Detection of Wheat-Rye Chromosomal Translocations Using an Antisecalin Monoclonal Antibody[1]

ROBERT GRAYBOSCH,[2] YONG WEON SEO,[3] and C. JAMES PETERSON[2]

Cereal Chem. 70(4):458-463

ABSTRACT

Hybridoma lines derived from mice immunized with a secalin preparation from the rye (*Secale cereale* L.) cultivar Rymin were analyzed for differential reactions with rye secalins and wheat (*Triticum aestivum* L.) gliadins. A single cell line, secreting a monoclonal antibody (MAB) with high affinity for rye secalin but comparatively low cross-reactivity with gliadin, was identified. After electrophoretic separation of unreduced proteins, immunoblotting experiments demonstrated specific binding to omega-secalins encoded by genes located on rye chromosome 1RS. When used in indirect enzyme-linked immunosorbent assay, the antisecalin monoclonal antibody allowed the discrimination of rye from wheat, the estimation of the percentage of rye in mechanical mixtures of wheat and rye, and the identification of wheat lines carrying wheat-rye chromosomal translocations involving 1RS.

Monoclonal antibodies (MABs) raised against the endosperm storage proteins of cereal grains have been used in a number of investigations to demonstrate immunological similarities among wheat (*Triticum aestivum* L.) endosperm proteins of various market classes (Skerritt and Underwood 1986) and to examine homologies among endosperm storage proteins of various cereal species (Skerritt and Lew 1990). Ellis et al (1989) used MABs to investigate antigenic affinities among protein fractions of various grain crops known to be toxic to individuals with coeliac disease. Additional applications of MABs include wheat varietal identification (Dawood et al 1989), assignment of chromosomal locations of genes encoding wheat proteins containing specific epitopes (Skerritt et al 1991), and the development of rapid assays for potential use in the prediction of wheat end-use quality (Skerritt 1991a,b).

Wheat, an allohexaploid species, is able to tolerate the addition or substitution of chromosomes or chromosomal fragments (the latter as chromosomal translocations) from related alien species. Numerous additions and translocations from rye (*Secale cereale* L.), species of the genus *Aegilops,* and the perennial wheatgrasses (*Elymus, Agropyron*) have been reported (Shepherd and Islam 1988). In terms of utility to wheat-breeding programs, the most useful source of alien genetic material to date has been rye. Several wheat-rye chromosomal translocations, in which portions of rye chromosomes have replaced the homeologous arms of wheat chromosomes, have been produced (Zeller and Hsam 1983, Koebner and Shepherd 1988). Rye chromosome 1RS has proven especially advantageous and has contributed unique disease resistance, yield potential, and adaptation to stressful environmental conditions. Numerous wheat cultivars carrying either 1AL/1RS or 1BL/1RS have been released; 1BL/1RS is now global in distribution among wheat-breeding programs (Pena et al 1990). However, detrimental effects on various end-use quality parameters have been associated with 1RS (Dhaliwal et al 1987, Graybosch et al 1990).

As specific binding of MABs to grain proteins of cereal species has been demonstrated (Skerritt and Lew 1990), the question arises as to whether MABs could be employed to recognize and quantify alien proteins expressed in wheat genetic backgrounds and, subsequently, to indirectly confirm the presence of alien chromosomal segments. Previous researchers (Howes et al 1989) described the utilization of a MAB raised against wheat gamma gliadin 45 in the identification of wheats carrying 1BL/1RS. The gene encoding gliadin 45 is located on chromosome 1BS. Wheat lines carrying 1BL/1RS do not produce gliadin 45 and, hence, give a negative reaction in assays using this MAB. However, the antigliadin 45 MAB can not be used to detect 1RS present at alternative positions in the wheat genome (e.g., 1AL/1RS or 1DL/1RS). Development of a positive test for the presence of 1RS would allow both universal identification of this chromosome arm and a means to objectively quantify production of rye proteins originating from 1RS. This report describes the development of such a system through the production of an antisecalin monoclonal antibody.

