Assessment of Enzyme-Linked Immunoassay of Rye Secalins as a Tool in the Prediction of 1RS Wheat Quality[1]

Y. W. SEO,[2] R. A. GRAYBOSCH,[3,4] C. J. PETERSON,[3] and D. R. SHELTON[2]

Cereal Chem. 72(3):252–254

ABSTRACT

Incorporation of rye chromatin into the wheat genome often has resulted in a subsequent loss of dough quality. A monoclonal antibody, specific for secalins (rye endosperm storage proteins) was tested as a means of consistently identifying wheats carrying rye chromosome arm 1RS and predicting their end-use quality. Mean absorbances (A) in enzyme-linked immunosorbent assays (ELISA) of 1AL/1RS or 1BL/1RS wheats from 16 production environments were significantly higher than those observed with non-1RS wheats. Significantly higher absorbances also were observed in 1AL/1RS wheats as compared to 1BL/1RS wheats. Among 1AL/1RS wheats, however, ELISA absorbances were associated with increased total flour protein content, a factor contributing to higher wheat quality scores. Among 1BL/1RS wheats, where absorbances were independent of total protein content, significant negative correlations with quality were detected. ELISA using this antisecalin monoclonal antibody has more utility as a means of identifying 1RS wheats in breeding programs than as a tool for predicting flour quality in the grain trade.

Over the past two decades, wheat (*Triticum aestivum* L.) breeders have used the short arm of rye (*Secale cereale* L.) chromosome 1R as a source of genes for disease- and pest-resistance and improved agronomic performance. Both 1AL/1RS and 1BL/1RS translocations are now common in breeding programs in the United States. Unfortunately, pronounced defects in various end-use quality parameters have been associated with breeding lines and cultivars carrying 1RS (Law and Payne 1983; Zeller and Hsam 1984; Moonen and Zeven 1984; Martin and Stewart 1986; Dhaliwal et al 1987, 1988; Graybosch et al 1990). In the United States, commercial bread bakeries cannot utilize flour solely from cultivars carrying 1RS translocations.

Increased proportions of water-soluble proteins and the presence of rye endosperm storage proteins (secalins) have been suggested as factors contributing to the poor end-use quality of 1RS wheats (Dhaliwal et al 1988). The weak and sticky dough properties of the translocation lines, alternatively, may arise from lower quantities of polymeric glutenin proteins (Dhaliwal and MacRitchie 1990; Graybosch et al 1990, 1993a) caused by the loss of the low molecular weight glutenin subunits encoded by the short arms of wheat chromosome 1A or 1B (MacRitchie et al 1988). The simultaneous loss of glutenin and addition of secalins in 1RS wheats complicates a definition of the true quality effects of rye proteins in wheat.

The presence of secalins in 1RS wheats might serve as both a marker for this particular chromosome arm, as well as an indicator of quality. If secalin concentration in grain or flour was negatively correlated with grain and flour quality, grain millers and flour bakers would be able to estimate flour quality before shipments reached production plants. Recently, an enzyme-linked immunosorbent assay (ELISA) procedure for the identification of secalins in flour and grain samples was developed (Graybosch et al 1993b). The system was capable of identifying wheats carrying 1RS in segregating breeding populations and amongst a limited sampling of the cultivars from five locations. In the present study, ELISA with antisecalin monoclonal antibody was used to characterize flours of 11 wheat genotypes obtained from diverse Nebraska locations to: 1) determine the interaction effects of genotype, environment, and genotype by environment on secalin concentrations as measured by ELISA; 2) assess the relationship between ELISA absorbances and end-use quality; and 3) test the ELISA on samples from more diverse environments than used in previous studies (Graybosch et al 1993b) to verify its capacity for consistent identification of 1RS wheats.

