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2008

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Using the Solvent Retention Capacity Test When Breeding Wheat for Diverse Production Environments

Carl Walker, Kimberly Garland Campbell, Brady Carter, and Kimberlee Kidwell*

ABSTRACT

The solvent retention capacity (SRC) test is used to predict commercial baking performance of soft wheat (*Triticum aestivum* L.) by measuring the capacity of flour to retain each of four solvents—water, Na_2CO_3 , sucrose, and lactic acid—to assess overall absorption capacity, starch damage, pentosan and gliadin content, and glutenin quality, respectively. Our objectives were to determine sources of variation in the test, repeatability, and optimum scale and resource allocation needed to maximize efficiency. Duplicate SRC tests were conducted for each solvent using two flour sample sizes (5 and 0.2 g) from two field replications of each of 8 soft white spring and 16 soft white winter genotypes grown in five and three environments, respectively. We conducted ANOVAs and used variance components to assess the consistency with which genotypic differences were detected. The interactions of genotype $\times$ environment and genotype $\times$ field replication within environment were significant ($P < 0.05$) for most solvent and sample weight combinations. Repeatability values were high and consistent for all solvents (0.86–0.96) when 5-g samples were used, indicating that selection based on any solvent should result in gains from selection at this scale. Only lactic acid and sucrose were accurately predictive at the 0.2-g scale, limiting its utility. Repeatability values improved with increased numbers of environments, field replications, or laboratory replications; however, this may be cost prohibitive when evaluating early-generation breeding material on a large scale, especially since the magnitude of increase in predictability diminished with each additional unit.

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Abbreviations: AACC, American Association of Cereal Chemists; E, environment; F(E), field replication within environment; G, genotype; L[G $\times$ F(E)], lab replication within the interaction of genotype and field replication within environment; SRC, solvent retention capacity.

THE SOLVENT RETENTION CAPACITY (SRC) test (AACC, 2000, Method 56-11), which is used to predict commercial baking performance of soft wheat (*Triticum aestivum* L.) flours (Gaines, 2000), was adapted from the alkaline water retention capacity test (AACC, 2000, Method 56-10) by Slade and Levine (1994). The test is conducted using a set of four solvents—water, 5% Na_2CO_3 solution, 5% lactic acid solution, and 50% sucrose solution—to assess overall absorption capacity, starch damage, glutenin quality, and pentosan and gliadin content, respectively, of wheat flour (Gaines, 2000). Solvent retention capacity results are reported as percentages of the mass of flour gel after exposure to the solvent divided by the original flour weight. Results are compared with those of standard flours as a means of predicting flour quality.

The SRC test can be used as a cultivar enhancement tool for selecting soft wheat genotypes with acceptable product-making potential and to characterize the basis of superior soft wheat quality (Guttieri et al., 2001). Soft wheat flours with low water retention are considered to have superior quality, since decreased

Published in Crop Sci. 48:495–506 (2008).

doi: 10.2135/cropsci2007.06.0348

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baking times are required for cookie and cracker production, resulting in more tender products and decreased manufacturing costs (Slade and Levine, 1994). In multiple research studies, significant, negative correlations between SRC values and cookie diameter were detected (Gaines, 2000; Guttieri and Souza, 2003; Guttieri et al., 2001, 2002). The magnitude of associations varied among studies and solvents, with significant correlations for lactic acid ranging from -0.33 to -0.65 , Na_2CO_3 from -0.55 to -0.86 , sucrose from -0.71 to -0.78 , and water from non-significant to -0.88 . These results demonstrate the ability of the test to consistently identify genotypes with superior baking performance.

The SRC test, as conducted using Method 56-11 of the American Association of Cereal Chemists (AACC, 2000), is limited to applications where a total of 20 g of flour (5 g per solvent) is available, which is often not the case in early generations of advancement when the grain of experimental breeding genotypes is limited (Bettge et al., 2002). Bettge et al. (2002) evaluated modifications to AACC Method 56-11, using mechanical agitation, 1 g of flour, 1 g of whole meal, and 0.2 g of whole meal to scale down the test for use in early-generation selection. Scale reduction, as well as the use of whole meal, reduced the magnitude of correlations between the results of the modified methods and the original 5-g flour scale, indicating that the small-scale tests may be less accurate at predicting flour quality than the full-scale tests. The 0.2-g whole-meal method had sufficient resolution, however, to be useful for selecting among experimental breeding lines since genotype rankings based on highest and lowest values were consistent. In early stages of the breeding process, the goal is to eliminate poor genotypes and to advance the best genotypes. For making gains from selection, consistently identifying genotypes at the extremes of the distribution is more important than discerning differences in the midrange when selecting among early-generation material. Using 0.2 g of flour instead of 0.2 g of whole meal may improve the correlation between the results of the small-scale test and the results of the 5-g method since the obscuring effect associated with the inclusion of bran is avoided.

For the SRC test to be suitable for use as a selection tool for wheat improvement, it must detect significant differences among genotypes (G) and genotype $\times$ environment (G $\times$ E) interactions must be nonsignificant or too small to interfere with selection. In several studies, SRC values were significantly influenced by differences among genotypes, and genotype accounted for a majority of the variation, indicating that the SRC test can be used to detect significant differences among genotypes (Guttieri and Souza, 2003; Guttieri et al., 2001, 2002).

Limited research has been conducted to determine the extent of the impact of G $\times$ E interactions on SRC test results. All previously reports indicated that the G $\times$ E effect was nonsignificant or too small to interfere with

genotype selection. Two of the studies included grain grown in Idaho with irrigation (Guttieri and Souza, 2003; Guttieri et al., 2001), and the third included irrigated and rain-fed samples from Idaho and Montana (Guttieri et al., 2002). It is unclear how well the results of previous studies apply to other wheat production regions with a broader range of environmental diversity, such as those found in eastern Washington. Soft wheat was planted on approximately 722,367 ha in Washington in 2006 (Messer and Bilderback, 2006) and a majority of this wheat was grown without irrigation in areas receiving from 250 to 600 mm of average annual precipitation (Peterson et al., 2001). Evaluating SRC test results across these environments is required to validate its utility as a selection tool for soft wheat cultivars targeted for commercial production in soft wheat production regions with widely varying precipitation ranges.