MATERIALS AND METHODS

Plant Materials

Seed of the rye cultivar Rymin and the wheat cultivar Bezostaya-1 were obtained from samples maintained in the Department of Agronomy, University of Nebraska, Lincoln. Samples of 30 wheat varieties and experimental lines, some known to carry 1RS, were obtained from field plantings sown at Lincoln, Nebraska, in 1990. The samples included five 1AL/1RS lines (TAM107, TAM202, TAM200, TXGH12588, and Century), three 1BL/1RS wheats (Siouxland, Rawhide, and KS8010-72), and 22 normal wheats (Abilene, Redland, RedChief, Lancota, Cheyenne, Karl, Vona, Arkan, Scout66, Eagle, N86L177, Lamar, Centurk78, Newton, N86L022, Plainsman V, Cimarron, TAM101, Centura, NE 83407, Chisholm, and Arapahoe). Additional samples of six wheat cultivars, two known to carry 1AL/1RS, two with 1BL/1RS, and two lacking rye chromosomal segments, were obtained from five Nebraska locations (Lincoln, Clay Center, North Platte, Sidney, and Alliance). Seed of 16 experimental lines derived from the cross TX86V6610 × Siouxland were obtained from plants grown in 1990 at both Lincoln, Nebraska, and Vernon, Texas. TX86V6610 carries 1AL/1RS, and Siouxland bears 1BL/1RS. The 16 progeny of TX86V6610 × Siouxland were selected such that four representatives of each of the following classes were obtained: 1AL/1RS, 1BL/1RS, DT (double-translocation lines; both translocations present) and N (normal lines; no translocation present). Five seeds of each line were germinated, and karyotypes were evaluated by C-banding (Lukaszewski and Gustafson 1983). Seed of isolines of the wheat cultivar Gabo, carrying either 2BL/2RS or 1DL/1RS, were obtained from Ken Shepherd, Waite Agricultural Research Institute, Adelaide, Australia. Whole grain rye samples were ground on a Udy Cyclone mill. Wheat samples (with the exception of the Gabo isolines) were tempered to 15.2% moisture and milled on a Brabender Quadraplex experimental mill. Flour and bran were separated using a strand shaker at 225 rpm with a U.S. standard sieve no. 70. Seed of the Gabo isolines were ground in a mortar and pestle, as only limited quantities were available.

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

Preparation of Antigen

Rye secalin proteins were obtained by extraction of 10 g of rye meal with 100 ml of 70% ethanol. Extracts were dialyzed against phosphate-buffered saline (PBS: 136.9 m*M* NaCl, 2.7 m*M* KCl, 10.1 m*M* Na_2HPO_4, 1.8 m*M* KH_2PO_4, pH 7.0). The resulting protein suspension was used for immunizations. Gliadin proteins were obtained in a similar manner from Bezostaya-1. Protein determinations used the bicinchoninic acid method (Smith et al 1985).

Production of Monoclonal Antibodies

The secalin protein preparation from Rymin was used to immunize BALB/C mice. Immunization procedures and production of hybridomas followed the protocols of Dawood et al (1989). Secalin and gliadin preparations (diluted in PBS) were added to microtitre plates at a concentration of 500 ng per well. Hybridoma cells secreting MABs, as determined by ELISA (see below), were screened against both secalin and gliadin preparations to isolate cell lines with the desired differential reaction to secalins. Antibody isotyping was accomplished with the Isotype Ab-STAT II kit (Sangstat Medical, Menlo Park, CA).

