Dissertation

Relationship of Glutenin Loci and Rye Translocations with Dough Mixing Properties of Wheat Grown in Colorado Environments

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY SHUSONG ZHENG ENTITLED RELATIONSHIP OF GLUTENIN LOCI AND RYE TRANSLOCATIONS WITH DOUGH MIXING PROPERTIES OF WHEAT GROWN IN COLORADO ENVIRONMENTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

RELATIONSHIP OF GLUTENIN LOCI AND RYE TRANSLOCATIONS WITH DOUGH MIXING PROPERTIES OF WHEAT GROWN IN COLORADO ENVIRONMENTS

The unique breadmaking properties of wheat (*Triticum aestivum* L.) flour are largely determined by its glutenin proteins, encoded by genes at the *Glu-1* and *Glu-3* loci. Whereas the quality effects of the *Glu-1* loci have been intensively studied, the effects of the *Glu-3* loci are not fully understood. The wheat-rye (*Secale cereale* L.) chromosomal translocations 1AL.1RS and 1BL.1RS have been associated with a decrease in hard winter wheat breadmaking quality. This project was undertaken to evaluate the effects of glutenin allelic variation and rye translocations on dough rheology and to compare the effects between irrigated and dryland environments.

We evaluated two populations in dryland and irrigated Colorado locations: (1) 190 recombinant inbred lines (RIL) from the cross between TAM 107-R7 and Arlin; and (2) a population of 96 cultivars and advanced lines, mainly from the U.S. Great Plains. Dough rheology was measured with a 10-g mixograph. Associations between genotype (glutenin loci and rye translocations) and phenotype (mixograph properties) were calculated with a simple *t*-test for the RIL population and a unified mixed model to reduce false positive associations for the cultivar population. Results indicated that

(1) quality effects of glutenin loci and rye translocations were significantly affected by growing environments ($P<0.05$); (2) in the RIL population, poorer dough mixing properties were observed with *Glu-D1a* vs. *Glu-D1d*, *Glu-A1a* vs. *Glu-A1b*, *Glu-B1c* vs. *Glu-B1b*, *Glu-A3e* vs. *Glu-A3c*, and the combination of *Glu-A1a*/*Glu-A3e* vs. other allelic combinations at the *Glu-A1* and *Glu-A3* loci; (3) in the cultivar population, *Glu-A1c*, *Glu-B1e*, *Glu-D1a*, and *Glu-B3c* and *i* were associated with poorer mixograph properties, whereas *Glu-A1b*, *Glu-B1b*, *Glu-D1b* and *d*, and *Glu-B3f* and *b* were associated with higher values of mixograph properties; (4) the presence of 1RS translocations was significantly ($P<0.05$) associated with lower values for most mixograph properties, with 1BL.1RS more detrimental than 1AL.1RS; *Glu-D1d* partially compensated for the negative effects of 1AL.1RS in the RIL population. These results suggest that selection of appropriate glutenin alleles and rye translocations can facilitate the development of cultivars with desirable dough rheology in wheat breeding programs in Colorado and similar environments.

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LIST OF ABBREVIATIONS

Abbreviation	Description
HMW-GS	High-molecular-weight glutenin subunits
LMW-GS	Low-molecular-weight glutenin subunits
SDS-PAGE	Sodium-dodecylsulfate-polyacrylamide gel electrophoresis
SDSSV	Sodium-dodecylsulfate sedimentation value
RIL	Recombinant inbred line
NIL	Near isogenic line
DH	Double haploid
GW	Grain weight per plot
KW	Kernel weight
NSPH	Number of seeds per head
DTH	Days to heading date
DPM	Days to physiological maturity
GFD	Grain filling duration=DPM-DTH
PH	Plant height
gprot12	Grain protein concentration at 12% moisture content
fprot14	Flour protein concentration at 14% moisture content
MPT	Mixograph peak time
MLW	Mixograph width at one minute before MPT
MLV	Mixograph midline height at one minute before MPT
MPW	Mixograph band width at MPT
MPV	Mixograph midline peak height
MRW	Mixograph band width at 2 minutes after MPT
MRV	Mixograph midline height at 2 minutes after MPT
logMLW	Log_e (ln) transformation of MLW
logMPW	Log_e (ln) transformation of MPW
logMRW	Log_e (ln) transformation of MRW
kD	KiloDalton
SSR	Simple-sequence repeat marker
P	Alveograph traits, the tenacity
L	Alveograph traits, extensibility
W	Alveograph traits, strength

PREFACE

Wheat (*Triticum aestivum* L.) is one of the primary cereal grains for human food and animal feed. Whereas wheat has very diversified uses around the world, the major use for U.S. wheat is for bread production. In my dissertation research I have concentrated on the breadmaking quality. As critical aspects of breadmaking quality, dough mixing properties have been found to mainly associate with the storage proteins of the wheat endosperm, especially glutenin proteins, which are mainly encoded by genes located on the group 1 chromosomes of wheat (Singh and Khatkar, 2005). The wheat-rye (*Secale cereale* L.) chromosomal translocations in the form of 1AL.1RS and 1BL.1RS were introduced for pest and disease resistance genes, but have been associated with a decrease in breadmaking quality (Graybosch, 2001). The effects of glutenin subunits and 1RS translocations have been found to be influenced by the growing environment of wheat.

Wheat is a major winter crop for Colorado and to improve wheat quality is one of the important goals for the wheat breeding program of Colorado State University. However, the quality effects of glutenin loci and 1RS translocations have not been systematically evaluated in Colorado environments. Thus, the objectives of this research were (1) to evaluate the effects of glutenin allelic variation and 1RS translocations on dough mixing properties measure by mixograph, (2) to determine the consistency of their effect on mixing properties in both irrigated and dryland Colorado environments. Two

wheat populations were used to achieve these objectives: a recombinant inbred line (RIL) population (TA population) derived from the cross between TAM 107-R7 and Arlin and a 'cultivar population' that included 96 cultivars and advanced lines mainly from the U.S. Great Plains. For TA population, we used simple single marker analysis, whereas for cultivar population, we used single maker analysis corrected for multiple-level relatedness of individuals with the mixed model (Q+K) proposed by Yu et al. (2006).

There are three major chapters in this dissertation. Each chapter could stand alone in the format of a 'Crop Science' article. In Chapter 1, some important topics which relate to this study are reviewed, including the glutenin allelic diversity in wheat cultivars, the effects of glutenin alleles on various quality properties measured by different methods, mapping of wheat quality properties, rye translocations and their effects on wheat quality, environmental effects on wheat quality, the mixograph methodology, and association mapping. In chapter 2, the study of TA population is reported. In Chapter 3, the study with the 'cultivar population' is reported. The major conclusions, significance and shortcomings of this research, and future perspectives are provided in the 'Afterword'. For the sake of some readers who might be interested, the detailed information of cultivar population, the statistical approaches, and the weather data for the experiment environments are given in the appendix (Table A1 to A8 and Figure A1 to A3).

CHAPTER 1
Literature Review

Wheat (*Triticum aestivum* L.) is one of the primary cereal grains for human food and animal feed, grown in an area of over 200 million hectares worldwide and now yielding almost 600 million tons annually (FAO; http://www.fao.org). Since the early 1960s, global wheat yields have increased almost three-fold without a significant increase in the area sown to wheat. This increase has been achieved through improved agricultural practices and the breeding of new cultivars (Marshall et al., 2001). Meanwhile, quality improvement has been an important goal for all breeding programs. The challenges for wheat breeding programs around the world are to maintain or improve agronomic performance of wheat cultivars and pest/disease resistance, and at the same time to improve their quality. Wheat quality has been a primary study objective for many research groups and is the major focus of my current research. In this chapter, some aspects of wheat quality relevant to my study are reviewed.

Glutenin and Wheat Quality

Wheat quality is a complex collection of traits influenced by both genetic factors and plant growth conditions (Ma et al., 2005; Perretant et al., 2000). Bekes et al. (2006) defined the quality of a wheat sample as suitability for producing an end-product, so the quality 'value' of a wheat sample depends on its potential uses: baking bread, making biscuits, producing noodles, baking cakes, etc. In some cases, an excellent breadmaking

wheat can have low quality for noodle-making and even weaker characteristics for cake-making. In my dissertation, 'quality' will refer to breadmaking quality unless otherwise specified.

One of the most important goals of cereal science is to understand the genetic control of wheat quality. Most of our knowledge about this subject comes from two major approaches: studying the changes in quality characteristics on samples with systematically altered chemical compositions, and relating quality characteristics to genetic components of large sample populations using statistical methods (Bekes et al., 2006). The second method is most interesting to my research.

Bread wheat is a hexaploid species consisting of three different but related genomes of seven chromosome pairs named arbitrarily the A, B, and D genomes. Each chromosome is identified with a number from 1 to 7 followed by the genome assignment (Payne, 1987). Quantitative trait loci (QTL) mapping has revealed that different quality aspects are controlled by loci distributed across all wheat genomes (see review section of 'QTL Mapping and Wheat Quality' in this literature review). Among these loci, those for wheat kernel storage proteins are of major importance for wheat quality.

Wheat Protein

Wheat storage proteins were among the first proteins to be studied, as early as 1745 (reviewed by Singh and Khatkar, 2005). Osborne (cited by Singh and Khatkar, 2005) classified wheat protein into four components according to their solubility in different solvents: albumins (soluble in water), globulins (soluble in dilute salt solutions), prolamins (soluble in aqueous alcohols), and glutelins (soluble in dilute acids or alkalis). Among them, prolamin is quantitatively the major protein in wheat and is further

classified into two groups, the gliadins and the glutenins. Upon hydration and during processing, gliadins and glutenins interact to form a unique viscoelastic gluten network, which is necessary for holding the gases produced by fermentation and for producing a light-texture porous structured bread during baking (reviewed by Khatkar and Schofield, 1997). The elastic properties of gluten are due to the glutenin fraction, while the viscous properties come from the gliadin fraction. An appropriate balance in the amount of these two major protein components of wheat gluten is required for achieving the desired breadmaking quality (as reviewed by Khatkar and Schofield, 1997).

Glutenins are believed to be formed of polymers linked by intermolecular disulphide bonds, and are further classified into two groups: the high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), based on their mobility in the sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions (reviewed by Singh and Khatkar, 2005). By analyzing nullisomic, tetrasomic, ditelocentric and inter-varietal chromosome substitution lines generated from the bread wheat cultivar, 'Chinese Spring', the HMW-GS and the LMW-GS were found to be encoded by genes at the complex *Glu-1* and *Glu-3* loci on the long and short arms of the group 1 chromosomes, respectively (Jackson et al., 1983; Payne, 1987; Payne et al., 1984). Some of the LMW-GS are also encoded by genes on the short arms of group 6 chromosomes (Jackson et al., 1983; Payne et al., 1984). These discoveries were confirmed in several mapping populations (Breseghello et al., 2005; Campbell et al., 2001; Joppa et al., 1997; McCartney et al., 2006; Nelson et al., 2006; Perretant et al., 2000; Zanetti et al., 2001).

The HMW-GS of wheat have been studied in detail because of their important role in determining breadmaking quality, including water absorption, peak dough mixing time and loaf volume, and their easy separation in SDS-PAGE. HMW-GS account for about 10 to 20% of total prolamins and only 5 to 15% of total flour proteins (reviewed by Singh and Khatkar, 2005). Glutenin component surveys have showed that up to 25 different HMW-GS are present in different cultivars (see the review section of 'Glutenin Allele Diversity and Wheat Quality' of this literature review). As determined by SDS-PAGE, the molecular weight of HMW-GS ranges between 80 and 160 kilodaltons (kD) depending on the particular subunit and the SDS-PAGE system used. This estimation is higher than their actual molecular weight determined by sedimentation equilibrium and amino acid sequencing, which varies from 63 to 88 kD. The subunits encoded at each locus are subdivided into '*x*' and '*y*' types with the single 1A encoded subunit being of the *x* type (Shewry et al., 1992). In general, *x*-type subunits migrate more slowly than their *y*-type counterparts. A numbering system to identify HMW-GS according to their electrophoretic mobility and chromosomal location was suggested by Payne and Lawrence (1983) and is widely accepted. Each HMW-GS is identified with its genetic locus (1A, 1B or 1D), type (*x* or *y*), and a number that reflects its order in a gel. For example, HMW-GS encoded by the *Glu-D1d* allele is named 1Dx5+1Dy10. Normally this nomenclature is abbreviated just to keep the reference number in the gel (e.g., 1D*x*5+1D*y*10 is shortened to 5+10).

The LMW-GS represent about one-third of the total seed protein and around 60% of total glutenins (reviewed by D'Ovidio and Masci, 2004). They have a molecular weight ranging from 20 to 45 kD. Despite their abundance, LWM-GS have not been

studied as thoroughly as HMW-GS, because LMW-GS are relatively difficult to separate in gels (reviewed by Gianibelli et al., 2001). A procedure to simultaneously separate HMW-GS and LMW-GS from wheat flour was reported by Gupta and Shepherd (1990), and was improved by Singh et al. (1991). However, due to the complex patterns of LMW-GS, there is no commonly accepted naming system. The nomenclature used by Gupta and Shepherd (1990) is used by many research groups (Bekes et al., 2001; He et al., 2005; Huang et al., 2006; Martin et al., 2001b; Martinez et al., 2005). It identifies LMW-GS by the genetic locus followed by an alphabetical letter (e.g., the null allele of LMW-GS at 1A is identified as *Glu-A3e*).

Wheat Protein and Wheat Quality

Researchers realized as long as a half century ago that baking quality is highly correlated with protein content and its components. As summarized by Weegels et al. (1996), wheat quality is associated with protein content of flour at various levels (R^2 ranged from 1 to 86% in independent studies). By comparing many studies, Weegels et al. (1996) concluded that, in general, the glutenin content of flour, especially its HMW-GS and LMW-GS content, was more important than the protein content in determining loaf volume. Much effort has been devoted to relating individual glutenin subunits or the genes encoding them to variation in breadmaking quality or less direct indicators of wheat quality such as SDS-sedimentation volume (SDSSV), mixograph properties, alveograph properties, etc. Based on association of many glutenin subunits to SDSSV in various cultivars, Payne et al. (1987) assigned each of the glutenin alleles a quality score to facilitate its use in breeding programs. This scoring system was updated by Pogna in

1992 and more glutenin alleles were included (Table 1.1) (reviewed by Singh and Khatkar, 2005).

Table 1.1. Quality scores assigned to high-molecular-weight glutenin subunits on SDS-sedimentation volume and *W* of Alveograph (Singh and Khatkar, 2005)

Locus	Allele	Subunit	SDS-sedimentation score † (Payne's quality Score)	Alveograph *W* score ‡
Glu-A1	*a*	1	3	3
	c	null	1	2
	b	2*	3	5
Glu-B1	*a*	7	1	2
	e	20	-§	1
	d	6+8	1	1
	b	7+8	3	1
	c	7+9	2	5
	i	17+18	3	6
Glu-D1	*a*	2+12	2	2
	b	3+12	2	-
	c	4+12	1	1
	h	5+12	-	2
	d	5+10	4	6

†Scores are ranked from 1 to 4, indicating poor and good breadmaking quality, respectively.

‡Scores are ranked from 1 to 6, indicating poor and good breadmaking quality, respectively.

§ '-', not available.

Some of the newer studies after Payne (1987) are summarized in Table 1.2. These studies were done in broad genetic backgrounds, including materials from Australia (Bekes et al., 2006; Bekes et al., 2001; Eagles et al., 2002a; Eagles et al., 2002b; Eagles et al., 2004; Ma et al., 2005; Vawser and Cornish, 2004), China (He et al., 2005; Liu et al., 2005), India (Ram, 2003), France (Branlard et al., 2001), Japan (Nagamine et al., 2000;

Tabiki et al., 2006), Canada (Huang et al., 2006), Spain (Martinez et al., 2005), Finland (Sontag-Strohm and Juuti, 1997), Italy (Blanco et al., 1998), USA (Dong et al., 1992), New Zealand (Luo et al., 2001), and Portugal (Brites and Carrillo, 2001). These studies used two major types of populations, collections of cultivars and advanced breeding lines (Bekes et al., 2006; Branlard et al., 2001; Eagles et al., 2002a; Eagles et al., 2002b; Eagles et al., 2004; Liu and Rathjen, 1996; Liu et al., 2005; Nagamine et al., 2000; Ram, 2003; Sontag-Strohm and Juuti, 1997; Vawser and Cornish, 2004), and experimental populations including doubled haploid (DH) lines, near-isogenic lines (NIL), and inbred backcross lines or recombinant inbred lines (RIL) (Blanco et al., 1998; Brites and Carrillo, 2001; Gupta et al., 1994; Huang et al., 2006; Luo et al., 2001; Ma et al., 2005; Martinez et al., 2005; Tabiki et al., 2006).

HMW-GS and Wheat Quality

Wheat dough properties and baking performance have been related to variation in HMW-GS by many studies (Table 1.2) (Bekes et al., 2006; Blanco et al., 1998; Branlard et al., 2001; Brites and Carrillo, 2001; Dong et al., 1992; He et al., 2005; Huang et al., 2006; Kolster et al., 1991; Liu and Rathjen, 1996; Liu et al., 2005; Luo et al., 2001; Ma et al., 2005; Martinez et al., 2005; Nagamine et al., 2000; Ram, 2003; Sontag-Strohm and Juuti, 1997; Tabiki et al., 2006; Vawser and Cornish, 2004). In general, the *Glu-D1* locus has the largest effect on dough properties, followed by the *Glu-B1* and *Glu-A1* loci.

Table 1.2. The comparison of glutenin allelic effects on wheat quality characteristics from various publications (not all indicated relationships are statistically significant)

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	Rmax					*d>c*	
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	Extensibility		*i>e*				
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	MPT	*a=b*	*i>e*	*d>a*	*b>e*	*d>c*	*a=c*
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	MPV			*a>d*		*d>c*	
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	MPW						
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	MRV						
Bekes et al., 2001	Bread wheat	Australia	Cranbrook×Halberd (DH)	172	MRW		*e>i*			*c>d*	
Bekes et al., 2006	Bread wheat	Australia	5 DH populations	174	Dough extensibility	*a>c>b*	*b>i>f>e>al>c*	*a>c>d*	*d>e=f=b>c*	*b>d>c=h>g*	*b>a=c*
Bekes et al., 2006	Bread wheat	Australia	Cultivars and advanced lines	394	Rmax	*b>a*	*al>i>c>f>e>b*	*d>a>c*	*b>e>d>f>c*	*b>h>d>g>c*	*b>c>a*
Branlard et al., 2001	Bread wheat	France	Cultivars and advanced lines	162	Strength	*b=a>c*	*i≥f≥c=b≥a=d*	*d≥b=a≥c*	*a=d=f≥e*	*b'≥d=c=c' =b=g>i>f≥j*	*a≥b=d=c*
Branlard et al., 2001	Bread wheat	France	Cultivars and advanced lines	162	Extensibility	*a=b=c*	*f≥b=c=i≥a≥d*	*a=b=c=d*	*d=a=f≥e*	*i≥b'≥c=c' =g>b=f=d>j*	*a=b=d=c*
Branlard et al., 2001	Bread wheat	France	Cultivars and advanced lines	162	zeleny SDSSV	*b=a>c*	*f≥i≥c=b=a=d*	*d>b=a>c*	*d≥a≥f>e*	*b'=c'=d=b =i=g=c>f>j*	*a=b=d=c*
Deng et al., 2005	Bread wheat	China	'Lumai 16'×'PH85-9' (NILs)	4	Glutenin elasticity and strength		*h>c*	*d>m*			
Deng et al., 2005	Bread wheat	China	'Lumai 16'×'PH85-9' (NILs)	4	Loaf volume		*h>c*	*d>m*			
Deng et al., 2005	Bread wheat	China	'Lumai 16'×'PH85-9' (NILs)	4	SDSSV		*h>c*	*d>m*			
Dong et al., 1992	Bread wheat	USA	Lines generated from multiple crosses	135	Loaf volume	*b>c>a*	*i>c>b=e>a>d*	*d>a>b*			
Dong et al., 1992	Bread wheat	USA	Lines generated from multiple crosses	135	Crumb grain score	*c>b>a*	*e>c>a>b>i>d*	*d>a>b*			
Dong et al., 1992	Bread wheat	USA	Lines generated from multiple crosses	135	Mixing time	*b>c>a*	*b>e>c>a>i>d*	*d>a>b*			

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Dong et al., 1992	Bread wheat	USA	Lines generated from multiple crosses	135	Mixing tolerance	*b>c>a*	*b>c>e>a>i>d*	*d>a>b*			
Dong et al., 1992	Bread wheat	USA	Lines generated from multiple crosses	135	Absorption	*c>b=a*	*i>a>c>b>e>d*	*a>b>d*			
Eagles et al., 2002a	Bread wheat	Australia	Cultivars and advanced lines	2377	Milling yield	*a=b>c*	*c>b>i*	*a=d*	*b=c=d=e*	*b>h>g*	*c>b>a*
Eagles et al., 2002a	Bread wheat	Australia	Cultivars and advanced lines	2377	PSI	*c>b>a*	*c>b>i*	*a>d*	*c>d>b>e*	*g>b>h*	*b>c>a*
Eagles et al., 2002a	Bread wheat	Australia	Cultivars and advanced lines	2377	Water absorption	*a>b>c*	*i>b>c*	*a>d*	*e>b>c>d*	*h>b>g*	*c>b>a*
Eagles et al., 2002a	Bread wheat	Australia	Cultivars and advanced lines	2377	Dough development time	*b>a>c*	*c>b>i*	*d>a*	*d>b>c>e*	*h>b>g*	*b>a>c*
Eagles et al., 2002b	Bread wheat	Australia	Cultivars and advanced lines	1926	Rmax	*a=b>c*	*h>f>i>b>c >e>a>d>l*	*d>c>a*	*d>b>c>e*	*f>g>b>d >h>i>j>c*	*a>b>c*
Eagles et al., 2002b	Bread wheat	Australia	Cultivars and advanced lines	1926	Extensibility	*a=b>c*	*e>l>b>f>c >i>a>d>h*	*c>a>d*	*d>b>c>e*	*f>i>c>h >b>g>d>j*	*b>a>c*
Eagles et al., 2002b	Bread wheat	Australia	Cultivars and advanced lines	1926	Flour protein content	*a=b=c*	*d>e>l>a>i >c>f>b>h*	*d>a>c*	*c>b>e>d*	*c>d>b>j >i>h>g>f*	*b>a>c*
Eagles et al., 2004	Bread wheat	Australia	Cultivars and advanced lines	902	Rmax	*b>a>c*	*al>f>i=b>c>e*	*d>a*	*d>b>c>e*	*b>h>g>d*	*c>b>a*
Eagles et al., 2004	Bread wheat	Australia	Cultivars and advanced lines	902	Extensibility	*b>a>c*	*al>e>b>c=f=i*	*a>d*	*d=b>c>e*	*g>b=d>h*	*a=b>c*
Eagles et al., 2004	Bread wheat	Australia	Cultivars and advanced lines	902	Flour protein content	*b>a>c*	*al>b=c>i>f>e*	*d>a*	*d>b>c>e*	*b>h>g>d*	*b>a>c*
Gupta et al., 1994	Bread wheat	Australia	Halberd×(W1xMMC)W1/10 ($F_{3:6}$)	74	Rmax	*a=b*	*i>e*	*d>a*	*c>e*	*b>C*	*b=c*
Gupta et al., 1994	Bread wheat	Australia	Halberd×(W1xMMC)W1/10 ($F_{3:6}$)	74	Extensibility	*a=b*	*i=e*	*a>d*	*c=e*	*c>b*	*b=c*
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Protein content	*a=c*	*h=b>c*	*d=a=c*	*d=c=a*	*d=b>f=j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	SDSSV	*a=c*	*h=b>c*	*d>c=a*	*d>a=c*	*d=b=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Stability	*a>c*	*h=b=c*	*d>a=c*	*d>a=c*	*d>b=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Rmax	*a>c*	*b>h=c*	*d>c=a*	*d>c=a*	*d=b=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Extensibility	*a>c*	*h>b>c*	*a=c=d*	*d=c=a*	*d=b=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Loaf volume	*a>c*	*b>h>c*	*d>c=a*	*d>a=c*	*d>b=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	76	Noodle score	*a=c*	*h=b=c*	*c=d=a*	*d=c=a*	*d>b=f>j*	

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Protein content	*a=c>b*	*b=c*	*d=a=c*	*d=c=a*	*d=b=f=j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	SDSSV	*a>c=b*	*b>c*	*d>c=a*	*d>c>a*	*b>d=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Stability	*a>b=c*	*b>c*	*d>c=a*	*d>c=a*	*b=d=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Rmax	*a>b=c*	*b>c*	*d>c=a*	*d>c=a*	*b=d=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Extensibility	*a=b>c*	*b>c*	*a=d=c*	*d=c=a*	*b>d=f>j*	
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Loaf volume						
He et al., 2005	Bread wheat	China	Cultivars and advanced lines	66	Noodle score	*a=b=c*	*b=c*	*d=a=c*	*d>c>a*	*b=f=d>j*	
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	MPT			*d>a*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	MPV	*b>a*	*b>c*	*a>d*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	Energy to peak	*b>a*	*b>c*	*d>a*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	First minute slope			*a>d*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	MPW			*a>d*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	Slope after peak			*a>d*			
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	Total energy		*b>c*				
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	Bandwidth energy		*b>c*				
Huang et al., 2006	Bread wheat	Canada	'AC Karma'×87E03-S2B1 (DH)	185	SDSSV		*c>b*				
Kolster et al., 1991	Bread wheat	Netherlands	breeding lines	226	Loaf volume						
Liu et al., 2005	Bread wheat	China	Cultivars and advanced lines	251	Protein content	*a>b=c*	*h>c=b*	*d=a=c*	*e>c; e=d=a; d=a=c*	*d=b>j=f*	
Liu et al., 2005	Bread wheat	China	Cultivars and advanced lines	251	SDSSV	*a>c=b*	*h=b>c*	*d>c=a*	*d>a>c>e*	*d>j; d=b=f; b=f=j*	
Liu et al., 2005	Bread wheat	China	Cultivars and advanced lines	251	MPT	*a>c=b*	*b>c; b=h; h=c*	*d>c=a*	*d>a>c=e*	*d>b=f>j*	
Liu et al., 2005	Bread wheat	China	Cultivars and advanced lines	251	Mixing tolerance	*a>c=b*	*b>c; b=h; h=c*	*d>c=a*	*d>a=c>e*	*d>f=b>j*	
Luo et al., 2001	Bread wheat	New Zealand	Morahi×Tui	34	Flour protein content	*b=c*		*a=d*	*d>d+c>c*		*b>a>a+b*

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Luo et al., 2001	Bread wheat	New Zealand	Morahi×Rongotea	31	hardness	*b+c>c=b*		*a=d*	*d=c*		*a=b*
Luo et al., 2001	Bread wheat	New Zealand	Otane×Karamu	33	SDSSV	*b+c>b>c*		*d>a+d>a*	*d>c>d+c*		*a=b*
Luo et al., 2001	Bread wheat	New Zealand	Otane×Pernel	51	MPT	*b=c*		*a=d*	*d=c*		*a+b>a>b*
Luo et al., 2001	Bread wheat	New Zealand	Rongotea×Tui	44	MPV	*b+c>b>c*		*a=d*	*d=c>d+c*		*b>a>a+b*
Luo et al., 2001	Bread wheat	New Zealand	MOrahi×Tui	34	Flour protein content	*a>b>a+b*			*d>e>d+e*		
Luo et al., 2001	Bread wheat	New Zealand	Morahi×Rongotea	31	Hardness	*a=b*			*d=e*		
Luo et al., 2001	Bread wheat	New Zealand	Otane×Karamu	33	SDSSV	*a=b*			*d=e*		
Luo et al., 2001	Bread wheat	New Zealand	Otane×Pernel	51	MPT	*a=b*			*d=e*		
Luo et al., 2001	Bread wheat	New Zealand	Rongotea×Tui	44	MPV	*a+b>a>b*			*d>e=d+e*		
Ma et al., 2005	Bread wheat	Australia	Cranbrook×Halberd (DH)	160	Dough extensibility	*a=b*	*i>e*	*d>a*	*b>e*	*d>c*	*a>c*
Ma et al., 2005	Bread wheat	Australia	Cranbrook×Halberd (DH)	160	Rmax	*a=b*	*i>e*	*d>a*	*b>e*	*d>c*	*a>c*
Nagamine et al., 2000	Bread wheat	Japan	DH of Norin61x Chinese Spring, Norin 61×Asakazekomugi, Norin 61×Gogatsukomugi	61	Farinographic valorimeter volue	*b>c*	*c>b*	*a=f*			
Nagamine et al., 2000	Bread wheat	Japan	Norin61x Chinese Spring, Norin 61×Asakazekomugi, Norin 61×Gogatsukomugi (DH)	61	Gluten relaxation test	*c>b*	*b=c*	*a=f*			
Sontag-Strohm and Juuti, 1997	Bread wheat	Finland	Cultivars	75	Pelshenke test			*d>a*	*e=least*		
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Flour protein content (1997)	*a=b*	*c=i*	*d=a*	*f=c*	*h=b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Wet gluten content (1997)	*b>a*	*c=i*	*d<a*	*f=c*	*h>b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Gluten index (1997)	*a=b*	*c=i*	*d>a*	*f=c*	*h<b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Dry gluten content (1997)	*b>a*	*c=i*	*d<a*	*f=c*	*h=b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Flour protein content (1998)	*a=b*	*c=i*	*d=a*	*f=c*	*h=b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Wet gluten	*b>a*	*c=i*	*d<a*	*f=c*	*h>b*	

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
					content (1998)						
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Gluten index (1998)	*b<a*	*c=i*	*d>a*	*f<c*	*h<b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	Dry gluten content (1998)	*b>a*	*c=i*	*d<a*	*f=c*	*h=b*	
Tabiki et al., 2006	Bread wheat	Japan	Grandin×Kitamiharu 57 (DH)	59	MPT (1998)	*b<a*	*c=i*	*d>a*	*f=c*	*h<b*	
Vawser and Cornish, 2004	Bread wheat	Australia	Cultivars and advanced lines	33	Rmax		*al>i>c=f> u>ak>e>d*				
Blanco et al., 1998	Durum wheat	Italy	Messapia×MG4343	65	SDSSV	*1*>null*	*d=7*+22**				
Ram, 2003	Bread wheat	Indian	Cultivars	172	SDSSV	*b>a>c*	*i>c>b*	*d>a*			
Ram, 2003	Durum wheat	Indian	Cultivars	25	SDSSV		*f>e; d>e*				
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	SDSSV	*a>c*				*a>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	MPT	*a=c*				*a>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	MPV-MRV(3m)	*a=c*				*a=b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	W	*a=c*				*a>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	P	*a=c*				*a=b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Raspinegro	70	L	*a=c*				*a=b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Faia×Mourisco Fino	65	SDSSV		*d>e*			*j>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Faia×Mourisco Fino	65	MPT		*d>e*			*j>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Faia×Mourisco Fino	65	MPV-MRV(3m)		*d<e*			*j<b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Faia×Mourisco Fino	65	W		*d>e*			*j>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Faia×Mourisco Fino	65	P		*d=e*			*j>b*	
Brites and Carrillo,	Durum wheat	Portugal	Faia×Mourisco Fino	65	L		*d=e*			*j>b*	

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
2001											
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	74	SDSSV		*h>b*			*c>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	74	MPT		*h=b*			*c>b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	74	MPV-MRV(3m)		*h=b*			*c<b*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	80	SDSSV		*h>e*			*c>k*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	80	MPT		*h>e*			*c>k*	
Brites and Carrillo, 2001	Durum wheat	Portugal	Celta×Lobeiro	80	MPV-MRV(3m)		*h<e*			*c<k*	
Liu and Rathjen, 1996	Durum wheat	Australia	Cultivars and advanced lines	79	SDSSV	*v>c>a*	*f>b>d>e*				
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	SDSSV	*c <a*	*b<d*			*b>l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	MPT	*c<a*	*b=d*			*b>l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	MPV	*c<a*	*b>d*			*b=l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	MRV	*c<a*	*b=d*			*b=l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	*W*	*c=a*	*b=d*			*b>l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	*p*	*c=a*	*b=d*			*b=l*	
Martinez et al., 2005	Durum wheat	Spain	Anton×Blancal de Nules ($F_{2:4}$)	150	*L*	*c=a*	*b=d*			*b>l*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	SDSSV		*e<d*		*a>a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	MPT		*e<d*		*a>a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	MPV		*e=d*		*a>a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	MRV		*e=d*		*a=a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	*W*		*e=d*		*a=a+i*	*a>b*	

Reference	Materials	Source	Population	# of lines	Traits†	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	*p*		*e=d*		*a=a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Senatore Capelli×Fanfarron ($F_{2:4}$)	48	*L*		*e=d*		*a=a+i*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Alcala la Real×Bidi-17 ($F_{2:4}$)	64	SDSSV		*e<d*				
Martinez et al., 2005	Durum wheat	Spain	Alcala la Real×Bidi-17 ($F_{2:4}$)	64	MPT		*e<d*				
Martinez et al., 2005	Durum wheat	Spain	Alcala la Real×Bidi-17 ($F_{2:4}$)	64	MPV		*e=d*				
Martinez et al., 2005	Durum wheat	Spain	Alcala la Real×Bidi-17 ($F_{2:4}$)	64	MRV		*e<d*				
Martinez et al., 2005	Durum wheat	Spain	Bidi17×Atalaya ($F_{2:4}$)	11	SDSSV		*e<aq*		*a=e*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Bidi17×Atalaya ($F_{2:4}$)	11	MPT		*e=aq*		*a=e*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Bidi17×Atalaya ($F_{2:4}$)	11	MPV		*e=aq*		*a=e*	*a>b*	
Martinez et al., 2005	Durum wheat	Spain	Bidi17×Atalaya ($F_{2:4}$)	11	MRV		*e=aq*		*a=e*	*a>b*	

† NIL, near isogenic line; RIL, recombinant inbred line; DH, doubled haploid.

‡ MPT, mixograph (midline) peak time; MLW, mixograph (midline) width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph (midline) band width at MPT; MPV, mixograph midline peak height; MRW, mixograph (midline) band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; SDSSV, sodium-dodecylsulfate-sedimentation volume; *W*, alveograph traits, strength; *L*, alveograph traits, extensibility; *P*, alveograph traits, the tenacity; PSI, particle size index.

At the *Glu-A1* locus, there are three major alleles: *a* (subunit 1), *b* (subunit 2*), and *c* (null allele). There is no consistent association between the *Glu-A1* alleles and quality measurements (Table 1.2). There is no significant difference for protein content for the *Glu-A1* alleles (Table 1.2) (Eagles et al., 2002a; Eagles et al., 2004; He et al., 2005; Liu et al., 2005; Luo et al., 2001; Tabiki et al., 2006). Generally, there is no difference in *Glu-A1* alleles for mixograph peak time (MPT) (Table 1.2, Fig. 1.1) (Bekes et al., 2001; Liu et al., 2005; Luo et al., 2001). Two studies showed that *Glu-A1c* was associated with shorter MPT than *Glu-A1a* (Liu et al., 2005; Martinez et al., 2005). Two studies showed that *Glu-A1b* gave a higher MPT than other *Glu-A1* alleles in American and Australian materials (Dong et al., 1992; Eagles et al., 2002a), whereas *Glu-A1b* was associated with lower MPT than other *Glu-A1* alleles in Chinese and Japanese materials (Liu et al., 2005; Tabiki et al., 2006). For mixograph midline peak height (MPV, Fig. 1.1), lines containing two alleles at the *Glu-A1* locus had a higher MPV than homogeneous lines (Table 1.2) (Luo et al., 2001). *Glu-A1c* had a lower mixograph tolerance value than the other two *Glu-A1* alleles (Dong et al., 1992; Liu et al., 2005). For maximum resistance (Rmax) measured by extensigraph, *Glu-A1a* and *b* might have an equal contribution, whereas *Glu-A1c* is an inferior allele (Table 1.2) (Bekes et al., 2006; Dong et al., 1992; Eagles et al., 2002b; Eagles et al., 2004; He et al., 2005; Ma et al., 2005). *Glu-A1a* and *GluA1c* had the same values for alveograph traits, the tenacity (*P*), extensibility (*L*), and strength (*W*) (Brites and Carrillo, 2001; Martinez et al., 2005). Except for one study on durum wheat (Liu and Rathjen, 1996), *Glu-A1c* always gave a lower value of SDS-sedimentation volume (SDSSV) than *Glu-A1a* and *b*. The *Glu-A1a* and *b* alleles were not consistently superior to one another for SDSSV (He et al., 2005; Liu et al., 2005; Ram, 2003) (Table 1.2). This result agrees with the assignment of glutenin quality score 3 to both

Glu-A1a and *b* (Table 1.1). Wheat with the *Glu-A1c* allele produces significantly lower dough extensibility than other *Glu-A1* alleles (Table 1.2) (Branlard et al., 2001; Eagles et al., 2002b; Eagles et al., 2004; He et al., 2005). The combination of inextensible dough, with low SDSSV, tolerance and Rmax of *Glu-A1c* suggests that *Glu-A1c* is an inferior allele for the breadmaking wheats (Table 1.2).

At the *Glu-B1* locus, more than 10 alleles were compared in the studies listed in Table 1.2. The most common ones are *Glu-B1a* (subunit 7), *b* (subunits 7+8), *c* (subunits 7+9), *d* (subunits 6+8), *e* (subunits 20*x*+20*y*), *f* (subunits 13+16), *h* (subunits 14+15), and *i* (subunits 17+18). For mixograph properties, the lines carrying *Glu-B1e* had the lowest MPT compared to other alleles (Table 1.2) (Bekes et al., 2001; Brites and Carrillo, 2001; Martinez et al., 2005). *Glu-B1b*, *c*, *h*, and *i* alleles had similar MPT in three populations (Table 1.2) (Brites and Carrillo, 2001; Liu et al., 2005; Tabiki et al., 2006). *Glu-B1b* had a higher MPV than *Glu-B1c* and *d* in two mapping populations (Huang et al., 2006; Martinez et al., 2005), whereas *Glu-B1e* and *d* had similar MPV in two RIL populations (Table 1.2) (Martinez et al., 2005). *Glu-B1b*, *e*, and *d* produced similar mixograph (midline) width at two minutes after peak (MRV, Fig. 1.1) in two RIL populations (Table 1.2) (Martinez et al., 2005) and also had similar values for the *P*, *L*, and *W* of alveograph in three mapping populations (Table 1.2) (Brites and Carrillo, 2001; Martinez et al., 2005). *Glu-B1a*, *d*, and *e* produced low Rmax values, whereas *Glu-B1b*, *f*, and *i* had high Rmax values in various genetic backgrounds (Table 1.2) (Bekes et al., 2006; Eagles et al., 2002b; Eagles et al., 2004; Vawser and Cornish, 2004). This agrees with Payne's quality scores based on SDSSV (Table 1.1). *Glu-B1d* had consistently higher SDSSV than *Glu-B1e* in different populations (Brites and Carrillo, 2001; Liu and Rathjen, 1996; Martinez et al., 2005; Ram, 2003). In agreement with Payne's quality score (Table 1.1), *Glu-B1b* produced

higher SDSSV than *Glu-B1c* in Chinese materials (He et al., 2005; Liu et al., 2005). However, opposite results were found in Canadian materials (Huang et al., 2006) and Indian materials (Ram, 2003). In general, *Glu-B1e* produces weak dough in various genetic backgrounds and should not be selected in breeding programs if strong dough is advisable.

In the studies listed in Table 1.2, the associations of allele variation and quality characteristics were analyzed at the *Glu-D1* locus, including the alleles *Glu-D1a* (subunits 2+12), *b* (subunits 3+12), *c* (subunits 4+12), *d* (subunits 5+10) and *e* (subunits 2+10). In general, there was no difference for protein content among the *Glu-D1* alleles (He et al., 2005; Liu et al., 2005; Luo et al., 2001; Tabiki et al., 2006). For mixograph properties, *Glu-D1d* had significantly ($P<0.05$) higher MPT than *Glu-D1a* both in mapping populations and cultivar collections (Bekes et al., 2001; Liu et al., 2005; Tabiki et al., 2006) with one exception in the study by Luo et al. (2001). Luo et al. (2001) found no significant difference for MPT between *Glu-D1d* and *a*. *Glu-D1a* showed higher MPV than *Glu-D1d* in two mapping populations (Brites and Carrillo, 2001; Huang et al., 2006). However, they had the same MPV in the study by Luo at al. (2001). *Glu-D1d* always produced higher Rmax values than *Glu-D1a* in various genetic backgrounds (Eagles et al., 2002b; Eagles et al., 2004; He et al., 2005; Ma et al., 2005). This confirmed the assignment of Payne's quality score to *Glu-D1a* with 2 and *d* with 4 (Table 1.1). The superiority of *Glu-A1d* to *a* for SDSSV were observed in various genetic backgrounds (He et al., 2005; Luo et al., 2001; Payne et al., 1987; Ram, 2003). For extensibility measured by extensigraph, *Glu-D1a* and *c* had a value higher or equal value than *Glu-D1d* in many studies (Bekes et al., 2006; Branlard et al., 2001; Eagles et al., 2002b; Eagles et al., 2004; Gupta et al., 1994; He et al., 2005). The quality superority of *Glu-D1d* to other alleles at the *Glu-D1* locus

might explain why its frequency has increased in the U.S. Great Plains wheat cultivars since 1991 (Shan et al., 2007).

LMW-GS and Wheat Quality

Although HMW-GS have major impacts on the quality characteristics discussed above, LMW-GS also play an important role. However, to achieve the same dough resistance, nearly twice as much LMW-GS was needed as compared with HMW-GS as determined by rheological measurements and baking tests on a micro-scale (Wieser and Kieffer, 2001). In general, LMW-GS are associated with dough resistance and extensibility (reviewed by D'Ovidio and Masci, 2004; Singh and Khatkar, 2005).

At the *Glu-A3* locus, the effects of alleles *a*, *b*, *c*, *d*, *e*, *f*, and *i* on various quality measurements have been compared (Table 1.2). For the mixograph property MPT, *Glu-A3a* and *e*, *c* and *d*, *d* and e, *c* and *e*, and *c* and *f* showed similar effects in independent populations (Table 1.2) (Liu et al., 2005; Luo et al., 2001; Martinez et al., 2005; Tabiki et al., 2006). *Glu-A3a* and *e*, and *d* and *c* had similar MPV in two populations (Luo et al., 2001; Martinez et al., 2005), whereas wheats with *Glu-A3d* gave a higher MPV than those with *Glu-A3e*, the null allele, in two RIL populations (Luo et al., 2001). Wheats with *Glu-A3a* and *e* had the same value of MRV in a double haploid population (Martinez et al., 2005). Wheats with *Glu-A3b* gave consistently higher Rmax than those with *Glu-A3e* in multiple populations (Bekes et al., 2001; Eagles et al., 2002a; Eagles et al., 2002b; Eagles et al., 2004; Ma et al., 2005; Vawser and Cornish, 2004). *Glu-A3d* and *c* were superior to *Glu-A3e* for Rmax in some studies (Bekes et al., 2006; Eagles et al., 2002b; Eagles et al., 2004; Gupta et al., 1994). Wheats with *Glu-A3d* had a higher SDSSV than those with *Glu-A3c* in collections of Chinese cultivars (He et al., 2005; Liu et al., 2005). *Glu-A3a* had the

same effects on SDSSV as *Glu-A3e* in a doubled haploid population (Martinez et al., 2005). There was no difference between *Glu-A3d* and *e* for SDSSV in a RIL population (Luo et al., 2001). In general, *Glu-A3e*, the null allele at the *Glu-A3* locus, exerts lower extensibility and strength than the other alleles. It is an allele that should be avoided in breeding wheat for good breadmaking quality.

Allelic variation at the *Glu-B3* locus has significant effects on dough rheology and breadmaking properties (reviewed by Martinez et al., 2005). It was shown that *Glu-B3b* had a lower MPT than *Glu-B3a* (Brites and Carrillo, 2001; Martinez et al., 2005), *c* (Brites and Carrillo, 2001), *j* (Brites and Carrillo, 2001) and *d* (Bekes et al., 2001), but a higher MPT than *Glu-B3h* (Huang et al., 2006). *Glu-B3b* had a lower MPV and MRV than *Glu-B3a* in two RIL populations (Martinez et al., 2005). *Glu-B3b* was found to be associated with lower alveograph values of *P*, *L* and *W* and SDSSV than *Glu-B3a* and *j* in three RIL populations (Brites and Carrillo, 2001; Martinez et al., 2005). *Glu-B3b* had a higher Rmax than *Glu-B3c* in a DH population (Ma et al., 2005) and three cultivar populations (Bekes et al., 2006; Eagles et al., 2002b; Eagles et al., 2004). Other *Glu-B3* alleles varied in their order for values of Rmax in different populations (Bekes et al., 2006; Eagles et al., 2002b; Ma et al., 2005). *Glu-B3b* was associated with higher extensibility measured by extensigraph than *Glu-B3g*, *c*, *j*, and *d* in several studies (Bekes et al., 2006; Branlard et al., 2001; Eagles et al., 2002b). *Glu-B3j*, the null allele associated with the 1BL.1RS translocation, decreased most of the quality characteristics, including protein content, MPT, mixing tolerance, extensibility, SDSSV, Rmax, stability, and loaf volume (Table 1.2). It is an allele that should be avoided in breeding programs.

