GENETICS OF END-USE QUALITY CHARACTERISTICS IN SPRING WHEAT

(*TRITICUM AESTIVUM* L.)

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important crops consumed by humans around the world. Improving the end-use quality traits is one of the major objectives in wheat breeding programs. However, little is known about the genomic regions controlling these traits. Discovering the genetic architecture underlying important end-use quality traits can accelerate breeding via marker-assisted selection (MAS) in addition to providing genomic and biological information. Therefore, for this dissertation, a quantitative trait loci (QTL) mapping and a genome-wide association study (GWAS) were conducted to identify QTL for 16 end-use quality traits, including the grain protein content, flour extraction rate, eight mixograph-related parameters, and six baking-related properties. A population of 127 recombinant inbred lines (RILs) from a cross between Glenn (PI-639273) and Traverse (PI-642780) was developed for the QTL mapping study, and an association panel of 355 elite spring wheat lines was used for the GWAS study. The phenotyping of these traits was performed in nine environments in North Dakota, USA, over a three-year period. The genotyping for both the RIL population and association panel was conducted using the wheat Illumina iSelect 90K SNP assay. A total of 76 additive QTL (A-QTL) and 73 digenic epistatic QTL (DE-QTL) were found for the 16 end-use quality traits in the QTL mapping study. These QTL were distributed across all wheat chromosomes except chromosome 3D. Overall, 12 stable major A-QTL and three stable DE-QTL were identified for the end-use quality traits in the QTL mapping study, indicating that both A-QTL and DE-QTL played an important role in controlling end-use quality traits. In addition to the QTL mapping study, a total of 91 significant marker–trait associations (MTA) were identified for the end-use quality traits in the GWAS study. These MTA were distributed across all wheat chromosomes except chromosome 4D. Overall, the current study identified multiple

novel stable QTL that could be used in MAS for end-use quality trait improvement in wheat breeding programs.

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DEDICATION

I dedicate my dissertation work to my loving parents.

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LIST OF ABBREVIATIONS

AE .....Across environments

FE.....Flour extraction rate

GPC.....Grain protein content

MELS.....Mixograph envelope left slope

MMLPT .....Mixograph MID line peak time

MMLPI .....Mixograph MID peak integral

MIXOPA.....General mixograph pattern

RIL…… .....Recombinant inbred line

TKW..... .....Thousand kernel weight

KSB.....Sandstedt, Kneen, and Blish

PPM.....Parts per million

MTA.....Marker trait association

QTL.....Quantitative trait locus/loci

DE-QTL..... .....Digenic epistatic QTL

GWAS.....Genome-wide association study

MERS.....Mixograph envelope right slope

MMLPV.....Mixograph MID line peak value

MMLTV.....Mixograph MID line peak value

MMLPW..... .....Mixograph MID line peak width

MMLPI..... .....Mixograph MID line peak width

DO.....Dough character

BA..... .....Baking absorption

CHAPTER 1. INTRODUCTION

1.1. General Introduction

Wheat (*Triticum aestivum* L., $2n = 2x = 42$, AABBDD genomes), with an annual harvest of ~700 million tons, is one of the most important grain crops consumed by humans in the world and accounts for ~20% of human caloric consumption (Food and Agriculture Organization (FAO), 2010). Food products made from wheat grain, such as bread, cake, noodles and pasta, are consumed daily around the world (Food and Agriculture Organization (FAO), 2010). Wheat grown in the United States is categorized into the eight following classes: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), durum (*T. turgidum* L. var. *durum* Desf.), white (soft white winter (SWW) and club), hard white winter (HWW), mixed, and unclassified (Delwiche and Norris, 1993). HRS wheat produced in the Northern Great Plains of the United States is known around the world for its high protein content and excellent end-use quality traits. Genetic improvements and breeding programs in HRSW focus on three major areas: enhancing yield, overcoming environmental stresses, and improving end-use quality traits (Mann et al., 2009).

The end-use quality traits of wheat, such as kernel characteristics, grain protein content (GPC), flour quality, dough quality, milling quality, and bread baking quality, are complex characteristics influenced by a combination of environmental conditions and genetic factors (Rousset et al. 1992; Peterson et al., 1998). Overall, knowledge is limited on the genetic and genomic control of end-use quality traits in wheat. The discovery of molecular markers associated with the genes governing the phenotypic characteristics of end-use quality traits will result in a better understanding of the genetic makeup of such complex traits. More specifically, genomic and genetic investigation to find the genomic-flanking regions and genes associated

with these critical traits should be done. This knowledge can be applied in breeding programs for wheat quality improvement. Molecular markers can be identified through bi-parental population or linkage disequilibrium-base association panel studies.

1.2. Objectives

1.2.1. General Objective

The aim of this study was to investigate and enhance the understanding of the genetic basis of end-use quality traits in HRSW to facilitate the breeding of improved wheat cultivars. The goal was to clarify and discover major loci associated with these important traits by using quantitative trait loci (QTL) mapping and association mapping (AM) approaches.

1.2.2. Specific Objectives

The objectives of this study were:

- To assess the phenotypic variations and correlations among the end-use quality traits in two HRSW populations, an RIL population, and an association panel.
- To investigate QTL and/or SNP marker associated with important end-use quality traits in HRSW, such as GPC, milling, and bread-baking, using QTL mapping and AM.

1.3. Literature Review

1.3.1. Molecular Markers

It is widely accepted that molecular markers can be very powerful tools for crop improvement by promoting the efficiency of conventional plant breeding programs (Eagles et al., 2001; Kasha, 1999). Recent developments in DNA markers, such as single nucleotide polymorphism (SNP), have largely facilitated genetic studies.

It is believed that SNP is a ubiquitous form of genetic variation in genomes of eukaryotic organisms. Even though most of the SNP genotyping assays and the SNP data analysis algorithms are designed and developed for diploid organisms, newly developed technologies, such as the Illumina GoldenGate Genotyping assays, are able to use SNP markers for genotypingpolyploid organisms (Akhunov et al., 2009). Based on recent studies, SNP markers are excellent molecular markers for investigating the genetic architecture of complex traits in polyploid wheat and its wild relatives (Akhunov et al., 2009). In addition, SNP markers are becoming the molecular marker of choice for studying complex traits owing to high genome density, low mutation rate, and appropriate amenability to high-throughput detection systems (Syvanen, 2005). SNP markers are very powerful tools for the construction of high-density genetic maps, QTL mapping and AM studies (Zhao et al., 2007; Aranzana et al., 2005). Both QTL mapping and AM are approaches that can be used to identify associations between QTL and traits in wheat, including kernel characteristics, GPC, flour-quality parameters, milling-quality traits, and bread-baking quality characteristics.

1.3.2. QTL Mapping Studies

One of the most common genetic analysis methods is QTL mapping (linkage analysis), which is based on the principle that genes and markers segregate through chromosome recombination. Genes and markers that are closely linked will co-segregate and transmit together from parent to progeny more frequently compared to genes and markers that are located far apart (Paterson, 1996). QTL mapping is commonly performed by using a population derived from a cross between two inbred lines. The power and accuracy of QTL detection are highly dependent on choosing the two parental lines (Jansen, 2001).

In plant species, conventional QTL mapping is analyzed using well developed and validated methods, including single-marker analysis, interval mapping, multiple-interval mapping, and Bayesian interval mapping (Doerge, 2002; Zeng, 2005). In general, these methods have been efficient in detecting major QTL, which may suggest a candidate gene and permit accelerated marker-assisted selection (MAS) (Osborn et al., 1997; Lagercrantz et al., 1996).

QTL mapping usually localizes QTL to 10 to 20 centimorgan (cM) intervals due to the limited number of recombination events that occur during the construction of mapping populations and the cost for estimating a large number of lines (Doerge, 2002; Holland, 2007). A main limitation of QTL mapping in a biparental population is that the QTL results are specific to that population and are usually not applicable to other populations (Holland, 2007; Bernardo, 2008).

1.3.3. Association Mapping Studies

In contrast to QTL mapping, AM has the advantage of identifying QTL in breeding populations that are of direct relevance for breeders to improve crops through knowledge-based breeding. Association mapping studies should also allow stronger estimates of QTL effects across populations (Würschum, 2012). In addition, AM based on elite lines and breeding populations has the advantage of detecting loci for traits with low heritability, such as yield and its components (Breseghello and Sorrells, 2006).

In summary, AM has three major strengths compared to QTL mapping: its high mapping resolution, shorter research time to develop populations, and greater allele numbers (Yu and Buckler, 2006). However, population structure has long been considered a barrier to AM analyses. In addition, the high molecular and biochemical cost of AM, as well as the technical challenges of the method, make it hard to replicate results in independent studies. Currently,

several statistical methods have been proposed to account for population structure, such as structure association (SA) (Pritchard et al., 2000), genomic control (GC) (Devlin and Roeder, 1999), EIGENSTRAT or principal component analysis (PCA) (Price et al., 2006), stepwise regression (SWR) (Setakis et al., 2006), and the mixed linear model (MLM) (Yu et al., 2006). Wang et al. (2012) demonstrated that the MLM is the most promising method for analyzing population structure in AM analyses.

1.3.4. Grain Protein Content

Grain protein content (GPC) is a major quality trait in bread wheat. GPC was reported to have a negative correlation with grain yield (Simmonds, 2006). Thus, QTL with less of a negative influence on yield are required. QTL for this characteristic are thought to be distributed on at least a dozen chromosomes in tetraploid and hexaploid wheat (Kuspira and Urau, 1957; Law et al., 1978; Morris et al., 1978; Levy and Feldman, 1989; Joppa and Cantrell, 1990; Stein et al., 1992; Snape et al., 1995; Blanco et al., 1996; Joppa et al., 1997; Mesfin et al., 1999; Prasand et al., 1999; Dholakia et al., 2001; Harjit-Singh et al., 2001). Blanco et al. (2006) reported three major QTL associated with GPC on chromosome arms 2AS, 6AS, and 7BL that explain all the genetic variation of the trait. A major QTL was mapped on chromosome 6BS of a *Triticum turgidum* ssp. *dicoccoides* accession with an average increase in GPC of 14 g Kg^{-1} (Distelfeld et al., 2006). Mann et al. (2009) reported that GPC had a high heritability (ranging from 0.69 to 0.93), and they also found that GPC was influenced by QTL on chromosomes 1A, 3A, 7A, and 1B. A QTL for GPC that accounts for 53% of the phenotypic variance was identified on chromosome 5A (Li, 2012). Table 1.3 shows a summary of QTL results based on previous studies for GPC in wheat.

Table 1.1. Summary of QTL results based on previous studies for grain protein content (GPC) in bread wheat.

Trait	Authors	Chromosomal Location of QTL	Population	Marker
GPC	Sourdille et al. (2003)	1BL, 6AS	DH	RFLP, AFLP
	Kulwal et al. (2005)	1AS, 1BL, 1DL, 2AS, 2AL, 2BL, 2DS, 2DL, 3BS, 4AS, 5BL, 5DL, 6DL, 7AL, 7DS	RIL	RFLP, SSR
	Huang et al. (2006)	4DS, 7BL	DH	SSR
	Kunert et al. (2007)	3AL, 4AL, 4BL, 5DL, 7BS, 7DS	BC_2F_3	SSR
	Mann et al. (2009)	1B, 3A	DH	SSR
	Nelson et al. (2006)	2A, 2D,	RIL	RFLP
	Raman et al. (2009)	4A	DH	DArTs
	Sun et al. (2010)	3AS, 4B	RIL	SSR
	Tsilo et al. (2010)	2BS, 5A, 6D	RIL	SSR, DArT
	Zhao et al. (2010)	3A, 3B, 5D, 6DS	DH	EST, ISSR, RFLP, SSR
	Conti et al. (2011)	1BS, 2AL, 2BS, 3BS, 3BL, 4AL, 5AS, 5BL, 7AS, 7BL	RIL	SSR, SNP, RFLP, STS
	Li et al. (2012a)	1A, 1B, 2A, 2B, 2D, 3A, 4A, 4B, 4D, 5A, 5B, 5D, 6B, 7A, 7B, 7D	RIL	G-SSR, EST-SSR
	Li et al. (2012b)	1AS, 2DL, 4BL, 5DL, 6AS, 6BL, 6D, 7B	BC_5, RIL	SSR
	Carter et al. (2012)	3BL	RIL	SSR, SNP
	Maphosa et al. (2013)	2B, 2D, 3A, 4A, 6A, 7A	DH	DArT, SSR
	El-Feki et al. (2013)	5B, 6A, 7B, 7D	DH	SSR, STS
	Maphosa et al. (2015)	2B, 2D, 3B, 5A	RIL	SSR, DArT
	Deng et al. (2015)	1B, 1D, 2A, 2B, 2D, 3B, 4B, 5B, 6D, 7A	DH, RIL	SSR

Table 1.1. Summary of QTL results based on previous studies for grain protein content (GPC) in bread wheat (continued).

Trait	Authors	Chromosomal Location of QTL	Population	Marker
GPC	Deng et al. (2015)	1B, 1D, 2A, 2B, 2D, 3B, 4B, 5B, 6D, 7A	DH, RIL	SSR
	Echeverry-Solarte et al. (2015)	5B, 6B, 7B	RIL	DArT
	Tiwari et al. (2016)	1A	DH	SSR, DArT

1.3.5. Milling Quality

The most common instruments used to test dough rheology are Farinograph, Glutograph, Mixograph, Extensograph, and Alveograph (Brabender, 1932; Sietz, 1987; Shelke and Walker, 1990; Panozoo and Eagles, 2000; Trethowan et al., 2001; Mann et al., 2007). Mann et al. (2009) reported major dough rheology QTL associated with the Glu-B1 and Glu-D1 loci in a double haploid population derived from a cross of Kukri x Jans. In the same study, Mann et al. (2009) identified major QTL for unextractable polymeric protein (UPP). UPP has been suggested by Gras et al. (2001) as a predictor of dough strength; these researchers believed the UPP QTL are located on chromosomes 1B and 2B. It is interesting to note that Mann et al. (2009) also showed time to peak dough development (TPDD) was associated with the Glu-B1, Glu-B3, and Glu-D1 loci, and peak resistance (PR) was influenced by two QTL detected on chromosome 1A.

Kuchel et al. (2006) reported a major QTL on chromosome 1A for dough development time and several QTL for dough stability time on chromosomes 1A and 1B. In the same study, Kuchel et al. (2006) identified QTL on chromosomes 1A and 2D for water absorption. Recently, a major QTL for water absorption was detected on the short arm of chromosome 5D (Li et al., 2009). In another study, Li et al. (2012) detected a main QTL for water absorption on chromosome 5B in a population derived from crosses among three Chinese wheat: Weimai8, Jimai20, and Yannong19. Martinannt et al. (1998) reported a QTL for water extractable arabinoxylans of wheat endosperm on the long arm of chromosome 1B. In 2006, Arbelbide and Bernardo identified four QTL for dough strength on chromosomes 1A, 1B, 1D, and 5B.

Flour characteristics, such as flour color and flour extraction rate (FE) are important traits for many end-use quality products of common wheat, especially for Asian noodles and Chinese steamed bread (He et al., 2004). Many recent studies conclude that genetic factors affect flour

color. In a study using a population derived from a cross of Schomburgk x Yarralinka (Parker et al. 1998), two QTL for flour color b* were detected on chromosomes 3A and 7A that explain 13% and 60% of phenotypic variation (PV), respectively. Zhang et al. (2006) reported a major QTL on chromosome 7A associated with flour color b* and kernel yellow pigment content that account for 12.1% to 37.6% of the PV across five environments using a biparental population. In another study, Mares and Campbell (2001) identified two QTL for yellow pigment content, flour color b*, yellow alkaline noodle, and the yellowness of Chinese white salted noodles on chromosomes 3B and 7A in a double haploid population derived from a cross of Sunco x Tasman. Tsilo et al. (2011) reported three QTL on chromosomes 5D and 5B for flour yellowness in a biparental population. In the same study, Tsilo et al. (2011) detected four QTL for flour protein content on chromosomes 1B, 2B, 5A, and 6D that account for 42.3% of the total phenotypic variance. Table 1.4 shows a summary of QTL results based on previous studies for milling quality characteristics in wheat.

Table 1.2. Summary of QTL results based on previous studies for milling quality characteristics in bread wheat.

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Flour extraction	Campbell et al. (2001)	3BS, 5AS, 5BS, 5DS	RIL	RFLP
	Kuchel et al. (2006)	1A, 2A, 6A	DH	SSR, STS, Proteins
	Nelson et al. (2006)	4A	RIL	RFLP
Mixogram pattern	Tsilo et al. (2011)	1B, 1D, 3B, 6D	RIL	SSR, DArT
Mixogram midline peak time, min.	Campbell et al. (2001)	1DL, 4AL, 7AS, 7DS	RIL	RFLP
	Tsilo et al. (2011)	1B1, 1D, 2A, 6D, 7D	RIL	SSR, DArT
	Li et al. (2012b)	2DL, 4A	BC_5, IL	SSR
Mixogram midline peak time, min.	Simons et al. (2012)	1DL	RIL	SSR
	Mergoum et al. (2013)	2B, 7BS	RIL	DART, SSR
Mixogram midline peak value, %	Tsilo et al. (2011)	1A, 1B, 1D, 6D	RIL	SSR, DArT
Mixogram midline peak value, %	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 3AL, 4BL, 5AS, 5B, 6AL, 7B	BC_5, IL	SSR

Table 1.2. Summary of QTL results based on previous studies for milling quality characteristics in bread wheat (continued).

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Mixogram midline peak value, %	Simons et al. (2012)	1BS, 1DL, 5BL	RIL	SSR
Mixogram line peak width, %	Tsilo et al. (2011)	1A, 1B, 6D	RIL	SSR, DArT
	Li et al. (2012b)	1AS, 1BS, 1DS, 2B, 2DL, 3AL, 4BL, 5AS	BC_5, IL	SSR
	Simons et al. (2012)	1BS, 1DL	RIL	SSR
Mixogram line peak integral	Tsilo et al. (2011)	1B, 1D, 6D, 7D	RIL	SSR, DArT
	Simons et al. (2012)	1DL	RIL	SSR
Mixogram mixing tolerance	Campbell et al. 2001	1AL, 1BL	RIL	RFLP
	Li et al. (2012b)	1BS, 2DL, 4A, 5AS, 6AL	BC_5, IL	SSR
Mixogram weakening slope	Li et al. (2012b)	4A, 6AL	BC_5, IL	SSR
Mixogram mixing development time	Huang et al. (2006)	1B, 1DL, 3B	DH	SSR
	McCartney et al. (2006)	1B, 4D	DH	SSR

Table 1.2. Summary of QTL results based on previous studies for milling quality characteristics in bread wheat (continued).

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Mixogram peak height	Huang et al. (2006)	1B, 1DL, 4DS	DH	SSR
Mixogram peak height	McCartey et al. (2006)	4D	DH	SSR
Mixogram energy to peak	Huang et al. (2006)	1B, 1DL, 3B	DH	SSR
	McCartney et al. (2006)	1B, 4D	DH	SSR
Mixogram first minute slope	Huang et al. (2006)	1DL, 4DS	DH	SSR
	McCartey et al. (2006)	1B, 4D, 7B, 7D	DH	SSR
Mixogram peak bandwidth	Huang et al. (2006)	1DL	DH	SSR
	McCartey et al. (2006)	2B, 4D, 7D	DH	SSR
Mixogram slope after peak	Huang et al. (2006)	1DL, 4DS	DH	SSR
	McCartey et al. (2006)	1B, 4D	DH	SSR
Mixogram total energy	Huang et al. (2006)	1B, 5DS	DH	SSR
	McCartey et al. (2006)	1B, 2B, 4D, 7D	DH	SSR
Mixogram bandwidth energy	Huang et al. (2006)	1B, 5DS	DH	SSR
Mixogram bandwidth energy	McCartey et al. (2006)	1B, 2A, 2B, 6A, 7D	DH	SSR

Table 1.2. Summary of QTL results based on previous studies for milling quality characteristics in bread wheat (continued).

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Resistance break down	Mann et al. (2009)	1D, 7B	DH	SSR
Mixogram midline time x=8 width	Li et al. (2012b)	1BS, 1DS, 2DL, 3AL, 4A, 4BL, 5AS, 5DL, 6AL	BC_5, IL	SSR
Mixogram midline time x=8 min value	Li et al. (2012b)	1AS, 1BS, 1DS, 2B, 3AL, 4A, 4BL, 5AS, 5B, 6AL	BC_5, IL	SSR
Mixogram midline right of peak width	Li et al. (2012b)	1AL, 1BS, 2B, 2DL, 3AL, 4A, 4BL, 5AS, 6AL	BC_5, IL	SSR
Mixogram midline right of peak value	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 4BL, 5B, 6AL	BC_5, IL	SSR
Mixogram midline left of peak width	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 3AL, 4BL, 5AS, 6AL	BC_5, IL	SSR
Mixogram midline left of peak value	Li et al. (2012b)	1AL, 1BS, 1DS, 2B, 2DL, 4BL, 5AS, 6AL	BC_5, IL	SSR
Mixing time	Mann et al. (2009)	1A, 1B, 1D	DH	SSR
Maximum band width	Mann et al. (2009)	1A, 1B, 4D, 5D, 7B	DH	SSR

1.3.6. Bread-Baking Quality

Limited information appears to be available on the genetic control of baking characteristics, such as sponge and dough-baking performance. Mann et al. (2009) found a QTL associated with sponge and dough baking on the 5D chromosome. Zanetti et al. (2001) detected 10 QTL for dough strength on chromosomes 5B, 5D, 5A, and 5B, together accounting for 39% of the PV. Kunert et al. (2007) reported two major QTL for the loaf-volume trait in a BC_2F_3 population B22. Recently, Simons et al. (2012) identified QTL on the long arm of chromosome 1D for bake-mixing time and bake-mixing water absorption traits in a population derived from a cross between BR34 x Grandin. In the same study, Simons et al. (2012) could not identify any QTL for flour brightness and bake-ware absorption and suggested that these characteristics may be controlled by small effect QTL. Table 1.5 shows a summary of QTL results based on previous studies for bread-baking characteristics in wheat.

Table 1.3. Summary of QTL results based on previous studies for bread-baking quality characteristics in wheat.

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Loaf volume	Campbell et al. (2001)	1AL, 2B, 2DL, 4AL, 7AS, 7DS	RIL	RFLP
	Kuchel et al. (2006)	2A, 3A	DH	SSR, STS
	Kunert et al. (2007)	4B, 6B, 7B	BC_2F_3	SSR
	Groos et al. (2007)	1A, 1B, 3A, 5B, 7A, 7B	RIL	SSR
	Mann et al. (2009)	1D, 4D, 5D	DH	SSR
	Tsilo et al. (2011)	1B, 2D, 6D	RIL	DArT
	Simons et al. (2012)	1DL	RIL	SSR, TRAP
	Maphosa et al. (2013)	5D	DH	SSR, DArT
	Maphosa et al. (2015)	5D	RIL	SSR, DArT
Water absorption	Campbell et al. (2001)	1DL, 2A, 2B, 2DL, 3L, 5DS, 7AS	RIL	RFLP
	Kuchel et al. (2006)	1A, 1B, 2A, 2D	DH	SSR, STS
	Tsilo et al. (2011)	1A, 1B, 5D	RIL	DArT

Table 1.3. Summary of QTL results based on previous studies for bread-baking quality characteristics in wheat (continued).