Immunoblot Analysis

Proteins were solubilized by extracting 25 mg of grain or flour samples with 1 ml of either 70% ethanol or 0.04*M* NaCl. Samples were extracted with constant shaking (200 rpm, 4°C, 1 hr). After centrifugation, samples were dried. Protein reduction, alkylation, and separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was as reported by Graybosch and Morris (1990). Unreduced protein samples were obtained through omission of steps involving dithioerythritol and 4-vinylpyridine. After electrophoresis, proteins were transferred to 15- × 15-cm nitrocellulose membranes using a Sartoblot dry-transfer apparatus (Sartorius, Long Island, NY), following procedures as outlined by the manufacturer. After transfer, membranes were washed once in 100 ml of Tris-buffered saline (TBS: 100 m*M* TRIS-HCl, 0.25*M* NaCl, pH 7.4) and allowed to air-dry overnight. Membranes were blocked by incubation in 100 ml of 1% polyvinylalcohol in TBS-Tween (0.3% Tween 20 in TBS) with constant agitation at ambient temperatures for 1 hr. Blocking solution was removed, and membranes were incubated in 100 ml of primary antibody solution (1:100 dilution of antibody-containing media in TBS) for 1 hr at ambient temperature. Membranes were subjected to four 20-min washes with TBS-Tween, followed by one wash in incubation buffer (100 m*M* Tris-HCL, 100 m*M* NaCl, 50 m*M* $MgCl_2$, pH 9.5) and incubated in protein-A-alkaline phosphatase conjugate, 1 mg/ml (Sigma Chemical Co., St. Louis, MO), diluted 1:1,000 in incubation buffer. After two washes in TBS-Tween, cross-reacting bands were detected by incubation in 0.4% (w/v) nitro blue tetrazolium chloride and 0.2% (w/v) 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine in incubation buffer.

Enzyme-Linked Immunosorbent Assay (ELISA)

DynaTech Immulon 2 microtitre plates (Dynatech Laboratories, Chantilly, VA) were used for all ELISAs. The 70% ethanol and 0.04*M* NaCl-soluble proteins (see Preparation of Antigen above) were incubated (50 µl per well) in microtitre plates at 37°C overnight. Plates were flicked dry and blocked for 1 hr with 1% polyvinylalcohol (150 µl per well) in PBS. Blocking solution was removed, and appropriate dilutions of antisecalin MAB were added (50 µl per well) for 1 hr at 37°C. Antisecalin MAB solution was removed, and plates were washed four times with PBS + 0.5% Triton X-100 and twice with deionized water. Wells were incubated with a solution of goat antibody to mouse immunoglobulin (Sigma) in PBS + 2% bovine serum albumin (BSA, Sigma) for 90 min. Plates were washed as above, and rabbit antigoat alkaline-phosphatase conjugate (Sigma), diluted in diluent buffer (1 m*M* $MgCl_2$, 50 m*M* Tris-HCl, pH 8.0, 0.04% NaN_3, 2% BSA) was added for 90 min. After washing as described above, 150 µl of substrate (0.04% *p*-nitrophenyl phosphate in 0.1*M* diethanolamine buffer, pH 9.5) was added. Reactions were stopped by addition of 50 µl of 4*N* NaOH. After 30 min, absorbances (405 nm) were recorded using a Bio-Tek ELISA plate reader (Bio-Tek Instruments, Winooski, VT). Mean absorbances were determined from a minimum of four microtitre wells and two separate microtitre plates; differences among sample means were tested by calculation of least significant difference (LSD) using mean squares from the appropriate error terms (Steel and Torrie 1980).

ELISA was also performed using protein extracted from single kernels to verify homogeneity of specific samples. Kernels were sliced into quarters with a razor blade and extracted with 1 ml of 0.04*M* NaCl at room temperature with constant agitation for 1 hr. After centrifugation, 40 µl of supernatant was removed and added to each microtitre plate well. Plates were processed as described above.

RESULTS AND DISCUSSION

Out of 800 sample hybridoma lines of cellular fusions, one secreted a MAB with a high affinity for secalin proteins and a comparatively low cross-reaction with gliadin proteins. This MAB, 3C1F2, belonged to the IgG_1 class of immunoglobulins. SDS-PAGE and immunoblot analysis of reduced and unreduced 70% ethanol-soluble proteins of Rymin rye and representative wheat cultivars are illustrated in Figures 1 and 2. The Rymin sample (Fig. 1, lanes 13 and 14) contained three groups of secalins that are typical of rye (Shewry et al 1983): 75K gamma-secalins, 45K omega-secalins, and 40K gamma-secalins. Rye chromosome 1RS carries genes encoding both the 45K omega-secalins and the 40K gamma-secalins; the 75K gamma-secalins arise from genes on chromosome arm 2RS (Shewry et al 1986). In the two 1AL/1RS wheat lines (TAM202, lane 7; TXGH12588, lane 9) included in Figure 1, 45K omega-secalins and 40K gamma-secalins were evident in the unreduced samples. The remaining wheats illustrated in Figure 1 are normal wheats, lacking both rye chromosome 1RS and secalins. Figure 2 illustrates the results of probing of an immunoblot of the same samples present on the SDS gel (Fig. 1). In the unreduced samples, positive reactions were detected only in the samples of Rymin rye (Fig. 2, lane 14) and the two 1AL/1RS wheats (Fig. 2, lanes 8 and 10). In Rymin, the antisecalin MAB bound to the 45K omega-secalins and to some high

