Association Mapping Reveals Effects of Allelic Variation at the Wheat *Glu-B3* Locus on Dough Properties

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Introduction

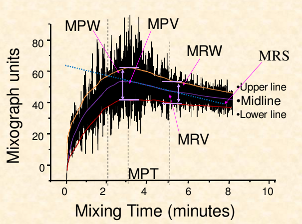
Wheat (*Triticum aestivum* L.) flour is unique because it has the ability to form a dough that can be used to produce leavened bread and other food. This ability is largely determined by its gluten proteins mainly encoded by the *Glu-1* and *Glu-3* loci (Gras et al., 2001). Due to the complexity of the *Glu-B3* locus, the effects of different alleles at this locus have not been fully understood. Association mapping is a method to associate alleles of glutenin loci with quality characteristics. However, the presence of population structure and an unequal distribution of alleles within a population can result in spurious associations (Price et al, 2006). Yu et al., (2006) suggested a mixed model to control for multiple relatedness of individuals. The effects of glutenins on quality are known to vary across environments. However, their effects on dough mixing properties has not been reported by evaluating a collection of cultivars and advanced lines in irrigated and dryland environments.

Objectives

- To evaluate the effects of allelic variation at the *Glu-B3* locus on wheat dough mixing properties using an association mapping approach.
- To determine the consistency of those effects on wheat flour mixing properties in one irrigated and two dryland field conditions in Colorado.

Materials and Methods

- Ninety-six cultivars and advanced lines mainly from the U.S. Great Plains.
- Plants were grown in two replications in an alpha-lattice incomplete block design at Fort Collins (irrigated with a sprinkler system), Dailey (dryland) and Akron (dryland), Colorado in 2005-06.
- Population structure (Q-matrix) was estimated with 60 microsatellite marker loci with the program STRUCTURE (Pritchard, 2000). The relative kinship (K-matrix) was estimated with 69 microsatellite marker loci with the program SPAGeDi (Hardy and Vekemans, 2002).
- The low-molecular-weight (LMW) glutenin subunits were extracted and resolved on SDS-PAGE according to Singh (1991) and Gupta and MacRitchie (1991). The nomenclature follows Gupta and Shepherd (1990).
- Milling followed the AACCI method 26-50 using a Brabender Quadrumat Jr experimental mill.
- Dough mixing properties were evaluated with a 10-g Mixograph connected to a computer (National Manufacturing, Lincoln, NE, USA) according to the AACCI method 54-40A. Data were analyzed with the Mixsmart®, V. 3.8. Selected mixograph properties are shown in the following mixogram.



MPT, Time to reach highest point of mixograph midline; MPW, band width (distance between the upper and lower line) at MPT; MPV, mixograph midline height at MPT; MRW, band width (the distance between the upper and lower line) at two minutes after MPT; MRV, mixograph midline height at two minutes after MPT; and MRS, the slope of the line across MPV and MRV.

- The associations of *Glu-B3* alleles and dough mixing properties were obtained with PROC MIXED of SAS (Cary, NC, USA) with the mixed model (Q+K) developed by Yu et al. (2006) .

$$y = X\beta + Q\nu + K\mu + \varepsilon$$

where, y is mixograph properties. xβ is the effect of *Glu-B3; Qv* is the effects of population structure, $K\mu$ is the polygene effects; ε is the residual effects.

Results

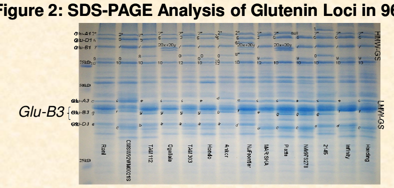
Figure 1. Plant Performance at the Maturity Stage in Three Environments

Fort Collins, CO: irrigated — Akron, CO: dryland — Dailey, CO: dryland

Table 1. Comparison of Trait Means in Three Environments

Characteristics	Fort Collins	Akron	Dailey	$LSD_{0.05}$
Yield ($kg.ha^{-1}$)	2867	1313	1440	127
Grain Fill Duration (days)	26.3	23.2	23.4	0.3
Flour protein concentration (gkg^{-1})	156.2	164.2	130.9	2.4
MPT (min)	2.88	3.31	4.56	0.2
MPW (Mu)	21.61	19.11	19.14	1
MRW(Mu)	10.81	10.46	14.98	1
MPV (Mu)	50.56	48.61	43.54	1.2
MRV (Mu)	43.06	42.21	40.82	1.1
MRS (Mu/min)	-3.8	-1.4	-3.2	0.3

