was extracted from seedling leaf tissue of each RIL and the parents using a protocol described by Riede and Anderson (1996)[10]. Parental DNA was screened for polymorphisms at both SNP (*BobWhite_c22638_135*) on chromosome 6B and SNP (*IWA5913_7A*) on chromosome 7A. To confirm the parental lines of the RIL population were polymorphic for the *BobWhite_c22638_135* SNP, targeted Polymerase Chain Reactions (PCR) were performed to amplify the region surrounding this SNP. PCR were performed using forward primer 5'-ACAAGTTTCGTGTGCCTGTG-3' and reverse primer 5'- CCAGGTTTGTGGCAAGATG-3', using 35 cycles of a touchdown program with a final annealing temperature of 57 ℃. Amplified PCR products were sequenced using Sanger sequencing to confirm the parental genotypes.

To screen for the *BobWhite_c22638_135* SNP in the RIL population, a three-primer KASP assay adopted from the Laboratory of the Government Chemist (LGC) was conducted with two allele-specific forward primers and one common reverse primer. The allele-specific primers were labelled with a fluorescence resonant energy transfer (FRET) cassette, one with FAM™ dye and the other with HEX™ dye. The KASP assay was conducted in 96-well microplates using 10 cycles of a touchdown program with a final annealing temperature of 63 ℃. The amplification profile consisting of 26 cycles of a touchdown program with a final annealing temperature of 55 ℃. Final extension occurred for seven minutes at 72 ℃. Fluorescence was observed and the RIL individuals were genotyped.

To screen for *IWA5913_7A* SNP among the RIL population, a restriction enzyme assay was used. A 472 bp PCR product was amplified using the forward primer 5'-CAGCAAATCTGTTTTCAGACTTCA-3' and the reverse primer 5'-AAGGGTGTTTCCGAACTTGA-3' in a PCR reaction, using 35 cycles of a touchdown program with a final annealing temperature of 55 ℃. Amplified products were digested for two hours at 37 ℃ with the restriction enzyme *Pvu* I I and separated by electrophoresis on a 1% agarose gel. PCR products amplified from individuals homozygous for the Platte parental allele showed a single undigested band, 472 bp in size, whereas lines homozygous for the CO940610 allele showed two digested products (279bp/193bp). Heterozygous lines displayed all three bands (472bp/279bp/193bp).

3. Results

The RIL population proved to be segregating for SPS. The observed trait-environment combinations had only a slight deviation from a normal distributed population. The small anomaly was approximated based on visual evaluation and calculated biallelic SNS means (data not shown). N-values were calculated to determine how many individuals were needed to justify a slight abnormal distribution. The RIL population was sufficient in that a population size of 224 individuals was larger than the necessary n-values for all allelic combinations.

Single-factor ANOVA analysis revealed the marker-trait association of the SNP markers with the respective observed traits (Kw, Gw, Kl, SPS) (Table 2). The allelic class means of the observed traits were determined by the least squares mean data of the two replicates of the RIL population (n=224). The least squares mean data gave a better representation of the two populations. The calculated P value was based on the significance between the Platte and CO940610 allelic class means of the observed trait and the corresponding marker. In other words, the significance of the marker-trait association. The R^2 (%) is the percent of phenotypic variation explained by the corresponding marker. A is the average additive effect. A positive value indicates an increasing effect of the Platte allele and a negative value indicates an increasing effect of the CO940610 allele.

Table 2. Marker-trait associations detected with single-factor ANOVA analysis of variance of the two allelic classes in the CO940610/Platte RIL population in Fort Collins, CO in the 2017 growing season

			ANOVA Results		Allelic Class Means		Avg. additive effect
Trait	Marker	Chr.	P	R^2(%)	Platte	CO940610	A
Grain width (mm)	*BobWhite_c22638_135*	6B	0.195	0.8	3.273	3.291	-0.009
Grain width (mm)	*IWA5913_7A*	7A	<0.0001	9.0	3.238	3.303	-0.033
Kernel weight (mg)	*BobWhite_c22638_135*	6B	0.581	0.1	35.810	36.065	-0.128
Kernel weight (mg)	*IWA5913_7A*	7A	0.0002	6.0	34.839	36.555	-0.858
Kernel length (mm)	*BobWhite_c22638_135*	6B	0.713	0.1	6.739	6.728	0.006
Kernel length (mm)	*IWA5913_7A*	7A	0.006	3.4	6.687	6.768	-0.041
Spikelets/spike (no.)	*BobWhite_c22638_135*	6B	<0.0001	11.8	17.560	16.780	0.390
Spikelets/spike (no.)	*IWA5913_7A*	7A	<0.0001	15.6	17.900	16.984	0.458