Less allelic variation was observed at the *Glu-D3* locus compared to the *Glu-A3* and *Glu-B3* loci. *Glu-D3a* had a higher MPT than *Glu-D3b* (Luo et al., 2001), and

similar MPT compared with *Glu-D3c* (Table 1.2) (Bekes et al., 2001). On the other hand, *Glu-D3b* produced a higher MPV than those with *Glu-D3c* (Luo et al., 2001). The values of Rmax for three alleles at the *Glu-D3* locus varied in three independent populations, with the order of *Glu-D3b>c>a* (Bekes et al., 2006), *Glu-D3a>b>c* (Table 1.2) (Eagles et al., 2002b), and *Glu-D3c>b>a* (Eagles et al., 2004). There was no significant difference among four alleles, namely *Glu-D3a*, *b*, *c*, and *d*, in a collection of 162 French cultivars on extensibility and Zeleny SDSSV (Branlard et al., 2001).

Interactions among Glutenin Loci and Their Effects on Wheat Quality

In most cases, there is no consistent order of glutenin allelic affects on wheat quality (Table 1.2). This phenomenon indicates the environmental dependence of glutenin effects on wheat quality (see the review section of 'Environment and Wheat Quality' of this literature review), and the lack of independence among the six glutenin loci and among glutenin loci and non-storage protein loci. With a huge population size (952 wheat lines), Eagles et al. (2002b) were able to study all two-way interactions among the 18 most frequent glutenin alleles. The results showed that epistasis was important for both Rmax and extensibility. The epistasis terms explained a further 7.6% and 6.0% of the variation of Rmax and extensibility, respectively, in addition to the variation explained by additive glutenin effects. In a population of 172 DH lines, Bekes et al. (2001) associated different combinations of glutenin loci with some mixograph properties. Their results showed that some combinations of glutenin loci gave extra high or low mixograph property values. Kolster et al. (1991) observed significant interactions between *Glu-D1* and *Glu-A1* and *Glu-B1* loci. In the presence of *Glu-D1a* or *b*, the rank of the loaf volume of lines with *Glu-A1a*, *b*, and *c* and *Glu-B1c*, *b*, and *d* changed. Interestingly, Ma et al. (2005) observed not only interactions

among glutenin loci, but also among glutenin loci and non-storage protein loci in a DH population segregating for all glutenin loci. For example, the most significant interaction for extensibility was found between the *Glu-B3* locus and a microsatellite marker, *gwm044*, on chromosome 7D, but the reason for this epistasis was not known.

Glutenin Quantity and Wheat Quality

Even though it is convincing that glutenin subunits have a different impact on wheat quality (as show in Table 1.1 and Table 1.2), some have argued that it is the quantity and not the composition of glutenin subunits that is important in determining breadmaking quality of wheat (reviewed by Singh and Khatkar, 2005). For example, in a RIL population from a cross between parents with high and low protein contents, one of the RILs possessing *Glu-D1a* had superior breadmaking quality compared to other lines with *Glu-D1d*, only because that line had high protein content (Garg et al., 2006). In the same population, Garg et al. (2006) found that grain protein content above a certain minimum value was a relatively more important determinant of breadmaking quality than *Glu-D1d*. Many cultivars of good breadmaking quality possess *Glu-D1a* (Luo et al., 2001), which is generally considered an inferior allele. Furthermore, wheat lines with the 1BL.1RS wheat-rye translocation consistently produced weak, sticky doughs irrespective of their HMW-GS composition (reviewed by Luo et al., 2001).

In summary, glutenin loci have significant impacts on wheat quality. A better understanding of the effects of glutenin alleles on wheat quality depends on an understanding glutenin function in various genetic backgrounds and various growing environments. Some glutenin alleles identified as good in one population might not be as favorable in other populations. However, some findings hold true for most genetic backgrounds. For example, the null alleles at glutenin loci, such as *Glu-A1c*, *Glu-A3e*

and *Glu-B3j*, are often associated with poor breadmaking quality. Therefore, once the effects of glutenin loci are identified in a pool of germplasm, they are relatively consistent and can be used as selection markers in a breeding program. For example, the results from multiple studies in Australia were similar (Bekes et al., 2006; Eagles et al., 2002a; 2002b; 2004; Gupta et al., 1994). Wheat quality is a series of complex traits controlled by loci distributed across the whole wheat genome and strongly influenced by growing environments. Thus, it is not surprising to see complex results from many similar studies.

Allelic Diversity at Glutenin Loci

Proteins are essential to many functional properties of cereal grains, and interaction between the protein components is a principal operative mechanism by which such functions occur. Gluten, the viscoelastic mass that gives wheat dough its unique properties, has been a research target for more than two centuries (see review section of 'Glutenin and Wheat Quality' in this literature review). Without the range of interactions essential to gluten structure, it would not be possible to produce the range of leavened breads, noodles and related products that are characteristic of wheat-based foods (reviewed by Singh and Khatkar, 2005).

General Description of the Analysis of Glutenin

Each wheat cultivar contains three to five HMW-GS and three to six LMW-GS with different electrophoretic mobility. Based on their mobility in the gel, glutenin subunits were classified and standard cultivars were identified (Gupta et al., 1994; Payne et al., 1987). In many breeding programs, crosses were made between parents with different HMW-GS and progenies were screened with markers for the HMW-GS (Gale, 2005).

There is a growing body of literature on the genetic characterization of glutenin proteins in hexaploid wheats as a result of improved methods for protein fractionation and greater availability of genetic resources (Nakamura, 2000a). The following section is a review of some of the investigations (listed in Table 1.3.1) on different materials from various areas and countries (Table 1.3.2 and 1.3.3).

Table 1.3.1: List of papers surveyed for glutenin allelic frequency in wheat cultivars from various areas and countries.

Paper	References	Origin and # of entries tested
Shan	Shan et al., 2007	101 U.S. germplasm
Gray_1	Graybosch, 1992	125 U.S. soft winter wheat
Gray_2	Graybosch, 1992	164 U.S. hard winter wheat
Branlard	Branlard et al., 2003	200 French germplasm
Redaelli	Redaelli et al., 1997	55 U.S. white wheat
Nagamine	Nagamine et al., 2000	85 Japanese common wheat
Naka_1	Nakamura, 2000	274 Chinese wheat
Naka_2	Nakamura, 2000	131 Japanese wheat
Naka_3	Nakamura, 2001	174 Japanese landraces
Naka_4	Nakamura et al., 1999	131 Japanese Norin wheats
Shahnejat	Shahnejat-Bushehri et al., 2006	95 Iranian bread wheats
Bahraei	Bahraei et al., 2004	43 Iranian current bread wheat
Gregova_1	Gregova et al., 1997	24 old Slovakian wheat
Gregova_2	Gregova et al., 1997	34 modern Slovakian wheat
Hua	Hua et al., 2005	282 Xinjiang wheat (China)

Table 1.3.2. Comparison of allele frequencies (%) for the *Glu-1* loci reported in 15 surveys (see Table 1.3.1 for list of papers).

Paper			Shan	Gray_1	Gray_2	Branlard	Redaelli	Nagamine	Naka_1	Naka_2	Naka_3	Naka_4	Shahnejat	Bahraei	Gregova_1	Gregova_2	Hua	overall
Glu-A1	*a*	1	21.2	53.6	18.4	15	40		5.2	12.2	4.6	12.2	15.7	22	54.2	29.4	2.1	14.79
	b	2*	76.1	37.2	76.1	15.5	34.55	29.4	14.4	13.7	8.6	13.7	37.7	59	4.2	8.8	22.3	29
	c	Null	2.7	8.7	4.9	69.5	25.45	70.6	80.4	74.1	86.8	74.1	46.4	19	41.6	61.8	75.5	57.64
		1*		0.8	0.6													0.1
Glu-B1	*a*	7		5.7		20.5	12.73		12.3		1.7		0.09	7			3.5	5.98
	b	7+8	33.3	32.8	27.2	30.5	10.91	89.4	71.9	83.2	94.1	83.2	52	39	8.3	20.6	90.8	61.77
	c	7+9	47.7	30.8	64.2	19	41.82	8.2	8.1	12.9	1.2	12.9	12.3	22	76	70.6	2.5	20.58
	an	6					12.73											0.36
	d	6+8	0	15	5.5	24.5	8.18		3.9	0.8	1.2	0.8	0.9		8.3	8.8	0.7	5.41
	e	20x+20y	7.7			0.5	8.18	2.4	1.4	1.5	0.6	1.5	1.8	2	4.2			1.49
	f	13+16		10.9	1.8	0.5	3.64		0.7				4.4	5				1.49
	g	13+19		3.2	1.2				0.4	0.8		0.8	41.8					0.58
	h	14+15											0.9	2				0.1
	i	17+18	6.3	1.6		4	1.82		0.7	0.8	1.2	0.8	18.4	23	4.2		1.4	2.13
	k	22							0.7								1.1	0.26
	w	6*+8*	5															0.29
	s	18*				0.5												0.05
	ah	null												0.1				0
Glu-D1	*a*	2+12	11.3	57.3	29.6	53	67.27	41.2	84.6	55	70.1	55	61.4	57	20.8	26.5	72	58.34
	b	3+12	7.2	10.1	8.3	5	12.73	58.8	1.1	1.5		1.5	0.8		37.5			6.15
	c	4+12				7			2.1	6.9	1.2	6.9					0.4	2.13
	d	5+10	80.6	31.9	61.4	34.5	20		10.5	1.5	3.4	1.5	30.7	41	41.7	73.5	1.1	22.83
	e	2+10	0.9														2.1	0.36
	e	2+11			0.6	0.5												0.1
	f	2.2+12							1.8	35.1	25.3	35.1						7.35
	bp(t)	2.6+12															24.5	3.6
		2***+12'											5.3	2				0.31
	i	null		0.8									0.8					0.1
	m	10											0.8					0.05

Table 1.3.3. Comparison of allele frequencies (%) for the *Glu-3* loci reported in three surveys (see Table 1.3.1 for list of papers).

Paper		Shan	Branlard	Redaelli	overall
Glu-A3	*a*	12.6	44.5	43.6	35.7
	b			27.2	4.2
	c	46.8	1.5		15.4
	d	13.5	34.5	10.9	25.3
	e	23.4	1.0	7.3	9.0
	e/f		18.5		10.4
	g	3.6			1.1
Glu-B3	*a*	5.4	0.5		2.0
	b	12.2	10.5	36.4	15.3
	b'		4.0		2.2
	c	4.5	8.0		5.9
	c'		5.5		3.1
	d	0.9	3.5	61.8	11.8
	e	9.0			2.8
	f	12.6	7.0		7.9
	g	41.0	49.0		40.3
	h	5.4	1.0		2.2
	i	9.0	4.5		5.3
	j		6.5		3.7
Glu-D3	*a*	30.2	11.5	62.7	25.6
	b	27.0	10.0		14.0
	c	26.6	78.0	37.3	57.9
	d	6.3	0.5		2.2
	e	9.0			2.8

Glutenin Allele Distribution in Different Epochs and Regions or Countries

In these surveys, the allele frequencies varied among the years of release and the countries where they were developed (Table 1.3.1) (Branlard et al., 2003; Caballero et al., 2004; Graybosch, 1992; Gregova et al., 1997; Gupta et al., 1994; Nagamine et al., 2000; Nakamura, 2000b; Nakamura, 2001; Nakamura et al., 1999; Redaelli et al., 1997; Shahnejat-Bushehri et al., 2006; Shan et al., 2007). HMW-GS 1, 2*, and null are encoded by *Glu-A1a*, *Glu-A1b*, and *Glu-A1c*, respectively. The frequency of subunit 1 ranged from 0 to 54% in materials from different countries (Table 1.3.2). No entry in a group of 85 modern cultivars from southern Japan had subunit 1, whereas 30 out of 55 cultivars from Slovakia had subunit 1 (Gregova et al.,

1997; Nagamine et al., 2000). The frequency of subunit 2* varied from 4.2 to 76.1% in the surveys. The lowest frequency was from the survey of Slovakian wheat, and the highest was from the survey of U.S. cultivars released after 1991 (Gregova et al., 1997; Shan et al., 2007). The mean frequency of the null allele *Glu-A1c* was 57.6% with a range of 2.7 to 86.8%. The lowest frequency was observed in current hard winter wheat cultivars from the U.S. Great Plains, and the highest frequency was from Japanese landraces (Nakamura, 2001; Shan et al., 2007).

There were 14 alleles (*Glu-B1a* to *Glu-B1k*) on chromosome 1B in the materials that were surveyed (Table 1.3.2). The mean frequency of these alleles ranged from nearly 0 to 61.8%, with *Glu-B1b* (subunits 7+8, 61.8%) and *Glu-B1c* (subunits 7+9, 20.58%) having the highest frequencies. Other alleles were present in less than 6% of the tested materials.

Eleven alleles were found at the *Glu-D1* locus (Table 1.3.2). The frequency of these alleles varied from 0.05 to 58.34%. The most common alleles were *Glu-D1a* (subunits 2+12) and *Glu-D1d* (subunits 5+10) with mean frequencies of 58.34% and 22.83%, respectively. Glutenin subunits 2.2+12 and subunits 2***+12' were only observed in Japanese materials (Nagamine et al., 2000; Nakamura, 2000a; Nakamura et al., 1999).

Due to the complexity of the LMW-GS patterns with some of the bands overlapping in one dimensional SDS-PAGE, only a few studies reported surveys of LWM-GS components in large collections of wheat cultivars (Branlard et al., 2003; Redaelli et al., 1997; Shan et al., 2007). At the *Glu-A3* locus, there were seven alleles identified in 358 cultivars (Table 1.3.3). These alleles were *a*, *b*, *c*, *d*, *e*, *e/f*, and *g*. Their frequency ranged from 1.1 to 35.7%, with *Glu-A3a* the most common. At the *Glu-B3* locus, 12 alleles were identified in 358 cultivars, with *Glu-B3g* the most

frequent (Table 1.3.3). Relatively fewer alleles were observed at the *Glu-D3* locus in 358 cultivars. They were *a*, *b*, *c*, *d* and *e* with allele frequencies of 2.2, 2.8, 14.0, 25.6 and 57.9%, respectively (Table 1.3.3).

Reasons for Glutenin Allele Distribution in Different Epochs and Regions or Countries

The frequency variation of the glutenin alleles is largely related to the epochs in which cultivars were released. In all the studies that compared the glutenin patterns of historical and modern cultivars, older genotypes had higher genetic diversity at glutenin loci than current cultivars (Graybosch, 1992; Gregova et al., 1997; Shan et al., 2007). Continuously intensive selection on wheat quality in the breeding process decreased allelic variability of glutenin patterns, especially at the *Glu-1* loci, and increased uniformity of modern cultivars (Gregova et al., 1997; Shan et al., 2007). This process is partially due to the breeding effort and spread of high-yielding cultivars in the last three decades. The breeding progress in modern cultivars has been based on the use of a limited number of common ancestors. For example, in the survey of Shan et al. (2007), 11 out of 101 cultivars had 'Jagger' in their pedigree and seven out of 101 cultivars had 'Abilene' in their pedigree. Eight out of 32 Slovakian modern cultivars had in their parentage the cultivar 'Mironovskaja 808' (Gregova et al., 1997). The trend toward decreasing genetic diversity of glutenin loci is also associated with breeding efforts to meet the breadmaking quality needs in recent decades (Gregova et al., 1997). This is evident from the composition of HMW glutenin alleles, mainly at the *Glu-D1* locus. The ratio of subunits 5+10, with a positive effect on quality, to subunit pairs with negative effects (2+12, 3+12) changed

from about 2:3 to 4:1 in the Slovakia cultivars and from 3:2 to 4:1 in U.S. hard winter wheat cultivars (Gregova et al., 1997; Shan et al., 2007).

The glutenin allele frequency varied in the collections of wheat cultivars from different countries (Table 1.3). This is largely due to the diverse requirements for flour properties for different end-products consumed in those countries. For example, the frequencies of the null allele at the *Glu-A1* locus and the subunits 5+10 at the *Glu-D1* locus were 27.1% and 78.7% in the 645 U.S. and European wheats, respectively, whereas they were 78.7% and 4.9%, respectively, in 795 Asian wheats. It would be reasonable to consider Europe and America as a bread-consumption zone and Asia as a noodle-consumption zone, where noodles are made from hexaploid wheats, rather than tetraploid durum wheats, which are preferred in Western countries. Stronger dough is required for breadmaking, whereas intermediate dough strength is needed for noodle production (Nakamura, 2000a). The major end-product for Iranian wheats is flat bread, which requires moderate dough strength, in contrast to pan breads (the major product for U.S. wheats), which require higher dough strength. This might explain why the ratio of subunits 5+10 to 2+12 was 1:2 in Iranian wheat and 8:1 for the U.S. wheats (Shahnejat-Bushehri et al., 2006; Shan et al., 2007). HWM glutenin subunits 2***+12' at the *Glu-D1* locus are unique to Iranian wheat and are correlated with good flat breadmaking quality (Shahnejat-Bushehri et al., 2006). Similarly, the subunits 2.2+12 was associated with good Japanese noodle-making quality, so the wheat lines having subunits 2.2+12 are preferred, particularly in southern Japan (Nakamura, 2000a). This might be the reason that the subunits 2.2+12 was relatively frequent in Japanese cultivars, whereas it is hard to find in materials from other countries (Nakamura, 2000a). The prevalence of the null allele *Glu-A1c* at the *Glu-A1* locus of 95 Iranian wheats was believed to be due to the Iranian baking requirement

for medium-elastic dough rather than strong gluten that results in less extensible dough (Shahnejat-Bushehri et al., 2006).

The high frequency of some alleles in the entries surveyed may be the result of pedigree relatedness among cultivars. For example, in the absence of any selective advantage of subunit 2* over subunit 1 at the *Glu-A1* locus among hard red winter wheat, the increased frequency of subunit 2* can only be attributed to a more frequent use of subunit 2*-carrying parents in wheat breeding programs (Graybosch, 1992). Although most cultivars were not selected based on protein pattern of SDS-PAGE, the high mean frequencies of *Glu-D1d* (34.5%), *Glu-A3a* (44.5%) and *Glu-A3d* (34.5%) in French wheats may be due to their positive effects on dough properties (strength and extensibility) as well as on the Zeleny value (Branlard et al., 2003).

The allelic identification of HMW and LMW glutenin loci in wheat cultivars revealed considerable diversity, especially among the materials from different countries. Analyzing glutenin patterns in wheats from different regions together with the knowledge of end-products in those regions improves the understanding of the functions of glutenin alleles.

QTL Mapping of Wheat Quality characteristics

Wheat quality is a very important and complex collection of traits and has been the target of wheat breeding programs for more than a century. Several empirical procedures have served the wheat industry well for selecting new wheat cultivars for favorable quality characteristics, such as near-infrared spectroscopy (NIR), mixograph, extensigraph, farinograph, SDS-sedimentation test, milling test, noodle-making test, steam breadmaking test, and baking test. These small-scale tests are suitable for meeting the increased and improved requirements of the industry and providing the basis for many studies in cereal science (Bekes et al., 2006). Although the hexaploid nature of bread wheat complicates QTL mapping in wheat, there is a growing body of literature on identifying QTLs that associate with the characteristics of small-scale wheat quality tests. The following is a review of some of the QTL mapping studies on a variety of measurements of wheat quality (Table 1.4).

Grain Protein Content

Grain protein content is a trait of primary importance for bread wheat quality. Due to its importance to breadmaking and its simplicity of measurement, the genetic components of grain protein content have been extensively investigated in bread wheat (Breseghello et al., 2005; Campbell et al., 2001; Groos et al., 2003; Huang et al., 2006; Ma et al., 2007; McCartney et al., 2006; Nelson et al., 2006; Prasad et al., 2003; Prasad et al., 1999)

Table 1.4. QTLs for wheat quality characteristics from the literature.

Reference	Population†	# of lines	Traits‡	QTL location
Ma et al., 2005	Cranbrook × Halberd (DH)	160	Dough extensibility	5A (*PAAG/MCCC4*), *Glu-1*, *Glu-3*
Ma et al., 2005	Cranbrook × Halberd (DH)	160	Rmax	*Glu-1*, *Glu-3*
Bekes et al., 2006	5 populations (DH)	174	Dough extensibility	*Glu-1*, *Glu-3*
Bekes et al., 2006	174 cultivars and 220 breeders lines	174	Rmax	*Glu-1*, *Glu-3*
Ma et al., 2007	W21MMT70 × Mendos (DH)	92	Water absorption	*B1* on 5AL (Awned locus)
Ma et al., 2007	W21MMT70 × Mendos (DH)	92	Protein content	*B1* on 5AL (Awned locus)
Perretant et al., 2000	Courtot × Chinese Spring (DH)	187	Hardness	5DS, 1A, 6D
Perretant et al., 2000	Courtot × Chinese Spring (DH)	187	Protein content	1B, 6A
Perretant et al., 2000	Courtot × Chinese Spring (DH)	187	W of alveograph	1A, 5D, 3B
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Kernel texture	5DS (*Ha*)
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Protein content	6DS(*Gli-D2*), 2DS(*Ppd1*)
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Dough strength	5DS(*Ha*)
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Dough tenacity	1BS(*Xgwm550*)
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Dough extensibility	1AS(*Glu-A3*)
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Dough viscosity	5DS
Nelson et al. 2006	ITMI, W7985 × Opata 85 (RILs)	114	Mixing tolerance	*Gli-B1*
Kuchel et al., 2006	Trident × Molineux (DH)	182	Flour protein	1B, 6A, 6D, 7A, 7D
Kuchel et al., 2006	Trident × Molineux (DH)	182	Flour color	7B, 1BS(*Glu-B3*), 1AS (*Glu-A3*)
Kuchel et al., 2006	Trident × Molineux (DH)	182	Rmax	1A (*Glu-A3*), 1B(*Glu-B3*)
Kuchel et al., 2006	Trident × Molineux (DH)	182	Dough extensibility	1A (*Glu-A3*)
Kuchel et al., 2006	Trident × Molineux (DH)	182	Water absorption	1A (*Glu-A3*), 1B(*Glu-B3*)
Kuchel et al., 2006	Trident × Molineux (DH)	182	Loaf volume	2A, 3A
Kuchel et al., 2006	Trident × Molineux (DH)	182	Flour paste viscosity	2B, 7A and 7D

Reference	Population†	# of lines	Traits‡	QTL location
Prasad et al., 2003	WL711 × PH132 (RIL)	100	Protein content	2BL, 2DL, 3DS (*Xgwm1204*), 7AS, 3DS (*Xgwm892*)
Groos et al., 2003	Reman × Recital (RIL)	194	Protein content	2A, 3A, 4D and 7D
Gross et al., 2004	Reman × Recital (RIL)	194	Hardness	1A (*Gli-A1/Glu-A3*), 5B, 6A, 6D, *gwm130*
Gross et al., 2004	Reman × Recital (RIL)	194	Dough strength (w)	3A, 5A, 7D
Gross et al., 2004	Reman × Recital (RIL)	194	Tenacity (p)	1B, 3B, 4A, 5A, 6A, 6D, 7D.
Gross et al., 2004	Reman × Recital (RIL)	194	Extensibility (L)	3B, 4B
Gross et al., 2004	Reman × Recital (RIL)	194	Protein content	3A, 4D, 7D
Prasad et al., 1999	PH132 × WL711 (RIL)	100	Protein content	2DL (*wmc41*)
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Protein content	2D (*Xwmc453*), 4D (*Xwmc52*), 7B(*Xwmc662*)
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Mixing development time	1B (*Glu-B1*), 1D(*Glu-D1*), 3B(*Xbarc164*)
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	MPV	1B*(Xgdm126b)*,4D*(Xwmc52)*,1D*(Glu-D1)*
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Energy to peak	1B*(Glu-B1)*,3B*(Xbarc164)*,*1D(Glu-D1)*
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	MLS	1D*(Glu-D1)*,4D*(Xwmc52)*
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Peak bandwidth	1D*(Glu-D1)*
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	MRS	1D*(Glu-D1)*, 4D*(Xcfd71a)*
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Total energy	1B(*Xgdm126b*), 5D(*Xcfd57*)
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	Bandwidth energy	1B(*Xgdm126b*), 5D(*Xcfd57*)
Huang et al., 2006	AC Karma × 87E03-S2B1 (DH)	185	SDSSV	1B(*STS-Bx7.2*),5D(*Xgwm639a*), 2D(*Xwmc453*)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Softness equivalent	1AS(*Xbcd808*), 1BL(*Xgwm403a*), 1AD(*Xgcat7*)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Flour protein content	2AS(*Xbcd855*), 2BL(*Xggat12*), 4B(*Xggat27*), 6B(*Xcdo524*), 4B(Xwmc48c)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Quadrumat flour yield	2BL(*Xccat8*), 3ABS(*Xccag4*), 2DS(*Xccac3*), 4B(*Xggta12*), 5BL(*Xwmc235*)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Alkaline water retention capacity	4B(*Xgwm6*)

Reference	Population†	# of lines	Traits‡	QTL location
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	MPT	1BS(*Xwmc216*)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	MPV	1BS(*Xwmc216*), 3ABS, 2AS
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Mixograph curve area	1BS(*Xwmc216*)
Breseghello et al., 2005	AC Reed (soft) × Grandin (DH)	101	Mixograph area under curve	1BS(*Xwmc216*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Grain protein content	2B(*Xgwm210–Xwmc25*), 4D(*Xwmc617–Xwmc48*), 4A(*Xgwm397–Xwmc650*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Flour protein content	1B(*Xwmc611*), 6B(*Xwmc398–Xgwm88*), 6A(*Xwmc672*), 4D(*Xwmc617–Xwmc48*), 2B(*Xgwm210–Xwmc25*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Flour yield	1B(*Glu-B1*), 7D(*Xgwm130*), 3B(*Xwmc446*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Particle size index	7D(*Xgwm130*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	MPV	4D(*Xwmc617–Xwmc48*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	MPT	1B(*Glu-B1*), 4D(*Xwmc617–Xwmc48*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Energy to peak	1B(*Glu-B1*), 4D(*Xwmc617–Xwmc48*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	MPW	2B (*Xgwm210–Xwmc25*), 4D (*Xwmc617–Xwmc48*), 7D (*Xgwm130–Xwmc405*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Total energy	1B(*Glu-B1*), 2B(*Xgwm210–Xwmc25*), 4D(*Xgwm608*), 7D(*Xgwm130*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Band width energy	1B(*Glu-B3*),*1B*(*Xwmc694*), 2A(*Xgwm294–Xwmc658*), 2B(*Xgwm210–Xwmc25*), 6A(*Xwmc748*), 7D(*Xgwm130–Xwmc405*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	MLS	1B(*Glu-B1*), 4D(*Xwmc617–Xwmc48*), 7B(*Xgwm400–Xwmc182*), 7D(*Xgwm130*)
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Absorption	2B(*Xwmc764–Xwmc661*), 4D(*Xwmc473*), 6B(*Xwmc398–Xgwm88*), 7D(*Xgwm130*)

Reference	Population†	# of lines	Traits‡	QTL location
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	MRS	1B*(Xgwm131–Glu-B1)*, 4D*(Xwmc617–Xwmc48)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Dough development time	1B*(Glu-B1)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Time to stability	1B*(Xbarc137)*, *4B(Xwmc617–Xgwm540)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Mixing tolerance index	1B*(Xgwm131–Glu-B1)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Time to breakdown	1B*(Xgwm131–Glu-B1)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Proof height	3D*(Xwmc492)*, 4D*(Xwmc617–Xwmc48)*, 6B*(Xwmc398-Xgwm88)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Loaf height	4D*(Xwmc617–Xwmc48)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Loaf volume	4D*(Xwmc617–Xwmc48)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	Baking mix time	1B*(Glu-B3)*, 2A*(Xgwm71.1)*
McCartney et al., 2006	RL4452 × AC Domain (DH)	182	SDSSV	1B*(Xgwm131–Glu-B1)*, 2A*(Xgwm296)*, 6A*(Xbarc23–Xwmc398)*
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Zeleny sedimentation volume	1B (*Glu-1*), 2A, 5A, 5D, 1D, 5B
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Protein content	1B, 5A, 7B, 3B, 4A, 5B, 6B, 7A, 7D
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Kernel hardness	2A, 5A, 3B, 4A, 4D, 6B, 7A, 7B, 7D
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Tenacity (P)	2A, 5A, 7B, 5B, 7D
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Extensibility (L)	2B, 3B, 7B
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Baking strength (w)	1B, 3DL, 3A, 5B, 5D
Zanetti et al., 2001	Forno (wheat) × Overkulmer (spelt) (RIL)	226	Elasticity index (Ie)	2D, 5D
Zanetti et al., 2001	Forno × Overkulmer(spelt) (RIL)	226	Configuration ratio (P:L)	1A, 1B, 2A, 4A, 5D, 7A, 7B
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Flour yield	3S(ksuG53), 5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Flour protein	1A(*abc156b*), 2B(*cdo1445b*), 2B(*bcd1688a*), 2B(*bcd1307a*), 7AL:7AL, 7BL, 7DL(ksuG12b)

Reference	Population†	# of lines	Traits‡	QTL location
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Softness equivalent	5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	MPT	1DL(*Glu-Dy1*), 4AL:4AL, 7AS, 7DS(*ksuERI*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Bread mixing time	1B(*ksuF43c,e*), 1DL(*Glu-Dy1*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	MPV	1AL(*GluAx1*), 1AL(*bcd592a*), 1BL(*Glu-By1*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Mixograph tolerance	1A(*mwg938a*), 1AL(*mwg695Hda*), 1BL(*Glu-By1*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Dough water absorption	3L(*mwg69*), 5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Damaged starch	4DL:4BL, 4DL, 5AL(*bcd1431b*), 5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	AWRC	4DL:4BL, 4DL, 5AL(*bcd1431b*), 5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Cookie diameter	5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Cookie top grain	3B(*cdo718*), 5BL:5AL, 5BL, 5DL(*cdo412*); 5DS:5AS, 5BS, 5DS(*PinB*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Loaf volume	2B(*Pkaba1c*), 2B(*rz753RV*), 5L(*bcd873b*), 7AL:7AL, 7BL, 7DL(*wg466*)
Campbell et al., 2001	NY6432-18 (soft) × Clarks Cream (DH)	78	Crumb grain	4DL:4BL, 4DL, 5AL(*bcd402*)

† NIL, near isogenic line; RIL, recombinant inbred line; DH, doubled haploid.

‡ MPT, mixograph (midline) peak time; MLW, mixograph (midline) width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph (midline) band width at MPT; MPV, mixograph midline peak height; MRW, mixograph (midline) band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; MLS, mixograph (midline) left slope; MRS, mixograph (midline) right slope; SDSSV, sodium-dodecylsulfate sedimentation volume; W, alveograph properties, strength; L, alveograph properties, extensibility; P, alveograph properties, the tenacity.

Grain protein content was found to be influenced by both environmental and genetic factors, so different QTLs were identified in different environments for the same set of wheat lines (Nelson et al., 2006; Prasad et al., 2003). For example, Prasad et al. (2003) identified 13 QTLs for grain protein content in a set of 100 RILs grown in five environments (Table 1.4). None of these QTLs was detected consistently in all the environments indicating a significant environmental interaction effect on grain protein content. Among these 13 QTLs, four QTLs on 2BL, 2DL, 3DS (marker *Xgwm456*), and 7A were identified either in more than one environment or by multiple mapping methods. Another QTL was on 3DS (marker *Xgwm892*) and was associated with mean grain protein content in all environments. These five QTLs were considered to be important and they were verified in NIL populations. In the study of Groos et al. (2003), 10 chromosomal regions were associated with grain protein content in a population of 194 RILs of a cross between parents with high and low protein content. These QTLs were on chromosomes 1A, 2A, 3A, 3B, 4A, 4D, 5B, 6A, 7A, and 7D. Among them, QTLs on chromosomes 2A, 3A, 4D, and 7D were identified in at least four of the six locations and were called 'stable' QTLs.

The size of QTL effects varied in different mapping populations. The largest QTL for grain protein content was found on chromosome 6B, which accounted for 66% of the variance for grain protein content in a cross of tetraploid wheat (Table 1.4) (Joppa et al., 1997). A major QTL for grain protein content was mapped to chromosome 4D in a DH population and explained 29.8% of the variance for grain protein content (McCartney et al., 2006). Two smaller QTLs in this population were mapped to chromosomes 2B and 4A, each accounting for around 6% of the variation for grain protein content (Table 1.4). Zanetti et al. (2001) found a quite large QTL (R^2=25%) for grain protein content on chromosome 5A in a set of 226 RILs from a

cross between bread wheat and spelt wheat (Table 1.4). In the study of Prasad et al. (1999), a QTL for grain protein content on chromosome arm 2DL was associated with SSR marker *wmc41*, which explained 18.7% of the total variation of 100 RILs from a cross between two bread wheats with high and low protein content (Table 1.4). In the study of Groos et al. (2003), each of five 'stable' QTLs explained about 10% of the phenotypic variance of grain protein content, whereas other QTLs each explained less than 5% of the variance for grain protein content.

Some QTLs were mapped in several populations, whereas others only mapped in one population or one environment. As expected, QTLs for protein content were mapped to the storage protein loci located on chromosome group 1 (*Glu-1*, *Glu-3*, *Gli-1* and *Gli-3* loci) and group 6 (*Gli-2* loci) in multiple populations (Breseghello et al., 2005; Campbell et al., 2001; Joppa et al., 1997; McCartney et al., 2006; Nelson et al., 2006; Perretant et al., 2000; Zanetti et al., 2001). Beside storage protein loci, other significant QTLs for grain protein were identified in multiple populations. For example, a highly significant QTL for grain protein content was found on 2AS in several populations (Breseghello et al., 2005; Groos et al., 2003; Prasad et al., 2003). It explained around 20% of the phenotypic variance of protein content of two populations (Breseghello et al., 2005; Prasad et al., 2003) and around 10% of the variation in another population (Groos et al., 2003) (Table 1.4). A QTL on chromosome 7D explained around 10% of the variation for grain protein content (Groos et al., 2003). The same region was also detected as a QTL for protein content in the populations of Kuchel et al. (2006) (R^2=13%), Campbell et al. (2001) (R^2=20%), and Zanetti et al. (2001) (R^2 is small) (Table 1.4). Zanetti et al. (2001) described a major QTL on 5A for their population which was consistent in all the

environments they investigated. However, this QTL was a minor and inconsistent one in the population of Groos et al. (2003) (Table 1.4).

QTLs for agronomic traits have been mapped to the same regions as grain protein content. For example, one major QTL for grain protein content was mapped to chromosome 4D and was in the same interval as the largest QTL for plant height (McCartney et al., 2006). This QTL was believed to be due to *Rht-D1*, a major gene controlling plant height (Hedden, 2003). This region was also associated with baking quality and dough rheology characteristics in their population. The peak significance level for QTL for grain protein content was found close to the awned locus (*B1*) (Ma et al., 2007). This QTL explained 7% and 19% of the variance in protein content from two environments.

A significant negative correlation between grain protein content and yield was observed in many studies (reviewed by Groos et al., 2003). However, Groose et al. (2003) observed that favorable alleles for grain protein content and yield co-localized on some chromosomal regions, such as chromosome 3A, 5B, 7A, and 7D. This observation contradicts the hypothesis that QTLs for high grain protein content are indirect QTLs for low yield. The absence of a strong negative effect due to linkage suggested that it would be possible to improve these two economically important traits in the same breeding scheme (Groos et al., 2003).

Mixograph properties

The mixograph is one of the most important methods to test wheat quality in breeding programs (see the review section on 'Rheology Test: the Mixograph' in this literature review). QTLs that control some of the mixograph properties have been identified in several studies (Breseghello et al., 2005; Campbell et al., 2001; Huang et al., 2006; McCartney et al., 2006). In all these studies, the glutenin loci on group 1

chromosomes had significant effects on mixograph properties when the research populations were segregating at glutenin loci. The *Glu-B1* locus was associated with MPT in three populations (Breseghello et al., 2005; Huang et al., 2006; McCartney et al., 2006) and the *Glu-D1* locus was associated with MPT in two populations (Campbell et al., 2001; Huang et al., 2006). These different findings were mainly due to segregation of glutenin alleles in the research populations. For example, lines with subunits 5+10 at the *Glu-D1* locus had longer MPT than those with subunits 2+12 (Campbell et al., 2001; Huang et al., 2006). The subunits 7 (overexpressed)+8 at the *Glu-B1* locus had longer MPT than subunits 7+9 at the *Glu-B1* locus (Huang et al., 2006). Mixograph (midline) peak value (MPV, Fig. 1.1) was reported to be associated with the *Glu-B1* locus (subunits 7+8 vs. 7+9) (Campbell et al., 2001; Huang et al., 2006) and the *Glu-A1* locus (subunit 1 vs. 2) (Campbell et al., 2001) and the *Glu-D1* locus (subunits 2+12 vs. 5+10) (Huang et al., 2006). Mixograph (midline) left width (MLW, Fig. 1.1) was mapped to the *Glu-D1* locus (Huang et al., 2006). Mixograph (midline) peak width (MPW, Fig. 1.1) was associated with the *Glu-D1* locus (subunits 2+12 vs. 5+10) (Huang et al., 2006). Mixograph slope after peak was mapped to the *Glu-B1* locus (subunits 7+8 vs. 7+9) (Campbell et al., 2001) and the *Glu-D1* locus (subunits 2+12 vs. 5+10) (Huang et al., 2006). Mixograph bandwidth energy was related to the *Glu-B3* locus (McCartney et al., 2006). Mixograph energy to peak was associated with the *Glu-B1* locus (subunits 7+8 vs. 7+9) (Campbell et al., 2001; McCartney et al., 2006) and the *Glu-D1* locus (subunits 2+12 vs. 5+10) (Huang et al., 2006).

Beside these significant QTLs that mapped to known storage protein loci, some QTLs for mixograph properties were mapped to other chromosome regions. For example, MPT was mapped to chromosome 3B (Huang et al., 2006), 4A (Campbell et

al., 2001), and 4D (McCartney et al., 2006). The propertiy, MPV was associated with regions on chromosome 4D (Huang et al., 2006; McCartney et al., 2006). Regions on chromosome 4D and 7D had effects on MPW and MLW (McCartney et al., 2006). Total energy of the mixograph was mapped to chromosomes 2B, 4D, and 7D (McCartney et al., 2006) and 5D (Huang et al., 2006). Mixograph band width energy was mapped to chromosomes 1B, 2A, 2B, 6A, and 7D (McCartney et al., 2006), and 5D (Huang et al., 2006).

Most of the mixograph properties were highly correlated to each other (Campbell et al., 2001; Huang et al., 2006; McCartney et al., 2006). As expected, some of the QTLs for different mixograph properties were located at the same chromosome region. This is particularly true in the studies of Huang et al. (2006) and McCartney et al. (2006). For example, a significant QTL for protein content on chromosome 4D (peak at *Xwmc52*) was also associated with MPV, the mixograph first minute slope, and the mixograph slope after peak in the population of Huang et al. (2006). The same region on chromosome 4D was associated with MPT, MPV, MPW, the mixograph left slope, and the mixograph right slope in the population of McCartney et al. (2006).

Alveograph Properties

The alveograph is another method used to estimate dough rheological properties. Some QTL analyses revealed that alveograph properties were controlled by many chromosome regions (Table 1.4) (Groos et al., 2004; Nelson et al., 2006; Perretant et al., 2000; Zanetti et al., 2001). Just as storage protein loci were associated with many mixograph properties, they were also associated with alveograph properties. For example, alveograph *L* was mapped to the *Glu-A3* locus and alveograph *W* was associated with *Gli-B1* in the study of Nelson et al. (2006). Beside

loci for storage proteins, some of the chromosome regions were important for alveograph properties (Table 1.4). For example, in the population of Nelson et al. (2006), alveograph *L* was mapped to chromosomes 1A, 1B, 3B, and 7A; alveograph *W* was mapped to chromosomes 1B, 3A, 3B, 4A, and 7B; and alveograph *P* was mapped to chromosome 1B, 3B, and 5D. In the study by Zanetti et al. (2001), alveograph *P* was associated with chromosomal regions on chromosomes 2A, 5A, 5B, 7B, and 7D, whereas alveograph *L* was mapped to chromosomes 2B, 3B, and 7B. For alveograph *W*, six QTLs were identified in the population of Perretant et al. (2000): on chromosomes 1A, 1B, 3B, 5A, 5D, and 6B. The one on chromosome 5D co-localized with the *Ha* locus for grain hardness.

SDS Sedimentation Test

The SDS sedimentation test is a simple and rapid test used in most breeding programs. SDSSV has shown to be significantly associated with the *Glu-B1* locus on chromosome 1B (Table 1.4) (Huang et al., 2006; McCartney et al., 2006). It also has been associated with regions on chromosomes 2D and 5D in the population of Huang et al. (2006). The region on chromosome 2D was the same region detected for flour protein content, whereas the region on chromosome 5D was near the *Ha* locus. In the population of McCartney et al. (2006), two chromosome regions on the distal end of chromosomes 2A and 6A had significant effects on SDSSV.

Baking Tests

The straight dough, "pup" loaf bake test is the final test for wheat quality in western breeding programs. Loaf volume is of major interest in the baking test. It was reported to be controlled by many chromosome regions (Table 1.4) (Campbell et al., 2001; Kuchel et al., 2006; McCartney et al., 2006). Campbell et al. (2001) reported that loaf volume was associated with some regions on chromosomes 2B, 2DL, and

group 7 chromosomes. McCartney et al. (2006) reported loaf volume, loaf height, and proof height were all associated with one region on chromosome 4D. The QTLs for loaf volume were mapped to chromosomes1A, 1B, 1D, 3A, 3B, 7A, and 7B in many studies (reviewed by Campbell et al., 2001).

Kernel Texture

In bread wheat, kernel texture has long been recognized to be important for breadmaking quality. Increased kernel hardness contributes to higher flour yield, better flowing and sifting properties, higher water absorption, and improved hydrolysis of starch into fermentable sugar (reviewed by Nelson et al., 2006). Kernel hardness is known to be controlled by two genes (*PinA* and *PinB*) at the *Ha* locus on chromosome arm 5DS (Martin et al., 2001a). Although the genetic basis of the difference between soft wheat and hard wheat is well established, little is known about the residual variation within each class of hardness and its genetic components (Nelson et al., 2006). Some populations were developed through crosses either of soft to hard wheat or hard to hard wheat to study the genetic components of grain hardness (Table 1.4) (Campbell et al., 2001; Groos et al., 2004; McCartney et al., 2006; Nelson et al., 2006; Perretant et al., 2000; Zanetti et al., 2001).

In all populations that segregated at the *Ha* locus, a major QTL on the distal end of chromosome 5DS (the *Ha* region) was identified. This QTL explained more than 50% phenotypic variance for kernel hardness in three populations (Campbell et al., 2001; Nelson et al., 2006; Perretant et al., 2000). Besides this major QTL, other minor QTLs for kernel hardness were identified on chromosomes 1A and 6D that explained only 3% and 5.5%, respectively, of the phenotypic variance in the population of Perretant et al. (2000). A region on chromosome 4D consistently

explained around 15% of the variance of kernel hardness in more than one environment in the population of Nelson et al. (2006).

For those populations that did not segregate at the *Ha* locus, other QTLs for kernel hardness were identified. Zanetti et al. (2001) found that regions on chromosomes 2A, 3B, 4A, 4D, 5A, 6B, 7A, 7B, and 7D had effects on kernel hardness. These regions together explained 54% of the variance of kernel hardness of the population. In the study of Groos et al. (2004), kernel texture was evaluated with two methods, near infrared spectroscopy (NIR) and SKCS 4100. Kernel hardness from both measurements was associated with regions on chromosomes 2D, 3A, 4A, 5A, 5B, and 6D, and marker *gwm130*, which did not link to other markers. Beside those regions, kernel hardness measured with NIR was mapped to chromosomes 1A, 2A, 5D, and 6A, whereas kernel hardness measured with SKCS 4100 was mapped to chromosomes 1B, 2B, 3B, and 6B.

Interactions

In the study of Ma et al. (2005) with 160 DH lines from a cross between wheat cultivar 'Cranbrook' (high dough extensibility) and 'Halberd' (low dough extensibility), the main additive QTLs for dough extensibility and strength were located at the HMW and LMW glutenin loci and also a new QTL located on chromosome 5A (at the location of marker PAAG/MCCC4). Significant interactions among glutenin loci and among glutenin loci and other chromosome regions were also identified. The results suggested that the genetic background might be the reason for the variation of some glutenin effects on dough properties. For example, the full expression of the superiority of *Glu-D1d* (subunits 5+10) depended on the presence of the Halberd allele at the locus defined by marker PAAT/MCAT3 or PACT/MCTG6 on chromosome 2B. The significant change of the *Glu-B1* effect on dough Rmax was

observed when the Cranbrook type allele of marker PAGT/MCAG2 (located on the long arm of chromosome 3A) was present. Significant epistasis was also detected between SSR marker *gwm044* and *Glu-B3* for dough extensibility.