Figure 2: SDS-PAGE Analysis of Glutenin Loci in 96 Cultivars and Advanced lines



Nine alleles were identified at the *Glu-B3* locus: *a, b, c, d, e, f, g, h, i*. Alleles *a, d*, and *h* were present in less than five genotypes and were pooled together in the '*p*' class for analysis.

Figure 3. STRUCTURE Group Probability for 96 Cultivars and Advanced Lines

STRUCTURE parameters: 60 SSR loci, K=8, admixture model, correlated allele frequency, 1 millions burnin times and 2 millions iterations

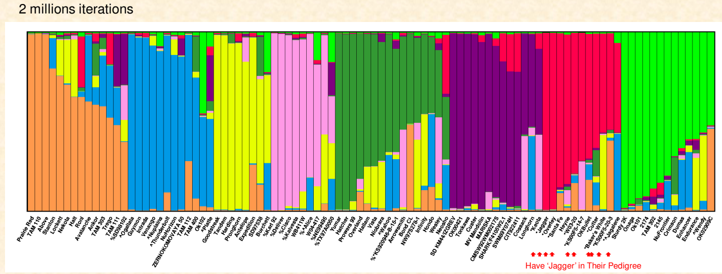


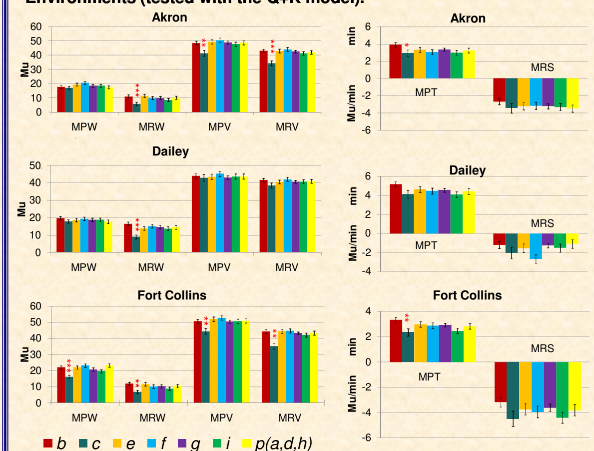
Table 2. Comparison of the Significance Levels of Associations among the *Glu-B3* Locus and Mixograph Properties Tested by Basic Model and the Q+K Model.

Location	Basic Model (not Accounting for Q+K)						Q+K Model					
	MPW	MRW	MPT	MPV	MRV	MRS	MPW	MRW	MPT	MPV	MRV	MRS
Fort Collins	***	***	***	***	***	*	***	**	**	**	***	
Dailey		***	**	*	***	*		***				
Akron	*	***	***	***	***	**		***	*	**	***	

*, **, ***: significant at P<0.05, <0.01, and <0.001, respectively.

Results

Fig 4. The Effects of *Glu-B3* Alleles on Mixograph Properties in Three Environments (tested with the Q+K model).



*, **, ***: significant at P<0.05, <0.01, and <0.001, respectively.

Conclusions

- Wheat dough mixing properties measured by mixograph were significantly affected by growing environments. Wheat from dryland location Dailey had longer MPT and more tolerance to overmixing than wheat from the other two locations.
- The presence of multiple relatedness of individuals caused possible false associations. The Q+K model was able to control for it.
- *Glu-B3c* was associated with the lowest values of mixograph properties except MRS and should be avoided in breeding materials.
- There was no significant difference among *Glu-B3b, e, f, g, i*, and combined group *p*. There was a trend that *Glu-B3b* and *f* were associated with high values of mixograph properties.
- The *Glu-D1* locus and 1RS translocation were also significantly associated with mixograph properties in this set of genotypes (data not shown).

Acknowledgements

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