Previous research evaluated the effectiveness of the SRC test as a selection tool using the method of comparing genotype and the interaction of genotype and environment in terms of significance levels based on analysis of variance (Guttieri et al., 2001, 2002) or variance components (Guttieri and Souza, 2003). Repeatability is another statistic used to evaluate the effectiveness of a testing method and is similar to the comparison of standardized variances. Both of these methods allow the determination of the degree to which significant G $\times$ E and other interactions involving genotype reduce the effectiveness of genotypic selection. The advantage of repeatability calculations is that the relative interference of interactions on genotype selection can be compared. Repeatability was used to evaluate the effectiveness of selection methods for traits such as fusarium head blight resistance (Campbell and Lipps, 1998) and starch concentration (Hucl and Chibbar, 1996) in wheat. Campbell and Lipps (1998) also evaluated the allocation of resources, in terms of numbers of environments and replication, to optimize selection.

The objectives of this research were (i) to evaluate the sources of variation for SRC values when tests were conducted on soft white wheat cultivars grown in distinct production regions in the state of Washington, (ii) to evaluate the repeatability of the 5- and 0.2-g flour versions of the SRC test, and (iii) to determine the optimum allocation of resources to maximize the efficacy of selecting for end-use quality enhancement based on SRC results. Repeatability was used to compare the utility of the 5- and 0.2-g flour versions of the SRC test to distinguish among genotypes for each solvent.

MATERIALS AND METHODS

Sample Material

In 2005, grain of eight soft white spring wheat genotypes, including one soft white club, was collected from field trials in Pullman, Reardan, Dayton, St. John, and Lind, WA, and from

16 soft white winter genotypes, including five soft white clubs, grown at Dusty, Pullman, and Colton, WA (Table 1). These genotypes included named cultivars and advanced breeding lines that were grown in Washington State University Extension uniform cereal variety testing trials (<http://variety.wsu.edu>; verified 6 Jan. 2008).

These locations encompassed the range of average annual precipitation levels for dryland wheat production in the state of Washington, including 230 mm (Lind), 280 to 380 mm (Dusty), 400 to 450 mm (Dayton, Reardan, and St. John), and 500 to 600 mm (Pullman and Colton). Monthly averages of daily maximum and minimum temperatures, along with precipitation levels for the September 2004 through August 2005 crop year are listed in Table 2. Data are from National Weather Service cooperators. Planting conditions, soil type, fertility management, and agronomic data for each location can be found at <http://variety.wsu.edu/>.

Grain samples were collected from two field replications per genotype per location, and samples were tempered to a target moisture of 14% by tumbling for 20 min followed by overnight storage in glass jars before milling (AACC, 2000, Method 26-10). The samples were milled using a modified Brabender Quadramat milling system (Jeffers and Rubenthaler, 1979). Flour moisture was determined by AACC Method 44-16 before and following completion of the SRC tests. The SRC results for four flours from the winter sample set were not included due to accidental mixing during sample collection and milling.

Data Collection

Solvent retention capacity evaluations were conducted using two scales: 5 and 0.2 g of flour. Five-gram tests were conducted as described by Bettge et al. (2002), using mechanical agitation. The 0.2-g tests were conducted as described by Bettge et al. (2002) for the 0.2-g wheat meal SRC, with the modification of replacing the wheat meal with flour. Four solvents were used: water, 5% (w/w) Na_2CO_3 solution, 5% (w/w) lactic acid solution, and 50% (w/w) sucrose solution. The SRC tests were conducted in batches of 18 and 28 for the 5- and 0.2-g scales, respectively. Two lab replications per flour sample were randomly assigned to batches, and each batch included two samples of a standard cookie flour as controls for identifying batches in which operator error impacted results.

Batches were repeated when control values deviated from the mean by ± 2 standard deviations. Individual samples within batches for which a major operator error occurred, such as the gel falling out of the tube during drainage, were repeated in the final batch for each solvent. The SRC results were reported as a gel weight percentage relative to flour weight, on a 14% moisture basis (AACC Method 56-11). Moisture levels of flour samples decreased nonuniformly by up to 1% during the time required to conduct all of the SRC tests. Flour moistures determined before conducting the SRC tests were used to calculate SRC values when reporting the results of the 0.2-g scale tests, whereas flour moistures determined after conducting the SRC tests were used to report the results from the 5-g scale.

Statistical Analyses

Pearson's linear correlation coefficients among SRC values were calculated using flour sample means (PROC CORR, SAS Institute, 2006). Analysis of variance was conducted on SRC data using the SAS GLM procedure (SAS Institute, 2006). Separate ANOVA calculations were conducted for each testing scale–growth habit–solvent combination using the following model:

$$\text{SRC}_{\text{gefl}} = \mu \dots + G_g + E_e + F_{f(e)} + \text{GE}_{g \times e} + \text{GF}_{g \times f(e)} + L_{[l \times g \times f(e)]}$$

in which SRC_{gefl} is the SRC data point, $\mu \dots$ is the grand mean of the sample set, G_g is the genotype ($g = 1, \dots, 8$ or 16), E_e is the environment ($e = 1, \dots, 3$ or 5), $F_{f(e)}$ is the field replication ($f = 1$ or 2) within environment e , $\text{GE}_{g \times e}$ is the interaction between genotype g and environment e , $\text{GF}_{g \times f(e)}$ is the interaction between genotype g and field replication f within environment e , and $L_{[l \times g \times f(e)]}$ is the laboratory replication ($l = 1$ or 2) within genotype g , field replication f , and environment e . All factors were considered

Table 1. Descriptions of soft white wheat genotypes evaluated using two scales of the solvent retention capacity test.

Sample set [†]	Germplasm name or number	Market class [‡]	Reference no. or pedigree
Spring	'Alturas'	common	CV-950 [§]
Spring	'Eden'	club	CV-953
Spring	'Fielder'	common	CV-554
Spring	'Louise'	common	CV-987
Spring	'Nick'	common	PVP no. 200500144 [¶]
Spring	WA7952	common	Strelinskaja Mestnaja (unavailable [#])/4* Centennial (CV-760)//Wakanz sib (unavailable)//Wadual sib (unavailable)
Spring	WA7964	common	Sprite (unavailable)//Wakanz (unavailable)/Treasure (CV-731)
Spring	'Zak'	common	CV-914
Winter	'Bruehl'	club	CV-912
Winter	'Brundage96'	common	CV-929
Winter	'Coda'	club	CV-874
Winter	'Dune'	common	Norman (unavailable)/VHO 88262 (unavailable)//Lambert (CV-803)
Winter	'Edwin'	club	CV-882
Winter	'Eltan'	common	CV-766
Winter	'Finch'	common	CV-966
Winter	'Hiller'	club	CV-871
Winter	ID587CF	common	CV-990
Winter	'Madsen'	common	CV-746
Winter	'Masami'	common	CV-977
Winter	'ORCF-101'	common	PVP no. 200300286
Winter	'Rely'	club	CV-777
Winter	'Simon'	common	PVP no. 200500001
Winter	'Stephens'	common	CV-614
Winter	WA7935	common	Madsen (CV746)/3* Eltan (CV766)

[†]Set of flour samples from spring or winter genotypes grown in the same environments.

