RESEARCH

Sources of Variation for Long-Flow Experimental Milling

E.J. Souza,* C. Griffey, M. Kweon, and M. Guttieri

ABSTRACT

Flour milling quality of new wheat cultivars routinely is measured on long-flow experimental flour mills with multiple break and reduction rolls that produce many mill streams. Virginia State wheat trials were evaluated to determine the relative contributions of genotypes, production years, and error to the variation in long-flow milling traits using the Allis-Chalmers long-flow flour mill at the USDA Soft Wheat Quality Laboratory. In one study, 11 cultivars were evaluated for five years. Variation due to error, which was based on the cultivar × year interactions, was half the size of genotypic variance for straight-grade and break-flour yield. In the second study, 27 cultivars were evaluated for two years. Genotype was a more important source of variance for milling traits than error. Error variances were approximately one-half of the genotype variances for straight-grade flour yield and break-flour yield. One paired evaluation between an experimental line and a standard cultivar may be sufficient to detect large differences in milling performance between an experimental and a check cultivar. However other milling and flour characteristics tended to have smaller ratios of genotype to error variance, suggesting that greater levels of replication may be required than for straight-grade flour yield.

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Abbreviations: ESI, endosperm separation index; SRC, solvent retention capacity; SWQL, Soft Wheat Quality Laboratory.

Flour milling yield is an important measure of quality for soft-wheat cultivars (*Triticum aestivum* L.). Flour milling can be assessed using simple short-flow experimental mills with single pairs of rollers or by more elaborate long-flow experimental mills that closely approximate commercial flour yields (Yamazaki and Andrews, 1982). Long-flow experimental mills have multiple break and reduction rolls with sophisticated sifting procedures. Therefore, long-flow mills can quantify extended characteristics of the milling process for cultivars, including break-flour yields, cumulative ash curves, the efficiency of separation of endosperm from the bran, and the reduction of particle size during flour milling.

Experimental flour milling quality is controlled by the genetics of the cultivars that compose the sample, the characteristics of the environments in which grain was grown, and the interaction of the genetic factors with the environment (Baenziger et al., 1985; Bassett et al., 1989). Measurements of the percent of variation in milling yield due to genotype across all types of wheat, hard and soft, demonstrate that the majority of the variation often

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is attributed to genetic factors (Souza et al., 2004). Variation in milling yield and flour characteristics, such as damaged starch, can be directly related to a single locus, *Ha*, which codes for the puroindoline proteins and controls the physical hardness of wheat kernels (Hogg et al., 2005). Additional loci have minor affects on milling yield but are difficult to detect without correcting for the overwhelming effects of the *Ha* grain hardness locus on chromosome arm 5DS (Breseghello et al., 2005). Rye translocations within soft wheat, which are present on the Group 1 chromosomes of wheat, are among the loci that appear to affect milling yield (McKendry et al., 2001). In one study with near-isogenic lines, the 1AL.1RS translocation, on average, decreased milling yield, and the 1BL.1RS translocation, on average, reduced break-flour yield as estimated using a modified Quadrumat Junior flour mill (C.W. Brabender Instrument Inc., South Hackensack, NJ). However the effect of the rye translocations was dependent on background and could be mitigated by other genetic effects (McKendry et al., 2001). Among soft wheat genotypes, the relative contribution to variation in milling due to genotype compared to environment is less than observed when both hard and soft genotypes are included (Baenziger et al., 1985; Bassett et al., 1989). Depending on the genetic background, Guttieri and Souza (2003) found that genotype contributed 32 to 95% of the total variation in flour yield and 61 to 81% of break-flour yield, after blocking for year effects.

Detailed estimations of sources of variation in flour milling have used short-flow experimental mills (Baenziger et al., 1985; Bassett et al., 1989, Guttieri and Souza, 2003; Souza et al., 2004). The performance of wheat samples on short-flow flour mills generally is considered predictive of performance on long-flow experimental mills and commercial mills (Finney and Andrews, 1986). The correlation r values commonly exceed 0.5 between short-flow and long-flow mills. Using multiple measurements of flour quality from a short-flow, modified Quadrumat Junior mill, Gaines et al. (2000) were able to predict flour yield on a long-flow Allis-Chalmers mill with a R^2 of approximately 90%. Based on this relationship and the strong influence of genotype on short-flow experimental milling, variation in flour yield from long-flow experimental mills should have large genetic contributions that explain the majority of sample-to-sample variation.

Location and year, however, repeatedly have been shown to influence variation in milling quality (Baenziger et al., 1985, Bassett et al., 1989, Gaines et al., 1996; Guttieri and Souza, 2003; Souza et al., 2004). Disease and pre-harvest sprouting are common sources of variation in milling quality between locations and years. Even excess nitrogen fertilization can reduce milling quality of soft-wheat cultivars (Johnson et al., 1999; Guttieri et al., 2006). The USDA-ARS Soft Wheat Quality Laboratory at Wooster, Ohio routinely uses separation into various fractions based on kernel size followed by aspiration to remove diseased or shriveled kernels. This procedure minimizes the effects of environment on milling quality by using primarily sound, well-formed kernels for long-flow milling procedures. Consequently, long-flow milling performance on the Allis-Chalmers mill routinely is presented as a measure of genotypic milling quality without adjustment for location or year. These milling evaluations routinely are used by the Eastern U.S. milling industry for selection of cultivars for end-use studies.