MATERIALS AND METHODS

Plant Materials and Experimental Design

Grain samples were obtained from five 1AL/1RS wheats (TAM-107, TAM-200, TAM-202, TXGH12588, and Century), three 1BL/1RS wheats (Siouxland, KS8010-72, and Rawhide) and three non-1RS wheats (Karl, Scout 66, and Arapahoe). Rawhide is heterogeneous for 1BL/1RS; the remaining 1RS wheats are homogeneous. Genotypes were assigned to three chromosome classes: 1AL/1RS, 1BL/1RS, and non-1RS. The lines were seeded at eight Nebraska locations in 1990 and 1991: Lincoln, Clay Center, McCook, North Platte, Grant, Sidney (two plantings, dryland and irrigated), Scottsbluff, and Alliance. Crop failures occurred at Sidney (irrigated) in 1990 and Alliance in 1991, restricting the analysis of data to 16 of the 18 possible production environments. The experimental design was a randomized complete block design with five replicates. Grain harvested from two replicates of each production environment were used for the ELISA experiments.

ELISA

Methods used for the production and characterization of antisecalin monoclonal antibody were described elsewhere (Graybosch et al 1993b). Proteins were extracted from 25 mg of flour with 1 ml of 0.04*M* NaCl by agitating 200 rpm at 4°C for 30 min. After centrifugation at 14,000 × *g*, supernatants were saved for determination of total extracted protein and for use in ELISA. Each sample extract was diluted 1:20 for determination of 0.04*M* NaCl saline extractable protein (SEP) content using the Bradford protein assay (Bio-Rad, Richmond, CA) using bovine serum albumin as a standard. Protein assays were conducted in 96-well polyvinylchloride assay plates (Costar, Cambridge, MA). Protein concentration was measured as absorbance at 595 nm. The samples were further diluted to a final concentration of 1:42 and added to Immulon-2 plates (Dynatech, Chantilly, VA) for ELISA. ELISA was conducted as previously reported (Graybosch et al 1993b). Results were expressed as A/25 mg of flour, and as A/ng of SEP.

[1]Joint contribution of the USDA-ARS and the Department of Agronomy, University of Nebraska-Lincoln as journal series paper 10781.
Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture or the University of Nebraska over other firms or products not mentioned.

[2]Department of Agronomy, University of Nebraska, Lincoln.
[3]USDA-ARS, Department of Agronomy, University of Nebraska, Lincoln.
[4]Corresponding author.

Quality Parameters

Grain samples were composited for each genotype over replicates to provide adequate quantities for milling and baking. Grain was tempered to 15.2% moisture and milled on a laboratory mill (Buhler, Minneapolis, MN) to yield white flour for baking. Samples of grain (50 g) from each of two replicates also were milled on a Brabender Quadrumat experimental mill (C. W. Brabender, South Hackensack, NJ) for ELISA and for analysis of mixograph properties and sodium dodecyl sulfate (SDS) sedimentation volumes.

Flour protein content (0% mb) was measured using near-infrared reflectance spectroscopy (NIR). Dough mixing characteristics were evaluated using a 10-g mixograph (National Mfg., Lincoln, NE) (AACC 1983). Mixogram variables were scored by using the Mixsmart computer program (National). Mixograph peak time was measured as time (minutes) to peak dough development. Mixograph tolerance was measured as the width of the mixogram curve at 2 min past peak dough development. SDS sedimentation volume was measured on a 2-g flour sample (14% mb) (AACC 1983).

Bake water absorption (%) was determined by a skilled baker, while 200 g of flour was mixed in a pin-type mixer (National). The baking method was a standard 100-g pup loaf method (AACC 1983).

Data Analyses

ELISA absorbances were normalized using a standard curve developed with dilutions of a common flour sample of TAM-107. Statistical Analysis System (SAS 1985) procedures and programs were used for data analyses. Analysis of variance (ANOVA) was calculated considering each year-location as a separate environment. Environment was treated as a random effect, and genotype was considered a fixed effect. Significance of mean squares associated with environments was tested using replication (environment) as the error term; the genotype by environment mean square was used to test significance of genotypic mean squares. Single degree of freedom contrasts were used to detect differences between major chromosome classes (1AL/1RS, 1BL/1RS, and non-1RS). Mean squares also were partitioned within each chromosome class. Simple correlations were calculated between absorbances and flour quality parameters, and were compared with those observed between flour protein content and the remaining quality parameters.