[‡]Federal Grain Inspection Service classification based on kernel characteristics.

[§]CV = Crop Science registration number.

[¶]PVP = Plant Variety Protection number.

[#]Unavailable indicates that no reference number was available for this parental line.

random since the goal was to predict the performance of the SRC test when used to evaluate any soft white wheat cultivar grown in any production region in eastern Washington.

Homogeneity of the environmental variances was tested using Levene's test (Levene, 1960) in the HOVTEST option of the SAS GLM procedure (SAS Institute, 2006). When environmental variances were heterogeneous, SRC results from each environment were weighted by the reciprocal of the mean square error within each environment (Yates and Cochran, 1938). Variance components and their standard errors were calculated using restricted maximum likelihood with the SAS MIXED procedure (SAS Institute, 2006). The relative values of variance components were determined by the estimated value expressed as a proportion of the total variation. The repeatability of genotype means of values generated from the test was calculated using variance components in the following equation:

$$\text{Repeatability} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g \times e}^2}{e} + \frac{\sigma_{g \times f(e)}^2}{fe} + \frac{\sigma_{l[g \times f(e)]}^2}{lfe}} \quad [1]$$

where σ_g^2 is the genotypic variance component, $\sigma_{g \times e}^2$ is the G×E variance component, $\sigma_{g \times f(e)}^2$ is the variance component of the interaction of G and F(E), $\sigma_{l[g \times f(e)]}^2$ is the variance component for lab error or lab within G×F(E), e is the number of environments, f is the number of replications within the field, and l is the number of lab replications for each sample.

A repeatability value of 1 indicates that the test values are only influenced by genotype; therefore, these values reflect consistent genotypic differences. Increasing values of genotype interaction variance components ($\sigma_{g \times e}^2$, $\sigma_{g \times f(e)}^2$, and $\sigma_{l[g \times f(e)]}^2$) results in lower repeatability values since these terms serve as part of the denominator in the repeatability calculation. Lower repeatability values indicate less consistent performance, and

indicate that genotype performance differs among environments, field replications, or lab replications. Repeatability values can be compared among tests or replication conditions, and higher repeatability values indicate greater gains from selection. To predict the effect of varying the number of test environments, field replications, or lab replications on repeatability, the observed variance components for each data set were entered into Eq. [1] with a range of values for e , f , and l , in a manner similar to that described by Campbell and Lipps (1998). This allowed the comparison of the predicted repeatability values for each SRC testing scale and solvent combination at varying levels of resource allocation. Increasing values for e , f , and l results in higher repeatability values by an amount dependent on variance component values.

RESULTS

The standard deviations of the 0.2-g SRC values for the control flour were 3.69, 1.53, 2.44, and 2.11 for 5% lactic acid, 50% sucrose, 5% Na_2CO_3 , and water, respectively. The standard deviations for the 5-g controls were 3.33, 0.93, 0.74, and 1.03 for lactic acid, sucrose, Na_2CO_3 , and water, respectively. Correlations between results from the 0.2- and 5-g scales of the SRC test across sample sets using 5% lactic acid, 50% sucrose, 5% Na_2CO_3 , and water were 0.99, 0.81, 0.75, and 0.60 respectively, all of which were highly significant ($P < 0.001$). Correlations between test results within each sample set exhibited similar trends (data not shown).

Table 2. Monthly average of daily maximum and minimum recorded temperatures and total precipitation levels for the September 2004 through August 2005 crop year, by location in the state of Washington. Data are from the National Weather Service and cooperators.

Date	Statistic	Lind	Pullman [†]	St. John	Dayton (Pomeroy) [‡]	Dusty (Lacrosse)	Rearden (Davenport)
Temperature, °C							
Sept. 2004	max.	23.4	22.2	22.3	22.3	22.5	20.6
	min.	6.9	6.9	6.4	8.1	7.1	5.3
Oct. 2004	max.	18.3	16.0	17.5	16.3	17.8	16.3
	min.	3.9	3.4	3.1	3.9	3.4	1.6
Nov. 2004	max.	9.2	7.9	7.9	8.3	9.7	6.4
	min.	-0.4	-0.1	-1.1	-1.6	-0.7	-3.2
Dec. 2004	max.	4.6	5.4	5.1	6.5	5.6	2.1
	min.	-2.2	-1.9	-2.5	-1.7	-2.4	-3.1
Jan. 2005	max.	3.6	4.6	4.6	5.4	4.9	0.0
	min.	-4.6	-3.0	-4.9	-3.5	-3.8	-7.1
Feb. 2005	max.	9.5	8.8	9.4	9.4	9.9	6.9
	min.	-4.1	-4.3	-5.5	-5.2	-5.9	-6.1
Mar. 2005	max.	14.4	12.4	13.3	13.2	16.8	11.2
	min.	0.1	-0.1	-0.9	0.4	-1.6	-2.2
Apr. 2005	max.	17.7	14.8	16.2	15.5	17.3	14.9
	min.	2.9	1.6	2.2	3.3	2.5	0.6
May 2005	max.	22.6	19.4	20.7	19.7	22.4	19.4
	min.	7.3	6.7	6.7	7.3	7.0	6.1
June 2005	max.	26.9	20.7	23.5	22.0	26.1	21.2
	min.	8.8	7.6	8.3	8.9	8.6	6.8
July 2005	max.	32.6	28.9	30.5	30.0	33.7	28.3
	min.	12.1	10.6	10.4	12.6	11.0	9.7
Aug. 2005	max.	31.7	29.9	30.6	30.2	33.8	28.8
	min.	11.6	8.7	9.1	10.3	11.2	8.3
Rainfall, mm							
2004–2005		147.6	390.1	395.5	294.4	246.6	265.4
Snowfall, mm							
2004–2005		292.1	304.8	538.5	185.4	154.9	599.4

[†]The National Weather Service cooperator station with complete data that is nearest to Colton is the Pullman station.