Long-flow milling, which is used in public research laboratories, is valued by the milling and baking industry for predicting cultivar milling quality. However, it is time consuming, and often only a single long-flow evaluation is presented for forums such as industry panel reviews before cultivar release. Consequently, it is important to measure the environment, genotype, and genotype × environment interaction as sources for variation for long-flow flour milling characteristics, including both total flour yield and the break-flour and reduction milling characteristics that can be difficult to assess with short-flow mills. Our goal was to estimate the sources of variation for long-flow milling quality as a means of predicting the reliability of long-flow milling for assessing genotypic milling quality using only one to several milling evaluations. This information will be useful for wheat breeders and members of quality councils that interpret the results of long-flow mill studies as part of the final stages of cultivar release. We investigated a set of soft red winter wheat cultivars produced in various locations in Virginia over a five-year and two-year period that were milled on a long-flow Allis-Chalmers mill.

MATERIALS AND METHODS

Wheat Samples

Wheat grain samples of soft red winter wheat were produced in replicated, randomized field plots (4.2 m^2) as part of Virginia Tech's statewide testing program of new cultivars and elite prerelease lines. Grain samples harvested from Painter, VA (2002 and 2003), Blacksburg, VA (2004), and Warsaw, VA (2005 and 2006) having no obvious pre-harvest sprouting present or grain diseases such as Fusarium head blight (causal organism *Fusarium graminearum* Schwabe) were submitted for quality analyses. In 2005, initial grain inspection and production conditions supported an absence of pre-harvest sprouting in the samples. However, additional inspection of the grain indicated that some sprouting had occurred in the 2005 nursery. Grain samples were cleaned after harvest to remove residual chaff and non-threshed heads and spikelets. A single composite grain sample derived from four replicated plots of each genotype in each year was submitted for quality evaluations. Genotypes included both released cultivars adapted to Virginia and experimental lines from the Virginia Tech program. For simplicity, the genotypes in this study will all be referred to collectively as cultivars.

At the USDA-ARS Soft Wheat Quality Laboratory (SWQL) grain samples were sized over two or three round-holed aluminum screens (aperture size: 3.6 mm, 3.4 mm, and

3.2 mm) on the modified Carter Day dockage tester. The selection of screens depended on the estimated kernel size. The screens were used to produce either three or four fractions. Individual size fractions were aspirated to remove shriveled seed and non-seed material. The aspirated size fractions then were recombined to form milling stock.

Sample Milling

Kernel weight was determined on each cleaned sample and grain volume weight measured. Following grain measurements, samples were tempered to 15% moisture. Tempered grain was milled on the SWQL Allis-Chalmers flour mill using the AACC method 26–32 as modified by Yamazaki and Andrews (1982). The Allis-Chalmers mill is a long flow experimental milling system with adjustable roll gaps. Grain is initially milled with six break-roll passes then reduced in seven reduction-roll passes to produce straight-grade flour. The roll settings, sifting screen sizes, and mill flow were as diagramed in Yamazaki and Andrews (1982).

For each grain sample, straight-grade flour yield (hereafter referred to as flour yield) and break-flour yield were recorded. Endosperm separation index (ESI) was calculated as described by Yamazaki and Andrews (1982). ESI is the estimated endosperm adhering to bran and bran pieces after the third through fifth break passes and first reduction pass, expressed as a percentage of the weight of milled grain. Lower ESI values indicate better bran separation from endosperm and better milling quality than higher ESI values. Friability (Gaines et al., 2000) estimates the ease with which mill stock is reduced to flour. Friability is calculated by dividing the weight of flour recovered during milling by the summed weight of mill stock passed through all roll stands, break and reduction, after the first break. The earlier in the break and reduction process that flour is recovered, the lower the weight of mill stock that passes to the later break and reduction rolls. Higher values of friability indicate better milling efficiency and reduced energy requirements to recover flour.

Flour samples were evaluated for baking quality in soft-wheat products. Each sample was evaluated using the sugar-snap cookie method (AACC, 2000; Method 10–52). Lactic acid solvent retention capacity (SRC) test was used to measure gluten strength of the flour samples (AACC, 2000; Method 56–11).

Twenty-seven cultivars included in the two-year study were classified for the presence of the rye translocations 1AL.1RS and 1BL.1RS. DNA was extracted from the milling fraction "shorts" using DNEasy Mini Kit (Qiagen, Hilden, Germany). Rye fragments were amplified using primers developed by Saal and Wricke (1999). The primer sequences amplified a micro-satellite on the 1RS arm, locus *XScm9* using the sense and antisense primers 5′-TGACAACCCCCTTTC-CCTCGT-3′ and 5′-TCATCGACGCTAAGGAGGACCC-3′, respectively. The sense primer was modified with a 5′ M13 tail (5′-CACGACGTTGTAAAACGAC-3′). A third primer, (5′-CACGACGTTGTAAAACGAC-3′), the M13 tail labeled 5′ with an infrared dye WellRed D4-PA (Sigma Proligo, The Woodlands, TX) was added to the reaction. Amplification products were visualized on a Beckman CEQ8800 capillary electrophoresis instrument (Beckman Coulter, Fullerton, CA). Amplification products of 244 bp were diagnostic for 1AL.1RS translocation and 227 bp were diagnostic for the 1BL.1RS translocation. The genotype for the rye translocations were compared against published reports for the cultivars in USDA regional nurseries (R.A. Graybosch, personal communication, 2008 http://www.ars.usda.gov/Research/docs.htm?docid=11932), pedigree information, and disease reaction. The consensus genotype for each cultivar is presented with the discussion of the two-year study.

