

Optimizing the SDS Sedimentation Test for End-Use Quality Selection in a Soft White and Club Wheat Breeding Program

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ABSTRACT

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Soft white and club wheat (*Triticum aestivum* L.) market subclasses have specific end-use characteristics. Among the most important of these characteristics are weak dough mixing and handling properties as a result of weak gluten. The SDS sedimentation test has gained wide acceptance as a useful, small-scale test in bread wheat breeding programs to predict gluten strength and baking quality. To optimize its use for soft white or club wheat breeding, variations of the SDS sedimentation test were performed on grain from winter wheats grown at eight locations in the U.S. Pacific Northwest, and the effects of lines, environment, and their interactions on SDS sedimentation volumes were determined. Using different

sample weights and substituting whole meal for flour did not affect the ability of the SDS sedimentation test to differentiate among lines. Changes in protein concentration and sample weight caused proportional changes in SDS sedimentation volumes; however, the response was not consistent among all lines. Line had a greater effect on the SDS sedimentation volumes than any other source of variation. If differential effects of protein to SDS sedimentation among lines are taken into account, the SDS sedimentation test should be an effective small-scale test for end-use quality assessment in soft white and club wheat breeding programs.

New wheat cultivars must possess end-use quality characteristics that typify their market class while maximizing agronomic performance if they are to be acceptable to both the producer and the processor. The various U.S. market classes of wheat are distinguished by kernel hardness (soft or hard), bran color, grain morphology, and growth habit (spring or winter). Soft white and club wheats typically have high flour extraction rates, weak gluten, and low protein concentration. These characteristics make them the preferred market subclasses for use in baking pastries, cookies, cakes, flat breads, and similar products (Yamamoto et al 1996, Lin and Czuchjowska 1997).

Increasing the efficacy of wheat cultivar development requires the assessment of end-use quality in early generations, thus avoiding the wasted expense and time associated with replicated field testing of undesirable lines. Unfortunately, only small volumes of grain are available for testing in the F₂, F₃, and F₄ generations. Therefore, small-scale tests are necessary to predict end-use quality of grain from early generation breeding material. To be effective, a small-scale test must accurately predict end-use quality across environments for selections to be made using grain produced in only one environment (Blackman and Gill 1980).

The SDS sedimentation test is a small-scale test, the results of which are highly correlated with the breadbaking quality of hard wheat (Axford et al 1978). The SDS sedimentation test is a modification of the Zeleny (1947) sedimentation test that requires only a small sample of whole meal flour, is simple to perform, and is highly reproducible. Since its development, wheat breeders have explored the feasibility of using the SDS sedimentation test for hard wheat cultivar improvement and during this process, several slight modifications were made to the procedure (Preston et al 1982, Dick and Quick 1983, Mansur et al 1990).

The end-use quality of wheat flour is highly influenced by both protein concentration and protein type (Carillo et al 1990). Grain protein is a complex mixture of many different protein components. However, gluten gives wheat flour its characteristic cohesiveness and elastic characteristics. Gluten consists of two major components, glutenin and gliadin. The sediment in the SDS solution theoretically results from the swelling of the glutenin strands (Eckert et al 1993), and high SDS sedimentation volumes have been associated with stronger gluten and superior breadbaking quality (Axford et al 1978, 1979; Blackman and Gill 1980; Dexter et al 1980; Preston et al 1982; Dick and Quick 1983; Lorenzo and Kronstad 1987; Ayoub et al 1993). Soft white and club wheats should have low SDS sedimentation volumes due to their weak gluten. Cultivars with different protein quality, as expressed by their gluten characteristics, should be differentiated by the SDS sedimentation test.