TABLE I
Mean Squares from Analysis of Variance of Enzyme-Linked Immunoassay Absorbances of Flour from 11 Hard Red Winter Wheats Grown in 16 Environments

Source of variation	df	A/25 mg of Flour	A/ng of Saline Extractable Protein
Environment	15	0.1256**[a]	2.538**
Replicate (Environment)	16	0.0007	0.095
Genotype	10	0.6221**	20.565**
Non-1RS vs. 1RS	1	4.3493**	133.2034**
1AL/1RS vs. 1BL/1RS	1	1.6878**	69.9350**
Within 1AL/1RS	4	0.0317**	0.448
Within 1BL/1RS	2	0.0406**	0.55
Genotype × environment	150	0.0042**	0.243**
Environment × (1AL/1RS vs. 1BL/1RS)	15	0.0150**	0.9204**
Environment × 1AL/1RS	60	0.0017**	0.112**
Environment × 1BL/1RS	30	0.0013*	0.07
Pooled Error	159	0.0008	0.065

[a]** = Significant at $P = 0.01$, * = significant at $P = 0.05$.

RESULTS

ANOVA (Table I) mean squares of A/25 mg of flour and A/ng of SEP were highly significant for environments, for genotype, and for genotype by environment interaction. Therefore, secalin production, as measured by ELISA, was subject to modification by both the genetic background in which 1RS resided, and the cultural environment in which the wheat was grown. 1AL/1RS wheats displayed significantly higher means of both A/25 mg of flour and A/ng of SEP than did 1BL/1RS wheats. Mean responses of all 1RS wheats were significantly higher than those of non-1RS wheats. This conclusion is supported by significant mean squares for the contrasts of non-1RS versus 1RS wheats and 1AL/1RS versus 1BL/1RS wheat. Within each environment (data not shown), all 1RS lines displayed significantly higher absorbances than did non-1RS wheats, with the exception of one sample of Rawhide, the heterogeneous 1BL/1RS genotype. Mean squares were highly significant for A/25 mg of flour, but not for A/ng of SEP, for genotypic differences, and for the interaction of genotypic and environmental effects within both 1AL/1RS and 1BL/1RS classes. The lack of differences within each class for A/ng of SEP suggests differences in A/25 mg of flour are a consequence of genotypic differences in the protein content of the 0.04*M* NaCl extracts.

Mean quality characteristics for 1AL/1RS and 1BL/1RS wheats, averaged across both genotypes and environments, are listed in Table II. Mean values of flour protein content, mixograph time, bake absorption, and loaf volume were similar in the two classes. 1AL/1RS wheats displayed higher mean values for mixograph tolerance and SDS sedimentation volumes than did 1BL/1RS wheats. A broad range of response for each trait was detected in each class.

Since both A/25 mg of flour and A/ng of SEP of 1AL/1RS were significantly higher than those of 1BL/1RS wheats, correlations with quality parameters were calculated separately for each class. Within the 1AL/1RS class, A/25 mg of flour and A/ng of SEP were significantly correlated with several quality parameters (Table III). All observed correlations, with the exception of the correlations with mixograph time, were positive. Both A/25 mg of flour and A/ng of SEP were significantly correlated to flour protein content. Hence, all correlations of A/25 mg of flour and A/ng of SEP with quality parameters were nearly identical to those observed with total flour protein.

Among 1BL/1RS samples (Table III), no significant correlations were observed between either A/25 mg of flour or A/ng of SEP, and increased flour protein content. Both A/25 mg of flour and A/ng of SEP were negatively correlated with mixograph time and mixograph tolerance. Flour protein, conversely, displayed no correlation with mixograph time, and a significant positive correlation with mixograph tolerance among 1BL/1RS samples. Within both 1AL/1RS and 1BL/1RS wheats, correlations were never of a magnitude that would justify use in selection of samples on the basis of quality potential.