For the QTL for Gw and Kw, CO940610 conferred the higher value allele associated with wider grains and heavier kernel weight, resulting in the negative additive effect for SNP 6B (A = -0.009, -0.128, respectively; Table 2). Neither the QTL for Gw or Kw at SNP 6B were statistically significant (p = 0.195, 0.581, respectively; Table 2), meaning the marker at 6B did not significantly influence the observed allelic class means. The correlation values for the marker-trait associations of SNP 6B for the QTL for Gw and Kw were very low (R^2 (%) = 0.8, 0.1, respectively; Table 2), indicating these traits were not significantly associated with the SNP at 6B.

SNP 7A was significantly associated with both Gw and Kw with the positive allele for both traits provided by CO940610 (A = -0.033, -0.858, respectively; Table 2). SNP 7A showed a significant influence on the observed allelic means (p = <0.0001; Table 2) for the QTL for Gw and was correlated with the Gw trait (R^2 (%) = 9.0; Table 2). SNP 7A was also significantly associated with Kw (p = 0.0002; Table 2).

For the QTL for Kl, Platte provided the beneficial allele at SNP 6B (A = 0.006; Table 2), and CO940610 for SNP 7A (A = -0.041; Table 2). Only the association of SNP 7A and Kl was statistically significant (p = 0.006; Table 2), indicating SNP 7A was responsible for the allelic class means for Kl, but there was little correlation between the phenotype and SNP 6B and 7A (R^2 (%) = 0.1, 3.4, respectively; Table 2).

Both 7A and 6B QTL were significantly associated with SPS (p = <0.0001, R^2 (%) = 11.8, 15.6, respectively; Table 2) with Platte conferring the beneficial allele at both SNP 6B and 7A (A = 0.390, 0.458, respectively; Table 2). The phenotypic variability for SPS was highly correlated with both QTL regions and was statistically significant. After looking at single-factor ANOVA analysis it was important to analyze whether there was correlation between any of the four traits to test for the presence of trade-offs between yield components (Table 3).

Table 3. Pearson correlation coefficients among four traits of the CO940610/Platte RIL population (n=224). * *P*<0.05; ** *P*<0.0001; ns, not significant

	Grain width	Kernel length	1000 kernel weight
Spikelets per spike	-0.269**	-0.099^{ns}	-0.168*
Grain width		0.421**	0.869**
Kernel length			0.622**

Gw and Kl both showed a significant positive correlation with Kw (r = 0.869, 0.622, respectively, P < 0.0001; Table 3). Gw was significantly positively correlated with Kl (r = 0.421, P < 0.0001; Table 3), but significantly negatively correlated with SPS (r = -0.269, P < 0.0001; Table 3). Kl showed no significant correlation with SPS. SPS showed a significant negative correlation with 1000 Kw (r = -0.168, P < 0.05; Table 3). Since the p-values for SPS at both SNPs (p = <0.0001; Table 2) were highly significant, meaning the allele at each SNP had a great influence on the phenotype, the allelic combinations for SPS were compared (Figure 1).

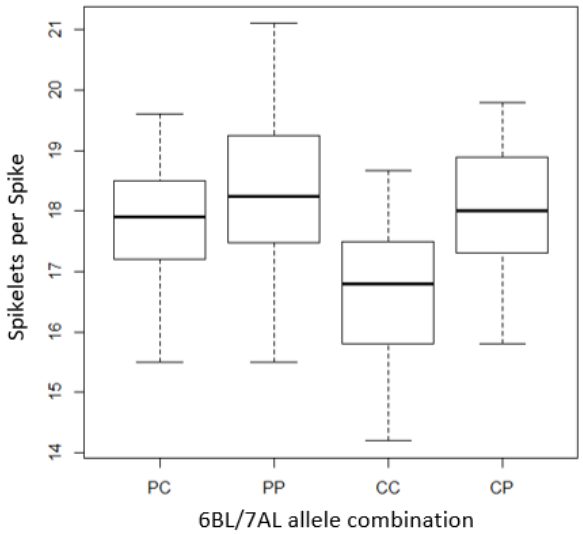

Figure 1. Additive effect of 6B loci and 7A loci QTL for spikelet number per spike

Figure 1. The effect of different 6BL/7AL allelic combinations on spikelet number per spike. PC means Platte allele for 6BL and CO940610 allele for 7AL. PP means Platte allele for both loci. CC means CO940610 allele for both loci. CP means CO940610 allele for 6BL and Platte allele for 7AL.