[‡]Data are from the National Weather Service cooperator station nearest to each location and are listed in parentheses.

Analysis of Variance

Significance levels of factors influencing SRC values differed among solvents, test scale, and sample sets (Table 3). When SRC tests were conducted at the 0.2-g scale using 5% lactic acid, all factors were significant ($P < 0.01$) sources of variation for results from the spring sample set (Table 3). When tested at the 5-g scale, results were similar, except that the effect of F(E) was not significant ($P > 0.05$). For the winter sample set, G, $G \times E$, and $G \times F(E)$ were significant ($P < 0.001$) sources of variation at both testing scales.

When SRC tests were conducted using 50% sucrose solution at the 0.2-g scale, G and E were significant sources of variation ($P < 0.01$; Table 3). The only other significant source of variation at this scale was $G \times E$, which was only significant ($P < 0.05$) for the spring sample set. When SRC tests were conducted at the 5-g scale, all sources of variation were significant ($P < 0.05$) except F(E).

When SRC tests were conducted at the 0.2-g scale using 5% Na_2CO_3 solution, E, $G \times E$, and $G \times F(E)$ were significant sources of variation for the spring sample set (Table 3). When conducted at the 5-g scale, all factors except F(E) were significant sources of variation for the spring sample set. Genotype was the only significant source of variation for the winter sample set when tested at the 0.2-g scale. In contrast, all factors except F(E) were significant at the 5-g scale, which aligns with what was detected for the spring set at the 5-g scale.

When tested with water at the 0.2-g scale, E was the only significant factor for the spring sample set (Table 3). In contrast, G, E, and $G \times F(E)$ were significant for the winter sample set. When SRC tests were conducted with water at the 5-g scale, all factors significantly influenced SRC values of the spring sample set. Genotype and $G \times E$ were significant sources of variation for the winter sample set at the 5-g scale.

Variance Components

Estimated and relative values for variance components varied widely among solvents, testing scales, and sample sets (Table 4). Relative values of the variance component for genotype (σ_g^2) were consistently higher for the winter sample set, since lower estimated values for σ_g^2 or higher environment variance component (σ_e^2) values were calculated for the spring sample set. Additionally, relative values of σ_g^2 were higher for the 5-g scale than the 0.2-g scale, due in most cases to lower $\sigma_{[g \times f(e)]}^2$ values. Repeatability values, calculated for the number of environments, lab replications, and field replications used in this study, were >0.4 for all solvent-scale-sample set combinations.

For lactic acid at both testing scales for both sample sets, σ_g^2 values were at least three times greater than $\sigma_{g \times e}^2$ values, the largest of the interaction terms, resulting in high (≥ 0.9) repeatability values. Scale made little difference in

repeatability levels or relative variance. The repeatability values for sucrose were high at both testing scales for both sample sets, since all four had high σ_g^2 values, and relatively low interaction values. At the 0.2-g scale, relative variance values for $\sigma_{[g \times f(e)]}^2$ were similar to those of σ_g^2 ; however, this only resulted in minimal reduction in repeatability for the 0.2-g scale compared with the 5-g scale.

When the spring sample set was evaluated at the 0.2-g scale with Na_2CO_3 , σ_g^2 was similar in value or lower than the interactions, resulting in relatively low repeatability (0.63) compared with the other scales and sample sets. The value of $\sigma_{[g \times f(e)]}^2$ for the winter set at the 0.2-g scale was higher than the value for σ_g^2 ; however, this only resulted in a 0.14 reduction in repeatability. When tested at the 5-g scale, both sample sets had high σ_g^2 values that were greater than the variance components of the interactions, and therefore, resulted in high repeatability values.

The lowest repeatability (0.42) was calculated for the spring sample set tested with water at the 0.2-g scale, which was due to a σ_g^2 value that was less than those of all of the interaction terms. Repeatability for the winter sample set was much higher (0.75), since the relative value of σ_g^2 was similar to that of the interaction terms. When tested at the 5-g scale, both sample sets had high repeatability, since the σ_g^2 values were greater than the interaction variance components.

Predicted Repeatability

Predicted repeatability values were calculated to demonstrate the impact of varying numbers of environments, field replications, or lab replications on SRC results (Fig. 1, Eq. [1]). Predicted repeatability values varied based on the variance components as well as the number of environments, field replications, and lab replications entered into Eq. [1] for e , f , and l , respectively. The variation in predicted repeatability values for each solvent was greater for the 0.2-g scale than the 5-g scale.

DISCUSSION

The standard deviations of the SRC values for the control samples provide some indication of the relative amounts of lab error among the solvents and scales. As is apparent from the further analyses, however, these values alone do not provide any information on how laboratory variation compares with genotypic or environmental variation, which is necessary to accurately determine the utility of each test. The nearly perfect correlation ($r = 0.99$, $P < 0.001$) between the lactic acid SRC test results at the 0.2- and 5-g scales indicates that these methods can be used interchangeably. The correlations between results of the two scales for the sucrose and Na_2CO_3 SRC tests were not perfect; however, they were high ($r = 0.81$ and 0.75 , respectively, $P < 0.001$). The reduced correlation compared with lactic acid indicates that a breeder using the

Table 3. Analysis of variance of the effect of genotype (G), environment (E), field replication within environment [F(E)], their interactions, and laboratory replication within G×F(E) (designated L[G×F(E)]) on solvent retention capacities (SRCs) using four solvents: 5% (w/w) lactic acid; 50% (w/w) sucrose; 5% (w/w) Na₂CO₃; and water. Two scales of the SRC test were conducted on eight spring and 16 winter soft white wheat genotypes grown in five and three environments, respectively, in eastern Washington in 2005. Grain samples were collected from two field replications per genotype in each environment, and duplicate SRC tests were conducted on flour extracted from each grain sample.