Statistical Analysis

Cultivars were divided into two groups for statistical analysis. The first analysis had a larger sampling of years, and the second analysis had a larger sampling of cultivars. Both types of analyses were included to improve the estimations of variances of traits across years and genotypes. Eleven cultivars were included in the analysis of results from 2002 to 2006. The second group used data from 2005 and 2006, but included a larger set of 27 cultivars.

In both analyses, years and genotypes were considered to be random variables. Although cultivars were selected before entry into these trials, they were not necessarily selected on the basis of soft-wheat quality. Cultivars selected for Allis quality testing represented the predominantly grown cultivars, or elite breeding lines, having desirable grain yield and disease resistance characteristics. Therefore, we considered them to be valid random estimators of the variances of traits in the larger pool of elite soft-wheat germplasm. Expected mean square terms for sources of variation were estimated as previously described (Baenziger et al., 1985; Guttieri et al., 2003; Guttieri and Souza, 2003). The expected mean square term for genotype is: $\sigma_e^2 + y\sigma_g^2$. The expected mean square for year is $\sigma_e^2 + g\sigma_y^2$. In these estimates, σ_e^2 is the residual variance (error), σ_g^2 is the variance for genotypes, σ_y^2 is the variance for years, and g and y are the number of cultivars (genotypes) and years, respectively.

All statistical analyses were calculated using PROC GLM, Type III sums of squares, within the Statistical Analysis System software (SAS, Raleigh, NC). This analysis focused on estimation of variances. Means were generated for individual years and cultivars to assist in the discussion of the range in variation. In 2004, lactic acid was not recorded on three of the cultivars. Means were corrected for missing values using the least squares estimate of means in PROC GLM. Analysis of effect of the rye translocation was measured using a model of random year effects, fixed genotype effects (three genotypic classes: no translocations, 1AL.1RS, and 1BL.1RS), and an error term for genotypic effects of cultivars nested within genotypic classes. The two cultivars containing mixed genotypes for the translocation events were excluded from the analysis. A total of 25 cultivars were included in the test of effects of the rye translocation.

RESULTS

Five-Year Study

In the first analysis of 11 soft wheat cultivars milled from 2002 to 2006, years were a significant source of variance for all measured traits (Table 1). Endosperm separation index and friability had the smallest *F*-values for year effects within this analysis ($p < 0.01$); all other traits had year effects significant at $p < 0.001$ (Table 1).

Table 1. Analysis of variance of soft red winter wheat grain and flour characteristics from Virginia, 2002 to 2006, milled on Allis Chalmer flour mill, Soft Wheat Quality Laboratory, Wooster OH.

Trait	Type III mean squares			Variances from EMS†		Variance ratios‡	
	Year	Genotype	Error	Year	Genotype	σ_y^2/σ_g^2	σ_e^2/σ_g^2
Degrees of Freedom	4	10	40	–	–	–	–
Grain wt. vol.	3303***	473***	24	298.1	89.8	3.3	0.3
Kernel wt.	117***	43***	1.5	10.5	8.3	1.3	0.2
Break-flour yield	1718***	2328***	143	143	437	0.3	0.3
Straight-grade flour yield	72**	246***	17	5.0	45.8	0.1	0.4
Endosperm separation index	1.85**	3.44***	0.34	0.14	0.62	0.2	0.5
Friability	0.93**	3.19***	0.18	0.07	0.60	0.1	0.3
Flour protein	455***	64***	14	40.1	10.0	4.0	1.4
Flour ash	0.389***	0.269***	0.016	0.03	0.05	0.7	0.3
Cookie diameter	1.798***	0.114*	0.045	0.16	0.01	17.1	6.0
Lactic acid SRC	62100***	41300***	1529	5515	7952	0.7	0.2

*F-ratio significant at $P < 0.05$.

**F-ratio significant at $P < 0.01$.

***F-ratio significant at $P < 0.001$.

†Variances for sources derived from expected mean square terms.

‡σ^2_g = Estimated variance for genotype, σ^2_y = estimated variance for year, σ^2_e = estimated variance for error.

Year variances for grain volume weights, kernel weights, and cookie diameter were all two-fold or more the size of the error variances. These traits were much more sensitive to year-to-year variation than the milling traits of the Allis-Chalmers mill. As an example, kernel weight varied from an average of 35.3 mg in 2004 to approximately 24% larger in 2006 at 43.7 mg (Table 2). In contrast, flour yield varied 6 mg from the lowest year in 2006 (771 g kg^{-1}) to the highest flour yield in 2003 (777 g kg^{-1}). Pre-harvest sprouting reduces sugar-snap cookie diameter. The presence of pre-harvest sprouting in some samples in 2005 may have elevated the variance attributed to year. However, removing 2005 from the analysis did not appreciably change the cookie diameter variance estimate for years (0.107 without 2005, 0.118 with 2005). This suggests that the pre-harvest sprouting in this year was relatively minor and did not significantly distort the variances beyond the other factors modulating cookie diameter. Across years, as grain weight-volume increased, ESI decreased in value ($r = -0.91$, $p < 0.01$), generally considered to indicate improved milling quality. Kernel weight also was negatively correlated to flour ash ($r = -0.97$, $p < 0.01$). No other correlations were observed among the year means.