Trait	Authors	Chromosomal Location of QTL	Population	Marker
Mixing time	Tsilo et al. (2011)	1A, 1B, 1D, 6D	RIL	DArT
	Simons et al. (2012)	1DL, 4BL	RIL	SSR, TRAP
	Maphosa et al. (2013)	7B	DH	SSR, DArT
	Maphosa et al. (2015)	2B, 2D	RIL	SSR, DArT
Crumb score	Kuchel et al. (2006)	2A, 3A	DH	SSR, STS
	Groos et al. (2007)	5B, 6B	RIL	SSR

1.4. References

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CHAPTER 2. END-USE QUALITY TRAITS IN BREAD WHEAT: IDENTIFICATION OF MULTIPLE STABLE ADDITIVE AND DIGENIC EPISTATIC QTL USING A HIGH-DENSITY SNP-BASED LINKAGE MAP

2.1. Abstract

Improving the end-use quality traits is one of the primary objectives in wheat breeding programs. In the current study, a population of 127 recombinant inbred lines (RILs) derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) was developed and used to identify quantitative trait loci (QTL) for 16 end-use quality traits, including grain protein content (GPC), flour extraction rate (FE), eight mixograph-related parameters, and six baking-related properties. The phenotyping of these traits was performed in nine environments in North Dakota, USA over a three-year period. The genotyping for the RIL population was conducted using the wheat Illumina iSelect 90K SNP assay. A high density genetic linkage map consisting of 7,963 SNP markers, with an average marker density of 0.33 cM/marker, identified a total of 76 additive QTL (A-QTL) and 73 digenic epistatic QTL (DE-QTL) associated with 16 end-use quality traits. These QTL were distributed across all wheat chromosomes except chromosome 3D. Overall, 12 stable major A-QTL and three stable DE-QTL were identified for the end-use quality traits in bread wheat, suggesting that both A-QTL and DE-QTL played an important role in controlling end-use quality traits. The most significant A-QTL (*AQ.MMLPT.ndsu.1B*) was detected on chromosome 1B for mixograph middle line peak time (MMLPT). The *AQ.MMLPT.ndsu.1B* A-QTL was located very close to the position of the *Glu-B1* gene encoding for a subunit of high molecular weight (*HMW-GS*) and explained up to 24.43% of phenotypic variation (PV) for MMLPT. A total of 23 co-localized QTL or pleiotropic loci were detected, suggesting the possibility of the simultaneous improvement of the end-use quality traits through selection

procedures in bread wheat breeding programs. Overall, the information provided in the current study could be used in marker-assisted selection (MAS) to increase selection efficiency and to improve the end-use quality in bread wheat.

2.2. Introduction

Bread wheat (*Triticum aestivum* L.) produced in the Northern Great Plains of the USA is known around the world due to its high protein content and outstanding end-use quality traits. In wheat breeding programs, the end-use quality traits are not usually evaluated until late in the breeding program. This is because the end-use quality evaluations are expensive and a large amount of grain is needed to conduct the evaluations. Performing these evaluations at a late stage in the breeding program often results in ostensibly promising wheat lines that cannot be released due to poor end-use quality traits, such as a low level of grain protein content (GPC) and weak performance for milling parameters and baking properties. To address these challenges, many studies have been conducted to identify quantitative trait loci (QTL) and associated markers for end-use quality traits, with the aim to use such markers for marker-assisted improvement of quality traits (Parker et al., 1999; Campbell et al., 2001; Mares and Campbell, 2001; Groos et al., 2003; Prasad et al., 2003; Sourdille et al., 2003; Schmidt et al., 2004; Breseghello et al., 2005; Kulwal et al., 2005; Arbelbide and Bernardo, 2006; Breseghello and Sorrells, 2006; Huang et al., 2006; Kuchel et al., 2006; McCartney et al. ,2006; Nelson et al., 2006; Groos et al., 2007; Kunert et al., 2007; Ma et al., 2007; Mann et al., 2009; Raman et al., 2009; Sun et al., 2010; Tsilo et al., 2010; Zhao et al., 2010; Conti et al., 2011; Carter et al., 2012; Li et al., 2012; Simons et al., 2012; El-Feki et al., 2013; Maphosa et al., 2013; Mergoum et al., 2013; Maphosa et al., 2014; Cabreral et al., 2015; Deng et al., 2015; Echeverry-Solarte et al., 2015; Maphosa et al., 2015; Tiwari et al., 2016; Jin et al., 2016).

Grain protein content has received special attention among end-use quality traits because it is an indication of the performance of wheat products such as bread, cake, noodles, and pasta (Zhao et al., 2010). Moreover, wheat markets are determined by the amount of protein in the grain (Regional Quality Report, 2011). Several studies reported the existence of genes associated with GPC across all wheat chromosomes (Galande et al., 2001; Gross et al., 2003; Prasad et al., 2003; Sourdille et al., 2003; Kulwal et al., 2005; Huang et al., 2006; Kunert et al., 2007; Mann et al., 2009; Nelson et al., 2006; Raman et al., 2009; Sun et al., 2010; Tsilo et al., 2010; Zhao et al., 2010; Conti et al., 2011; Li et al., 2012a; Li et al., 2012b; Carter et al., 2012; Maphosa et al., 2013). In some of these studies, molecular markers associated with genes regulating gluten proteins have also been reported. Gluten is the coherent mass formed when glutenin and gliadin (storage protein) bind after water is added to flour (Stone and Savin, 1999). Glutenins are responsible for dough strength and are conformed by subunits of high molecular weight (HMW) and subunits of low molecular weight (LMW). The major genes controlling HMW Glutenins are Glu-1, Glu-A1, Glu-B1, and Glu-D1, whereas the major genes controlling LMW Glutenins are Glu-A3, Glu-B3, and Glu-D3 (Payne, 1987).

Mixograph-related properties govern the performance of wheat flour dough during mechanical treatment (Alamri 2009a; 2009b). Mann et al. (2009) reported major dough rheology QTL associated with the Glu-B1 and Glu-D1 loci in a double haploid population derived from a cross of Kukri × Jans. The same study also identified a major QTL for unextractable polymeric protein (UPP). Unextractable polymeric protein were located on chromosomes 1B and 2B and were suggested as a predictor of dough strength (Gras et al., 2001). Mann et al. (2009) also showed time to peak dough development (TPDD) was associated with the Glu-B1, Glu-B3, and

Glu-D1 loci, while peak resistance (PR) was influenced by two QTL detected on chromosome 1A.

Kuchel et al. (2006) identified a major QTL for dough development time on chromosome 1A and several QTL for dough stability time on chromosomes 1A and 1B. The same study identified QTL for water absorption on chromosomes 1A and 2D (Kuchel et al. 2006). Recently, a major QTL for water absorption was detected on the short arm of chromosome 5D (Li et al., 2009). In another study, Li et al. (2009) detected a major QTL for water absorption on the short arm of chromosome 5D. Further Li et al. (2012) identified a main effect QTL for water absorption on chromosome 5B in two populations derived from crosses among three Chinese wheat cultivars: Weimai8, Jimai20, and Yannong19. Martinannt et al. (1998) reported a QTL for water extractable arabinoxylans of wheat endosperm on the long arm of chromosome 1B. Arbelbide and Bernardo (2006) identified four QTL for dough strength on chromosomes 1A, 1B, 1D, and 5B.

Limited information appears to be available on the genetic control of baking properties. Mann et al. (2009) found a QTL associated with sponge and dough baking on chromosome 5D. In another study, Zanetti et al. (2001) detected 10 QTL for dough strength on chromosomes 5B, 5D, 5A, and 5B. Kunert et al. (2007) reported two major QTL for loaf volume trait in the BC_2F_3 population B22. Simons et al. (2012) identified a QTL on the long arm of chromosome 1D for bake-mixing time and water absorption traits in a population derived from a cross between BR34 × Grandin. In the same study, Simons et al. (2012) found no significant QTL for flour brightness and bake-mixing water absorption, suggesting that these characteristics may be controlled by small effect QTL.

Although several studies were conducted in the past to dissect the genetics of wheat end-use quality traits, almost all of these studies were based on low-density genetic linkage maps containing only several hundred molecular markers. This limits the successful application of associated markers in breeding programs. In the current study, for the first time, the wheat Illumina 90K iSelect assay (Wang et al., 2014) was used to detect marker-trait associations for end-use quality traits in bread wheat. Kumar et al. (2016) reported using the wheat Illumina 90K iSelect assay to create a genetic linkage map, indicating that it had a much higher resolution compared to most of the previous genetic linkage maps for the dissection of grain shape and size traits. Thus, the aims of this study were to: (1) construct a high-density linkage map using the wheat Illumina 90K iSelect assay, (2) provide comprehensive insight into the genetic control of end-use quality traits, and (3) identify SNP markers closely linked to QTL associated with end-use quality traits to augment molecular breeding strategies in wheat breeding programs.

2.3. Materials and Methods

2.3.1. Plant Material

A population of 127 RILs derived from a cross between Glenn (PI-639273; Mergoum et al., 2006) and Traverse (PI-642780; Karl et al., 2006) was used in this study. Glenn and Traverse are both hard red spring wheat (HRSW) cultivars. Glenn was developed by the Hard Red Spring Wheat Breeding Program at North Dakota State University (NDSU) in Fargo, ND, USA, in 2005. It is well-known in domestic and export markets due to its high level of resistance to *Fusarium* head blight (FHB), high GPC, and excellent end-use quality characteristics. Traverse was developed and released by the South Dakota Agricultural Experiment Station in 2006. It is a high yielding, FHB-tolerant cultivar with a low GPC. The RIL population was advanced by the single seed descent (SSD) method from the F_2 generation through F_{10}. This study also used 12

HRSW cultivars as checks, including: Alsen “PI-615543” (Frohberg, 2006), Faller “PI-648350” (Mergoum, 2008), Granite “CN-106418”, Howard “PI-642367” (Mergoum, 2006), ND901CLPLUS “PI-659776”, Parshall “PI-613587”, Prosper “PI-662387” (Mergoum, 2012), RB07 “PI 652930” (Anderson, 2009), Reeder “PI-613586”, Saturn, Polaris, and Mott.

2.3.2. Field Experiment Design

The RILs, parental lines, and check varieties were grown under field conditions at three locations in ND for three years from 2012 to 2014 (Table 2.1). In 2012, the three sites were Prosper, Carrington, and Casselton; whereas in 2013 and 2014 the Casselton site was replaced with a Minot site. A detailed description of the environments is given in Table 1. In 2012, lines were grown in a randomized complete block design (RCBD) with two replicates; however, in 2013 and 2014, a 12 × 12 partially balanced square lattice design with two replicates (simple lattice design) was used to reduce experimental error and increase precision in the experiment. In 2012 and 2013, each plot was 2.44 m long and 1.22 m wide; whereas in 2014 the plots were 2.44 m long and 1.42 m wide. All plots consisted of seven rows. Sowing rate was 113 kg ha^{-1} in all environments.

Table 2.1. Description of the environments and planting date to evaluate spring wheat end-use quality traits in a recombinant inbred lines (RIL) population derived from a cross between Glenn and Traverse (NDAWN, 2000-2016).

Location	Year	LAT[a]	LNG[b]	ALT (m)[c]	Planting date	TGS (^{0}C)[d]	PGS (mm)[e]
Prosper	2012	46°57'46.90"N	97°1'11.31"W	275	05.15.2012	21	148.8
Carrington	2012	47°27'11.56"N	99°9'15.15"W	491	04.23.2012	19	225.0
Casselton	2012	46°51'18.26"N	97°12'39.83"W	283	05.10.2012	21	144.0
Prosper	2013	46°57'46.90"N	97°1'11.31"W	275	05.30.2013	20	318.0
Carrington	2013	47°27'11.56"N	99°9'15.15"W	491	04.30.2013	18	83.2
Minot	2013	48°13'58.68"N	101°17'32.25"W	514	05.14.2013	19	425.0
Prosper	2014	46°57'46.90"N	97°1'11.31"W	275	05.24.2014	19	216.9
Carrington	2014	47°27'11.56"N	99°9'15.15"W	491	05.02.2014	17	203.2
Minot	2014	48°13'58.68"N	101°17'32.25"W	514	05.22.2014	17	347.7

[a] Latitude in degrees and minutes; [b] Longitude in degrees and minutes; [c] Altitude in meters; [d] Mean temperature during growing season in degrees Celsius (May-October); [e] Mean precipitation in growing season in millimeters.

2.3.3. Phenotypic Data Collection

The grain samples harvested from the field experiments were cleaned in two steps before evaluating quality traits. First, the samples were cleaned using a clipper grain cleaner machine. Second, the samples were cleaned using a carter dockage tester machine. One replicate was used to create a 200-g grain sample per line in each location for evaluating end-use quality characteristics. Quality characteristics analyzed in this study were: GPC, FE, eight mixograph-related parameters, and six baking-related properties.

Grain protein content (%) was measured based on 12% moisture using the Near-Infrared Reflectance (NIR) method for protein determination in small grains and following the American Association of Cereal Chemists (AACCI)-approved method 39.10.01 (AACC International Method, 1999). Flour extraction (%) was determined using 150 g of thoroughly cleaned wheat grain per sample tempered to 16.0% moisture, using the Brabender Quadrumat Jr. Mill and following the AACCI-approved method 26-50.01 (AACC International Method, 1999).

Mixograph parameters include the mixograph envelope left slope (MELS), mixograph envelope right slope (MERS), mixograph MID line peak time (MMLPT), mixograph MID line

peak value (MMLPV), mixograph MID line time * value (MMLTV), mixograph MID line peak width (MMLPW), mixograph MID line peak integral (MMLPI), and general mixograph pattern (MIXOPA). Mixograph measurements were obtained from 10 g of flour per sample on a 14% moisture basis using the National Manufacturing Mixograph (National Manufacturing, TMCO Division, Lincoln, NE) and following the AACCI-approved method 54-40.02 (AACC International Method, 1999). Mixsmart software was used to collect data of MELS (%/min), MERS (%/min), MMLPT (min), MMLPV (%), MMLPW (%), MMLPI (%/min), and MMLTV (%). The general mixograph pattern was based on a 0 to 9 scale (0 = weakest and 9 = strongest) according to the NDSU Wheat Quality and Carbohydrate Research Lab protocol (https://www.ndsu.edu/faculty/simsek).

Baking properties include bake-mixing time (BMT), baking absorption (BA), dough character (DO), bread loaf volume (BLV), crumb color (CBCL), and crust color (CTCL). Baking parameters were determined from 100 g of flour per sample on a 14% moisture basis according to the AACCI-approved method 10-09.01 with a little modification in baking ingredients (AACC International Method, 1999). The baking ingredients were modified as follows: (1) malt dry powder was replaced with fungal amylase (15 SKB); (2) compressed yeast was replaced with instant dry yeast; (3) ammonium phosphate was increased from 0.1 to 5 ppm; (4) two percent shortening was added. Bake mixing time (minutes) was determined as time to full dough development. Baking absorption was evaluated as a percent of flour weight on a 14% moisture basis for the amount of water required for optimum dough baking performance. Dough character was assessed for handling conversion at panning based on a scale of 1 to 10, with higher scores preferred. Bread loaf volume (cubic centimeters) was measured by rapeseed (*Brassica napus* L.) displacement 30 minutes after the bread was removed from the oven. Crumb color and CTCL

were valued according to visual comparison with a standard by using a constant illumination source based on a 1 to 10 scale, with higher scores preferred.

2.3.4. Phenotypic Data Analysis

Data collected from the first replicate of each environment was used to analyze phenotypic data. The experimental design employed was a randomized complete block design (RCBD). End-use quality traits were analyzed for only a single replicate in each environment, thus data from each environment was considered as a replicate. Variance components were estimated using restricted maximum likelihood (REML) in the MIXED procedure of SAS software Version 9.3 (SAS Institute, Inc., Cary, NC, USA). Blocks (environments) and genotypes were considered random effects. Best linear unbiased predictor (BLUP) values were estimated using the *solution option* of the random statement of the Proc Mixed procedure in SAS. Broad-sense heritability and genetic correlations were calculated using the Proc Mixed procedure in SAS (Holland et al., 2003; Holland et al., 2006). Broad-sense heritability coefficients were classified according to Hallauer and Miranda (1988): VH = very high = $h^2 > 0.70$, HI = high = $0.50 < h^2 < 0.70$, M = medium = $0.30 < h^2 < 0.50$, and L = low = $h^2 < 0.30$. Pearson correlations between quality traits were evaluated using BLUP values across all environments. The CORR procedure in SAS was used to calculate Pearson correlations. Trait values collected from the first replicate of each environment and BLUP values were used for the QTL mapping analysis.

2.3.5. Genotyping and Genetic Linkage Map Construction

Lyophilized young leaves were used to isolate genomic DNA for RILs and parental lines following a modified Doyle and Doyle (1987) protocol described by Diversity Arrays Technology Pty., Ltd.

(http://www.diversityarrays.com/sites/default/files/resources/DArT_DNA_isolation.pdf accessed August 2014). DNA quality was checked via visual observation on 0.8% agarose gel. DNA concentrations were determined with a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). DNA samples were diluted to the concentration of 50 ng/µl, and 20 µl of the diluted samples were sent to the USDA Small Grains Genotyping Lab in Fargo, ND, for SNP analysis using the wheat Illumina 90K iSelect SNP assay (Wang et al., 2014). SNP markers were called as described by Wang et al. (2014) using Genome Studio Polyploid Clustering Module v1.0 software (www.illumina.com).

Out of a total 81,587 SNP markers from the wheat Illumina 90K iSelect assay (Wang et al., 2014), 8,553 polymorphic SNP markers between parents were identified. Markers with a high number of missing values (≥ 15%), inconsistent results in three replicates of each parental genotype, or significant segregation distortion (χ^2 goodness-of-fit statistic, $p < 0.001$) were excluded from the following map construction. Linkage analysis for 8,553 SNP markers was performed using a combination of MAPMARKER/EXP software version 3.0 (Lander et al., 1989) and MSTmap software (Wu et al., 2008). In the first step, a high-density SNP consensus map was used (Wang et al., 2014) as a reference to select 210 anchor SNP markers for all 21 wheat chromosomes. For each chromosome, 10 SNP markers that covered the whole length of each chromosome were selected. By using MAPMARKER/EXP software version 3.0 (Lander et al., 1989) and the 210 anchor SNP markers, 7,963 out of 8,553 SNP markers were placed into the 21 wheat chromosomes based on a minimum LOD score of 5.0 and a maximum distance of 40 centimorgans (cM). In the second step, the marker orders and genetic distances of each linkage group were estimated using MSTmap software (Wu et al., 2008), with a cut-off at $p < 0.000001$, the maximum distance of 15 cM between markers, grouping LOD criteria of 5.0, and a minimum

linkage group size of 2 cM. Genetic distances between markers were calculated using Kosambi's genetic mapping function (Kosambi, 1944). To check the accuracy of the marker orders, the genetic linkage groups were compared by inspection with the high-density SNP consensus map of Wang et al. (2014). The final genetic linkage maps and corresponding graphs were drawn using Mapchart software version 2.2 program (Voorrips, 2002).

2.3.6. Quantitative Trait Loci Mapping

Inclusive composite interval mapping with additive effects (ICIM-ADD) was implemented to identify additive QTL (A-QTL) for each trait within each of the nine environments, as well as across all environments, using QTL IciMapping software version 4.1 (Wang et al., 2012). In QTL IciMapping, stepwise regression ($p < 0.001$) with simultaneous consideration of all marker information was used. The step size chosen for all A-QTL was kept at the default value, 1.0 cM. Left and right confidence intervals were calculated by one-LOD drop off from the estimated A-QTL (Wang et al., 2016). The LOD threshold values to detect significant A-QTL were calculated by performing a permutation test with a set of 1,000 iterations at a Type I error of 0.05; all A-QTL identified above the LOD threshold value were reported in this study. In addition, those A-QTL detected in more than two environments or associated with at least two traits were reported. Furthermore, an A-QTL with an average LOD value above the LOD threshold value and an average phenotypic variance (PV) contribution over 10% was considered a major A-QTL. Moreover, A-QTL which were identified in at least three environments were defined as stable QTL.

Digenic epistatic QTL (ICIM-EPI) method, available in QTL IciMapping software version 4.1 (Wang et al., 2012), was employed to identify additive-by-additive epistatic interactions or digenic epistatic QTL (DE-QTL) for each of the end-use quality characteristics

within each environment, as well as across all environments. For the convenience of illustration, the digenic epistatic QTL were named as DE-QTL. The step size chosen for DE-QTL was 5.0 cM. The probability used in stepwise regression for DE-QTL was 0.0001. To detect DE-QTL, the LOD threshold values were kept at the default value of 5.0. Additionally, the LOD value of 3.0 was also used as another threshold to declare the presence of a putative DE-QTL. Those DE-QTL that were identified in at least two environments were reported in this study. Furthermore, a DE-QTL detected in at least three environments was defined as a stable DE-QTL. It should be noted that in order to represent the most relevant data, only the highest values observed across environments for LOD score, additive effect, epistatic effect, and PV were reported in this study.

2.4. Results

2.4.1. Phenotypic Variation, Heritability, and Genetic and Pearson Correlations

The RIL population showed variation for all of the end-use quality characteristics (Fig 1; Table 2.2). The parental lines showed significantly different values for GPC, BMT, BA, BLV, MIXOPA, MELS, MMLPT, MMLTV, MMLPW, and MMLPI; the values differed slightly for CBCL, CTCL, FE, MERS, MMLPV, and DO across all environments, but this difference was statistically insignificant (Table 2.2). All traits showed approximately normal distributions (Figure 1), demonstrating the polygenic nature and quantitative inheritance of these traits (Fatokun et al., 1992). Transgressive segregation in both directions was observed for GPC, BA, BLV, CBCL, FE, MELS, MERS, MMLPT, and MMLPV across all environments, indicating positive alleles were present in both parents. Transgressive segregation for CTCL, MMLTV, and DO was observed in the direction of the better parent (Glenn cultivar); several RILs showed better performance than the Glenn cultivar for these traits. For FE and ENLSMIN, transgressive

segregation in the direction of Traverse was observed, with several RILs showing higher values than the Traverse cultivar for these characteristics (Table 2.2).

The broad-sense heritability coefficients varied from low to high for different traits. The highest broad-sense heritability was estimated for MMLPT (0.77), and the lowest for CTCL (0.05) (Table 2.2). Among baking properties, BMT and BA showed high and moderate broad-sense heritability (0.65 and 0.40, respectively); while BLV, CBCL, CTCL, and DO showed low broad-sense heritability (0.26, 0.11, 0.05, and 0.22, respectively). Among milling and mixograph traits, FE, MIXOPA, MELS, MERS, MMLPT, MMLPV, MMLTV, and MMLPI showed moderate to high broad-sense heritability (0.55, 0.42, 0.38, 0.50, 0.77, 0.31, 0.41, and 0.43, respectively), but MMLPW had low broad-sense heritability (0.23). High to very high broad-sense heritability coefficients for BMT, FE, MMLPT, and MMLPV indicated stability of these traits, and the PV of these characteristics was mainly due to genetic effects (Table 2.2).