Parameter	0.2-g scale			5-g scale			Parameter	0.2-g scale			5-g scale		
	df	MS	F test	df	MS	F test		df	MS	F test	df	MS	F test
Lactic acid							Na₂CO₃						
Spring [†]							Spring ^{††}						
G	7	179.34	14.63***	7	3651.11	24.32***	G	7	9.55	2.05	7	134.55	8.89***
E	4	733.24	30.46***	4	7651.72	42.00***	E	4	208.87	57.02***	4	1306.08	50.38***
F(E)	5	13.24	4.62***	5	64.08	2.00	F(E)	5	0.63	0.36	5	15.47	2.48
G×E	28	14.23	5.14***	28	150.13	4.69***	G×E	28	4.77	2.76**	28	17.08	2.85**
G×F(E)	35	2.86	2.86***	35	32.04	5.38***	G×F(E)	35	1.74	1.74*	35	6.23	6.23***
L[G×F(E)]	80	1.00		80	5.95		L[G×F(E)]	79	1.00		80	1.00	
		<i>R</i> ² = 0.98			<i>R</i> ² = 0.99				<i>R</i> ² = 0.93			<i>R</i> ² = 0.99	
Winter [†]							Winter						
G	15	185.64	10.05***	15	1742.14	10.81***	G	15	60.46	5.53***	15	41.63	17.85***
E	2	46.22	2.56	2	452.32	2.85	E	2	89.67	7.15	2	235.13	55.96***
F(E)	3	1.72	0.52	3	15.05	0.89	F(E)	3	10.64	1.18	3	3.20	2.44
G×E	28	19.70	6.30***	28	160.45	9.49***	G×E	28	10.92	1.21	28	2.33	1.78*
G×F(E)	41	3.41	3.41***	41	16.90	8.09***	G×F(E)	41	9.00	1.19	41	1.31	2.82***
L[G×F(E)]	90	1.00		90	2.09		L[G×F(E)]	90	7.54		90	0.46	
		<i>R</i> ² = 0.98			<i>R</i> ² = 0.99				<i>R</i> ² = 0.85			<i>R</i> ² = 0.97	
Sucrose							Water						
Spring [†]							Spring [†]						
G	7	164.91	14.57***	7	181.68	28.83***	G	7	1.83	1.31	7	24.23	14.35***
E	4	487.31	36.42***	4	319.13	26.98***	E	4	20.20	14.77**	4	119.72	37.42***
F(E)	5	8.19	1.34	5	6.72	2.19	F(E)	5	1.15	0.85	5	2.05	3.79**
G×E	28	11.32	1.85*	28	8.50	2.94**	G×E	28	1.55	1.17	28	1.69	3.12***
G×F(E)	35	6.13	1.52	35	3.07	3.07***	G×F(E)	35	1.35	1.35	35	0.54	2.16**
L[G×F(E)]	80	4.04		80	1.00		L[G×F(E)]	80	1.00		80	0.25	
		<i>R</i> ² = 0.92			<i>R</i> ² = 0.97				<i>R</i> ² = 0.71			<i>R</i> ² = 0.97	
Winter							Winter [†]						
G	15	56.18	6.63***	15	82.93	11.58***	G	15	26.99	3.83**	15	71.47	12.81***
E	2	89.93	21.63**	2	115.34	10.17*	E	2	117.38	14.82**	2	3.23	0.44
F(E)	3	1.81	0.29	3	7.94	2.15	F(E)	3	5.78	1.18	3	3.20	2.16
G×E	28	8.47	1.38	28	7.14	1.93*	G×E	28	7.04	1.44	28	5.66	3.85***
G×F(E)	41	6.15	1.26	41	3.70	2.28***	G×F(E)	41	4.88	2.23***	41	1.48	1.48
L[G×F(E)]	90	4.90		90	1.62		L[G×F(E)]	90	2.19		90	1.00	
		<i>R</i> ² = 0.78			<i>R</i> ² = 0.93				<i>R</i> ² = 0.83			<i>R</i> ² = 0.93	

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]Analysis at the 0.2-g scale conducted using environmental weighting to equalize variation among environments.

^{††}Analysis at the 5-g scale conducted using environmental weighting to equalize variation among environments.

0.2-g sucrose and Na₂CO₃ SRC tests instead of the full-scale versions would not rank the genotypes exactly the same between scales. In early-generation selection programs, however, the identification of extreme values is more important than discerning differences among genotypes with similar values (Bettge et al., 2002). Therefore, the correlations between the scales for the sucrose and Na₂CO₃ SRC tests suggest that the 0.2-g test has sufficient resolution for use in early-generation selection.

The moderate correlation ($r = 0.60$, $P < 0.001$) between test scales for water indicates that the 0.2-g SRC

test results do not agree with the 5-g results. This limits the ability to use the SRC test to accurately select for water absorption capacity using 0.2 g of flour. The magnitude of the correlations between the 0.2- and 5-g flour SRC values were similar to those observed by Bettge et al. (2002) for the correlation between the 0.2-g wheat meal and 5-g flour SRC versions. This indicates that the use of flour instead of whole meal for the 0.2-g scale test provides little to no improvement in the ability of the reduced-scale test to approximate results of the 5-g flour version. Differing correlations among the four solvents between the two

A: Lactic Acid Predicted Repeatabilities

B: Sucrose Predicted Repeatabilities

C: Sodium Carbonate Predicted Repeatabilities

D: Water Predicted Repeatabilities

Figure 1. Predicted repeatabilities of solvent retention capacities (SRCs) using four solvents: (A) 5% (w/w) lactic acid; (B) 50% (w/w) sucrose; (C) 5% (w/w) Na_2CO_3 ; and (D) water. Two scales of the SRC test were conducted on eight spring and 16 winter soft white wheat genotypes grown in five and three environments, respectively, in eastern Washington in 2005. Grain samples were collected from two field replications per genotype in each environment, and duplicate SRC tests were conducted on each grain sample. Predicted repeatabilities were calculated using data from the spring sample set according to Eq. [1], using variance components in Table 4. L, number of lab replications entered into Eq. [1]; F, number of field replications entered into Eq. [1]; Env, number of environments entered into Eq. [1].

Table 4. Repeatability calculations and variance components of genotype (σ_g^2), environment (σ_e^2), field replication within environment ($\sigma_{f(e)}^2$), the interaction of genotype and environment ($\sigma_{g \times e}^2$), the interaction of genotype and field replication ($\sigma_{g \times f(e)}^2$), and replication within $g \times f(e)$ (designated $\sigma_{f(g \times f(e))}^2$) for solvent retention capacities (SRCs). Two scales (0.2 and 5 g) of the SRC test were conducted with four solvents on eight spring and 16 winter soft white wheat genotypes grown in five and three environments, respectively, in eastern Washington in 2005. Grain samples were collected from two field replications per genotype in each environment, and duplicate SRC tests were conducted on each grain sample for all solvents at each test scale.