It has been well established that the SDS sedimentation test accurately assesses protein quality. However, results also are highly influenced by protein concentration (Preston et al 1982, Dick and Quick 1983, Lorenzo and Kronstad 1987, Ayoub et al 1993). The effect of protein concentration on SDS sedimentation volume may be eliminated by multiplying SDS sedimentation volumes by the grain protein concentration of the sample divided by 10 (Baik et al 1994). SDS sedimentation volumes also have been highly influenced by environment, crop year, and their interactions with cultivar (Bassett et al 1989, Peterson et al 1992, Graybosch et al 1996). Nevertheless, SDS sedimentation volumes are highly heritable and can be used for selecting among early generation progeny (Matuz 1998).

To date, the majority of published studies on the use of the SDS sedimentation test in breeding programs have been in the context of hard wheats with the goal of identifying strong gluten types. Our goal was to adapt this test to select weak gluten types in a soft white or club wheat breeding program. Specifically, our objectives were to examine the effects of sample weight, whole meal versus flour, and protein concentration on the ability of the SDS sedimentation test to differentiate among lines and to determine the variation in the SDS sedimentation test due to line, environment, growing year, and their interactions.

MATERIALS AND METHODS

Thirty-four club and soft white winter wheat lines from the 1997 USDA-ARS club wheat breeding elite nursery were planted with three replicates at eight locations in Washington State including: Harrington, Pomeroy, Lind, Walla Walla, Hartline, Connell, and two sites at Pullman. A subset of 21 lines was grown in 1998 with three replicates at the same locations as 1997, with the excep-

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tion of substituting Ritzville for Hartline. The wheat was combine-harvested, and grain from the three replicates was blended before analysis in 1997. In 1998, the grain from each field replicate was analyzed separately. A 600-g grain sample of each cultivar grown at each location in 1997 was milled on a Quadrumat mill (Jeffers and Rubenthaler 1979), and in 1997 and 1998, a 10-g grain sample of each line at each location was ground on a Udy grinder (0.5-mm screen) (Udy Co., Boulder, CO). Each field replicate from 1998 was ground separately.

The SDS sedimentation test was conducted according to the procedure outlined by Dick and Quick (1989) and modified by Mansur et al (1990). Samples were placed in glass tubes (150 mm long \times 14 mm i.d.), the tubes were placed in racks (single row of 20 tubes per rack) with each rack having a measurement scale background. Two stock solutions were prepared: 1) 85% lactic acid (LA) in water (1:8, v/v) and 2) 20%, w/v, SDS solution. The two stock solutions were mixed to create a working solution of 1:48, v/v, LA/SDS. Distilled water (4 mL) was added to each sample. The samples were mixed for 20 sec on a high speed vortex mixer, allowed to hydrate for 5 min, mixed again on the high speed vortex for 10 sec, and then allowed to hydrate for another 5 min. LA/SDS solution (12 mL) was added to each sample and the tubes were agitated on a Zeleny type rocker (40 cycles/min) for 40 sec, rested for 2 min, and agitated again for 40 sec. The racks were left in an upright position for 10 min and the height (mm) of the sediment was recorded. Each SDS sedimentation test was performed twice to account for error in procedure, with control samples of cultivars Paha and Hyak included in each rack of 20 samples as checks. The duplicate tests were performed on two flour and whole meal samples taken from the blended grain samples in 1997 and from two individual replicates in 1998.

Experiment 1. To examine the effect of sample weight on the SDS sedimentation test, sedimentation volumes of four club wheat cultivars, Paha, Hyak, Rohde, and Coda, grown in 1997 at Connell and Hartline, Washington, were recorded at weights of 0.35–0.80 g in 0.05-g increments. The SDS sedimentation test was performed on both whole meal and flour to compare the ability of the SDS sedimentation test to differentiate among cultivars or lines when either sample type is used. These cultivars were chosen because they represented a range of sedimentation volumes based on preliminary analyses.