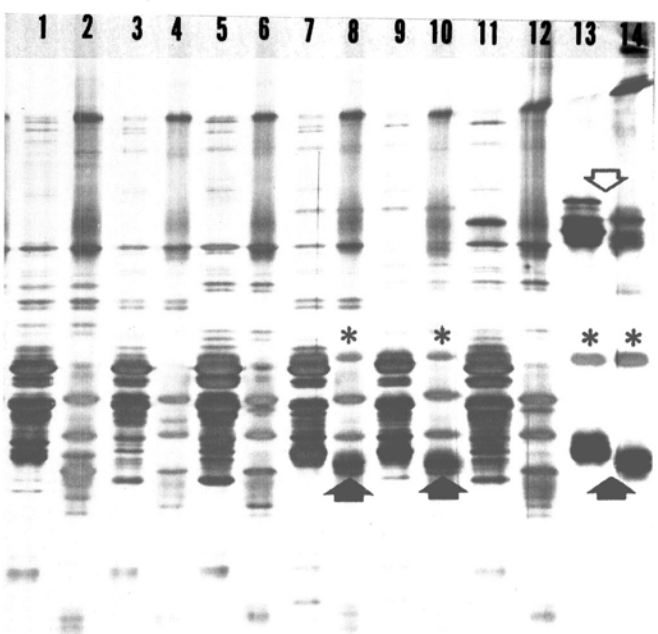

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis separations of 70% ethanol-soluble flour proteins. Odd-numbered lanes contain reduced and alkylated proteins, even-numbered lanes contain unreduced proteins. Lanes 1 and 2, RedChief; 3 and 4, NE83407; 5 and 6, Karl; 7 and 8, TAM202 (1AL/1RS); 9 and 10, TXGH12588 (1AL/1RS); 11 and 12, Scout66; 13 and 14, Rymin rye. * = 45K omega-secalins, closed arrows = 40K gamma-secalins, open arrow = 75K secalins.

molecular weight proteins. The latter likely represent polymers of the 75K gamma-secalins that form multimers through intermolecular disulfide bonds (Field and Shewry 1987). The 45K omega-secalins lack cysteine residues and, consequently, do not form polymers (Shewry et al 1983). Curiously, the monomeric, unreduced 75K secalins showed little cross-reactivity in this experiment. After reduction and alkylation, proteins from all samples, including the nontranslocation wheats, showed cross-reactivity. Bound proteins included all secalins from Rymin rye (Fig. 2, lane 13), both 45K omega-secalins and 40K gamma-secalins in the 1AL/1RS wheats (Fig. 2, lanes 9 and 11), and gliadins with M_r of 36K–45K in the normal wheat samples. Comparison of the results of reduced and unreduced samples indicates that the epitope recognized by the antisecalin MAB is available in unreduced omega-secalins. Similar epitopes evidently exist in some wheat gliadins and additional secalins, but the conformation of the proteins in unreduced form is such that the epitope is either unrecognizable or not present on the surface of the protein molecules.