The genetic and Pearson correlation analyses showed most of the quality traits were associated with each other (Table 2.3). High positive significant genetic and phenotypic correlations, where correlation coefficient value lies between + 0.50 and + 1 and is significant at $P < 0.01$, were observed between GPC and BLV; GPC and MELS; GPC and MMLPV; BMT and MIXOPA; BMT and MERS; BMT and MMLPT; BMT and MMLPI; BA and MMLPV; BLV and MELS; MIXOPA and MMLTV; MIXOPA and MMLPW; MIXOPA and MMLPI; MERS and MMLPT; MMLPT and MMLPI; MMLPV and MMLPI; MMLTV and MMLPW; and MMLTV and MMLPI. In contrast, high negative significant genetic and phenotypic correlations, where correlation coefficient value lies between - 0.50 and – 1 and is significant at $P < 0.01$), were found between BMT and MELS; MELS and MMLPT; and MERS and MMLPV. Moderate positive significant genetic and phenotypic correlations, where correlation

coefficient value lies between + 0.30 and + 0.50 and is significant at $P < 0.01$, were identified between GPC and MMLTV; GPC and MMLPW; BMT and MMLTV; BMT and MMLPW; BA and MELS; BLV and CTCL; NLV and MIXOPA; BLV and MMLPV; CTCL and MIXOPA; CTCL and MMLPV; CTCL and MMLTV; CTCL and MMLPW; MIXOPA and MMLPT; MIXOPA and MMLPV; MERS and MMLPI; MMLPT and MMLTV; and MMLPW and MMLPI. However, moderate negative significant genetic and phenotypic correlations (correlation coefficient value lies between - 0.30 and - 0.50; significant at $P < 0.01$) were detected between GPC and MERS; GPC and MMLPT; BMT and MELS; BA and MMLPT; MMLPT and MMLPV. In other pairs of traits genetic and phenotypic correlations were either low or not statistically significant at $P < 0.05$. Correlations between the end-use quality traits are shown in more detail in Table 2.3. Differences between genetic and phenotypic correlation coefficients (Table 2.3) could be due to low heritability values; Hill and Thompson (1978) suggested higher heritability values could result in the accuracy of genetic correlation estimates and greater similarity of genetic and phenotypic correlation coefficients. The overall level of genetic correlation was greater than phenotypic correlation, but the magnitude and pattern of genetic and phenotypic correlations were similar, suggesting phenotypic correlations would likely be fair estimates of their genetic correlations in end-use quality traits (Table 2.3).

Table 2.2. Phenotypic performance of Glenn, Traverse and their recombinant inbred lines (RILs) based on BLUP/average values and broad-sense heritability (h2) for end-use quality traits across all environments.

Trait	Parental lines				RIL population						
	Glenn		Traverse		Mean		S.D.	Range	Q_2	h^2	Class of trait h^2
GPC	15.76 /	0.51*	14.49 /	-0.76	15.25 /	0	0.5	-1.12 to 1.52	-0.02	0.29	L
BMT	4.08 /	0.98*	2.68 /	-0.42	3.10 /	-0.03	0.26	-0.53 to 0.76	-0.01	0.65	HI
BA	62.44 /	1.42*	60.33 /	-0.69	61.02 /	-0.02	0.75	-1.44 to 2.93	-0.09	0.4	M
BLV	200.83 /	6.37*	185.86 /	-8.6	194.46 /	-0.13	4.67	-10.56 to 17.77	-0.26	0.26	L
CBCL	7.68 /	-0.01	7.65 /	-0.04	7.69 /	0.01	0.12	-0.40 to 0.28	0.02	0.11	L
CTCL	9.63 /	-0.01	9.53 /	-0.11	9.64 /	0	0.04	-0.11 to 0.06	0.01	0.05	L
FE	53.51 /	0.87	54.07 /	1.43	52.64 /	-0.01	1.21	-2.91 to 2.89	0.07	0.55	HI
MIXOPA	6.22 /	2.93*	2.19 /	-1.1	3.29 /	-0.04	0.39	-1.19 to 0.82	-0.05	0.42	M
MELS	23.68 /	-0.40*	23.70 /	-0.38	24.08 /	0.19	2.4	-4.64 to 7.18	-0.25	0.38	M
MERS	-10.07 /	0.24	-12.44 /	-2.13	-10.31 /	-0.08	1.21	-3.45 to 2.35	0.01	0.5	HI
MMLPT	5.68 /	1.53*	3.10 /	-1.05	4.15 /	-0.05	0.7	-1.53 to 2.08	-0.09	0.77	VH
MMLPV	60.45 /	1.73	55.94 /	-2.78	58.72 /	0.05	1.85	-6.82 to 5.50	0.16	0.31	M
MMLTV	56.72 /	4.23*	45.63 /	-6.86	52.49 /	-0.06	2.38	-6.52 to 6.48	-0.47	0.41	M
MMLPW	20.79 /	2.81*	15.93 /	-2.05	17.98 /	-0.01	0.96	-2.18 to 2.19	-0.12	0.23	L
MMLPI	185.17 /	43.41*	114.29 /	-27.47	141.76 /	-0.61	13.7	-30.86 to 35.98	-0.77	0.43	M
DO	8.88 /	-0.35	8.71 /	-0.52	9.23 /	0.01	0.16	-0.44 to 0.27	0.01	0.22	L

[a] GPC: grain protein content, BMT: bake mixing time, BA: baking absorption, BLV: bread loaf volume, CBCL: crumb color, CTCL: crust color, FE: flour extraction rate, MIXOPA: the general mixograph pattern, MELS: mixograph envelope left slope, MERS: mixograph envelope right slope, MMLPT: mixograph MID line peak time, MMLPV: mixograph MID line peak value, MMLTV: mixograph MID line time * value, MMLPW: mixograph MID line peak width, MMLPI: mixograph MID line peak integral; DO: dough character; [b*]A significant difference between parental lines at $P < 0.05$; [c] Standard deviation; [d] The second quartile or median; [e] broad-sense heritability coefficient according to Holland (2006); [f] Class of broad-sense heritability according to Hallauer and Miranda (1988), VH = very high = h2 > 0.70, HI = high = 0.50 < h2 < 0.70, M = medium = 0.30 < h2 < 0.50, L = low = h2 <0.30.

Table 2.3. Genetic and Pearson's rank correlations of end-use quality traits for the recombinant inbred lines (RILs) population derived from a cross between Glenn and Traverse across all environments. Values in bold displayed above the diagonal indicate genetic correlation coefficients, and values under the diagonal show Pearson correlation coefficients.

Trait	GPC	BMT	BA	BLV	CBCL	CTCL	FE	MIXOPA	MELS	MERS	MMLPT	MMLPV	MMLTV	MMLPW	MMLPI	DO
GPC	-	**-0.29**[a]	**0.42****	**0.76****	**0.18**	**0.48****	**-0.31****	**0.25****	**0.70****	**-0.49****	**-0.35****	**0.74****	**0.34****	**0.40****	**0.11**	**0.10**
BMT	-0.29**[b]	-	**-0.17**	**-0.29****	**-0.05**	**0.27****	**-0.02**	**0.73****	**-0.60****	**0.69****	**0.90****	**-0.11**	**0.69****	**0.50****	**0.89****	**0.27****
BA	0.33**	-0.12	-	**0.22***	**0.21***	**0.14**	**-0.53****	**0.31****	**0.61****	**-0.36****	**-0.46****	**0.80****	**0.37****	**0.32****	**0.01**	**0.07**
BLV	0.59**	-0.24**	0.16	-	**0.29****	**0.97****	**-0.08**	**0.43****	**0.76**	**-0.37****	**-0.22***	**0.70****	**0.38****	**0.48****	**0.24****	**0.01**
CBCL	0.13	-0.06	0.10	0.23**	-	**0.39****	**-0.41****	**0.10**	**0.24****	**0.13**	**-0.05**	**0.08**	**0.10**	**-0.03**	**0.13**	**-0.30****
CTCL	0.21*	0.06	0.06	0.34**	0.07	-	**-0.49****	**0.65****	**0.48****	**0.10**	**0.16**	**0.62****	**0.71****	**0.71****	**0.65****	**-0.65****
FE	-0.24**	-0.04	-0.36**	-0.05	-0.20*	-0.16	-	**-0.20***	**-0.18**	**-0.02**	**0.07**	**-0.25****	**-0.25****	**-0.35****	**-0.16**	**-0.13**
MIXOPA	0.24**	0.57**	0.22*	0.30**	0.07	0.37**	-0.14	-	**-0.13**	**0.58****	**0.58****	**0.45****	**0.97****	**0.83****	**0.92****	**0.08**
MELS	0.57**	-0.48**	0.41**	0.46**	0.14	0.17	-0.11	0.01	-	**-0.87****	**-0.79****	**0.79****	**-0.03**	**0.09**	**-0.43****	**0.03**
MERS	-0.48**	0.55**	-0.27**	-0.26**	0.01	0.02	-0.03	0.25**	-0.67**	-	**0.83****	**-0.67****	**0.33****	**0.16**	**0.61****	**-0.02**
MMLPT	-0.35**	0.85**	-0.39**	-0.19*	-0.06	0.05	0.04	0.44**	-0.64**	0.68**	-	**-0.48****	**0.45****	**0.36****	**0.97****	**0.24****
MMLPV	0.62**	-0.11	0.48**	0.39**	0.01	0.30**	-0.14	0.42**	0.61**	-0.54**	-0.31**	-	**0.49****	**0.83****	**-0.03**	**-0.21***
MMLTV	0.33**	0.48**	0.26**	0.24**	0.02	0.33**	-0.17	0.79**	0.08	0.11	0.36**	0.67**	-	**0.96****	**0.80****	**0.11**
MMLPW	0.35**	0.31**	0.20*	0.27**	0.02	0.35**	-0.19*	0.66**	0.13	-0.04	0.18*	0.60**	0.71**	-	**0.71****	**-0.17**
MMLPI	0.04	0.67**	0.03	0.10	0.04	0.14	-0.17	0.62**	-0.29**	0.41**	0.75**	0.01	0.53**	0.34**	-	**0.56****
DO	0.13	0.09	0.02	0.03	-0.03	-0.09	-0.04	0.11	0.05	-0.11	0.14	0.05	0.15	0.04	0.24**	-

[a] GPC: grain protein content, BMT: bake mixing time, BA: baking absorption, BLV: bread loaf volume, CBCL: crumb color, CTCL: crust color, FE: flour extraction rate, MIXOPA: the general mixograph pattern, MELS: mixograph envelope left slope, MERS: mixograph envelope right slope, MMLPT: mixograph MID line peak time, MMLPV: mixograph MID line peak value, MMLTV: mixograph MID line time * value, MMLPW: mixograph MID line peak width, MMLPI: mixograph MID line peak integral; DO: dough character; [b] Genetic correlation coefficient according to Holland (2002); [c] Pearson correlation based on BLUP values. *, ** Significant at $P < 0.05$ and 0.01; [ns] Not significant at $P < 0.05$.

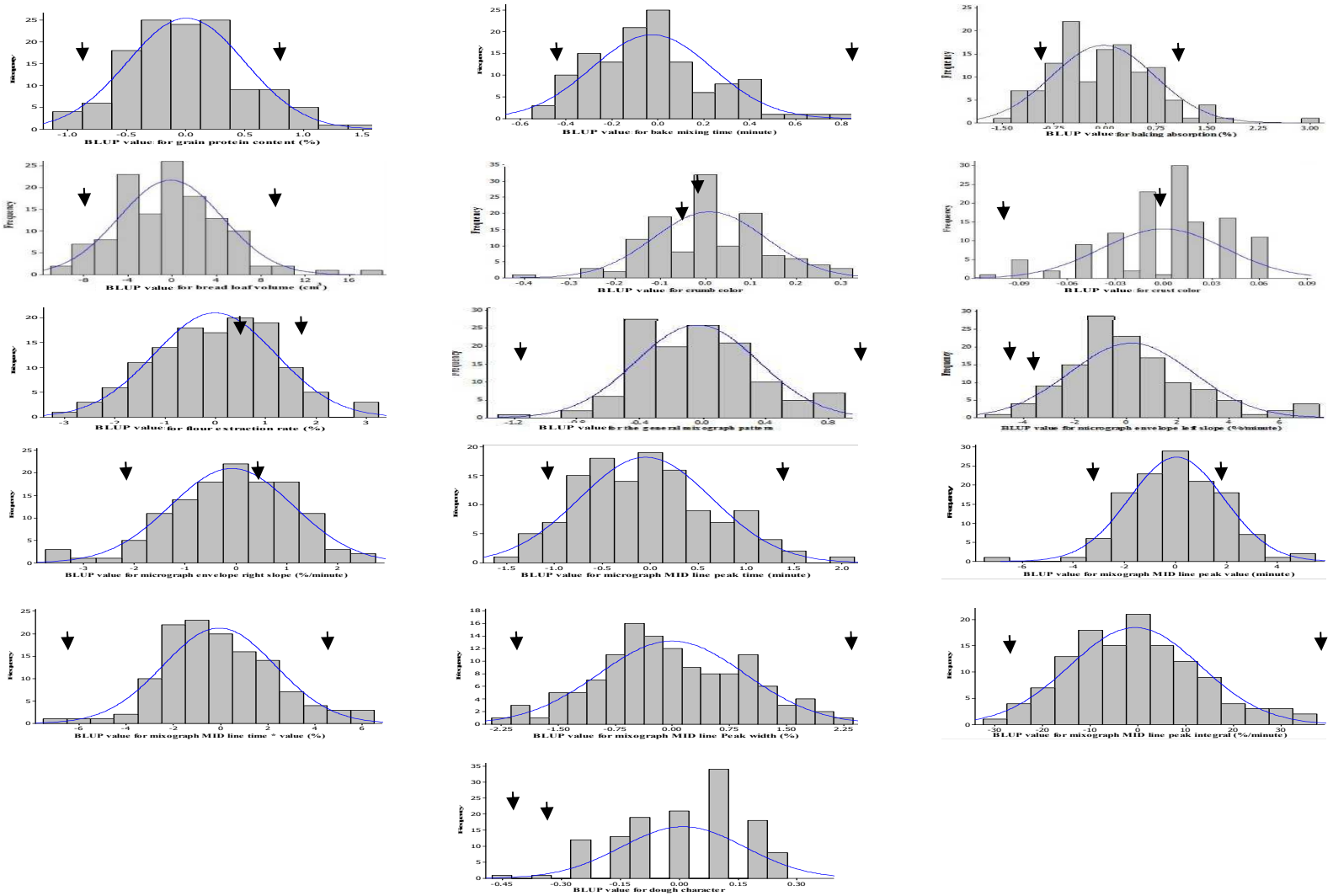

Figure 2.1. Frequency distribution of BLUP values for end-use quality characteristics of a population of 127 recombinant inbred lines (RILs derived from a cross between Glenn and Traverse across all environments. Estimates of the parental lines are indicated by arrows.

2.4.2. Genetic Linkage Map

Out of a total of 8,553 SNP markers, 7,963 markers were selected for genetic linkage mapping according to criteria described in the materials and methods section. These markers were mapped onto 41 linkage groups covering all 21 wheat chromosomes (Table 2.4). The linkage maps covered a total genetic length of 2,644.82 cM, with an average distance of 0.33 cM between any two markers (Table 2.4). The linkage map consisted of 1,427 unique loci (~18%), with an average genetic distance of 1.85 cM between any two unique loci. Altogether, the B-genome contained considerably more markers (4,807) than the A-genome (2,549); notably fewer markers were mapped on the D-genome (607). The number of markers on individual linkage groups varied from 10 (1B2) to 770 (3B1). Furthermore, the number of unique loci in a linkage group ranged from 2 (3D1) to 113 (7A1) (Table 2.4). Compared to the high-density SNP consensus map of Wang et al. (2014), the marker orders were fairly consistent.

Table 2.4. Distribution of markers and marker density across linkage groups in the bread wheat (*Triticum aestivum* L.) genetic map developed by using the recombinant inbred line (RIL) population of a cross between Glenn (PI-639273) and Traverse (PI-642780).

Linkage group	No. of markers	No. of unique loci	Map distance (cM)	Map density (cM/marker)	Map density (cM/locus)
1A1	345	70	131.08	0.38	1.87
1A2	108	24	30.79	0.29	1.28
2A1	215	74	142.28	0.66	1.92
2A2	52	11	14.30	0.28	1.30
3A1	221	41	87.52	0.40	2.13
3A2	91	18	60.99	0.67	3.39
4A1	278	57	150.56	0.54	2.64
5A1	78	21	80.58	1.03	3.84
5A2	197	42	59.79	0.30	1.42
5A3	29	14	32.84	1.13	2.35
6A1	173	33	72.94	0.42	2.21
6A2	173	23	16.24	0.09	0.71
7A1	525	113	196.80	0.37	1.74
7A2	64	18	17.14	0.27	0.95
1B1	529	58	68.48	0.13	1.18
1B2	10	5	19.69	1.97	3.94
1B3	43	10	11.10	0.26	1.11
2B1	461	54	40.33	0.09	0.75
2B2	614	106	181.12	0.29	1.71
3B1	770	70	77.38	0.10	1.11
3B2	78	21	31.31	0.40	1.49
3B3	27	9	16.27	0.60	1.81
3B4	103	29	18.45	0.18	0.64
4B1	273	58	111.08	0.41	1.92
5B1	395	88	241.74	0.61	2.75
6B1	794	103	144.16	0.18	1.40

Table 2.4. Distribution of markers and marker density across linkage groups in the bread wheat (*Triticum aestivum* L.) genetic map developed by using the recombinant inbred line (RIL) population of a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Linkage group	No. of markers	No. of unique loci	Map distance (cM)	Map density (cM/marker)	Map density (cM/locus)
6B2	104	22	73.09	0.70	3.32
7B1	555	88	134.67	0.24	1.53
7B2	51	14	11.12	0.22	0.79
1D1	111	24	78.26	0.71	3.26
2D1	131	7	13.48	0.10	1.93
2D2	47	16	14.09	0.30	0.88
2D3	11	10	22.03	2.00	2.20
3D1	33	2	9.62	0.29	4.81
4D1	17	7	6.21	0.37	0.89
5D1	118	12	21.32	0.18	1.78
6D1	40	14	73.50	1.84	5.25
6D2	31	10	10.75	0.35	1.08
7D1	31	14	35.44	1.14	2.53
7D2	22	5	9.89	0.45	1.98
7D3	15	12	76.40	5.09	6.37
A genome	2549	559	1093.86	0.43	1.96
B genome	4807	735	1179.99	0.25	1.61
D genome	607	133	370.97	0.61	2.79
Whole genome	7963	1427	2644.82	0.33	1.85

2.4.3. Quantitative Trait Loci Analysis

A total of 76 A-QTL and 73 DE-QTL were identified for the 16 end-use quality traits evaluated in this study (Table 2.5 and Table 2.6). These A-QTL and DE-QTL were distributed across all wheat chromosomes except chromosomes 3D and 6A for A-QTL, and 3D for DE-QTL. In terms of the genome-wide distribution of QTL, the B-genome had the highest number of A-QTL (36), while the A-genome had the most DE-QTL (46). This was followed by the A-genome with 25 A-QTL, the D-genome with 15 A-QTL, the B-genome with 23 DE-, and the D-genome with four DE-QTL (Table 2.5 and Table 2.6). All of the A-QTL and DE-QTL were identified in at least two environments and/or were associated with at least two different end-use

quality traits (Table 2.5 and Table 2.6). Out of the 76 A-QTL, a total of 43 A-QTL (~57%) explained more than 10% of PV and were considered major A-QTL, while the remaining 32 A-QTL explained less than 10% of PV and were considered minor QTL (Table 2.5). Furthermore, a total of 12 A-QTL and three DE-QTL were identified in at least three environments and were considered stable QTL.

2.4.3.1. Quantitative Trait Loci for Grain Protein Content

A total of 11 A-QTL and 18 DE-QTL were detected for GPC (Table 2.5; Table 2.6; Figure 2.2). The 11 A-QTL were located on chromosomes /linkage groups 1A1, 1B1, 2A1, 2B2, 3A2, 3B1, 4B, 5B, and 7A2. No A-QTL was found on the D-genome for GPC in this study. Five A-QTL individually explained over 10% of PV and were considered major A-QTL. The major A-QTL were located on chromosomes/linkage groups 1A1, 2A1,3B1,4B, and 5B (Table 2.5; Figure 2.2). Three A-QTL were detected in more than three environments and were considered stable A-QTL. Two of these stable A-QTL, *AQ.GPC.ndsu.1A* and *AQ.GPC.ndsu.5B*, explained up to13.69% and 20.18% of PV for GPC, respectively, and were also considered major QTL. For this trait, both parental genotypes contributed positive alleles, although the majority of the alleles (including the three stable A-QTL) were contributed by the cultivar Glenn (Table 2.5; Figure 2.2). The QTL *AQ.GPC.ndsu.7A* showed sequence similarity with wheat HMGB1 mRNA for high mobility globular protein. Christov et al. (2007) suggested the wheat HMGB1 protein may have a specific function as a general regulator of gene expression during cold acclimation in wheat.

The results of digenic epistatic effects for GPC are shown in Table 2.6. The accumulated contribution of these nine epistatic interactions for GPC was ~16.38%. These DE-QTL were located on pairs of linkage groups 1A1/7D3, 1A1/7D3, 2B2/5B1, 3B1/2D2, 4A1/7B1, 4A1/6D2,

5A3/2B2, 5B/6D1, and 6B1/2D2. Unlike A-QTL, DE-QTL for GPC were identified on the D-genome. The majority of these DE-QTL showed negative values for digenic epistatic effects indicating the positive effects of recombinant genotypic combinations on GPC. The *AQ.GPC.ndsu.5B* had the most important main effect on GPC, and the *AQ.BA.ndsu.6D* had a significant main effect on BA; the epistatic interaction between these A-QTL had a positive effect on GPC. The parental genotypic combinations increased GPC through this interaction (Table 2.6).

2.4.3.2. Quantitative Trait Loci for FE and Mixograph-related Parameters

A total of 32 A-QTL and 51 DE-QTL were identified for FE and mixograph-related parameters (Table 5; Table 6; Figure 2). These 32 A-QTL were located across all 21 wheat chromosomes except chromosomes 1D, 2B, 3D, 5A, 6A, and 6D. A total of 19 A-QTL individually explained more than 10% of PV and were considered major A-QTL. Out of these A-QTL, five stable A-QTL were found for these traits: one stable A-QTL for FE (*AQ.FE.ndsu.3B*) and four stable A-QTL for MMLPT (*AQ.MMLPT.ndsu.1B*, *AQ.MMLPT.ndsu.5D*, *AQ.MMLPT.ndsu.3B.2*, and *AQ.MMLPT.ndsu.2D*). For all of these stable A-QTL, except the *AQ.MMLPT.ndsu.1B*, the alleles were contributed through the Traverse cultivar. The *AQ.MMLPT.ndsu.1B* A-QTL was identified in six out of nine environments and explained up to 24.35% of PV for MMPLT. This A-QTL was considered the most stable A-QTL, which had the highest effect on MMLPT (Table 2.5).

