

Relationship of Dough Extensibility to Dough Strength in a Spring Wheat Cross

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ABSTRACT

Cereal Chem. 83(3):255–258

A negative relationship between dough strength and dough extensibility would pose a problem for breeding hard wheats, as both dough strength and dough extensibility are desirable. We derived 77 recombinant inbred lines (RIL) from a cross between hard red spring wheat cultivars McNeal and Thatcher. McNeal produces flour with stronger dough and lower extensibility than does Thatcher. RIL were evaluated for strength-related properties using mixograph analysis and extensibility parameters using the Kieffer attachment to the TA.XT2 texture analyzer. Additionally, the RIL were test baked. Measurements using the mixograph and the Kieffer attachment were highly heritable. Maximum

dough extensibility (Ext_{max}) was negatively correlated with resistance to extension (R_{max}) ($r = -0.74$) and with mixograph tolerance ($r = -0.45$). Loaf volume was correlated with both R_{max} ($r = 0.42$) and area under the extensigraph curve ($r = 0.44$) based on partial correlation analysis adjusted for protein differences. Ext_{max} was negatively correlated with loaf volume ($r = -0.26$). The McNeal allele for polymorphism at the *Gli-B1* locus on chromosome 1BS caused high dough-mixing tolerance and low dough extensibility. Our results suggest that traditional selection criteria in hard red spring wheat, including tolerance to dough mixing and high loaf volume, may result in reduced dough extensibility.

Spring wheat production in the arid regions of the Great Plains of the United States is characterized by low yield potential and high protein. Growers depend on premium prices for high protein to augment lower production. Asian buyers are the largest users of the wheat and generally blend the high protein wheat with lower protein and less costly wheat from other sources. Besides high protein, there has been selection in the region for wheat cultivars with strong gluten, primarily to increase the value of the crop for blending with weaker gluten types. While strong gluten leads to increased mixing tolerance, it may also lead to low extensibility. Adequate extensibility is required for proper dough handling and baking performance.

Several studies have shown that breadmaking quality, especially final loaf volume, improves with higher dough strength (Campbell et al 1987; Cressey et al 1987; Branlard et al 1991). Selection programs for hard red spring wheat rely on measurements of dough strength. Mixograph measurements require small flour amounts and graphically record dough strength as resistance offered by dough to a set of mixing pins. Important mixograph parameters are the time to peak resistance and the time from peak resistance to dough breakdown, referred to as tolerance. In general, hard red spring wheat selection in the Great Plains has favored greater values for each of these measurements. However, dough that is too strong may lack extensibility and is sometimes referred to as being “bucky”, with low extensibility per unit of dough resistance to extension.

A traditional method of assessing dough extensibility is with the extensigraph. Parameters including maximum resistance (R_{max}) and maximum extensibility (Ext_{max}) at the time of dough rupture are related to dough handling properties. However, the requirement for large amounts of flour has precluded its use as a testing tool in segregating breeding populations. The alveograph also provides a measure of dough extensibility. Similar to the extensigraph, time requirements and the need for relatively large samples have precluded its general use for breeding selection in the United States. The recent development of an attachment for the TA.XT2 texture analyzer for measuring extensibility (Kieffer et al

1998; Suchy et al 2000) on smaller samples may allow breeders to devote more attention to this parameter.

Gluten storage proteins have large effects on dough-mixing and breadmaking properties. Two classes of these proteins are present in the wheat kernel, including monomeric gliadin proteins and polymeric glutenin proteins that are joined by disulfide bonds. Dissolution of these bonds reveals two classes of glutenins, referred to as high molecular weight and low molecular weight. The high molecular weight glutenins (HMW) are encoded by homoeologous loci on the long arm of wheat group 1 chromosomes. The glutenin subunits 2+12 encoded by *Glu-D1* have been found in several studies to be deleterious for dough strength (Branlard and Dardevet 1985; Lawrence et al 1988) and are generally not found in North American hard wheat cultivars selected for breadmaking. Low molecular weight (LMW) glutenins are encoded by genes on the short arm of chromosome 1 and are closely linked to gliadin loci. Recent reports demonstrate that alternative alleles of the LMW glutenin-gliadin loci can alter breadmaking traits, especially dough strength (Nieto-Taladriz et al 1993; Manifesto et al 1998; Branlard et al 2001; Ikeda et al 2003).