Regression and Pearson's correlation coefficients between sample weight and SDS sedimentation volume were calculated using the Microsoft Excel program. Analysis of covariance using the estimate option, with weight as the covariate and SDS sedimentation volume as the response variable, was performed using SAS methods (SAS Institute, Cary, NC) to determine whether the regression

coefficients for each cultivar from each location were significantly different. Pearson's and Spearman's rank correlation coefficients were used to compare slopes of regression lines with both whole meal and flour SDS sedimentation volumes using the Microsoft Excel program. Whole meal and flour SDS sedimentation volumes, as well as slopes of regression lines for whole meal and flour, were compared using Spearman's rank correlation coefficients. To determine whether sample weight had a significant effect on SDS sedimentation volume, analysis of variance (SAS) was performed with line considered a fixed effect, and weight and environment considered random effects. This data set was also used to estimate the percentage of total variation accounted for by each effect based on variance components. PROC VARCOMP was used to calculate the variance components under the assumption that all effects were random. To compare percentage of total variation due to line, environment, and their interaction for each weight (both whole meal and flour), PROC VARCOMP by weight with environment and line considered random effects was performed (SAS).

Experiment 2. The SDS sedimentation test was performed on whole meal and flour from all 34 lines from 1997 and the 21 lines from 1998 grown at all eight locations to examine the effects of protein concentration, environment, year, and their interactions on SDS sedimentation volumes. Based on the results of Experiment 1, a 0.50-g sample of whole meal and flour was used for all SDS sedimentation tests in this experiment, and each test was duplicated. The replicated tests performed on flour and whole meal from the field replicates in 1998 were considered the same as the duplicated tests performed on flour and whole meal from the blended grain in 1997.

Protein concentration of whole grain wheat was measured using near-infrared (NIR) spectroscopy (Infratec IA450, Hoganas, Sweden). Regression coefficients between protein concentration and SDS sedimentation volume for both whole meal and flour were calculated using the Microsoft Excel program. Pearson's and Spearman's rank correlation coefficients were used to compare slopes of regression lines with both whole meal and flour SDS sedimentation volumes using the Microsoft Excel program. Spearman's rank correlation coefficients also were used to determine whether the slopes of regression lines were similar for whole meal and flour.

To compare the percentage of total variation explained by line at different average protein concentrations, PROC VARCOMP by protein concentration with line considered a random effect was performed. The variance components were calculated using data from Pomeroy 1998, Pullman (late planting) 1998, Pullman 1998, Harrington 1997, Pullman (late planting) 1997, Connell 1997, Lind

TABLE I
Mean Squares, Percentages of Total Variance, and Tests of Significance from Analysis of Variance of SDS Sedimentation Volumes for 10 Sample Weights of Four Club Wheat Cultivars (Lines) Grown at Two Washington Environments in 1997^{a-c}

Source of Variation	df	Mean Squares		% of Total Variance	
		Flour	Whole Meal	Flour	Whole Meal
Line (L)	3	7008.6	3456.4	42.2	39.5
Environment (E)	1	3080.0	1506.7	8.2	6.0
Weight (W)	9	2007.1	670.3	31.0	20.1
L \times E	3	611.6	636.5	8.1	17.6
L \times W	27	119.0	76.4	7.3	8.6
E \times W	9	49.6	39.4	1.3	1.7
L \times E \times W	27	10.8	15.3	0.9	2.1
Error	80	3.9	7.6	1.1	4.4

^a All mean squares were significant at $P \leq 0.01$.

^b All SDS sedimentation tests were performed in duplicate.

^c Protein concentrations for four cultivars at two locations were: Coda at Connell 11.9%, Coda at Hartline 8.7%, Hyak at Connell 10.8%, Hyak at Hartline 8.7%, Paha at Connell 10.4%, Paha at Hartline 9.2%, Rohde at Connell 11.3%, and Rohde at Hartline 9.5%.