Solvent	Scale	Sample set	σ_g^2			σ_e^2			$\sigma_{f(e)}^2$			$\sigma_{g \times e}^2$			$\sigma_{g \times f(e)}^2$			$\sigma_{f(g \times f(e))}^2$			Repeat [†]
			Estimate [†]	SE [‡]	RV [§]	Estimate	SE	RV	Estimate	SE	RV	Estimate	SE	RV	Estimate	SE	RV	Estimate	SE	RV	
Lactic acid	0.2	spring	103.02	59.07	0.33	165.59	121.73	0.53	4.33	3.79	0.01	29.49	9.99	0.09	9.35	3.48	0.03	0.98	0.15	0.00	0.94
		winter	114.40	46.96	0.70	5.61	8.44	0.03	0.00	0.00	0.00	33.90	10.87	0.21	7.89	2.65	0.05	1.02	0.16	0.01	0.90
5	spring	spring	175.05	97.60	0.38	233.42	169.09	0.51	2.00	2.58	0.00	29.52	10.21	0.06	13.05	3.86	0.03	5.95	0.94	0.01	0.96
		winter	137.99	55.61	0.73	5.78	8.59	0.03	0.00	0.00	0.00	36.14	10.79	0.19	7.32	1.79	0.04	2.09	0.31	0.01	0.91
Sucrose	0.2	spring	7.68	4.41	0.26	14.81	10.77	0.51	0.13	0.34	0.00	1.30	0.84	0.04	1.04	0.80	0.04	4.04	0.64	0.14	0.93
		winter	4.36	1.90	0.37	1.34	1.48	0.11	0.00	0.00	0.00	0.67	0.68	0.06	0.49	0.73	0.04	4.90	0.73	0.42	0.86
5	spring	spring	24.91	13.87	0.33	44.28	32.16	0.58	0.96	1.01	0.01	3.56	1.50	0.05	1.86	0.75	0.02	1.04	0.17	0.01	0.96
		winter	6.52	2.61	0.55	1.77	1.95	0.15	0.12	0.20	0.01	0.86	0.55	0.07	1.07	0.44	0.09	1.62	0.24	0.14	0.92
Na ₂ CO ₃	0.2	spring	1.39	1.32	0.05	23.30	16.90	0.77	0.00	0.00	0.00	3.16	1.36	0.10	1.49	0.85	0.05	0.97	0.15	0.03	0.63
		winter	4.45	2.03	0.31	1.22	1.46	0.08	0.08	0.31	0.01	0.61	0.91	0.04	0.66	1.12	0.05	7.54	1.12	0.52	0.83
5	spring	spring	4.91	3.06	0.08	48.91	35.20	0.81	0.75	0.68	0.01	2.81	1.12	0.05	1.93	0.57	0.03	1.01	0.16	0.02	0.86
		winter	3.65	1.42	0.42	3.80	3.87	0.44	0.06	0.08	0.01	0.25	0.18	0.03	0.43	0.15	0.05	0.46	0.07	0.05	0.95
Water	0.2	spring	0.11	0.24	0.02	5.15	3.91	0.73	0.00	0.00	0.00	0.21	0.49	0.03	0.56	0.59	0.08	1.02	0.15	0.15	0.42
		winter	1.81	0.95	0.23	1.77	1.91	0.23	0.03	0.16	0.00	0.63	0.57	0.08	1.31	0.55	0.17	2.19	0.33	0.28	0.75
5	spring	spring	1.13	0.65	0.20	3.64	2.65	0.66	0.09	0.08	0.02	0.29	0.12	0.05	0.15	0.07	0.03	0.25	0.04	0.05	0.93
		winter	1.53	0.61	0.53	0.00	0.00	0.00	0.01	0.02	0.00	0.27	0.10	0.09	0.07	0.05	0.02	1.00	0.15	0.35	0.89

[†]Estimate of the variance component.

[‡]Standard error of the variance component.

[§]Relative value of variance component.

^{††}Repeatability calculated according to Eq. [1].

scales may reflect differences in the way the reduced scale or altered tube geometry influence the formation of the flour gel among the solvents.

Analysis of Variance

Analysis of variance was used to identify sources of variation that significantly affect SRC results as was previously reported (Guttieri et al., 2001, 2002). In our study, genotype did not significantly ($P < 0.05$) affect results for the spring sample set using Na₂CO₃ and water at the 0.2-g scale, but was significant ($P < 0.001$) at the 5-g scale. The sensitivity of the 0.2-g scale was insufficient for detecting differences among the genotypes tested. Therefore, the 0.2-g scale of the Na₂CO₃ and water SRC tests may not be suitable for selecting for starch damage and water absorption differences, respectively, among elite wheat genotypes.

Results using 50% sucrose at the 5-g scale were significantly influenced by $G \times E$ and $G \times F(E)$ effects, whereas the 0.2-g results were only significantly affected by $G \times E$ for the spring sample set. Guttieri et al. (2001, 2002) suggested that the lack of a significant interaction involving genotype (i.e., $G \times E$) indicates that these effects are too small to necessitate the use of multiple environments or replications when using the SRC test for selection. The risk of using this approach to justify the use of fewer experimental units arises in situations for which a large interaction term is determined to be nonsignificant due to an equally large error term. Since the F test compares the mean squares of the treatment to the mean squares of the error, a large interaction could be nonsignificant if the mean squares of the error are of similar magnitude (Dean and Voss, 1999). When compared with the interaction terms, the mean squared values for the error terms were higher and the R^2 values were lower for the 0.2-g sucrose SRC results than for the 5-g results.

The larger error terms may have obscured the interactions at the 0.2-g scale when the interaction was observed at the 5-g scale. Therefore, the lack of significant interaction terms does not justify the use of fewer environments or replicates, and further analysis is necessary to determine if the interactions would interfere with genotypic selection.

The effects of $G \times E$ and $G \times F(E)$ on the Na_2CO_3 SRC results were significant for the spring sample set at the 0.2-g scale and both sample sets at the 5-g scale but were not significant for the winter sample set tested at the 0.2-g scale. This is probably due to a greater effect of error, as reflected by the lower R^2 values. As described for the sucrose data, the large effect of error may have obscured the effects of the interactions. Similarly, many of the factors that had significant effects on water SRC tests conducted at the 5-g scale were nonsignificant at the 0.2-g scale. As described for the sucrose and Na_2CO_3 tests, this may have resulted from the relatively large mean square errors observed for the 0.2-g results. This does not completely explain the differing results between the scales, however, since E and $G \times F(E)$ were found to be significant for the winter sample set at the 0.2-g scale and were not significant at the 5-g scale. Further study is required to determine why the same flour samples responded differently at the two testing scales.