Significant cultivar variation was observed for all traits (Table 1). In all but the case of cookie diameter, cultivar effects were significant at the $p < 0.001$ level. Cookie diameter had the smallest F-value for cultivar variance and was significant at $p < 0.05$. Among the flour evaluations, lactic acid SRC had the largest genetic component with a ratio of error to genotype of only 0.2 (Table 1). The values reported in this manuscript were unadjusted for flour protein as protein was not a significant covariate in the analysis of variance after fitting year and cultivar (data not shown). Flour protein and cookie diameter had very large ratios of error variance to cultivar variance, because the calculated cultivar variances for these two traits approached zero (Table 1). These were the least reliable traits measured for cultivars. The problems of the two traits are intertwined. Flour protein is correlated to cookie diameter ($r = -0.62$, $p < 0.001$). If flour protein is used as a covariate in the analysis of variance for cookie diameter, the F-test significance for genotypes improved from the $p < 0.05$ level to the $p < 0.01$ level.

Table 2. Means of soft red winter wheat grain and flour characteristics from Virginia, 2002 to 2006, milled on Allis-Chalmer flour mill, Soft Wheat Quality Laboratory, Wooster OH.

Year	Grain weight volume	Kernel weight	Break flour yield	Straight-grade flour yield	Endosperm separation index	Friability	Flour protein	Flour ash	Cookie diameter	Lactic acid SRC
	kg m^{-3}	mg	g kg^{-1}	g kg^{-1}	Score	Score	g kg^{-1}	g kg^{-1}	cm	g kg^{-1}
2002	812	38.7	292	775	9.5	28.8	80.4	4.17	17.8	886
2003	808	36.4	297	777	9.8	28.4	98.3	4.18	16.9	1027
2004	796	35.3	317	772	10.0	28.3	91.6	4.28	17.2	1038
2005	843	39.1	285	775	8.9	29.0	91.4	4.02	16.8	1026
2006	815	43.7	288	771	9.7	28.5	90.1	3.80	17.1	1088
Standard error†	2	0.4	3	1	0.2	0.1	1.1	0.04	0.06	13†

†Standard error for years varies between years due to missing values for 3 cultivars 2004. The largest standard error (2004) is reported.

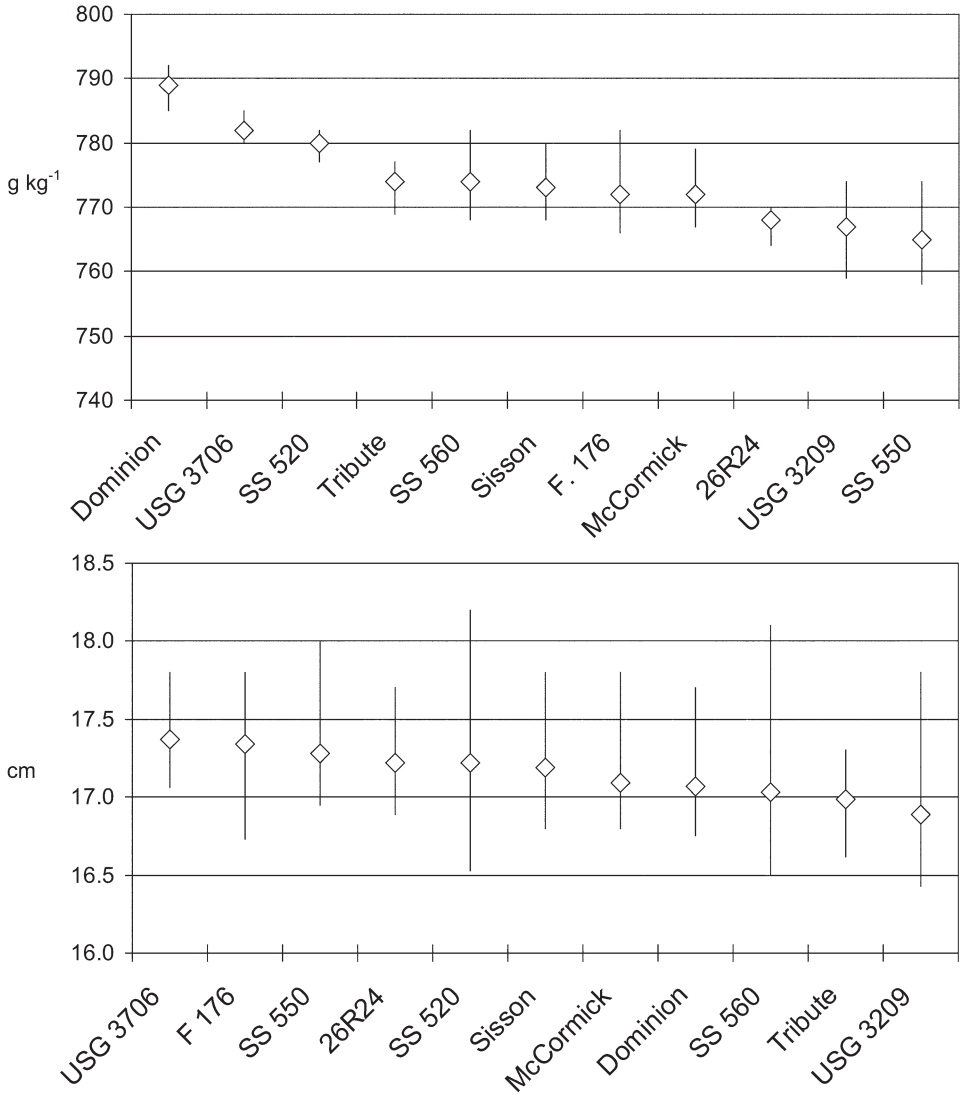

Figure 1. Comparison of two soft wheat quality traits that differ for ratios of error variance to genetic variance, 11 soft red winter wheat cultivars, grown in Virginia, 2002 to 2006. Cultivars are sorted from highest to lowest by average value for the quality trait (◊) with the range in values for quality trait marked by the lines on either side of the mean (◊). Figure 1a. Straight-grade flour yield, a quality trait with a small ratio of error variance to genetic variance. Figure 1b. Sugar-snap cookie diameter, a quality trait with a large ratio of error variance to genetic variance.