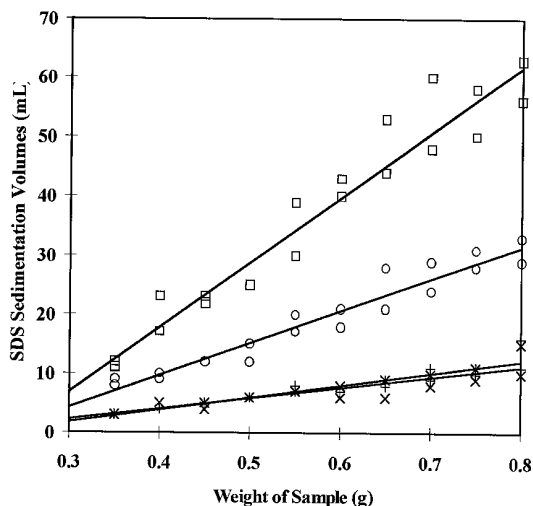


Fig. 1. Regression of SDS sedimentation volume on sample weight (0.35–0.80 g) for wheat whole meal. Hyak grown at Connell (\square) and Hartline (\circ); Coda grown at Connell ($+$) and Hartline (\times).

1997, and Walla Walla 1997 because these locations represented a broad range of average protein concentrations. All 21 lines at each location were used to determine the level of variation in SDS sedimentation test results associated with line.

Analysis of covariance using the estimate option with protein concentration as the covariate and SDS sedimentation volume as the response variable was performed to determine whether the regression coefficients for each line were significantly different. Analysis of variance with line considered a fixed effect and environment considered a random effect was performed on the subset of 21 lines grown in both 1997 and 1998. Each environment-by-year combination was considered as a separate environment resulting in a total of 16 environments for the analysis. The percentage of total variation due to each effect was estimated using variance components. To calculate the variance components, PROC VARCOMP was used under the assumption that both cultivar and environment were random. Analysis of covariance was performed on the same subset of data with line as a fixed effect, environment as a random effect, and protein as the covariate.

RESULTS AND DISCUSSION

Results from the analysis of variance on the four cultivars (lines) examined in Experiment 1 indicated that sample weight, line, environment, and their interactions all had a significant effect on SDS sedimentation volumes, with line having the greatest effect and the three-way interaction having the least (Table I). A deviation of just 0.05 g/sample resulted in significantly different sedimentation volume means, and increasing sample weight was positively correlated ($P \leq 0.05$) with SDS sedimentation volume for both whole meal ($r = 0.51$) and flour ($r = 0.61$) across lines (Fig. 1). Only whole meal values are shown in Fig. 1 because results for whole meal and flour were nearly identical. Although linear, the effect of changing sample weight on SDS sedimentation volume was not consistent for all lines when using either whole meal or flour, and the magnitude of the effect for each line depended on the environment (Fig. 1). The Spearman's rank correlation coefficient between regression line slope and SDS sedimentation volume for individual lines was significant ($P \leq 0.05$) for both whole meal ($r = 0.57$) and flour ($r = 0.69$), indicating that lines with higher SDS sedimentation volumes

also generally had the greatest regression slopes for sample weight by SDS sedimentation volume.

The percentages of total variation in SDS sedimentation volume attributed to line, environment, and their interaction were fairly constant across the range of sample weights (Fig. 2). The percentage of variation due to line was always much greater than environment and line-by-environment interaction at all weights for both whole meal and flour. Although the percentage of variation attributed to line did vary among different weights, no overall trend in gain or loss of ability to differentiate among lines at 0.35–0.80 g was detected when using either whole meal or flour.

The weight of material used to conduct the SDS sedimentation test varies widely depending on the laboratory (Axford et al 1978, Preston et al 1982, Dick and Quick 1983). Initially, 6 g of whole meal or 5 g of flour were used in the SDS sedimentation test (Axford et al 1978). The sample weight was changed to 4 g of flour, and bromophenol blue was added to the supernatant to better distinguish the sediment from the liquid (Preston et al 1982). The amount of flour used for the test was later decreased to 1.0 g, and the name of the test was changed to the SDS micro-sedimentation test (Dick and Quick 1983). While there are limits to the amount of sample that can be used with the SDS sedimentation test, it would seem that the sample weight can be adjusted for individual convenience. However, the sample weight must be measured accurately to eliminate the significant effect of varying sample weight as described above. In theory, larger sample weights should decrease sampling error, thus reducing the effects of imprecise measurements and errors in reading sediment height.