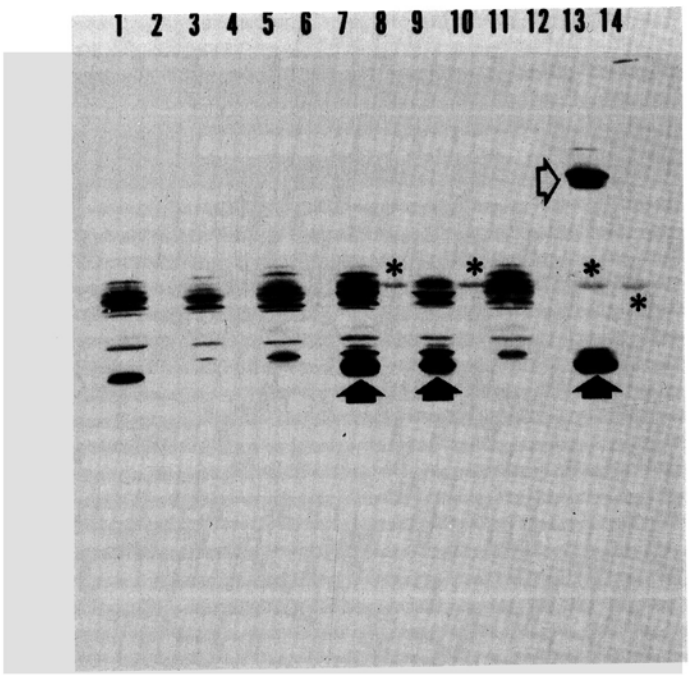

Fig. 2. Immunoblot of gel illustrated in Fig. 1. with same lane designations and symbols.

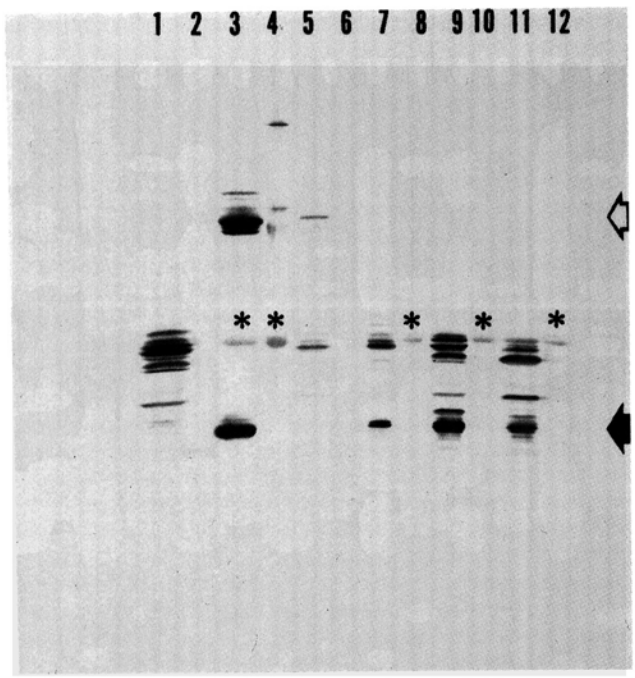

Fig. 3. Immunoblot analysis of 70% ethanol-soluble flour proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Odd-numbered lanes contain reduced and alkylated proteins, even-numbered lanes contain unreduced proteins. Lanes 1 and 2, Scout66; 3 and 4, Rymin rye; 5 and 6, Gabo (2BL/2RS); 7 and 8, Gabo (1DL/1RS); 9 and 10, TAM107 (1AL/1RS); 11 and 12, Siouxland (1BL/1RS). * = 45K omega-secalin, closed arrow = 40K gamma-secalins, open arrow = 75K secalins.

Additional wheats carrying wheat-rye chromosomal translocations were also examined by immunoblotting, with similar results (Fig. 3). In the unreduced protein samples, the antisecalin MAB bound to 45K secalins of Rymin (lane 4), Gabo (1DL/1RS) (lane 8), TAM107 (1AL/1RS) (lane 10), and Siouxland (1BL/1RS) (lane 12). Again, binding of the antisecalin MAB to a number of proteins was observed in the reduced samples. Positive reaction with protein from Gabo (2BL/2RS) was detected only after reduction and alkylation (Fig. 3, lanes 5 and 6). Chromosome 2RS carries only genes encoding 75K gamma-secalins; 45K secalins would be expected to be lacking in a wheat line carrying this chromosome arm (Shewry et al 1986). Thus, rye chromosome arm 1RS may be identified by a specific positive response when unreduced 70% ethanol-soluble proteins are probed with the antisecalin MAB. The absence of any similarly detected reduced wheat gliadins in the same M_r range as 75K secalins also allows for specific identification of 2RS.