The results of DE-QTL for FE and mixograph-related parameters are shown in Table 6. A total of 49 DE-QTL were detected on all wheat chromosomes expect chromosome 3D. The individual epistatic interactions explained ~0.77% to ~8.15% of PV for FE and mixograph parameters. Three stable digenic epistatic interactions were found for these traits: one DE-QTL

(*DEQ.FE.ndsu.5A1/1D1*) for FE and two DE-QTL (*DEQ.MMLPT.ndsu.2A2/4B1* and *DEQ.MMLPT.ndsu.4A1/5A1*) for MMLPT. The *DEQ.FE.ndsu.5A1/1D1* DE-QTL explained up to 3.84% of PV for FE. The parental genotypic combinations of this DE-QTL had a positive effect on the increase of FE. The *DEQ.MMLPT.ndsu.2A2/4B1* and *DEQ.MMLPT.ndsu.4A1/5A1* DE-QTL explained up to 2.19% and 1.66% of PV for MMLPT, respectively. The parental genotypic combinations increased MMPLT through the *DEQ.MMLPT.ndsu.4A1/5A1* stable DE-QTL, whereas recombinant genotypic combinations increased MMPLT through the *DEQ.MMLPT.ndsu.2A2/4B1* stable DE-QTL. Overall, both parental and recombinant genotypic combinations almost equally contributed to the increase of FE and improvement of the mixograph-related parameters (Table 2.6).

2.4.3.3. Quantitative Trait Loci for Baking-related Properties

A total of 31 A-QTL and 15 DE-QTL were detected for baking-related properties in this study (Table 2.5; Table 2.6; Figure 2.2). These 31 A-QTL individually explained ~2.14% to ~28.06% of PV for the associated traits. These A-QTL were located on 17 wheat chromosomes excluding 1A, 2B, 3D, and 6A. A total of 19 major A-QTL with PV values over 10% were found for the baking-related properties. Three stable A-QTL were identified in this study: two A-QTL for BA (*AQ.BA.ndsu.4D.1* and *AQ.BA.ndsu.1B*) and one A-QTL (*AQ.BMT.ndsu.5D*) for BMT. Although the Glenn cultivar contributed over 60% of the desirable alleles for the baking-related properties in this study, the cultivar Traverse contributed the desirable alleles for these three stable A-QTL. The *AQ.BA.ndsu.4D.1* stable A-QTL associated with BA had the highest PV (~28.06%) for end-use quality traits in this study (Table 2.5).

The results of digenic epistatic interactions for the baking-related properties are presented in Table 2.6. Out of the six baking-related properties evaluated in this study, digenic epistatic

effects were only identified for BA, BLV, and BMT traits with one, one, and 13 digenic epistatic interactions, respectively. The DE-QTL, *DEQ.BA.ndsu.1A1/1A1* and *DEQ.BLV.ndsu.6D1/7D3*, explained ~6.94% and ~3.37% of PV for BA and BLV, respectively. The accumulated contribution of the 13 DE-QTL for BMT was ~26.29%. Both parental and recombinant genotypic combinations contributed to the increase of BMT, whereas only the parental genotypic combinations had positive effects on BA and BLV (Table 2.6).

2.4.3.4. Co-Localized or Pleiotropic Quantitative Trait Loci

A total of 19 additive co-localized QTL or pleiotropic loci, and four epistatic co-localized QTL or pleiotropic loci were found in this study (Table 2.5; Table 2.6; Figure 2.2). These 19 additive co-localized QTL or pleiotropic loci were mainly located on the A- and B-genomes (Table 2.5; Figure 2.2). Positive pleiotropy was shown in 14 out of 19 additive co-localized QTL or pleiotropic loci, where the additive effects of a locus on multiple traits were of the same sign. In contrast, negative pleiotropic effects were observed for five co-localized QTL or pleiotropic loci on chromosomes/linkage groups 1A1, 2A1, 2A1, 4A, and 4D harboring major A-QTL, respectively, for GPC and FE; GPC and BMT; GPC and MMLPT; FE, MMLTV, and BA; and MELS, MERS, and BA. Overall, approximately 63% of A-QTL with close linkage or pleiotropic effects on the integrated set of traits (Table 2.5; Figure 2.2) were considered major A-QTL. Additive co-localized QTL or pleiotropic loci for the end-use quality traits are shown in more detail in Table 2.5.

In addition to additive co-localized QTL or pleiotropic loci, four epistatic co-localized QTL or pleiotropic loci ("epistatic pleiotropy," Wolf et al., 2005) were identified in this study (Table 6). These co-localized QTL or epistatic pleiotropies were located on pairs of linkage groups 1A1/7A1, 5A1/7D3, 1A1/7D3, and 1B1/7B1 associated with MIXOPA and MMLTV;

MMLPT, MMLPI, and MMLTV; GPC and MERS; and MMLPV and MMLTV, respectively (Table 6). All co-localized QTL or epistatic pleiotropies except one (1A1/7D3 for the integrated set of GPC and MERS traits) showed positive pleiotropic effects (Table 2.6).

Table 2.5. QTL detected for end-use quality traits in a bread wheat (*Triticum aestivum* L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780).

Trait[a]	A-QTL name[b]	Other associated traits	Env.[c]	Chromosome/linkage group	Left marker	Right marker	Position (cM)[d]	LOD[e]	Additive effect[e]	PV(%)[g]	Confidence interval
FE	AQ.FE.ndsu.1A.1	-	I, X	1A1	BS00084022_51	RAC875_c9700_989	50	8.7788	-0.4935	14.4911	48.5-50.5
FE	AQ.FE.ndsu.1A.1	GPC	VII	1A1	wsnp_Ra_c15564_23999084	wsnp_BG263358A_Ta_2_3	94	7.6547	-1.061	19.4012	92.5-95.5
GPC	AQ.GPC.ndsu.1A	FE	III, V, VIII, VIIII	1A1	wsnp_Ra_c15564_23999084	wsnp_BG263358A_Ta_2_3	95	4.6476	0.2376	13.6941	93.5-96.5
BMT	AQ.BMT.ndsu.1B	MMLPI	IV, VIII, VIIII	1B1	TA015141-0717	wsnp_JD_c4444_5575748	13	4.7945	0.1736	12.9075	12.5-13.5
BMT	AQ.BMT.ndsu.1B.1	-	VI, X	1B1	Kukri_c33561_564	wsnp_Ku_c16938_25916260	14	13.6184	0.1303	12.085	13.5-14.5
BMT	AQ.BMT.ndsu.1B.2	MMLPT	I, V	1B1	RAC875_c75885_302	Tdurum_contig28305_106	20	6.5489	0.1804	12.5043	19.5-20.5
GPC	AQ.GPC.ndsu.1B.1	MIXOPA	VII	1B1	BS00064162_51	Excalibur_rep_c101787_89	57	3.9039	0.2683	8.1766	56.5-58.5
MIXOPA	AQ.MIXOPA.ndsu.1B.1	GPC	IV	1B1	BS00064162_51	Excalibur_rep_c101787_89	57	3.9039	0.2683	7.7358	56.5-58.5
MMLPI	AQ.MMLPI.ndsu.1B.1	BMT	VI, VIII, X	1B1	TA015141-0717	wsnp_JD_c4444_5575748	13	7.5203	10.7587	15.9048	12.5-13.5
MMLPI	AQ.MMLPI.ndsu.1B.2	MMLPT; MMLTV; BMT	IV	1B1	RAC875_c75885_302	Tdurum_contig28305_106	20	14.3296	33.5754	16.6441	19.5-20.5
MMLPT	AQ.MMLPT.ndsu.1B	BMT	I, IV, V, VI, VII, VIII, VIIII, X	1B1	RAC875_c75885_302	Tdurum_contig28305_106	20	15.2002	0.3698	24.4267	19.5-20.5
MMLPW	AQ.MMLPW.ndsu.1B	-	V, X	1B1	wsnp_Ex_c2569_4780450	Tdurum_contig65853_534	62	4.6175	0.3643	11.4578	60.5-65.5
MMLTV	AQ.MMLTV.ndsu.1B	MMLPI; MMLPT; BMT	IV	1B1	RAC875_c75885_302	Tdurum_contig28305_106	20	4.3062	12.1355	1.6784	19.5-20.5
BA	AQ.BA.ndsu.1B	-	I, IV, VIII, III	1B3	BS00093275_51	BobWhite_c12960_138	0	3.6756	-0.4042	8.1774	0-2.5
BMT	AQ.BMT.ndsu.1D	-	VIII, X	1D	RAC875_rep_c105196_532	BS00038418_51	76	25.0366	0.1984	27.7923	74.5-76.5
BMT	AQ.BMT.ndsu.2A.1	GPC	I	2A1	Excalibur_c27279_699	Kukri_c44255_832	37	8.2391	-0.204	12.8403	34.5-38.5
FE	AQ.FE.ndsu.2A.1	MMLPT	V	2A1	BS00022903_51	Ra_c34214_1320	20	7.9438	0.8736	10.3544	19.5-22.5
GPC	AQ.GPC.ndsu.2A	BMT	IV,V	2A1	Kukri_c44255_832	RAC875_c13861_1248	38	6.2687	0.4351	13.19	37.5-39.5
GPC	AQ.GPC.ndsu.2A	MMLPT	III, X	2A1	wsnp_Ex_c28204_37349164	Kukri_c77188_798	18	4.939	0.1596	8.0024	17.5-19.5
MMLPT	AQ.MMLPT.ndsu.2A	GPC	I, III	2A1	wsnp_Ex_c28204_37349164	Kukri_c77188_798	18	5.2543	-0.5361	16.3459	17.5-19.5
MMLPT	AQ.MMLPT.ndsu.2A.1	FE	III	2A1	BS00022903_51	Ra_c34214_1320	20	7.9438	0.8736	10.0223	19.5-22.5
GPC	AQ.GPC.ndsu.2B	-	I, III	2B2	BS00064658_51	RAC875_c1755_971	27	4.6386	-0.1599	8.7567	23.5-27.5

Table 2.5. QTL detected for end-use quality traits in a bread wheat (*Triticum aestivum* L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	A-QTL name[b]	Other associated traits	Env.[c]	Chromosome/linkage group	Left marker	Right marker	Position (cM)[d]	LOD[e]	Additive effect[e]	PV(%)[g]	Confidence interval
BLV	AQ.BLV.ndsu.2D.1	-	II, X, III	2D2	Kukri_c31121_1460	Kukri_c44769_750	7	3.8365	4.4342	9.7413	5.5-8.5
BLV	AQ.BLV.ndsu.2D.2	-	VII, VIII	2D2	BobWhite_c6365_965	D_GDS7LZN02FDZX8_269	4	3.6217	8.5516	12.8348	3.5-4.5
MMLPT	AQ.MMLPT.ndsu.2D	-	II, IV, VII, X	2D3	BS00011109_51	wsnp_Ku_c8712_14751858	20	4.3893	-0.1918	6.5246	13.5-22
BMT	AQ.BMT.ndsu.3A	MMLPT	I, V,VIIII, X	3A2	BobWhite_c38444_238	Kukri_c10751_1031	47	12.0827	0.1218	10.2537	46.5-48.5
GPC	AQ.GPC.ndsu.3A	-	III, V, X	3A2	BS00022058_51	Excalibur_c39808_453	26	5.9339	-0.334	9.3796	21.5-28.5
MMLPT	AQ.MMLPT.ndsu.3A.1	BMT	IV, VIIII, X	3A2	Kukri_c10751_1031	wsnp_Ex_c1533_2930233	49	6.8915	0.2345	9.5047	47.5-51.5
GPC	AQ.GPC.ndsu.3B.1	MMLPV	X	3B1	wsnp_Ex_c47078_52393295	D_GB5Y7FA01EIDVZ_263	25	7.5082	0.206	13.0023	22.5-27.5
MMLPV	AQ.MMLPV.ndsu.3B.1	GPC	VIII	3B1	RFL_Contig1456_842	wsnp_Ex_c47078_52393295	24	5.3548	2.4546	7.5943	22.5-27.5
BMT	AQ.BMT.ndsu.3B.1	-	II, V, X	3B2	Tdurum_contig12455_385	Excalibur_c21708_555	0	4.9225	0.0716	3.5988	0-0.5
BMT	AQ.BMT.ndsu.3B.2	MMLPI; MMLTV	I, VIII	3B2	Excalibur_rep_c102270_677	Kukri_c2227_583	6	7.7153	0.1939	11.5294	5.5-6.5
FE	AQ.FE.ndsu.3B	-	I, V, VII, X	3B2	Tdurum_contig82214_79	wsnp_BE499016B_Ta_2_1	68	8.5226	-0.5046	15.2959	64.5-69.5
MMLPI	AQ.MMLPT.ndsu.3B.2	BMT; MMLTV;	IV	3B2	Excalibur_rep_c102270_677	Kukri_c2227_583	6	4.9406	8.976	9.6693	5.5-6.5
MMLPT	AQ.MMLPT.ndsu.3B.2	-	IV, VI, VIII, X	3B2	Tdurum_contig15928_135	BobWhite_c9424_243	5	3.8931	0.1712	5.1946	4.5-5.5
MMLTV	AQ.MMLTV.ndsu.3B.2	BMT; MMLTV;	V	3B2	Excalibur_rep_c102270_677	Kukri_c2227_583	6	3.4132	2.3331	9.894	5.5-6.5
BA	AQ.BA.ndsu.4A	FE; MMLTV	IV, VI, X	4A	BS00022395_51	BS00021957_51	147	6.6931	0.2547	11.552	144.5-148.5
MMLPV	AQ.MMLPV.ndsu.4A	-	VII, X	4A	TA004912-0408	Kukri_c17417_797	150	5.821	0.8158	13.7424	149.5-150
MMLTV	AQ.MMLTV.ndsu.4A	FE; BA	IV, V, X	4A	Kukri_c35451_857	BS00022395_51	143	3.5732	0.7363	7.8228	141.5-145.5
FE	AQ.FE.ndsu.4A.1	MMLTV;BA	X	4A1	Kukri_c18346_556	Kukri_c35451_857	142	4.5021	-0.3776	6.9089	141.5-144.5
BLV	AQ.BLV.ndsu.4B.2	BMT	VI, X	4B	RAC875_c39339_400	RAC875_c17026_714	97	4.0885	-1.3594	7.4436	94.5-97.5
BMT	AQ.BMT.ndsu.4B.1	BLV	III, X	4B	RAC875_c39339_400	RAC875_c17026_714	97	4.0885	-1.3594	6.7181	94.5-97.5
GPC	AQ.GPC.ndsu.4B	-	I, II	4B	BobWhite_c47144_153	Tdurum_contig10302_187	94	6.6325	-0.2086	15.0008	93.5-94.5
BA	AQ.BA.ndsu.4B.1	MIXOPA	V	4B1	Excalibur_c39876_403	Kukri_c19909_733	70	4.7301	-0.6243	11.2395	69.5-73.5
MIXOPA	AQ.MIXOPA.ndsu.1B.1	BA	II	4B1	Excalibur_c39876_403	Kukri_c19909_733	70	5.0876	-0.2838	12.3347	69.5-70.5
BA	AQ.BA.ndsu.4D.1	MELS; MERS	I, III, V, VIIII, X	4D	wsnp_JD_rep_c51623_35119179	Ra_c350_837	1	14.2653	-0.3725	28.0617	0-1.5

Table 2.5. QTL detected for end-use quality traits in a bread wheat (Triticum aestivum L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	A-QTL name[b]	Other associated traits	Env.[c]	Chromosome/linkage group	Left marker	Right marker	Position (cM)[d]	LOD[e]	Additive effect[e]	PV(%)[g]	Confidence interval
MELS	AQ.MELS.ndsu.4D.2	BA; MERS	III, X	4D	wsnp_JD_rep_c51623_35119179	Ra_c350_837	1	6.6917	-3.0005	18.0403	0-1.5
MERS	AQ.MERS.ndsu.4D.1	BA; MELS	IV, X	4D	wsnp_JD_rep_c51623_35119179	Ra_c350_837	1	3.6362	0.4349	13.0994	0-2.5
BLV	AQ.BLV.ndsu.5A	-	IV,VI	5A1	Kukri_c28555_114	wsnp_Ku_c18023_27232712	36	6.9598	-5.0049	15.8001	30.5-42.5
BLV	AQ.BLV.ndsu.5B	GPC	X	5B	BS00064297_51	wsnp_BE499835B_Ta_2_5	25	5.5542	18.5451	2.1438	11.5-35.5
FE	AQ.FE.ndsu.5B	-	V, X	5B	Kukri_c3070_72	BS00021993_51	240	3.4037	0.2971	5.1324	238.5-241
GPC	AQ.GPC.ndsu.5B	BLV	I, II, IV, V, VII, VIIII, X	5B	BS00032003_51	wsnp_BE499835B_Ta_2_5	14	10.3662	0.3196	20.1838	9.5-20.5
MIXOPA	AQ.MIXOPA.ndsu.5B	-	II, III	5B	wsnp_Ex_c2582_4804223	Tdurum_contig10268_1000	153	3.5364	0.3448	12.2996	152.5-153.5
MMLPT	AQ.MMLPT.ndsu.5B	-	I, VII	5B	RAC875_c33933_350	JD_c9261_426	49	3.7684	-0.2447	7.2642	48.5-63.5
BMT	AQ.BMT.ndsu.5D	MMLPT	IV, V, X	5D1	BS00110953_51	Excalibur_c16573_197	18	4.5987	-0.0698	3.4365	9.5-19.5
MMLPT	AQ.MMLPT.ndsu.5D	BMT	IV, VI, VIII, VIIII, X	5D1	BS00110953_51	Excalibur_c16573_197	19	7.4008	-0.1963	15.3925	12.5-19.5
BLV	AQ.BLV.ndsu.6B	CTCL	II, III	6B1	BobWhite_c10140_297	BobWhite_c8571_699	52	6.1493	5.56	15.4305	51.5-52.5
CBCL	AQ.CBCL.ndsu.6B	-	II, X	6B1	CAP8_c1678_709	Kukri_c23433_416	46	4.4927	0.0378	3.1303	44.5-46.5
CTCL	AQ.CTCL.ndsu.6B.1	BLV	III	6B1	BobWhite_c10140_297	BobWhite_c8571_699	52	5.5319	0.2905	16.3676	51.5-52.5
FE	AQ.FE.ndsu.6B	-	II, IV, X	6B1	BobWhite_c30500_527	Excalibur_c31379_71	95	5.4465	-0.3753	8.367	94.5-95.5
BA	AQ.BA.ndsu.6D	-	II, VIII	6D1	wsnp_Ex_c23383_32628864	BobWhite_c13435_700	43	4.6326	-1.3204	3.7619	41.5-44.5
BLV	AQ.BLV.ndsu.7A.1	-	IV, X	7A1	Excalibur_rep_c109881_701	Tdurum_contig16202_319	59	4.5713	1.439	8.3454	58.5-59.5
BLV	AQ.BLV.ndsu.7A.2	-	IV, X	7A1	RAC875_c9012_276	BobWhite_c15497_199	118	6.5815	1.7646	12.6133	116.5-118.5
BMT	AQ.BMT.ndsu.7A	-	IV, X	7A1	Excalibur_c44794_122	RAC875_c55351_223	5	5.5287	0.0764	4.1206	1.5-6.5
CTCL	AQ.CTCL.ndsu.7A	MMLPV	III, X	7A1	Excalibur_c33589_373	RAC875_rep_c111778_387	86	5.6857	0.016	15.7116	85.5-86.5
GPC	AQ.GPC.ndsu.7A.1	MMLPT	II	7A1	BobWhite_c23261_226	BS00022970_51	24	4.2443	0.1848	6.5514	22.5-24.5
MMLPT	AQ.MMLPT.ndsu.7A.1	GPC	VIII	7A1	BobWhite_c23261_226	BS00022970_51	24	3.6069	-0.2228	5.9423	23.5-24.5
MMLPV	AQ.MMLPV.ndsu.7A.1	CTCL	IV	7A1	Excalibur_c33589_373	RAC875_rep_c111778_387	86	4.1338	1.8898	11.2755	84.5-86.5
GPC	AQ.GPC.ndsu.7A	-	IV, VII,VIII, X	7A2	BobWhite_c55693_396	BS00023003_51	16	4.6188	0.1507	7.1353	15.5-17
BLV	AQ.BLV.ndsu.7B	-	V, X	7B1	BobWhite_c41356_62	wsnp_CAP7_c44_26549	33	3.7635	3.4251	10.7091	31.5-39.5

Table 2.5. QTL detected for end-use quality traits in a bread wheat (Triticum aestivum L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	A-QTL name[b]	Other associated traits	Env.[c]	Chromosome/linkage group	Left marker	Right marker	Position (cM)[d]	LOD[e]	Additive effect[e]	PV(%)[g]	Confidence interval
MMLPT	AQ.MMLPT.ndsu.7B	-	I, III	7B1	BobWhite_c44404_312	CAP12_c1816_325	42	4.3413	-0.3644	3.6894	41.5-50.5
BMT	AQ.BMT.ndsu.7D	-	I,V	7D1	Kukri_c23468_590	Kukri_c16416_647	12	3.4443	0.1253	4.8285	7.5-13.5
FE	AQ.FE.ndsu.7D	-	IV, VI	7D2	RAC875_c39217_314	Excalibur_c16580_388	1	3.518	0.7611	11.1963	0-3.5
DO	AQ.DO.ndsu.7D	-	VI, X	7D3	wsnp_BE490643D_Ta_2_1	BobWhite_rep_c65034_450	71	4.1343	-0.0572	13.6687	70.5-72.5
MMLPT	AQ.MMLPT.ndsu.7D	-	I, III	7D3	IAAV6265	BobWhite_c7263_337	27	3.544	0.315	3.0233	25.5-32.5

Table 2.6. Digenic epistatic QTL (DE-QTL) detected for end-use quality traits in a bread wheat (*Triticum aestivum* L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780).