In summary, wheat quality is a collection of complex traits. Specific characteristics that are indicators of wheat quality have been mapped across the entire genome. Some of the QTLs for wheat quality co-localized with QTLs for agronomic traits, such as yield, plant height, awn type, and maturity. Since characteristics for different tests are intercorrelated, some of the characteristics shared the same QTLs. In the future, intensive interaction analysis among quality QTLs and other chromosome regions will help improve understanding of wheat quality characteristics for a variety of end-uses.

The 1RS Translocation and Wheat Quality

Prevalence of 1RS Translocations

A number of genes of agronomic importance have been transferred from rye (*Secale cereale* L.) to its relative, common bread wheat, through the production of interspecific chromosomal translocation and substitution lines (reviewed by Graybosch, 2001). 1RS (the short arm of rye chromosome 1) has been transferred to wheat in the form of 1AL.1RS, 1BL.1RS, and 1DL.1RS wheat-rye chromosomal translocations (1AL, 1BL and 1DL stand for the long arms of group 1 wheat chromosomes). Among these, the 1AL.1RS and 1BL.1RS translocation are most common in wheat cultivars (Friebe et al., 1996; Graybosch, 2001). In the survey of the 1RS translocations of more than 2500 cultivars and germplasm lines by USDA-ARS in 2006, 331 carried 1BL.1RS and 207 carried 1AL.1RS, and most were released since 1970 (Graybosch, 2006). The 1BL.1RS translocation probably has had more

worldwide impact on wheat breeding and production than any other piece of alien chromatin, whereas the 1AL.1RS is more limited to U.S. wheats (Graybosch, 2006).

Origin of the 1RS Translocations

Prominent wheat cultivars with the 1AL.1RS and 1BL.1RS translocations are from cultivars 'Amigo' and 'Kavkaz', respectively (Berzonsky and Francki, 1999). The Amigo translocation was initiated by hybridizing triticales (*Triticosecale* Wittmack) to wheat, then backcrossing to wheat. The backcross progeny were irradiated, so the breakpoint in Amigo may not be centromeric as a whole arm translocation (Berzonsky and Francki, 1999). Kavkaz was originally derived from a cross between wheat and 'Petkus' rye in Krasnodar, Russia (Graybosch, 2001).

Benefits of the 1RS Translocations

Rye translocations have been used to transfer genes for resistance to fungal pathogens (especially rust and powdery mildew), insects, and, in some cases, to enhance grain yield, grain yield stability, and grain protein content (Table 1.5) (Graybosch, 2001; Kumlay et al., 2003). The Amigo translocation (1AL.1RS) carries genes for resistance to powdery mildew (*Erysiphe graminins* DC.) (Heun et al., 1990) and greenbug (*Schizaphis graminum* Rond.) (Sebesta et al., 1995) and provides tolerance to the wheat curl mite (*Eriophyes tulipae* Keifer), the vector of wheat streak mosaic virus (Conner et al., 1991) (reviewed by Friebe et al., 1996; Graybosch, 2001). In some studies, wheat with the 1AL.1RS translocation had increased yield, kernel weight, and test weight compared to non-1RS lines (Table 1.5) (Kumlay et al., 2003; Villareal et al., 1996). However, lines with the Amigo translocation (1AL.1RS) in a 'Nekota' background had a higher protein content, but were not better for either agronomic performance or stability than sister lines without the 1RS translocation in eight Nebraska environments (Espitia-Rangel et al., 1999b).

Table 1.5.1. The effect of the 1AL.1RS translocation on recipient wheat compared to wheat without the translocation.

Authors and Year	Material†	Traits ‡										
		YLD	KW	HLW	SPE	KPE	HT	PROT	FYD	SDSSV	MPT	TR	LV
Graybosch et al., 1993	RIL							0		-	-	0	
Graybosch et al., 1999b	RIL	-	+					0		-	-	-	0
Villareal et al., 1996	RIL	+	+,0§	+	+		0						
Espitia-Rangel et al., 1999a	RIL	0	+	+	-	0	-						
Espitia-Rangel et al., 1999b	RIL							+	-		-	-	
Kumlay et al., 2003	NIL	+	0					0		-	0	-	

† 'BL', random breeding lines; 'CV', cultivars; 'NIL', near isogenic lines.

‡ 'YLD', yield; 'KW', kernel weight; 'HLW', hectoliter weight; 'SPE', spikelets per ear; 'KPS', kernels per ear; 'HT', plant height; 'PROT', protein content; 'ZELY', Zeleny sedimentation volume; 'SDSSV', SDS sedimentation volume.

'+', trait expression in the translocation lines is higher; '-', trait expression in the translocation lines is lower; '0', no difference between translocation and non-translocation wheats.

§ Reported two experiments.

Table 1.5.2. the effect of the 1BL.1RS translocation on recipient wheat compared to wheat without the translocation.

Authors and Year	Material†	Traits‡								
		YLD	KW	HLW	SPE	KPE	HT	PROT	ZELY	SDSSV
Villareal et al., 1991	CV, BL	0	+	+		0	0			
Carver and Rayburn, 1994	BL, NIL	+,+§	+	0		0	0,+			
Fenn et al., 1994	CV, BL		+	+				-	-	
Schlegel and Meinel, 1994	BL	+,+	+,0		0, 0	+,+	+,0	0,0	0,0	
Burnett et al., 1995	NIL							+		-
Carver and Rayburn, 1995	BL, NIL							+,+		-,-
Lee et al., 1995	BL							0,+,-		-
Moreno-sevilla et al., 1995b	BL	+	+	0		0	-			
Moreno-sevilla et al., 1995a	CV, BL	0	+	0		0	0	+		
Villareal et al., 1995	BL	+	+	+		+	-			
McKendry et al., 1996a	BL	0	0				-,-			
McKendry et al., 1996b	BL			-,0				0		
Bullrich et al., 1998	NIL	0	+		+,0	0	-	0		-
Singh et al., 1998	NIL	-,0	0	+		0	-			
Villareal et al., 1998	NIL	+	+,0	+		+,0	0			
Martin and Carrillo, 1999	BL							0		-
Lelley et al., 2004	NIL	+	+	+,0			-	-		-

† 'BL', random breeding lines; 'CV', cultivars; 'NIL', near isogenic lines.

‡ 'YLD', yield; 'KW', kernel weight; 'HLW', hectoliter weight; 'SPE', spikelets per ear; 'KPS', kernels per ear; 'HT', plant height; 'PROT', protein content; 'ZELY', Zeleny sedimentation volume; 'SDSSV', SDS sedimentation volume. Some literatures reported results of two experiments.

'+', trait expression in the translocation lines is higher; '-', trait expression in the translocation lines is lower; '0', no difference between translocation and non-translocation wheats.

§ Those cells contained two symbols reported two experiments.

The Kavkaz (1BL.1RS) translocation conferred resistance to stem rust (*Puccinia graminis* Pers), leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis* Westend), and powdery mildew (*Erysiphe graminins* DC.) (Graybosch, 2001). In addition to disease resistance, 1BL.1RS has been shown to increase grain protein content (Moreno-sevilla et al., 1995a) and grain yield (Kumlay et al., 2003; Moreno-sevilla et al., 1995b), at least in some genetic backgrounds and environments (Bullrich et al., 1998).

Quality Effects of the 1RS Translocation

Unfortunately, the 1RS translocation often results in significant alterations of wheat processing quality (reviewed by Graybosch, 2001). Severe quality problems are recognized when 1BL.1RS is present in hard wheat backgrounds, including low loaf volumes, the production of 'sticky' doughs, a lack of tolerance of doughs to overmixing, and low SDSSV (Table 1.5.2). In addition, when dough strength is measured with a mixograph, there is a pronounced lack of correlation between time to peak dough resistance and tolerance to overmixing (Graybosch et al., 1999a; He et al., 2005; Kumlay et al., 2003; Martin and Stewart, 1990). When tested with commercial-scale equipment, problems with dough handling and dough stickiness also were noted (Graybosch, 2001).

Some studies found that wheats with the 1AL.1RS translocation appeared to have better end-product quality than those with the 1BL.1RS translocation (Table 1.5.1) (Espitia-Rangel et al., 1999a; Graybosch, 2001; Graybosch et al., 1993; Kumlay et al., 2003). However, McKendry et al. (2001) found 1AL.1RS is more detrimental than 1BL.1RS for soft wheat quality. In all studies, mixing strength and SDSSV were reduced in 1AL.1RS lines compared to non-1RS lines (Amiour et al., 2002; Espitia-Rangel et al., 1999a; Graybosch et al., 1999b; Graybosch et al., 1993;

Kumlay et al., 2003). Elevated flour protein contents were reported when 1AL.1RS lines were compared to non-1RS sibs (Espitia-Rangel et al., 1999a; Graybosch et al., 1999b). Certain 1AL.1RS cultivars, especially TAM 107, have long been criticized by the American milling and baking industry for poor commercial dough mixing and breadmaking performance (Espitia-Rangel et al., 1999a; Graybosch, 2001; Graybosch et al., 1993).

Reasons for Quality Effects of the 1RS Translocations

The quality defects associated with the 1RS translocations may be the consequence of a change in the protein composition of the recipient wheats because incoming genes, such as secalin genes, are detrimental for wheat quality. In addition, 1RS at least replaces the short arm of one wheat chromosome pair, leading to a permanent loss of some potentially important wheat genes, such as LMW glutenin genes and genes encoding gliadins (Graybosch, 2001; Kumlay et al., 2003). The LMW glutenins can form intermolecular disulfide bonds, and participate in the formation of the gluten protein matrix, the primary determinants of dough strength (D'Ovidio and Masci, 2004; Singh et al., 1990). The presence of *Sec-1*, a complex rye locus, results in the production of two types of rye secalin proteins, ω-secalins and γ-secalins (Shewry et al., 1983). The ω-secalins are extremely water soluble. They replace gliadin proteins, which are more hydrophobic. Many studies have shown that there was a measurable decrease in the amount of polymeric glutenin protein and an increase in the amount of water-soluble proteins in wheats with a 1RS translocation (Graybosch, 2001; Graybosch et al., 1993; Lee et al., 1995). The loss of glutenin protein led to a reduction in the amount of insoluble gel protein, which was important for dough mixing tolerance (Pritchard and Brock, 1994). Sulfhydryl-disulfide interchange experiments, on the other hand, suggest that dough stickiness is due to the

water-soluble fraction (reviewed by Berzonsky and Francki, 1999). The combined effects of the presence of 1RS and loss of the specific LMW-GS are the reasons for reduced quality of wheat with a 1RS translocation.

Reducing the Quality Effects of the 1RS Translocation

A long-term goal of breeders and geneticists has been to mitigate the undesirable quality effects associated with the presence of 1RS whole-arm translocations (Berzonsky and Francki, 1999; Jiang et al., 1994). Efforts have been made to introduce a smaller 1RS segment to improve quality and at the same time keep beneficial genes (Amiour et al., 2002; Berzonsky and Francki, 1999; Ren and Zhang, 1997). Another strategy is to look for other translocation schemes, such as a 2BL.2RS translocation to avoid loss of *Glu-3* genes (Hysing et al., 2007). Encouraging results have been found for both strategies (Hysing et al., 2007).

In conclusion, the presence of the 1RS translocations in wheat enables recipient wheat to resist multiple diseases and pests, and improve some agronomic properties, but also reduces wheat quality. Looking for different rye germplasm resources and introducing selected segments to suitable wheat genetic backgrounds will increase its usage in wheat breeding. The 1RS translocations will continue to impact wheat breeding for many years to come.

Wheat Quality and Environmental Conditions

Environment affects overall performance of wheat, particularly grain yield and end-use quality. It has long been recognized that productivity and quality vary considerably as a result of environmental variations during grain fill (reviewed by Dupont and Altenbach, 2003). Prior to anthesis, environment affects germination, photosynthesis, tiller formation, and inflorescence development, thereby impacting

grain number. After anthesis, environmental conditions primarily affect kernel size and composition, and then finally determine grain yield and wheat end-use quality.

In all cases, environment was an important factor that affected wheat end-use quality in the 14 studies reviewed in this dissertation. In these studies, environments were a combination of location × year and location × agronomic management treatments. Agronomic management included different irrigation levels (Ames et al., 1999; Guttieri et al., 2000), nitrogen (N) fertilization rates (Johansson et al., 2004; Johansson et al., 2003; Robert, 1997; Souza et al., 2003), timing of fertilization (Johansson et al., 2004), sowing times (Baric et al., 2004), seeding rates (Geleta et al., 2002), and even fungicide treatments (Robert, 1997). Among all environmental factors, location was the most important component, followed by variation among crop years. Within one year, temperature during anthesis and grain filling (Peterson et al., 1998), irrigation and precipitation (Guttieri et al., 2000), and N fertilization were the main variables affecting quality (Johansson et al., 2004; Johansson et al., 2003; Robert, 1997; Souza et al., 2003).

Temperature

Temperature is a critical factor for wheat end-use quality. The initiation of glutenin synthesis has been reported to occur about five days after anthesis, varying among the genotypes and growing conditions (reviewed by D'Ovidio and Masci, 2004). Temperature, particularly high temperatures during grain filling, can significantly elevate protein content while decreasing the functionality of protein, ultimately changing the rheological properties of flour (Tahir et al., 2006). Cultivars differed significantly in end-use quality due to the detrimental effects of high temperature stress (Guttieri et al., 2000). High temperature during grain filling increased SDSSV, MPV, and the descending slope at two minutes after peak, but

decreased MPT and MPW (Tahir et al., 2006). Loaf volume was positively correlated with flour protein contents regardless of temperature regimen whereas mixing tolerance was highest for wheat growing under 24/17 °C regimens (day/night) (DuPont et al., 2007).

Effects of temperature on storage protein composition are unclear, and may vary among genotypes because of gene regulation during grain fill (reviewed by Dupont and Altenbach, 2003). Temporal expression of gluten protein genes was influenced by temperature during grain fill. Transcripts within all major gene families accumulated earlier and disappeared earlier in kernels developing under high temperature regimens or high temperatures combined with drought, when compared to kernels produced under a moderate temperature regimen (reviewed by Dupont and Altenbach, 2003). Similar changes in the timing of protein deposition during grain development were observed by Altenbach et al. (2003). However, there has been no convincing evidence thus far that temperature alters the levels of transcripts of any gluten protein gene families despite the presence of putative heat shock consensus elements in the upstream regions of several gliadin genes (Blumenthal et al., 1990).

High temperature was reported to increase the proportion of gliadins to glutenins and decrease the proportion of large polymers in flour from several wheat cultivars grown in controlled environment experiments (Blumenthal et al., 1995; Corbellini et al., 1997; Panozzo and Eagles, 2000). It was reported that genotypes having *Glu-D1d* (HMW-GS 5+10) generally showed less variability in storage protein composition in response to high temperature than genotypes with *Glu-D1a* (HMW-GS 2+12) (Blumenthal et al., 1995). However, the gliadin-to-glutenin ratio also increased, and flour breadmaking quality decreased, when plants of the U.S. wheat 'Karl' carrying *Glu-D1d* were exposed to a 35/20 °C (day/night) rather than a 20/20

°C temperature regimen from 10 days after anthesis until maturity (reviewed by Dupont and Altenbach, 2003).

Irrigation and Moisture

Soil moisture is an important environmental factor affecting agronomic performance of wheat cultivars. Cultivars differed in magnitude of end-use quality response to moisture stress applied from tillering through anthesis, which changed cultivar rankings for flour protein, flour yield, MPV, mixograph tolerance, mixing time, and loaf volume (Guttieri et al., 2000). However, properties such as MPT and the ratio of loaf volume to protein were stable in six different irrigation treatments.

Fertilizer

Nitrogen fertilization strongly influences wheat flour protein content (reviewed by Dupont and Altenbach, 2003). In a detailed study of the protein composition of 13 wheat cultivars grown under differential fertilization, Wieser and Seilmeier (1998) found that increased N fertilization decreased the proportion of hydrophobic proteins (γ-gliadins, LMW glutenins) and increased the proportions of hydrophilic proteins (ω-gliadins, HMW glutenins). This phenomenon was confirmed by Johansson et al. (2001). The effect of fertilization on protein content and composition varied significantly with different wheat cultivars (Guttieri et al., 2000; Labuschagne et al., 2006). N fertilization influenced only Chinese noodle color in irrigated environments, and impacted test weight, flour ash, loaf volume, and bake absorption in moisture-limited environments (Souza et al., 2003). Large amounts of N fertilizer increased protein content in wheat flour (Johansson et al., 2003). The timing of applying N fertilizer also affected flour quality in the study by Johansson et al. (2004). They found that early N fertilizer application led to higher gluten strength

than late N fertilizer application, because early application gave a higher percentage of large and unextractable polymeric protein in the total polymeric protein.

Seeding Rate

Differences among seeding rates were observed for agronomic performance and end-use quality characteristics even though the size of the effect is smaller than the effect from environmental factors such as temperature (Geleta et al., 2002). In the study by Geleta et al. (2002), kernel weight, flour yield, and mixing time increased with increased seeding rate up to a seeding rate of 65 kg/ha, but mixing tolerance significantly decreased with seeding rate up to 65 kg/ha. Most quality characteristics were low when grain yields were high. At higher seeding rates, more fertilizer could be required for better grain yield and end-use quality of wheat. Higher seeding rate decreased the proportion of secondary tillers. Seeding rate is a predictable environmental factor that affects some agronomic and end-use quality characteristics of wheat (Geleta et al., 2002).

In summary, to study the relationship of wheat quality and environments, we can evaluate the effects of temperature variations, fertilizer applications, moisture stresses and plant density on wheat quality. Meaningful information about genotype and environment effects on wheat quality comes from testing over a sufficient number of environments and evaluating an appropriate range of genotypes (Ames et al., 1999).

Dough Rheology Test: the Mixograph

Traditionally, dough is made by combining flour, water, and energy through mixing. Undeveloped dough is homogeneous, fully hydrated wheat flour where the energy input phase of the dough has not been initiated. The addition of sufficient mechanical energy causes the distribution and hydration of flour particles, allowing

the formation of a continuous protein matrix to hold starch and other components (Campos et al., 1997). Dough development is thought to result from the disaggregation/depolymerization of glutenin and the realignment and aggregation of glutenins and gliadin. Both processes of disruption and formation reach a balance to form a continuous protein network. The physical mechanism of dough development involves the breaking of covalent bonds, specifically disulfide bonds, the disruption of noncovalent interactions and the alignment of molecules by the shear created during mixing (Letang et al., 1999).

The mixograph was developed in the late 1920s to determine the mixing characteristics of flour during dough development (Shogren, 1990; Swanson and Working, 1933). The procedure for using a mixograph is described in AACCI Method 54-40A (AACCI, 2004) and by Finney and Shogren (1972). It involves (1) putting flour in a bowl, holding the bowl on a station that can move according to the strength of the mixing dough and can record its movement on a rolling strip of paper, (2) adding optimum water to the flour, (3) mixing the flour, and (4) recording the movement of the station on the paper. A series of steps or phases occur during the dough mixing process regardless of mixer type (described by Hamer and Hoseney, 1998). The first stage is the pickup stage of mixing. In this stage, the ingredients blend into a reasonably homogeneous mixture. At the end of this stage, the dry and wet materials are mixed only to the point of combining together. The mass is sticky, cold, and lumpy. The second stage is the initial development stage. In this stage, the dough is getting warmer, smoother and drier. The third stage is the cleanup stage. In this stage the water is being taken up by the dry material and forms a stiff mass that is wet in spots. The fourth stage is the development stage. In this stage, the dough becomes elastic with signs of dryness. The fifth stage is the final development stage. In this

stage, the dough reaches optimum development. Dough is at its correct temperature and handling quality. It has a silky and dry appearance. It has no evidence of wetness or roughness. It is smooth and mellow. A gluten film can be easily obtained by extending a piece of dough. If dough is mixed beyond this stage, it will reach the letdown stage. In this stage, the dough is too warm. It lacks elasticity and is forming thin strings that are wet and sticky. Dough that has been excessively over-mixed reaches the breakdown stage. In this stage, the dough is slack and translucent and shows signs of liquefaction (Hamer and Hoseney, 1998; Singh and MacRitchie, 2001).

In order to apply mixograph analysis to early generation selection for wheat quality, sample size has been reduced from 400 g flour to 2 g flour over the years (Gras and Obrien, 1992; Ohm and Chung, 1999; Shogren, 1990). Since the introduction of the 10-g mixograph (Finney and Shogren, 1972), the mixograph has been widely used to evaluate the quality of early generation lines in wheat breeding programs because it utilizes a small amount of sample and requires a short analysis time because it generates more intensive shear force than any other mixer (Chung et al., 2001). More recently a 2-g mixograph was introduced by Rath et al. (1990), which allows the cereal quality lab to determine the dough properties of grain from F_2 plants (Gras and Obrien, 1992).

Traditional mixograph characteristics used in quality evaluation, including mixing time, water absorption, and mixing tolerance, were manually or subjectively scored. As early as the mid-1960s, a potentiometer (digital sensor) was placed at the bottom of the mixograph station to record the digital mixograph characteristics (Navickis et al., 1990). In either method of interpreting data, a curve is drawn relative to the midpoint of the band. The peak, slope, and width characteristics are measured relative to this line. Computer-analyzed mixograph characteristics had significant

correlations with the conventional analysis. For example, MPT from the computer analysis was significantly correlated with conventional mixograph mixing time (r=0.89, P<0.001, n=642) and mixograph bandwidth at six minutes was significantly correlated with mixing tolerance (r=0.81, P<0.001, n=642) (Chung et al., 2001). Thus, the computer-analyzed mixograph characteristics may be used to evaluate flour quality instead of the conventional characteristics to reduce human subjectivity and analysis time (Chung et al., 2001).

Using the Mixsmart® software version 3.80 (National Manufacturing Co., Lincoln, NE), 43 characteristics can be extracted from each mixogram. The most often used characteristics are mixograph (midline) peak time (MPT), mixograph midline left height at one minute before peak (MLV), mixograph (midline) left width at one minute before peak (MLW), mixograph midline peak height (MPV), mixograph (midline) peak width (MPW), mixograph midline peak height at two minutes after peak (MRV) and mixograph (midline) peak width at two minutes after peak (MPW) (Amiour et al., 2002; Chung et al., 2001; Dobraszczyk et al., 2005; Khatkar et al., 1996). Fig. 1.1 adapted from Dr. B. Seabourn, USDA-ARS, Manhattan, KS, illustrates these characteristics.

Mixograph characteristics have been correlated with baking characteristics, such as loaf volume. For example, MPV (in a range from 33.6 to 70.2 mixograph units in 0 to 100 scale) was highly correlated with loaf volume (r=0.82, P<0.001, n=32) in a range of flours and glutens obtained from the UK, Canada, and France (Khatkar et al., 1996). By using partial least squares multiple regression analysis in a study of 54 wheat cultivars, mixograph characteristics and protein contents explained a very high percentage of variation for baking volume (R^2=0.999) (Dobraszczyk and Schofield, 2002). Thus, mixograph characteristics can be very useful in predicting

baking quality and can be used to select good quality wheat lines in wheat breeding programs (Chung et al., 2001; Gras and Obrien, 1992; Khatkar et al., 1996; Ling and Sanchez, 1986; Swanson and Working, 1933; Wikstrom and Bohlin, 1996). Due to its extensive use, the mixograph was approved as an official physical test in 1961 (AACCI Method 54-40), revised in 1988 and included in AACCI methods 10th edition (AACCI, 2004; Martinant et al., 1998).

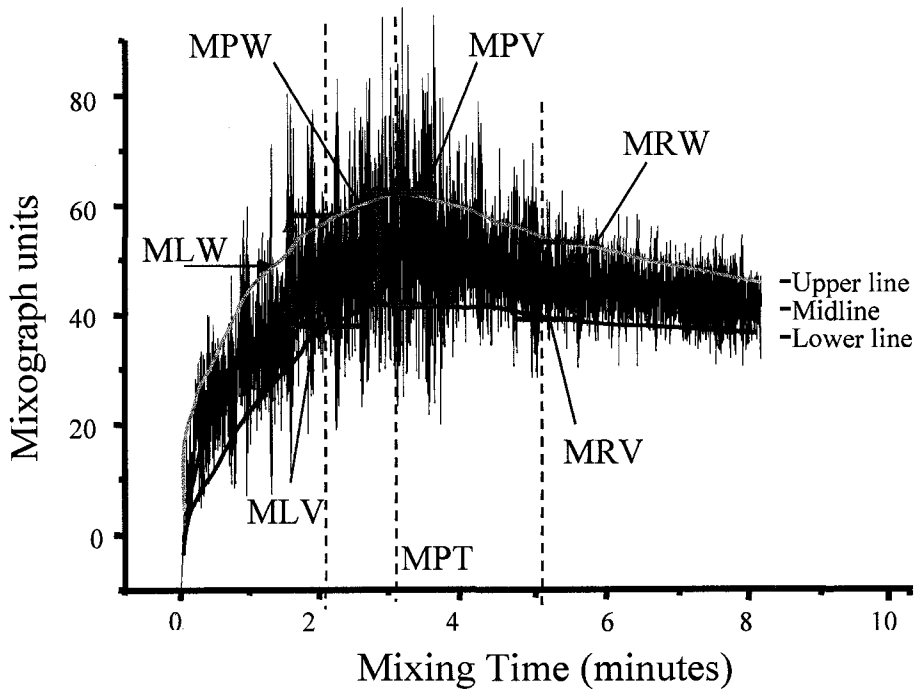


Fig. 1.1 Illustration of mixograph characteristics†

† MPT, Time to reach highest point of mixograph midline; MLW, band width (distance between the upper and the lower line) at one minute before MPT; MLV, mixograph midline height (value) at one minute before MPT; MPW, band width (distance between the upper and lower line) at MPT; MPV, mixograph midline height (value) at MPT; MRW, band width (the distance between the upper and lower line) at two minutes after MPT; and MRV, mixograph midline height (value) at two minutes after MPT.

Association Mapping in Plants

Most traits of agricultural or economical importance are controlled by multiple quantitative trait loci (QTL) (Holland, 2007). In the past decade, marker-assisted selection (MAS) has become a tool in some breeding programs to improve some quantitative traits (Collard et al., 2005). The success of MAS is highly dependent on the discovery of markers linked to traits. The two most commonly used methods for dissecting complex traits are linkage analysis and association mapping (Mackay, 2001). In plants, QTL mapping relies principally on linkage analysis which includes development of segregating populations derived from crosses between inbred lines (F_2, backcross, recombinant inbred lines, DH, etc), genotyping the individuals with genetic markers across the genome, phenotyping the individuals for the trait of interest, and then analyzing the results via linkage mapping (Flint-Garcia et al., 2005). Although designed segregating populations are straightforward and have certainly proven useful in identifying a number of important genes and QTLs for important traits in many plant species, they have several disadvantages (Malosetti et al., 2007). First, in a diploid species, each population segregates at most for two alleles per locus at those loci that are polymorphic between the parents, whereas in the absence of allele polymorphisms between parents, no QTL can be identified. Second, due to small population sizes and the modest degree of recombination within the population, mapping resolution is often in the range of 10-30 cM. Intermating among sibling lines increased the exchange rate and has improved the mapping resolution to a few cM in maize, but this still corresponds to millions of bases and hundreds of genes (Lee et al., 2002). Third, the genetic backgrounds in which mapping studies take place are generally not representative of backgrounds used in elite germplasm and QTL effects observed might not be reproducible (Jannink et al., 2001). Due to these limitations of

linkage analysis, an alternative mapping strategy, association mapping, also known as linkage disequilibrium (LD) mapping or association analysis, has been employed in plants in the last decade and has received wide recognition (reviewed by Flint-Garcia et al., 2003; Flint-Garcia et al., 2005). Association mapping was originally developed for mapping human disease genes, because natural and ethical constraints make it impossible to use linkage mapping in humans. Association mapping utilizes ancestral recombination events in natural populations to detect marker-trait associations. These recombination events break down LD, initially present in a population, at a rate determined by the genetic distance between loci and the number of generations since the genetic distance arose. Essentially, association mapping exploits historical and evolutionary recombination at the population level (reviewed by Flint-Garcia et al., 2003; Flint-Garcia et al., 2005; Gupta et al., 2005).

Association mapping has several advantages over linkage mapping as reviewed by many authors (Gupta et al., 2005; Malosetti et al., 2007; Whitt and Buckler, 2003; Yu and Buckler, 2006). First, it uses "natural" populations, including collections of landraces, current and historical cultivars, breeding lines, core collections of gene bank accessions, etc. Second, it simultaneously evaluates more than two alleles per locus. Third, QTL alleles can be mapped in the genetic backgrounds relevant to current breeding materials. Fourth, it gives a higher mapping resolution (e.g., 2000 bp in diverse maize inbred lines (Remington et al., 2001)). The genetic basis of a phenotype can even be mapped to a single nucleotide mutation (e.g., a nucleotide mutation caused translational inhibition of a myostatin gene that contributes to the muscular hypertrophy of Texel sheep (Clop et al., 2006)). Fifth, association mapping can use the large amounts of historical phenotypic data that are

available for mapping efforts at no or little extra costs (e.g., for traits evaluated in the regional nursery trials of breeding programs) (Malosetti et al., 2007).

However, due to geographic isolation, natural selection, or artificial selection, population stratification is common for plant germplasm (Yu and Buckler, 2006). This population stratification and an unequal distribution of alleles within a population can result in spurious associations (Knowler et al., 1988; Price et al., 2006). The success of association mapping efforts depends on the possibility of separating LD due to linkage from LD due to other causes, because population structure is seen as a second major cause of marker-trait associations in addition to linkage (Flint-Garcia et al., 2003). In order to reduce the number of false associations, a number of association methods have been developed. In plants, the control is adopted in two stages. The first stage is to control stratification in the population composition by selecting or creating materials with minimal population stratification. Populations used for linkage analysis are the extreme of this control method. Other methods suggested by Hamilton et al. (2002) may include selection of populations with the same phenotype from different geographical origins or selection of populations with contrasting phenotypes from the same geographical origin, and repeating this from a number of different locations. The second stage is to account for population stratification with statistical models. The most popular statistical methods are the transmission disequilibrium test (TDT), genomic control, structured association, and principal component analysis approach (Mackay and Powell, 2007).

The following review will be focused on how to reduce false associations caused by population stratification in plant association mapping.

Control of False Associations through Composition of the Mapping Population

Linkage analysis

Linkage analysis can be regarded as a special case of association mapping in which LD is generated by establishing a population segregating for the traits of interest through a small number of founders in the recent past (Mackay and Powell, 2007). This is considered the most effective way to control false association. In this type of population, there is no population stratification, because the genetic background is homogenized during recombination and there is no confounding effect of low allele frequency in detecting QTL effects. Even if the frequency of some alleles might be reduced because of unintended selection and genetic drift, they should exist in a sufficient number of individuals to test their effects on quantitative traits. Marker-trait association through linkage analysis can be established through a simple *t*-test. The mapping resolution of linkage analysis is highly dependent on the recombination rate of F_1 plants and the number of their offspring (Peleman et al., 2005). Numerous QTLs have been identified through linkage analysis. It is a powerful and indispensable method, even though it has some disadvantages as mentioned above (Mackay and Powell, 2007).

Multiparent advanced generation intercross

The multiparent advanced generation intercross (MAGIC) method was first applied to mice and has not yet been reported in plants (Mackay and Powell, 2007; Mott and Flint, 2002). This method is designed to increase allele diversity and mapping resolution in a single population. To develop a MAGIC mapping population, multiple crosses are made between widely distinct multiple parents, F_2 individuals of these crosses are intermated for several generations, and then recombinant inbred lines are developed from these intermated lines (Valdar et al., 2006). As it does in bi-

parent populations, the successive rounds of recombination cause LD to decay, to increase the precision of QTL location, and more importantly, to diminish population stratification. In plants, the MAGIC method could be easily adopted in a crop which is readily outcrossing and tolerant to inbreeding, such as rapeseed (*Brassica napus* L.) and could also use dominant genetic-nuclear male sterility in an autogamous crop (Sorrels and Pritz, 1982).

Control of False Associations with Statistical Approaches

The transmission disequilibrium test (TDT) and derivatives

The TDT was first developed to control spurious association between a marker and a disease locus in humans (Spielman et al., 1993). TDT uses ascertained affected individuals and their parental marker information to determine if a specific allele is transmitted preferentially. For outbred species, the test population consists of many families with parents that are heterozygous at the putatively associated marker locus and with progeny that express the trait of interest. From each parent, one allele must be transmitted to the progeny and one is not transmitted. From all families, a count is than made of the number of transmissions and non-transmissions. If the progeny carrying the trait show any significant distortion frequency of marker alleles from their parents compared to the unlinked expectation of 50%, the marker should be linked to the trait (Jannink et al., 2001). Later on, the TDT was extended to study haplotype transmissions, quantitative traits, the use of sib pairs rather than parents and progeny, and information from extended pedigrees (Allison, 1997; Guo et al., 2007). Because of random Mendelian segregation, alleles transmitted to different progeny are independent of each other, conditional on the parental genotypes. Neither linkage alone nor disequilibrium alone will generate a positive result, and therefore, the TDT is robust against population admixture and stratification (reviewed by Jannink et al.,

2001; Stich et al., 2006). Even though the TDT is a valid method for association analysis in plants, directly applying the TDT in plants has not been reported. This may be because it is relatively easier to develop and evaluate a mapping population for linkage analysis than to create a population for TDT analysis.

Genomic control

Genomic control (GC) is one of the most common methods utilized in both human and plant studies to address the issue of population stratification in population-based samples. With GC, a group of "null" loci are used to estimate the impact of the confounding observed in population-based samples. These "null" loci are defined as unlikely to have a functional effect on the trait under investigation, loosely matching the test markers in allele frequency, and distributed over the whole genome. The same individuals in a population are genotyped at all loci including "null" loci and test markers. Assuming that population structure has a similar effect on all loci, the null hypothesis is that there is no greater association between the candidate markers and the traits than for null loci and the traits, rather than that there is simply no association between the candidate markers and the traits (Bacanu et al., 2002; Devlin and Roeder, 1999). The test statistic is an adjusted chi-squared value obtained by dividing chi-square for candidate markers and traits by the mean chi-square for control marker associations. The *P* value is determined as in the normal chi-square method (Mackay and Powell, 2007).

By the nature of its design, GC accounts for population structure and corrects for unknown kinship among collections of lines (Devlin and Roeder, 1999; Devlin et al., 2004). However, the correction of the false positive rate using GC reduces its statistical power (Price et al., 2006; Yu et al., 2006; Zhao et al., 2007). This loss of power can be great when traits of interests arise in only one subpopulation (Setakis et

al., 2006). Furthermore, because some "null" loci differ in their allele frequencies across ancestral populations more than others, a uniform overall adjustment of GC might be insufficient at loci having unusually strong differentiation across ancestral populations and overcorrect at other loci without such differentiation (reviewed by Mackay and Powell, 2007; Price et al., 2006).

Structured association

The structured association (SA) method is another very commonly used method to control spurious association in association analysis. It is more sophisticated than genomic control, and is computationally more demanding (Falush et al., 2003; Pritchard et al., 2002; Pritchard et al., 2000). The SA assumes that the genome of the individuals under investigation came from a fixed number of ancestral populations which were in linkage equilibrium and Hardy-Weinberg equilibrium for the alleles at the sampled loci (Falush et al., 2003; Pritchard et al., 2000). The well known program, "STRUCTURE" (http://pritch.bsd.uchicago.edu/software/structure2_2.html), uses a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. By trial and error, it allocates each individual's genome to one or more subpopulations such that disequilibrium within populations is minimized, and creates a so-called 'Q matrix'.

To allocate individuals to populations, it is necessary to know in advance how many ancestral populations there are. Contrary to human and animal structure, the genetic structure of plant species is generally poorly known *a priori*. Plant association panels often consist of related and/or admixed accessions that are of high interest for breeders but may artificially increase the number of groups (Falush et al., 2003). Estimation of the *a priori* population number (k) is notoriously difficult. Pritchard et al. (2000) suggested a method based upon marginal likelihoods to determine the

number of populations needed to explain the observations. The method is applied several times to the data, with varying numbers of populations for each treatment. The marginal likelihood can be calculated as the harmonic mean of the likelihoods sampled from the output of the Markov Chain Monte Carlo (MCMC) method used to approximate the probability of assigning individuals to populations. In plants, due to the complexity of the population structure, the estimation with this method is not straightforward and even problematic. Huelsenbeck and Andolfatto (2007) suggested a method to estimate *a priori* k based on a Dirichlet process model. It allows the assignment of individuals to populations and the number of populations both to be considered as random variables, and automatically infers population number k. An implementation of this method is available in the software StructuRama (http://www.structurama.org/).

The Q matrix estimated from STUCTURE can be included in the statistical model to test marker-trait associations while controlling for spurious associations (Breseghello and Sorrells, 2006b; Pritchard and Rosenberg, 1999; Remington et al., 2001; Simko et al., 2004; Thornsberry et al., 2001). The principle of this control method is that variation attributable to population membership is accounted for first, and then the presence of any residual association between the marker and phenotype is tested (Mackay and Powell, 2007). The SA is effective in detecting and adjusting for the presence of population structure, but does not deal with consanguinity within populations arising from the first cousin-level relationships. Recently, Ed Buckler's group (USDA-ARS, Ithaca, NY) developed a unified mixed model (Q+K) to account for both population structure and the correlation between individuals (Yu et al., 2006). The population membership (Q) is estimated using STRUCTURE, and kinship (K) among cultivars is estimated empirically from a set of control markers using SPAGedi

(http://www.ulb.ac.be/sciences/ecoevol/spagedi.html) (Hardy and Vekemans, 2002; Yu et al., 2006). The key to their approach is using a random effect to estimate the fraction of the phenotypic variation that can be explained by genome-wide correlations. The individual random deviations from the population mean are constrained by assuming that the phenotypic covariance between individuals is proportional to their relative relatedness (or kinship), which is estimated using genome-wide marker data. This method was implemented in the software TASSEL (http://www.maizegenetics.net/index.php?page=bioinformatics/tassel/index.html).

Principal component analysis

Due to the difficulty of inferring *a priori* k with STRUCTURE as discussed above, a new approach termed EIGENSTRAT was proposed (Price et al., 2006). EIGENSTRAT is a method based on principal component analysis (PCA). It summarizes the variation of a large number of biallelic markers (single nucleotide polymorphisms, SNP) with genome-wide distribution into a small number of principal component variables (default is 10 variables). These variables can be interpreted as subpopulations from which the individuals originated. The genome of individual cultivars is composed of a series of principal components (PCA loadings) relating to certain ancestral populations (Patterson et al., 2006). The PCA loadings can be used to adjust individual candidate marker genotypes and phenotypes for their ancestry. The adjusted values are independent of estimated ancestry, so a statistically significant correlation between an adjusted candidate marker and adjusted phenotype is therefore evidence of close linkage of a trait locus to the marker (Price et al., 2006). The PCA loadings can also be simply used as a Q matrix from STRUCTURE, replacing Q in the mixed model of Yu et al. (2006) to account for the population stratification (Zhao et al., 2007).

Comparing the EIGENSTRAT approach with SA, it has the following advantages. First of all, EIGENSTRAT is less dependent on assessing the number of ancestral populations, and it runs extremely fast on large datasets which are impractical for STRUCTURE (Patterson et al., 2006). Second, EIGENSTRAT outputs each individual's coordinates along the PCA axis instead of assigning its genome to discrete populations, which may not always be the correct model for population history (Patterson et al., 2006). Third, EIGENSTRAT automatically tests the presence of population stratification (Patterson et al., 2006). Fourth, when the outputs of EIGENSTRAT are used in a mixed model (Q+K, Yu et al., 2006) or a GC model, the mixed model gave higher power (Price et al., 2006; Zhao et al., 2007). However, EIGENSTRAT was developed for a human genetics background and requires hundreds of thousands of SNP markers covering the whole genome to achieve a relatively accurate estimation of population stratification. Therefore, it might not be applicable for most plant species until the genotyping cost is dramatically reduced.

In summary, there is always population stratification in crops. In order to test QTLs of agronomic or quality characteristics with molecular markers, population stratification should be controlled by some methods or combination of methods, including selecting discrete materials to form the study population and using GC, SA or PCA to control spurious associations. A population with the least amount of population stratification, but harboring most of the genetic variation for traits of interest, is a good starting point even though it is hard to achieve. If the population has obvious stratification, GC can be used. It controls for Type I error rate, but has poor power because of its universal adjustment. If the study population follows the STUCTURE assumptions (a fixed k, in Hardy-Weinberg-equilibrium, unlinked markers), SA is a good model to control Type I error rate and has a higher power than

GC, but it is a serious computational challenge. With the introduction of a new generation of sequencing techniques (e.g., Solexa, http://www.solexa.com/), the genotyping costs will be dramatically reduced in the near future. Then, the PCA approach may be a suitable method for all plant species, since it is robust and straightforward. However, no matter which approach is used in association analysis, one should always check how much of the variance is explained by the model and the candidate marker, because that is the major interest of association analysis (E. Buckler, lecture note from Summer Institute of Statistics Genetics, University of Washington, June 27-29, 2007).

CHAPTER 2

Effects of Glutenin Subunit Variation and 1AL.1RS Translocation on Dough Rheology of Wheat Grown in Colorado Environments

ABSTRACT

Wheat (*Triticum aestivum* L.) flour is unique because it has the ability to form a dough that can be used to produce leavened bread and other food products. This ability is largely determined by its gluten proteins. Studies have shown that allelic variation at the *Glu-1* and *Glu-3* loci plays an important role in determining wheat dough properties and breadmaking quality. The 1AL.1RS translocation from rye (*Secale cereale* L.) to wheat confers disease and insect resistance and increased grain yield, biomass and grain weight and, therefore, has been used extensively in North America. On the other hand, 1AL.1RS has been associated with a decrease in hard winter wheat end-use quality. Although the effects of glutenins and rye translocations on quality are known to vary across environments, their effects on dough mixing properties of a segregating population of recombinant inbred lines (RILs) in irrigated and dryland environments have not been reported.

To evaluate the effects of alleles at glutenin loci and the presence of 1AL.1RS on dough mixing properties of hard winter wheat, we evaluated the RIL population from the

cross between two hard winter wheat parents that differ at all glutenin loci and 1AL.1RS, namely TAM 107-R7 (*Glu-A1a*, *Glu-B1b*, *Glu-D1a*, *Glu-A3e*, *Glu-B3g*, *Glu-D3b*, 1AL.1RS) and Arlin (*Glu-A1b*, *Glu-B1c*, *Glu-D1d*, *Glu-A3c*, *Glu-B3e*, *Glu-D3a*, non-1RS). The 190 RILs were grown at two Colorado locations, Akron (dryland) and Fort Collins (irrigated) in the 2004-05 growing season. Dough mixing properties were measured by 10-g mixograph. The results indicated that (1) wheat dough mixing properties were significantly affected by the growing environments ($P<0.0001$) with wheat from the dryland location having higher mixing tolerance than wheat from the irrigated location; (2) the presence of 1AL.1RS was associated ($P<0.01$) with reduced mixograph mixing tolerance; (3) longer mixograph peak time were observed in both environments with *Glu-D1d* (subunits 5+10) compared to *Glu-D1a* (2+12), *Glu-A1b* (2*) compared to *Glu-A1a* (1), *Glu-B1b* (7+8) compared to *Glu-B1*c (7+9), and *Glu-A3c* compared to *Glu-A3e*; (4) significant interaction effects ($P<0.05$) between *Glu-A1* (*a/b*) and *Glu-A3* (*e/c*) were observed. These results can be useful for assessing the potential quality of parental and early generation breeding lines in wheat breeding programs in Colorado and similar environments, by analyzing glutenin composition and the presence of the 1AL.1RS translocation.

INTRODUCTION

Wheat (*Triticum aestivum* L.) flour is unique because it has the ability to form a dough that can be used to produce leavened bread and other food products. This ability is largely determined by its gluten proteins consisting of glutenin and gliadin (reviewed by Singh and Khatkar, 2005). Glutenins are composed of polymers linked by disulphide bonds and are further classified into two groups, the high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), based on their mobility in sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions (reviewed by Singh and Khatkar, 2005). The HMW-GS and the LMW-GS have been found to be encoded by genes at the complex *Glu-1* and *Glu-3* loci on the long and short arms of the group 1 chromosomes, respectively (Jackson et al., 1983; Payne, 1987; Payne et al., 1984). Some LMW-GS are also encoded by genes on the short arms of group 6 chromosomes (Jackson et al., 1983; Payne et al., 1984).