Hard red spring wheat has traditionally been selected for strength with less attention given to extensibility parameters. This is due to both the high correlation between strength and breadmaking parameters such as water absorption and loaf volume, and to the relative ease of measuring strength. However, both strength and extensibility are important in breadmaking (Cauvin and Young 1998; Anderssen et al 2004). In particular, Asian wheat buyers are increasingly concerned with extensibility parameters of imported wheat. Information is lacking on the genetic interplay between strength as measured on a mixograph and measurements of extensibility. This information would be useful to breeders in developing cultivars acceptable for both characteristics. In this experiment, we developed recombinant inbred lines from a cross between a hard red spring wheat cultivar with strong gluten and one with moderate strength. The lines were tested in two environments for dough strength using the mixograph, extensibility parameters using the texture analyzer, and baking quality using traditional test bake methods. Our results have implications regarding selection of wheat genotypes for breadmaking properties.

MATERIALS AND METHODS

Line Development

A cross was made between hard red spring wheat cultivars McNeal (Lanning et al 1995) and Thatcher (CI 10003) in 1999. These lines differ in dough strength as indicated by mixograph

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measurements of tolerance and mixing time, with McNeal being stronger than Thatcher. Single-seed descent was conducted from the F₂ through F₅ generation, at which time seed was bulked from a single plant per line as source material for recombinant inbred lines (RIL). The lines differed for height due to segregation at *Rht2*, and only semi-dwarf lines were included in this study. A total of 77 RIL were grown in a three-replicate trial under both irrigated and dryland conditions in Bozeman, MT, in 2004. Seed was bulked across replicates within environments to provide sufficient quantity for quality testing.

Quality Analysis

Grain harvested from field trials was analyzed using Approved Methods (AACC 2000). Whole grain protein was determined according to Approved Method 39-21 by near-infrared transmittance using a grain analyzer (Infratec 1225, Foss North America, Silver Springs, MD). A mill (Quadromat Sr., C.W. Brabender Instruments, South Hackensack, NJ) was used to obtain straight-grade flour. Wheat was tempered to 15% moisture before milling (Approved Method 26-10A). Whole wheat meal was obtained by grinding wheat through a cyclone mill (Udy Corporation, Fort Collins, CO) equipped with a 0.5-mm screen. Flour protein (14% moisture basis) was determined according to Approved Method 39-11 by near-infrared reflectance (InfraAnalyzer 400, Technicon Industrial Systems, Tarrytown, NY). Moisture was determined by Approved Method 44-15A (oven method) and results were adjusted to 14% moisture basis (Approved Method 44-01). Dough properties were measured using the mixograph (Approved Method 54-40). A standard bake test methodology was used to measure breadmaking properties (Approved Method 10-10B).

The Kieffer attachment to the TA.XT2 texture analyzer (Texture Analysis Corporation, Scarsdale NY) was used for extensibility parameters (Kieffer et al 1998). Parameters measured included R_{max} (peak force, or resistance to extension) and the distance of dough extension at which the peak force occurs (Ext_{max}). The absolute value (positive number) of Ext_{max} was used in correlation analyses that followed.

Assessment of Glutenin Genotypes

High molecular weight glutenins were assayed following the method of Payne et al (1981) except that di-thiothreitol was used as a reducing agent rather than 2-mercapto-ethanol. Additionally, several primer sets for polymerase chain reaction (PCR) previously reported to be diagnostic for LMW glutenin/gliadin genes were tested for polymorphism between McNeal and Thatcher. These included *pspl* for the *Glu-A3* locus (Devos et al 1995), *psp2* for the *Gli1-1B* locus (Devos et al 1995), and gene-specific primer sets for LMW glutenin loci on 1A, 1B, and 1D as described by Van Campenhout et al (1995). Polymorphic markers were assayed

on the entire RIL population. Marker-trait associations (r^2) were determined for polymorphic marker-trait combinations using single factor analysis of variance.