It has been casually suggested that either whole meal or flour can be used in the SDS sedimentation test (Axford et al 1979, Baik et al 1994). Whole meal is more desirable than flour due to ease of preparation. However, whole meal contains bran which may reduce the effectiveness and accuracy of the SDS sedimentation test. Across the sample weight range (0.35–0.80 g) included in Experiment 1, the use of flour versus whole meal consistently resulted in a higher proportion of variation explained by line, indicating a greater ability to differentiate among lines when using flour. However, flour SDS sedimentation volume rankings were highly correlated with whole meal SDS sedimentation volume rankings ($r = 0.96$) when all 21 lines averaged across locations, were considered in Experiment 2.

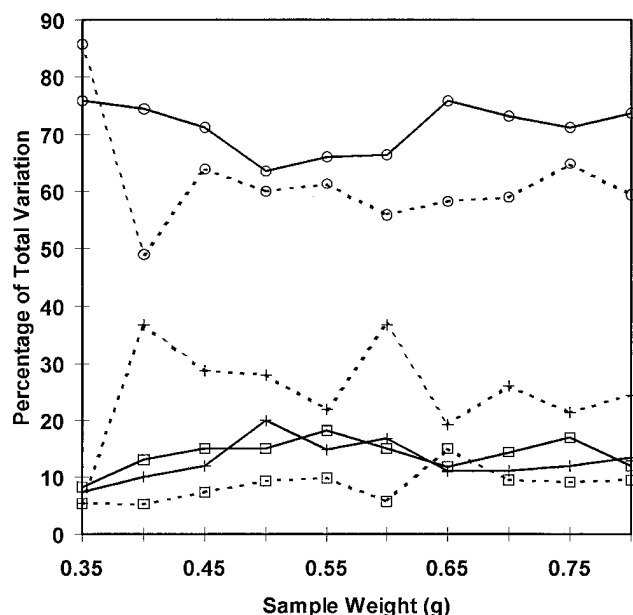


Fig. 2. Percentage of total variation from analysis of variance of wheat SDS sedimentation volumes by sample weight due to line (cultivar) (○), environment (□), and their interaction (+) using flour (—) or whole meal (---).

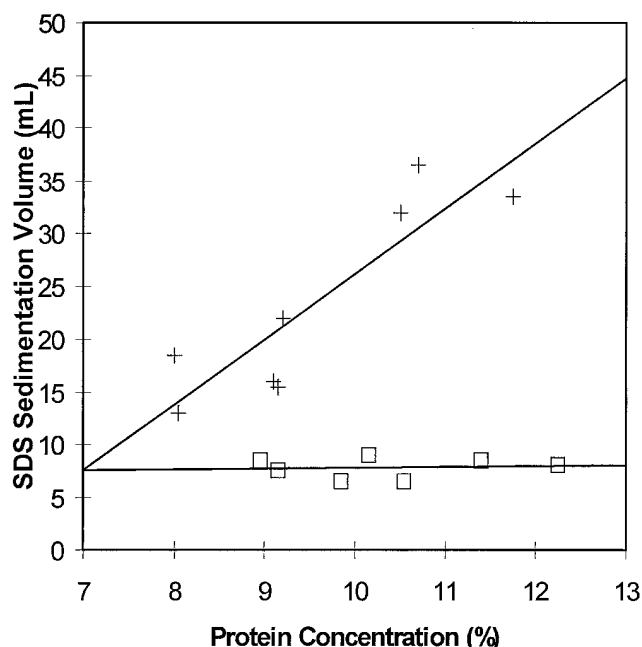


Fig. 3. Regression of wheat whole meal SDS sedimentation volumes on protein concentration for Eltan (+) and A9660 (□).