In previous work, the influence of genotype, environment (location or year), and their interaction on SRC results was evaluated (Guttieri and Souza, 2003; Guttieri et al., 2001, 2002). The SRC method used in these studies differed from the AACC method (AACC, 2000) and the 5-g method described by Bettge et al. (2002) in terms of method of mechanical agitation, centrifuge speed, and the duration of each step. These differences in methods were described as minor, and conclusions made from evaluations utilizing one version are likely to apply to the other (Guttieri and Souza, 2003). Genotype was a significant source of variation for SRC results at the 5-g scale for all four solvents in our study, as was detected in previous work (Guttieri and Souza, 2003; Guttieri et al., 2001); however, genotype significantly influenced only SRC results for water and Na_2CO_3 in Guttieri et al. (2002).

Environment was significant for all four solvents for the spring set in this study and for sucrose and Na_2CO_3 for the winter set and in Guttieri et al. (2002). In contrast, E was not a significant source of variation for the water and lactic acid results from our winter set and in Guttieri et al. (2001). A possible explanation for this contrast is that the grain used in Guttieri et al. (2001) and Guttieri and Souza (2003) was grown with irrigation, whereas our samples and those used in Guttieri et al. (2002) were grown under a wide range of natural precipitation levels. The winter samples were grown in three environments with similar precipitation levels, which may explain the lack of significant environmental effects for the lactic acid and water

SRC results. Guttieri et al. (2001, 2002) and Guttieri and Souza (2003) indicated that $G \times E$ had little or no effect on SRC results. In contrast, $G \times E$ significantly affected SRC values for both sample sets using all four solvents in this study. This difference in conclusions may have resulted from the differing environments and genotypes included in our study, since with all studies of heritability or repeatability of a trait, the estimates are most applicable to the genotypes and environments tested (Hucl and Chibbar, 1996). Therefore, the results of our study are most applicable to soft wheat produced in eastern Washington or in other regions with similar precipitation ranges.

The significant effects of environment noted above indicate that when using the SRC test in a breeding program, control genotypes must be grown with and tested with the genotypes under evaluation. These allow a breeder to measure and control for the average effect of environment on SRC values for that site and year. Often end-product producers will have set SRC values that they require in flour that they use. Therefore, breeders selecting genotypes with specific end products in mind might desire to select based on these set values. Unfortunately, the significant effects of environment indicate that absolute SRC values are not a valid measure of a genotype's performance in all environments. The only way a breeder could be certain of the absolute value would be to test the genotype in the range of environments where it would be grown for multiple years, thus determining both the average value and range. A more efficient approach would be to test the genotype against control genotypes for which this work has already been done, and to select for SRC values superior to those of the controls. Even so, since significant $G \times E$ interactions occur, it is still necessary to test a line grown in multiple locations and years before releasing it. To determine if the significant interaction components would prevent effective selection in the generations before release, it is necessary to compare the relative effects of the interactions against the genotypic variation.

Variance Components

The calculation of variance components allowed the comparison of the relative influence of G , E , $F(E)$, $G \times E$, $G \times F(E)$, and $L[G \times F(E)]$ on SRC results. The lower relative values of $\sigma^2_{l[g \times f(e)]}$ for most of the 5-g results indicate that the use of this scale results in less relative lab error when conducted using Na_2CO_3 , sucrose, and, for the spring sample set, water. The greater relative values of σ^2_e for the spring results compared with the winter results indicate that the environments used for the spring sample set had a wider range of effects on the SRC values than did the winter environments. This is to be expected, since the spring environments encompass a wider range of precipitation levels than the winter environments. Variance components were used by Guttieri and Souza (2003), after standardizing as

a proportion of the nonenvironmental variation using the formula $\sigma_{gg}^2 / [\sigma_g^2 + (\sigma_{g \times e}^2 / Y) + (\sigma_{\text{error}}^2 / RY)]$, where Y is the number of years the experiment was repeated and R is the number of replications within a year. This calculation is similar to the equation we used for repeatability, and both calculations allow the determination of the effect of genotypic variance relative to the interaction terms.

Repeatability values allow the estimation of the degree to which selection based on genotypic means within each environment is confounded by differing performance in varying environments, field replications, and lab replications. The calculation (Eq. [1]) compares the variation that is due to consistent differences among genotypes (σ_g^2) to itself (σ_g^2) plus the variation due to differences among the performance of genotypes in varying environments ($\sigma_{g \times e}^2$), field replications ($\sigma_{g \times f(e)}^2$), and lab replications ($\sigma_{l[g \times f(e)]}^2$). The high repeatability values for the lactic acid SRC tests conducted at both scales on both sample sets, along with the high correlation between the scales, indicate that the 0.2- and 5-g scales are equivalent and are equally effective for use in selection, although the reduced flour requirement of the 0.2-g scale makes it better suited for use in early-generation selection. A breeder can use the 0.2-g scale of the lactic acid SRC test to select for protein quality with little loss of sensitivity.

The results of the sucrose SRC test conducted at both scales on both sample sets had high repeatability values. These results show that when used in selection under similar conditions to those tested in this study, the ability to select consistently superior genotypes is not lost when the 0.2-g scale is used. This is despite the high relative values of $\sigma_{l[g \times f(e)]}^2$ for the 0.2-g data, since the use of multiple environments and lab and field replications reduced the effect of this factor. The high relative values of $\sigma_{l[g \times f(e)]}^2$ for the 0.2-g scale, however, would have a greater effect when fewer environments, lab replications, and field replications are used, as is described below.

The 5-g scale of the Na_2CO_3 SRC test was more repeatable than the 0.2-g scale, and this difference was more pronounced when evaluating the spring samples, which were collected from a wider range of precipitation levels. This suggests that the 0.2-g scale has a reduced ability to discern consistent genotypic differences when used to evaluate samples from diverse environments.

Similar to the other solvents, the 5-g scale of the water SRC test exhibited close to 90% repeatability, indicating that the 5-g scale can identify consistent genotypic differences in overall absorption when used under similar conditions to those tested. The use of the 0.2-g-scale water SRC, however, resulted in the greatest reduction in repeatability of all the solvents, most dramatically for the spring sample set. Therefore, the use of the 0.2-g scale water SRC in a selection program might result in reduced gains from selection compared with the use of the other

solvents or the 5-g scale. Individual breeders must decide if the reduced flour requirement of the 0.2-g scale justifies the reduced gain from selection. The greater reduction for the spring sample set was due to a relative value of σ_g^2 one-tenth the value of the 5-g scale, and a relative value of σ_e^2 greater than in the 5-g scale. The measurement of environmental differences was preserved at the reduced scale, and genotypic differences were lost. This suggests that the reduced sensitivity of the 0.2-g scale may be compounded by large environmental differences.