RESULTS

McNeal and Thatcher had similar flour protein percentages, but differed significantly for most other quality-related traits (Table I). Mixograph analysis showed that McNeal had greater tolerance to mixing, higher water absorption, and a longer mixing time. Extensibility data showed a greater R_{max} and lower Ext_{max} for McNeal compared with Thatcher (Table I, Fig. 1). Relative to Thatcher, McNeal had a longer bake mix time, higher water absorption, and a larger final loaf volume (Table I). RIL had significant genetic

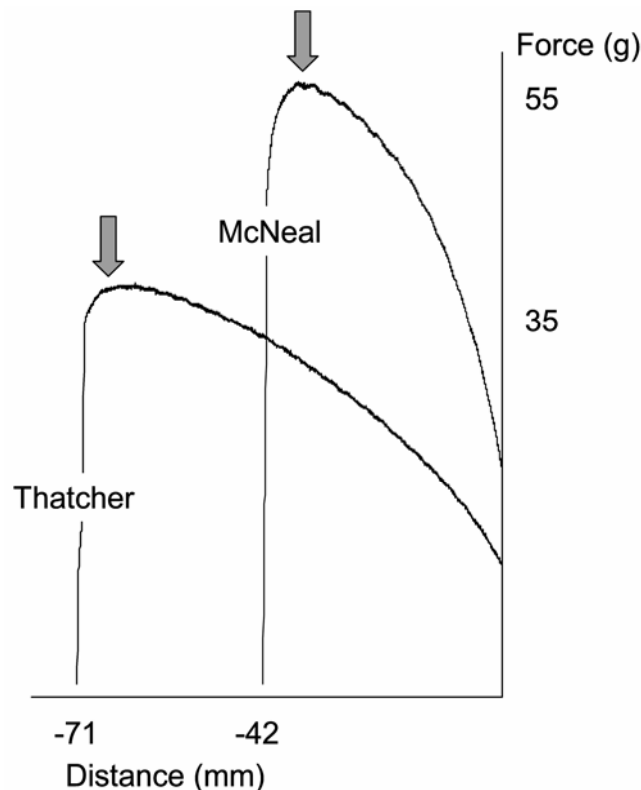


Fig. 1. Dough extensibility graphs for McNeal and Thatcher obtained using the Kieffer rig attachment to the TA.XT2 texture analyzer. Arrows represent point of maximum resistance. R_{max} is shown on the y axis and Ext_{max} is shown on the x axis. McNeal has a relatively high R_{max} and a relatively low Ext_{max}.

TABLE I
Mean Performance of Parents and Recombinant Inbred Lines for Tolerance, Extensibility, and Baking Quality Traits from Irrigated and Dryland Locations in 2004

Trait ^a	McNeal	Thatcher ^b	RIL Mean	RIL Range	Heritability
Fl. Protein (%)	11.7	11.7	12.0	10.7–13.6	0.76
SDSS (mm)	4.0	3.6	3.4	2.7–4.4	0.76
Tolerance (1–7)	5.7	4.2*	4.1	1.0–5.5	0.58
Mix Time (min)	5.3	3.4*	3.66	2.1–6.3	0.91
Mix Abs (%)	62.5	60.7*	61.3	57.8–64.3	0.46
R _{max} (g)	48.9	34.4*	38.3	27.1–53.4	0.82
Ext _{max} (mm)	39.9	55.2*	44.4	27.5–63.8	0.67
Area (mm ²)	1,778	1,668*	1,497	1,216–1,876	0.54
Bake Time (min)	7.5	4.3*	5.1	2.4–12.5	0.92
Bake Abs (%)	74.3	70.8*	71.4	67.7–74.3	0.69
Loaf Volume (cm ³)	1,097	1,063	1,072	1,227–967	0.64

^a Fl. Protein, flour protein; SDSS, SDS sedimentation; Mix Time, mixograph peak time; Mix Abs, mixograph water absorption; Bake Abs, bake water absorption; R_{max}, resistance at peak extensibility; Ext_{max}, maximum dough extension.

^b *, indicates values for McNeal and Thatcher are significantly different ($P < 0.05$) based on LSD.

variation ($P < 0.05$) for all traits presented in Table I and all heritability values were >0.46 . These values may represent upper bounds for heritability because no estimate for genotype-by-environment interaction variation was obtained. However, these results show a strong genetic component to all of the quality measurements.

Correlations among the extensibility traits were calculated from the means of the two environments (data not shown). Means over environments were used because the genotype-by-environment interaction was not important. As expected, R_{\max} (resistance to extension) was negatively correlated with Ext_{\max} ($r = -0.74$). Area under the curve was positively correlated with R_{\max} ($r = 0.56$) but not correlated with other variables.