The ratio of error variance to genotype variance is difficult to capture graphically; however, plotting means and ranges of traits approximates the contrast between traits like straight-grade flour yield and cookie diameter (Fig. 1). Both traits have significant genetic effects. However, the year and genotype × year interaction are so small that the range in yield for the three best-milling wheat cultivars does overlap with the three poorest-milling wheat cultivars (Fig. 1a). In contrast, the ranges in cookie diameters for all eleven cultivars overlap with each other (Fig. 1b). In this case, only by blocking for year and using multiple observations in time and space can the best lines be distinguished from the poorest lines.

'Sisson' is adapted to the mid-Atlantic region of the eastern U.S. and is a soft wheat with known quality that is generally acceptable to the milling and baking industry (Griffey et al., 2003). None of the cultivars in this study had values more than two standard errors different from Sisson for all four of the milling traits: break-flour yield, flour yield, ESI, and friability (Table 3). This is due in part to a negative correlation between break-flour yield and straight-grade yield for the average values of the cultivars ($r = -0.70$, $p < 0.05$, $n = 11$). Few of the cultivars with the best milling yield also had good break-flour yield. 'SS 520' had the best overall milling quality with break-flour yield similar to Sisson, yet flour yield and friability values more than two standard errors greater than Sisson, and ESI more than two standard errors less than Sisson (Table 3). No other cultivars matched SS 520 with three or more milling traits that were more than two standard errors better than Sisson.

In this study, cultivar means for grain weight-volumes and kernel weights were not correlated to any of the milling characteristics ($p > 0.05$). Cultivars such as SS 520 had good milling characteristics and low grain volume weight while conversely 'USG 3209' had poor milling characteristics and relatively good grain volume weight (Table 3).

Two-Year Study

The two-year study included 27 cultivars, some of which were included in the five-year study. In comparing the analyses of variance for the two studies (Tables 1, 4), years were significant sources of variation in the five-year study for break-flour yield and flour protein, but were not significant sources of variation in the two-year study. This suggests that two years at one location per year may be insufficient to fully estimate the variation of these two traits in flour quality studies. Other traits measured in these two studies had similar sized mean square terms and F-test levels of significance.

Lactic acid SRC, as in the five-year study, had a small ratio of error to genotype (0.1), confirming that the trait has a large genetic component to variation that can be measured with limited locations of testing. In the two-year study, protein was a significant covariate ($p < 0.01$) for lactic acid. Lactic acid increased 7 g kg^{-1} for each 1 g kg^{-1} increase in flour protein. A similar value is used within the SWQL to adjust lactic acid values for flour protein concentration (Table

Table 3. Means of soft red winter wheat grain and flour characteristics from Virginia, 2002 to 2006, milled on Allis Chalmer flour mill, Soft Wheat Quality Laboratory, Wooster OH.

Year	Grain weight volume	Kernel weight	Break-flour yield	Straight-grade flour yield	Endosperm separation index	Friability	Flour protein	Flour ash	Cookie dia.	Lactic acid SRC
	kg m^{-3}	mg	g kg^{-1}	g kg^{-1}	score	Score	g kg^{-1}	g kg^{-1}	cm	g kg^{-1}
'Dominion'	816	35.5	260	789	8.30	29.12	95.0	4.16	17.07	1064
'Featherstone 176'	816	40.8	278	772	9.76	28.62	90.3	3.96	17.34	1077
'McCormick'	829	35.2	314	772	9.50	28.56	95.4	4.28	17.09	1064
'Pioneer 26R24'	817	39.4	330	768	9.62	28.54	88.3	4.18	17.22	1075
'Sisson'	816	39.2	301	773	9.90	28.98	88.5	4.00	17.19	848
'SS 520'	799	41.2	300	780	8.56	29.68	85.5	4.08	17.22	1024
'SS 550'	808	38.5	318	765	10.58	28.28	87.1	4.00	17.28	860
'SS 560'	802	35.7	310	774	9.78	28.08	90.2	4.06	17.03	950
'Tribute'	831	39.7	279	774	9.76	28.34	88.4	4.24	16.99	1067
'USG 3209'	814	44.4	291	767	11.00	26.66	89.0	4.48	16.89	973
'USG 3706'	815	35.6	273	782	8.60	29.38	96.2	3.56	17.37	1142
Standard error	2.2	0.5	5	2	0.26	0.19	1.7	0.06	0.09	20

4). However, despite the greater number of cultivars in the two-year study, the ratio of error variance to cultivar variance was 0.7 for flour protein and 1.1 for cookie diameter. This confirms that, even with a larger sample of cultivars, these variances for error remains large relative to the cultivar variances. Variance ratios for error to cultivar terms are fractions for all other traits. In general, these ratios are smaller in the two-year study with 27 cultivars as compared to the five-year study with 11 cultivars (Tables 1, 4). This suggests that 11 cultivars may be too small a pool to fully measure the true variance in milling and baking traits among elite soft red genotypes. The cultivar variance in the two-year study is inflated relative to the five-year study by the cultivar × year variance that is not partitioned from cultivar effects in the shorter duration study (Hallauer and Miranda Fo, 1981). In the five-year study, cultivar × environment variance includes both cultivar × year and cultivar × location interactions, whereas, in the two-year study only cultivar × year interaction is included as all grain samples were produced in adjoining fields at Warsaw, Virginia in 2005 and 2006.