Trait[a]	DE-QTL Name[b]	Env.	Other associated traits	Chrom. 1 name	Position 1	Left Marker1	Right Marker1	Chrom. 2 name	Position 2	Left Marker2	Right Marker2	Associated A-QTL	LOD	PV(%)	Additive by Additive Effects
BA	DEQ.BA.ndsu.1A1/1A1	II, VI, X	-	1A1	5	Kukri_c13513_759	RAC875_c50463_808	1A1	30	RFL_Contig1703_695	Excalibur_rep_c92985_618	-	3.86	6.94	1.28
BMT	DEQ.BMT.ndsu.1A1/1A1	VI, X	-	1A1	0	Kukri_c13513_759	RAC875_c50463_808	1A1	120	BobWhite_c27541_67	IAAV2729	-	3.64	2.10	0.06
BMT	DEQ.BMT.ndsu.1A1/4D1	V, X	-	1A1	120	BobWhite_c27541_67	IAAV2729	4D1	0	wsnp_JD_rep_c51623_35119179	Ra_c350_837	AQ.BA.ndsu.4D.1	3.58	1.90	-0.12
MMLPT	DEQ.MMLPT.ndsu.1A1/4D1	I, VIIII, X	-	1A1	5	Kukri_c13513_759	RAC875_c50463_808	4D1	0	wsnp_JD_rep_c51623_35119179	Ra_c350_837	AQ.BA.ndsu.4D.1	4.54	2.32	-0.22
MMLPW	DEQ.MMLPW.ndsu.1A1/5A1	II, X	-	1A1	35	RFL_Contig1703_695	Excalibur_rep_c92985_618	5A1	60	IAAV3916	RAC875_c54693_298	-	5.08	2.56	-1.20
MIXOPA	DEQ.MIXOPA.ndsu.1A1/7A1	VIII, X	MMLTV	1A1	125	BobWhite_c27541_67	IAAV2729	7A1	170	wsnp_Ex_c6354_11053460	BS00053365_51	-	4.87	1.27	0.15
MMLTV	DEQ.MMLTV.ndsu.1A1/7A1	VIII, VIIII	MIXOPA	1A1	130	BobWhite_c27541_67	IAAV2729	7A1	180	Excalibur_c48973_1688	IACX6080	-	3.60	2.23	2.13
MMLPW	DEQ.MMLPW.ndsu.1A1/7B1	I , X	-	1A1	0	Kukri_c13513_759	RAC875_c50463_808	7B1	0	Tdurum_contig57324_104	Excalibur_c21252_227	-	3.51	1.35	0.81
GPC	DEQ.GPC.ndsu.1A1/7D3	II, V	MERS	1A1	15	Excalibur_c5139_198	wsnp_Ex_c1358_2601510	7D3	20	Kukri_c37793_135	Kukri_c9804_462	-	4.73	1.30	-0.30
GPC	DEQ.GPC.ndsu.1A1/7D3	I, X	-	1A1	30	RFL_Contig1703_695	Excalibur_rep_c92985_618	7D3	25	IAAV6265	BobWhite_c7263_337	-	3.51	1.90	-0.13
MERS	DEQ.MERS.ndsu.1A1/7D3	V, X	GPC	1A1	15	Excalibur_c5139_198	wsnp_Ex_c1358_2601510	7D3	20	Kukri_c37793_135	Kukri_c9804_462	-	5.74	3.16	1.30
MMLPV	DEQ.MMLPV.ndsu.1B1/7B1	VII, VIII	MMLTV	1B1	0	RAC875_c4385_1628	wsnp_Ra_c23758_33291657	7B1	50	CAP12_c1816_325	BobWhite_c14812_828	-	3.88	8.15	2.56
MMLTV	DEQ.MMLTV.ndsu.1B1/7B1	VII, VIII	MMLPV	1B1	5	RAC875_c4385_1628	wsnp_Ra_c23758_33291657	7B1	45	CAP12_c1816_325	BobWhite_c14812_828	-	4.80	3.46	3.20

Table 2.6. Digenic epistatic QTL (DE-QTL) detected for end-use quality traits in a bread wheat (Triticum aestivum L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	DE-QTL Name[b]	Env.	Other associated traits	Chrom. 1 name	Position 1	Left Marker1	Right Marker1	Chrom. 2 name	Position 2	Left Marker2	Right Marker2	Associated A-QTL	LOD	PV(%)	Additive by Additive Effects
MMLPT	DEQ.MMLPT.ndsu.1D1/5D1	V, X	-	1D1	20	RAC875_c16352_594	CAP8_c2401_433	5D1	0	wsnp_Ku_c44483_51751682	wsnp_JD_c825_1223454	-	3.96	1.90	0.33
MMLPI	DEQ.MMLPI.ndsu.2A1/2B1	IV, VIIII	-	2A1	5	Excalibur_c51876_189	wsnp_Ku_c10302_17079851	2B1	30	TA002233-0872	Ku_c36209_204	-	4.06	0.92	7.74
MMLPT	DEQ.MMLPT.ndsu.2A1/2B2	I, II, X	-	2A1	10	wsnp_JD_rep_c48914_331685 44	wsnp_Ex_rep_c102538_876822 73	2B2	20	GENE-0592_352	BS00064658_51	-	5.59	1.87	-0.61
FE	DEQ.FE.ndsu.2A1/3A2	II, X	-	2A1	105	BobWhite_rep_c50285_616	Tdurum_contig67827_98	3A2	0	Ex_c35861_1382	Tdurum_contig42150_3190	-	3.35	1.72	-0.27
MIXOPA	DEQ.MIXOPA.ndsu.2A1/3A2	VIIII, X	-	2A1	45	Excalibur_c65910_246	RAC875_c81899_216	3A2	45	BobWhite_c38444_238	RAC875_c15109_106	AQ.BMT.ndsu.3A	3.75	1.20	-0.41
MIXOPA	DEQ.MIXOPA.ndsu.2A1/5B	VIII, X	-	2A1	115	IAAV880	CAP12_c575_105	5B	225	GENE-2471_259	Kukri_c9285_762	-	4.20	2.59	-0.31
MMLPT	DEQ.MMLPT.ndsu.2A1/6D1	I, X	-	2A1	0	wsnp_Ex_c5412_9565527	Ra_c10616_265	6D1	35	wsnp_Ex_c23383_32628864	BobWhite_c13435_700	AQ.BA.ndsu.6D	4.13	1.91	-0.63
MERS	DEQ.MERS.ndsu.2A1/7A1	IV, X	-	2A1	125	CAP8_c3129_381	Tdurum_contig92425_3144	7A1	185	Excalibur_c1142_724	Tdurum_contig54832_139	-	4.04	2.71	0.42
MMLPT	DEQ.MMLPT.ndsu.2A2/4B1	II, III, VII, VIIII	-	2A2	0	Excalibur_c29231_932	RAC875_c8069_1709	4B1	55	wsnp_Ex_c26285_35532440	RAC875_rep_c119568_203	-	5.00	2.19	-0.21
MELS	DEQ.MELS.ndsu.2B1/2B2	I, II	-	2B1	10	BobWhite_c19554_544	Kukri_c9785_1557	2B2	95	BobWhite_c23046_293	wsnp_Ex_c3695_6740339	-	5.49	1.87	-6.74
BMT	DEQ.BMT.ndsu.2B2/1D1	VI, VIII	-	2B2	15	BobWhite_rep_c64429_660	Kukri_c53810_315	1D1	60	CAP8_c1305_148	BS00022168_51	-	3.37	0.89	-0.13
MMLPT	DEQ.MMLPT.ndsu.2B2/1D1	II, VI	-	2B2	100	BobWhite_c23046_293	wsnp_Ex_c3695_6740339	1D1	45	CAP8_c1305_148	BS00022168_51	-	4.37	1.99	-0.74
FE	DEQ.FE.ndsu.2B2/2D2	I, X	-	2B2	170	Excalibur_c15671_87	Excalibur_c29221_311	2D2	5	Kukri_c9478_2764	Kukri_c65380_490	-	3.11	1.75	0.27
BMT	DEQ.BMT.ndsu.2B2/5B	IV, VI	-	2B2	100	BobWhite_c23046_293	wsnp_Ex_c3695_6740339	5B	30	BS00064297_51	wsnp_BE499835B_Ta_2_5	AQ.GPC.ndsu.5B	8.45	2.50	-0.20
GPC	DEQ.GPC.ndsu.2B2/5B	II, X	-	2B2	0	BS00070900_51	GENE-1343_315	5B	125	Kukri_c34173_169	wsnp_Ku_c3201_5970486	-	5.09	1.51	-0.28
BMT	DEQ.BMT.ndsu.2B2/6B1	V, X	-	2B2	25	GENE-0592_352	BS00064658_51	6B1	135	wsnp_Ex_c9038_15058444	Tdurum_contig43335_1397	-	4.27	3.25	-0.16
FE	DEQ.FE.ndsu.2B2/7D1	II, X	-	2B2	65	Excalibur_c45094_602	BS00040959_51	7D1	15	wsnp_Ex_c17914_26681837	RAC875_c11933_885	-	4.13	2.45	-0.31
MMLPT	DEQ.MMLPT.ndsu.2B2/7D3	V, X	-	2B2	50	RFL_Contig996_818	Tdurum_contig30989_79	7D3	15	Kukri_c37793_135	Kukri_c9804_462	-	3.44	1.82	0.17
MMLPT	DEQ.MMLPT.ndsu.3A1/2D1	IV, VIIII, X	-	3A1	0	Tdurum_contig74920_757	CAP8_rep_c3652_80	2D1	10	RAC875_c110838_423	Kukri_c12032_508	-	4.35	1.69	-0.17
MMLPT	DEQ.MMLPT.ndsu.3A1/6A1	I, II	-	3A1	65	BS00077819_51	Kukri_c51666_401	6A1	55	BobWhite_c1131_328	Excalibur_c29639_65	-	3.52	2.33	0.33
MMLPT	DEQ.MMLPT.ndsu.3A1/7A1	I, IV	-	3A1	50	TA002540-0938	RAC875_c52195_324	7A1	45	BS00065020_51	tplb0024a09_2106	-	4.03	1.31	0.51
GPC	DEQ.GPC.ndsu.3B1/2D2	VII, X	-	3B1	45	wsnp_Ex_c26128_35374652	Excalibur_c45968_83	2D2	10	Excalibur_rep_c104620_183	wsnp_BE426620D_Ta_2_2	-	5.42	2.23	0.15
BMT	DEQ.BMT.ndsu.3B2/4B1	V, X	-	3B2	30	CAP12_c1468_114	JD_c37202_67	4B1	45	wsnp_CAP12_c1101_569783	BS00042105_51	-	5.54	2.13	0.07
FE	DEQ.FE.ndsu.3B3/4B1	II, X	-	3B3	5	BS00087695_51	BS00003884_51	4B1	100	wsnp_Ra_c10988_17932922	RAC875_rep_c82932_428	-	3.41	1.92	0.29
BMT	DEQ.BMT.ndsu.3B4/5B	II, VIII	-	3B4	5	BS00022154_51	wsnp_Ex_rep_c66766_65123941	5B	180	Excalibur_c12395_467	wsnp_Ex_c32488_41134388	-	3.25	1.44	0.15

Table 2.6. Digenic epistatic QTL (DE-QTL) detected for end-use quality traits in a bread wheat (Triticum aestivum L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	DE-QTL Name[b]	Env.	Other associated traits	Chrom. 1 name	Position 1	Left Marker1	Right Marker1	Chrom. 2 name	Position 2	Left Marker2	Right Marker2	Associated A-QTL	LOD	PV(%)	Additive by Additive Effects
MIXOPA	DEQ.MIXOPA.ndsu.4A1/1B1	I, X	-	4A1	10	BS00035307_51	RAC875_c16277_737	1B1	60	RAC875_c61512_173	wsnp_Ex_c9091_15135511	-	3.56	1.21	-0.15
MERS	DEQ.MERS.ndsu.4A1/1D1	IV, VI, X	-	4A1	95	wsnp_Ku_c4924_8816643	Tdurum_contig42526_994	1D1	10	Excalibur_c35316_137	RAC875_c16352_594	-	5.03	5.59	1.69
MMLPI	DEQ.MMLPI.ndsu.4A1/2D2	IV, VI	-	4A1	55	RFL_Contig5998_745	RAC875_c65221_438	2D2	5	Kukri_c9478_2764	Kukri_c65380_490	-	4.78	1.44	11.39
MMLPT	DEQ.MMLPT.ndsu.4A1/5A1	I, III, IV, V	-	4A1	90	Tdurum_contig47148_651	RAC875_c25124_182	5A1	30	Kukri_c28555_114	wsnp_Ku_c18023_27232712	AQ.BLV.ndsu.5A	4.19	1.66	0.55
GPC	DEQ.GPC.ndsu.4A1/6D2	III,VIIII	-	4A1	85	Ex_c66324_1151	wsnp_Ex_c5470_9657856	6D2	0	BS00022523_51	Kukri_rep_c105352_281	-	3.29	1.04	-0.19
BMT	DEQ.BMT.ndsu.4A1/7B1	I, VI	-	4A1	35	wsnp_Ex_c22913_32130617	CAP12_c2677_138	7B1	40	BobWhite_c41356_62	wsnp_CAP7_c44_26549	-	4.63	1.03	-0.20
GPC	DEQ.GPC.ndsu.4A1/7B1	VII, VIIII	-	4A1	5	BS00035307_51	RAC875_c16277_737	7B1	80	BobWhite_c6580_361	wsnp_Ex_c10550_17231294	-	3.60	3.49	0.30
MMLPW	DEQ.MMLPW.ndsu.4A1/7B1	VIIII, X	-	4A1	80	Kukri_c27874_515	Ex_c66324_1151	7B1	5	Excalibur_c21252_227	Excalibur_c8486_471	-	3.97	1.63	0.30
MMLPT	DEQ.MMLPT.ndsu.4B1/2D1	IV, VII	-	4B1	70	Excalibur_c39876_403	Kukri_c19909_733	2D1	10	RAC875_c110838_423	Kukri_c12032_508	-	4.03	1.00	0.18
BMT	DEQ.BMT.ndsu.4B1/5B	V, VII, X	-	4B1	90	wsnp_Ex_c15490_23776560	IAAV8499	5B	0	BS00032003_51	BS00064297_51	AQ.GPC.ndsu.5B	5.65	2.58	0.20
MMLTV	DEQ.MMLTV.ndsu.4B1/5D1	VII, X	-	4B1	60	RAC875_rep_c119568_203	Tdurum_contig59914_323	5D1	20	wsnp_Ex_c5185_9189184	D_GDS7LZN02F4FP5_176	-	3.70	1.96	2.38
FE	DEQ.FE.ndsu.5A1/1D1	II, IV, VI, VII	-	5A1	35	Kukri_c28555_114	wsnp_Ku_c18023_27232712	1D1	25	RAC875_c16352_594	CAP8_c2401_433	AQ.BLV.ndsu.5A	4.65	3.84	1.07
MMLPI	DEQ.MMLPI.ndsu.5A1/5A2	IV, VI	-	5A1	35	Kukri_c28555_114	wsnp_Ku_c18023_27232712	5A2	10	BS00022683_51	BobWhite_c17440_130	AQ.BLV.ndsu.5A	4.61	1.85	-13.09
MMLPI	DEQ.MMLPI.ndsu.5A1/7B1	IV, VI, X	-	5A1	20	wsnp_Ex_c31672_40435001	Kukri_c28555_114	7B1	65	Kukri_c18749_968	Tdurum_contig12064_92	-	3.58	1.42	11.23
MMLPI	DEQ.MMLPI.ndsu.5A1/7D3	IV, VIIII	MMLPT, MMLTV	5A1	75	BS00020605_51	BobWhite_c11539_336	7D3	50	Tdurum_contig46368_632	RAC875_c68368_99	-	4.72	1.52	-9.66
MMLPT	DEQ.MMLPT.ndsu.5A1/7D3	I, IV	MMLTV, MMLPI	5A1	70	BS00020605_51	BobWhite_c11539_336	7D3	45	Tdurum_contig46368_632	RAC875_c68368_99	-	4.69	1.63	-0.23
MMLTV	DEQ.MMLTV.ndsu.5A1/7D3	IV, X	MMLPT, MMLPI	5A1	70	BS00020605_51	BobWhite_c11539_336	7D3	55	Tdurum_contig46368_632	RAC875_c68368_99	-	3.17	2.98	-0.64
MMLPI	DEQ.MMLPI.ndsu.5A2/7A1	VI, X	-	5A2	25	Kukri_c41797_393	Ex_c19057_965	7A1	80	wsnp_Ex_c5939_10417052	wsnp_Ex_c39221_46569987	-	3.88	4.13	-4.30
GPC	DEQ.GPC.ndsu.5A3/2B2	I, X	-	5A3	5	BS00099534_51	Excalibur_c6714_246	2B2	5	IAAV5802	GENE-1676_1048	-	3.91	1.89	-0.16
MMLPT	DEQ.MMLPT.ndsu.5A3/3B4	III, VII,X	-	5A3	5	BS00099534_51	Excalibur_c6714_246	3B4	5	BS00022154_51	wsnp_Ex_rep_c66766_65123941	-	3.62	1.64	-0.15
BMT	DEQ.BMT.ndsu.5B/2D1	V, VII, X	-	5B	105	CAP12_c1419_574	RAC875_c14780_54	2D1	0	RAC875_c110838_423	Kukri_c12032_508	-	3.79	2.90	-0.07
GPC	DEQ.GPC.ndsu.5B/6D1	VI, VIII	-	5B	30	BS00064297_51	wsnp_BE499835B_Ta_2_5	6D1	45	wsnp_Ex_c23383_32628864	BobWhite_c13435_700	AQ.GPC.ndsu.5Bx AQ.BA.ndsu.6D	5.73	0.79	0.98
MELS	DEQ.MELS.ndsu.5B1/6B1	I, X	-	5B1	170	BobWhite_rep_c50349_139	Kukri_c10508_755	6B1	100	BS00037933_51	BS00063217_51	-	3.86	1.51	-0.74
BMT	DEQ.BMT.ndsu.5D1/6D1	IV, X	-	5D1	15	BS00110953_51	Excalibur_c16573_197	6D1	35	wsnp_Ex_c23383_32628864	BobWhite_c13435_700	AQ.BMT.ndsu.5Dx AQ.BA.ndsu.6D	3.98	1.64	0.17

Table 2.6. Digenic epistatic QTL (DE-QTL) detected for end-use quality traits in a bread wheat (Triticum aestivum L.) RIL population derived from a cross between Glenn (PI-639273) and Traverse (PI-642780) (continued).

Trait[a]	DE-QTL Name[b]	Env.	Other associated traits	Chrom. 1 name	Position 1	Left Marker1	Right Marker1	Chrom. 2 name	Position 2	Left Marker2	Right Marker2	Associated A-QTL	LOD	PV(%)	Additive by Additive Effects
MMLPT	DEQ.MMLPT.ndsu.6A1/4B1	IV, VI	-	6A1	5	RAC875_c32053_291	BobWhite_c44549_83	4B1	110	wsnp_Ku_c7838_13435765	Excalibur_c26571_370	-	4.43	0.77	0.40
MMLPT	DEQ.MMLPT.ndsu.6A2/5B	I, X	-	6A2	10	BS00110512_51	BS00065028_51	5B	40	BS00064297_51	wsnp_BE499835B_Ta_2_5	AQ.GPC.ndsu.5B	4.88	2.05	-0.59
GPC	DEQ.GPC.ndsu.6B1/2D2	II, VIII	-	6B1	100	BS00037933_51	BS00063217_51	2D2	0	wsnp_RFL_Contig2659_2346243	RAC875_c78404_242	-	4.89	2.22	-0.18
BLV	DEQ.BLV.ndsu.6D1/7D3	II, X	-	6D1	5	BobWhite_c14066_403	Ra_c32572_334	7D3	20	Kukri_c37793_135	Kukri_c9804_462	-	4.09	3.37	1.43
MIXOPA	DEQ.MIXOPA.ndsu.7A1/7B1	VIII, X	-	7A1	50	tplb0024a09_2106	Tdurum_contig98029_517	7B1	5	Excalibur_c21252_227	Excalibur_c8486_471	-	3.84	1.44	0.41
MMLPT	DEQ.MMLPT.ndsu.7A1/7D1	I, VII	-	7A1	65	wsnp_Ex_c13337_21022241	RAC875_c28842_99	7D1	20	BS00066128_51	BS00083421_51	-	4.04	2.20	-0.32
BMT	DEQ.BMT.ndsu.7A1/7D3	V, X	-	7A1	25	BS00106739_51	Excalibur_rep_c68458_1536	7D3	70	wsnp_BE490643D_Ta_2_1	BobWhite_rep_c65034_450	-	5.08	2.28	0.07
MMLPT	DEQ.MMLPT.ndsu.7A1/7D3	I, X	-	7A1	55	BS00011330_51	Tdurum_contig67992_238	7D3	75	BobWhite_rep_c65034_450	wsnp_CAP8_rep_c9647_4198594	-	4.32	1.81	-0.17
MIXOPA	DEQ.MIXOPA.ndsu.7A2/7B1	VIII, X	-	7A2	10	Kukri_c40353_179	Excalibur_c59653_238	7B1	5	Excalibur_c21252_227	Excalibur_c8486_471	-	6.97	1.22	0.17
BMT	DEQ.BMT.ndsu.7B1/7D2	IV, VII	-	7B1	110	wsnp_Ra_c39394_47110214	BobWhite_c26534_532	7D2	5	Excalibur_c16580_388	Kukri_c19321_416	-	3.62	1.65	0.14
MMLPI	DEQ.MMLPI.ndsu.7D1/7D3	IV, VIII	-	7D1	0	BS00051338_51	IAAV5917	7D3	40	BobWhite_c7263_337	Tdurum_contig46368_632	-	4.74	1.96	-13.80

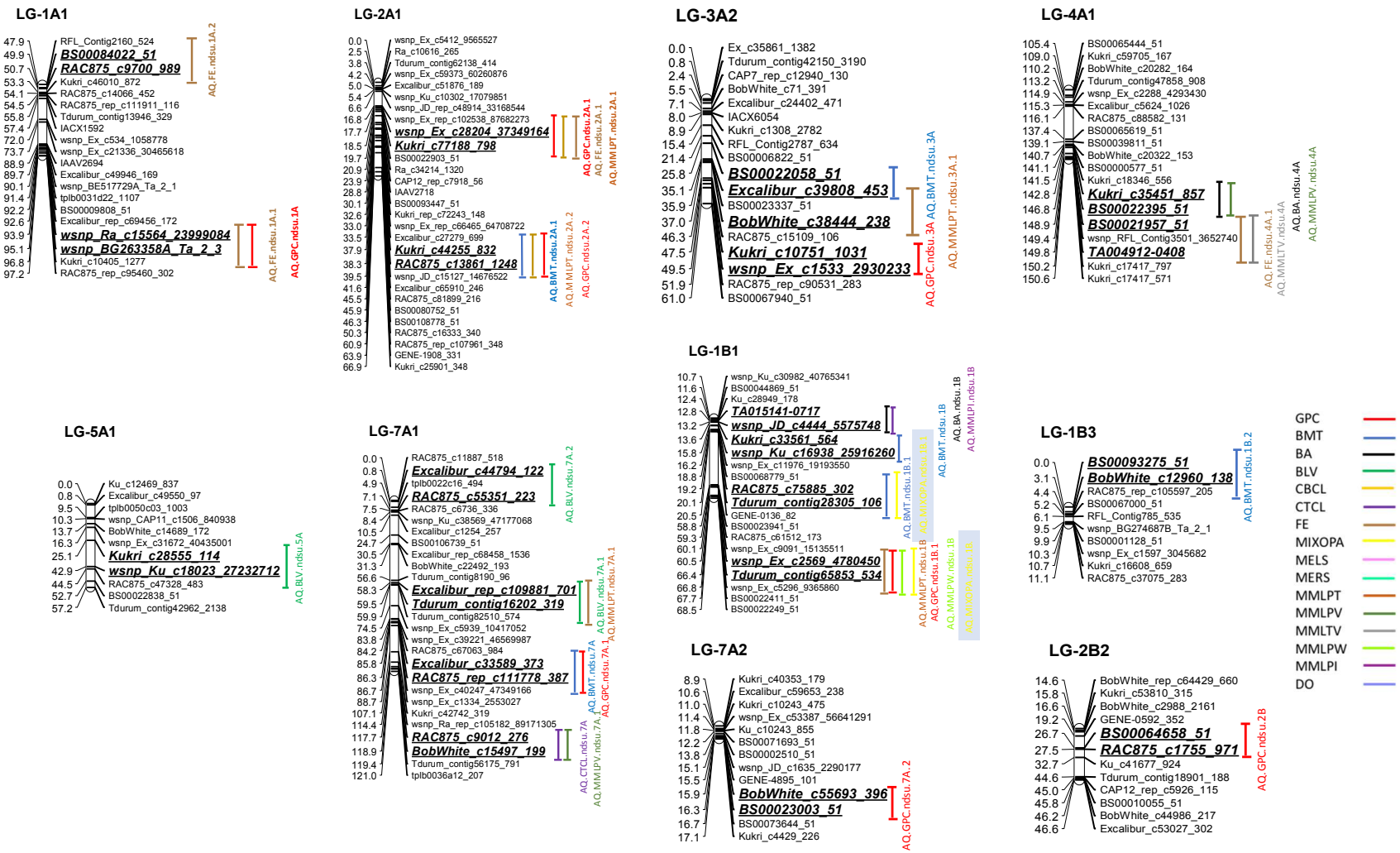

Figure 2.2. Additive and additive co-localized QTL for end-use quality traits in the Glenn × Traverse RIL population. QTL confidence intervals are indicated by vertical bars and bold and italic scripts.