Table II presents correlations of extensibility measurements with other traits related to breadmaking. R_{\max} was positively correlated with mixograph tolerance ($r = 0.63$). This shows that genotypes producing dough with greater resistance to extension also maintained strength during mixing. R_{\max} was also correlated with mixograph mix time, bake mix time, and bake water absorption. Ext_{\max} was negatively correlated with these same variables. Of the extensibility parameters, only area under the curve (a function of both R_{\max} and Ext_{\max}) showed a significant positive correlation with final loaf volume.

A significant positive correlation existed between flour protein and R_{\max} , while a negative correlation existed between flour protein and Ext_{\max} . This suggested that protein level may confound interpretation of the correlation between extensibility traits and other variables. Thus, a partial correlation analysis was conducted to account for protein differences (Table II). The partial correlation measures the correlation between two variables, while holding the effect of a third variable (protein) constant (Steel et al 1997). In general, simple correlation and partial correlation values were similar. However, correlations of all three extensibility-related measurements (R_{\max} , Ext_{\max} , and Area) to loaf volume were

greater and reached statistical significance ($P < 0.05$) in the partial correlation analysis. R_{\max} and Area were both positively correlated with loaf volume ($r = 0.42$ and 0.44 , respectively), while Ext_{\max} was slightly negatively correlated ($r = -0.26$).

Storage protein alleles varied between McNeal and Thatcher. McNeal contained HMW glutenin subunits 1 and 17+18 for *Glu-A1* and *Glu-B1*, respectively. Thatcher contained subunits 2 and 7+9 at the respective loci. Both cultivars contained the 5+10 subunits at *Glu-D1*. The cultivars also differed for the LMW glutenin/gliadin alleles based on marker polymorphism with microsatellite *psp1* for *Glu-A3*, and microsatellite *psp2* for *Gli-1B*. Markers for the *Glu-1D* locus were not polymorphic. The population of 77 RIL was screened for HMW subunit composition by SDS-PAGE and with the two polymorphic microsatellite markers using PCR. Single-factor analysis of variance analysis measured the proportion of variance for each trait attributed to the marker polymorphism. The HMW glutenin proteins and the *Glu-A3* locus revealed by primer set *psp1* did not predict variation among the RIL for any of the quality traits (data not shown). Polymorphism at the *Gli-1B* locus (as revealed by *psp2*) was predictive of differences among the RIL for R_{\max} ($r^2 = 0.27$), mixograph tolerance ($r^2 = 0.17$), Ext_{\max} ($r^2 = 0.08$), and mixograph mix time ($r^2 = 0.20$) (Table III). This result indicates that a significant portion of the variation for these key traits is controlled by the *Gli-1B* locus or a linked locus. The *Gli1* locus is closely linked to the *Glu3* on chromosome 1B (Manifesto et al 1998). This polymorphism had no predictive value for RIL loaf volume.

DISCUSSION

Wheat genotypes vary for most dough-handling and breadmaking properties. Several microscale techniques are employed by breeders to evaluate lines for properties that may influence final quality. One commonly used tool is the mixograph, which measures the resistance of the dough to mixing after addition of water to flour. Key measurements include the time required for the dough to reach optimal resistance, the amount of water needed to achieve optimal resistance, and the time that dough will remain at peak resistance before breakdown (tolerance). High water absorption is a desirable property, and long tolerance allows a long window of opportunity for breadmaking. An extended time to peak mixing is not desirable as this is an added cost to baking. Much of the hard red wheat crop is exported to Asia, where it is blended to add strength to weaker classes of wheat. Thus, strong gluten with long tolerance is generally a desirable trait in hard red spring wheat. High tolerance and high water absorption are generally associated with a high final loaf volume (Campbell et al 1987; Cressey et al 1987; Branlard et al 1991).

In addition to strength, breadmaking requires dough that is extensible, allowing ease of handling and the rising of bread to form a large loaf (Anderssen et al 2004). Intuitively, extensibility may be expected to be negatively associated with strength; that is, resistance to mixing may equate to difficulty in extending the dough. This presents a challenge, in that selection for tolerance as measured by the mixograph may equate to selection for poor extensibility. Genetic analyses of the relationship between these traits are lacking.