Although the amount of bran in flour was greatly reduced, the higher proportion of variation was accounted for by line, and lower line-by-environment percentages for flour were likely the result of the absence of the bran, the overall ability of the SDS sedimentation test to differentiate among lines was only slightly lower for whole meal. Additionally, the high Spearman's rank correlation coefficients between whole meal and flour when slope of regression lines for both weight ($r = 0.98$) and protein concentration ($r = 0.94$) were compared indicates that whole meal and flour provided similar line rankings. Any loss in the ability to distinguish among lines when using whole meal was offset by the convenience of its use. Additionally, for breeding purposes, the separation of means that differ by small volumes may not be essential.

Results from Experiment 2 indicate that, as with increasing weight, SDS sedimentation volumes increased linearly with increasing protein concentration when cultivars were analyzed individually, however the rate of increase was different for each line (Figs. 3 and 4). The two lines in Fig. 3 had the lowest (A9660) and highest (Eltan) regression line slopes for both whole meal and flour among all lines evaluated in this study. There was an obvious difference in the response, with Eltan SDS sedimentation volumes increasing markedly as protein concentration increased, whereas A9660 volumes increased only slightly with increasing protein concentrations. Additionally, the difference between the SDS sedimentation volumes of the two lines was greatest at high protein concentrations. The Spearman's rank correlation coefficient between slope of regression lines and SDS sedimentation volume were significant ($P \leq 0.05$) for both whole meal ($r = 0.91$) and flour ($r = 0.90$), indicating that lines with the highest SDS sedimentation volumes also had the largest protein concentration-SDS sedimentation volume response (Fig. 4).

An increase in both protein concentration and sample weight resulted in an increase in the amount of protein available to swell in the SDS sedimentation test. Results of Experiments 1 and 2 confirmed that SDS sedimentation volumes of lines did not respond uniformly to increasing protein concentration, and this difference was likely the cause of the significant line-by-environment interaction detected in analysis of variance. It is important to emphasize that these results indicated that the SDS sedimentation test responded to protein quantity as well as protein quality.

Lorenzo and Kronstad (1987) suggested that the SDS sedimentation test should only be used when accompanied by protein con-

centration because of its large effect on SDS sedimentation test results. Baik et al (1994) attempted to overcome the effect of protein concentration by running the SDS sedimentation test on a constant protein basis. Results of our study indicate that the SDS sedimentation test is affected by protein concentration and that the magnitude of the effect is not the same for all lines. A differential line response to protein also was reported for the Zeleny sedimentation test (Kitterman and Barmore 1969). This differential response must be considered if any correction for protein concentration is to be undertaken. For example, when using the whole meal SDS sedimentation test to differentiate among lines, a stronger gluten soft wheat line at 9% protein concentration may have the same SDS sedimentation volume as a weaker gluten line at 13% protein concentration, and it will be impossible to differentiate between them. In theory, lines could be grown in multiple environments to produce grain with different protein concentrations, and the SDS sedimentation volumes for all lines could be corrected using the adjusted SDS sedimentation volume means from analysis of covariance. However, in early generation testing, the response trend of a particular line would not be known, and multiple location testing is not possible due to limited grain supplies. The protein effect may not be as important in soft white and club wheats because lines with high protein concentrations are undesirable and are eliminated from further testing. This narrows the range of protein concentrations among lines to be tested with the SDS sedimentation test, which decreases the effect of protein concentration on SDS sedimentation volumes.