These experiments were conducted with proteins solubilized by extraction with 70% ethanol. Rye secalins can also be extracted with dilute saline solutions (Dhaliwal et al 1988, Lookhart et al 1991), so additional experiments were conducted with wheat-flour proteins solubilized by 0.04*M* NaCl. Probing of 0.04*M* NaCl proteins separated by SDS-PAGE with the antisecalin MAB demonstrated a more specific reaction than probing 70% ethanol-soluble proteins (Fig. 4). In unreduced samples, 45K omega-secalins were detected in Rymin rye (lane 2), Century (1AL/1RS) (lane 8), and Siouxland (1BL/1RS) (lane 16); no proteins were detected in unreduced samples of normal wheat. The 1BL/1RS wheat, Siouxland, also displayed an additional protein that migrated to a slightly higher apparent molecular weight than did the 45K omega-secalins. All remaining 1BL/1RS wheats examined (data not shown) displayed this additional band, but 1AL/1RS

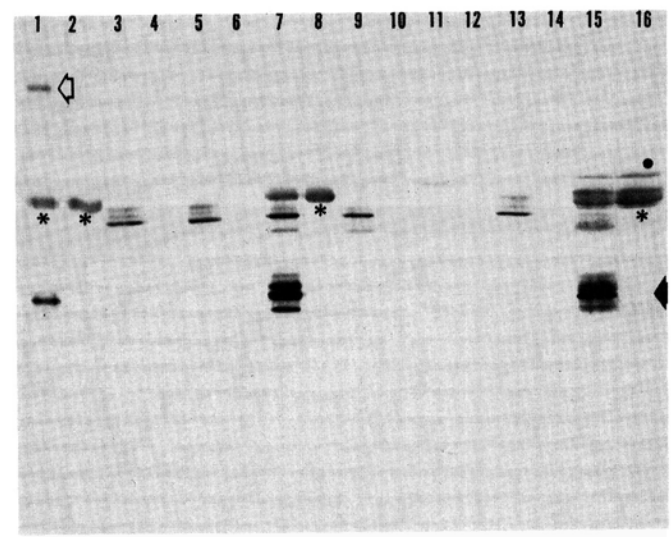

Fig. 4. Immunoblot analysis of 0.04*M* NaCl-soluble flour proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Odd-numbered lanes contain reduced and alkylated proteins, even-numbered lanes contain unreduced proteins. Lanes 1 and 2, Rymin rye; 3 and 4 Scout66; 5 and 6, Newton; 7 and 8, Century; 9 and 10, Cimarron; 11 and 12, Chisholm; 13 and 14, Cheyenne; 15 and 16, Siouxland. * = 45K omega-secalin, closed arrow = 40K gamma secalins, open arrow = 75K secalins, closed circle = additional cross-reacting band in 1BL/1RS lines.

wheats did not. Berzonsky et al (1991) observed similar differences in secalins produced by wheats carrying these two chromosomal translocations. In reduced samples, all three types of secalin were detected in Rymin rye; both 45K omega-secalins and 40K gamma secalins were recognized in the 1RS wheats. Reduced 0.04*M* NaCl-soluble proteins of normal wheat were also bound; such proteins probably represent gliadins that were partially soluble in 0.04*M* NaCl.

ELISA experiments further characterized the antisecalin MAB. Absorbance values of secalin obtained by 70% ethanol extraction of Rymin rye meal were compared to those of gliadin isolated from Bezostaya-1 over a range of protein concentrations (10, 50, 100, 250, 500, and 1,000 ng per well) and at two antibody dilutions. At an antibody dilution of 1:10 (hybridoma cell media supernatant in PBS), significantly higher absorbances were recorded for the secalin preparation up to and including the 250 ng per well antigen concentration. At the lower antibody dilution (1:100), significantly higher responses were observed for all secalin and gliadin comparisons.