Studies have shown that allelic variation at the glutenin loci plays an important role in determining wheat dough properties and breadmaking quality (Gras et al., 2001; Payne, 1987). The HMW-GS of wheat have been studied in detail because of their important role in determining breadmaking quality and easy separation on SDS-PAGE. HMW-GS account for about 6 to 16% of total grain proteins (reviewed by Singh and Khatkar, 2005). Based on the association of glutenin subunits to SDS-sedimentation volume (SDSSV) in various cultivars, Payne et al. (1987) assigned some alleles at the *Glu-1* locus a quality score to facilitate their use as indirect selection criteria in breeding programs. This scoring system was updated and more glutenin alleles were included

(reviewed by Singh and Khatkar, 2005). In this scoring system, *Glu-D1d* was assigned the highest score of 4 and *Glu-A1b* and *Glu-B1i* were assigned a score of 3. The lowest score of 1 was assigned to *Glu-A1c*, *Glu-B1a*, *Glu-B1e* and *Glu-D1c*. The LMW-GS represent about one-third of the total grain protein and around 60% of total glutenins (reviewed by D'Ovidio and Masci, 2004). Despite their abundance, LWM-GS have not been studied as thoroughly as the HMW-GS, because LMW-GS are relatively difficult to separate in gels (reviewed by Gianibelli et al., 2001).

The 1AL.1RS translocation from rye (*Secale cereale* L.) to wheat confers disease and insect resistance and increased grain yield, biomass and grain weight and, therefore, has been extensively used in North American wheat breeding programs (Espitia-Rangel et al., 1999b; Graybosch et al., 1999b; McKendry et al., 2001). On the other hand, 1AL.1RS has been associated with a decrease in hard winter wheat quality (Espitia-Rangel et al., 1999b; Graybosch et al., 1999b; Graybosch et al., 1993; McKendry et al., 2001).

Dough properties are evaluated by various methods. Mixograph tests are most commonly used in hard wheat breeding programs for quality selection in the early stages, and in some instances by bakers for predicting dough-handling requirements (Finney and Shogren, 1972). The mixograph records a time curve of dough resistance to mixing in a diagram known as a mixogram. A mixogram can be interpreted by more than 40 computer-analyzed mixograph characteristics (Navickis et al., 1990). These characteristics have been highly correlated with baking characteristics, such as loaf volume (Dobraszczyk and Schofield, 2002; Khatkar et al., 1996), and can be very useful in selecting good quality wheat lines in wheat breeding programs (Chung et al., 2001).

In Colorado, irrigated and dryland winter wheat are both important. According to Colorado agricultural statistics (http://www.nass.usda.gov/co/pub/alwhtce04.pdf), in Colorado, the acreage of irrigated winter wheat was 170,000 hectare, which was one tenth of total winter wheat acreage in 2004. However, because of the high yield of irrigated wheat, the total production of irrigated winter wheat was 9,910,000 bushels which was one fifth of total wheat yield for Colorado winter wheat in 2004.

The effects of glutenins and rye translocations on quality are known to vary across environments (reviewed by Dupont and Altenbach, 2003). However, their effects on dough mixing properties of a segregating population of recombinant inbred lines (RILs) in irrigated and dryland environments have not been reported. Therefore, the objective of this study was to evaluate the additive and interaction effects on flour mixing properties due to allelic variation at the *Glu-1* and *Glu-3* loci and the presence of the 1AL.1RS translocation in the common genetic background of a RIL population.

MATERIALS AND METHODS

Plant Materials

TAM 107-R7/Arlin population

A RIL population consisting of 190 lines was used in this study. This was a $F_{5:6}$ population developed from the cross between TAM 107-R7 and 'Arlin', advanced by the single seed descent method. After the last generation of selfing, each line was bulk-harvested to provide seeds for replicated trials and for glutenin protein analysis. TAM 107-R7 is an unreleased sister line of the hard red winter wheat cultivar 'Prairie Red', which has the pedigree of CO850034/PI372129//5*'TAM 107', and was developed by the Colorado State University (CSU) wheat breeding program (Quick et al., 2001). TAM 107-R7 is resistant to Biotype 1 of the Russian Wheat Aphid (*Diuraphis noxia* Mordvilko), has poor breadmaking quality (http://www.colostate.edu/Depts/SoilCrop/extension/CropVar/Wheat/1997/97wheatb.pdf), and possesses the 1AL.1RS translocation. Arlin is a hard white winter wheat cultivar developed cooperatively by the Kansas Agricultural Experiment Station and the Agricultural Research Service, United States Department of Agriculture (USDA) (Sears et al., 1997). It has good milling and baking quality (http://www.oznet. ksu.edu/library/crpsl2/l882.pdf). Besides differing in end-use quality, TAM 107-R7 and Arlin differ in various agronomic traits (http://www.colostate.edu/Depts/SoilCrop/extension/CropVar/Wheat/1997/97wheatb.pdf). All six HMW and LMW glutenin loci are polymorphic between the two parental lines (Table 2.1). Hence, a primary purpose of this cross was to study the genetic control of quality measured by the 10-g mixograph.

Table 2.1. *Glu-1* and *Glu-3* alleles (subunits) and the 1AL.1RS translocation status of the TAM 107-R7/Arlin population.†

Parent	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	1AL.1RS
TAM 107-R7	*a* (1)	*b* (7+8)	*a* (2+12)	*e*	*g*	*b*	yes
Arlin	*b* (2*)	*c* (7+9)	*d* (5+10)	*c*	*e*	*a*	no

† Letters in italics are allele designations (McIntosh et al., 2003) and numbers in parentheses are the corresponding subunits.

Field experiments

The 190 RILs, their parents, and check cultivars 'Longhorn' and 'Trego' were grown in two locations in Colorado: the Agricultural Research, Development and Education Center of CSU, Fort Collins and the Central Great Plains Research Station, USDA Agricultural Research Service, Akron, in the 2004-05 growing season. In both locations, the fields were arranged in an alpha-lattice incomplete block design with two replications, and each plot consisted of two rows with 25 cm spacing between rows. Plots at Fort Collins were 3.4 m long and plots at Akron were 3.7 m long. Ten grams of seeds per plot were planted in each trial. The trial at Fort Collins was irrigated with a linear overhead sprinkler system to maintain optimum moisture conditions until three weeks before harvest. Akron was a dryland location and no additional water was applied to the trial there. All plots were hand harvested and threshed separately with a stationary thresher. Seeds were cleaned manually.

Phenotypic Data

Agronomic traits

Phenotypic data were recorded for days to heading (DTH) and kernel weight (KW). The DTH was the number of days from January 1, 2005 to the day on which 50%

of the spikes were fully visible above the flag leaf collar. The KW was estimated from the weight of 200 random seeds.

Mixograph properties

Dough mixing properties were analyzed in the wheat quality lab of the CSU wheat breeding program at room temperature (around 25 °C). Fifty grams of seed from each plot were weighed, tempered to 15.5% moisture content, and then ground to flour with a Brabender Quadrumat® Jr. Mill. (C. W. Brabender Instruments, Inc. South Hackensack, NJ) following the AACCI Method 26-50 (AACCI, 2004). Grain and flour moisture, grain protein concentration (gprot12) and flour protein concentration (fprot14) were determined by near-infrared spectroscopy (NIR) with a Foss NIRSystems model 6500 (FOSS NIRSystems, Inc., Laurel, MD) according to AACCI Method 39-10 and 39-11 (AACCI, 2004). Mixing tests were performed on 10 g flour with a 10-g mixograph (National Manufacturing Division, TMCO, Lincoln, NE). Samples were weighed on a 14% moisture basis to an accuracy of 0.01 g, and all mixing experiments were carried out following the AACCI Method 54-40A (AACCI, 2004). The water absorption W (mL) which is the actual amount of water added to 10 g dry flour to perform a mixograph, was determined with the following formula:

$$W = \frac{1.5 \times p + 43.6}{10}$$

where p=percent flour protein concentration (14% moisture basis) measured by NIR.

Mixograph characteristics were determined with the commercial software program Mixsmart® version 3.80 (National Manufacturing Division, TMCO, Lincoln, NE). All mixing characteristics were measured from the center line (mixograph midline) of the mixogram, and all characteristics except mixing time are reported on a 100-unit

scale (Mu). Mixograph (midline) peak time (MPT) was visually determined and other characteristics were automatically estimated by Mixosmart®. Selected mixograph characteristics (MPT, mixograph midline left value at one minute before peak (MLV), mixograph left width at one minute before peak (MLW), mixograph peak width (MPW), mixograph midline peak height (MPV), mixograph midline right value at two minutes after peak (MRV) and mixograph right width at two minutes after peak (MRW)) are shown in the following mixogram (Fig. 2.1). The optimum MPT for hard red winter wheat is 3 to 6 minutes and the target for mixing tolerance (traditional score) is at least 3 on a scale of 0 to 6, as suggested by the U.S. Hard Winter Wheat Quality Committee, 2006 (personal communication, Brad Seabourn, USDA-ARS, Manhattan, Kansas). In the TAM 107-R7/Arlin RIL population, the higher value of mixograph properties, the better for the breadmaking quality.

Genotypic Data

Glutenin analysis

The glutenin proteins of each entry were extracted and analyzed by SDS-PAGE according to the protocols of Gupta and MacRitchie (1991) and Singh et al. (1991). HMW-GS were scored using the nomenclature of Payne and Lawrence (1983). LMW-GS were scored using the nomenclature of Gupta and Shepherd (1990). The allele designations follow those listed by McIntosh et al. (2003). Ten random seeds from each entry were bulked for each extraction. Reduced and alkylated glutenin subunits were analyzed by one-dimensional-three-stacking-layer SDS-PAGE with 4, 12, and 15% layers arranged from top to bottom. Gels were run at 12.5 mA per gel (Bio-Rad 2-gel system) for 18 h so that the HMW-GS would be resolved by the 12% layer and the

LMW-GS would be resolved by the 15% layer in the same gel (as described by Shan et al., 2007).

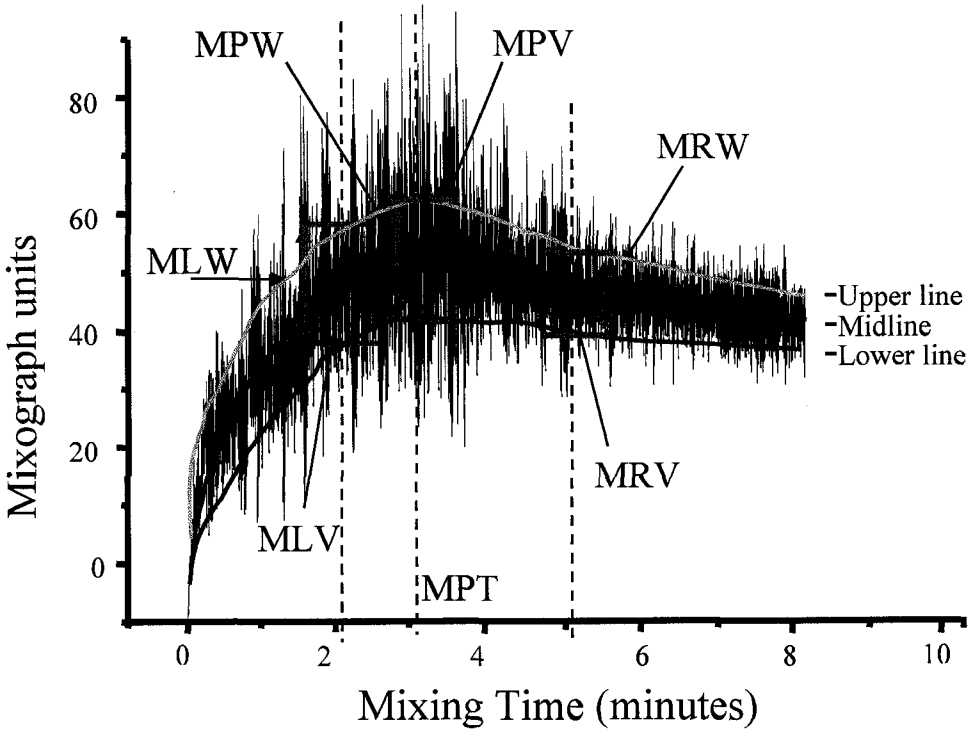


Fig. 2.1. Illustration of mixograph characteristics†

† MPT, Time to reach highest point of mixograph midline; MLW, band width (distance between the upper and the lower line) at one minute before MPT; MLV, mixograph midline height (value) at one minute before MPT; MPW, band width (distance between the upper and lower line) at MPT; MPV, mixograph midline height (value) at MPT; MRW, band width (the distance between the upper and lower line) at two minutes after MPT; and MRV, mixograph midline height (value) at two minutes after MPT.

Rye translocation

DNA was extracted from young leaves of a single plant for each entry using the method described by Riede and Anderson (1996). The 1AL.1RS translocation was identified with the polymerase chain reaction (PCR) method developed by Katto et al. (2004), which targets a widely dispersed rye specific repetitive sequence, and confirmed with the marker 'SCM9' (Saal and Wricke, 1999), which discriminates 1AL.1RS and 1BL.1RS.

Data Analysis

Analysis of variance was conducted with the GLM procedure of SAS version 9.1 (SAS Institute Cary, NC, USA). Data were analyzed for each location separately, and then combined over locations. In the combined analysis, the effects of environment, genotype, and genotype × environment interaction were highly significant ($P<0.01$) for most traits. Therefore, results are presented for each location separately, rather than for the combined data set. In each location, the MIXED procedure of SAS was used to obtain least squares means of each RIL with 'incomplete block' as a random effect. The following analyses were conducted with the least squares means. First, the homogeneity of error variance of the traits was evaluated with the Kolmogorov-Smirnov test of the UNIVARIATE procedure of SAS. Second, the association of loci with mixograph properties was determined with the GLM procedure including one glutenin locus or 1AL.1RS at a time in the model. Third, the interactions among the glutenin loci and the presence of 1AL.1RS were ascertained with the GLM procedure including two target loci and their interaction term in one model. At each locus, the mean for each glutenin allele and the presence or absence of 1AL.1RS was calculated and compared with the 'lsmean /pdiff' statement of the GLM procedure. Fourth, the Pearson correlation coefficients among mixograph properties, protein concentrations, DTH and KW were obtained with the CORR procedure of SAS. To test for deviation from expected segregation in an F_5-derived population, the chi-square tests for allelic frequency were carried out with the Excel program of Microsoft® Office 2007 (Microsoft ® Corporation, Redmond, WA).

RESULTS

Trait Means

There were highly significant ($P<0.0001$) differences among the RILs for all the traits at both locations (data not shown). Highly significant differences ($P<0.0001$, n=189) in trait means were observed between Fort Collins and Akron with the exception of DTH ($P=0.015$, n=189) (Table 2.2). Lines grown at Akron had higher trait values than those grown at Fort Collins with the exception of KW, which had lower values at Akron than Fort Collins. All the traits were approximately normally distributed within each location (data not shown). There was no obvious weather difference at the two experimental locations, Fort Collins and Akron, in the wheat growing season (see Table A8), so the moisture condition due to the irrigation should be the major environmental difference between the two experimental locations.

Correlation among characteristics

The characteristics gprot12, fprot14, DTH, KW, and mixograph properties were mostly significantly ($P<0.05$) correlated with each other in the 189 RILs of the TAM 107-R7/Arlin population (Table 2.3). The DTH was negatively correlated with KW in both Fort Collins ($r=-0.30$, $P<0.001$) and Akron ($r=-0.51$, $P<0.001$). It was negatively correlated with gprot12 ($r=-0.51$, $P<0.001$) and fprot14 ($r=-0.50$, $P<0.001$) at Fort Collins, but was positively correlated with gprot12 ($r=0.40$, $P<0.001$) and fprot14 ($r=0.40$, $P<0.001$) at Akron. The gprot12 and fprot14 were negatively correlated with MPT, MLV, MRV, and MRW at Fort Collins ($r=-0.18$ to -0.37, $P<0.05$), and they were negatively correlated with MPT, MLV, and MRW ($r=-0.16$ to -0.35, $P<0.05$) and were

positively correlated with MLW and MPV (r=0.20 to 0.29, P<0.01) at Akron. All the mixograph properties were highly positively correlated with each other at Fort Collins (r=0.30 to 0.94, P<0.001), whereas at Akron weaker correlation among mixograph properties was observed than at Fort Collins. In general, most mixograph properties were positively correlated with each other (r=0.19 to 0.89, P<0.01) at Akron, with the exception that MPV was negatively correlated with MPT (r=-0.25, P<0.001).

Table 2.2. Means for observed quality and agronomic characteristics (n=189) of the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.

Location	Fort Collins				Akron				*P* for Difference between locations
Variable†	Mean	Std Dev	Min	Max	Mean	Std Dev	Min	Max	
DTH (days)	139.8	2.0	136.1	145.1	140.2	2.6	135.8	146.9	0.015
KW (mg)	36.10	2.91	28.07	44.34	29.23	2.82	22.20	34.90	<0.0001
gprot12 (gkg^{-1})	149.0	11.6	119.1	181.4	156.6	6.9	138.5	176.9	<0.0001
fprot14 (gkg^{-1})	145.1	11.9	115.8	175.4	155.6	7.1	133.0	175.3	<0.0001
MLV (Mu‡)	40.82	7.06	18.72	55.59	58.28	4.90	42.65	70.18	<0.0001
MLW (Mu‡)	24.15	5.22	9.75	37.50	32.58	4.68	21.84	45.31	<0.0001
MPT (min)	2.27	0.45	1.28	3.40	3.79	0.92	2.33	6.51	<0.0001
MPV (Mu‡)	58.54	3.87	45.59	68.03	65.64	3.95	52.13	76.01	<0.0001
MPW (Mu‡)	19.02	2.57	11.5	24.85	23.45	2.79	17.06	31.39	<0.0001
MRV (Mu‡)	44.52	4.51	30.46	54.56	58.13	3.33	48.50	66.01	<0.0001
MRW (Mu‡)	4.64	1.32	2.88	12.34	12.8	3.59	5.27	22.34	<0.0001

† DTH, Days to heading; KW, kernel weight; fprot14, flour protein concentration at 14% moisture content; gprot12, grain protein concentration at 12% moisture content; MLV, mixograph midline left height at one minute before peak; MLW, mixograph left band width at one minute before peak; MPT, mixograph peak time; MPV, mixograph midline peak height; MPW, mixograph peak width; MRV, mixograph midline right height at two minutes after peak; MRW, mixograph right band width at two minutes after peak.

‡ Mu, mixograph units in 100-unit scale.

Table 2.3. Significant Pearson correlation coefficients (*r*) among quality characteristics, heading date, and kernel weight (n=189) of the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.†

Loc		Akron										
	Variable‡	fprot14	gprot12	DTH	MLV	MLW	MPT	MPV	MPW	MRV	MRW	KW
Ft Collins	fprot14		0.90	0.24	-0.16		-0.33	0.23			-0.32	-0.20
	gprot12	0.96		0.40		0.20	-0.31	0.29			-0.35	-0.26
	DTH	-0.5	-0.51					0.15			-0.18	-0.51
	MLV‡	-0.21	-0.26	0.16		0.41	0.52	0.52	0.48	0.89	0.56	
	MLW‡				0.80			0.55	0.57	0.53		
	MPT‡	-0.29	-0.34	0.24	0.89	0.62		-0.26	0.19	0.39	0.71	
	MPV‡				0.67	0.79	0.36		0.43	0.67		
	MPW‡				0.77	0.80	0.59	0.76		0.63	0.37	
	MRV‡	-0.18	-0.23	0.18	0.94	0.82	0.77	0.81	0.81		0.51	
	MRW‡	-0.33	-0.37	0.28	0.64	0.46	0.66	0.3	0.43	0.65		
	KW	0.16	0.18	-0.3			-0.14				-0.17	

† The upper right half of the table is the correlation of traits from Akron; the lower left half of the table is the correlation of traits from Fort Collins; divided by shaded cells. Only significant values are listed. For absolute value of r, when $0.14<r<0.18$, $0.01<P<0.05$; when $0.18<r<0.24$, $0.001<P<0.01$; when $r>0.24$, $P<0.001$.

‡ fprot14, flour protein concentration at 14% moisture content; gprot12, grain protein concentration at 12% moisture content; DTH, days to heading; MLV, mixograph midline left height at one minute before peak; MLW, mixograph left band width at one minute before peak; MPT, mixograph peak time; MPV, mixograph midline peak height; MPW, mixograph peak width; MRV, mixograph midline right height at two minutes after peak; MRW, mixograph right band width at two minutes after peak; KW, kernel weight.

Genotyping of Glutenin Loci and the 1AL.1RS Translocation for the TAM 107-R7/Arlin Population

The TAM 107-R7/Arlin population segregates at all the glutenin loci (Fig. 2.2) and the 1AL.1RS translocation. There were striking distortions from expected segregations at the *Glu-A1*, *Glu-B1*, *Glu-D1*, *Glu-A3* and 1AL.1RS (Table 2.4). About twice the expected number (12) of lines were heterozygous at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci (Table 2.4). The number of lines possessing *Glu-A1b* and *Glu-A3c* (both from Arlin) were 12 and 32% higher than expected, respectively (Table 2.4). Only one third of lines have 1AL.1RS (Table 2.4).

Table 2.4. Segregation ratio of glutenin loci and 1AL.1RS translocation in the TAM 107-R7/Arlin population

Locus & 1AL.1RS	Alleles†			Total	Chi-square	*P*
	Arlin	TAM 107-R7	Heterozygote			
Glu-A1	100	61	28	189	32.2	<0.0001
Glu-B1	79	83	25	187	16.3	0.0003
Glu-D1	94	73	19	186	7.5	0.0233
Glu-A3	126	63	-‡	189	20.9	<0.0001
Glu-B3	70	114	-	184	2.9	0.2303
Glu-D3	80	98	9	187	2.5	0.2855
1AL.1RS	158	32	-	190	83.6	<0.0001

† TAM 107-R7 (*Glu-A1a*, *Glu-B1b*, *Glu-D1a*, *Glu-A3e*, *Glu-B3g*, *Glu-D3b*, 1AL.1RS) and Arlin (*Glu-A1b*, *Glu-B1c*, *Glu-D1d*, *Glu-A3c*, *Glu-B3e*, *Glu-D3a*, non-1RS).
‡'-' indicates that we were not able to distinguish heterozygous from homozygous lines.

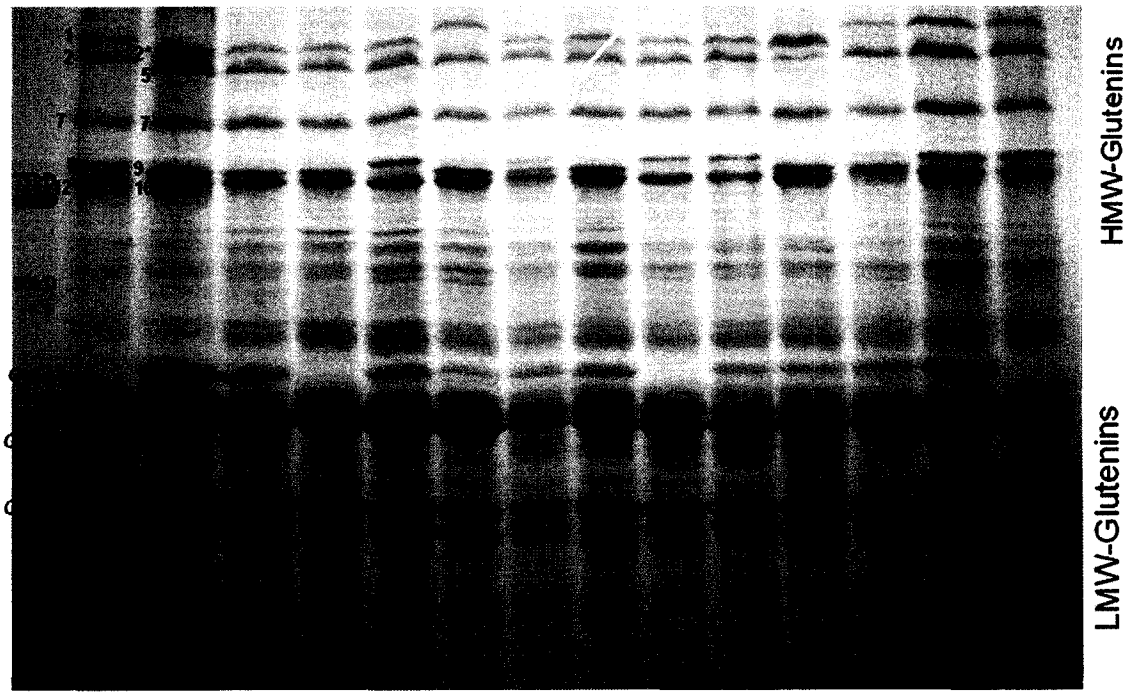


Fig. 2.2. Glutenin subunit patterns of 12 RILs and their parents of the TAM 107-R7/Arlin population analyzed by one-dimensional SDS-PAGE.
The HMW-GS are labeled according to the nomenclature of Payne and Lawrence (1983), and the LMW-GS are labeled according to the allelic designation of Gupta and Shepherd (1990)

Effects of Glutenin Loci and the 1AL.1RS Translocation and Their Interaction on Dough Rhelology Properties

The effects of *Glu-D1*, 1AL.1RS translocation, *Glu-B1* and *Glu-A1* × *Glu-A3* were of major importance for the variation of most mixograph properties in both locations for this population (Table 2.5). Other glutenin loci were important for some mixograph properties (Table 2.5).

Glu-A1

As can be seen from Table 2.5, the variation between lines with *Glu-A1a* and those with *Glu-A1b* had significant effects on MPV and MRV at both locations. In addition, it was important for MPW at Fort Collins and for MLV at Akron. The *Glu-A1b* allele, from the parent Arlin, always gave higher values of mixograph properties than *Glu-A1a* from the parent TAM 107-R7 (Table 2.6, 2.7 and 2.8).

Table 2.5. Mean squares from the analysis of variance for the relationship among mixograph properties and glutenin loci, 1AL.1RS and their interaction in the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.†

Locus & 1AL.1RS	Ft Collins							Akron						
	MPT	MLV	MPV	MRV	MLW	MPW	MRW	MPT	MLV	MPV	MRV	MLW	MPW	MRW
Glu-A1			193.2***	119.1*		25.8*			187**	153.1**	104.2**			
Glu-B1	1.1*	416.7**		181.8**			11.5*	5.1*	154.5*		91.9**			175.8***
Glu-D1	13.1***	2602.6***	93.3**	480.9***	589***	105.2***	11.1*	72.1***	322.8***	377.1***		95.4*		343.2***
Glu-A3			244.6***	109.3*	293***	63**			263.5***	133.4**	111.7**	152.7**	60.9**	
Glu-B3												104.5*		
Glu-D3	0.7*	210*												
1AL.1RS		517.5**	509.6***	387.3***	699.9***	136.7***		4.3*	435.6***	191.1***	254.6***	292.1***	185.6***	162***
Glu-A1 × Glu-A3	0.8*	302.7*	80.7*	143.9**	316***	34.3*	13.6**	3.2*	95.9*		92.2**	194.9**	79.5**	71.7*
Glu-A1 × Glu-B3	1*	226.2*												
Glu-A1 × Glu-D3	1.1*	258*		92.4*	161.3*	27.8*	10.3*							
Glu-B1 × Glu-D1		192.7*							90.7*					
Glu-B1 × Glu-B3										88.6*				
Glu-B1 × Glu-D3													49.9*	
Glu-D1 × Glu-B3										59.9*				
Glu-A3 × Glu-B3		213.4*			155.4*	54.4**								
1AL.1RS × Glu-D1									136.5**	58.5*				
1AL.1RS × Glu-A3												125*		
1AL.1RS × Glu-B3										69.2*				
1AL.1RS × Glu-D3	0.8*	268.3*	60*	91*	193.6**	47.2**								

*, **, ***, significant at the 0.05, 0.01, and 0.001 levels, respectively.

† MLV, mixograph midline left height at one minute before peak; MLW, mixograph left band width at one minute before peak; MPT, mixograph peak time; MPV, mixograph peak value; MPW, mixograph peak width; MRV, mixograph midline right height at two minutes after peak; MRW, mixograph right band width at two minutes after peak.

Table 2.6. The effects of glutenin loci and 1AL.1RS on mixograph peak time (MPT) in the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.

Location	Locus			MPT (min)		
		df	R^2 (%)	A†	T‡	P §
Akron	1AL.1RS	188	1.2	3.9	3.5	NS
	Glu-A1	159	0.2	3.8	3.7	NS
	Glu-B1	160	3.8	3.6	3.9	*
	Glu-D1	165	50.0	4.4	3.1	***
	Glu-A3	187	1.8	3.9	3.6	NS
	Glu-B3	181	0.1	3.8	3.8	NS
	Glu-D3	176	0.2	3.8	3.7	NS
Fort Collins	1AL.1RS	187	1.1	2.3	2.2	NS
	Glu-A1	158	0.0	2.3	2.3	NS
	Glu-B1	159	3.3	2.2	2.3	*
	Glu-D1	164	38.5	2.5	2.0	***
	Glu-A3	186	0.6	2.3	2.2	NS
	Glu-B3	181	0.0	2.3	2.3	NS
	Glu-D3	175	2.2	2.3	2.2	*

*, **, ***, significant at the 0.05, 0.01, and 0.001 levels, respectively.

† A, least squares means of lines with the Arlin (A) allele.

‡ T, least squares means of lines with the TAM 107-R7 (T) allele.

§ *P*, the least squares mean difference between the alleles from Arlin and TAM 107-R7.

Table 2.7. The effects of glutenin loci and 1AL.1RS on mixograph midline heights in the TAM 107-R7/Arlin population.

Location	Locus			MLV† (Mu) ‡					MPV† (Mu)					MRV †(Mu)		
		df	R^2 (%)	A§	T¶	*P*#	df	R^2 (%)	A	T	*P*#	df	R^2 (%)	A	T	*P*#
Akron	1AL.1RS	188	9.4	58.9	54.9	***	188	6.3	66.0	63.4	***	188	11.9	58.6	55.5	***
	Glu-A1	159	4.4	59	56.8	**	159	5.6	66.4	64.4	**	158	5.3	58.7	57	**
	Glu-B1	160	3.8	57.2	59.2	*	160	0.0	65.9	65.7	NS	159	5	57.3	58.9	**
	Glu-D1	165	7.8	59.4	56.6	***	165	14.0	64.2	67.3	***	164	1.5	58.4	57.6	
	Glu-A3	187	5.7	59.1	56.6	***	187	4.5	66.2	64.4	**	186	5.2	58.6	57	**
	Glu-B3	181	0.2	58.1	58.6	NS	181	0.1	65.9	65.7	NS	180	0.1	58.1	58.3	NS
	Glu-D3	185	0.0	58.2	58.2	NS	176	0.6	65.4	66.0	NS	175	0	58.1	58.1	NS
Fort Collins	1AL.1RS	187	5.5	41.6	37.1	**	187	18.1	59.3	54.8	***	187	10.1	45.2	41.3	***
	Glu-A1	158	0.9	41.1	39.8	NS	158	7.8	59.3	57.1	***	158	3.6	45.1	43.3	*
	Glu-B1	159	5.2	38.8	42.0	**	159	1.5	57.9	58.8	NS	159	5.5	43.2	45.3	**
	Glu-D1	164	30.8	44.4	36.5	***	164	4.0	59.1	57.6	**	164	14.4	46.1	42.7	***
	Glu-A3	186	1.7	41.4	39.4	NS	186	8.7	59.3	56.9	***	186	2.9	45	43.4	*
	Glu-B3	181	0.1	40.6	41.0	NS	181	0.0	58.6	58.7	NS	181	0.1	44.4	44.7	NS
	Glu-D3	175	2.4	41.8	39.6	*	175	0.8	58.9	58.2	NS	175	1.2	44.9	44	NS

*, **, ***, significant at the 0.05, 0.01, and 0.001 levels, respectively.

† MLV, mixograph midline left height at one minute before peak; MPV, mixograph peak value; MRV, mixograph midline right height at two minutes after peak.

‡ Mu, mixograph units.

§ A, least squares means of lines with the Arlin (A) allele.

¶ T, least squares means of lines with the TAM 107-R7 (T) allele.

P, the least squares means difference between the alleles from Arlin and TAM 107-R7.

Table 2.8. The effects of glutenin loci and 1AL.1RS on mixograph band widths in the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.

Location	Locus			MLW† (Mu) ‡					MPW † (Mu)					MRW † (Mu)		
		df	R^2 (%)	A§	T¶	*P* #	df	R^2 (%)	A	T	*P* #	df	R^2 (%)	A	T	*P* #
Akron	1AL.1RS	188	7.0	33.1	29.8	**	88	12.3	23.9	21.2	***	188	6.6	13.2	10.7	***
	Glu-A1	159	1.9	33.0	31.7	NS	159	2.1	23.8	22.9	NS	158	0.3	12.8	12.4	NS
	Glu-B1	160	0.2	32.7	32.2	NS	160	0.8	23.2	23.7	NS	159	8.3	11.5	13.5	***
	Glu-D1	165	2.4	32.0	33.5	*	165	0.0	23.4	23.3	NS	164	15.5	14.0	11.2	***
	Glu-A3	187	3.6	33.2	31.3	**	187	4.1	23.8	22.6	**	186	0.8	12.9	12.3	NS
	Glu-B3	181	2.4	31.8	33.3	*	181	0.4	23.3	23.6	NS	180	0.5	12.4	12.9	NS
	Glu-D3	185	0.0	32.5	32.6	NS	176	0.0	23.5	23.4	NS	175	0.0	12.7	12.6	NS
Fort Collins	1AL.1RS	187	13.6	25.0	19.8	***	187	11.0	19.4	17.1	***	187	7.6	4.7	4.4	NS
	Glu-A1	158	1.9	24.7	23.2	NS	158	2.4	19.3	18.4	*	158	1.6	4.8	4.4	NS
	Glu-B1	159	0.8	23.6	24.5	NS	159	2.0	18.5	19.2	NS	159	3.8	4.3	4.9	*
	Glu-D1	164	13.1	25.8	22.0	***	164	9.5	19.7	18.1	***	164	3.6	4.9	4.4	*
	Glu-A3	186	5.8	25.0	22.3	***	186	5.1	19.4	18.2	**	186	0.0	4.6	4.7	NS
	Glu-B3	181	0.3	23.9	24.4	NS	181	0.3	18.9	19.2	NS	181	0.1	4.7	4.6	NS
	Glu-D3	175	0.6	24.6	23.7	NS	184	1.1	13.3	18.8	NS	176	0.1	4.5	4.6	NS

*, **, ***, significant at the 0.05, 0.01, and 0.001 levels, respectively.

† MLV, mixograph midline left height at one minute before peak; MPV, mixograph peak value; MRV, mixograph midline right height at two minutes after peak.

‡ Mu, mixograph units.

§ A, least squares means of lines with the Arlin (A) allele.

¶ T, least squares means of lines with the TAM 107-R7 (T) allele.

P, the least squares means difference between the alleles from Arlin and TAM 107-R7.

Table 2.9. Mean values of mixograph characteristics for selected combinations of alleles of two glutenin loci or one glutenin locus and 1AL.1RS in the TAM 107-R7/Arlin population at Fort Collins and Akron in the 2004-05 growing season.†

Locus 1	Locus 2	Fort Collins (irrigated)							Akron (dryland)						
		MLV (Mu)	MLW (Mu)	MPT (min)	MPV (Mu)	MPW (Mu)	MRV (Mu)	MRW (Mu)	MLV (Mu)	MLW (Mu)	MPT (min)	MPV (Mu)	MPW (Mu)	MRV (Mu)	MRW (Mu)
Glu-A1b (2*)	*Glu-A3e*	40.64a‡	24.23a	2.25ab		18.65a	44.75a	5.26a	57.82a	33.12a	3.67ab	65.62a	23.80a	58.28a	12.95a
	Glu-A3c	41.26a	24.83a	2.27a		19.45a	45.19a	4.62b	59.34a	32.94a	3.83a	66.65a	23.74a	58.78a	12.84a
Glu-A1a (1)	*Glu-A3e*	36.13b	19.48b	2.07b		16.89b	40.80b	4.07b	54.09b	29.15b	3.29b	62.98b	21.25b	54.93b	10.88b
	Glu-A3c	42.84a	26.31a	2.41a		19.74a	45.45a	4.71ab	59.01a	33.82a	4.07a	65.60a	24.30a	58.76a	13.71a
Glu-A1b (2*)	*Glu-D3a*	40.87a	24.32a	2.21b		19.19a	44.81a	4.36b							
	Glu-D3b	40.40a	24.72a	2.23b		19.22a	44.87a	4.91a							
Glu-A1a (1)	*Glu-D3a*	43.01a	25.33a	2.44a		19.41a	45.07a	4.71ab							
	Glu-D3b	37.13b	21.45b	2.11b		17.66b	41.89b	4.18b							
Glu-A3c	*Glu-B3e*	37.48a	20.58b			17.18b									
	Glu-B3g	40.88ab	23.76a			19.00a									
Glu-A3e	*Glu-B3e*	42.26a	25.54a			19.78a									
	Glu-B3g	40.91a	24.68a			19.20a									
Non-1RS	*Glu-D1d*								59.79a			64.47b			
	Glu-D1a								57.77b			68.02a			
1AL.1RS	*Glu-D1d*								57.38b			63.07b			
	Glu-D1a								50.47c			63.42b			
Non-1RS	*Glu-D3a*	41.77a	24.81a	2.31a	59.29a	19.39a	45.13a								
	Glu-D3b	40.79a	25.08a	2.24a	59.28a	19.38a	44.91a								
1AL.1RS	*Glu-D3a*	41.64a	22.93a	2.42a	56.65b	18.75a	43.68a								
	Glu-D3b	33.78b	17.35b	1.98b	53.38c	15.85b	39.45b								

† Means followed by different letters indicate a significant difference at $P<0.05$. Empty cells indicate their F tests are not significant at $P<0.05$ level.

‡ MLV, mixograph midline left height at one minute before peak; MLW, mixograph left band width at one minute before peak; MPT, mixograph peak time; MPV, mixograph peak value; MPW, mixograph peak width; MRV, mixograph midline right height at two minutes after peak; MRW, mixograph right band width at two minutes after peak.

Glu-B1

The variation due to the *b* (subunits 7+8) and *c* (subunits 7+9) alleles at the *Glu-B1* locus had significant effects on MLV, MPT, MRV and MRW at both locations (Table 2.5). The allele *Glu-B1b*, from the parent TAM 107-R7, gave higher values for mixograph properties than *Glu-B1c*, from the parent Arlin (Table 2.6, 2.7 and 2.8).

Glu-D1

The results of our population confirmed the significantly beneficial effects of *Glu-D1d* (subunits 5+10) on mixograph properties compared to that of *Glu-D1a* (subunits 2+12) (Table 2.5, Table 2.6, Table 2.7 and Table 2.8). Based on R^2 values, *Glu-D1* explained 50.0% of the variation for MPT at Akron and 38.5% of the variation for MPT at Fort Collins.

Glu-A3

In our population, the null allele *Glu-A3c* had higher values of MLW, MPV, MPW and MRV at both locations and MLV at Fort Collins than *Glu-A3e*. However, the amount of variation for these traits explained by the *Glu-A3* locus was low (R^2<8.7%).

Glu-B3

The allelic variation between *g* and *e* at the *Glu-B3* locus appeared to be less important than variation at all other loci (Table 2.5). It only significantly affected MLW at Akron (R^2=2.4%) (Table 2.6, Table 2.7, Table 2.8).

Glu-D3

The variation between *b* and *a* at the *Glu-D3* locus had significant impacts on MLV (R^2=2.4%) and MPT (R^2=2.2%) at Fort Collins (Table 2.6, 2.7 and 2.8). *Glu-D3a* gave higher values of MLV and MPT than *Glu-D3b*.

Interactions among glutenin loci

As can be seen from Table 2.5, some interactions between the *Glu-1* and *Glu-3* loci had significant effects on some mixograph properties. Among them, the interaction between *Glu-A1* and *Glu-A3* had significant ($P<0.05$) influence on all the mixograph properties with the exception of MPV at Akron. In the four combinations of alleles at the *Glu-A1* and *Glu-A3* loci, the combination of *Glu-A1a*/*Glu-A3e* had significantly lower values for mixograph properties than other allelic combinations at these two loci (Table 2.8). Other important interactions among glutenin loci were between *Glu-A1* and *Glu-D1*, and between *Glu-A3* and *Glu-B3* at Fort Collins.

1AL.1RS translocation

The presence of 1AL.1RS in wheat lines significantly reduced the values of most mixograph properties with the exceptions of MPT and MRW at Fort Collins. For these significantly affected traits, the amount of variation explained by the 1AL.1RS status differed widely, ranging from 5.5 to 18.1% (see R^2 in Table 2.6, 2.7 and 2.8). The smallest effect of the presence of 1AL.1RS was on MLV at Fort Collins (R^2=5.5%) and the largest effects was on MPV at Fort Collins (R^2=18.1%).

Significant interactions were observed between 1AL.1RS and *Glu-D1*, *Glu-A3*, *Glu-B3*, and *Glu-D3* for some mixograph properties (Table 2.5). For the combinations of the 1AL.1RS status and the alleles at the *Glu-D1* locus, there is a trend that the value of mixograph properties of lines is in the order of non-1RS/*Glu-D1d*>non-1RS/*Glu-D1a*>1AL.1RS/*Glu-D1d*>1AL.1RS/*Glu-D1a* (Table 2.9). For the combination of 1AL.1RS status and *Glu-D3* alleles, lines with 1AL.1RS had lower values of mixograph properties than non-1RS lines. The lines with the combination of 1AL.1RS/*Glu-D3b* always had the lower values of mixograph properties than other combinations (Table 2.9).

DISCUSSION

Distorted Segregation of Glutenin Loci and 1AL.1RS.

There were highly significant distortions in the distribution of glutenin alleles and 1AL.1RS in the TAM 107-R7/Arlin population (Table 2.4). This population was derived from several F_1 crosses involving several parental plants as part of the CSU wheat breeding program (J. Quick, personal communication). The generation for genotyping was $F_{5:6}$, so the expected proportion of heterozygous lines should be one sixteenth of the total number of RILs (about 12 lines in this population for each locus). The distorted distribution of glutenin alleles and 1AL.1RS might have arisen in different stages of population development. For example, the crosses were developed for breeding purposes, so the purity of parents and cross-pollination control were likely less than optimum for developing a mapping population. We observed that although the TAM 107-R7/Arlin population segregates for *Glu-A1a* and *b*, one of the seed stocks of parent TAM 107-R7 we have now has *Glu-A1b* rather than the expected *Glu-A1a*. This may explain why *Glu-A1b* is predominant in this population (Table 2.4). The frequency of lines with the 1AL.1RS translocation is very low in the TAM 107-R7/Arlin population, only 32 lines out of 189 lines (Table 2.4). This was confirmed by a probe targeting rye-specific chromatin (Katto et al., 2004) and the 'SCM9' marker (Saal and Wricke, 1999) that detects the presence of 1AL.1RS. However, both primers are dominant markers, so we could not distinguish heterogeneous lines for 1AL.1RS from lines that were homogeneous for 1AL.1RS. When we consider the pedigree of TAM 107-R7 (CO850034/PI372129//5*'TAM 107', with selection for Russian Wheat Aphid resistance from the line PI372129), it is possible that our parent TAM 107-R7 plants were heterogeneous for 1AL.1RS or

some TAM 107-R7 plants were heterozygous at 1AL.1RS. If the TAM 107-R7 plants used as parents had equal representation of 1AL.1RS and non-1RS chromosomes, the expected number of RILs that carry 1AL.1RS is about 45, which is close to the observed number 32. The reduced transmission of 1RS to progenies was previously observed in other studies (Graybosch et al., 1999b; Koebner and Shepherd, 1986).

Segregation at the *Glu-A3* locus, located on the short arm of chromosome 1A, is expected to match segregation for 1AL.1RS. Lines with 1AL.1RS should lack any *Glu-A3* alleles and protein bands, based on the assumption that 1AL.1RS is a whole short arm translocation. However, seven lines with 1AL.1RS also were found to have protein bands for the allele *Glu-A3c*. Although the direction of the distortion was similar (158:32 for 1AL.1RS and 126:63 for *Glu*-A3), there was considerable disagreement between these two factors. When we evaluated the *Glu-A3* locus, we could not distinguish the true *Glu-A3e* (null allele) from absence of protein bands because of the presence of 1AL.1RS. We do not have a good explanation for the distorted distribution of alleles at the *Glu-A3* locus. When we evaluated the presence of the *Glu-A3c* allele with a sequence-based marker (Zhang et al., 2004), 16 lines had protein bands for the *Glu-A3c* allele that were not detected by the *Glu-A3c* primer. On the other hand, six lines that did not have protein bands for the *Glu-A3c* allele were amplified by the *Glu-A3c* primer. The disagreement of the protein score and the DNA score based on PCR for *Glu-A3c* can be explained in many ways, including the non-expression of *Glu-A3c*. We need to test the expression of the *Glu-A3c* allele and the absence of *Glu-A3c* allele due to the presence of the 1AL.1RS translocation in this population. For this dissertation, the analysis was based on protein scores rather than the DNA scores for the *Glu-A3* locus.