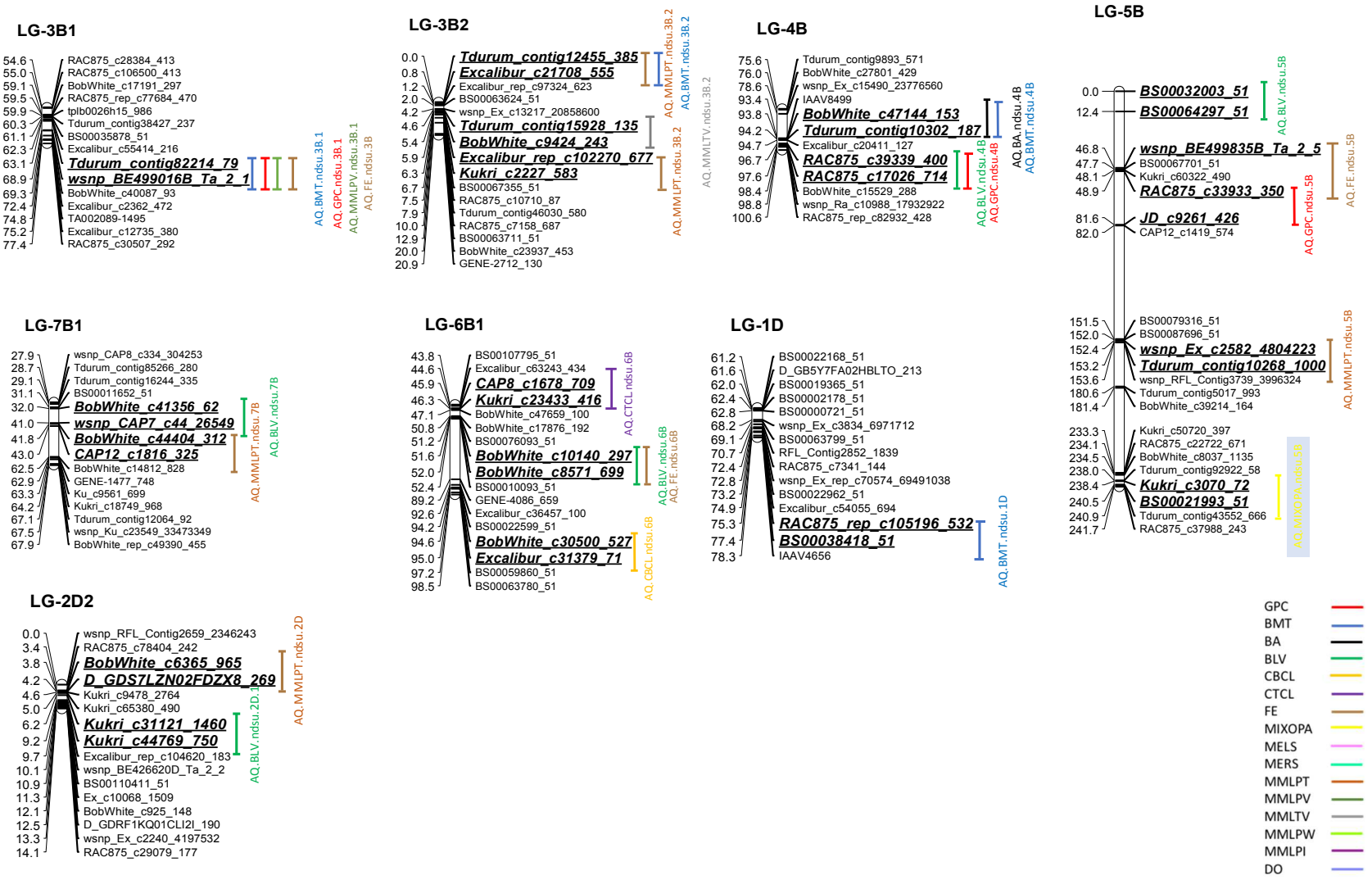

Figure 2.2. Additive and additive co-localized QTL for end-use quality traits in the Glenn × Traverse RIL population. QTL confidence intervals are indicated by vertical bars and bold and italic scripts (continued).

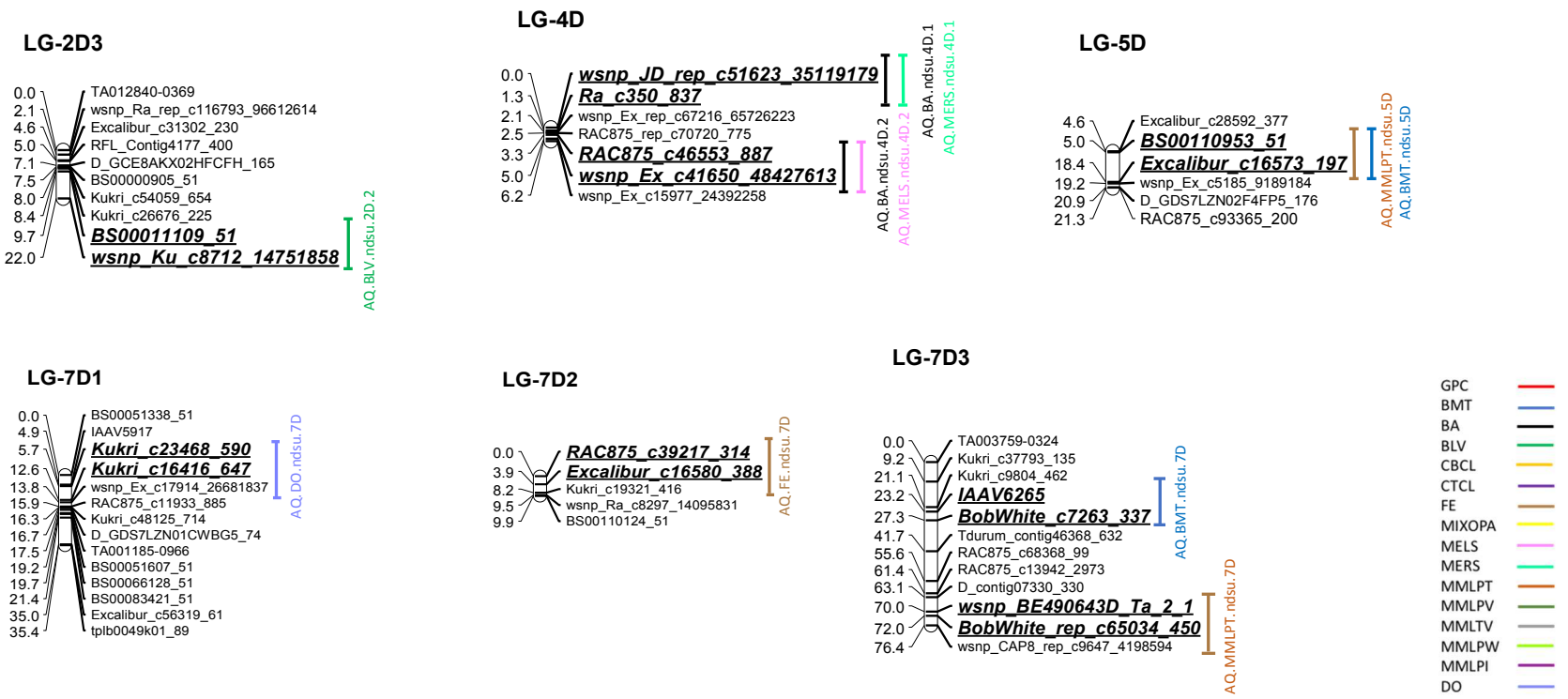

Figure 2.2. Additive and additive co-localized QTL for end-use quality traits in the Glenn × Traverse RIL population. QTL confidence intervals are indicated by vertical bars and bold and italic scripts (continued).

2.5. Discussion

2.5.1. Phenotypic Evaluation

It is well-known that end-use quality traits in bread wheat are complex and are influenced by a combination of environmental conditions and genetic factors (Rousset et al., 1992; Peterson et al., 1998; Sun et al., 2009; Tsilo et al., 2011; Simons et al., 2012). The power and accuracy of QTL detection are highly dependent on choosing the parental lines (Jansen, 2001). In other words, both power and accuracy depend on allelic polymorphism and phenotypic variation between parental lines (Mason et al., 2013). In the current study, the RIL population was developed from a cross between Glenn (PI 639273) and Traverse (PI 642780). Glenn has excellent end-use quality characteristics. By comparison, Traverse has a high grain yield but poor end-use quality characteristics. As expected, our results showed significantly different values between the parental lines for most of the end-use quality traits. The RIL population showed continuous variation and transgressive segregation for all the end-use quality characteristics, suggesting the polygenetic inheritance and contribution of positive alleles for the end-use quality traits by both parental lines.

Our results showed a wide range of broad-sense heritability (0.23 – 0.77) for mixograph-related parameters, suggesting environmental effects had a wide range of influences on the phenotypic values of the mixograph-related parameters. These results corresponded with those of Patil et al. (2009), who also reported a wide heritability range of 0.17 to 0.96 for mixograph-relative parameters. In contrast to our results, Tsilo et al. (2011) and Prashant et al. (2015) found high broad-sense heritability for most of the end-use quality traits in bread wheat. Also similar to the current study, Echeverry-Solarte et al. (2015) reported very high broad-sense heritability for FE and MMLPT.

The genetic and Pearson correlation analyses revealed most of the end-use quality traits were associated with each other. Previous studies have also reported these traits were correlated with each other (Patil et al., 2009; Tsilo et al., 2011; Prashant et al., 2015; Echeverry-Solarte et al., 2015). Our results showed differences between genetic and phenotypic correlation coefficients for end-use quality traits. These differences could be due to low heritability values for these traits (Hill and Thompson 1978). Notably, although there were differences between the genetic and phenotypic correlation coefficients, the pattern and magnitude of these coefficients were similar. These similarities suggest the phenotypic correlation could be a fair estimate of the genetic correlation for end-use quality traits in bread wheat.

2.5.2. Genetics of Grain Protein Content

Improving GPC is one of the principal objectives of every wheat breeding program in the world. Similar to our study, previous studies have reported a few major and several minor QTL for GPC, suggesting the polygenetic nature and quantitative inheritance of this trait (Jonhson et al., 1978; Noel and Robert, 1992; Snape et al., 1955; McCartney et al., 2005; Grag et al., 2006; Bogard et al., 2013; Echeverry-Solarte et al., 2015; Li et al., 2016). The most significant A-QTL in this study, *AQ.GPC.ndsu.5B*, identified on chromosome 5B, was also involved in a digenic epistatic interaction. Previous studies have reported an A-QTL associated with GPC on the long arm of chromosome 5B (Kulwal et al., 2005; Conti et al., 2011; Bordes et al., 2013; Echeverry-Solarte et al., 2015). However, unlike previous studies, this study identified the *AQ.GPC.ndsu.5B* A-QTL on the short arm of chromosome 5B, suggesting the novelty of this major A-QTL. Similar to our results, Prasad et al. (2003) and Groos et al. (2003) reported an A-QTL for GPC on chromosome 7A. It is worthwhile to note that the minor stable A-QTL, *AQ.GPC.ndsu.7A,* showed nucleotide sequence similarity with the wheat HMGB1 protein. Christov et al. (2007)

reported the wheat HMGB1 protein may play a major role in controlling general aspects of gene expression through chromatin structure modification. In addition to this significant role, Christov et al. (2007) also mentioned this protein possibly has a specific function as a general regulator of gene expression during cold stresses. Further studies are needed to elucidate the similarity between the *AQ.GPC.ndsu.7A* A-QTL and the wheat HMGB1 protein. As it was expected, most of the alleles for increased GPC were contributed by the cultivar Glenn.

2.5.3. Genetics of Flour Extraction Rate and Mixograph-related Parameters

Flour extraction rate and mixograph-related parameters are important end-use quality traits for the milling industries. Both FE and mixograph-related parameters are quantitative traits controlled by multiple genes (Campbell et al., 2001; Breseghello et al., 2005; Breseghello and Sorrells, 2006; Nelson et al., 2006; Simons et al., 2012; Echeverry-Solarte et al., 2015). This study found one stable A-QTL (*AQ.FE.ndsu.3B*) on chromosome 3B for FE. Similarly, Carter et al. (2012) and Ishikawa et al. (2015) also reported a stable A-QTL with a minor effect on chromosome 3B for FE. Besides the A-QTL, this study also identified a stable DE-QTL (*DEQ.FE.ndsu.5A1/1D1*) for FE. In addition, the *AQ.BLV.ndsu.5A* A-QTL, which showed a significant main effect for BLV, was involved in the epistatic interaction of the *DEQ.FE.ndsu.5A1/1D1* DE-QTL. Xing et al. (2014) indicated epistatic interactions could play an important role in the genetic basis of complex traits. Xing et al. (2002) and Yu et al. (1997) mentioned epistatic effects should be much more sensitive to environmental effects than to main effects, making the detection of a stable QTL with an epistatic effect more difficult. This study is likely the first time that a stable QTL with an epistatic effect has been reported for FE. As expected, the majority of the positive alleles for FE were contributed from the Traverse cultivar.

Previous studies have shown the effects of *HMW-GS* and *LMW-GS* on mixograph-related parameters (Payne et al., 1981; Brett et al., 1993; Gupta and MacRitchie, 1994; Ruiz and Carrillo, 1995; Maucher et al., 2009; Zhang et al., 2009; Branlard et al., 2001; He at el., 2005; Liu et al., 2005; Nelson et al., 2006; Mann et al., 2009; Jin et al., 2013; Echeverry-Solarte et al., 2015; Jin et al., 2016). In the current study, a stable A-QTL (*AQ.MMLPT.ndsu.1B*) with a major effect on MMLPT was detected on chromosome 1B, close to the location of the *Glu-B1* gene encoding for *HMW-GS*. Similarly, a recent study reported a major stable A-QTL for MMLPT in the same position close to the *Glu-B1* gene (Jin et al., 2016). The favorable alleles for this A-QTL were contributed through the Glenn cultivar. The three stable A-QTL (*AQ.MMLPT.ndsu.2D*, *AQ.MMLPT.ndsu.3B.1, and AQ.MMLPT.ndsu.5D*) for MMLPT on chromosomes 2D, 3B, and 5D, respectively, seem to be novel, with Traverse contributing the desirable alleles. In addition to the A-QTL, this study identified two novel stable epistatic DE-QTL (*DEQ.MMLPT.ndsu.2A2/4B1* and *DEQ.MMLPT.ndsu.4A1/5A1*) for MMLPT on pairs of linkage groups 2A2/4B1 and 4A1/5A1, respectively. In another study, El-Feki et al. (2013) identified a significant epistatic interaction between the *Glu-B1* locus on chromosome B1 and a QTL region near SSR marker *Xwmc76* on chromosome 7B for MMLPT in a doubled haploid hard winter wheat population.

2.5.4. Genetics of Baking Properties

Baking quality evaluations are the final assessments to determine the appropriateness of a wheat line in a bread wheat breeding program. Despite the importance of baking quality, limited information is available on the genetic control of baking properties. Previous studies have indicated the effects of *HMW-GS* on baking properties (Campbell et al., 2001; Rousset et al., 2001; Haung et al., 2006; Mann et al., 2009; Tsilo et al., 2010). In the current study, the locations

of two major A-QTL (*AQ.BMT.ndsu.1B* and *AQ.BMT.ndsu.1B.2*) for BMT were close to the location of the *Glu-B1* gene. Besides these two A-QTL, three stable A-QTL were detected for baking properties, *AQ.BA.ndsu.4D.1*, *AQ.BA.ndsu.1B*, and *AQ.BMT.ndsu.3A*. Similar to the *AQ.BMT.ndsu.1B* and *AQ.BMT.ndsu.1B.2* A-QTL for BMT, the favorable allele for the *AQ.BMT.ndsu.3A* A-QTL was contributed through the Glenn cultivar. Conversely, the favorable alleles for the *AQ.BA.ndsu.4D.1* and *AQ.BA.ndsu.1B* A-QTL were contributed through the Traverse cultivar. Similar to our results, Kuchel et al. (2006) and Tsilo et al. (2011) reported A-QTL for BA on chromosome 1B. To our knowledge, there is no information about the digenic epistatic interaction effects for baking properties. A total of 15 DE-QTL were detected in the current study.

2.5.5. Closely Linked or Pleiotropic Effects

Co-localized QTL or pleiotropic QTL could be valuable in the simultaneous improvement of several traits. Our results showed most of the end-use quality traits were associated with each other. Thus, it was expected to be able to identify closely linked or pleiotropic loci controlling these traits. A total of 19 closely linked or additive pleiotropic loci were identified for the end-use quality traits in the current study. In accordance with previous studies (Cheverud, 2000; Leamy et al., 2002; Wolf et al., 2006), most of these additive pleiotropic loci (~74%) showed positive pleiotropy. The loci controlling functionally integrated groups of traits are known to show positive pleiotropy (Cheverud, 2000; Leamy et al., 2002; Wolf et al., 2006). However, five additive closely-linked or pleiotropic loci showed negative pleiotropy in the current study. These five additive closely-linked or pleiotropic loci harbored A-QTL for GPC and FE; GPC and BMT; MMPLT and GPC; FE, BA, and MMLTV; and BA, MERS, and MELS on chromosomes 1A, 2A, 2A, 4A, and 4D, respectively. Similar to these

results, Echeverry-Solarte et al. (2015) found a co-localized QTL or pleiotropic locus with negative pleiotropy on chromosome 5B for three integrated sets of traits (GPC, mixograph envelope peak time (MEPT), and MMLPT, where alleles from the exotic parent (WCB617) increased GPC, but decreased MEPT and MMLPT. In the current study, the most important co-localized QTL or pleiotropic locus was identified on chromosome 1B, which harbored two major A-QTL (*AQ.BMT.ndsu.1B.2* and *AQ.MMLPT.ndsu.1B*) for BMT and MMLPT, respectively. Moreover, this co-localized QTL or pleiotropic locus was located very close to the location of the *Glu-B1* gene. Furthermore, this co-localized QTL or pleiotropic locus showed positive pleiotropy, where the desirable alleles were contributed through the Glenn cultivar. This positive pleiotropy indicated the simultaneous improvement of BMT and MMPLT would be possible through selection. Besides the additive co-localized QTL or pleiotropic loci, four epistatic co-localized QTL or pleiotropic loci were identified in the current study. It is generally accepted that additive pleiotropic effects are more common than epistatic pleiotropic effects (Wolf et al., 2005 and 2006). Thus, as expected, the frequency of epistatic co-localized QTL or pleiotropic loci was less than the frequency of additive co-localized QTL or pleiotropic loci. The current study appears to be the first to report for epistatic co-localized QTL or pleiotropy for end-use quality traits in wheat. Furthermore, all epistatic co-localized QTL or pleiotropic loci showed positive pleiotropy except one, which harbored A-QTL on pairs of linkage group 1A1/7D3 for GPC and MERS. This negative pleiotropy is in contrast with previous findings; Wolf et al. (2005) suggested positive pleiotropy might be generally expected in epistatic pleiotropic analyses of integrated sets of traits.

2.6. Conclusion

The current study suggests FE, MERS, MMLPT, and BMT can be used for the evaluation of the end-use quality traits in bread wheat breeding programs due to their high broad-sense heritability values. Overall, both parental lines (Glenn and Traverse) contributed desirable alleles that had positive effects on the end-use quality traits, suggesting both parental lines could be excellent resources to improve end-use quality traits in bread wheat breeding programs.

In the current study, for the first time, a high-density SNP-based linkage map was constructed and used to identify QTL for the full-scale end-use quality traits in bread wheat. It is worthwhile to note the use of the wheat Illumina 90K iSelect assay resulted in a large improvement in genome coverage, marker density, and identification of QTL compared to previous studies for end-use quality traits in bread wheat.

This study found 12 stable major main effect QTL and three stable digenic epistatic interactions for the end-use quality traits in bread wheat. This suggests both additive and digenic epistatic effects should be taken into consideration for these traits in molecular wheat breeding programs, such as MAS. Furthermore, a total of 23 closely-linked or pleiotropic loci were identified in the current study. The pleiotropic loci could be valuable simultaneously improving the end-use quality traits via selection procedures in bread wheat breeding programs. The information provided in the current study could be used in molecular wheat breeding programs to enhance selection efficiency and to improve the end-use quality traits in bread wheat.

2.7. References

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CHAPTER 3. GENOME-WIDE ASSOCIATION STUDY OF END-USE QUALITY TRAITS IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

3.1. Abstract

The main goal of the current study was to investigate the genetic basis of end-use quality traits in spring wheat using the genome-wide association mapping approach to detect linked molecular markers for marker-assisted selection (MAS). A total of 355 elite spring wheat lines were evaluated for 15 end-use quality traits including: grain protein content, flour extraction rate, eight mixograph-related parameters, and five baking-related properties, in nine environments. These elite lines were genotyped using the wheat Illumina iSelect 90K SNP assay, of which 17,514 SNP polymorphic markers were selected to perform genome-wide association mapping analyses. Marker–trait associations (MTA) were conducted using different statistical models. The most appropriate model was the fixed and random model circulating probability unification. This model could effectively control false positives and negatives. A total of 91 significant MTA were identified for these 15 end-use quality traits. These MTA were distributed across all wheat chromosomes except chromosomes 4D and 6D. The most significant MTA was detected on chromosome 7D for an association between baking absorption and the *D_contig20831_166* marker. Overall, the current study identified multiple novel stable markers that could be used in MAS for end-use quality traits improvement in wheat breeding programs.