TABLE II
Means, Standard Deviations (SD), and Ranges of Quality and Biochemical Parameters for 1AL/1RS and 1BL/1RS Chromosome Wheat Classes

	1AL/1RS (n = 80)				1BL/1RS (n = 48)			
Variable	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Flour protein content (%)	11.9	1.7	8.4	17.3	12.5	1.7	9.3	18.3
Sodium dodecyl sulfate sedimentation volume (ml)	32.4	5.5	20.0	44.5	25.6	4.7	12.5	34.5
Mixograph time (min)	3.9	1.3	1.9	9.1	4.0	1.2	2.5	7.7
Mixograph tolerance (mm)	17.3	3.1	10.1	24.8	13.7	4.5	6.6	24.6
Bake absorption (%)	61.8	2.1	57.0	65.0	60.6	1.9	54.5	63.0
Loaf volume (ml)	897	105	645	1,080	899	81.9	740	1,100

TABLE III
Statistically Significant Simple Correlations Between Enzyme-Linked Immunoassay Variables and Quality Traits for 1AL/1RS and 1BL/1RS Chromosome Wheat Classes

Variable	1AL/1RS			1BL/1RS		
	A/25 mg Flour	A/ng Saline Extractable Protein	Flour Protein Content	A/25 mg Flour	A/ng Saline Extractable Protein	Flour Protein Content
Flour protein content	0.24*[a]	0.58**	...			...
Sodium dodecyl sulfate sedimentation volume		0.30**	0.34**			0.51**
Mixograph time	−0.55**	−0.42**	−0.46**	−0.59**	−0.44**	
Mixograph tolerance	0.32**	0.52**	0.60**	−0.47**	−0.30**	0.53**
Bake absorption	0.45**	0.29**	0.29**			0.52**
Loaf volume		0.32**	0.56**			0.53**

[a] * = Significant at $P = 0.05$, ** = significant at $P = 0.01$.

DISCUSSION

ELISA using the antisecalin monoclonal antibody again proved to be a reliable method for the identification of flour samples derived from both 1AL/1RS and 1BL/1RS wheats. Samples of eight 1RS genotypes from 16 production environments consistently exhibited higher absorbances than did the non-1RS wheats. The ELISA did not, however, separate wheat samples on the basis of intrinsic quality potential. Among 1AL/1RS wheats, the association of increased ELISA absorbance with flour protein content, a factor correlated with higher quality scores, masked any possible negative effects of secalin on quality. The significant positive correlations of both A/25 mg of flour and A/ng of SEP to total flour protein content, and the positive correlation of A/ng of SEP to SDS sedimentation volumes, suggest secalin expression was tightly coupled to increasing flour gluten protein content and is likely regulated in a manner similar to that of the gliadin and glutenin proteins of wheat. In other words, better quality 1AL/1RS samples possessed more total protein, more gluten protein, more secalin, and gave higher ELISA absorbances. Environmental conditions favoring wheat quality evidently favor secalin production as well.

Comparison of the responses of the two types of translocations suggests that secalins may, in fact, negatively contribute to quality. In 1BL/1RS wheats, where secalin expression was independent of total protein content, negative correlations with quality were observed. Diminished secalin synthesis, effected either by selection or genetic engineering, could result in improved quality of 1BL/1RS wheats. Whether such a strategy would result in improved quality 1AL/1RS wheats is less clear. Attempts at such manipulations, however, should be accompanied by simultaneous efforts to increase glutenin contents. Loss of glutenin, the flour proteins responsible for the visco-elastic properties of wheat, has been identified as a major defect of 1BL/1RS wheats (Graybosch et al 1993a).