The parental lines chosen for this study are different for strength and extensibility parameters, and these differences are reflected in the range of values observed in progeny lines (Table I). Heritability estimates for the microscale quality measurements obtained by the mixograph and the texture analyzer suggest a large genetic component to the trait variance. These results are similar to previous reports (Martin et al 2001; O'Brien and Ronalds 2003). Effectiveness for the end user requires that traits selected using small-scale tests be related to baking performance. Our results (not shown) showed that mixograph tolerance was significantly correlated with bake water absorption ($r = 0.38$).

TABLE II
Pearson Correlation of R_{\max} , Ext_{\max} , and Area with Mixograph and Test-Bake Traits in a Set of 77 Spring Wheat RIL Grown in Two Environments^a

Trait ^b	R_{\max}	Ext_{\max}	Area
Fl. Protein	-0.26	0.30	0.07
SDSS	0.42 (0.42)	-0.22 (-0.21)	0.07 (0.50)
Tolerance	0.63 (0.61)	-0.45 (-0.41)	0.43 (0.46)
Mix time	0.85 (0.83)	-0.61 (-0.57)	0.53 (0.57)
Mix abs	0.06 (0.48)	0.13 (-0.18)	0.30 (0.40)
Bake time	0.85 (0.84)	-0.56 (-0.52)	0.58 (0.61)
Bake Abs	0.51 (0.71)	-0.24 (-0.43)	0.52 (0.54)
Loaf Volume	0.09 (0.42)	0.04 (-0.26)	0.35 (0.44)

^a Values >0.22 are significant ($P < 0.05$). Partial correlations adjusted for protein variation are in parentheses.

^b Fl. Protein, flour protein; SDSS, SDS sedimentation; Mix Time, mixograph peak time; Mix Abs, mixograph water absorption; Bake Abs, bake water absorption; R_{\max} , resistance at peak extensibility; Ext_{\max} , maximum dough extension.

TABLE III
Mean Performance of RIL from a McNeal-by-Thatcher Cross Containing Alternative Alleles for Microsatellite Marker *psp2* at the *Gli1* Locus on Chromosome 1B^{a-c}

	R_{\max}	Ext_{\max}	Tolerance	Mix Time	LV
Population mean	38.3	44.4	4.1	3.7	1,072
McNeal allele	42.4	42.1	4.4	4.0	1,081
Thatcher allele	34.2	46.4	3.8	3.3	1,065
r^2	0.27**	0.08*	0.17**	0.20**	0.02

^a R_{\max} , resistance at peak extensibility; Ext_{\max} , maximum dough extension; Mix Time, mixograph peak time; LV, loaf volume.

^b *, ** indicates significance at $P < 0.05$ and 0.05 , respectively.

^c Proportion of variance explained by allele differences at the *psp2* locus.

R_{\max} as measured by the texture analyzer also was correlated with bake water absorption ($r = 0.51$), while Ext_{\max} was negatively correlated with this trait ($r = -0.24$) (Table II). None of these measurements were correlated with final loaf volume. However, area under the extensibility curve (Area) was positively correlated with loaf volume ($r = 0.35$). These correlations were much improved after adjustment for protein differences among lines (Table II) and, in fact, area appears to be a good predictor of final loaf volume.

Market demands dictate that hard red spring wheat breeding programs develop cultivars with both good tolerance to mixing and with good extensibility. Breeding programs have relied heavily on measures of strength as indicated by the mixograph to select cultivars with strong gluten. The potential exists that selection for strength may negatively impact extensibility. In this study, we found a significant negative correlation between tolerance and Ext_{\max} ($r = -0.45$). In addition, marker trait analysis further indicates that variation at the *Glu-3/Gli-1* complex on chromosome 1B assayed by PCR marker *psp2* has a positive effect on strength and a negative effect on extensibility (Table III). Thus, it appears that the same locus that confers tolerance to mixing also causes low extensibility. Breeders may wish to avoid using the McNeal allele if both strength and extensibility are desired. The result of using the Thatcher allele is likely to be somewhat lower dough strength coupled with greater dough extensibility. Area under the extensibility curve incorporates both resistance to extension and extensibility into a single measurement, and is correlated with final loaf volume. While it may not be possible to completely break the negative relationship between strength and extensibility observed in this study, Area may provide a valuable measure to breeders in joint selection for optimal bread quality.

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[Received November 7, 2005. Accepted February 8, 2006.]