3.2. Introduction

Bread wheat (*Triticum aestivum* L.) is a primary staple crop worldwide and accounts for about 20% of human calorie consumption (http://faostat.fao.org). Improving end-use quality traits is one of the main goals of wheat breeding programs. In the past, many studies have been conducted to detect marker-trait associations (MTA) for end-use quality traits in wheat

(Campbell et al., 2001; Raman et al., 2009; Sun et al., 2010; Tsilo et al., 2010; Zhao et al., 2010; Conti et al., 2011; Carter et al., 2012; Li et al., 2012; Simons et al., 2012; El-Feki et al., 2013; Maphosa et al., 2013; Cabrera1 et al., 2015; Deng et al., 2015; Echeverry-Solarte et al., 2015; Maphosa et al., 2015; Tiwari et al., 2016; Jin et al., 2016). However, these studies were based on bi-parental populations and low-resolution genetic linkage maps. In the current study, for the first time, a genome-wide association study (GWAS) was conducted by applying the wheat Illumina 90K iSelect assay (Wang et al., 2014) to identify MTA for full-scale end-use quality traits in bread wheat. Yu and Buckler (2006) suggested GWAS had at least three major strengths compared to linkage mapping: 1) a higher mapping resolution, 2) a shorter time to develop populations, and 3) a greater allele number. In contrast to linkage mapping, Würschum (2012) mentioned GWAS had an advantage of identifying quantitative trait loci (QTL) in breeding populations. This advantage could allow breeders to improve crops via knowledge-based breeding, which could allow stronger estimates of QTL effects across populations (Würschum, 2012). Furthermore, GWAS based on elite lines and breeding populations was better at identifying loci for traits with low heritability (Breseghello and Sorrells, 2006). However, population structure has been considered a barrier to GWAS analyses. Therefore, several statistical methods have been proposed to account for population structure, such as structure association (SA; Pritchard et al., 2000), genomic control (GC; Devlin and Roeder, 1999), principal component analysis (PCA; Price et al., 2006), stepwise regression (SWR; Setakis et al., 2006), and the mixed linear model (MLM; Yu et al., 2006). Wang et al. (2012) demonstrated that the MLM is the most promising method for analyzing population structure in GWAS analyses.

In bread wheat, GPC is a major quality trait. Quantitative trait loci for GPC are distributed throughout the wheat genome (for review, see Kumar et al., 2017). Blanco et al.

(2006) reported three major QTL associated with GPC on chromosome arms 2AS, 6AS, and 7BL, which explain most of the genetic variation for the trait. A major QTL was mapped on chromosome 6BS of a *Triticum turgidum* ssp. *dicoccoides* accession, with an average increase in GPC of 14 g Kg^{-1} (Distelfeld et al., 2006). Mann et al. (2009) reported GPC had high heritability (ranging from 0.69 to 0.93) and they also found GPC was influenced by QTL on chromosomes 1A, 3A, 7A, and 1B.

Other important quality traits are dough-related properties. The most common instruments used to test dough rheology are Farinograph, Glutograph, Mixograph, Extensograph, and Alveograph (Brabender, 1932; Sietz, 1987; Shelke and Walker, 1990; Panozoo and Eagles, 2000; Trethowan et al., 2001; Mann et al., 2007). Mann et al. (2009) reported a major dough rheology QTL associated with the *Glu-B1* and *Glu-D1* loci. Limited information is available on the genetic control of baking properties such as loaf volume, bake-mixing time, and bake-mixing water absorption. Therefore, for the first time in the current study, a genome-wide association study (GWAS) and the Illumina 90K iSelect wheat SNP assay were used to detect marker-trait associations for the full-scale end-use quality traits in bread wheat. Thus, the objectives of this study were to: (1) provide a comprehensive insight into the genetic control of the full-scale end-use quality traits using a genome-wide association mapping approach, and (2) identify SNP markers, which are closely linked to QTL associated with the end-use quality traits for molecular breeding strategies.

3.3. Materials and Methods

3.3.1. Plant Material

A collection of 333 advanced breeding lines and 22 cultivars with varying end-use quality characteristics were used for association mapping analysis in this study (Supplementary

Table. 1). The advanced breeding lines were developed by the NDSU Hard Red Spring Wheat Breeding Program and involved 128 different crosses. The cultivars used in this study were: Advance "PI 664482" (Glover et al., 2014); Albany (Limagrain Cereal Seeds, 2009); Barlow "PI 658018" (Mergoum et al., 2011); Prosper "PI-662387" (Mergoum, 2012); RB07 "PI 652930" (Anderson, 2009); Glenn "PI-639273" (Mergoum et al., 2006); Traverse "PI-642780" (Karl et al., 2006); Brennan (Syngenta, 2009); Brick (Golver et al., 2010); Briggs (Golver and Hall, 2004); Elgin "PI 668099" (Mergoum et al.; 2016); Faller "PI-648350" (Mergoum, 2008); Forefront, Howard "PI-642367" (Mergoum, 2006); Kelby, Linkert, Mott (Mergoum, 2009); Norden, Reeder, Rowyn (Syngenta); Steele-ND "PI 634981" (Mergoum et al., 2005); and Velva (Mergoum, 2012).

3.3.2. Field Experiment Design

The germplasm was grown under field conditions at three locations in ND, USA, from 2012 to 2014. In 2012, the three sites were Prosper, Carrington, and Minot; in 2013, the Carrington site was replaced with a Williston site, and in 2014, it was replaced with a Hettinger site (Table. 3.1). In 2012, the genotypes were grown in a randomized complete block design (RCBD) with two replicates; however, in 2013 and 2014, a 12 × 12 simple lattice design was used to reduce experimental error and increase precision in the experiment. In 2012 and 2013, each plot was 2.44 m long and 1.22 m wide and consisted of seven rows. In 2014, the plot size was a little wider, at 1.42 m, and consisted of seven rows. The sowing rate was 113 kg ha^{-1} in all environments.

Table 3.1. Description of the environments and planting date to evaluate spring wheat traits in an association panel developed by the North Dakota State University hard red spring wheat program in the USA (NDAWN, 2000-2016).

Location	Year	LAT[a]	LNG[b]	ALT (m)[c]	Planting date	TGS (^{0}C)[d]	PGS (mm)[e]
Prosper	2012	46°57'46.90"N	97°1'11.31"W	275	05.15.2012	21	148.8
Minot	2012	48°13'58.68"N	101°17'32.25"W	491	04.23.2012	19	225.0
Casselton	2012	46°51'18.26"N	97°12'39.83"W	283	05.10.2012	21	144.0
Prosper	2013	46°57'46.90"N	97°1'11.31"W	275	05.30.2013	20	318.0
Williston	2013	48° 9′23.00″N	103°37′41.00″W	491	05.01.2013	18	319.3
Minot	2013	48°13'58.68"N	101°17'32.25"W	514	05.14.2013	19	425.0
Prosper	2014	46°57'46.90"N	97°1'11.31"W	275	05.24.2014	19	216.9
Hettinger	2014	46°0′3.00″N	102°38′ 0.00″W	491	05.14.2014	17	200.3
Minot	2014	48°13'58.68"N	101°17'32.25"W	514	05.22.2014	17	347.7

[a] Latitude in degrees and minutes; [b] Longitude in degrees and minutes; [c] Altitude in meters; [d] Mean temperature during growing season in degrees Celsius (May-October); [e] Mean precipitation in growing season in millimeters.

3.3.3. Phenotypic Data Collection

The grain samples harvested from the field experiments were cleaned in two steps using a clipper grain cleaner machine and then a carter dockage tester machine. To evaluate end-use quality traits, a 200-g grain sample per line from the first replicate in each location was used. End-use quality traits evaluated in this study were: GPC, flour extraction rate (FE), eight mixograph-related parameters, and five baking-related properties. These traits were evaluated following the American Association of Cereal Chemists (AACCI)-approved method 39.10.01 and NDSU Wheat Quality and Carbohydrate Research Lab protocol (AACC International Method, 1999; https://www.ndsu.edu/faculty/simsek). Mixograph-related parameters were: mixograph envelope left slope (MELS), mixograph envelope right slope (MERS), mixograph MID line peak time (MMLPT), mixograph MID line peak value (MMLPV), mixograph MID

line time * value (MMLTV), mixograph MID line peak width (MMLPW), mixograph MID line peak integral (MMLPI), and general mixograph pattern (MIXOPA). The baking-related properties evaluated in this study were: bake mixing time (BMT), baking absorption (BA), bread loaf volume (BLV), crumb color (CBCL), and crust color (CTCL). The phenotypic data evaluations were described in more detail in Chapter 2.

3.3.4. Phenotypic Data Analysis

The grain samples collected from the first replicate of each environment were used to evaluate phenotypic data. The experimental design used was a randomized complete block design (RCBD); each environment was considered a replicate. Variance components were estimated using restricted maximum likelihood (REML) in the MIXED procedure of SAS software Version 9.3 (SAS Institute, Inc., Cary, NC, USA). Blocks (environments) and genotypes were considered random effects. Best linear unbiased predictor (BLUP) values were estimated by using the *solution option* of the random statement of the proc mixed procedure in SAS. Pearson correlation between quality traits were analyzed using BLUP values across all environments. The CORR procedure of SAS was used to calculate Pearson's rank correlation. Phenotypic data collected from the first replicate of each environment and BLUP values were used for GWAS.

3.3.5. Genotyping Data

Lyophilized young leaves were used to isolate genomic DNA for the association panel following a modified Doyle and Doyle (1987) protocol described by Diversity Arrays Technology Pty., Ltd. (http://www.diversityarrays.com/sites/default/files/resources/DArT_DNA_isolation.pdf accessed August 2014). DNA quality was tested via visual observation on 0.8 % agarose gel. DNA

concentrations were determined with a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). DNA samples were diluted to the concentration of 50 ng/µl, and a 20 µl of each sample was sent to the USDA Small Grains Genotyping Lab in Fargo, ND, for SNP analysis using the wheat Illumina 90K iSelect SNP assay (Wang et al., 2014). SNP marker calling was performed as described by Wang et al. (2014) using Genome Studio Polyploid Clustering Module v1.0 software (www.illumina.com).

Out of a total 81,587 SNP markers from the wheat Illumina 90K iSelect assay (Wang et al., 2014), 17,555 polymorphic SNP markers were identified and used for GWAS analysis. Markers with a high number of missing values (≥ 50%) and the minor allele frequency (MAF) <5% were excluded from the analysis. Missing data were imputed using imputation by best linear unbiased prediction (iBLUP) with the default setting Yang and Pan (2014) described. Yang and Pan (2014) used linkage disequilibrium (LD) and identity by descent (IBD) simultaneously to develop a genotypic imputation algorithm for non-randomized missing values.

3.3.6. Population Structure, Kinship, and Linkage Disequilibrium

Both population structure and kinship were calculated using markers with pairwise $R^2 <$ 0.5 for all pairwise comparisons. STRUCTURE software version 3.2 was used to assign the subpopulation membership for each genotype and to calculate the structure matrix (Q-matrix). This study used an admixture model with independent allele frequencies, a burn-in of 100,000, and an MCMC replication of 500,000 for K = 1 to 10 with five replications. The delta k calculated from the STRUCTURE software was used to select the optimum number of subpopulations. The Principal Component Analysis (PCA) using PRINCOMP in SAS 9.3 (SAS institute, 2011) was employed to control for population structure in genome-wide association mapping (GWAS) (Price et al., 2006). To account for individual relatedness, an identity-by-state

kinship matrix was generated by the fixed and random model circulating probability unification (FARMCPU) algorithm run in FARM-CPU (Liu et al., 2016) using the complete SNP data set with the minor allele frequency (MAF) ≥ 5%. Pairwise linkage disequilibrium (LD) between markers in the null model was calculated as the squared allele frequency correlation in the R package (Lipka et al., 2012) after filtering for minor allele frequency (MAF) ≥ 5%.

3.3.7. Genome-Wide Association Mapping Analysis

A total of 17,514 SNP markers with minor allele frequency (MAF) ≥ 5% were used for GWAS analyses. GWAS analyses were implemented using GAPIT and FARM-CPU, R packages developed by Lipka et al. (2012) and Liu et al, (2016), respectively. Multiple models were used for association analysis, including: the null general linear model; general linear models with fixed effects to control for population structure; the univariate unified mixed linear model (Yu et al., 2006) using the population parameters previously determined (P3D) (Zhang et al., 2010) to control both relatedness and population structure; the efficient mixed model association (EMMA) (Lipka et al., 2012); and the fixed and random model circulating probability unification (FARMCPU) (Liu et al., 2016). A Bonferroni-corrected threshold probability of 0.05/N was employed to verify the significance levels for the results, where N was the number of trait-SNP combinations tested. Quantile-quantile (Q-Q) plots and Manhattan plots were created by R 3.1.1 software.

3.3.8. Identification and Annotation of Candidate Genes

Gene identification information was downloaded from the International Wheat Genome Sequencing Consortium (IWGSC) database (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies) to define candidate genes.

3.4. Results

3.4.1. Phenotypic Variation and Pearson Correlation

The association panel showed variation for all the end-use quality characteristics. The Pearson correlation analysis showed most of the end-use quality traits were associated with each other (Table 3.2). Highly positive significant phenotypic correlations (correlation coefficient value lies between + 0.50 and + 1 and is significant at $P < 0.01$) were detected between GPC and MMLPV; BMT and MMLPT; BMT and MMLPI; MERS and MMLPT; and MMLPT and MMLPI. Moderately positive significant phenotypic correlations (correlation coefficient value lies between + 0.30 and + 0.50 and is significant at $P < 0.01$) were identified between GPC and MMLTV; GPC and MMLPW; BMT and MERS; BMT and MIXO; MELS and MMLPV; MIXOPA and MMLPV; MIXOPA and MMLPV; MIXOPA and MMLTV; MIXOPA and MMLPW; and MIXOPA and MMLPI. Conversely, moderately negative significant phenotypic correlations (correlation coefficient value lies between - 0.30 and - 0.50 and is significant at $P <$ 0.01) were observed between MERS and MMLPV; MERS and MMLPI; MMLPT and MMLPV; and MMLPT and MMLP.

3.4.2. Population Structure and Linkage Disequilibrium Analyses

The number of subpopulations (k) were plotted against the delta k calculated using STRUCTURE software. The peak of the broken line graph was observed at k = 7, indicating the natural population can be divided into seven subpopulations (Figure 3.1). Linkage disequilibrium (LD) heat maps were created for each chromosome separately (Figure 3.3). The LD pattern was varied by chromosome even after controlling for population relatedness (Figure 3.3). Overall, the A and B genomes showed high LD compared to the D genome (Figure 3.3).

3.4.3. Genome-Wide Association Study (GWAS) Analyses

In the current study, the FARM-CPU model using principle component analysis (PCA) and kinship (K) was selected for GWAS analyses based on results of the Q-Q plots (Figure 3.4). This study detected genomic regions underlying 15 end-use quality traits using 17,514 SNP markers with a MAF ≥ 5%. Based on the Bonferroni-corrected threshold, the *P*-value less than 2.85×10−6 was considered significant.

The implementation of the FARM-CPU model showed out of the 17,514 SNP markers, only 91 SNP markers showed significant association (*P*-value < 2.85×10−6) with end-use quality traits (Table 3). A total of 57 significant markers were located on the B-genome, while 25 and 9 markers were located on the A- and D-genomes, respectively.

A total of 23 SNP markers showed significant association with GPC (*P*-value < 2.85×10−6). These MTA were located on chromosomes 1A, 1B, 2B, 2D, 4B, 5B, and 7B. The majority of these MTA for GPC (~70%) were found on chromosome 5B (Table 3). The MTA, wsnp_BE495277B_Ta_2_5 and GPC association, showed a synthetic relationship with the *Bradi4g34980.1* gene in *brachypodium* and its orthologues *Os09g0512900* and *Sb02g029670.1* in rice and sorghum, respectively.

A total of 11 MTA were identified for FE, with the *P*-value ranging from 5.95×10−6 (Tdurum_contig28598_245) to 3.64×10−10 (Kukri_c37212_1286), and the MAF ranging from 0.06 (Kukri_c37212_1286) to 0.42 (BobWhite_c5276_631). These MTA were located on chromosomes 1A, 1B, 1D, 3B, 4B, 6A, and 7A (Table 3.3). The MTA, Kukri_c37212_1286 and FE association, was identified to be significant in five out of eight environments for FE. This MTA was considered the most stable MTA for FE.

A total of 27 MTA were found to be significant for mixograph-related parameters on chromosomes 1A, 1B, 2A, 2B, 3B, 3D, 4A, 5A, 6A, and 7B (Table 3). A total of nine MTA were identified to be significant for MMLPI, with the *P*-value ranging from 8.02×10−6 (wsnp_Ex_c54003_57045475) to 3.64×10−10 (IACX6064) (Table 3). The SNP marker, wsnp_Ex_c9842_16228523, showed a synthetic relationship with the *Bradi4g01080.1* gene in *brachypodium*, which in turn showed gene ontology with the Noc2p family. The function of this protein is not known.

A total of 30 MTA were detected to be significant for baking-related properties. These MTA were located on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5D, 6A, 6B, 7A, 7B, and 7D. A total of 19 MTA were found to be significant for BA. The SNP markers BobWhite_c1214_798, D_contig28615_96, Kukri_rep_c118476_63, and Tdurum_contig54704_176 were identified to be significant in more than four environments for BA and considered stable MTA. Furthermore, all of these markers were located on chromosome 2B (Table 3.3).

These results showed most of the end-use quality characteristics were correlated. Therefore, the identification of co-localized or pleiotropic loci controlling these characteristics was expected. A total of four co-localized or pleiotropic loci were detected to be significant. These four co-localized or pleiotropic loci were located on chromosomes 1A and 2B, harboring SNP markers respectively for MMLPV and CBCL, and MMLPI and BMT.

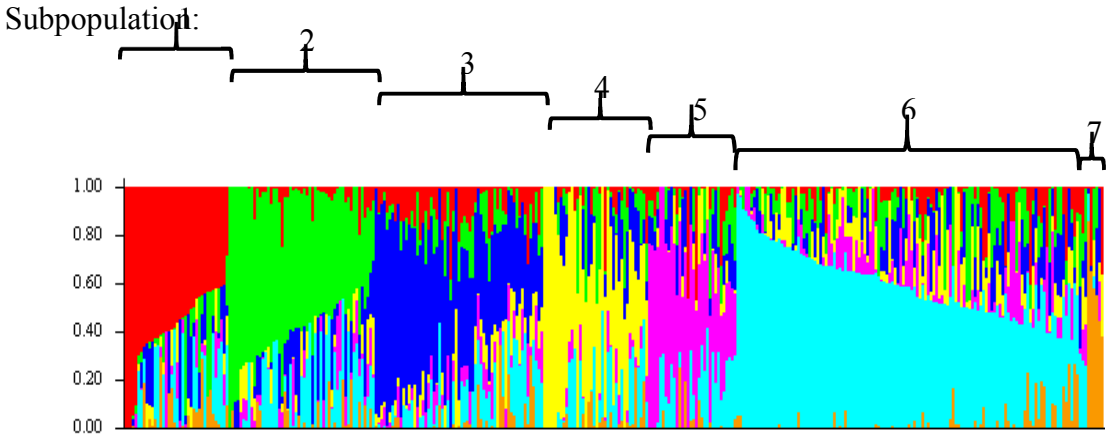

Figure 3.1. The structure analysis of the association panel from K=7.

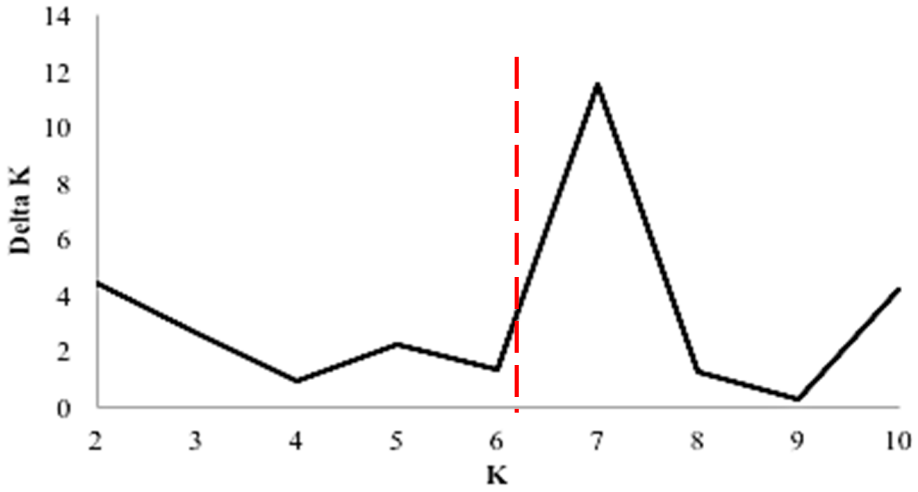

Figure 3.2. Plot of delta K against putative K ranging from K=1 to K=10.

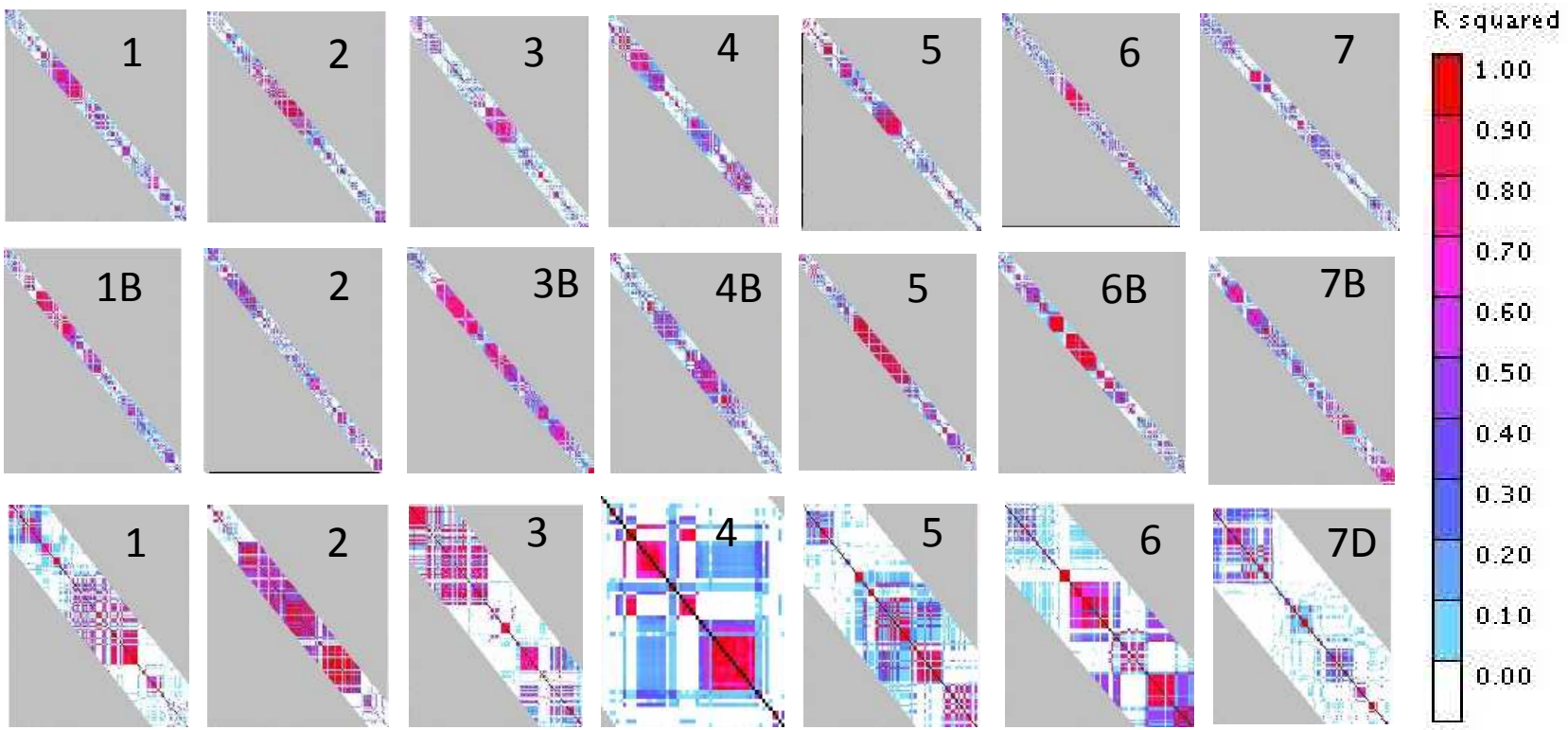

Figure 3.3. The linkage disequilibrium (LD) heatmap plot for a pairwise genome-wide LD between SNP markers in each wheat chromosome. Each box represents a chromosome in bread wheat. Each pixel illustrates the r^2 of the corresponding pairs of markers, as shown in the color code at the upper right.