The negative correlation among cultivars for break-flour yield and straight-grade flour yield found in the five-year study was not observed in the two-year study ($r = -0.35$, $p > 0.05$). As a result, it was possible to identify cultivars better than Sisson (more than two standard errors) for all milling traits (Table 5). These were the experimental lines VA01W-205, VA03W-235, and VA03W-409. As in the five-year study, in the two-year study SS 520 also was similar to Sisson for break-flour yield and better than Sisson for the milling traits. Milling traits measured in the

Table 4. Analysis of variance of soft red winter wheat grain and flour characteristics from Virginia, 2005 to 2006, milled on Allis Chalmers flour mill, Soft Wheat Quality Laboratory, Wooster OH.

	Type III mean squares			Variances from EMS†		Variance ratios‡	
Trait	Year	Genotype	Error	Year	Genotype	σ_y^2/σ_g^2	σ_e^2/σ_g^2
Degrees of Freedom	1	26	26	–	–	–	–
Grain wt. vol.	9245***	265***	16	342	125	2.7	0.1
Kernel wt.	301***	27.1***	1.3	11	13	0.9	0.1
Break-flour yield	34.2	984***	70	0§	457	0.0	0.2
Straight-grade flour yield	137**	148***	12	5	68	0.1	0.2
Endosperm separation index	4.51***	2.05***	0.13	0.16	0.96	0.2	0.1
Friability	2.08***	1.37***	0.10	0.07	0.64	0.1	0.2
Flour protein	2.2	26.3***	6.7	0§	9.80	0.0	0.7
Flour ash	0.712***	0.078***	0.011	0.03	0.03	0.8	0.3
Cookie diameter	1.167***	0.171**	0.060	0.04	0.06	0.7	1.1
Lactic acid SRC	732300***	27700***	1205	27079	13227	2.0	0.1

**F-ratio significant at $P < 0.01$.

***F-ratio significant at $P < 0.001$.

†Variances for sources derived from expected mean square terms.

§Not defined. The expected mean square term is less than zero and the ratio also is not defined. Both are assumed to be zero.

Table 5. Means of soft red winter wheat grain and flour characteristics from Virginia, 2005 to 2006, milled on Allis Chalmer flour mill, Soft Wheat Quality Laboratory, Wooster OH.

Year	Rye trans-location	Grain weight volume	Kernel weight	Break-flour yield	Straight-grade flour yield	Endosperm separation index	Friability	Flour protein	Flour ash	Cookie dia.	Lactic acid SRC
		kg m^{-3}	mg	g kg^{-1}	G kg^{-1}	score	score	g kg^{-1}	g kg^{-1}	cm	g kg^{-1}
'Chesapeake'	1BL.1RS	834	49.4	312	759	10.7	27.6	92.4	4.05	16.85	930
'Dominion'	1AL.1RS	828	38.0	262	787	7.9	29.3	93.1	4.00	16.93	1083
'Featherstone 176'	None	831	44.0	264	770	9.4	28.5	90.8	3.75	17.15	1128
'Jamestown'	None	839	39.1	285	765	10.3	28.3	93.9	3.95	16.75	1061
'McCormick'	1AL.1RS	844	37.5	305	771	9.7	28.7	96.1	4.00	16.93	1113
'Pioneer 26R15'	None	814	41.8	313	773	8.4	29.4	91.8	3.85	17.17	1168
'Pioneer 26R24'	None	831	42.1	321	767	9.4	28.9	88.3	3.95	17.10	1136
'Pioneer 26R31'	1AL.1RS	824	44.6	257	789	8.1	29.4	89.2	3.90	16.96	948
'Renwood 3260'	None	831	36.1	287	772	8.7	29.3	99.9	3.65	16.53	1278
'Sisson'	1BL.1RS	825	41.1	297	769	10.1	28.7	85.3	3.90	17.04	874
'SS MPV-57'	1BL.1RS	812	42.6	286	782	7.8	29.6	89.8	4.10	17.12	844
'SS 520'	None	815	44.8	288	781	8.2	29.8	87.8	4.00	16.65	1067
'SS 550'	1BL.1RS	820	40.0	319	762	10.6	28.1	86.7	3.80	17.04	907
'SS 560'	None	816	38.8	298	776	9.2	28.7	91.0	3.80	16.83	1030
'SS 8404'	1BL.1RS	852	42.9	298	781	8.3	29.6	90.0	4.20	17.76	860
'Tribute'	1AL.1RS	848	42.1	272	772	9.45	28.8	90.6	4.05	16.82	1095
'USG 3209'	1BL.1RS	833	48.3	275	768	10.2	27.1	90.4	4.30	16.58	993
'USG 3706'	None	832	38.6	255	783	8.4	29.4	98.4	3.45	17.19	1202
'USG 3555'	1BL.1RS	819	46.5	293	761	10.3	27.2	88.9	4.25	16.82	1031
'V 9412'	None	831	40.8	276	767	10.7	28.1	89.0	3.70	17.07	1100
VA01W-205	None	836	43.1	327	775	9.0	29.9	86.1	3.75	17.48	1065
VA02W-398	None	810	43.6	306	783	8.0	30.5	88.5	3.95	17.15	1101
VA02W-713	Mixed	846	41.5	306	761	10.8	28.1	86.7	3.55	17.22	920
VA03W-235	1BL.1RS	830	46.9	330	781	8.2	29.4	91.1	3.90	17.53	889
VA03W-409	1BL.1RS	807	45.0	326	778	8.9	29.4	85.5	4.05	17.51	822
VA03W-412	Mixed	839	43.9	303	770	9.4	29.4	88.1	3.90	17.08	965
VA03W-434	1BL.1RS	818	33.7	321	760	10.7	28.4	87.7	3.85	17.26	977
Standard error		3	0.8	6	2	0.3	0.2	1.8	0.08	0.18	24