Trait Means and Correlation among Traits

Grain protein concentration was previously found to be influenced by environmental and genetic factors (Nelson et al., 2006; Prasad et al., 2003). This is also true for our population. The flour protein concentration of RILs at the dryland location, Akron, was higher than that at Fort Collins, the irrigated location, which might explain why flour from Akron produced higher means for all the mixograph properties (Table 2.2). This finding agrees with many studies, summarized by Weegels et al. (1996), showing that higher protein concentration was associated with higher SDSSV and stronger dough. However, within a location, flour protein concentration was negatively correlated with most of the mixograph properties (Table 2.3). This seems to contradict the previous findings. However, this is a reflection of the wide difference of fprot14 (ranging from 115.8 to 175.4 gkg^{-1} at Fort Collins and from 133.0 to 175.3 gkg^{-1} at Akron) and the formula we used to calculate water absorption for the mixograph (see formula in Material and Methods). In our formula, as suggested in AACCI Method 54-40A (AACCI, 2004), the higher the protein concentration, the more water was added in the dough developing system. Extra water above optimum water absorption likely will reduce the strength of bonds formed in the flour system.

As observed by Nelson et al. (2006) in the International *Triticeae* Mapping Initiative population, most of the mixograph properties in our population were positively correlated with each other with a few exceptions. For example, MPT was negatively correlated with MPV at Akron. This negative correlation was also observed in a group of European cultivars grown in France (Martinant et al., 1998).

Effects of Glutenin Loci and 1AL.1RS Translocation and Their Interaction on Dough Rheology

This study confirmed previous research on the importance of the glutenin loci on mixograph properties. In this population, *Glu-D1*, *Glu-B1*, *Glu-A1* × *Glu-A3*, and the 1AL.1RS translocation, had major impacts on mixograph properties, and other loci or interactions had important effects on some mixograph properties (Table 2.5).

Glu-A1

In our population, the *Glu-A1b* allele (subunit 2*) from the parent Arlin always gave higher values of mixograph properties than the *Glu-A1a* allele (subunit 1) from the parent TAM 107-R7 (Table 2.6, 2.7 and 2.8). This agreed with the previous report on the analysis of various cultivars by Moonen et al. (1983). They found that *Glu-A1b* produced higher loaf volume and SDSSV than *Glu-A1a*. However, some studies have found that *Glu-A1a* and *Glu-A1b* had similar effects on SDSSV and mixograph properties (Payne, 1987; Tabiki et al., 2006). Such a discrepancy could be due to the difference in the genetic background of the cultivars studied or the environmental conditions in which they were grown.

Glu-B1

The *Glu-B1b* allele (subunits 7+8) exerted higher values on MLV, MPT, MRV and MRW in both locations than *Glu-B1c* (subunits 7+9) in this population (Table 2.5). This agreed with results from the study of various collections of cultivars from Australia (Eagles et al., 2004) and China (He et al., 2005), and also agreed with assignment of the gluten quality scores (Payne et al., 1987) in which *Glu-B1b* was assigned a score of 3 and *Glu-B1c*, 2. However, as measured by SDSSV, opposite results were found in Canadian materials (Huang et al., 2006) and Indian materials (Ram, 2003).

Glu-D1

The results of our population confirmed the significantly beneficial effects of *Glu-D1d* on breadmaking quality compared to that of *Glu-D1a* (Table 2.6, 2.7 and 2.8), as shown by previous studies (Eagles et al., 2004; He et al., 2005; Payne, 1987; Tabiki et al., 2006). This result corresponds to the increased frequency of *Glu-D1d* in U.S. Great Plains cultivars and advanced lines since 1991 (Shan et al., 2007).

Glu-A3

In our population, lines with *Glu-A3c* (the presence of a protein band) had higher mean values of MLW, MPV, MPW, and MRV at both locations and MLV at Fort Collins than *Glu-A3e* (the absence of a protein band). The R^2 values associated with the *Glu-A3* locus were low, only 2.9 to 8.7% (Table 2.6, 2.7 and 2.8). *Glu-A3e* was associated with lower dough strength and extensibility than other *Glu-A3* alleles in some studies (Eagles et al., 2004; Liu et al., 2005; Luo et al., 2001). However, Bekes et al. (2006) found *Glu-A3e* was associated with higher Rmax and extensibility than *Glu-A3c* in five doubled haploid populations and collections of cultivars in Australia.

Glu-B3

To the best of our knowledge, the *g* and *e* alleles at the *Glu-B3* locus have not been directly compared in the same population in previous studies. In our population, these two alleles exerted similar effects on mixograph properties.

Glu-D3

Previously, the allele *Glu-D3b* was found to have a higher Rmax than *Glu-D3a* in collections of cultivars from Australia (Bekes et al., 2006; Eagles et al., 2004) and lower MPT in Canadian materials (Luo et al., 2001). In our population, *Glu-D3a* had a higher MPT and MLV than *Glu-D3b* at Fort Collins, but showed no significant

difference in other mixograph properties (Table 2.6, 2.7 and 2.8). The genetic background and different environmental conditions could be the reason for this discrepancy.

Interactions among glutenin loci

The lack of independence among the six glutenin loci and also among glutenin loci and non-storage protein loci has been previously reported (Eagles et al., 2002a; Liu et al., 2005; Ma et al., 2005). In our population, certain interactions between *Glu-1* and *Glu-3* had significant effects on some mixograph properties (Table 2.9). Among them, the interaction between *Glu-A1* and *Glu-A3* was of major importance. The combination of *Glu-A1a/Glu-A3e* had significantly lower values for mixograph properties than the other three allelic combinations at these two loci. Liu et al. (2005) and Eagles et al. (2002a) detected a significant interaction between *Glu-A1* and *Glu-A3* for MPT and Rmax, respectively. Unfortunately, they did not specify which alleles were involved in those interactions.

1AL.1RS translocation

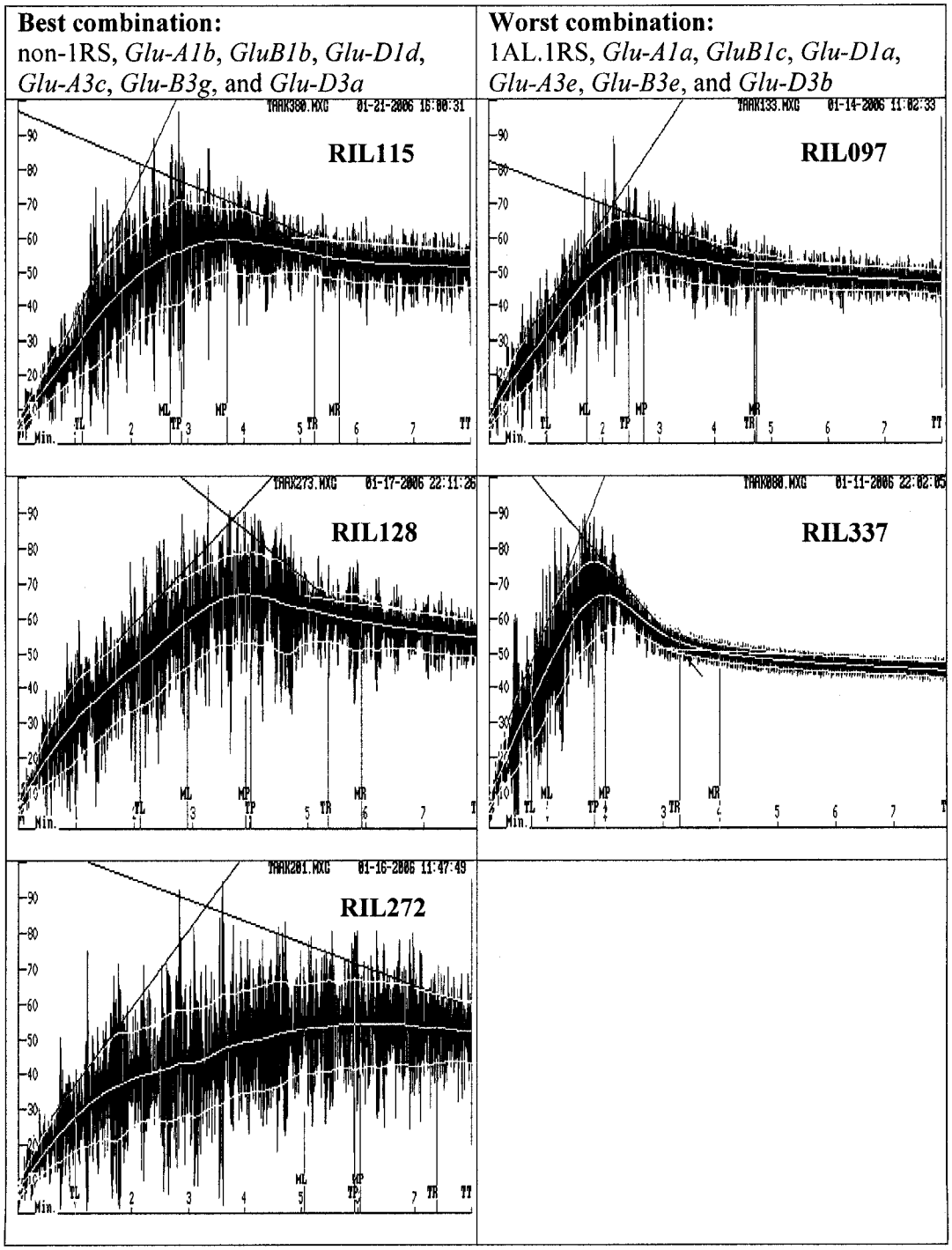
In our population, the presence of 1AL.1RS in wheat lines significantly reduced the values of most mixograph properties with the exceptions of MPT and MRW at Fort Collins (Table 2.6, 2.7 and 2.8). Our results confirmed the negative quality effects of the introduction of 1RS into wheat. The presence of 1AL.1RS in wheat lines reduced dough mixing strength and SDSSV compared to non-1RS lines (Espitia-Rangel et al., 1999a; Graybosch et al., 1999b; Kumlay et al., 2003).

In our population, significant interactions were observed between 1AL.1RS and *Glu-D1* and *Glu-D3* for some mixograph properties (Table 2.5). For the combinations of the 1AL.1RS status and the alleles at the *Glu-D1* locus, there was a trend that the value of mixograph properties of lines was in the order of non-1RS/*Glu-*

D1d>non-1RS/*Glu-D1a*>1AL.1RS/*Glu-D1d*>1AL.1RS/*Glu-D1a* (Table 2.9). This indicates that the presence of *Glu-D1d* in wheat lines with 1AL.1RS can partially compensate for the negative effects of 1AL.1RS on wheat quality, as suggested by Graybosch (2001). In the background of *Glu-D3b*, the presence of 1AL.1RS appeared to be more detrimental in our population (Table 2.9). In wheat breeding programs, careful selection of glutenin makeup could be a way to reduce the negative effects of 1AL.1RS.

Based on the results we obtained, in order to select for high breadmaking quality wheat in our population regardless of other traits, we should choose RILs without 1AL.1RS, and with *Glu-A1b*, *GluB1b*, *Glu-D1d*, *Glu-A3c*, *Glu-B3g*, and *Glu-D3a*. There are three RILs in the total of 190 RILs fitting the criteria: RIL115, RIL128 and RIL272. We should also try to avoid the opposite combinations (with 1AL.1RS, and with *Glu-A1a*, *GluB1c*, *Glu-D1a*, *Glu-A3e*, *Glu-B3e*, and *Glu-D3b*). There are two RILs out of 190 RILs having those combinations, RIL 97 and RIL 337. Comparing the mixograms for these entries grown at Akron, the RILs with the favorable combination of glutenin alleles and the 1AL.1RS status (RIL115, RIL128 and RIL272) had obviously wider band widths than those with the inferior combination (RIL 97 and RIL 337) (Fig. 2.3).

Fig. 2.3. The sample mixograms of the best and worst combinations of glutenin loci and the 1AL.1RS status for the TAM 107-R7/Arlin population at Akron in the 2004-05 growing season.



In summary, to evaluate the effects of the glutenin loci and the presence of 1AL.1RS on dough mixing properties of hard winter wheat, we evaluated 190 $F_{5:6}$ RILs from the cross between TAM107-R7 and Arlin at two Colorado locations, Akron (dryland) and Fort Collins (irrigated) in 2004-05. Gluten strength and dough mixing properties were measured by 10-g mixograph. The results indicated that (1) wheat dough mixing properties were significantly effected by growing environments; (2) wheat from the dryland location produced higher dough mixing tolerance than wheat from the irrigated location; (3) the presence of 1AL.1RS was associated with reduced dough mixing tolerance; (4) better dough mixing properties were observed in both environments with *Glu-D1d* (5+10) vs. *Glu-D1a* (2+12), *Glu-A1b* (2*) vs. *Glu-A1a* (1), *Glu-B1b* (7+8) vs. *Glu-B1c* (7+9), and *Glu-A3c* vs. *Glu-A3e*; (5) significant interaction effects between *Glu-A1* and *Glu-A3*, and *Glu-D1* and 1AL.1RS translocation were also observed. The results can be useful for assessing the potential quality of parental and early generation breeding lines in wheat breeding programs in Colorado and similar environments, by analyzing their glutenin compositions and the presence of the 1AL.1RS translocation.

CHAPTER 3
Association Analysis Reveals Effects of Allelic Variation at the Wheat Glutenin Loci and Rye Translocations on Dough Rheology

ABSTRACT

Allelic variations at the glutenin loci have been reported to play an important role in determining wheat (*Triticum aestivum* L.) dough properties and breadmaking quality. The effects of alleles at *Glu-1* loci have been intensively studied. However, due to the complexity of the *Glu-3* loci, the quality effects of different alleles at these loci are not yet fully understood. The wheat-rye (*Secale cereale* L.) chromosomal translocations in the form of 1AL.1RS and 1BL.1RS have been widely used to provide wheat resistance to diseases and insects. However, they have been associated with a decrease in hard winter wheat quality. We evaluated 96 cultivars and advanced lines, mainly from the U.S. Great Plains, at two dryland locations (Akron and Dailey) and one irrigated location (Fort Collins) in Colorado in the 2005-06 growing season. Gluten strength and dough mixing properties were measured with 10-g mixograph. Six characteristics, mixograph peak time (MPT), mixograph midline left height (MLV), mixograph left width, mixograph peak value (MPV), mixograph peak width (MPW), mixograph midline right height (MRV) and mixograph right width (MRW), were used as indicators of mixing time and dough mixing tolerance. Population structure was estimated on the basis of 60 simple sequence repeat (SSR) marker loci. The association of allelic variation and mixograph properties was analyzed with a linear regression model with one glutenin locus or rye translocation and

subpopulations as fixed factors, and the relatedness of individuals as a random effect. The results indicated that accounting for multiple-level relatedness of individuals in the statistical model reduced most associations among mixograph properties and genetic factors and detected additional associations in this population. Growing environments, all glutenin loci, and the presence of 1RS in wheat had significant ($P<0.05$) effects on mixograph properties. In general, *Glu-A1c*, *Glu-B1e*, *Glu-D1a*, and *Glu-B3c* and *i* were associated with low values of MPT and MRW, whereas *Glu-A1b*, *Glu-B1b*, *Glu-D1b* and *d*, and *Glu-B3f* and *b* were consistently associated with higher values of MPT and MRW than other alleles at the respective loci. *Glu-B1w* was associated with higher values of MPT and MRW, indicating longer peak dough mixing time and mixing tolerance in the irrigated location, Fort Collins and could be a beneficial allele at the *Glu-B1* locus for breadmaking quality. It was associated with intermediate values of MPT and MRW at dryland locations, Dailey and Akron. The presence of the 1RS translocation was significantly ($P<0.05$) associated with a decrease in all mixograph properties. The values of most mixograph properties were in the order of non-1RS≥1AL.1RS>1BL.1RS, except for MPT at all three locations, MPW at Akron, and MPV at Dailey. These results suggest that selection of appropriate alleles at glutenin loci and the 1RS translocation type can facilitate the development of cultivars with desirable dough properties for breadmaking quality.

INTRODUCTION

Improving quality to meet market needs has been the target of wheat (*Triticum aestivum* L.) breeding programs for more than a century. Wheat breeding programs in the U.S. Great Plains develop cultivars that can produce grain suitable for a wide range of domestic and international markets. A number of genetic, biochemical, and environmental factors have been related to wheat quality. Among them, protein concentration and its composition are both of primary importance (reviewed by Singh and Khatkar, 2005). In wheat, the prolamins are quantitatively the major storage protein. They are classified into two groups, gliadins and glutenins, which are the major components of gluten. Glutenins are formed of polymers linked by disulphide bonds and are further classified into two groups, the high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), based on their mobility in sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions (reviewed by Singh and Khatkar, 2005). The HMW-GS and LMW-GS are encoded by genes at the complex *Glu-1* and *Glu-3* loci on the long and short arms of wheat group 1 chromosomes, respectively (Jackson et al., 1983; Payne, 1987; Payne et al., 1984). Some of the LMW-GS are also encoded by genes on the short arms of group 6 chromosomes (Jackson et al., 1983; Payne et al., 1984). Each wheat cultivar contains three to five HMW-GS and three to six LMW-GS with different electrophoretic mobility. The HMW-GS have been studied in detail because of their important roles in determining breadmaking quality, as well as their ease of separation on SDS-PAGE. The HMW-GS account for only 6 to 16% of total seed storage proteins

(reviewed by Singh and Khatkar, 2005). As determined by SDS-PAGE, the molecular weight of HMW-GS ranges between 80 and 160 kilodaltons (kD) depending on the particular subunit and the SDS-PAGE system used. The LMW-GS represent about one-third of the total seed protein (reviewed by D'Ovidio and Masci, 2004). They have a molecular weight ranging from 20 to 45 kD. Despite their abundance, LWM-GS have not been studied as thoroughly as the HMW-GS because LMW-GS are relatively difficult to separate in gels (reviewed by Gianibelli et al., 2001)

A number of genes of agronomic importance, including disease and pest resistance genes, have been transferred from rye (*Secale cereale* L.) to its relative, common bread wheat, through the production of interspecific chromosomal translocation and substitution lines (reviewed by Graybosch, 2001). Rye chromosome 1RS (the short arm of rye chromosome 1) has been transferred to wheat in the form of 1AL.1RS, 1BL.1RS and 1DL.1RS wheat-rye chromosomal translocations (1AL, 1BL, and 1DL stand for the long arms of group 1 wheat chromosomes). Among these, the 1AL.1RS and 1BL.1RS translocations are most common in wheat cultivars (Friebe et al., 1996; Graybosch, 2001). The 1BL.1RS translocation probably has been utilized more widely worldwide in wheat breeding and production than any other piece of alien chromatin, whereas the 1AL.1RS is more limited to U.S. wheats (Graybosch, 2006).

Unfortunately, the 1RS translocation often results in significant alterations of wheat processing quality (reviewed by Graybosch, 2001). Severe quality problems are recognized when 1BL.1RS is present in hard wheat backgrounds, including low SDS sedimentation volumes (SDSSV), the production of 'sticky' doughs, a lack of tolerance of doughs to overmixing, and low loaf volumes (Graybosch, 2001). Some studies found

that wheats with the 1AL.1RS translocation appeared to have better end-product qualities than those with the 1BL.1RS translocation (Espitia-Rangel et al., 1999a; Graybosch, 2001; Graybosch et al., 1993; Kumlay et al., 2003), although it still reduced mixing strength and SDSSV compared to non-1RS sibling lines (Amiour et al., 2002; Espitia-Rangel et al., 1999a; Graybosch et al., 1999b; Graybosch et al., 1993; Kumlay et al., 2003).

In the past decade, marker-assisted selection (MAS) has become a tool for some breeding programs to improve some complex traits (Collard et al., 2005). The success of MAS depends on the discovery of markers linked to traits, and is enhanced by knowledge of gene functions. Two of the most commonly used methods for dissecting complex traits are linkage analysis and association mapping, the later also known as linkage disequilibrium mapping or association analysis (Mackay, 2001). The quality effects of some glutenin alleles have been studied with linkage analysis in structured populations, such as recombinant inbred line (RIL) populations (Dong et al., 1992; Gupta et al., 1994; Luo et al., 2001), doubled haploid (DH) populations (Bekes et al., 2001; Huang et al., 2006; Nagamine et al., 2000; Tabiki et al., 2006), and near isogenic line (NIL) populations (Deng et al., 2005). Although designed segregating populations are straightforward and have certainly proven useful in wheat to study the quality effects of glutenin alleles, the largest number of associations between glutenin alleles and quality characteristics have been established in collections of wheat cultivars and advanced lines (Bekes et al., 2006; Branlard et al., 2001; Eagles et al., 2002a; Eagles et al., 2002b; Eagles et al., 2004; He et al., 2005; Liu et al., 2005). Based on the association of glutenin

subunits to SDSSV in various cultivars, Payne et al. (1987) assigned some alleles at the *Glu-1* locus a quality score to facilitate their use in breeding programs.

A study based on a collection of cultivars has several advantages. First, it simultaneously evaluates many alleles at each locus in one population, whereas in structured wheat populations, each population segregates at most for two alleles per locus at those loci that are polymorphic between the parents. Second, allelic effects are assessed in genetic backgrounds that are representative of backgrounds of elite germplasm and therefore, the results can be applied to breeding materials. Third, multi-location and multi-year quality data are often available for analysis at no additional costs because these data are collected routinely in many wheat breeding programs (Breseghello and Sorrells, 2006a; Eagles et al., 2002b). Due to these advantages of association analysis, it has been employed to study the function of glutenin loci in recent years.

Nevertheless, there are certain precautions that must be followed with association analysis to avoid reaching incorrect conclusions. Due to geographic isolation, natural selection, or artificial selection, population stratification is common for plant germplasm (Yu and Buckler, 2006), and has been observed in collections of wheats (Breseghello and Sorrells, 2006b; Ravel et al., 2006). This population stratification and an unequal distribution of alleles within a population can result in spurious associations (Knowler et al., 1988; Malosetti et al., 2007; Price et al., 2006). In order to reduce the number of false associations, several association methods have been developed. Traditionally, pedigree information has been used to control population stratification with the identity by descent method (Crepieux et al., 2005), the quantitative inbred pedigree disequilibrium test (Stich et al., 2006), or the *in silico* mapping (Parisseaux and Bernardo, 2004) when complete

pedigree information is available. However, pedigree information is often incomplete or inaccurate (Kraakman et al., 2004; Yu and Buckler, 2006). Therefore, genome-wide-distributed molecular markers have been used to estimate the population structure and the relatedness of individuals. Descriptions of that relationship are incorporated in the statistical model to reduce false associations (Crepieux et al., 2005).

Structured association is a common method using molecular marker information to control false associations. In structured association, the Q-matrix estimated from the program STRUCTURE (Pritchard et al., 2000) is included in the statistical model to test marker-trait associations while controlling for spurious associations (Breseghello and Sorrells, 2006a; Remington et al., 2001; Thornsberry et al., 2001). The principle of this method is that variation attributable to subpopulation memberships is accounted for first and then the presence of any residual association between the marker and phenotype is tested (Mackay and Powell, 2007). Structured association is effective in detecting and adjusting for the presence of population structure, but does not deal with consanguinity within populations arising from the first cousin-level relationships (Yu et al., 2006). Recently, Ed Buckler's group (USDA-ARS, Ithaca, NY) developed a unified mixed model (Q+K) to account for both population structure and the correlation between individuals (Yu et al., 2006). In this model, the population membership (Q-matrix) is estimated with the program STRUCTURE (Pritchard et al., 2000) using a set of unlinked markers, and kinship (K-matrix) among varieties is also estimated empirically with a set of control markers using the program SPAGedi (Hardy and Vekemans, 2002; Yu et al., 2006). The key in their approach is using a random effect to estimate the fraction of the phenotypic variation that can be explained by genome-wide correlations among

individuals. The individual random deviations from the population mean are constrained by assuming that the phenotypic covariance between individuals is proportional to their relatedness (or kinship), which is estimated with genome-wide marker data (Yu et al., 2006).

One of the difficulties for structured association is to estimate *a priori* population number (k, which is different from the K-matrix). Pritchard et al. (2000) suggest a method based upon marginal likelihoods to determine the number of ancestral populations needed to explain the observations. A series of values of k were tested in the program STRUCTURE with the same setting for several times to get the likelihoods for the respective k. The marginal likelihood can be calculated as the harmonic mean of the likelihoods sampled from the output of the Markov Chain Monte Carlo (MCMC) method used to approximate the probability of assigning individuals to populations. In plants, due to the complexity of the population structure, the estimation with this method is not straightforward and even problematic. Huelsenbeck and Andolfatto (2007) have suggested a method to estimate *a priori* ancestral number k based on a Dirichlet process model. It allows both the assignment of individuals to populations and the number of populations to be considered as random variables, and automatically infers ancestral population number k.

Because of the importance of wheat quality, several empirical procedures to select new wheat varieties for good quality have been developed, such as near-infrared spectroscopy (NIR), mixograph, extensigraph, farinograph, SDS sedimentation test, milling test, and baking test. The mixograph is commonly used in hard wheat breeding programs for early-generation quality selection, in some instances by bakers for

predicting dough-handling requirements (Finney and Shogren, 1972). It records a time curve of dough resistance to mixing in a diagram known as a mixogram. A mixogram can be interpreted by more than 40 computer-analyzed mixograph characteristics (Navickis et al., 1990). These characteristics have been highly correlated with baking characteristics, such as loaf volume (r>0.65, P<0.001, n=13) (Khatkar et al., 1996) and can be very useful in selecting good quality wheat lines in breeding programs (Chung et al., 2001).

In Colorado, irrigated and dryland winter wheat are both important. According to Colorado agricultural statistics (http://www.nass.usda.gov/co/pub/alwhtce04.pdf), the acreage of irrigated winter wheat was 170,000 hectare, which was one tenth of total winter wheat acreage in 2004. However, because of the high yield of irrigated wheat, the total production of irrigated winter wheat was 9,910,000 which was one fifth of total wheat yield for Colorado winter wheat in 2004.

The effects of glutenin alleles and rye translocations on wheat quality are known to vary across environments (reviewed by Dupont and Altenbach, 2003). However, their effects on dough mixing properties have not been assessed in Colorado environments. The associations of glutenin alleles and the presence of the 1RS translocation in wheat with quality characteristics have been established in collections of wheat cultivars and advanced lines, but the possible false associations of glutenin alleles and 1RS translocation and quality characteristics due to multiple-level relatedness among individuals have not been examined. Thus, the objective of this study was to evaluate the effects of allelic variation at the *Glu-1* and *Glu-3* loci and the presence of the 1RS translocation on dough mixing properties in the collection of 96 wheat cultivars primarily from the U.S. Great Plains in both irrigated and dryland Colorado environments.

MATERIALS AND METHODS

Plant Materials

Cultivar population

A population of 99 hard red and hard white winter wheat cultivars and advanced lines was evaluated in this study, including 89 entries released or developed since 1991 in the U.S. Hard Winter Wheat Region, and 10 entries from other countries (Appendix, Table A1). The glutenin composition, pedigree information, and developing organizations for the 77 U.S. materials were given in the report of Shan et al. (2007). For the convenience of genotyping in 96-well plates, three entries (Venera, BEZOSTAYA1, and OR939526) were arbitrarily excluded from the population, mainly because of their late maturity in the Colorado environments. The associations among glutenin alleles and mixograph properties were then based on the remaining 96 entries.

Field experiments

The 99 genotypes were grown at three locations in Colorado in the 2005-06 growing season: the Agricultural Research, Development and Education Center of Colorado State University, Fort Collins; the Central Great Plains Research Station, USDA Agricultural Research Service, Akron; and a farmer's field near Dailey. At all locations, the fields were arranged in an alpha-lattice incomplete block design with two replications, and each plot consisted of two rows with 25 cm spacing between rows. Plots at Fort Collins were 3.4 m long, and plots at Akron and Dailey were 3.7 m long. Ten grams of seed per plot were planted in each trial. The trial at Fort Collins was irrigated with a linear overhead sprinkler system as needed to maintain optimum moisture

conditions until three weeks before harvest. Akron and Dailey were dryland locations with no additional water applied to those trials. All plots were hand harvested and threshed separately with a stationary thresher. Seeds were cleaned manually.

Weather reports from weather stations close to the experiment fields showed that there was very little difference in temperature among the three locations. Dailey received twice as much precipitation Akron in May coinciding with anthesis and the grain filling period, a critical time for wheat. However, the absolute values were all small 0.353 cm/day and 0.150 cm/day for Dailey and Akron, respectively (Appendix, Table A8).

Phenotypic Data

Agronomic traits

Phenotypic data were recorded for grain yield, kernel weight (KW), number of seeds per head (NSPH), days to heading (DTH), days to physiological maturity (DPM), grain fill duration (GFD), and plant height (PH). The KW was estimated from the weight of 200 random seeds. The NSPH was averaged over 10 heads per plot. The DTH was the number of days from January 1, 2006 to the day on which 50% of the spikes were fully visible above the flag leaf collar. The DPM was the number of days from January 1, 2006 to the date when 50% of the heads had peduncles showing complete loss of green color. The GFD was determined by subtracting DTH from DPM. The PH was measured as the height of the stem to the tip of the spike, excluding awns, measured one week before harvest.

Mixograph properties

To assess mixing properties, 50 g of seeds from each plot were weighed, tempered to 15.5% moisture content, and then milled to flour with a Brabender

Quadrumat® Jr. Mill. (C. W. Brabender Instruments, Inc., South Hackensack, NJ) following the AACCI Method 26-50 (AACCI, 2004). Seed and flour moisture, grain protein concentration (gprot12), flour protein concentration (fprot14), and grain hardness (hardness) were determined by NIR with Foss NIRSystems model 6500 (FOSS NIRSystems, Inc., Laurel, MD) according to AACCI methods 39-10 and 39-11 (AACCI, 2004). Mixing tests were performed on 10 g flour with 10-g mixograph (National Manufacturing Division, TMCO, Lincoln, NE, USA). Samples were weighed on a 14% moisture basis to an accuracy of 0.01 g, and all mixing experiments were carried out following the AACCI Method 54-40A (AACCI, 2004). The water absorption W (mL), which is the actual amount of water added to 10 g flour to perform a mixograph experiment was determined with the following formula, as suggested by the AACCI Method 54-40A (AACCI, 2004):

$$W = \frac{1.5 \times p + 43.6}{10}$$

where p=percent flour protein concentration (14% moisture basis) measured by NIR.

Mixograph characteristics were determined with the commercial software program Mixsmart® version 3.80 (National Manufacturing Division, TMCO, Lincoln, NE). All mixing characteristics were measured from the center line (mixograph midline) of the mixogram, and all characteristics except mixing time are reported on a 100-unit scale (Mu). Mixograph (midline) peak time (MPT) was visually determined and other characteristics were automatically estimated by Mixosmart®. Selected mixograph characteristics (MPT, mixograph midline left height at one minute before peak (MLV), mixograph left width at one minute before peak (MLW), mixograph peak width (MPW), mixograph midline peak height (MPV), mixograph midline right height at two minutes

after peak (MRV) and mixograph right width at two minutes after peak (MRW)) are shown in the following mixogram (Fig. 3.1). Hereafter these mixograph characteristics are referred to as 'mixograph properties'. The optimum MPT for hard red winter wheat is 3 to 6 minutes and the target for mixing tolerance (traditional score) is at least 3 on a scale of 0 to 6, as suggested by the U.S. Hard Winter Wheat Quality Committee, 2006 (personal communication, Brad Seabourn, USDA-ARS, Manhattan, Kansas). In this cultivar population, the higher the value of mixograph properties, the better for breadmaking quality.

Genotypic Data

Glutenin analysis

The glutenin proteins of each entry were extracted and analyzed by SDS-PAGE according to the protocols of Gupta and MacRitchie (1991) and Singh et al. (1991). The HMW-GS were scored using the nomenclature of Payne and Lawrence (1983) and the LMW-GS were scored using the nomenclature of Gupta and Shepherd (1990). The allele designations follow those listed by McIntosh et al. (2003). For each entry, heterogeneous loci were scored as the allele with the higher intensity, except for those at *Glu-A1a* and *b* whose intensities could not be distinguished. Ten random seeds from each entry were bulked for each extraction. Reduced and alkylated glutenin subunits were analyzed by one-dimensional-three-stacking-layer SDS-PAGE with 4, 12, and 15% layers arranged from top to bottom. Gels were run at 12.5 mA per gel (Bio-Rad 2-gel system) for 18 hours so that the HMW-GS would be resolved by the 12% layer and the LMW-GS would be resolved by the 15% layer in the same gel (as described by Shan et al., 2007). A sample gel image is shown in Fig. 3.2.

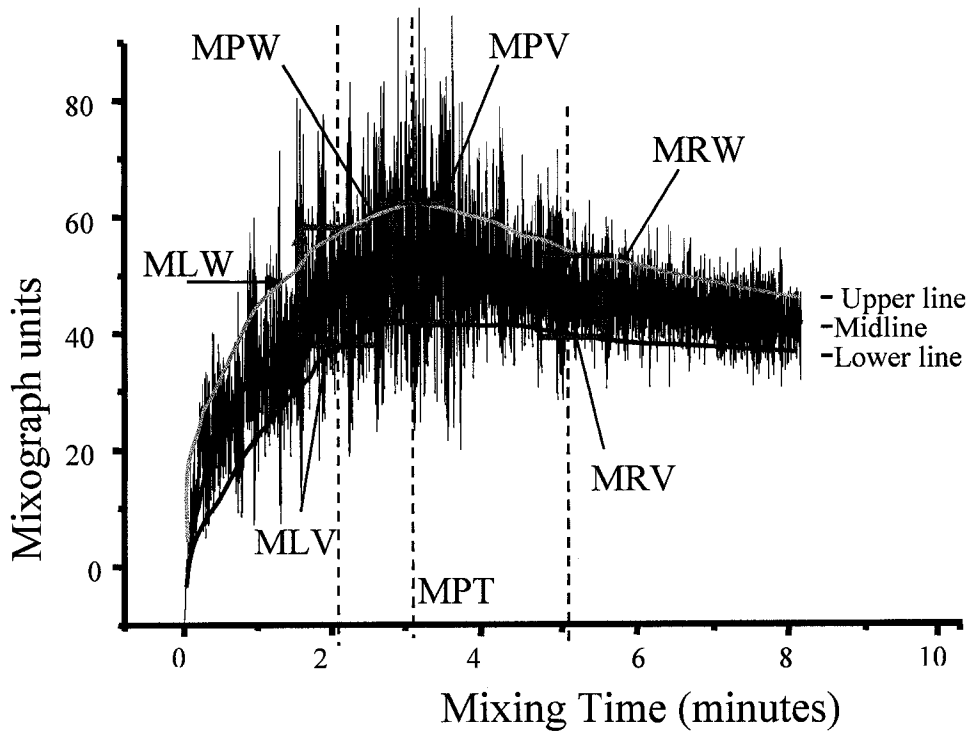


Fig. 3.1 Illustration of mixograph characteristics.†

† MPT, Time to reach highest point of mixograph midline; MLW, band width (distance between the upper and the lower line) at one minute before MPT; MLV, mixograph midline height (value) at one minute before MPT; MPW, band width (distance between the upper and lower line) at MPT; MPV, mixograph midline height (value) at MPT; MRW, band width (the distance between the upper and lower line) at two minutes after MPT; and MRV, mixograph midline height (value) at two minutes after MPT.

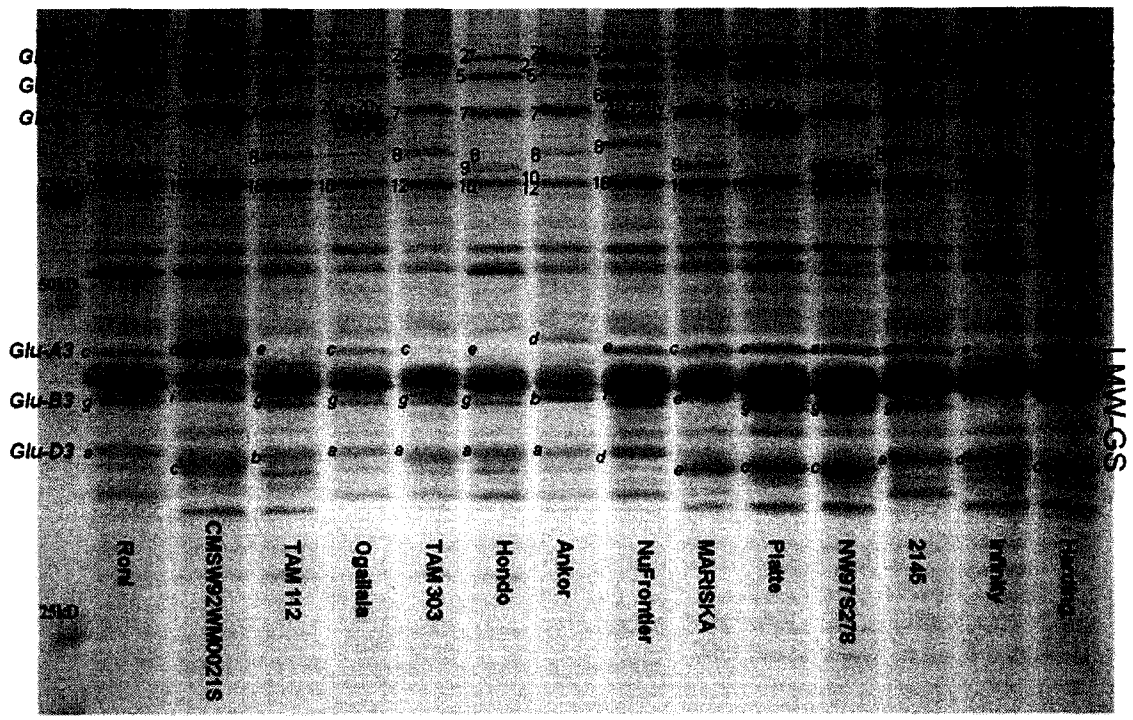


Fig. 3.2. Sample gel of SDS-PAGE analysis of glutenin subunit variation in 96 cultivars and advanced lines.†

† The first lane is the protein marker, followed by 14 cultivars/advanced lines; names are given at the bottom of the lane. High-molecular-weight glutenin subunits (HMW-GS) are identified by numerical number of the nomenclature of Payne and Lawrence (1983). Low-molecular-weight glutenin subunits (LMW-GS) are identified with English letters following the system proposed by Gupta and Shepherd (1990).

Rye translocation

DNA was extracted from bulked leaves of 10 seedlings of each entry using the method described by Riede and Anderson (1996). 1BL.1RS and 1AL.1RS were identified with the marker 'SCM9' which distinguishes 1AL.1RS and 1BL.1RS (Saal and Wricke, 1999) and confirmed with the polymerase chain reaction (PCR) method developed by Katto et al. (2004) which targets a widely dispersed rye specific repetitive sequence.

Genotyping with microsatellite markers (simple-sequence repeats, SSR)

All genotyping was carried out in the USDA Small Grain Genotyping Lab in Manhattan, Kansas following that lab's standard protocols (http://www.oznet.ksu.edu

/wheatgenotyping/). The same set of DNA used for testing 1RS was used for genotyping with SSR markers, which were selected based on the map of Somers et al. (2004). For each chromosome, we chose at least three SSR markers (two in both telomere regions and one in the centromere region) for testing, and had the primers synthesized according to information available in the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml, verified on September 5, 2007). Markers producing a single band were preferred. If markers produced two well-separated bands, those markers were scored as independent loci. A total of 63 SSR markers were applied to 96 entries including 13 BARC markers (Song et al., 2005), four CFA and seven CFD markers (Guyomarc'h et al., 2002), 15 GWM markers (Roder et al., 1998), and 24 WMC markers (Gupta et al., 2002). A total of 69 SSR loci were scored from these SSR markers, including one locus per marker from 57 SSR markers and two loci per marker from the other six markers. The list of markers is given in Appendix, Table A2.

All markers were analyzed with a three-primer system (Schuelke, 2000), including a modified forward primer (tailed with a M13 18-base oligonucleotides, 5' -ACG ACG TTG TAA AAC GAC -3'), a standard reverse primer, and a corresponding M13 dye labeled forward primer. The M13 primer has an 18-base tail, 5' -ACG ACG TTG TAA AAC GAC -3' labeled with one of four fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, Dye Set G5).

The PCR was performed in 12-μL reaction volumes containing 1x ASB buffer (16 mM ammonium sulfate, 67 mM Tris-HCl (pH 8.8), and 0.01% Tween-20), 50 to 150 ng of template DNA, 0.05 μM of forward tailed primer, 0.25 μM of reverse primer, and 0.2 μM of M13 dye-labeled primer, 0.05 μM (each) deoxyribonucleotides, and 2 units of *Taq*

polymerase. The PCR was performed by a program consisting of five stages: (1) 95 °C for 5 min (initial denaturation); (2) five cycles of denaturation at 96 °C for 1 min, annealing at 68 °C (lowered by 2 °C/cycle) for 5 min, extension at 72 °C for 1 min; (3) five cycles of denaturation at 96 °C for 1 min, annealing at 58 °C (lowered by 2 °C/cycle) for 2 min, extension at 72 °C for 1 min; (4) 25 cycles of denaturation at 96 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min; and (5) a final extension for 5 min at 72°C. Four PCR reactions with different dyes were pooled together and analyzed in an ABI 3730 sequencer (Applied Biosystems, Foster City, CA). Trace files were analyzed with GeneMarker® version 1.6 (Softgenetics®, LLC, State College, PA, http://www.softgenetics.com/).

Data Analysis

Multiple levels of relatedness of 96 entries

To control for spurious associations, the mixed model (Q+K) proposed by Yu et al. (2006) was used to account for population structure and cousin-level relatedness of individuals. The genetic structure among the 96 entries was inferred with the 60 SSR loci (at least two loci on each chromosome with less than 17% missing values), using admixture and correlated allele frequency options in the program STRUCTURE version 2.2 (Pritchard et al., 2000). One run with 1,000,000 'burnin' periods, followed by 2,000,000 MCMC iterations, was used to estimate the Q-matrix (Yu et al., 2006). Graphical presentations of the Q-matrix were generated with Distruct version 1.0 developed by Rosenberg (2002). The *a priori* subpopulation number (k) was estimated with the program StructuRAMA (Huelsenbeck and Andolfatto, 2007) with different sets of SSR loci and a full set and reduced set of entries, and an estimate of k was also

obtained with STRUCTURE (Pritchard et al., 2000), with three independent replicates each with 100,000 burnin periods and 200,000 MCMC iterations for k ranging from 1 to 11 on the entire data set (96 entries) (Pritchard et al., 2000).

The relative kinship (K) matrix (K-matrix, distinct from the *a priori* subpopulation number k) was calculated on the basis of 69 microsatellite loci (two or three loci per chromosome) using the method proposed by Ritland (1996), which is built into the program SPAGeDi (Hardy and Vekemans, 2002). Following the suggestion by Yu et al. (2006) and Hardy and Vekemans (2002), negative values between individuals were set to 0, as this indicates that they are less related than random individuals.

Least squares means, correlation, and association analysis

First, exploratory association analysis was performed on mixograph properties combining data from the three locations. This analysis revealed very significant ($P<0.0001$) genotype × environment interactions among most of the glutenin loci and growing locations (data not shown). Thus, our data were analyzed and presented separately by location. Second, at each location, least squares means (lsmeans) of mixograph properties (MLW, MPW, MRW, MLV, MPV, MRV and MPT), grain and flour characteristics (hardness, gprot12, fprot14), and agronomic traits (GW, KW, NSPH, DTH, DPM, GFD and PH) were calculated with the MIXED procedure of SAS version 9.1 (SAS Institute, Cary, NC). In the model, 'entry' was treated as a fixed effect and 'incomplete block' a random effect. Third, homogeneity of error variance of mixograph properties was evaluated by the Kolmogorov-Smirnov test of the UNIVARIATE procedure of SAS and visually with residual plots generated with the GPLOT procedure of SAS. The expected and residual values used in residual plots were obtained from the

MIXED procedure for calculating lsmeans. Fourth, at each location, the Pearson correlation coefficients among lsmeans were evaluated with the CORR procedure of SAS. Fifth, the association of glutenin loci and 1RS translocation with mixograph properties was tested with the Q+K model proposed by Yu et al. (2006). The SAS code for the Q+K model was adapted from Buckler's group (USDA-ARS, Ithaca, NY, http://www.maizegenetics.net/bioinformatics/tassel/QK.sas). The subpopulations together with one glutenin locus or 1RS translocation were treated as fixed effects, 'incomplete block' as random effects and 'entry' as random effects with K-matrix defining the degree of genetic covariance between a pair of individuals. Sixth, the LSMEAN statement in the MIXED procedure was used to estimate the lsmean for each allele of glutenin loci and 1RS status. The lsmeans for glutenin loci or presence/absence of 1RS were compared using the 'PDIFF/adjust=Tukey' option in the MIXED procedure, which gives probability values for the null hypothesis of no difference among lsmeans at a locus with Tukey-Kramer multiple range tests (as suggested by Phil Chapman, Department of Statistics, Colorado State University).