PP (Platte allele at both loci) had the highest number of SPS, whereas CC (CO940610 allele at both loci) had the lowest number of SPS. PC (Platte allele at 6BL/CO940610 allele at 7AL) and CP (CO940610 allele at 6BL/Platte allele at 7AL) had roughly the same number of SPS. PC and CP resulting in the same number of SPS indicates both positive alleles (7A and 6B) have about the same effect size. The statistical significance of comparing the allelic combinations of SNP 6B and SNP 7A for SPS were then calculated (Table 4).

Table 4. Single ANOVA analysis showing the significance of the allelic combinations for SPS compared to each other. Platte allele for 6BL and CO940610 allele for 7AL (PC). Platte allele for both loci (PP). CO940610 allele for both loci (CC). CO940610 allele for 6BL and Platte allele for 7AL (CP).

	Single ANOVA p-values		
PC	0.01162	6.54E-06	0.219204
PP		1.13E-08	0.3978
CC			0.000391
	PP	CC	CP

Only the difference between the allelic combination PC (Platte allele at 6BL/CO940610 allele at 7AL) and CC (CO940610 allele at both loci), and the difference between CC and PP (Platte allele at both loci) were statistically significant (p = 6.54E-06, 1.13E-08, respectively; Table 4). This does not mean there was not a positive effect for both alleles as the other comparisons just fell below the threshold for significance.

4. Discussion

The SSR 6B markers (*Xwmc397* and *Xwmc182a*) were significantly associated with grain width and kernel weight in Dao et al. 2017, hence the selection of the SNP 6B marker (*BobWhite_c22638_135*) as between those two markers. However, in the RIL population analyzed in the current study, no significant association was found between the SNP

6B and either grain width or kernel weight. Instead, significant associations were found with spikelets per spike. In genetic association studies, this pattern is common. When testing for association with traits that are negatively correlated with one another (as between grain size and grain number here), the same allele can have a significant positive effect on one trait in one population and be detected as a negative effect on the contrasting trait in a different population.

This pattern was observed for the significant association between the SNP 7A and spikelets per spike and grain width, which were both validated in this population. The CO940610 allele was associated with wider kernels but lower SPS and vice versa. When more SPS are produced, less photosynthate is available to fill those spikelets to produce a large grain width. The inverse relationship of spikelet per spike and grain width is further shown by the negative correlation. Grain width is positively correlated with kernel weight; As a larger width means fatter grains, so grain width and kernel weight are both negatively correlated with spikelets per spike. This means by having the CO940610 allele at SNP 7A, the number of spikelets per spike is lowered, attributing to the increase in kernel weight rather than having the CO940610 allele at SNP 6B causing the observed larger kernel weight. The observed negative correlation between kernel weight and spikelets per spike concurred with Grogan's comparisons in her paper[8].

SNP 7A was significantly associated with kernel length, but no significant correlation between the number of spikelets per spike and kernel length was observed. Kernel length was found to be significantly positively correlated with 1000 kernel weight and grain width. Kernel length may be associated with the number of spikelets per spike indirectly in that as kernel weight decreases because the increase in the spikelet number, kernel length also decreases. More importantly was the observation of both QTL being associated with spikelets per spike.

Based on the predicted effects on kernel weight and spikelet per spike, the allelic combination C-P (CO940610-Platte; 6BL-7AL) was hypothesized to result in plants with the highest overall effect on yield. However, the allelic combination PP (Platte at both loci) exhibited the highest number of spikelets, which concurs with spikelets per spike being highly correlated with both SNP 6B and SNP 7A and the positive addictive effects at both SNPs. The allelic combination PP compared to CC showed the greatest effect size, which accordingly follows the significant association of both SNP with spikelet per spike. The other allelic combination that was statistically relevant was the comparison between PC (Platte-CO940610; 6BL-7AL) and CC (CO940610 at both loci). The allelic combination PC compared to CC having a higher significance than CP (CO940610-Platte; 6BL-7AL) versus CC shows how strong the influence the Platte allele at SNP 6B is on the phenotype. Based on these findings, it is recommended that breeders interested in developing varieties with increased number of spikelet per spike select for the positive alleles at both 6B and 7A QTL using the markers described in this study.