The antisecalin MAB was also tested for the ability to specifically recognize secalin protein in secalin-gliadin mixtures. Rymin meal was mixed with flour from the wheat cultivar Scout66 at percentages ranging from 0 to 100% rye. Protein extracted from the mixtures with 70% ethanol was used to coat microtitre wells at a concentration of 200 ng per well. The correlation of absorbance with increasing percent rye in the mixture was highly significant ($r = 0.98$). As little as 10% rye mixed with wheat could be detected. Thus, the antisecalin MAB will allow accurate determination of the amount of rye in wheat-rye mechanical mixtures, assuming similar responses are obtained with all wheat and rye varieties.

Proteins solubilized by 0.04*M* NaCl from Rymin rye, Scout66 (normal wheat), and TAM107 (1AL/1RS) were also compared by ELISA. Over the range of protein dilutions tested (10, 100, 250, and 1,000 ng per well), the TAM107 samples displayed significantly higher absorbances than did Rymin, which, in turn, had much higher absorbances than did Scout66. These data suggest the proportion of 0.04*M* NaCl-soluble protein detected by the antisecalin MAB is actually higher in the wheat-rye translocation line than in Rymin rye.

To test whether the antisecalin MAB displayed consistent specificity for 1RS wheats, as compared to normal wheats of diverse origin, a collection of 30 wheat cultivars and experimental lines was examined. Both 1AL/1RS and 1BL/1RS wheats were included. No 1DL/1RS or 2BL/2RS wheats adapted to Great Plains growing environments have been produced to date and, hence, none could be analyzed. Absorbances were recorded from ELISA using both 70% ethanol-soluble proteins (coated at a concentration of 200 ng per well) and 0.04*M* NaCl (1,000 ng per well) (Fig. 5). Antibody containing cell culture media was diluted 1:100 in PBS. For 70% ethanol-soluble proteins, absorbances of all 1AL/1RS wheats were significantly higher than those of 1BL/1RS wheats and normal wheats; mean responses of 1BL/1RS wheats did not differ from those of normal wheats. No significant differences were observed among the normal wheats. Absorbance values of 70% ethanol-soluble proteins from all samples were relatively low. Increasing the amount of protein coated on the microtitre wells increased the absorbances, but specificity was lost due to nonspecific binding at high-protein concentrations.

Better discrimination of 1RS wheats was obtained among tested wheats using 0.04*M* NaCl-soluble proteins (Fig. 5). Again, the 1AL/1RS wheats displayed significantly higher absorbances than did the 1BL/1RS wheats and all normal wheats. Absorbances of the two homogeneous 1BL/1RS wheats (Siouxland and KS8010-72) were also significantly higher than those of all normal wheats. Again, no differences among normal wheats were detected, suggesting little genetic variation among non-1RS wheats for response in ELISAs with the antisecalin MAB. Rawhide, heterogeneous for 1BL/1RS (Baenziger et al 1992), did not differ from three of the normal wheats. Single-kernel ELISA of Rawhide established the level of heterogeneity (e.g., frequency of 1BL/1RS) to be approximately 50% (results not shown). Identification of 1RS using this antibody might be inconsistent in heterogeneous samples. However, the Rawhide planting was damaged by leaf rust, resulting in a lower than average flour protein concentration (11.9% for Rawhide vs. 14.62% for the location average). Perhaps this contributed to a low concentration of bound antigen.

The ability of the antisecalin MAB to recognize 1RS in wheat lines grown in diverse environments was also examined. Samples of six lines were obtained from five Nebraska locations. Results of ELISA are given in Figure 6. The two 1AL/1RS wheats (Century and TAM107) and the 1BL/1RS wheat (Siouxland) displayed significantly higher absorbances than did the two normal wheats (Redland and Scout66) in all five locations. The remaining 1BL/1RS wheat (OK83396) gave significantly higher responses than did normal wheats in all locations except Alliance. The