Predicted Repeatability

To evaluate the effect of varying numbers of environments, field replications, or lab replications on the repeatability of the SRC tests, predicted repeatability values were calculated using the variance component data from the spring sample set. The winter sample set was not chosen for analysis since conclusions derived from it could only be applied to narrow sets of environmental conditions. The predicted repeatability graphs demonstrate that the use of multiple environments, field replications, and lab replications in this study resulted in higher repeatability values than would have occurred with a lower number of experimental units (Fig. 1). Predicted repeatability values were higher with increasing values for e , f , and l ; however, with each additional unit (environment, field replication, or lab replication), the magnitude of increase diminished. The magnitude of increase also depended on the relative values of σ_g^2 and the interaction term involved. The greater the relative value of the interaction variance component compared with σ_g^2 , the greater the increase in predicted repeatability. Increasing e resulted in an equal or greater increase than increasing f or l by an equivalent amount, since it reduced the effects of $\sigma_{g \times e}^2$, $\sigma_{g \times f(e)}^2$, and $\sigma_{l[g \times f(e)]}^2$ on repeatability. The higher $\sigma_{g \times e}^2$ was, the greater the effectiveness of adding an environment rather than a field replication. The greater $\sigma_{g \times e}^2$ or $\sigma_{g \times f(e)}^2$ were, the greater the effectiveness of adding an environment rather than a lab replication. Adding an extra field replication was equal to or more effective than adding a lab replication, since it reduced the effects of both $\sigma_{g \times f(e)}^2$ and $\sigma_{l[g \times f(e)]}^2$ on repeatability. The greater $\sigma_{g \times f(e)}^2$ was, the greater the effectiveness of adding a field replication rather than a lab replication.

The predicted repeatability graphs or the variance components and Eq. [1], which these graphs were based on, can be used by breeders when selecting genotypes across wide ranges of environments to decide what SRC scale to use and how many environments, field replications, and lab replications are needed to maximize gains from selection. In terms of lactic acid, testing within a single environment using single field and lab replications would be predicted to achieve a repeatability value >0.7 for both scales. Selection using a second environment is predicted to result in an increased repeatability level;

however, this increase is not large enough to justify the increase in testing costs.

The use of reduced numbers of environments and replicates when selecting based on sucrose SRC test results is predicted to produce a greater decrease in repeatability at the 0.2-g scale than the 5-g scale. As for lactic acid, sufficient gain from selection based on the 5-g sucrose SRC test may be achieved using single environments and replications. For the 0.2-g scale, large increases in repeatability are predicted for the use of additional environments or replications. In an early-generation selection program, however, when using the 0.2-g scale may be the only option, the increased cost required to achieve higher repeatability may not be warranted since distinguishing intermediate values is less important than differentiating genotypes at the extremes of the distribution (Bettge et al., 2002).

Both scales of the Na_2CO_3 SRC test are predicted to have substantially lower repeatability values when a single environment is used. At a set number of environments and replications, the 5-g scale has greater repeatability than the 0.2-g scale, although this difference decreases as the number of environments and replications increases. When using the 5-g scale to evaluate advanced breeding material, the increased cost of evaluating a second or third environment may be justified by the large improvement in repeatability. For early-generation selection using the 0.2-g scale, a greater investment would be required to achieve the same predicted repeatability; therefore, a breeder should weigh the benefits of early-generation selection against the increased costs or reduced gain from selection.

The predicted repeatability values for the water SRC test show trends similar to Na_2CO_3 ; however, the difference between the scales is greater. For the 5-g scale, repeatability values >0.8 are achieved using three environments or two environments with multiple lab or field replications. To an even greater extent than the Na_2CO_3 SRC test, the use of the reduced-scale water test would result in little gain from selection or a large investment in multiple environments or replications.

This study evaluated released cultivars and advanced breeding lines that had undergone selection for improved soft wheat quality; therefore, the range of quality differences may be less than that found in unselected breeding populations. The repeatability of the SRC tests in a breeding population may then be greater than the predicted repeatabilities reported in this study. Further study is required to confirm that the relative performances of the scales and solvents are consistent among diverse sets of genotypes.

CONCLUSIONS

Significant interactions involving genotype [$G \times E$ and $G \times F(E)$] were detected when SRC tests were conducted on samples from the a wide range of wheat production environments in eastern Washington, in contrast to pre-

vious studies where significant genotype $\times$ environment interactions were not detected (Guttieri and Souza, 2003; Guttieri et al., 2001, 2002). These interactions, however, did not result in repeatability values of <0.8 for all solvent and scale combinations tested except the 0.2-g SRC tests conducted with water and Na_2CO_3 . Based on predicted repeatabilities, the 5-g scale of the SRC test is an effective tool for selecting superior soft white genotypes with the use of three or fewer environments, depending on the solvent used, and with little or no field or lab replication. The high predicted repeatabilities of the lactic acid and sucrose 0.2-g scale SRC tests, even when as few as one environment is used, justify their use for early-generation selection when large samples and multiple environments are not available. In contrast, the 0.2-g water and Na_2CO_3 SRC tests may not provide sufficient value to warrant their use, even in early stages of the breeding process when the use of the 5-g scale is not possible. The use of the SRC test allows the partitioning of a flour's absorption capacity in terms of specific attributes: lactic acid is associated with gluten quality, sucrose with pentosans, Na_2CO_3 with damaged starch, and water with overall absorption (Gaines, 2000; Slade and Levine, 1994). Since the water SRC test does not provide an evaluation of a specific source of absorption, early-generation selection based on this solvent may not be necessary. In addition, the reduced repeatability of the 0.2-g scale Na_2CO_3 SRC test may necessitate the use of other evaluations to determine a genotype's tendency toward damaged starch or may delay the evaluation of this trait until greater quantities of flour are available.

Acknowledgments

Funding for this project was provided by Kraft Food, the International Marketing Program for Agricultural Commodities and Trade (IMPACT) at Washington State University (Project no. 3024 3627), and the College of Agriculture, Human and Natural Resource Sciences at Washington State University.

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