The differential effect of protein concentration on SDS sedimentation volumes among lines was considered a prominent contributor to the significance level of the line-by-environment interaction (i.e., nonparallel slopes) detected in Experiment 1. A subset of 21 lines was used to further investigate this interaction. Only whole meal was used for this study because results described earlier indicated that whole meal produced results similar to those for flour. The error due to using duplicated samples from blended grain in 1997 did not differ significantly ($P \leq 0.05$) from the error associated with using field replicates in 1998. This indicated that the experimental error was not increased by field variation. Even when multiple environments and lines were included in the analysis, line again explained the largest percentage of total variance, followed by environment and line-by-environment interaction as in Experiment 1 (Table II). To determine whether protein concentration indirectly contributed to the large amount of environmental and line-by-environment variation detected, analysis of covariance with protein as the covariate was performed. All mean square values decreased when protein was included in the model, however, the most dramatic decrease occurred for the environmental mean squares, whereas the line mean squares were least affected. The mean square for protein (covariate) was particularly large, which confirmed the conclusion that the SDS sedimentation volumes are highly influenced by protein concentration (Preston et al 1982, Dick and Quick

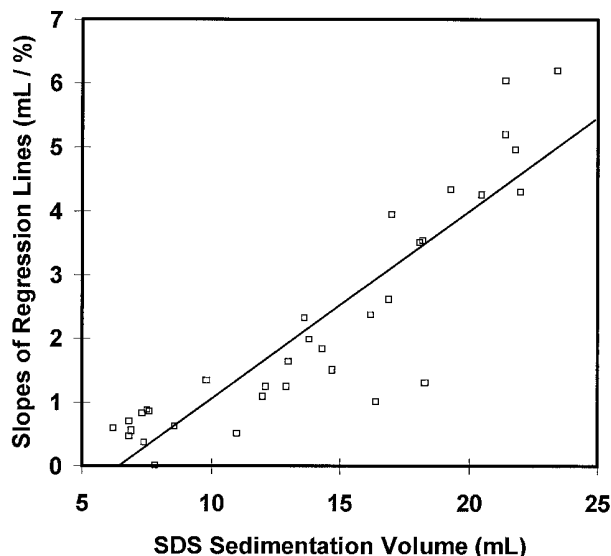


Fig. 4. Plot of slopes of regression lines (SDS sedimentation volume per percentage point of protein concentration) and SDS sedimentation volumes for whole meal for 34 lines (□) grown in eight environments during 1997. $R^2 = 0.81$, $y = 0.2946x - 1.8936$.

TABLE II
Percentages of Total Variation, Mean Squares, and Tests of Significance for Each Source of Variation from Analysis of Variance and Covariance (with protein as covariate) for SDS Sedimentation Volumes of 21 Soft White and Club Wheat Lines Grown in 16 Environments (8 locations in Washington in 1997 and 1998)^{a,b}

Source of Variation	df	% of Total Variation	Mean Squares	
			Analysis of Variance	Analysis of Covariance
Line (L)	20	45.4	589.6	586.5
Environment (E)	15	20.0	349.8	84.2
L × E	300	21.0	21.7	14.2
Error	336	13.6	5.3	4.0
Protein (covariate)	1		...	431.4

^a All mean squares were significant at $P \leq 0.01$.

^b All SDS sedimentation tests were performed in duplicate.

1983, Lorenzo and Kronstad 1987, Ayoub et al 1993). The dramatic decrease in environmental mean squares when protein concentration was considered a covariate indicated that the significant environmental effect that was detected was actually confounded by the protein effect. When the protein effect was accounted for, the ability of the SDS sedimentation test to differentiate among lines increased because the environment effect was greatly reduced and the line-by-environment effect also decreased. Accounting for the protein effect enhances the ability of the SDS sedimentation test to differentiate among lines, thereby facilitating a breeder's ability to select lines with weak gluten strength.

Results indicate that the SDS sedimentation test is a desirable, small-scale test that can be performed using whole meal on a range of different sample weights. Although SDS sedimentation volumes are affected by protein concentration, the effect either can be controlled as a covariate or may be of little practical significance because the range in protein concentration among lines may be small, and low protein, weak gluten types are desired. Consequently, the SDS sedimentation test should be effective for selection among soft white and club wheat lines.

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