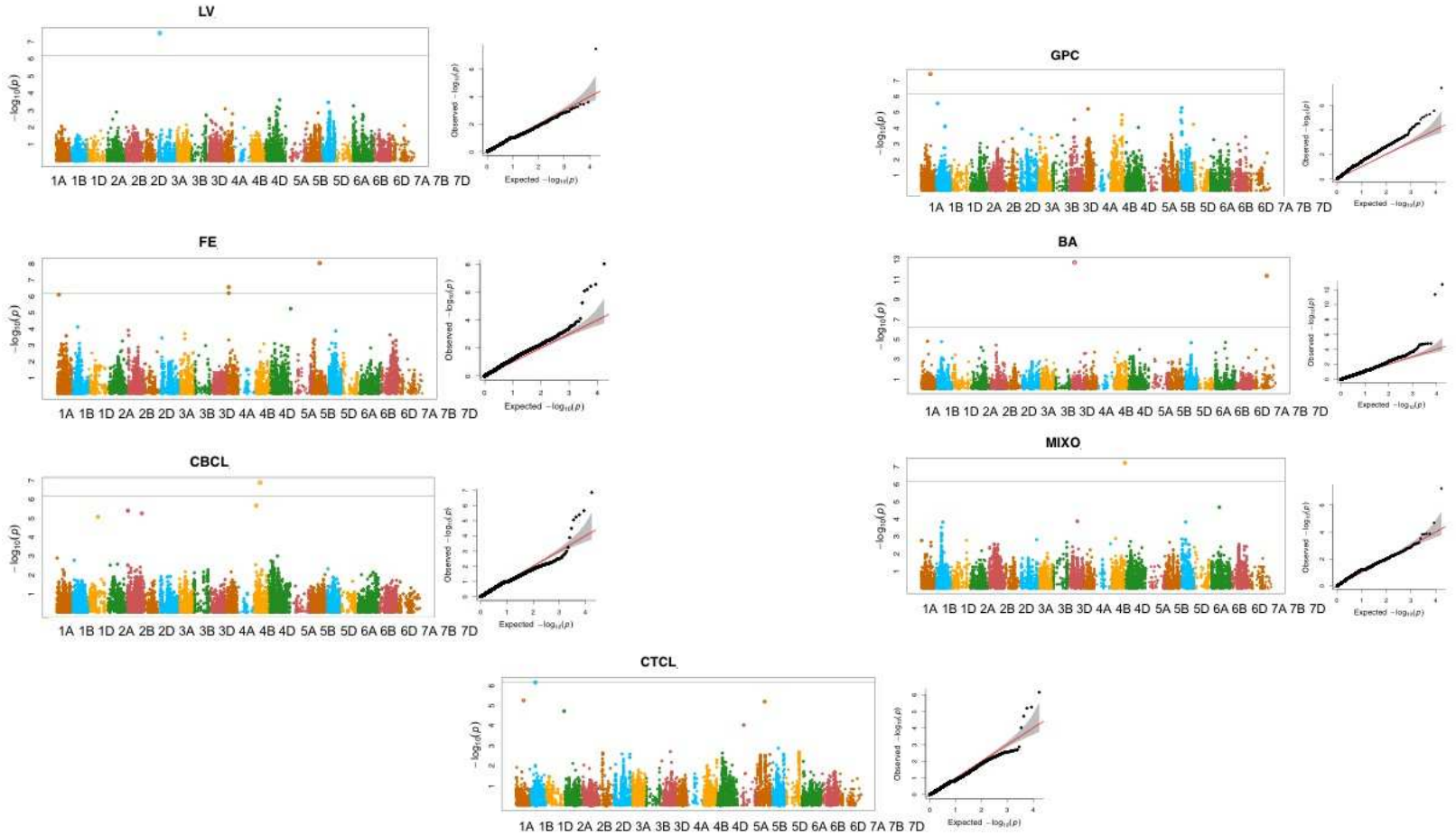

Figure 3.4. Manhattan plots of the best models for some of the end-use quality traits in an association panel of 355 elite spring wheat lines. GPC: grain protein content, BA: baking absorption, LV: bread loaf volume, CBCL: crumb color, CTCL: crust color, FE: flour extraction rate, MIXO: the general mixograph pattern. The green lines are the cutoff values to call a significant peak. The quantile–quantile plot (Q-Q plot) represents the goodness of the best model for BLUP values across all the locations in a wheat association panel.

Table 3.2. Pearson correlation of end-use quality traits for a genome-wide association panel across all environments.

	FE	GPC	BMT	BA	LV	CBCL	CTCL	MELS	MERS	MMLPT	MMLPV	MMLTV	MMLPW	MMLPI	MIXO
FE	1	-.173**	.031	-.013	.017	.008	-.076	.017	.011	-.012	.029	.039	.131*	-.041	.081
GPC		1	-.208**	.084	.246**	.086	.117*	.279**	-.197**	-.250**	.585**	.448**	.375**	-.051	.258**
BMT			1	-.078	-.067	-.149**	-.008	-.165**	.412**	.776**	-.177**	-.116*	.159**	.786**	.371**
BA				1	.100	.141**	.022	-.016	-.028	-.085	.049	.045	-.001	-.071	.100
LV					1	.113*	.138**	-.003	-.001	-.028	.167**	.130*	.118*	.023	.169**
CBCL						1	.293**	.076	-.055	-.222**	.238**	.322**	.132*	-.134*	.176**
CTCL							1	.058	.028	-.057	.175**	.208**	.113*	.028	.200**
MELS								1	-.235**	-.256**	.365**	.282**	.262**	-.123*	-.017
MERS									1	.516**	-.353**	-.215**	-.225**	.412**	.148**
MMLPT										1	-.380**	-.373**	-.062	.907**	.174**
MMLPV											1	.876**	.696**	-.035	.333**
MMLTV												1	.602**	-.042	.394**
MMLPW													1	.183**	.379**
MMLPI														1	.349**
MIXO															1

[a] GPC: grain protein content, BMT: bake mixing time, BA: baking absorption, BLV: bread loaf volume, CBCL: crumb color, CTCL: crust color, FE: flour extraction rate, MIXOPA: the general mixograph pattern, MELS: mixograph envelope left slope, MERS: mixograph envelope right slope, MMLPT: mixograph MID line peak time, MMLPV: mixograph MID line peak value, MMLTV: mixograph MID line time * value, MMLPW: mixograph MID line Peak width, MMLPI: mixograph MID line peak integral; [b] Genetic correlation coefficient according to Holland (2002); [c] Pearson correlation based on BLUP values. *, ** Significant at $P < 0.05$ and 0.01; [ns] Not significant at $P < 0.05$.

Table 3.3. Marker-trait associations identified across all environments for 15 end-use quality traits in spring wheat.

Trait	Marker	Environment	Other associated traits	Chromosome	Position(cM)	P-Value	MAF	Effect
BA	IAAV749	VII	_	1A	71.10	9.14E-07	0.485	-1.047
CBCL	Excalibur_c35316_154	IV, X	MMLPV	1A	16.67	4.75E-06	0.262	-0.038
FE	Kukri_c37212_1286	II, III, IV, VII, X	_	1A	26.60	3.64E-10	0.061	2.066
FE	BS00103478_51	III, IV, VI, X	_	1A	35.65	1.69E-06	0.076	-0.665
FE	Ku_c972_560	IV, VI, X	_	1A	21.55	5.84E-06	0.077	-1.588
GPC	Ex_c26688_969	II, V, X	_	1A	111.55	3.93E-08	0.116	0.276
LV	Ku_c28007_1398	I, IV, X	_	1A	21.55	8.15E-07	0.362	-5.312
MMLPV	Excalibur_c35316_154	VIII, X	CBCL	1A	17.67	1.27E-06	0.262	-0.494
MMLPW	wsnp_Ex_c9343_15514531	VII, X	_	1A	137.20	2.98E-06	0.177	0.254
BA	IAAV4349	I, IV, VII	_	1B	62.58	6.40E-06	0.244	-0.595
BMT	BS00022133_51	VII, X	_	1B	69.30	6.74E-06	0.086	-0.128
CTCL	BS00029345_51	II	_	1B	64.89	7.18E-07	0.192	-0.199
FE	RAC875_c865_1936	I	_	1B	70.00	4.94E-07	0.214	0.405
GPC	RAC875_c46093_492	III, VII, X	_	1B	109.00	1.26E-06	0.077	0.159
MMLPI	IACX6064	I, IV	_	1B	81.95	2.48E-11	0.056	-23.212
MMLPI	wsnp_Ex_rep_c66802_65172754	X	_	1B	65.42	1.77E-09	0.196	-6.051
MMLPI	Ra_c33845_794	VIII	_	1B	65.00	2.81E-07	0.194	-10.010
MMLPT	RAC875_c24895_311	V	_	1B	80.00	2.83E-11	0.054	-0.596
MMLPT	IACX6064	V	_	1B	81.95	5.67E-08	0.056	-0.266
MMLPV	BS00065487_51	VII, X	_	1B	30.34	1.93E-07	0.425	-0.556
MMLPW	IACX6064	I, VII	_	1B	81.95	8.63E-06	0.056	-1.550
FE	Excalibur_c6154_413	II, IV, V, X	_	1D	57.27	3.77E-07	0.062	-4.880
BA	Kukri_c26288_419	I, IV, VI, VII, X	_	2A	77.91	8.40E-07	0.056	0.004
MMLPI	BS00070693_51	V, X	_	2A	92.29	9.12E-07	0.224	10.214
MMLPT	Ra_c42714_1137	V	_	2A	109.00	5.67E-07	0.073	0.362
MMLPV	BS00065667_51	V, X	_	2A	47.26	5.22E-06	0.051	2.952
BA	BobWhite_c1214_798	I, IV, VI, VII, X	_	2B	73.75	8.09E-07	0.075	0.003
BA	D_contig28615_96	I, II, IIV, V, X	_	2B	73.75	8.25E-07	0.070	-0.003
BA	Kukri_rep_c118476_63	I, IV, VII, VIII, X	_	2B	76.00	1.78E-06	0.068	0.003
BA	Tdurum_contig54704_176	II, IV, VII, X	_	2B	73.75	2.08E-06	0.056	-0.003
BMT	BS00064055_51	I, III, IV, VI, VIII	MMLPI	2B	181.92	1.60E-06	0.238	-0.155
GPC	Kukri_c33299_519	I	_	2B	74.47	3.82E-08	0.183	0.387
MIXO	Kukri_c42244_809	III, X	_	2B	96.99	6.78E-07	0.227	-0.290
MMLPI	RFL_Contig2324_729	V, X	_	2B	182.00	3.95E-09	0.228	-5.274

Table 3.3. Marker-trait associations identified across all environments for 15 end-use quality traits in spring wheat (continued).

Trait	Marker	Environment	Other associated traits	Chromosome	Position(cM)	P-Value	MAF	Effect
MMLPI	BS00064055_51	V,VII	BMT	2B	181.92	1.38E-06	0.238	-10.154
GPC	RAC875_c39966_280	I, II, III, VI, X	_	2D	80.00	7.56E-07	0.350	-0.131
LV	BS00110921_51	V	_	3A	26.01	3.25E-08	0.254	-7.594
BA	wsnp_Ex_c4156_7507247	VII, X	_	3B	62.57	6.72E-08	0.206	0.002
BA	BS00064258_51	I, IV, VII	_	3B	178.60	9.29E-06	0.272	-0.587
FE	BobWhite_c5276_631	II, IV, V, VII, X	_	3B	122.52	3.68E-06	0.415	-0.798
MERS	Excalibur_c5977_383	IV	_	3B	70.70	3.65E-07	0.414	0.557
MMLPW	BS00094456_51	IV, X	_	3B	73.45	2.65E-08	0.341	0.214
MMLPW	IACX3169	IV	_	3B	81.20	6.21E-07	0.158	-1.134
MMLPW	GENE-1511_622	IV	_	3B	81.20	8.71E-07	0.159	1.128
GPC	Kukri_c55081_219	II, III, V, VII, X	_	3D	101.09	8.16E-06	0.132	-0.265
MMLPI	IAAV1578	V, VII, X	_	3D	0.00	7.75E-06	0.263	9.354
MMLPI	wsnp_Ex_c54003_57045475	VI, X	_	3D	67.15	8.02E-06	0.406	-3.489
BA	RAC875_c45385_212	I, IV	_	4A	49.00	2.04E-13	0.244	0.837
BA	BS00065137_51	I, IV	_	4A	150.71	3.74E-06	0.342	-0.496
BA	wsnp_Ex_rep_c68677_67531081	I, IV	_	4A	164.13	5.30E-06	0.314	0.510
MMLPV	RAC875_c88582_131	VIII, X	_	4A	91.00	1.93E-06	0.166	1.694
CBCL	RAC875_c2542_1197	VIII	_	4B	115.00	6.70E-07	0.246	-0.108
FE	GENE-3521_378	III, IV, X	_	4B	21.78	2.74E-07	0.063	6.099
FE	Kukri_rep_c112779_183	III, IV	_	4B	22.00	6.41E-07	0.066	0.112
GPC	Tdurum_contig42229_113	III, VII, X	_	4B	56.00	4.70E-06	0.387	-0.172
CBCL	Kukri_rep_c72329_163	V	_	5A	64.00	1.34E-07	0.214	-0.136
MMLPT	wsnp_Ex_c9842_16228523	I, II	_	5A	15.61	8.47E-07	0.082	0.271
GPC	RAC875_c4287_2984	I	_	5B	61.00	8.35E-09	0.182	0.406
GPC	Excalibur_c10444_2056	I	_	5B	61.38	1.23E-08	0.182	-0.399
GPC	RAC875_c24376_704	I	_	5B	61.00	3.82E-08	0.183	-0.387
GPC	RAC875_c39141_55	I	_	5B	61.00	3.82E-08	0.183	-0.387
GPC	Tdurum_contig57403_311	I	_	5B	61.84	3.82E-08	0.183	0.387
GPC	Tdurum_contig57403_589	I	_	5B	61.84	3.82E-08	0.183	-0.387
GPC	BS00066289_51	I	_	5B	61.84	3.82E-08	0.183	-0.387
GPC	Kukri_c46476_551	I	_	5B	62.60	3.82E-08	0.183	-0.387
GPC	Kukri_c46476_647	I	_	5B	61.84	3.82E-08	0.183	0.387
GPC	Tdurum_contig68472_291	I	_	5B	61.38	3.93E-08	0.180	-0.377
GPC	wsnp_BE495277B_Ta_2_5	I	_	5B	61.84	3.93E-08	0.180	-0.377
GPC	RAC875_c2437_1569	I	_	5B	61.00	4.23E-08	0.189	0.369

Table 3.3. Marker-trait associations identified across all environments for 15 end-use quality traits in spring wheat (continued).

Trait	Marker	Environment	Other associated traits	Chromosome	Position(cM)	P-Value	MAF	Effect
GPC	BS00021949_51	I	_	5B	61.84	1.44E-07	0.180	-0.378
GPC	RFL_Contig658_1166	I	_	5B	62.00	1.82E-07	0.186	-0.371
GPC	Tdurum_contig10338_566	I	_	5B	62.00	1.82E-07	0.186	-0.371
GPC	Kukri_c45713_151	I	_	5B	61.92	5.72E-07	0.323	-0.060
BA	BS00072464_51	I, VI	_	5D	46.8	2.25E-06	0.110	-0.916
BA	BS00000020_51	VII, X	_	5D	103.00	3.48E-06	0.121	0.711
CBCL	D_contig19916_460	I, IV	_	6A	159.97	2.11E-06	0.372	0.164
FE	wsnp_Ex_c17089_25709028	IV	_	6A	79.08	9.33E-09	0.265	-1.036
MMLPW	BS00058929_51	VI, X	_	6A	100.12	5.90E-06	0.093	-1.199
MMLPW	Excalibur_c25898_434	V, VII, X	_	6A	99.44	9.43E-06	0.096	-1.148
BA	BobWhite_c47831_87	I, IV, VII	_	6B	64.08	9.34E-06	0.263	-0.553
LV	RAC875_c10650_90	I, IV	_	6B	49.00	2.31E-07	0.132	-7.328
BA	wsnp_CAP11_c827_513472	I, IV	_	7A	136.43	6.33E-06	0.259	-0.581
BMT	wsnp_Ex_c4804_8579139	V, VI	_	7B	73.79	3.17E-06	0.314	-0.117
FE	BS00076622_51	IV,V,VII	_	7B	148.65	3.39E-07	0.190	-1.300
FE	Tdurum_contig28598_245	II, III, IV, V, VII, X	_	7B	152.00	5.95E-06	0.321	-0.333
GPC	wsnp_Ex_c27323_36528037	I, II	_	7B	77.13	4.62E-06	0.369	0.172
LV	wsnp_CAP11_rep_c4076_1926235	I, IV	_	7B	102.79	6.23E-06	0.470	5.690
MMLPI	Tdurum_contig19022_1555	V, X	_	7B	75.00	3.49E-07	0.370	-4.371
MMLPT	Excalibur_c17078_400	I, IV, VI, X	_	7B	73.39	2.91E-06	0.196	-0.108
BA	D_contig20831_166	I, II, IV, VI	_	7D	135.55	4.56E-12	0.270	-0.936
BA	D_F5XZDLF02H192C_184	I, IV, VIII	_	7D	22.85	6.79E-06	0.313	-0.529

3.5. Discussion

Understanding the genetic architecture controlling end-use quality traits is important in breeding programs. Earlier findings, mostly using biparental populations and QTL mapping analyses, have been conducted to uncover the genetic basis of end-use quality traits in wheat (McCartney et al., 2005; Grag et al., 2006; Patil et al., 2009; Tsilo et al., 2011; Bogard et al., 2013; Prashant et al., 2015; Echeverry-Solarte et al., 2015; Li et al., 2016). However, the QTL mapping studies more often detect QTL, which are limited to the biparental population and have

low resolution. On the other hand, a genome-wide association study has become a promising approach to genetic mapping based on LD. Some of the advantages of GWAS over QTL mapping studies are: increased QTL resolution, allele coverage, and potential use of natural germplasm (such as landraces and advanced breeding lines) (Buckler and Thornsberry 2002; Davey et al., 2011). Furthermore, a higher marker coverage enhances the accuracy of MTA studies that are important tools for analyzing the genetic architecture of any trait (Varshney et al., 2009; Deschamps and Campbell, 2009; Davey et al., 2011). In wheat, few studies have used association mapping to dissect the genetics of end-use quality traits (Breseghello and Sorrells 2006), yield component traits (Yao et al. 2009), disease resistance (Tommasini et al. 2007), and kernel weight-related traits (Chen et al., 2016). To our knowledge in this study, for the first time, a GWAS using the wheat Illumina iSelect 90K SNP assay was performed and used to detect MTA for the full-scale end-use quality traits in bread wheat.

In the current study, the Pearson correlation analysis showed most of the end-use quality traits were associated with each other. These results corresponded with the results of previous studies (Patil et al., 2009; Tsilo et al., 2011; Prashant et al., 2015; Echeverry-Solarte et al., 2015).

This study used a new statistical method developed by Liu et al. (2016) to identify MTA. Liu et al. (2016) proposed the Farm-CPU method to control false positives as well as false negatives in GWAS analyses. Farm-CPU iteratively performs marker tests with pseudo quantitative trait nucleotides (QTN) as covariates in a fixed-effect model and optimization on pseudo QTN in a random-effect model. Consequently, to some extent, this new statistical method simultaneously controls false negatives, controls false positives, and removes the confounding between testing markers and kinship.

Previous studies have reported a few major and several minor QTL for end-use quality traits, indicating the polygenetic nature and quantitative inheritance of these traits (McCartney et al., 2005; Grag et al., 2006; Bogard et al., 2013; Echeverry-Solarte et al., 2015; Li et al., 2016). In the current study, 23 MTA were identified to be significant for GPC. The majority of these MTA were identified on chromosome 5B for GPC. Similarly, Echeverry-Solarte et al. (2015), Deng et al. (2015), and El-Feki (2013) in separate studies found major QTL for GPC on chromosome 5B. In contrast to our results in the second chapter, where no QTL were detected on the D-genome, two QTL were found on chromosomes 2D and 3D for GPC. In the current study, a stable MTA for FE was found on chromosome 1A. Similarly, Kuchel et al. (2006) and Echeverry-Solarte et al. (2015) in separate studies reported a major QTL on chromosome 1A for FE. All of the MTA identified for mixograph-related traits had minor effects. This suggests a need to further study these QTL before any recommendations can be made to use them in improving wheat quality. Multiple stable MTA were found for BA on chromosomes 2A and 2B. Similar to these results, Campbell et al. (2001) and Kuchel et al. (2006) identified QTL on chromosomes 2A and 2B for BA.

Breeding wheat for end-use quality traits has been a difficult task for several reasons, including: the complex nature of these traits, the expense of end-use quality evaluations, and the large amount of grain needed to conduct the evaluations. For the first time, in the current study, a genome-wide study was constructed and used to identify QTL for the full-scale of end-use quality traits in bread wheat.

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CHAPTER 4. GENERAL CONCLUSIONS

A total of 73 QTL were identified through the QTL mapping study, whereas a total of 91 MTA were detected through the GWAS study. Compared to the QTL mapping study, the GWAS approach significantly increased the range of natural variation that resulted in a large number of significant regions associated with the end-use quality traits. Furthermore, the GWAS provided a higher resolution than the QTL mapping, facilitating fine-mapping and gene discovery.

In brief, this dissertation focused on the discovery of new genetic regions associated with end-use quality traits in bread wheat with the goal of facilitating the implementation of these new discoveries in wheat breeding programs.