five-year study were strongly correlated ($r > 0.93$) with the same traits measured in the two-year study of the same 11 cultivars. The ability to determine relative milling value of cultivars did not change greatly with the additional three years of evaluation. However, the precision of the measure did increase as indicated by a decrease in the size of the standard error for all four milling traits by about half.

The effect on milling quality due to rye translocations on the short arm of chromosome 1A and chromosome 1B was not significant within the two-year study (Table 5). All milling traits were similar between the non-translocation genotypes and translocation genotypes ($p > 0.1$) and similar between the 1A translocation and the 1B translocation genotypes ($p > 0.1$). The rye translocations cultivars had a greater flour ash concentration in this study. Ash concentration was 0.18 g kg^{-1} greater in 1AL.1RS translocation flours than in non-translocation line flours ($p < 0.05$), and the 1BL.1RS translocation genotypes produced flours with 0.24 g kg^{-1} greater ash than flours of non-translocation genotypes ($p < 0.01$). As is often measured in gluten strength studies, the 1BL.1RS translocation genotypes had weaker gluten strength (as measured in this study by lactic acid SRC) than the non-translocation genotypes. In this study the difference was 180 g kg^{-1} greater lactic acid SRC for the 1BL.1RS translocation than the non-translocation ($p < 0.001$) genotypes. No significant differences were observed for lactic acid SRC between the 1AL.1RS genotypes and the non-translocation genotypes.

DISCUSSION

The Allis-Chalmers mill for evaluation of wheat cultivars provides a prediction of cultivar performance in commercial milling. Because of the effort required for long-flow milling, it often is used after initial selection and characterization of wheat cultivars with simpler short-flow milling procedures involving one to several milling passes (Souza et al., 2002). In many cases, only one or several experimental

milling determinations are made for a cultivar using long-flow experimental milling before the cultivar is adopted by wheat growers. The SWQL presents the results of the long-flow milling annually in research reviews and quality councils. Comparisons of cultivars often are made using an approximate standard error based on pooling the variance of cultivar that is inflated upward by including variances for year, location, cultivar with environment interaction, and error variances. This is a valid error estimate; however, the significant year effects for all traits in the five-year study confirms that added precision can be gained by limiting comparison only to check cultivars grown in the same environment and year as the new cultivar.

In this study, we were not able to measure cultivar × year effects or cultivar × location effects as separate from error effects. However the relative size of cultivar variances to error variances for milling traits was similar in the two-year study to the pooled variance of cultivar × year and error effects found by Guttieri and Souza (2003). Cultivar × year effects were small, less than 10% of total variation for flour yield, when they were partitioned by Guttieri and Souza (2003). Based on the pooled error and cultivar x year effect in this study, no more than two years of evaluations within a region, such as Virginia, are needed to rate a cultivar's flour yield and break-flour yield relative to a known check, if sound samples are evaluated. A number of other studies have found significant cultivar × environment interactions for milling quality (Baenziger et al., 1985; Bassett et al., 1989; Gaines et al., 1996; Guttieri and Souza, 2003; Souza et al., 2004). The cultivar × year interaction was used as the error term in this study, which is not strictly the same as a cultivar × environment interaction since multiple environments were not used within a year. Baenziger et al. (1985) found that for micro-milling flour yield, within one year, across the eastern U.S., the ratio of cultivar × environment variance to cultivar variance was 0.24. That is about half the ratio for flour yield of cultivar × year variance to cultivar variance found in the five-year study for straight-grade flour yield. This suggests that the cultivar × environment effect for the Allis-Chalmers milling may not be large and is probably not much larger than the cultivar × year interactions found in this study.

Several flour traits also were measured in this study. Flour protein variation among cultivars was relatively small compared to year and error cultivar × year effects. This was consistent with the findings of Baenziger et al. (1985), where environment variance for grain protein was 16 times larger than cultivar variance, and the environment × cultivar variance was as large as the variance for cultivars. A number of studies have found significant genetic variation for flour protein concentration and cookie diameter (Guttieri et al., 2003; Souza et al., 2004; Breseghello et al., 2005). Yet, flour protein concentration is strongly influenced by a range of environmental effects that modulate the grain yield per area and the soil nitrogen fertility (Souza et al., 2004; Guttieri et al., 2006). Flour protein can be used in some cases as a covariate to reduce genotype × environment interaction for cookie diameter that may be caused by large environmental differences in flour protein concentration.