5. Conclusion

Both QTL for both SNPs were associated with spikelet per spike instead of the researched association of 6B being correlated with kernel weight. Since the increasing number of spikelets per spike decreased grain width and kernel weight because of a physical limitation, wheat cultivars that have high photosynthate accumulation are being considered. The positive alleles for spikelet per spike validated in this study are currently being introgressed into three wheat cultivars from CIMMYT that have been selected for their high amount of photosynthate. It is hypothesized that the combination of high spikelet per spike with high photosynthate phenotypes will result in varieties exhibiting higher overall yields per se. These further steps in wheat breeding attribute to achieving food security in the future.

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7. References

1. National Association of Wheat Growers, "Wheat Nutrition" Wheatworld.org, 2018. Washington, DC, https://www.wheatworld.org/wheat-101/wheat-industry/wheat-nutrition/.

2. Ray, Deepak K., Mueller, Nathaniel D., West, Paul C., Foley, Jonathan A, "Yield Trends Are Insufficient to Double Global Crop Production by 2050." *PLoS One* 8.6 (2013). Web. June 19, 2013. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3686737/

3. Simmonds, James, Scott, Peter, Leverington-Waite, Michelle, Turner Adrian S, Brinton, Jemima, Korzun Viktor, Snape John, Uauy Cristobal, "Identification and Independent Validation of a Stable Yield and Thousand Grain Weight QTL on Chromosome 6A of Hexaploid Wheat (*Triticum aestivum L.*)." *BMC Plant Biology* 14:191 (2014). Web. 18 July 2014. https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-014-0191-9

4. Tian, Jichun, Deng Zhiying, Zhang, Kunpu, Yu, Haixia, Jiang, Xiaoling. Genetic Analyses of Wheat and Molecular Marker-Assisted Breeding, Volume 1. Beijing, Heidelburg: Science Press, Springer Science+Business Media Dordrecht, 2015. 13-40. https://link-springer-com.proxy2.library.illinois.edu/book/10.1007%2F978-94-017-7390-4

5. Dao, Hung Quoc, Byrne, Patrick F., Reid, Scott D., Haley, Scott D, "Validation of Quantitative Trait Loci for Grain Quality-Related Traits in a Winter Wheat Mapping Population." *Euphytica.* 213.5 (2017). SpringerLink. Web. 08 December 2016. https://link.springer.com/article/10.1007%2Fs10681-016-1793-0

6. El-Feki, Walid, Byrne, Patrick F., Reid, Scott D., Lapitan, Nora L V, Haley, Scott D, "Quantitative Trait Locus Mapping for End-Use Quality Traits in Hard Winter Wheat Under Contrasting Soil Moisture Levels." *Crop Science* 53.5 (2013):1953–1967. Web. September 2013. https://www.researchgate.net/publication/270476602_Quantitative_Trait_Locus_Mapping_for_End-Use_Quality_Traits_in_Hard_Winter_Wheat_Under_Contrasting_Soil_Moisture_Levels

7. El-Feki Walid, Byrne Patrick F., Reid Scott D., Haley Scott D, "Registration of CO940610/ 'Platte' Wheat Doubled Haploid Mapping Population." *Journal of Plant Registrations* 9.3 (2015): 419-423. Web. September 2015. https://www.researchgate.net/publication/276100717_Registration_of_CO940610%27Platte%27_Wheat_Doubled_Haploid_Mapping_Population

8. Grogan, S.M, "Dissecting Drought Tolerance in Winter Wheat Using Phenotypic and Genetic Analyses of Agronomic and Spectral Traits." Ph.D. dissertation (2015). Colorado State University, Fort Collins

9. Wang, Shichen, Wong, Debbie, Forrest, Kerrie, Allen, Alexandra, Chao, Shiaoman, Huang, Bevan E., Maccaferri, Marco, Salvi, Silvio, Milner, Sara G., Cattivelli, Luigi, Mastrangelo, Anna M., Whan, Alex, Stephen, Stuart, Barker, Gary, Wieseke, Ralf, Plieske, Joerg, International Wheat Genome Sequencing Consortium, Lillemo, Morten, Mther, Diane, Appels, Rudi, Dolferus, Rudy, Guedira, Gina Brown, Korol, Abraham, Akhunova, Alina R., Feuillet, Catherine, Salse, Jerome, Morgante, Michele, Pozniak, Curtis, Luo, Ming-Cheng, Dvorak, Jan, Morell, Matthew, Dubcovsky, Jorge, Ganal, Martin, Tuberosa, Roberto, Lawley, Cindy, Mikoulitch, Ivan, Cavanagh, Colin, Edwards, Keith J., Hayden, Matthew, Akhunov, Eduard, "Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array." *Plant Biotechnology Journal*. 12(6) (2014): 787–796. Web. March 2014. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4265271/

10. Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909