RESULTS

Trait Means

In general, wheats from the irrigated location, Fort Collins, had higher values ($P<0.05$) of agronomic traits (Yield, KW, NSPH, DPM, GFD, and PH) than those from the dryland locations, Dailey and Akron (Table 3.1). Wheats from Akron had the highest grain protein concentration and flour protein concentration (mean of 170.1 gkg^{-1} and 164.2 gkg^{-1}, respectively), followed by those from Fort Collins (mean of 165.1 gkg^{-1} and 156.2 gkg^{-1}, respectively), and Dailey (mean of 142.7 gkg^{-1} and 130.9 gkg^{-1}, respectively). The MPT for flour from the three locations were in the order of Dailey>Akron>Fort Collins ($P<0.001$). For mixograph band width (MLW, MPW and MRW), wheats from Fort Collins had higher MLW and MPW than those from Dailey and Akron ($P<0.001$), whereas for MRW, wheats from Dailey had a higher value than those from Fort Collins and Akron ($P<0.001$), indicating flours from Dailey were more tolerant to overmixing. For the height of mixograph midline (MLV, MPV and MRV), wheat from the three locations had similar MLV, whereas Dailey had higher MPV and MRV than Fort Collins and Akron ($P<0.001$).

Homogeneity of Error Variance Test of Dough Rheology

From the residual plots of each location, MPT, MLV, MPV and MRV were considered to be homogeneous for error variance (Appendix, Fig. A2) and can be directly used in association analysis with the MIXED procedure. However, MLW, MPW, MRW were not consistent within an acceptable level of homogeneity of error variance across the three locations. In order to present data consistently, MLW, MPW and MRW were

Log_e (ln) transformed to achieve homogeneous variance in all three environments (Appendix, Fig. A2). Hereafter, these three traits are presented as Log_e (ln) transformed values, logMLW, logMPW, and logMRW.

Correlation among the Characteristics

The correlation of chracteristics was calculated with the lsmeans of 96 entries for each location (except KW and NSPH at Dailey, which were calculated from 95 entries). Significant correlations among many characteristics were observed (Table 3.2.1 and 3.2.2). Yield was negatively correlated with protein concentrations at Fort Collins and Dailey ($-0.43<r<-0.36$, $P<0.001$), and with DTH at the three locations ($-0.51<r<-0.21$, $P<0.05$), and positively correlated with PH at the three location ($0.30<r<0.36$, $P<0.01$). KW was negatively correlated with grain and flour protein concentrations ($-0.37<r<-0.28$, $P<0.01$) at Akron and Dailey and with DTH ($r=-0.44$ and -0.23, $P<0.05$) at the three locations, and positively correlated with MPT at Fort Collins ($r=0.21$, $P<0.05$). NSPH was positively correlated with PH ($r=0.21$ and 0.38, $P<0.05$), but negatively correlated with KW ($r=-0.31$ and -0.24, $P<0.05$) at Fort Collins and Akron. DTH was positively correlated with grain and flour protein concentrations at Akron and Dailey ($0.32<r<0.42$, $P<0.01$). GFD was negatively correlated with logMRW and MRV ($-0.33<r<-0.26$, $P<0.05$) at Fort Collins and Dailey. In general, grain and flour protein concentrations were positively correlated with MLV, MPV and MRV at all three locations ($0.21<r<0.43$, $P<0.05$). Most mixograph properties were highly positively correlated with each other at the three locations ($0.22<r<0.93$, $P<0.05$), except the negative correlation of MPT with logMPW and MPV at Akron.

Table 3.1. Least squares means of mixograph and agronomic characteristics of 96 genotypes in three Colorado locations in the 2005-06 growing season (n=96 except KW and NSPH from Dailey, for which, n=95).

Location	Fort Collins				Akron				Dailey				$LSD_{0.05}$
Variable†	Mean	Std Dev	Minimum	Maximum	Mean	Std Dev	Minimum	Maximum	Mean	Std Dev	Minimum	Maximum	
Yield ($kg.ha^{-1}$)	2867	655	1151	4365	1313	308	726	2046	1440	270	379	2082	127
KW (mg)	28.45	2.41	20.15	34.12	22.66	2.47	16.93	28.93	25.02	2.54	19.01	31.29	0.7
NSPH	25.9	4.1	15.7	38.2	24.0	3.5	14.7	32.0	29.8	3.7	21.5	38.9	1.1
DPM (days)	167.4	1.5	164.0	171.3	165.9	1.3	163.1	168.7	165.7	1.2	163.8	170.2	0.4
DTH (days)	141.2	1.5	138.5	145.4	142.7	1.5	140.1	146.8	142.3	1.7	138.7	147.4	0.4
GFD (days)	26.3	1.4	23.2	30.2	23.2	1.1	20.6	26.4	23.4	1.2	20.4	26.2	0.3
PH (cm)	74.8	4.7	63.3	84.8	53.0	3.9	43.9	66.7	57.5	4.0	45.6	73.0	1.2
gprot12 (gkg^{-1})	165.1	8.9	135.4	183.6	170.1	5.7	157.5	1872	142.7	8.0	118.0	159.4	2.2
fprot14 (gkg^{-1})	156.2	10.0	126.9	179.9	164.2	6.7	149.4	182.4	130.9	8.0	108.1	147.7	2.4
MPT (min)	2.88	0.62	1.47	4.68	3.31	0.75	1.79	5.10	4.56	0.89	2.58	7.11	0.2
MLW (Mu)‡	25.74	4.20	13.32	36.00	22.11	3.07	15.32	29.70	22.31	3.51	11.58	31.76	1.0
MPW (Mu)	21.61	3.72	13.29	33.82	19.11	3.58	12.56	30.68	19.14	3.14	10.48	27.47	1.0
MRW(Mu)	10.81	3.39	5.33	22.48	10.46	3.35	5.07	22.18	14.98	3.45	5.35	23.65	1.0
logMLW	3.23	0.17	2.59	3.58	3.09	0.14	2.73	3.39	3.09	0.16	2.45	3.46	0.05
logMPW	3.06	0.18	2.59	3.52	2.93	0.18	2.53	3.42	2.94	0.17	2.35	3.31	0.05
logMRW	2.33	0.31	1.67	3.11	2.30	0.30	1.62	3.10	2.68	0.26	1.68	3.16	0.08
MLV (Mu)	39.90	5.27	25.25	50.10	40.99	4.69	30.72	53.05	40.55	3.65	28.59	53.61	1.3
MPV (Mu)	50.56	4.17	41.28	60.68	48.61	4.14	38.05	61.59	43.54	3.88	34.54	57.13	1.2
MRV (Mu)	43.06	4.18	31.89	51.26	42.21	3.90	33.05	53.04	40.82	2.99	34.34	48.93	1.1

† Std Dev, standard deviation; yield, grain yield; KW, kernel weight; NSPH, number of seeds per head; DTH, days to heading; DPM, days to physiological maturity date; GFD, grain filling duration=DPM-DH; PH, plant height; gprot12, grain protein concentration at 12% moisture content; fprot14, flour protein concentration at 14% moisture content; MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline peak height; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

‡ Mu, mixograph units in 100-unit scale.

Table 3.2.1. Pearson correlation coefficients among quality and agronomic characteristics of 96 cultivars at Akron and Dailey in the 2005-06 growing season (n=96 except KW and NSPH from Dailey, for which, n=95). †

Loc‡		Akron																
	Traits	Yield	KW	NSPH	DPM	DTH	GFD	PH	gprot12	fprot14	Hardness	MPT	logMLW	logMPW	logMRW	MLV	MPV	MRV
Dailey	Yield		0.20	0.28	-0.45	-0.51		0.31										
	KW			-0.24	-0.36	-0.44			-0.30	-0.37			-0.22	-0.25				
	NSPH							0.38			0.22							
	DPM	-0.57	-0.24			0.69	0.22		0.42	0.36	0.30							
	DTH	-0.47	-0.29		0.75		-0.55		0.32	0.38								
	GFD					-0.74												
	PH	0.36													0.23			
	gprot12	-0.38	-0.28		0.36	0.40	-0.25			0.68			0.21					0.23
	fprot14	-0.38	-0.35		0.35	0.42	-0.28		0.93				0.27	0.24			0.25	0.21
	Hardness				0.35	0.23			0.26									
	MPT			-0.25			-0.24								0.69	0.69		0.51
	logMLW													0.67	0.36	0.55	0.77	0.66
	logMPW							0.36		0.22		-0.23	0.68		0.27	0.35	0.58	0.49
	logMRW	0.23					-0.33	0.25				0.44	0.27	0.31		0.75	0.45	0.76
	MLV		-0.24			0.23	-0.23		0.30	0.33			0.54	0.37	0.29		0.73	0.93
	MPV		-0.27			0.22			0.38	0.43		-0.21	0.65	0.53		0.90		0.86
	MRV						-0.23		0.32	0.35			0.66	0.58	0.45	0.86	0.92	

† Only significantly correlated values are listed. For absolute value of r, when $0.20<r<0.26$, $0.01<P<0.05$; when $0.26<r<0.33$, $0.001<P<0.01$; when $r>0.33$, $P<0.001$.

‡ The upper right half of the table is the correlation of traits from Akron; the lower left half of the table is the correlation of traits from Dailey.

§ Yield, grain yield; KW, kernel weight; NSPH, number of seeds per head; DTH, days to heading; DPM, days to physiological maturity; GFD, grain filling duration=DPM-DH; PH, plant height; gprot12, grain protein concentration at 12% moisture content; fprot14, flour protein concentration at 14% moisture content; MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline peak height; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

Table 3.2.2. Pearson correlation coefficients of quality and agronomic characteristics of 96 cultivars at Fort Collins in the 2005-06 growing season (n=96). †

Traits	Yield	KW	NSPH	DPM	DTH	GFD	PH	gprot12	fprot14	Hardness	MPT	logMLW	logMPW	logMRW	MLV	MPV	MRV
Yield																	
KW																	
NSPH	0.37	-0.31															
DPM																	
DTH	-0.21	-0.23		0.59													
GFD		0.23		0.44	-0.46												
PH	0.30		0.21														
gprot12	-0.36		-0.39				-0.28										
fprot14	-0.43		-0.34				-0.24	0.92									
Hardness		0.27															
MPT		0.22															
logMLW					0.21			0.20			0.22						
logMPW						-0.21	0.27				0.25	0.67					
logMRW						-0.28					0.68	0.46	0.56				
MLV								0.21	0.25	0.22	0.77	0.64	0.60	0.74			
MPV					0.28	-0.24		0.35	0.37		0.22	0.76	0.68	0.38	0.69		
MRV					0.24	-0.26		0.29	0.32		0.59	0.75	0.71	0.71	0.92	0.86	

†Only significantly correlated values are listed. For absolute value of r, when $0.20<r<0.26$, $0.01<P<0.05$; when $0.26<r<0.33$, $0.001<P<0.01$; when $r>0.33$, $P<0.001$.

‡Yield, grain yield; KW, kernel weight; NSPH, number of seeds per head; DTH, days to heading; DPM, days to physiological maturity; GFD, grain filling duration=DPM-DH; PH, plant height; gprot12, grain protein concentration at 12% moisture content; fprot14, flour protein concentration at 14% moisture content; MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline peak height; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

Genetic Diversity

Glutenin allelic diversity

A high level of diversity was observed for most glutenin loci in this set of materials. The glutenin compositions of the set of materials are listed in Appendix, Table A1 and the frequency distributions of glutenin alleles of 96 entries are given in Table 3.3. The glutenin allele frequencies of this set of materials generally agreed with the report of Shan et al. (2007), although this study included 77 genotypes from the study of Shan et al. (2007) and added other 19 other entries. The most common alleles at different glutenin loci were *Glu-A1b* and *a*, *Glu-B1b* and *c*, *Glu-D1d*, *Glu-A3c*, *Glu-B3g*, and *Glu-D3a*, *b* and *c*. Some alleles were present in less than five entries, including *Glu-A1c* (3, the total of three entries with the *Glu-A1c* allele), *Glu-B1a* (1), *Glu-D1e* (1), *Glu-A3b* (2), *Glu-A3g* (4), *Glu-B3a* (3), *Glu-B3d* (1), and *Glu-B3h* (4). These alleles were grouped in an artificial allele category '*p*' at each glutenin locus to reduce the level of multiple comparisons.

Microsatellite polymorphism

Ninety-six entries were genotyped with 63 SSR primer pairs and scored for 69 SSR loci. For these 69 SSR loci, the total number of alleles varied from two to 19 per locus, with an average of 6.17 alleles per locus. The mean frequency of missing data was 7.74%, with a range of 0 to 76% (Appendix, Table A3). For the reduced set of 60 SSR loci (excluding nine loci with more than 16% missing values) for estimating the Q-matrix with STRUCTURE, the total number of alleles varied from 2 to 19 per locus, with an average of 6.32 alleles per locus. The mean frequency of missing data was 3.21% with a range of 0 to 16% (Appendix, Table A2 and A3).

Table 3.3. The frequency of the glutenin alleles in 96 genotypes used in this study.†

Allele	*Glu-A1*‡		*Glu-B1* ‡		*Glu-D1* ‡		*Glu-A3*	*Glu-B3*	*Glu-D3*
a	**1**	15	7	1	**2+12**	10	14	3	30
b	**2***	72	**7+8**	33	**3+12**	6	2	14	27
c	**null**	3	**7+9**	38			51	5	28
d					**5+10**	79	7	1	6
e			**20x+20y**	10	**2+10**	1	18	8	5
f								11	
g							4	40	
h								4	
i			**17+18**	7				10	
w			**6*+8***	7					
a/b	**1/2***	6							
p (pool<5 counts)		3		1		1	6	8	
Total		96		96		96	96	96	96

† Alleles that were present in less than five cultivars (in the shaded cells) were pooled together in one group for each locus, identified as *p* to reduce the level of multiple comparisons.

‡ For the HMW glutenin loci, the left column lists the subunit designation and the right column contains the allele frequency.

Multiple-Level Relatedness of Entries

Estimation of prior population number k with STRUCTURE (Pritchard and Rosenberg, 1999) was computationally demanding and not straightforward. We tried combinations of factors that might be relevant to assign individuals to subpopulation, including (1) testing the scores of 21 (one SSR locus per chromosome), 42 (two SSR loci per chromosome), and 60 (two or three SSR loci per chromosome) SSR loci (Appendix, Table A2); and (2) testing the original 96 entries and a reduced set of 75 entries by keeping just one of the entries that share at least 15 SSR marker alleles out of 21 SSR markers. Although the posterior probability (Ln_P(D)) of the data did not peak in the range of one to eleven subpopulations, the magnitude of increase of Ln_P(D) peaked at eight subpopulations with 60 SSR loci (Appendix, Fig. A2). When the scores of 21, 42, and 60 SSR loci were tested on 96 entries and the reduced set of 75 entries with

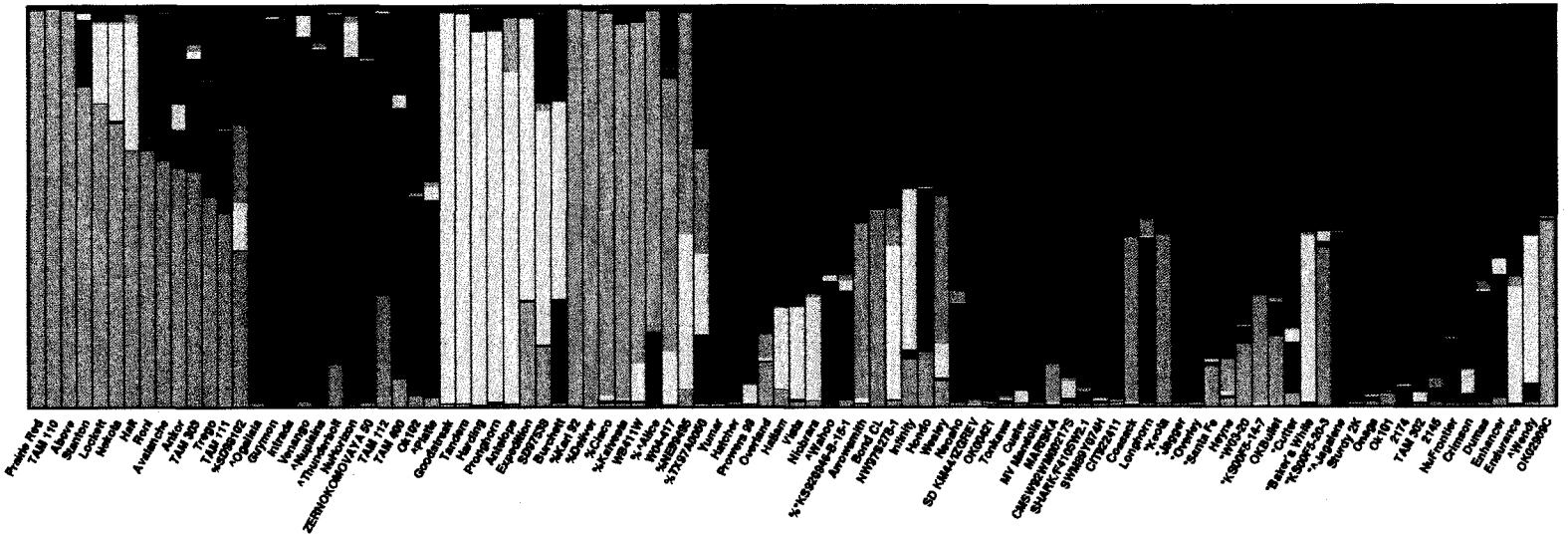
StructuRAMA (Huelsenbeck and Andolfatto, 2007), the program estimated that these entries were composed of genetic materials from approximately eight subpopulations (Appendix, Table A5). To calculate the Q-matrix, STRUCTURE was run with 60 SSR loci with a 1,000,000 burnin period, followed by 2,000,000 MCMC iterations to estimate the individual ancestral subpopulation components of 96 entries with the following options: admixture model, correlated allele frequency and k=8. The results showed that the F_{ST} (the inbreeding coefficient of a subpopulation relative to the whole population) values for eight ancestral populations were 0.42, 0.34, 0.29, 0.40, 0.32, 0.21, 0.41 and 0.22, respectively (Appendix, Table A4). The F_{ST} across subpopulations was averaged to 0.32 which is higher than 0.188 of 96 soft winter wheat cultivars from the eastern United States (Breseghello and Sorrells, 2006a). The graphical presentation of the compositions of ancestral population for all 96 entries is shown in Fig. 3.3 and the Q-matrix is given in Appendix, Table A4.

The results revealed that the structure of the 96 entries can be explained by different levels of admixture from eight ancestral populations. Eighty-five entries of 96 entries came mainly from one or two ancestral populations (>0.50 probability to assign it to one ancestral population) (Appendix, Table A4). For example, the cultivar 'Above' could be assigned to ancestral population one with a probability of 99%, and the cultivar 'Jagger', to ancestral population seven with a probability of 99%. However, 11 entries could not be clearly assigned to one or two of the ancestral populations (<0.50 probability to assign them to one ancestral population). For example, TX97A0050 was almost equally assigned to ancestral populations two, three, four, six, and eight with a probability of around 20% each. The assignment of cultivars to subpopulation was related

to their pedigree (Fig. 3.3). For example, 10 of 11 entries having 'Jagger' in their pedigree were grouped together and seven of nine entries having 'Karl' or 'Karl 92' were grouped together.

To estimate the K-matrix, SPAGeDi (Hardy and Vekemans, 2002) was run on 69 microsatellite loci. Negative values between individuals were set to 0, as this indicates that they are less related then random individuals (Hardy and Vekemans, 2002; Yu et al., 2006). Of 96 entries, 61% of them had a K value of 0 and 96% of them had a K value less than 0.3, indicating that most of these entries were not closely related to each other (Appendix, Fig. A2).

Fig. 3.3. STRUCTURE group probabilities for 96 cultivars and advanced lines. †



†The bar plot represents the genetic background of 96 cultivars. Genotypes are represented on the horizontal axis, each one by a bar composed of up to eight regions according to the proportion of the genetic background that was inferred to come from each of eight different ancestral population (indicated with different colors). Entries with '*' have Jagger in their pedigree; with '%' have either cultivar 'Karl' or 'Karl 92' in their pedigree; and with '^' have 'Abilene' in their pedigree. The program STRUCTURE was run with 60 SSR markers, an assigned *a priori* number of eight, admixture model and correlated allele frequency with 1,000,000 burnin times, followed by 2,000,000 iterations. The assignment of a cultivar/advanced line to eight subpopulations was then used in Distruct version 1.0 developed Rosenberg (2002) to generate this bar plot.

The Relationship of Glutenin Loci and 1RS Translocation with Dough Rheology

We tested the association of the six glutenin loci and 1RS translocation with mixograph properties while accounting for population structure (Q-matrix) alone, the relationship among individuals (K-matrix) alone, and the Q-matrix and the K-matrix (Q+K) in the same test with the MIXED procedure of SAS. The Q+K model gave the best model fit (Appendix, Table A7). Thus, in each location, we chose the Q+K model to associate glutenin alleles and the presence of the 1RS translocation to mixograph properties by analyzing one glutenin locus or 1RS at a time. When comparing the association obtained with the basic model (not accounting for Q+K) (Table 3.4.1) and the Q+K model (Table 3.4.2), the Q+K model reduced the significance levels of most associations in the basic association model (not adjusted to Q+K), because the variance was partitioned to the population structure (Table 3.4.2). For example, *Glu-D3* was significantly ($P<0.05$) associated with all the mixograph properties except MPT at Fort Collins in the basic model, whereas when Q+K was included in the model, these significant associations were not detected. On the other hand, the Q+K model detected additional significant ($P<0.05$) associations among glutenin loci and mixograph properties which were not detected with the basic model (Table 3.4.1). For example, in the Q+K model, there were significant ($P<0.05$) associations between *Glu-D1* and logMPW, and *Glu-A3* and MPT at Dailey, and *Glu-A1* and MPT and MRV, *Glu-A3* and MPT and MRV, and *Glu-D3* and MPT at Akron, but these associations were not detected in the basic model.

In general, all six glutenin loci had important impacts on MPT and *Glu-B3* and 1RS status affected all mixograph properties across the three locations with few exceptions (Table 3.4.2).

Table 3.4.1. The effects of glutenin loci and the presence of 1RS translocation on mixograph properties of 96 genotypes at Fort Collins, Dailey and Akron in the 2005-06 growing season (obtained from the basic model; tested one genetic factor at a time).

Location	Loci	Mixograph properties†						
		logMLW	logMPW	logMRW	MLV	MPT	MPV	MRV
Fort Collins	*Glu-A1*			***‡		*		
	Glu-B1					*		
	Glu-D1	*		**	***	***		*
	Glu-A3	*					**	
	Glu-B3	***	***	***	***	***	***	***
	Glu-D3	**	*	**	**		*	**
	1RS	***	***	***	***		***	***
Dailey	*Glu-A1*				*		*	*
	Glu-B1			**	**	**	*	**
	Glu-D1					***	*	
	Glu-A3						*	
	Glu-B3	*		***	*	**	*	***
	Glu-D3			*	*			
	1RS	***	***	***	***	*	***	***
Akron	*Glu-A1*	*					*	
	Glu-B1					***		
	Glu-D1		*			***	*	
	Glu-A3	*					**	
	Glu-B3	***	*	***	***	***	***	***
	Glu-D3							
	1RS	***	**	***	***		***	***

*, **, ***, F test significant at 0.05, 0.01 and 0.001 levels, respectively; empty cells indicate not significant ($P>0.05$) in the F test.

† MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline height at peak; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

‡ Shaded cells indicate the strength of associations was **reduced** in the Q+K model compared to the basic model.

Table 3.4.2. The effects of glutenin loci and the presence of 1RS translocation on mixograph properties of 96 genotypes at Fort Collins, Dailey and Akron in the 2005-06 growing season (obtained from the Q+K model; tested one genetic factor at a time).

Location	Locus	Mixograph properties†						
		logMLW	logMPW	logMRW	MLV	MPT	MPV	MRV
Fort Collins	*Glu-A1*							
	Glu-B1					*** ‡		
	Glu-D1				*	***		
	Glu-A3							
	Glu-B3	**	***	**	**	**	**	***
	Glu-D3							
	1RS	***	***	*	**		**	***
Dailey	*Glu-A1*							
	Glu-B1				*	**		
	Glu-D1		*			**	*	
	Glu-A3					*		
	Glu-B3			***				
	Glu-D3							
	1RS	*	**	***	*			**
Akron	*Glu-A1*					**	*	*
	Glu-B1					***		
	Glu-D1		*			***	*	
	Glu-A3					*	**	**
	Glu-B3			***	**	*	**	***
	Glu-D3					*		
	1RS	***		*	**		***	***

*, **, ***, F test significant at 0.05, 0.01 and 0.001 levels, respectively; empty cells indicate not significant ($P>0.05$) in the F test.

† MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline height at peak; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

‡ Shaded cells indicate that the strength of associations was **increased** in the Q+K model compared to the basic model.

Glu-A1

There were three alleles at the *Glu-A1* locus, *a* (subunit 1), *b* (subunit 2*), and *c* (null allele) (Table 3.3). The comparison of their lsmeans for mixograph properties is listed in Table 3.5.1, 3.5.2 and 3.5.3. In general, *Glu-A1c*, the null allele, produced the highest MPT and lowest values for other mixograph properties at all three locations. *Glu-A1c* had significantly ($P<0.05$) higher MPT than *Glu-A1a*, lower MPV than *Glu-A1b* and *a/b*, and lower MRV than *Glu-A1a* and *a/b* at Akron, but not at other locations. However, there were only three entries carrying *Glu-A1c* in the 96 genotypes, so the results might not be representative of all wheat from the U.S. Great Plains. *Glu-A1a*, *b* and *a/b* were not significantly different for all mixograph properties.

Glu-B1

Six alleles at the *Glu-B1* locus were observed in this set of materials: *a* (subunit 7), *b* (subunits 7+8), *c* (subunits 7+9), *e* (subunits 20x+20y), *i* (subunits 17+18), and *w* (subunits 6*+8*) (Table 3.3). Variation at the *Glu-B1* locus was significantly associated with MPT ($P<0.01$) at all three locations and with MLV ($P<0.05$) at Dailey (Table 3.4.1). In general, the trend was that *Glu-B1w* (subunits 6*+8*) was associated with lower MLV, MPV and MRV values than other *Glu-B1* alleles at dryland locations, Dailey and Akron, but had higher values of MLV, MPV and MRV than other *Glu-B1* alleles at the irrigated location, Fort Collins. However, the differences were not statistically significant. *Glu-B1e* (subunits 20x+20y) produced the lowest MPT at all three locations. *Glu-B1c* produced higher MLV, MPV and MRV, but lower logMPW and logMRW, than *Glu-B1b* at all three locations, although the difference was not statistically significant. Other alleles were not significantly different from each other for any of the mixograph properties.

Table 3.5.1. The least squares means of mixograph properties for glutenin allelic classes and 1RS translocations of 96 genotypes at Akron in the 2005-06 growing season.

Loci	Allele	n	Mixograph properties‡						
			logMLW	logMPW	logMRW	MLV	MPT	MPV	MRV
Glu-A1	*a*	15	3.04a†	2.95a	2.34a	40.73a	3.22a	48.26ab	42.23a
	a/b	6	3.16a	2.93a	2.40a	43.57a	3.88ab	50.17b	44.16a
	b	75	3.09a	2.92a	2.29a	41.15a	3.37ab	48.69b	42.3ab
	p (c)§	3	2.98a	2.77a	2.23a	38.39a	3.95b	43.93a	38.88b
Glu-B1	*b*	33	3.08a	2.93a	2.32a	40.94a	3.43b	48.12a	42.27a
	c	40	3.07a	2.91a	2.32a	41.78a	3.58b	48.64a	42.66a
	e	10	3.10a	2.95a	2.19a	40.87a	2.58a	51.03a	43.34a
	i	7	3.10a	3.02a	2.36a	41.21a	3.06ab	50.61a	43.38a
	p (a) §	1	3.05a	2.83a	2.28a	36.74a	3.41ab	44.07a	36.29a
	w	8	3.10a	2.84a	2.25a	41.66a	3.85b	47.52a	41.30a
Glu-D1	*a*	11	3.10a	2.96ab	2.34a	40.47a	2.47a	50.07ab	43.15a
	b	6	3.15a	3.12a	2.44a	41.4a	2.81ab	52.79a	45.31a
	d	81	3.07a	2.90b	2.29a	41.39a	3.50b	48.30d	42.24a
	p (e) §	1	3.20a	3.07ab	2.33a	37.01a	1.90ab	52.64ab	41.81a
Glu-A3	*a*	14	3.09a	2.91a	2.27a	39.22a	3.23a	47.90ab	40.57ab
	c	53	3.10a	2.95a	2.27a	41.69a	3.21a	49.64a	42.99a
	d	7	3.09a	2.96a	2.32a	42.34a	3.95a	48.84ab	42.74ab
	e	19	3.03a	2.89a	2.42a	41.50a	3.72a	47.22ab	42.46ab
	p (p, g) §	6	3.05a	2.83a	2.19a	38.78a	3.61a	45.29b	39.58b
Glu-B3	*b*	14	3.06a	2.88a	2.41a	43.14b	3.92b	48.61b	43.11b
	c	6	2.94a	2.83a	1.77b	33.89a	2.95ab	41.44a	34.34a
	e	9	3.11a	2.97a	2.44a	42.00b	3.32ab	49.41b	43.01b
	f	11	3.12a	3.03a	2.30a	42.05b	3.06ab	50.56b	44.11b
	g	40	3.09a	2.93a	2.30a	41.24b	3.36ab	49.00b	42.59b
	i	11	3.11a	2.92a	2.15ab	39.38ab	3.00a	47.79ab	41.27b
	p (a, d, h) §	8	3.05a	2.86a	2.32a	40.87b	3.25ab	48.81b	42.06b
Glu-D3	*a*	31	3.10a	2.93a	2.26a	40.46a	3.41ab	48.17a	41.58a
	b	27	3.06a	2.90a	2.36a	41.66a	3.66a	48.29a	42.33a
	c	29	3.09a	2.92a	2.33a	41.54a	3.30ab	49.21a	43.08a
	d	6	3.06a	2.93a	2.18a	41.93a	2.84b	50.31a	43.67a
	e	6	3.08a	3.09a	2.39a	42.67a	3.26ab	50.03a	44.26a
1RS	1AL/1BL.1RS	3	3.03ab	2.94a	2.15ab	42.01ab	3.95a	47.03ab	41.04ab
	1AL.1RS	16	3.04ab	2.91a	2.31ab	41.48a	3.32a	48.74a	42.45a
	1BL1RS	12	2.95b	2.82a	2.05b	35.84b	3.17a	43.64b	37.89b
	Non-1RS	65	3.12a	2.95a	2.35a	42.02a	3.39a	49.64a	43.21a

† Means followed by different letters indicate significant difference at $P<0.05$ level.

‡ MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline height at peak; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

§ The alleles in parentheses were grouped in one artificial category p.

Table 3.5.2. The least squares means of mixograph properties for glutenin allelic classes and 1RS translocations of 96 genotypes at Dailey in the 2005-06 growing season.

Loci	Allele	n	Mixograph properties‡						
			logMLW	logMPW	logMRW	MLV	MPT	MPV	MRV
Glu-A1	*a*	15	3.04a†	2.92a	2.60a	40.03a	4.77a	42.36a	39.91a
	a/b	6	3.11a	2.93a	2.73a	38.61a	4.47a	41.72a	40.37a
	b	75	3.09a	2.94a	2.69a	40.90a	4.54a	44.10a	41.23a
	p (c)§	3	2.98a	2.77a	2.64a	39.37a	4.99a	40.90a	38.83a
Glu-B1	*b*	33	3.07a	2.95a	2.74a	40.33ab	4.75ab	43.21a	40.8a
	c	40	3.09a	2.94a	2.69a	40.79ab	4.59ab	43.78a	41.25a
	e	10	3.2a	2.95a	2.54a	40.2ab	3.80b	44.50a	40.74a
	i	7	3.02a	2.90a	2.58a	42.3ab	4.20ab	45.38a	41.39a
	p (a) §	1	3.25a	3.14a	3.09a	49.36a	6.37a	49.91a	45.34a
	w	8	3.04a	2.88a	2.59a	38.2b	4.92a	40.80a	39.22a
Glu-D1	*a*	11	3.16a	3.00a	2.61a	42.02a	3.82a	45.19a	41.30a
	b	6	3.19a	3.06a	2.62a	42.63a	3.74a	47.08a	42.57a
	d	81	3.07a	2.92a	2.69a	40.24a	4.72b	43.18a	40.78a
	p (e) §	1	3.29a	3.15a	2.58a	44.17a	3.48ab	49.56a	42.86a
Glu-A3	*a*	14	3.07a	2.93a	2.59a	40.39a	4.24a	44.23a	40.94a
	c	53	3.11a	2.95a	2.67a	40.82a	4.46a	44.13a	41.19a
	d	7	3.06a	3.00a	2.69a	38.88a	4.60a	42.11a	39.98a
	e	19	3.05a	2.89a	2.75a	40.32a	4.97a	42.55a	40.87a
	p (p, g) §	6	3.07a	2.9a	2.75a	42.45a	5.20a	43.57a	40.19a
Glu-B3	*b*	14	3.05a	2.98a	2.80a	41.82a	5.17a	44.03a	41.63a
	c	6	2.98a	2.88a	2.20b	39.15a	4.12ab	42.92a	38.52a
	e	9	3.11a	2.93a	2.62a	40.81a	4.62ab	43.59a	40.62a
	f	11	3.13a	2.96a	2.72a	42.01a	4.47ab	45.31a	42.15a
	g	40	3.08a	2.93a	2.68a	40.14a	4.54ab	43.13a	40.72a
	i	11	3.12a	2.93a	2.62a	39.99a	4.08b	43.74a	40.76a
	p (a, d, h) §	8	3.07a	2.88a	2.67a	39.95a	4.41ab	43.79a	41.03a
Glu-D3	*a*	31	3.07a	2.92a	2.64a	41.01a	4.74a	43.52a	40.70a
	b	27	3.07a	2.96a	2.70a	40.64a	4.67a	43.58a	40.93a
	c	29	3.10a	2.92a	2.73a	40.65a	4.35a	44.28a	41.60a
	d	6	3.09a	2.93a	2.51a	37.57a	4.32a	41.66a	39.19a
	e	6	3.20a	3.05a	2.72a	41.80a	4.77a	44.39a	42.17a
1RS	1AL/1BL.1RS	3	3.04ab	2.86ab	2.78a	41.42ab	5.51a	42.69a	41.4ab
	1AL.1RS	16	3.12ab	2.95a	2.67a	42.01a	4.62a	44.76a	42.02a
	1BL.1RS	12	2.94b	2.75b	2.35b	37.7b	4.53a	40.80a	37.43b
	Non-1RS	65	3.11a	2.97a	2.73a	40.73ab	4.51a	43.91a	41.28a

† Means followed by different letters indicate significant difference at $P<0.05$ level.

‡ MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline height at peak; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

§ The alleles in parentheses were grouped in one artificial category p.

Table 3.5.3. The least squares means of mixograph properties for glutenin allelic classes of 96 genotypes at Fort Collins in the 2005-06 growing season.

Loci	Allele	n	Mixograph properties‡						
			logMLW	logMPW	logMRW	MLV	MPT	MPV	MRV
Glu-A1	*a*	15	3.18a†	3.06a	2.27a	40.21a	3.01a	50.52a	43.33a
	a/b	6	3.22a	3.00a	2.52a	43.68a	3.46a	50.38a	45.12a
	b	75	3.24a	3.05a	2.33a	39.99a	2.85a	50.76a	43.15a
	p (c)§	3	3.15a	3.02a	2.23a	40.88a	3.24a	48.56a	41.62a
Glu-B1	*b*	33	3.20a	3.05a	2.38a	40.47a	2.97a	49.86a	43.12a
	c	40	3.24a	3.04a	2.31a	40.95a	3.05a	51.02a	43.69a
	e	10	3.22a	2.97a	2.18a	37.02a	2.15b	50.81a	42.11a
	i	7	3.22a	3.15a	2.41a	38.69a	2.92ab	51.10a	43.03a
	p (a) §	1	3.38a	3.16a	2.60a	42.84a	3.72ab	51.31a	44.57a
	w	8	3.28a	3.09a	2.34a	42.70a	3.20a	51.56a	44.31a
Glu-D1	*a*	11	3.15a	3.02a	2.18a	35.73b	2.19a	50.87a	41.69a
	b	6	3.27a	3.07a	2.22ab	37.5ab	2.42ab	52.62a	43.34a
	d	81	3.23a	3.05a	2.35b	40.92a	3.03b	50.44a	43.45a
	p (e) §	1	3.36a	3.21a	2.33ab	37.68ab	2.17ab	56.63a	45.05a
Glu-A3	*a*	14	3.17a	3.05a	2.28a	38.69a	2.82a	50.51a	42.11a
	c	53	3.27a	3.05a	2.30a	40.46a	2.81a	51.42a	43.79a
	d	7	3.23a	3.07a	2.39a	39.78a	3.11a	48.74a	42.30a
	e	19	3.16a	3.04a	2.42a	41.43a	3.22a	49.89a	43.63a
	p (p, g) §	6	3.17a	3.06a	2.32a	39.37a	3.08a	48.75a	41.72a
Glu-B3	*b*	14	3.33a	3.09b	2.47a	42.22a	3.34a	50.69a	44.37a
	c	6	3.00b	2.78a	1.92b	31.98b	2.34b	44.38b	35.26a
	e	9	3.23ab	3.09b	2.44a	41.06a	2.99ab	52.01a	44.50b
	f	11	3.29a	3.14b	2.33ab	40.58a	2.89ab	52.72a	44.68a
	g	40	3.20ab	3.03b	2.33a	40.50a	2.93ab	50.39a	43.23a
	i	11	3.25ab	2.97ab	2.17ab	38.38ab	2.46b	50.71a	42.13a
	p (a, d, h) §	8	3.21ab	3.15b	2.35ab	39.50a	2.83ab	50.93a	43.32a
Glu-D3	*a*	31	3.24a	3.04a	2.30a	40.33a	3.00a	50.79a	42.93a
	b	27	3.22a	3.05a	2.37a	40.75a	3.05a	50.12a	43.52a
	c	29	3.26a	3.08a	2.37a	40.56a	2.90a	51.27a	43.91a
	d	6	3.16a	3.03a	2.22a	38.88a	2.42a	49.85a	42.56a
	e	6	3.13a	2.95a	2.23a	38.65a	2.79a	50.68a	42.85a
1RS	1AL/1BL.1RS	3	3.19ab	3.11a	2.29ab	40.45ab	2.86a	49.47ab	42.18ab
	1AL.1RS	16	3.24a	3.06a	2.36ab	41.79a	3.18a	51.25a	44.00a
	1BL.1RS	12	3.02b	2.82b	2.06b	34.71b	2.67a	46.36b	38.56b
	Non-1RS	65	3.27a	3.08a	2.37a	40.82a	2.91a	51.34a	44.00a

† Means followed by different letters indicate significant difference at $P<0.05$ level.

‡ MPT, mixograph peak time; MLW, mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; MPW, mixograph band width at MPT; MPV, mixograph midline height at peak; MRW, mixograph band width at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; logMLW, $\log_e$ (ln) transformation of MLW; logMPW, $\log_e$ (ln) transformation of MPW; and logMRW, $\log_e$ (ln) transformation of MRW.

§ The alleles in parentheses were grouped in one artificial category p.

Glu-D1

There were four alleles at the *Glu-D1* locus in 96 cultivars and advanced lines: *a* (subunits 2+12), *b* (subunits 3+12), *d* (subunits 5+10), and *e* (subunits 2+10) (Table 3.3). The variation at the *Glu-D1* locus was significantly ($P<0.01$) associated with MPT at all three locations, logMPW and MPV at Dailey and Akron ($P<0.05$), and MLV at Fort Collins ($P<0.05$) (Table 3.4.1). For MPT, *Glu-D1d* had a significantly higher value than *Glu-D1a* at all three locations and was higher than *Glu-D1b* at Dailey (Table 3.5.1, 3.5.2 and 3.5.3). *Glu-D1d* had lower logMPW and MPV values than *Glu-D1a* and *Glu-D1b* at Akron and Dailey, although the difference was not significant at Dailey due to the stringent adjustment of the Tukey-Kramer multiple comparison procedure (Table 3.5.1, 3.5.2 and 3.5.3). The variation at the *Glu-D1* locus was associated with logMLW, logMPW, MPV and MRV, in the order *Glu-D1b>a>d* at Akron and Dailey, although the association was not statistically significant.

Glu-A3

Six alleles were observed at the *Glu-A3* locus: *a*, *b*, *c*, *d*, *e*, and *g*. *Glu-A3b* was present in two cultivars and *Glu-A3g* in four cultivars; they were pooled into the artificial category *p* for multiple comparisons. Variation at the *Glu-A3* locus was significantly associated with MPT, MPV and MRV at Akron and MPT at Dailey (Table 3.4.1). The pooled category *p* (alleles *b* and *g*) had significantly lower MPV and MRV than other alleles at the *Glu-A3* locus at Akron. The lsmeans of allelic classes for MPT were in the order of *d>e>p>a>c* at Akron and *p>e>d>c>a* at Fort Collins. Although F tests were significant, the means of the alleles were not differentiated in the Tukey-Kramer multiple comparisons (Table 3.5.1, 3.5.2 and 3.5.3). *Glu-A3e*, a null allele at the *Glu-A3* locus produced lower logMLW and

logMPW, but higher logMRW, MPT and MLV than other alleles at all three locations (Table 3.5.1, 3.5.2 and 3.5.3).

Glu-B3

There were nine alleles at the *Glu-B3* locus, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h* and *i*. The alleles *a*, *d* and *h* were of low frequency in this population, present in 3, 1, and 4 entries, respectively, of 96 genotypes, and were pooled into the artificial allele category *p* (Table .3.3). The variation at the *Glu-B3* locus was associated with all the mixograph properties at Fort Collins, logMRW, MLV, MPT, MPV and MRV at Akron and only logMRW at Dailey (Table 3.41). In general, the trend was that *Glu-B3b* and *f* were associated with relatively higher values of all mixograph properties compared to other alleles at the *Glu-B3* locus, whereas *Glu-B3c* and *i* were associated with lower values of all mixograph properties at the three locations, although not all the differences were statistically significant. *Glu-B3c* was associated with lower values for all mixograph properties at all three locations (Table 3.5.1, 3.5.2 and 3.5.3). *Glu-B3i* produced lower MPT than other alleles at *Glu-B3*, but only significantly different from *Glu-B3b*.

Glu-D3

There were five alleles at the *Glu-D3* locus in 96 genotypes, *a*, *b*, *c*, *d*, and *e* (Table .3.3). Variation at the *Glu-D3* locus was only significantly ($P<0.05$) associated with MPT at Akron and Dailey (Table 3.41). This association was due to significantly ($P<0.05$) lower MPT of *Glu-D3d* compared to *Glu-D3b* (Table 3.5.1, 3.5.2 and 3.5.3). Other alleles produced similar mixograph property values across three locations (Table 3.5.1, 3.5.2 and 3.5.3).

1RS translocation

There were four classes of the 1RS translocations in the 96 genotypes. Sixty-five entries had no rye chromatin (non-1RS) and 31 entries had a 1RS translocation in the form of 1AL.1RS (16 entries), 1BL.1RS (12 entries), or containing both 1AL.1RS and 1BL.RS (denoted as 1AL/1BL.1RS) (3 entries) (Table 3.3). The 1BL.1RS was significantly ($P<0.05$) associated with the lowest mixograph properties, except for MPT at all three locations, logMPW at Akron, and MPV at Dailey (Table 3.5.1, 3.5.2 and 3.5.3). Among the other classes of the 1RS translocation (non-1RS, 1AL.1RS, and 1AL/1BL.1RS), there were no significant differences for any mixograph property.

DISCUSSION

Our results confirmed that wheat quality is a very complex collection of traits influenced by both genetic and environmental factors. There are two main methods to dissect quantitative traits: QTL mapping and association mapping. In wheat, since structured populations such as RIL, NIL, and DH populations are relatively straightforward to create, QTL mapping has been an important tool for studying the genetic architecture of complex traits. However, the effects of the *Glu-1* and *Glu-3* loci have been mainly studied with the association analysis approach because QTL mapping evaluates only two alleles at each locus, whereas association analysis has the advantage of testing multiple alleles at one locus at the same time. The advantages of association analysis in the study of the quality effects of glutenin loci have been demonstrated by Eagles et al. (2002b). In their study, three alleles at the *Glu-A1* locus, nine alleles at the *Glu-B1* locus, four alleles at the *Glu-D1* locus, four alleles at the *Glu-A3* locus, eight alleles at the *Glu-B3* locus, and three alleles at the *Glu-D3* locus were associated with two routinely collected traits, Rmax and dough extensibility in wheat breeding programs over five years and 90 environments. However, the study of Eagles et al. (2002b) did not take population structure into account. Population stratification in wheat has been observed previously (Breseghello and Sorrells, 2006a; Ravel et al., 2006). This population stratification and an unequal distribution of alleles within a population can result in spurious associations (Knowler et al., 1988; Malosetti et al., 2007; Price et al., 2006).

In this study, the 96 wheats were from eight ancestral groups and most entries maintained a high level of admixture as shown in Fig. 3.3. The history of domestication and breeding of wheat may account for this high level of admixture.

Alternatively, the admixture may be due to recent breeding efforts. Wheat breeders often cross plants from different origins to combine the desirable qualities of each ancestral line in a new cultivar in complex pedigrees (Appendix, Table A1), as observed by Ravel et al. (2006) in their materials.