Although previous studies (McKendry et al., 2001) found some reduction in milling yield because of the presence of rye translocations, their conclusion was that it could be at least partially offset by other genetic factors. The ability to breed combinations of high milling yield and rye translocations is consistent with previous findings (Johnson et al., 1999) and with the results of this study. In the two-year study, we found no consistent differences in milling for rye translocation groups. This suggests that the effect on milling from the rye translocation may be readily masked during normal breeding program selection or be of such small consequence that the differences would not be detected in a two-year study of this design. The elevation in flour ash due to the1BL.1RS translocation may cause concern for flour quality, if ash concentration is included as a flour specification. However the greater flour ash concentration in the 1BL1RS genotypes seem unlikely to be related to poor milling yield since we measured no differences in flour milling yield due to translocations. The greater flour ash concentration in the 1BL.1RS translocation more than likely is caused by changes in whole grain ash. This may derive from changes in root architecture or stress tolerance that subsequently affect mineral uptake (Ehdaie et al., 2003).

The relatively large effect of cultivar on the differences in milling performance on long-flow mills supports the use of this type of milling evaluation in industry quality conference evaluations before release of new cultivars. The significant year effects for all milling traits confirms that the long-flow mill evaluations need to compare experimental lines to known checks so that the milling yield of the new line can be appropriately interpreted. The error of these evaluations due to cultivar × environment interaction and experimental error can be relatively small for milling traits, yet quite large for flour evaluations such as cookie bakes. This suggests that supplemental information, such as previous baking evaluations during the cultivar selection, may be needed to confirm genetic characteristics of the new cultivar once it reaches the stage of long-flow milling evaluation.

References

AACC. 2000. American Association of Cereal Chemists. Approved methods of the AACC, 10th ed. The Association, St. Paul, MN.

Baenziger, P.S., R.L. Clements, M.S. McIntosh, W.T. Yamazaki, T.M. Starling, D.J. Sammons, and J.W. Johnson. 1985. Effect of cultivar, environment, and their interaction and stability analyses on milling and baking quality of soft red winter wheat. Crop Sci. 25:5–8.

Bassett, L.M., R.E. Allan, and G.L. Rubenthaler. 1989. Genotype × environment interactions on soft white winter wheat quality. Agron. J. 81:955–960.

Breseghello, F., P.L. Finney, C. Gaines, L. Andrews, J. Tanaka, G. Penner, and M.E. Sorrells. 2005. Genetic loci related to kernel quality differences between a soft and a hard wheat cultivar. Crop Sci. 45:1685–1695.

Ehdaie, B., R.W. Whitkus, and J.G. Waines. 2003. Root biomass, water-use efficiency, and performance of wheat-rye translocations of chromosomes 1 and 2 in spring bread wheat 'Pavon'. Crop Sci. 43:710–717.

Finney, P.L., and L.C. Andrews. 1986. A 30 minute conditioning method for micro-, intermediate-, and large-scale experimental milling of soft red winter wheat. Cereal Chem. 63:18–21.

Gaines, C.S., P.L. Finney, and L.C. Andrews. 2000. Developing agreement between very short flow and longer flow test wheat mills. Cereal Sci. 77:187–192.

Gaines, C.S., P.L. Finney, and G. Rubenthaler. 1996. Milling and baking qualities of some wheats developed for Eastern or Northwestern regions of the United States and grown in both locations. Cereal Chem. 73:521–525.

Griffey, C.A., W.L. Rohrer, T.H. Pridgen, W.S. Brooks, M.E. Vaughn, W.L. Sisson, A.M. Price, D.E. Brann, and A.E. Smid. 2003. Registration of Sisson wheat. Crop Sci. 43:1134–1135.

Guttieri, M.J., R. McLean, S.P. Lanning, L.E. Talbert, and E. Souza. 2003. Assessing environmental influences on solvent retention capacities of two soft white spring wheat cultivars. Cereal Chem. 79:880–884.

Guttieri, M.J., K. O'Brien, C. Becker, J.C. Stark, J.M. Windes, and E. Souza. 2006. Managing nitrogen fertility of irrigated soft white spring wheats for optimum quality. Can. J. Plant Sci. 86:459–464.

Guttieri, M.J., and E. Souza. 2003. Sources of variation in the solvent retention capacity test of wheat flour. Crop Sci. 43:1628–1633.

Hallauer, A.R., and J.B. Miranda Fo. 1981. Quantitative genetics in maize breeding. University of Iowa Press. Ames, IA.

Hogg, A.C., B. Beecher, J.M. Martin, F. Meyer, L. Talbert, S. Lanning, and M.J. Giroux. 2005. Hard wheat milling and bread baking traits affected by the seed-specific overexpression of puroindolines. Crop Sci. 45:871–878.

Johnson, J.M., C.A. Griffey, and C.H. Harris. 1999. Comparative effects of 1BL/1RS translocation in relation to protein composition and milling and baking quality of soft red winter wheat. Cereal Chem. 76:467–472.

McKendry, A.L., D.N. Tague, and K. Ross. 2001. Comparative effects of 1BL.1RS and 1AL.1RS on soft red winter wheat milling and baking quality. Crop Sci. 41:712–720.

Saal, B., and G. Wricke. 1999. Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42:964–972.

Souza, E., R.A. Graybosch, and M.J. Guttieri. 2002. Breeding for improved milling and baking quality in wheat—a review. J. of Crop Prod. 5:39–74.

Souza, E.J., J.M. Martin, M.J. Guttieri, K. O'Brien, D.K. Habernicht, S.P. Lanning, G.R. Carlson, and L.E. Talbert. 2004. Influence of genotype, environment, and nitrogen management on spring wheat quality. Crop Sci. 44:425–432.

Yamazaki, W.T., and L.C. Andrews. 1982. Experimental milling of soft wheat cultivars and breeding lines. Cereal Chem. 59:41–45.