Several possible hypotheses might explain the higher ELISA readings of 1AL/1RS versus 1BL/1RS wheats. 1AL/1RS wheats, based on experiments in our laboratory (data not shown) using alternative methods such as high-performance liquid chromatography or SDS polyacrylamide gel electrophoresis, do not appear to produce more secalin than 1BL/1RS wheats. The secalins produced by the two translocations more likely differ in either affinity for the antibody or binding to the ELISA plate surface. The higher concentrations of SEP proteins observed in 1BL/1RS wheats (Graybosch et al 1993a) also could be a factor, due to increased competition for binding sites on the plate surfaces. Finally, the 1AL/1RS and 1BL/1RS translocations were derived from different rye sources (Zeller and Hsam 1984) and very likely carry different alleles at the secalin-encoding locus. Given the high mutation rate of genes encoding cereal storage proteins (Metakovsky et al 1993), extensive polymorphisms for characteristics such as antibody epitopes within rye are quite probable.

In conclusion, ELISA using the antisecalin monoclonal antibody is best suited for the identification of 1RS wheats in breeding programs. Use of the ELISA in the grain trade also will identify grain or flour derived from 1RS wheats, but the observed absorbances will not correlate with lower quality, unless one is dealing only with 1BL/1RS wheats.

ACKNOWLEDGMENT

Financial support from ConAgra Grain Processing Company is gratefully acknowledged.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 56-61A approved April 1964, reviewed October 1982. The Association: St. Paul, MN.

DHALIWAL, A. S., and MacRITCHIE, F. 1990. Contributions of protein fractions to dough handling properties of wheat-rye translocation cultivars. J. Cereal Sci. 12:113-122.

DHALIWAL, A. S., MARES, D. J., and MARSHALL, D. R. 1987. Effect of 1B/1R chromosome translocation on milling and quality characteristics of bread wheats. Cereal Chem. 64:72-76.

DHALIWAL, A. S., MARES, D. J., MARSHALL, D. R., and SKERRITT, J. H. 1988. Protein composition and pentosan content in relation to dough stickiness of 1B/1R translocation wheat. Cereal Chem. 65:143-149.

GRAYBOSCH, R. A., PETERSON, C. J., HANSEN, L. E., and MATTERN, P. J. 1990. Relationships between protein solubility characteristics, 1BL/1RS, high molecular weight glutenin composition, and end-use quality in winter wheat germ plasm. Cereal Chem. 67:342-349.

GRAYBOSCH, R. A., PETERSON, C. J., HANSEN, L. E., WORRALL, D., SHELTON, D. R., and LUKASZEWSKI, A. 1993a. Comparative flour quality and protein characteristics of 1BL/1RS and 1AL/1RS wheat-rye translocation lines. J. Cereal Sci. 17:95-106.

GRAYBOSCH, R. A., SEO, Y. W., and PETERSON, C. J. 1993b. Detection of wheat-rye chromosomal translocations using an antisecalin monoclonal antibody. Cereal Chem. 70:458-463.

LAW, C. N., and PAYNE, P. I. 1983. Genetic aspects of breeding for improving grain protein content and type in wheat. J. Cereal Sci. 1:79-93.

MacRITCHIE, F., du CROS, D. L., and WRIGLEY, C. W. 1988. Flour polypeptides related to wheat quality. Pages 78-145 in: Advances in Cereal Science and Technology, Vol. X. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

MARTIN, D. J., and STEWART, B. G. 1986. Dough mixing properties of a wheat-rye derived cultivar. Euphytica 35:225-232.

METAKOVSKY, E., DAVIDOV, S. D., CHERNAKOV, V. M., and UPELNIEK, V. P., 1993. Gliadin allele identification in common wheat. III. Frequency of occurrence and appearance of spontaneous mutations at the gliadin-coding loci. J. Genet. Breed. 47:221-236.

MOONEN, J. H. E., and ZEVEN, A. C. 1984. SDS-Page of the high-molecular-weight subunits of wheat glutenin and the characterization of 1R(1B) substitution and 1BL/1RS translocation lines. Euphytica 33:3-8.

SAS. 1985. SAS User's Guide: Statistics. Ver. 5 ed. The Institute: Cary, NC.

ZELLER, F. J., and HSAM, S. L. K. 1984. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. Pages 161-173 in: Proc. Int. Wheat Genet. Symp., 6th Kyoto University: Kyoto, Japan.

[Received July 6, 1994. Accepted February 23, 1995.]