We used a candidate gene association analysis to study the glutenin and 1RS translocation effects on wheat quality as measured by the mixograph, accounting for multiple-level relatedness with the unified mixed model suggested by Yu et al. (2006). The results showed that multiple-level relatedness of individuals caused both false positive and false negative associations among the glutenin alleles and mixograph properties (Table 3.4.1 and 3.4.2). In most cases, the Q+K model reduced the strength of the association, but in some cases, it increased the association compared to the basic model (not accounting for Q+K).

Our results showed that the HMW-GS, LMW-GS and 1RS translocations play important roles in determining dough mixing properties. Among them, the 1RS translocation, the *Glu-B3* locus, and the *Glu-D1* locus were of major importance in this set of wheat cultivars and advanced lines, as discussed in detail below.

Glu-A1

In our set of wheats (mainly from the U.S. Great Plains), *Glu-A1c*, the null allele, was inferior compared to *Glu-A1a* (subunit 1) and *Glu-A1b* (subunit 2*). This is consistent with findings from Australia (Eagles et al., 2002b), France (Branlard et al., 2001), China (He et al., 2005), New Zealand (Luo et al., 2001) and Britain (Payne et al., 1987). There was no significant difference between *Glu-A1a* and *Glu-A1b* in this study, in agreement with previous studies on materials from Australia (Bekes et al., 2001; Eagles et al., 2002b; Ma et al., 2005) and France (Branlard et al., 2001). However, Moonen et al. (1983) found that *Glu-A1b* gave higher values for loaf

volume and SDSSV than *Glu-A1a* in wheat grown in the Netherlands. This discrepancy could be due to the different genetic background of the materials and their growing environments. In our TAM 107-R7/Arlin RIL population, we observed significantly ($P<0.05$) higher MPV and MRV for *Glu-A1b* than *Glu-A1a* at both the irrigated (Fort Collins) and dryland locations (Akron). In the current study, although *Glu-A1b* had higher values for most mixograph properties than *Glu-A1a*, the difference was not statistically different, possibly due to the stringency of the Tukey-Kramer multiple comparison procedure (Hayter, 1984).

Glu-B1

The quality effect of *Glu-B1w* (subunits 6*+8*) has not been previously reported to the best of our knowledge. In the present study with wheat mainly from the U.S. Great Plains, there was a trend that *Glu-B1w* produced similar mixograph properties to *Glu-B1e* in the dryland locations, Dailey and Akron, and could be considered as an inferior allele for breadmaking quality in those environments compared to alleles *b*, *c*, and *i* at the *Glu-B1* locus. However, the trend was that *Glu-B1w* had higher values of mixograph properties at the irrigated location, Fort Collins, and therefore could be considered as a good allele for breadmaking quality in that environment compared to alleles *b*, *c*, and *e* at the *Glu-B1* locus (Table 3.5.1, 3.5.2 and 3.5.3). This reflects the significant environmental effects on quality characteristics of *Glu-B1w*. Because the production of irrigated wheat amounts to one fifth of total wheat yield for Colorado (http://www.nass.usda.gov/co/pub/alwhtce04.pdf), selection for *Glu-B1w* for wheat cultivars sowed to irrigated field will improve the overall quality of Colorado winter wheat.

Glu-B1e (subunits 20x+20y) appeared to be an inferior allele compared to other alleles at the *Glu-B1* locus, especially for MPT in this study (Table 3.5.1, 3.5.2

and 3.5.3), which is in agreement with the score of Payne et al. (1987) and previous reports in bread wheat (Eagles et al., 2004; Ma et al., 2005) and durum wheat (Brites and Carrillo, 2001; Martinez et al., 2005). *Glu-B1b* was considered better than *Glu-B1c* for breadmaking quality in the gluten quality score of Payne et al. (1987). In our TAM 107-R7/Arlin RIL population, *Glu-B1b* was associated with higher mixograph properties than *Glu-B1c*. In the present study, *Glu-B1b* produced lower MLV, MPV and MRV, but higher logMPW and logMRW than *Glu-B1c* at all three locations, although the difference was not statistically significant. Since the mixograph band widths and heights are all positively correlated with SDSSV (Dobraszczyk and Schofield, 2002), we could not draw a conclusion that our results support or contradict the gluten quality scores. *Glu-B1i* was associated with higher MLV, MPV and MRV than *Glu-B1b* at dryland locations, Akron and Dailey (Table 3.5.1, 3.5.2 and 3.5.3), although the differences were not statistically different. This superiority of *Glu-B1i* to other alleles at the *Glu-B1* locus was previously reported (Eagles et al., 2002b; Payne et al., 1987).

Glu-D1

In the present study and also in the study of our TAM 107-R7/Arlin RIL population, we confirmed the beneficial effects of *Glu-D1d* over *Glu-D1a* (Table 3.5.1, 3.5.2 and 3.5.3). This agreed with the gluten quality score (Payne et al., 1987) and many other studies (Bekes et al., 2001; Eagles et al., 2002b; He et al., 2005; Huang et al., 2006). This might be the reason why the ratio between *Glu-D1d* and *Glu-D1a* changed from about 2:3 to 4:1 in Slovakian cultivars released before 1969 and after 1981, respectively (Gregova et al., 1997) and from 3:2 to 4:1 in U.S. cultivars since 1991 (Shan et al., 2007).

Glu-A3

Glu-A3e, a null allele produced lower logMLW and logMPW, but higher logMRW, MPT and MLV than other alleles at all three locations (Table 3.5.1, 3.5.2 and 3.5.3). Since all mixograph properties were positively associated with loaf volume and SDSSV (Dobraszczyk and Schofield, 2002), we could not draw a conclusion whether *Glu-A3e* is a favorable allele or an inferior allele. However, *Glu-A3e* was previously associated with a lower extensibility and Rmax than *Glu-A3d*, *b*, and *c* and was an inferior allele for breadmaking quality (Eagles et al., 2002b). In our TAM 107-R7/Arlin RIL population, *Glu-A3e* had lower mean values of MLW, MPV, MPW and MRV than *Glu-A3c* and was an inferior allele. In the current study, *Glu-A3d* was associated with intermediate values of mixograph properties relative to other alleles. However, *Glu-A3d* has been associated with higher SDSSV than other alleles at the *Glu-A3* locus (Branlard et al., 2001; He et al., 2005; Luo et al., 2001). This discrepancy might be due to the different genetic background and growing environments.

Glu-B3

Our study confirmed the important role of the *Glu-B3* locus for breadmaking quality. *Glu-B3b* and *f* produced higher mixograph properties in our materials than other alleles at the *Glu-B3* locus, consistent with the finding in Australian materials (Eagles et al., 2002b). In that study, *Glu-B3b* and *f* were associated with high Rmax and extensibility, indicating that they could be desirable alleles in cultivars bred for markets where strong, extensible dough is required. *Glu-B3c* was present in five genotypes in our population and four of these genotypes also carried 1BL.1RS. Within a group of 12 lines having 1BL.1RS, we compared lines with *Glu-B3c* to lines with other alleles at the *Glu-B3* locus. We found that *Glu-B3c* was consistently

associated with low values of mixograph properties in all three environments, which is in agreement with many studies (Bekes et al., 2001; Branlard et al., 2001; Eagles et al., 2002b; Ma et al., 2005). In those studies, *Glu-B3c* was associated with low Rmax and extensibility. Our results also suggest *Glu-B3c* is undesirable for breadmaking quality. *Glu-B3g* and *e* had similar mixograph properties in both the 96 cultivars and advanced lines and our TAM 107-R7/Arlin RIL population.

Glu-D3

The five alleles at the *Glu-D3* locus produced similar values for mixograph properties in our study. The only significant difference was observed for the higher MPT of *Glu-D3d* than that of *Glu-D3b*. Other studies have shown that the *Glu-D3* alleles had similar Rmax and extensibility in a large collection of wheats (Branlard et al., 2001; Eagles et al., 2002b). These results indicate that *Glu-D3* would have the lowest priority among glutenin loci in which to invest efforts to improve wheat quality because of its minor role in determining quality.

1RS translocation

The quality effects of 1RS in wheat have been investigated by many studies (Graybosch, 2001). In the current study, 1BL.1RS was the most detrimental form of the 1RS translocation in wheat, whereas wheat with 1AL.1RS had no significant difference with non-1RS wheat for all mixograph properties. This agrees with several studies (Espitia-Rangel et al., 1999a; Graybosch et al., 1993; Kumlay et al., 2003). The quality defects associated with the 1RS translocation may be the consequence of a change in the protein composition of the recipient wheats because incoming rye genes, such as secalin genes, are detrimental for wheat quality. In addition, 1RS replaces the short arm of at least one wheat chromosome pair, leading to a permanent

loss of some potentially important wheat genes, such as genes encoding the LMW glutenins (Graybosch, 2001; Kumlay et al., 2003).

Lack of independence between the six glutenin loci has been observed in other studies (Eagles et al., 2002b; He et al., 2005) and in our TAM 107-R7/Arlin RIL population. However, due to the low number of entries in the present study, we are not comfortable in inferring any interaction terms, although it is likely there will be significant interactions among glutenin loci and among glutenin loci and other non-glutenin loci as previously reported (Huang et al., 2006, Eagles et al., 2002b).

The Tukey-Kramer multiple range test is very conservative (Hayter, 1984). In some cases, a glutenin locus was significantly associated with a mixograph property in the *F*-test, whereas the lsmeans for the alleles at that locus were not differentiated with the Tukey-Kramer procedure. This was probably due to high levels of multiple comparisons and the low number of entries in allelic classes even after we pooled rare (<5) entries into an artificial category *p* at each locus. For example, in the *F*-test, *Glu-B3* was associated with MLV ($P<0.01$) at Akron and MRV ($P<0.001$) at Fort Collins, but the lsmeans for the alleles at *Glu-B3* were not statistically significantly different for those traits. However, when comparisons were possible, our results were remarkably similar to those of previous studies, especially for characteristics associated with dough mixing properties, indicating candidate gene association mapping can be efficient in estimating the effects of glutenin alleles.

In conclusion, we have completed one of the few association analysis studies in wheat that has taken the multiple-level relatedness into account. Controlling for population structure did indeed reduce the number of significant associations, presumably including many that were false positives. Our results confirmed the importance of *Glu-A1*, *Glu-B1*, *Glu-D1*, *Glu-B3* and 1RS translocations in

determining wheat breadmaking quality, with some of these association reported for the first time in U.S. hard winter wheat germplasm. In general, *Glu-A1c*, *Glu-B1e*, *Glu-D1a*, and *Glu-B3c* and *i* were associated with low values of mixograph properties, whereas *Glu-A1b*, *Glu-B1b*, *Glu-D1b* and *d*, *Glu-B3b* and *f* were associated with higher values for mixograph properties than other alleles. The presence of the 1BL.1RS translocation was significantly ($P<0.05$) associated with a decrease in all mixograph properties. These results suggest that selection of appropriate alleles at glutenin loci can facilitate the development of cultivars with desirable dough properties for breadmaking quality.

AFTERWORD

As a counterpart to the 'Preface', I wrote the following paragraphs to describe the major conclusions, significance and shortcomings, and future perspectives of this dissertation research.

Considering the studies with both populations, the TAM107-R7/Arlin RIL population (TA population) and the 'cultivar population' consisting 96 cultivars and advanced lines, we have the following major conclusions: (1) the environments in which wheats were grown played a major role in determining wheat dough mixing properties evaluated with 10g-mixograph; (2) multiple-level relatedness of individuals of cultivar populations caused potentially false associations as shown in the difference of significant levels of associations in the basic model (Table 3.4.1) and the mixed model (Q+K) (Table 3.4.2). The Q+K model showed the potential of correcting false associations and had higher statistical power as found in the study of Yu et al. (2006); (3) the study with the TA population had higher statistical power in comparing two alleles/locus, whereas the study with the cultivar population had the advantage of comparing multiple alleles/locus at the same time; (4) the presence of 1AL.1RS and 1BL.1RS in wheat is detrimental to wheat dough mixing properties. 1BL.1RS is more detrimental than 1AL.1RS; (5) *Glu-B3c* was an allele consistently associated with low values of mixograph properties and should be avoided in breeding for breadmaking wheat. However, this conclusion was inferred only from five entries grown in three environments and should be verified in further studies. In U.S. wheat, the frequency

of *Glu-B3c* is low, only existing in five cultivars (Wendy, SD97538, Cougar, Rowdy, and AP03-20) out of 111 cultivars from the U.S. Great Plains released after 1991, as reported by Shan et al. (2007); (6) *Glu-B1w* was associated with higher values of mixograph properties at the irrigated location, Fort Collins, whereas it was associated with higher MPT and intermediate or low values for other mixograph properties at the dryland locations, Akron and Dailey. The frequency of *Glu-B1w* in U.S. wheat is low, only 5% in 111 U.S. wheat cultivars released after 1991 in the U.S. Great Plains (Shan et al., 2007); (7) the presence of *Glu-D1d* could partially compensate for the negative effects of the presence of 1AL.1RS in the TA population. If breeders have to introduce some desirable genes with 1AL.1RS translocation, selection for glutenin alleles that are associated with desirable dough mixing properties could mitigate the detrimental quality effects of the 1AL.1RS translocation.

Here I discuss the significance of my study: (1) this study is the first to report the effects of glutenin and 1RS translocations on dough mixing properties in both irrigated and dryland Colorado environments. (2) This is the first attempt to control for multiple-relatedness of individuals in studying glutenin allelic functions with a collection of cultivars. (3) This is the first report on mixing properties of *Glu-B1w*; (4) our results support the conclusion of the inferior quality effects due to *Glu-B3c* reported for Australian wheats. (4) Since our study was done on a collection of cultivars and advanced lines from the U.S. Great Plains, our results could be applied to the hard winter wheat germplasm in the region.

However, toward the end of this project, we found that we paid inadequate attention to some factors that might lead to questionable results, especially for the cultivar population. (1) It is desirable to compare equal number of lines that carry interesting alleles at one glutenin loci. However, in our cultivar population, some

alleles were predominant at certain glutenin loci whereas some alleles only were represented by less than 10 lines. For example, *Glu-B1c* was represented by 38 lines whereas *Glu-B1w* was only represented by seven lines. (2) The effects of some alleles might be confounded with the effects of other glutenin alleles or genome regions, and we were not able to analyze and separate those effects because of the small sample size of the cultivar population. We observed 33 alleles at six glutenin loci. To study the interaction among those glutenin alleles, we need to infer 435 parameters with data from 96 entries, so we do not feel comfortable in inferring any interactions among glutenin loci and between glutenin loci and 1RS translocations. For example, in our study, *Glu-B3c* was associated with a very significant reduction of MRW at all locations. However, this result should be interpreted cautiously because four lines that carry *Glu-B3c* also had 1BL.1RS which also associated with a reduction of MRW. We compared *Glu-B3c* with a combination of other alleles (two lines with *Glu-B3b*, two lines with *Glu-B3i*, one line with *Glu-B3a* and one line with *Glu-B3d*) in the lines having 1BL.1RS and found that *Glu-B3c* was associated with a reduction of MRW compared to the combined allele group. However, the total number of lines we compared was only 12 lines. (3) The measurements of mixograph properties may not be at optimum values because we used a universal formula to calculate the water absorption for all flours as suggested by AACCI Method 54-40A (AACCI, 2004) (see formula on page 115). Some of the mixograph evaluations may not be done at optimum water absorption because of wide difference of protein concentration among the plots. Some lines have higher than 18% flour protein concentration or lower than 12% flour protein concentration. For those lines that had high protein concentration we might have added too much water whereas for those lines with lower protein concentration, we might have added too little water. We did not adjust water

absorption for every sample that was not at optimum water absorption because we do not have enough flour to repeat experiments and also because it was a formidable job to adjust water absorption for 800 samples for the TA population and 600 samples for the cultivar population.

Base on the results of this study, I would propose to pay more attention to the following aspects in doing similar studies: (1) I will not use the universal formula for water absorption, instead I will determine the coefficient for protein concentration in the formula based on the range of the flour protein concentration. If possible, mixograph evaluations should be done multiple times until samples are at optimum water absorption (the MPV is right at 50 Mu). (2) I will plant at least 3 rows/plot instead of 2 rows/plot, since it won't take too much effort to manage several extra rows. If we can harvest only middle rows and have enough seeds for mixograph, that will reduce the variance due to the boundary effect and interaction among the adjacent plots. We can harvest all the rows and we may have flour for small baking tests for a small random set of samples and analyze the correlation among mixograph properties and baking properties. This will help data interpretation. (3) For the cultivar population, I will compose my association panel based on the alleles I am interested in and worry less about the relation among the entries. I will enrich those alleles that have not been intensively studied. For example, at the *Glu-D1* locus, I will reduce the lines carrying *Glu-D1d*, because the superiority of *Glu-D1d* over other alleles is well known. I will include more entries that have *Glu-D1a*, *Glu-D1b*, and *Glu-D1e*, because they have not been intensively studied. (4) To control for multiple-level relatedness of individuals, I will use principal component analysis (PCA) (Price et al., 2006) instead of population structure to control for population stratification because it is so hard to find *a priori* number of ancestral populations for the population. With the

introduction of a new generation of sequence technique (e.g., Solexa, http://www.solexa.com), the cost of single nucleotide polymorphism (SNP) marker will be dramatically reduced in the near future. A larger number of SNP markers will be available in wheat to do PCA analysis.

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APPENDIX

Table A1. Glutenin allele composition and 1RS status of 99 cultivars and advanced lines.

ID	ORIGIN§	TYPE¶	Year#	PEDIGREE	*Glu-A1†*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	1RS‡
Neosho	Agripro		2005	W91-376-20/W9-084	*b*	*c*	*d*	*e*	*g*	*b*	Non
Baker's White	Agripro	HWW	2002	Ponderosa/Jagger	*a/b*	*c*	*d*	*e*	*a*	*c*	1BL
Burchett	Agripro	HWW	2002	W91-126/WI88-052-05	*b*	*c*	*a*	*e*	*e*	*c*	Non
Cutter	Agripro	HRW	2001	Jagger/WI89-189-14	*a*	*i*	*d*	*c*	*g*	*d*	Non
Dumas	Agripro	HRW	2000	WI90-425//N84-0758//WI81-297-3	*b*	*c*	*d*	*e*	*a*	*c*	Non
Hondo	Agripro	HRW	1998	W84-179/W81-171/3/Sturdy/Hawk//Vona/W76-1141	*b*	*b*	*d*	*e*	*g*	*a*	1AL
Jagalene	Agripro	HRW	2001	Abilene/Jagger	*a/b*	*i*	*d*	*c*	*g*	*c*	Non
Ogallala	Agripro	HRW	1993	TX81V6187/Abilene	*b*	*e*	*d*	*c*	*g*	*a*	1AL
Platte	Agripro	HWW	1995	N84-1104/Abilene	*b*	*e*	*d*	*c*	*g*	*c*	Non
Thunderbolt	Agripro	HRW	1999	Abilene/KS90WGRC10	*b*	*b*	*a*	*c*	*g*	*c*	Non
Postrock	Agripro	HRW	2005	Ogallala/KSU94U261//Jagger	*b*	*b*	*d*	*c*	*i*	*c*	Non
W04-417	Agripro	HRW		Bulk population	*a*	*b*	*d*	*c*	*g*	*c*	Non
NuFrontier	Agripro	HWW	2000	Pioneer bulk selection (HBK0927)	*b*	*w*	*d*	*c*	*g*	*a*	Non
NuHorizon	General Mills	HWW	2000	WI89-282/Arlin	*b*	*c*	*d*	*a*	*e*	*e*	1AL
Avalanche	CSU	HWW	2001	KS87H325/Rio Blanco	*b*	*b*	*d*	*g*	*g*	*a*	Non
Halt	CSU	HRW	1994	Sumner/CO820026,F1//PI372129,F1/3/TAM 107	*b*	*b*	*e*	*a*	*e*	*a*	1AL
Prairie Red	CSU	HRW	1998	CO850034/PI372129//5*TAM 107	*b*	*b*	*a*	*e*	*g*	*e*	1AL
Prowers 99	CSU	HRW	1999	CO850060/PI372129//5*Lamar	*b*	*c*	*d*	*c*	*g*	*b*	Non
Ankor	CSU	HRW	2002	Akron/Halt//4*Akron	*b*	*b*	*a*	*d*	*b*	*a*	Non
Bond CL	CSU	HRW	2004	Yumar//TXGH12588-120*4/FS2	*b*	*b*	*b*	*d*	*b*	*b*	Non
Hatcher	CSU	HRW	2004	Yuma/PI 372129//TAM 200/3/4*Yuma/4/KS91H184/Vista	*a/b*	*b*	*d*	*d*	*b*	*b*	Non
Yumar	CSU	HRW	1997	Yuma/PI372129//CO850034/3/4*Yuma	*a*	*b*	*b*	*d*	*b*	*b*	Non
Above	CSU-TX	HRW	2001	TAM 110*4/FS2	*b*	*b*	*a*	*e*	*g*	*b*	1AL
Ronl	KS-Hays	HWW	2005	TREGO/CO960293	*b*	*c*	*d*	*c*	*g*	*a*	Non
Stanton	KS-Hays	HRW	2000	PI220350/KS87H57//TAM-200/KS87H66/3/KS87H325	*b*	*b*	*d*	*c*	*e*	*b*	Non
Trego	KS-Hays	HWW	1999	KS87H325/Rio Blanco	*b*	*e*	*d*	*c*	*g*	*b*	Non
2145	KS-Manhattan	HRW	2001	HBA142A/HBZ621A//Abilene	*a/b*	*b*	*d*	*c*	*g*	*a*	Non
Heyne	KS-Manhattan	HWW	1998	KS82W422/SWM754308//KS831182/KS82W422	*a*	*i*	*d*	*d*	*i*	*c*	Non
Jagger	KS-Manhattan	HRW	1994	KS82W418/Stephens	*a*	*i*	*d*	*a*	*h*	*c*	Non
Karl 92	KS-Manhattan	HRW	1992	Karl reselection (Plainsman V/3/Kaw/Atlas 50//Parker *5/Agent)	*a*	*b*	*d*	*c*	*f*	*c*	Non
Fuller	KS-Manhattan	HRW	2006	unknown (likely has Jagger in it)	*a*	*b*	*d*	*c*	*i*	*e*	Non
KS00F5-20-3	KS-Manhattan	HRW		unknown (likely has Jagger in it)	*a*	*b*	*a*	*e*	*g*	*e*	Non
Overley	KS-Manhattan	HRW	2003	U1275-1-4-2-2/KS85W663-7-4-2//JAGGER	*a*	*i*	*d*	*a*	*g*	*c*	Non
Goodstreak	NE-UNL	HRW	2002	SD3055/KS88H164//NE89646 (=COLT*2/PATRIZANKA)	*b*	*c*	*d*	*a*	*i*	*a*	Non
Hallam	NE-UNL	HRW	2004	NE90461 (=BRL/BENN)/NIOBRARA	*b*	*c*	*d*	*c*	*g*	*b*	Non
Infinity	NE-UNL	HRW	2004	WINDSTAR/3/NE94481//TXGH125888-120*4/FS2	*b*	*c*	*d*	*e*	*f*	*d*	1AL/1BL
Overland	NE-UNL	HRW		NE94482 (=ARA/ABILENE//NE86488)/ND8974	*b*	*c*	*b*	*e*	*f*	*d*	Non
NE99495	NE-UNL	HRW		ALLIANCE/KARL 92	*a/b*	*b*	*d*	*c*	*f*	*c*	Non

ID	ORIGIN§	TYPE¶	Year#	PEDIGREE	*Glu-A1†*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	1RS‡
Niobrara	NE-UNL	HRW	1994	TAM 105*4/Amigo (TX80GH2679)//Brule Fsel #3	*b*	*c*	*d*	*c*	*g*	*b*	1AL/1BL
Pronghorn	NE-UNL	HRW	1995	Centura/Dawn//Colt sib	*a/b*	*c*	*d*	*e*	*g*	*b*	Non
Vista	NE-UNL	HRW	1992	NE68513/NE684457//Ctk/3/Brule	*b*	*b*	*d*	*a*	*h*	*c*	Non
Wahoo	NE-UNL	HRW	2000	Arapahoe/Abilene//Arapahoe	*b*	*e*	*d*	*c*	*g*	*b*	Non
Antelope	NE-USDA	HWW	2002	Pronghorn/Arlin	*b*	*c*	*d*	*c*	*b*	*b*	Non
Arrowsmith	NE-USDA	HWW	2002	KS87809-10/Arapahoe	*a*	*i*	*d*	*c*	*g*	*b*	Non
Nuplains	NE-USDA	HWW	1999	Abilene/KS831862	*b*	*e*	*d*	*c*	*g*	*c*	Non
Wesley	NE-USDA	HRW	1998	KS831936-3//Colt/Cody	*b*	*b*	*d*	*c*	*g*	*c*	Non
2174	OK-OSU	HRW	1997	IL71-5662/PL145//2165	*b*	*w*	*d*	*c*	*i*	*a*	Non
Custer	OK-OSU	HRW	1994	F-29-76/TAM-105//Chisholm	*b*	*c*	*d*	*d*	*d*	*d*	1BL
Deliver	OK-OSU	HRW	2004	OK91724/Karl	*a*	*b*	*d*	*c*	*f*	*c*	Non
Endurance	OK-OSU	HRW	2004	HBY756A/Siouxland//2180	*b*	*w*	*d*	*c*	*i*	*a*	1BL
Intrada	OK-OSU	HWW	2000	Rio Blanco/TAM 200	*b*	*e*	*d*	*g*	*g*	*a*	Non
OK00421	OK-OSU	HRW		Tonkawa/GK50 F4:10	*b*	*c*	*d*	*c*	*b*	*a*	Non
OK Bullet	OK-OSU	HRW	2005	KS93U206//KS82W418/Stephens F3:9	*a*	*i*	*d*	*a*	*e*	*b*	Non
Guymon	OK-OSU	HWW	2005	Intrada/WI89-163W F2:8	*b*	*e*	*d*	*c*	*g*	*a*	Non
Okfield	OK-OSU	HRW	2005	TXGH12588-120*4/FS4//2174 F2:8	*b*	*w*	*d*	*c*	*h*	*b*	1AL
Ok101	OK-OSU	HRW	2001	OK87W663/Mesa//2180	*b*	*w*	*d*	*c*	*g*	*a*	1AL
Ok102	OK-OSU	HRW	2002	2174/Cimarron	*b*	*w*	*d*	*c*	*g*	*a*	Non
Tonkawa	OK-OSU	HRW	1994	F-29-76/TAM-105//Chisholm	*b*	*c*	*d*	*g*	*b*	*a*	1BL
Crimson	SD-SDSU	HRW	1997	TAM-105/Winoka	*b*	*c*	*a*	*c*	*f*	*c*	Non
Expedition	SD-SDSU	HRW	2002	Tomahawk/Bennett	*b*	*c*	*d*	*e*	*g*	*d*	Non
Longhorn	Agripro	HRW	1991	NS2630-1/Thunderbird	*b*	*c*	*d*	*e*	*b*	*a*	1BL
Harding	SD-SDSU	HRW	1999	Brule//Bennett/Chisholm/3/Arapahoe	*b*	*c*	*d*	*c*	*f*	*c*	Non
SD97538	SD-SDSU	HRW		NE90518/MARTINOF93	*b*	*c*	*d*	*c*	*c*	*c*	1BL
Alice	SD-SDSU	HWW	2006	Abilene/Karl	*b*	*b*	*d*	*c*	*f*	*c*	Non
SD98102	SD-SDSU	HRW		2076-W12-11/KARL 92//NE89526	*b*	*b*	*d*	*c*	*f*	*c*	1AL
Tandem	SD-SDSU	HRW	1997	Brule/Agate	*b*	*c*	*d*	*c*	*f*	*c*	Non
Wendy	SD-SDSU	HWW	2004	SD89333/Abilene	*b*	*c*	*d*	*c*	*c*	*b*	1BL
Nekota	SD-SDSU/NE-UNL	HRW	1994	Bennett/TAM 107	*b*	*c*	*d*	*c*	*b*	*b*	1AL
Sturdy 2K	TX-Amarillo	NEW		Sinvalocho/Wichita//Hope/Cheyenne/3/Wichita/4/Seu Seun 27	*b*	*b*	*b*	*c*	*g*	*a*	Non
TAM 110	TX-Amarillo	HRW	1995	(TAM 105*4/Amigo)*5//Largo	*b*	*b*	*a*	*e*	*g*	*b*	1AL
TAM 111	TX-Amarillo	HRW	2002	TAM-107//TX78V3630/CTK78/3/TX87V1233	*b*	*c*	*a*	*a*	*h*	*a*	Non
TAM 302	TX-Dallas	HRW	1998	Pro 812/Caldwell//TX86D1310	*b*	*c*	*b*	*c*	*g*	*b*	Non
TAM 303	TX-Dallas	HRW	2005	TX89D1253*2/TTCC404	*b*	*b*	*b*	*c*	*g*	*a*	1AL
Lockett	TX-Vernon	HRW	1998	TX86V1540/TX78V2430-4	*b*	*b*	*d*	*c*	*g*	*b*	Non
TAM-400	TX-Vernon	HRW	2000	TAM 200/TX82D8556	*a/b*	*c*	*d*	*e*	*e*	*a*	1AL
TAM 112	TX-Vernon	HRW	2005	U1254-7-9-2-1/TXGH10440	*b*	*b*	*d*	*e*	*g*	*b*	1AL
Cisco	Westbred	HRW	2002	CG9119021/CG60725//KARL 92	*b*	*b*	*d*	*c*	*f*	*c*	Non
Cossack	Westbred	HRW	1998	BCD1828/83	*a*	*c*	*d*	*c*	*b*	*c*	Non
Keota	Westbred	HRW	2005	CUSTER/JAGGER	*c*	*b*	*d*	*g*	*a*	*b*	Non

ID	ORIGIN§	TYPE¶	Year#	PEDIGREE	*Glu-A1†*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	1RS‡
WB411W	Westbred	HWW	2005	G3006/ARLIN	*b*	*b*	*d*	*c*	*e*	*a*	Non
Kalvesta	Westbred	HRW	1999	Oelson/Hamra//Australia 215/3/Karl 92	*b*	*b*	*d*	*c*	*b*	*b*	Non
Onaga	Agseco	HRW	1998	unknown	*b*	*c*	*d*	*a*	*i*	*a*	Non
Santa Fe	Westbred	HRW	2004	G1878/JAGGER	*b*	*c*	*d*	*a*	*i*	*c*	Non
Venango	Westbred	HRW	2000	Random Mating Population	*b*	*e*	*d*	*a*	*g*	*a*	Non
Enhancer	Westbred	HRW	1998	1992 Nebraska Bulk Selection	*b*	*c*	*d*	*d*	*b*	*b*	Non
SD KM44/IZGREV	Sadovo	HRW		SD KM44/IZGREV	*c*	*c*	*d*	*b*	*c*	*a*	1BL
SHARK/F4105W2.1	TCI	HRW		SHARK/F4105W2.1	*b*	*b*	*d*	*e*	*b*	*a*	Non
MARISKA	MV	HRW		DTL/Spartanka//Martonvasar 19	*b*	*c*	*a*	*c*	*e*	*e*	1BL
Venera††	UKR-MIR	HRW		Venera	*b*	*c*	*d*	*c*	*c*	*a*	
MV Mandolin	MV	HRW		Martonvasar MA/Martonvasar12//Fundulea2099	*b*	*e*	*d*	*c*	*c*	*d*	1BL
CIT922411	TCI	HRW		CHAM4/TAM200/RSK/FKG15	*b*	*a*	*d*	*b*	*b*	*b*	Non
CMSW92WM00217S	MX-CIT	HRW		SPN/NAC/ATTILA	*b*	*c*	*d*	*c*	*i*	*c*	1BL
BEZOSTAYA1††	Eastern Europe	HRW		selection from Bezostaya 4 (Lutescens 17 / Skorospelka 2)	*b*	*c*	*d*	*c*	*e*	*e*	
SWM89Y074H	MXORTCI	HRW		MNCH/3/JUP/BJY//GA	*a*	*c*	*d*	*a*	*c*	*b*	1BL
OR939526††	OSU	SWW		Madsen/Malcom	*b*	*w*	*a*	*e*	*i*	*c*	
KS920946-B-15-1	KS	HRW		ABI86*3414/Jag'S'//KARL 92	*b*	*w*	*d*	*a*	*i*	*a*	1AL/1BL
TX97A0050	Texas A&M	HRW		TX86V1540/KARL 92	*a/b*	*e*	*d*	*c*	*g*	*a*	Non
NW97S278 (?)	NE-USDA	HWW		pronghorn/Arlin	*c*	*c*	*d*	*a*	*g*	*c*	1AL
ZERNOKOMOVAYA 50	KAZ	HRW		Bogarnaya56/(K-47100) Moldova	*b*	*c*	*d*	*e*	*g*	*a*	1AL

† Glutenin allele designations based on McIntosh et al. (2003);

‡ non, non-1RS translocation; 1AL, 1AL.1RS translocation; 1BL, 1BL.1RS translocation; 1AL/1BL, heterogenous with 1AL.1RS and 1BL.1RS translocations.

§ CSU, Colorado State University; KS, Kansas State University; OSU, Oklahoma State University; UNL, University of Nebraska-Lincoln; SDSU, South Dakota State University; TAM, Texas Agricultural and Mechanical University; USDA-NE, U.S. Dept. of Agriculture-Agricultural Research Service, Lincoln, NE; TCI, Turkey-CIMMYT-ICARDA, MV, Martonvásár, Hungary. MX, Mexico.

¶ HWM, hard white winter wheat; HRW, hard red winter wheat.

Year, the year of the cultivar release.

†† These entries were excluded from the genotyping and association analysis.

? NW97S278 was released as Antelope. However, this line has different glutenin protein compared to Antelope and it was not group with Antelope in the STRUCTURE groups. We assume it is line named NW97S278 but not Antelope.

Table A2. The list of microsatellite markers used in genotyping for 96 cultivars and advanced lines.

Std Name	number of Loci scored	Different SSR Sets†			Somers‡ Chrom	Physical Position	Somers #Alleles	Size§					
		60	42	21				CS	ACBarrie	Thatcher	Alsen	Grandin	Other
BAR0018	1	BAR018	BAR0018		6B(59.8)	2BS-1~c	9	228	238	268	258	258	
BAR0059	1	BAR059			5B (153.9)	5BL-6~t	4	181	189	189	211	189	
BAR0062	1	BAR062			1D(117.1)		2	131	150	150	150	150	
BAR0075	1	BAR075	BAR0075	BAR0075	3B(0)		5	111	127	130	Null	Null	
BAR0077	2	BAR077A			3B(111.2)	3BL-2~c	7	199	228	228	161	161	
BAR0080	1	BAR080	BAR0080	BAR0080	1B(106.3)	1BL-10~t	5	110	121	121	121	121	
BAR0081	1				1BL (85/111)	1BL-3~5							
BAR0090	1	BAR090			2D, 7B	7BL-7~c							
BAR0096	1	BAR096	BAR0096		6D(91.7)	6DL-6~t	3	190	206	203	203	203	
BAR0146	1	BAR146			6A(36.66)	6DL-6~c	4	130	174	178	178	178	
BAR0148	1	BAR148	BAR0148		1A(56.8), 1D(53.9)	1AS-1~2	15	196	214, 263	210, 265	214, 298	216, 298	
BAR0149	1	BAR149	BAR0149	BAR0149	1D(13.7)	1DS-1~2	3	249	146	146	150	150	
BAR0181	1				1B(38.3)		7	185	205	207	207	207	
CFA2019	1	CFA2019	CFA2019	CFA2019	7A(106.5)		4	217	Null	Null	235	Null	
CFA2028	1	CFA2028	CFA2028		7A(47.7)		2	261	272	272	274	272	
CFA2193	1	cfa2193			3A(73.8)		4	195	229	231	233	233	
CFA2219	1	CFA2219	CFA2219	CFA2219	1A(124.1)		5	223	265	265	235	235	
CFD0005	1	CFD005	CFD0005		5B (0)		2	195	222	222	197	197	
CFD0035	1				3D(0)		6	191	Null	Null	Null	216	
CFD0049	1	CFD049	CFD0049	CFD0049	6D(0)		8	214	Null	Null	239	239	
CFD0084	1	CFD084			4D(66.7)		3	193	Fail	204	200	Fail	
CFD0175	1	CFD175			7D(154.3)		3	281	293	295	293	Fail	
CFD0190	2	CFD190			6D(54.9)		2	191	190	190	190	190	

Std Name	number of Loci scored	Different SSR Sets†			Somers‡ Chrom	Physical Position	Somers #Alleles	Size§					
		60	42	21				CS	ACBarrie	Thatcher	Alsen	Grandin	Other
CFD0223	1	CFD223	CFD0223	CFD0223	3D(27.9)		4	153	187	187	187	191	
GWM0124	1	GWM124			1B(64.1)								
GWM0133	1				1B, 3A, 4D, 5B, 6B, 6D, 7B								
GWM0136	1				1A(11.6)		13		330	307	330	337	
GWM0149	1	GWM149	GWM0149	GWM0149	4B(28.4)		3	172	172	182	182		
GWM0156	1				5A(73.4)		5		306	336	336	336	
GWM0194	1	GWM194	GWM0194		4D(82)								
GWM0272	1	GWM272	GWM0272		5D(115.9)		4		154	154	152	152	
GWM0291	1	GWM291	GWM0291	GWM0291	5A(162.9)		7		180	Fail	184	169	
GWM0292	1	GWM292			5D(63.7)		7		234	234	228	228	
GWM0301	1	GWM301	GWM0301		2D(107)		9		Null	228	222	243	
GWM0333	1	GWM333	GWM0333	GWM0333	7B(65)		5		168	168	168	168	
GWM0334	1	GWM334	GWM0334		6A(1.8)		5		144	144	146	146	
GWM0443	1	GWM443	GWM0443		5A(23.1)		4		144	144	148	148	
GWM0518	2	GWM518A	GWM0518		6B(27.3)		10		175	175	200	188	
GWM0630	1	GWM630	GWM0630	GWM0630	2B(57.9)		2		140	122	122	122	
WMC0144	1	WMC144	WMC0144	WMC0144	2D(67.1)		3	143	161	159	161	161	143
WMC0149	1	WMC149			2B(99.6)								
WMC0160	2	WMC160A			5B (133.1)		4	169	137	137	135	191	169
WMC0216	2	WMC216A	WMC0216		1B(35.1), 1D(59.2)		4	123	109, 145	109, 145	109, 145	109, 145	123
WMC0233	1	WMC233	WMC0233	WMC0233	5D(0)		3	260	274	274	274	274	260
WMC0254	1				6A(148.206)		5	193	214	214	188	188	193
WMC0285	1	WMC285	WMC0285	WMC0285	4D(9.9)		5	289	314	312	312	Fail	289
WMC0313	1				4A(82.9)								

Std Name	number of Loci scored	Different SSR Sets†			Somers‡ Chrom	Physical Position	Somers #Alleles	Size§					
		60	42	21				CS	ACBarrie	Thatcher	Alsen	Grandin	Other
WMC0376	1	WMC376	WMC0376	WMC0376	5B (60)		4	222	228	228	236	236	222
WMC0413	1	WMC413			4B(17.3)		2	162	186	186	184	184	162
WMC0438	1	WMC438	WMC0438	WMC0438	7D(83.8)		6	256	276	276	276	276	256
WMC0468	1	WMC468	WMC0468	WMC0468	4A(37.7)		5	150	152	152	152	152	150
WMC0487	1	WMC487	WMC0487	WMC0487	6B(9.2)		7	160	Null	176	182	176	160
WMC0491	1	WMC491	WMC0491		4A(7.6)		9	192	226	210	220	232	192
WMC0506	1	WMC506	WMC0506		7D(21)		11	216	239	239	239	Fail	216
WMC0532	1	WMC532	WMC0532	WMC0532	3A(5.7)		8	176	193	193	177	201	176
WMC0533	2	WMC533A	WMC0533		3B(62.1)		3	147	171	169	171	171	147
WMC0553	1	WMC553	WMC0553	WMC0553	6A(52.03)		3	356	Null	Null	Null	380	356
WMC0594	1	WMC594	WMC0594		3A(105)		11		176	186	176	186	
WMC0606	1	WMC606	WMC0606		7B(0)		6		207	207	Null	Null	
WMC0658	1	WMC658	WMC0658		2A(139.9)		5		Null	Null	Null	Null	
WMC0667	1	WMC667	WMC0667	WMC0667	2A(10.4)		7		172	172	172	172	
WMC0710	1	WMC710	WMC0710		4B(48.2)		8		151	123	130	130	
WMC0764	1				2B(1.3)		8		157	165	Null	Null	

† Different SSR marker sets used to select cultivars (21 SSR markers), estimate *a priori* population number (k) in StructuRAMA (21, 42, 60 SSR markers) and estimate Q-matrix (60 SSR markers).

‡ Refers to the genetic map developed by Somers et al. (2004).

§ The size of the PCR fragments amplified with the corresponding SSR marker.

Table A3. The allele information of 69 microsatellite loci tested on 96 cultivars and advanced lines (obtained from SPAGeDi).

SSR Locus	Number of missing genotypes	Number of alleles	He †	Allele‡																		
BAR146	1	11	0.73	167	169	171	173	175	177	179	181	183	187	191								
BAR148	7	8	0.70	209	213	215	217	218	219	221	223											
BAR149	2	5	0.69	144	146	148	150	155														
BAR18	1	11	0.80	236	239	242	248	251	254	258	261	264	267	273								
BAR181	0	6	0.49	186	194	196	202	204	206													
BAR59	0	4	0.55	187	191	197	201															
BAR62	1	2	0.08	147	999																	
BAR75	14	2	0.35	125	128																	
BAR77A	1	4	0.50	158	171	175	999															
BAR77B	1	6	0.76	220	224	228	251	255	999													
BAR80	1	5	0.43	120	123	126	129	160														
BAR81	45	2	0.42	202	205																	
BAR90	0	2	0.42	142	161																	
BAR96	13	3	0.46	199	202	205																
CFA2019	2	9	0.49	233	250	251	252	253	484	485	501	999										
CFA2028	0	4	0.41	262	270	272	274															
CFA2193	2	7	0.75	212	225	227	229	231	233	257												
CFA2219	4	8	0.72	235	259	263	265	267	269	272	280											
CFD175	3	3	0.53	289	295	297																
CFD190A	1	2	0.48	176	178																	
CFD190B	1	2	0.08	186	188																	
CFD223	1	7	0.70	167	169	184	186	188	190	195												
CFD35	76	5	0.76	201	203	205	209	211														

SSR Locus	Number of missing genotypes	Number of alleles	He †	Allele‡																		
CFD49	8	4	0.67	180	182	212	253															
CFD5	1	4	0.60	196	219	221	223															
CFD84	2	6	0.73	194	196	198	200	201	204													
GWM124	0	2	0.02	211	224																	
GWM133	22	6	0.68	191	193	197	199	201	203													
GWM136	58	7	0.76	250	273	296	302	306	316	999												
GWM149	0	3	0.44	172	182	183																
GWM156	28	5	0.68	332	334	336	338	345														
GWM194	8	8	0.82	145	146	147	148	149	150	151	152											
GWM272	16	5	0.63	143	150	152	154	160														
GWM291	0	8	0.78	166	168	170	174	177	180	181	999											
GWM292	1	9	0.76	216	218	220	224	227	229	231	233	235										
GWM301	10	16	0.87	221	223	224	226	227	228	229	230	231	233	234	236	237	238	239	241			
GWM333	2	6	0.57	159	161	163	165	167	169													
GWM334	3	7	0.56	116	120	128	130	132	136	138												
GWM443	6	13	0.85	126	134	136	138	140	142	144	146	150	159	176	178	180						
GWM518A	0	3	0.30	139	141	999																
GWM518B	3	7	0.73	168	172	178	180	182	186	196												
GWM630	0	3	0.30	125	127	131																
WMC144	1	4	0.46	155	156	158	160															
WMC149	4	3	0.50	194	195	389																
WMC160A	2	4	0.18	133	135	136	138															
WMC160B	2	7	0.69	186	188	190	192	194	204	999												
WMC216A	4	2	0.42	106	109																	

SSR Locus	Number of missing genotypes	Number of alleles	He †	Allele‡																		
WMC216B	5	3	0.38	143	145	147																
WMC233	10	2	0.05	275	277																	
wmc254	20	7	0.70	208	212	214	216	218	220	227												
WMC285	9	5	0.58	292	309	311	313	314														
WMC313	58	3	0.49	205	226	232																
WMC376	1	4	0.60	226	228	230	233															
WMC413	2	2	0.10	182	184																	
WMC438	0	8	0.57	250	261	264	266	272	274	276	278											
WMC468	0	8	0.75	145	147	149	151	161	170	178	181											
WMC487	16	9	0.76	174	175	176	178	180	182	187	188	189										
WMC491	1	10	0.80	210	212	214	216	218	220	222	225	227	233									
WMC506	14	13	0.85	220	222	224	232	234	236	240	242	244	245	247	251	253						
WMC532	0	9	0.75	177	187	189	193	195	197	199	203	205										
WMC533A	1	2	0.25	146	148																	
WMC533B	0	6	0.64	158	162	164	166	168	170													
WMC553	0	9	0.81	363	365	367	372	374	378	380	381	383										
WMC594	0	12	0.89	162	166	168	172	174	176	182	188	189	191	193	195							
WMC606	1	14	0.71	140	154	157	172	187	189	191	193	198	202	203	205	208	999					
WMC658	6	19	0.82	197	199	203	205	207	211	213	219	221	223	225	227	229	231	237	239	241	249	251
WMC667	0	3	0.65	78	90	999																
WMC710	1	13	0.80	108	110	123	125	129	133	139	141	143	145	147	157	159						
WMC764	31	5	0.45	152	154	162	188	888														

† Gene diversity corrected for sample size, Nei 1978.

‡ The alleles identified by microsatellite markers; fragment sizes include 18 base M13 primer.

Table A4. The Q-matrix of 96 cultivars and advanced lines generated from STRUCTURE (Pritchard et al., 2000).

Entry	Inferred ancestry of individuals								Max‡	Min§
	pop1†	pop2†	pop3†	pop4†	pop5†	pop6†	pop7†	pop8†		
Neosho	0.008	0.249	0.004	0.028	0.353	0.018	0.330	0.012	0.353	0.004
Baker's White	0.002	0.007	0.418	0.008	0.002	0.004	0.554	0.005	0.554	0.002
Burchett	0.005	0.262	0.495	0.002	0.007	0.006	0.004	0.220	0.495	0.002
Cutter	0.030	0.129	0.031	0.003	0.148	0.012	0.642	0.004	0.642	0.003
Dumas	0.007	0.279	0.004	0.021	0.004	0.019	0.017	0.649	0.649	0.004
Hondo	0.134	0.410	0.003	0.004	0.426	0.002	0.004	0.017	0.426	0.002
Jagalene	0.002	0.433	0.001	0.002	0.002	0.002	0.500	0.058	0.500	0.001
Ogallala	0.007	0.984	0.001	0.001	0.002	0.002	0.002	0.001	0.984	0.001
Platte	0.020	0.497	0.034	0.012	0.054	0.257	0.049	0.077	0.497	0.012
Thunderbolt	0.102	0.883	0.001	0.002	0.002	0.002	0.006	0.002	0.883	0.001
Postrock	0.155	0.044	0.003	0.003	0.003	0.001	0.789	0.002	0.789	0.001
W04-417	0.002	0.002	0.133	0.684	0.003	0.003	0.021	0.153	0.684	0.002
NuFrontier	0.005	0.163	0.002	0.002	0.003	0.020	0.002	0.802	0.802	0.002
NuHorizon	0.002	0.873	0.088	0.016	0.003	0.002	0.003	0.012	0.873	0.002
Avalanche	0.615	0.370	0.002	0.001	0.003	0.002	0.005	0.002	0.615	0.001
Halt	0.641	0.002	0.320	0.021	0.005	0.003	0.007	0.001	0.641	0.001
Prairie Red	0.992	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.992	0.001
Prowers 99	0.002	0.003	0.048	0.002	0.938	0.002	0.003	0.001	0.938	0.001
Ankor	0.598	0.097	0.062	0.004	0.178	0.012	0.046	0.003	0.598	0.003
Bond CL	0.485	0.002	0.003	0.001	0.503	0.001	0.003	0.003	0.503	0.001
Hatcher	0.002	0.002	0.001	0.001	0.985	0.001	0.001	0.006	0.985	0.001
Yumar	0.004	0.003	0.002	0.001	0.987	0.001	0.001	0.002	0.987	0.001
Above	0.991	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.991	0.001
Ronl	0.641	0.030	0.001	0.001	0.019	0.004	0.282	0.021	0.641	0.001
Stanton	0.800	0.171	0.015	0.001	0.004	0.003	0.002	0.005	0.800	0.001
Trego	0.526	0.290	0.002	0.001	0.004	0.001	0.174	0.003	0.526	0.001
2145	0.010	0.035	0.002	0.022	0.004	0.018	0.066	0.843	0.843	0.002
Heyne	0.018	0.008	0.010	0.080	0.020	0.013	0.839	0.011	0.839	0.008
Jagger	0.001	0.001	0.001	0.001	0.001	0.001	0.993	0.001	0.993	0.001
Karl 92	0.001	0.001	0.001	0.993	0.001	0.001	0.001	0.001	0.993	0.001
Fuller	0.005	0.001	0.001	0.269	0.002	0.001	0.719	0.001	0.719	0.001
KS00F5-20-3	0.397	0.016	0.023	0.002	0.018	0.002	0.539	0.003	0.539	0.002
Overley	0.002	0.002	0.001	0.002	0.001	0.001	0.989	0.001	0.989	0.001
Goodstreak	0.003	0.004	0.979	0.002	0.004	0.002	0.005	0.002	0.979	0.002
Hallam	0.040	0.002	0.205	0.002	0.745	0.002	0.002	0.002	0.745	0.002
Infinity	0.117	0.024	0.402	0.002	0.446	0.003	0.002	0.004	0.446	0.002
Overland	0.107	0.005	0.010	0.057	0.815	0.002	0.002	0.002	0.815	0.002
NE99495	0.040	0.002	0.389	0.555	0.007	0.003	0.003	0.002	0.555	0.002

Entry	Inferred ancestry of individuals								Max‡	Min§
	pop1†	pop2†	pop3†	pop4†	pop5†	pop6†	pop7†	pop8†		
Niobrara	0.012	0.003	0.258	0.008	0.667	0.047	0.002	0.002	0.667	0.002
Pronghorn	0.002	0.010	0.930	0.001	0.004	0.007	0.044	0.003	0.930	0.001
Vista	0.009	0.010	0.230	0.002	0.742	0.002	0.002	0.003	0.742	0.002
Wahoo	0.002	0.312	0.014	0.001	0.663	0.002	0.001	0.005	0.663	0.001
Antelope	0.003	0.003	0.832	0.135	0.005	0.014	0.003	0.004	0.832	0.003
Arrowsmith	0.003	0.004	0.013	0.437	0.521	0.007	0.012	0.002	0.521	0.002
Nuplains	0.002	0.895	0.004	0.010	0.003	0.007	0.002	0.076	0.895	0.002
Wesley	0.062	0.008	0.084	0.372	0.378	0.037	0.027	0.033	0.378	0.008
2174	0.002	0.047	0.004	0.002	0.005	0.018	0.004	0.918	0.918	0.002
Custer	0.005	0.004	0.028	0.003	0.001	0.950	0.006	0.004	0.950	0.001
Deliver	0.001	0.001	0.001	0.990	0.001	0.004	0.001	0.001	0.990	0.001
Endurance	0.002	0.003	0.292	0.026	0.019	0.056	0.007	0.595	0.595	0.002
Intrada	0.002	0.969	0.001	0.001	0.001	0.001	0.022	0.002	0.969	0.001
OK00421	0.004	0.002	0.002	0.004	0.002	0.980	0.003	0.004	0.980	0.002
OKBullet	0.173	0.088	0.002	0.007	0.015	0.008	0.700	0.007	0.700	0.002
Guymon	0.002	0.971	0.004	0.001	0.002	0.008	0.010	0.002	0.971	0.001
Okfield	0.464	0.004	0.001	0.005	0.002	0.002	0.004	0.518	0.518	0.001
Ok101	0.028	0.005	0.001	0.001	0.031	0.001	0.001	0.930	0.930	0.001
Ok102	0.026	0.502	0.003	0.006	0.004	0.002	0.005	0.452	0.502	0.002
Tonkawa	0.004	0.011	0.003	0.007	0.002	0.967	0.002	0.004	0.967	0.002
Crimson	0.003	0.028	0.058	0.003	0.208	0.004	0.001	0.694	0.694	0.001
Expedition	0.259	0.007	0.705	0.002	0.003	0.002	0.019	0.002	0.705	0.002
Longhorn	0.005	0.417	0.003	0.043	0.005	0.519	0.003	0.005	0.519	0.003
Harding	0.002	0.002	0.936	0.001	0.055	0.002	0.001	0.001	0.936	0.001
SD97538	0.146	0.008	0.586	0.018	0.220	0.007	0.002	0.013	0.586	0.002
Alice	0.002	0.185	0.002	0.804	0.002	0.001	0.002	0.003	0.804	0.001
SD98102	0.39	0.002	0.119	0.194	0.004	0.284	0.004	0.003	0.390	0.002
Tandem	0.003	0.005	0.976	0.001	0.008	0.002	0.001	0.003	0.976	0.001
Wendy	0.008	0.049	0.366	0.005	0.005	0.006	0.002	0.559	0.559	0.002
Nekota	0.709	0.007	0.242	0.005	0.018	0.007	0.006	0.004	0.709	0.004
Sturdy 2K	0.001	0.002	0.003	0.001	0.003	0.003	0.002	0.984	0.984	0.001
TAM 110	0.992	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.992	0.001
TAM 111	0.484	0.209	0.003	0.001	0.005	0.289	0.004	0.007	0.484	0.001
TAM 302	0.003	0.006	0.023	0.002	0.004	0.041	0.045	0.876	0.876	0.002
TAM 303	0.587	0.284	0.020	0.015	0.064	0.023	0.002	0.004	0.587	0.002
Lockett	0.758	0.002	0.199	0.007	0.012	0.007	0.003	0.013	0.758	0.002
TAM-400	0.068	0.681	0.030	0.002	0.197	0.004	0.002	0.015	0.681	0.002
TAM 112	0.278	0.71	0.004	0.001	0.003	0.001	0.001	0.001	0.710	0.001
Cisco	0.005	0.01	0.013	0.959	0.004	0.005	0.003	0.003	0.959	0.003
Cossack	0.003	0.002	0.004	0.414	0.008	0.566	0.001	0.002	0.566	0.001
Keota	0.004	0.003	0.002	0.419	0.002	0.480	0.087	0.003	0.480	0.002

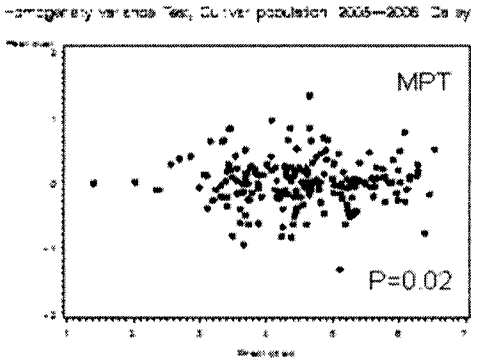
Entry	Inferred ancestry of individuals								Max‡	Min§
	pop1†	pop2†	pop3†	pop4†	pop5†	pop6†	pop7†	pop8†		
WB411W	0.008	0.006	0.095	0.855	0.009	0.004	0.019	0.004	0.855	0.004
Kalvesta	0.005	0.009	0.006	0.939	0.004	0.008	0.020	0.009	0.939	0.004
Onaga	0.009	0.012	0.004	0.001	0.003	0.002	0.009	0.959	0.959	0.001
Santa Fe	0.099	0.011	0.005	0.001	0.003	0.012	0.865	0.003	0.865	0.001
Venango	0.012	0.917	0.053	0.002	0.004	0.002	0.002	0.009	0.917	0.002
Enhancer	0.018	0.311	0.038	0.002	0.002	0.024	0.007	0.597	0.597	0.002
SD KM44/IZGREV	0.001	0.002	0.002	0.010	0.001	0.981	0.001	0.002	0.981	0.001
SHARK/F4105W2.1	0.004	0.032	0.002	0.006	0.002	0.792	0.159	0.003	0.792	0.002
MARISKA	0.003	0.002	0.004	0.096	0.003	0.887	0.002	0.004	0.887	0.002
MV Mandolin	0.001	0.002	0.002	0.003	0.001	0.938	0.039	0.014	0.938	0.001
CIT922411	0.007	0.004	0.002	0.001	0.013	0.754	0.215	0.004	0.754	0.001
CMSW92WM00217S	0.008	0.011	0.043	0.009	0.004	0.85	0.043	0.031	0.850	0.004
SWM89Y074H	0.005	0.008	0.005	0.002	0.003	0.761	0.208	0.009	0.761	0.002
KS920946-B-15-1	0.015	0.274	0.025	0.015	0.581	0.044	0.007	0.038	0.581	0.007
TX97A0050	0.003	0.176	0.202	0.263	0.006	0.183	0.004	0.164	0.263	0.003
NW97S278 (?)	0.013	0.003	0.385	0.096	0.475	0.008	0.004	0.017	0.475	0.003
ZERNOKOMOVAYA 50	0.007	0.860	0.003	0.002	0.002	0.094	0.018	0.014	0.860	0.002
Genetic diversity of ancestry populations										
F_{ST}	0.4222	0.3417	0.2918	0.3999	0.315	0.2142	0.4062	0.216		

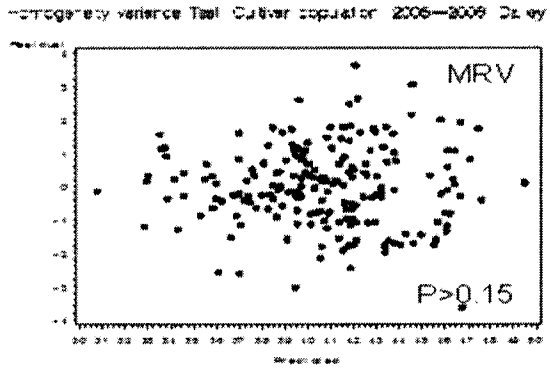
† Assigned name of ancestry population from pop1 to pop8.

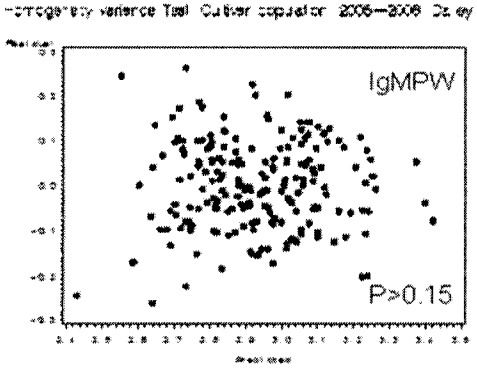
‡ Max, the maximum of the probability for eight ancestry populations.

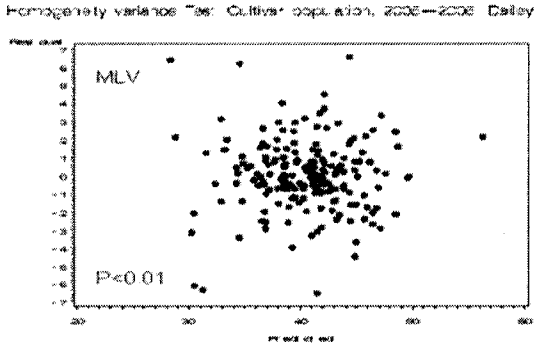
§ Min, the minimum of the probability for eight ancestry populations

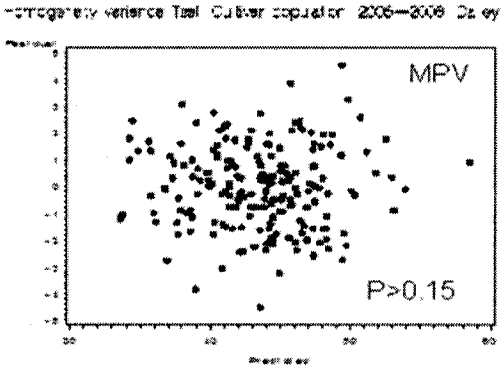
Fig. A1.1 Residual plots of mixograph properties for the cultivar population-Dailey†

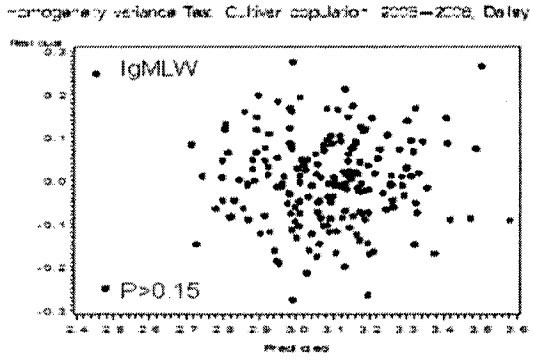


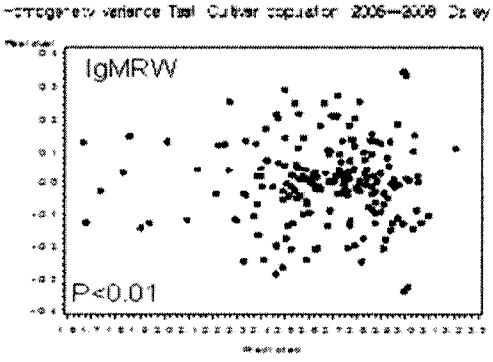






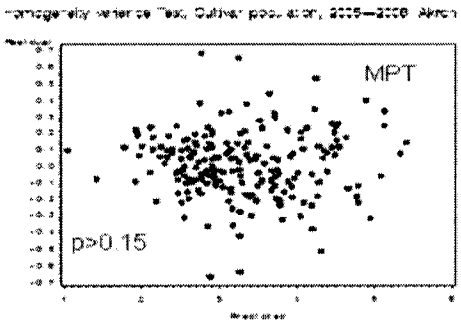


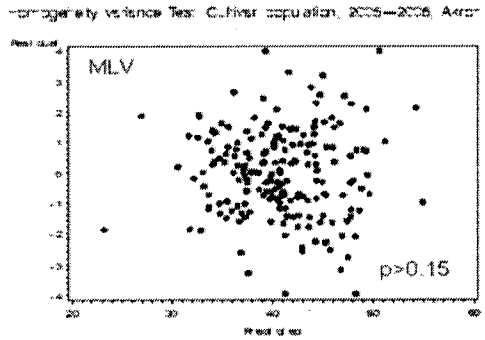


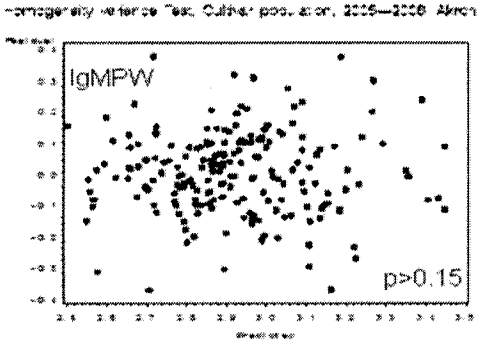


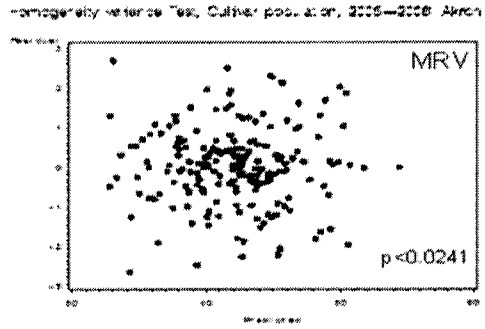
† Traits are labeled in the figure. The p-value of Kolmogorov-Smirnov test are also given in the figure.
MPT, mixograph peak time; lgMLW, $\log_e$ (ln) transformation of mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; lgMPW, $\log_e$ (ln) transformation of mixograph band width at MPT; MPV, mixograph midline peak height; lgMRW, $\log_e$ (ln) transformation of mixograph band width at two minutes after MPT; and MRV, mixograph midline height at two minutes after MPT.

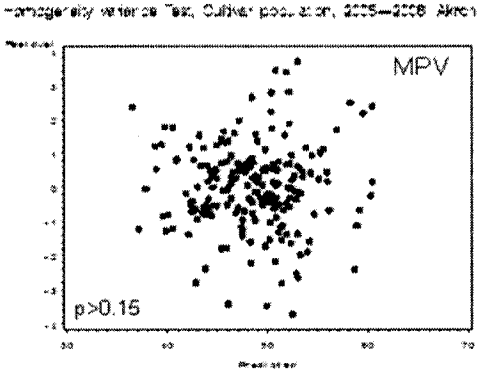
Fig. A1.2. Residual plots of mixograph properties of the cultivar population-Akron†

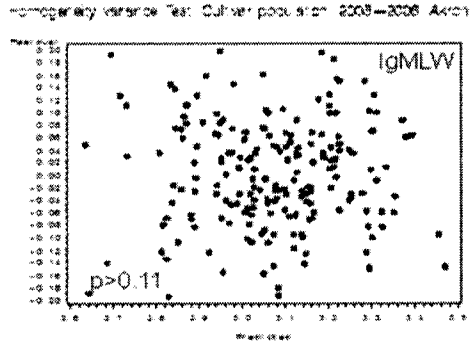


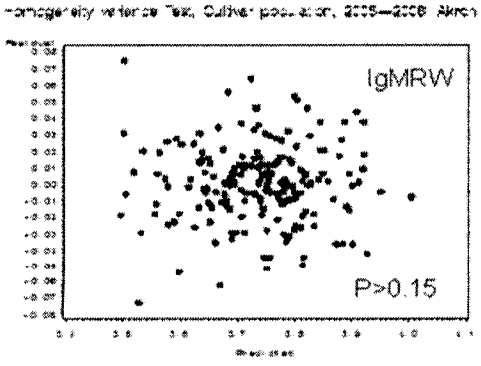






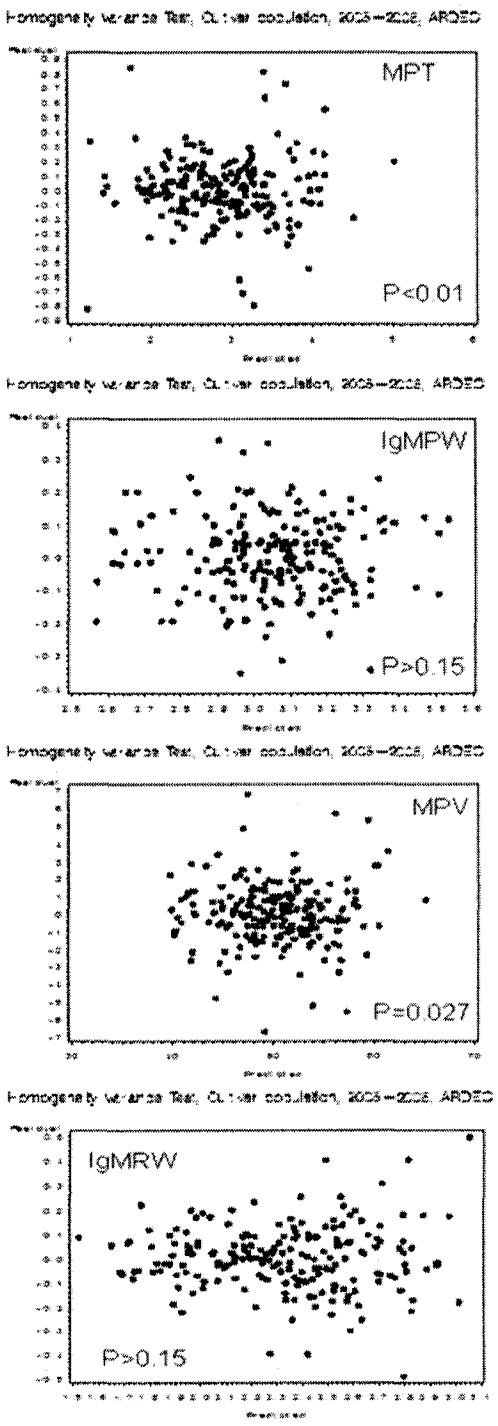






† Traits are labeled in the figure. The p-value of Kolmogorov-Smirnov test are also given in the figure.
MPT, mixograph peak time; lgMLW, $\log_e$ (ln) transformation of mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; lgMPW, $\log_e$ (ln) transformation of mixograph band width at MPT; MPV, mixograph midline peak height; lgMRW, $\log_e$ (ln) transformation of mixograph band width at two minutes after MPT; and MRV, mixograph midline height at two minutes after MPT.

Fig. A1.3. Residual plots of mixograph properties of the cultivar population-Fort Collins†



† Traits are labeled in the figure. The p-value of Kolmogorov-Smirnov test are also given in the figure.
MPT, mixograph peak time; lgMLW, $\log_e$ (ln) transformation of mixograph width at one minute before MPT; MLV, mixograph midline height at one minute before MPT; lgMPW, $\log_e$ (ln) transformation of mixograph band width at MPT; MPV, mixograph midline peak height; lgMRW, $\log_e$ (ln) transformation of mixograph band width at two minutes after MPT; and MRV, mixograph midline height at two minutes after MPT.

Table A5. Estimation of the number of clusters in the population 96 cultivars and advanced lines with StructuRAMA.†

Number of cultivars‡	96			72		
Number of SSR Markers§	60	42	21	60	42	21
E[K|X] ¶	8.783	8.0823	8.4314	6.1222	7.5686	7.0499
Var[K|X]	0.8855	0.6221	4.5652	0.7689	0.8772	0.5242
Alpha#	1.5615	1.5615	1.8922	2.0649	2.0649	2.0649

† The number of clusters/subpopulations for the cultivar population was estimated with StructuRAMA (Huelsenbeck and Andolfatto, 2007);

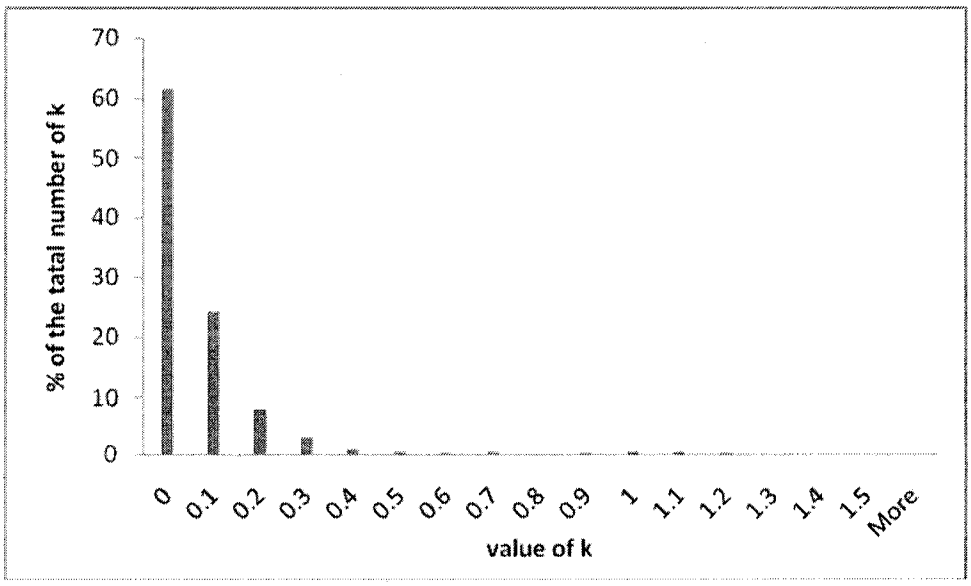
‡ The total number of entries genotyped was 96. One of the entries shared above 15 out of 21 SSR marker types (each chromosome one SSR marker) with other cultivars were kept to make a reduced number of cultivars, 72 cultivars.

§ A total of 60 SSR marker (two or three loci per chromosome), 42 SSR markers (two loci per chromosome) and 21 SSR markers (one locus per chromosome) were used to infer the number of clusters with StructuRAMA.

¶ E[K|X], the estimated number (K) of cluster of X observations.

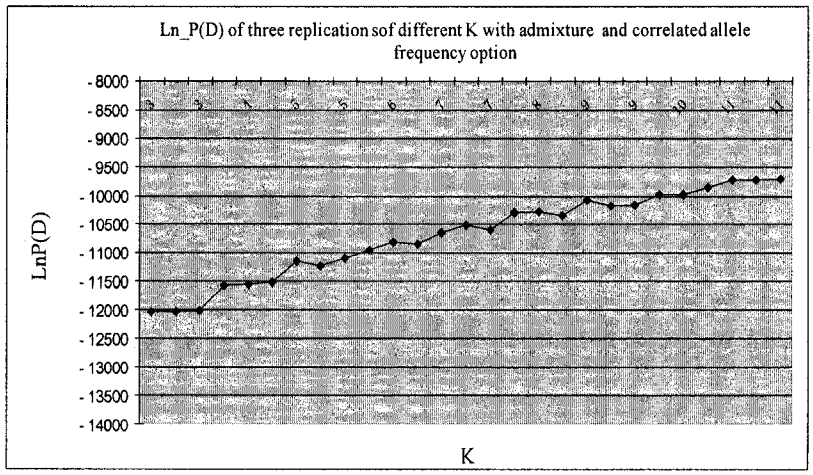
Alpha, the parameter of Dirichlet process.

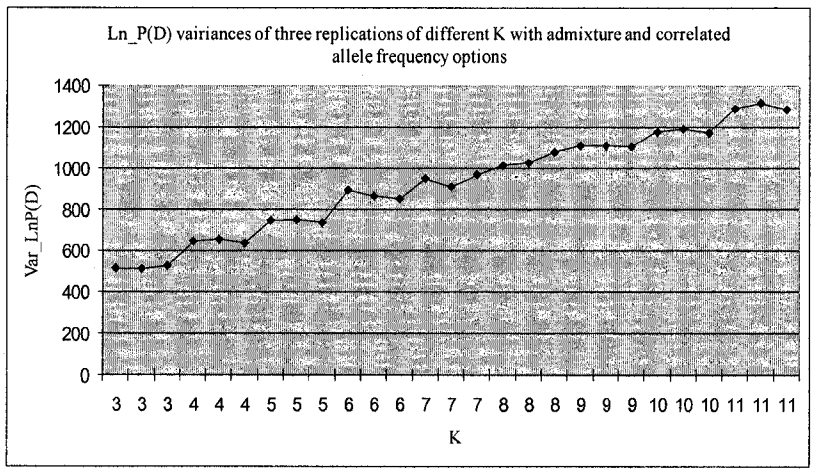
Fig. A2. The frequency distribution of individual relatedness levels (K) calculated from 69 microsatellite loci with SPAGeDi. †

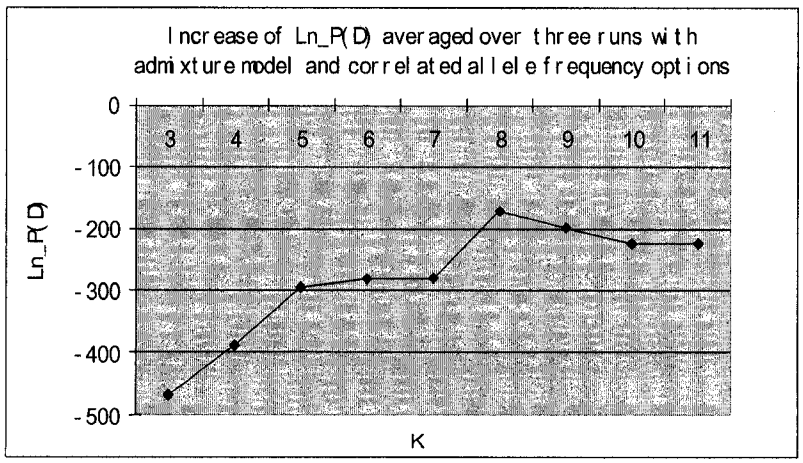


† From the histogram, we can see majority of entries are not closely related (K≤0.4).

Fig. A3. Estimation of *a priori* subpopulation number (k) with STRUCTURE.†







† Three figures show Ln_P(D), variance of Ln_P(D) and increase of Ln_P(D) from STRUCTURE runs with 60 microsatellite loci with three replications, admixture model and correlated allele frequency options. Although Ln_P(D) (first figure) and variance of Ln_P(D) (the second figure) keep on increasing with larger k, the magnitude of increase is peak at k=8 (last figure). This possibly indicates *a priori* k is eight.

Table A6. The distribution of glutenin alleles in eight subpopulations for 96 cultivars and advanced lines

(The different shade colors indicate different clusters/populations. 1AL, 1BL, and 1AL1BL indicate 1AL.1RS, 1BL.1RS and heterogenous lines respectively).

Entry	*GluA1*		*GluB1*		*GluD1*		*GluA3*		*GluB3*		*GluD3*		*1RS*	
Prairie Red	*b*		*b*		*a*		*e*		*g*		*e*		1AL	
TAM 110	*b*		*b*		*a*		*e*		*g*		*b*		1AL	
Above	*b*		*b*		*a*		*e*		*g*		*b*		1AL	
Stanton	*b*		*b*		*d*		*c*		*e*		*b*		Non	
Lockett	*b*		*b*		*d*		*c*		*g*		*b*		Non	
Nekota	*b*		*c*		*d*		*c*		*b*		*b*		1AL	
Halt	*b*		*b*		*p*	*a*			*e*		*a*		1AL	
KS03HW158	*b*		*c*		*d*		*c*		*g*		*a*		Non	
Avalanche	*b*		*b*		*d*			*p*	*g*		*a*		Non	
Ankor	*b*		*b*		*a*			*d*	*b*		*a*		Non	
TX98D1170	*b*		*b*		*b*		*c*		*g*		*a*		1AL	
Trego	*b*		*e*		*d*		*c*		*g*		*b*		Non	
TAM 111	*b*		*c*		*a*	*a*				*p*	*a*		Non	
SD98102	*b*		*b*		*d*		*c*		*f*		*c*		1AL	
Ogallala	*b*		*e*		*d*		*c*		*g*		*a*		1AL	
Guymon	*b*		*e*		*d*		*c*		*g*		*a*		Non	
Intrada	*b*		*e*		*d*			*p*	*g*		*a*		Non	
Venango	*b*				*d*	*a*			*g*		*a*		Non	
Nuplains	*b*		*e*		*d*		*c*		*g*		*c*		Non	
Thunderbolt	*b*		*b*		*a*		*c*		*g*		*c*		Non	
NuHorizon	*b*		*c*		*d*	*a*			*e*			*e*	1AL	
ZERNOKOMOVAYA 50	*b*		*c*		*d*		*e*		*g*		*a*		1AL	
TX98V9628	*b*		*b*		*d*		*e*		*g*		*b*		1AL	
TAM-400	*b*		*c*		*d*		*e*		*e*		*a*		1AL	
Ok102	*b*			*w*	*d*		*c*		*g*		*a*		Non	
Platte	*b*		*e*		*d*		*c*		*g*		*c*		Non	
Goodstreak	*b*		*c*		*d*	*a*				*i*	*a*		Non	
Tandem	*b*		*c*		*d*		*c*		*f*		*c*		Non	
Harding	*b*		*c*		*d*		*c*		*f*		*c*		Non	
Pronghorn	*a/b*		*c*		*d*		*e*		*g*		*b*		Non	
Antelope	*b*		*c*		*d*		*c*		*b*		*b*		Non	
Expedition	*b*		*c*		*d*		*e*		*g*			*d*	Non	
SD97538	*b*		*c*		*d*		*c*		*c*		*c*			1BL
Buchett	*b*		*c*		*a*		*e*		*e*		*c*		Non	
Karl 92	*a*		*b*		*d*		*c*		*f*		*c*		Non	
Deliver	*a*		*b*		*d*		*c*		*f*		*c*		Non	
Ciseo	*b*		*b*		*d*		*c*		*f*		*c*		Non	
Kalvesta	*b*		*b*		*d*		*c*		*b*		*b*		Non	
WB411W	*b*		*b*		*d*		*c*		*e*		*a*		Non	
SD97W609	*b*		*b*		*d*		*c*		*f*		*c*		Non	
W04-417	*a*		*b*		*d*		*c*		*g*		*c*		Non	
NE99495		*a/b*	*b*		*d*		*c*		*f*		*c*		Non	
TX97A0050		*a/b*	*c*		*d*		*c*		*g*		*a*		Non	
Yumar	*a*		*b*		*b*		*d*		*b*		*b*		Non	
Hatcher		*a/b*	*b*		*d*		*d*		*b*		*b*		Non	
Prowers 99	*b*		*c*		*d*		*c*		*g*		*b*		Non	
NE01643	*b*		*c*		*b*		*e*		*f*			*d*	Non	

Entry	*GluA1*				*GluB1*				*GluD1*				*GluA3*				*GluB3*								*GluD3*				*1RS*			
Hallam		*b*				*c*					*d*				*c*							*g*				*b*			Non			
Vista		*b*			*b*						*d*		*a*										*p*				*c*		Non			
Niobrara		*b*				*c*					*d*				*c*							*g*				*b*						1AL1BL
Wahoo		*b*					*e*				*d*				*c*							*g*				*b*			Non			
KS920946-B-15-1		*b*						*w*			*d*		*a*										*i*		*a*							1AL1BL
Arrowsmith	*a*						*i*				*d*				*c*							*g*				*b*			Non			
Bond CL		*b*			*b*				*b*							*d*	*b*									*b*			Non			
NW97S278				*p*		*c*					*d*		*a*									*g*					*c*			1AL		
Infinity		*b*				*c*					*d*			*e*						*f*								*d*				1AL1BL
Hondo		*b*			*b*						*d*			*e*								*g*			*a*					1AL		
Wesley		*b*			*b*						*d*				*c*							*g*					*c*		Non			
Neosho		*b*				*c*					*d*			*e*								*g*				*b*			Non			
SD KM44/TZGREV				*p*		*c*					*d*					*p*		*c*							*a*						1BL		
OK00421		*b*				*c*					*d*				*c*		*b*								*a*				Non			
Tonkawa		*b*				*c*					*d*					*p*	*b*								*a*						1BL	
Custer		*b*				*c*					*d*					*d*								*p*				*d*			1BL	
MV Mandolin		*b*					*e*				*d*				*c*			*c*										*d*			1BL	
MARISKA		*b*				*c*			*a*						*c*				*e*										*e*		1BL	
CMSW92WM00217S		*b*				*c*					*d*				*c*								*i*				*c*				1BL	
SHARK/F4105W2.1		*b*			*b*						*d*			*e*			*b*								*a*				Non			
SWM89Y074H	*a*					*c*					*d*		*a*					*c*								*b*					1BL	
CIT922411		*b*			*p*						*d*					*p*	*b*									*b*			Non			
Cossack	*a*					*c*					*d*				*c*		*b*										*c*		Non			
Longhorn		*b*				*c*					*d*			*e*			*b*								*a*						1BL	
HV9W98-143				*p*	*b*						*d*					*p*								*p*		*b*			Non			
Jagger	*a*							*i*			*d*		*e*											*p*			*c*		Non			
Overley	*a*							*i*			*d*		*a*									*g*					*c*		Non			
Santa Fe		*b*				*c*					*d*		*a*										*i*				*c*		Non			
Hayne	*a*							*i*			*d*					*d*							*i*				*c*		Non			
Postrock		*b*			*b*						*d*				*c*								*i*				*c*		Non			
Fuller					*b*						*d*				*c*							*g*	*i*					*e*	Non			
OK09314	*a*							*i*			*d*		*a*					*c*		*f*						*b*			Non			
Custer	*a*							*i*			*d*				*c*							*g*						*d*	Non			
Baker's White			*a/b*			*c*					*d*			*e*										*p*			*c*				1BL	
KS96FS-30-4		*b*				*b*					*d*			*e*								*g*						*e*	Non			
Jagalene	*a*		*a/d*					*i*			*d*					*p*						*g*					*c*		Non			
Sturdy 2K		*b*				*b*					*b*				*c*							*g*			*a*				Non			
Onaga		*b*				*c*					*d*		*a*										*i*		*a*				Non			
Ok101		*b*							*w*		*d*				*c*							*g*			*a*					1AL		
2174		*b*							*w*		*d*				*c*								*i*		*a*				Non			
TAM 302		*b*				*c*					*b*				*c*							*g*				*b*			Non			
2145	*a*				*b*						*d*				*c*							*g*			*a*				Non			
NuFrontier		*b*							*w*		*d*				*c*							*g*			*a*				Non			
Crimson		*b*				*c*				*a*					*c*					*f*							*c*		Non			
Dumas		*b*				*c*					*d*			*e*										*p*			*c*		Non			
Enhancer		*b*				*c*					*d*					*d*	*b*									*b*			Non			
Endurance		*b*							*w*		*d*				*c*								*i*		*a*						1BL	
Wendy		*b*				*c*					*d*				*c*			*c*								*b*					1BL	
OK02909C		*b*							*w*		*d*				*c*									*p*		*b*				1AL		

Table A7. Model fit tests for mixograph peak time for model with one location at a time and six glutenin loci for 96 genotypes. †

Location	ARDEC				Akron				Dailey			
Fit Statistics	**Base**	**Q**	**K**	**Q+K**	**Base**	**Q**	**K**	**Q+K**	**Base**	**Q**	**K**	**Q+K**
-2 Res Log Likelihood	327.1	317.5	298.9	294.7	388.5	376	323	315.2	463.7	447.6	451.2	439.6
AIC (smaller is better)	331.1	321.5	304.9	300.7	392.5	380	329	321.2	467.7	451.6	457.2	445.6
AICC (smaller is better)	331.2	321.6	305	300.8	392.6	380.1	329.1	321.3	467.8	451.7	457.4	445.7
BIC (smaller is better)	333.3	323.7	308.2	303.9	394.7	382.2	332.3	324.5	469.9	453.8	460.5	448.9
Effect	**ProbF‡**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**	**ProbF**
Q1§		0.0787		0.2204		0.7632		0.4764		0.3338		0.5437
Q2		0.7755		0.9065		0.3111		0.4061		0.5463		0.7823
Q3		0.4856		0.4423		0.0847		0.8136		0.8263		0.8349
Q4		0.0692		0.2766		0.6233		0.7860		0.9264		0.8813
Q5		0.2476		0.8421		0.0153		0.7459		0.3586		0.6513
Q6		0.5543		0.7992		0.9377		0.5575		0.0042		0.0660
Q7		0.0292		0.3075		0.0765		0.4216		0.7220		0.5727
Glu-A1	0.1722	0.2232	0.5272	0.4691	0.5770	0.7952	0.7930	0.8630	0.5351	0.4423	0.3383	0.3437
Glu-B1	0.0019	0.0184	0.0623	0.1148	0.0023	0.1320	0.0523	0.1768	0.0256	0.0656	0.2323	0.3173
Glu-D1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0012	0.0000	0.0000	0.0000	0.0001
Glu-A3	0.0212	0.0858	0.3064	0.3971	0.0470	0.0557	0.5226	0.6475	0.1854	0.1467	0.2574	0.2544
Glu-B3	0.0000	0.0006	0.0104	0.0505	0.0002	0.0000	0.0844	0.0538	0.0064	0.0040	0.1019	0.0612
Glu-D3	0.1247	0.1341	0.2937	0.4679	0.0533	0.1662	0.5848	0.7257	0.4770	0.1823	0.5457	0.3641

† Models tested include 'Base' model, no adjustment, 'Q' model, only adjusted to Q Matrix (k=8), 'K' model, only adjusted to kinship matrix and 'Q+K', adjust to 'Q'and 'K' matrices as proposed by Yu et al. (2006).

‡ The *P* value of *F*-test.

§ The subpopulations (Q matrix) effects.

Table A8. Weather information for Fort Collins, Dailey and Akron in January to July of 2005 and 2006. (Obtained from http://ccc.atmos.colostate.edu/~coagmet/)

Month	Location	Maximum temperature (C°)	Minimum temperature (C°)	Solar radiation (langleys)	Precipitation (cm)	Wind Speed (mile/hour)
January, 2005	Fort Collins	6.4	-8.5	184	0.015	115
	Akron				0.000	
February, 2005	Fort Collins	9.2	-6.8	255	0.010	131
	Akron	11.6	-5.8	197	0.018	213
March, 2005	Fort Collins	11.9	-4	327	0.061	180
	Akron	12.1	-4.8	281	0.028	222
April, 2005	Fort Collins	15.5	-0.4	334	0.140	183
	Akron	15.6	0.4	421	0.152	215
May, 2005	Fort Collins	20.5	4.9	359	0.183	141
	Akron	22.1	5.5	526	0.165	178
June, 2005	Fort Collins	25.7	9.6	477	0.279	140
	Akron	28.1	11.6	599	0.254	194
July, 2005	Fort Collins	32.7	12.9	551	0.023	123
	Akron	34.2	15.6	625	0.137	183
January, 2006	Fort Collins	10.6	-7.2	209	0.000	174
	Akron	11.1	-6	207	0.010	205
	Dailey				0.000	275
February, 2006	Fort Collins	6.8	-10.7	301	0.008	173
	Akron	7.7	-9.9	304	0.000	209
	Dailey				0.000	
March, 2006	Fort Collins	9.6	-5.2	365	0.043	167
	Akron	11.2	-4.9	375	0.028	210
	Dailey				0.000	
April, 2006	Fort Collins	19.3	0.4	477	0.013	206
	Akron	19.7	0.3	507	0.089	222
	Dailey	25.2	3	104	0.000	181
May, 2006	Fort Collins	23.6	6	514	0.048	162
	Akron	24.6	6.3	580	0.142	172
	Dailey	28.6	6.4	720	0.353	182
June, 2006	Fort Collins	31	12.1	560	0.020	152
	Akron	31.1	12.2	466	0.086	177
	Dailey	31.1	12.4	699	0.036	172
July, 2006	Fort Collins	31.3	14.7	465	0.048	125
	Akron	33.1	16.9	614	0.277	179
	Dailey	33.6	15.5	630	0.041	135

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