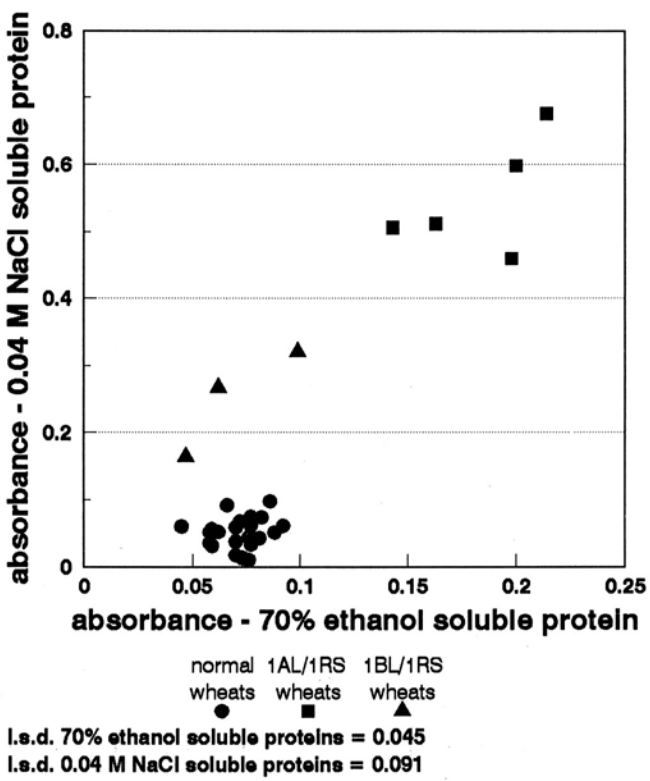

Fig. 5. Binding of antisecalin monoclonal antibody to proteins extracted from flour of 30 wheat varieties grown at Lincoln, Nebraska, 1990. 1AL/1RS and 1BL/1RS wheats carry respective wheat-rye translocation.

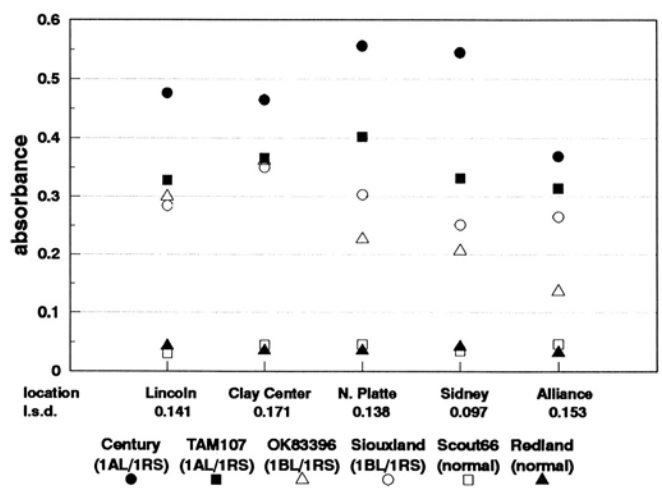

Fig. 6. Binding of the antisecalin monoclonal antibody to 0.04*M* NaCl-soluble flour proteins of six wheat cultivars grown at five Nebraska locations in 1989.

response of OK83396 at Alliance was abnormally low compared to all other locations. Single-kernel ELISA was performed on the OK83396 samples from Lincoln, Clay Center, and Alliance (20 seeds per location). The Lincoln and Clay Center samples produced uniform responses of magnitudes similar to the flour samples. However, the Alliance samples gave a mixed response: approximately 50% of the seed produced the high response typical of 1RS wheats, while the remaining seed gave the low response characteristic of normal wheats. The most likely conclusion is that the Alliance sample was contaminated by an unknown normal wheat during harvest or planting operations. Differences among translocation lines also were detected; therefore, genotypic, environmental, and genotypic × environmental interactions might influence secalin production in wheat lines.

Results of ELISA experiments with 0.04*M* NaCl-soluble proteins demonstrated a possible differential response among 1RS wheats: 1AL/1RS wheats often display significantly higher absorbances than do 1BL/1RS wheats. However, the genetic backgrounds of the 1RS wheats tested were quite different, and a limited number of 1BL/1RS lines were analyzed. To examine this putative differential response among 1RS wheats, 0.04*M* NaCl-soluble proteins, extracted from flour of 16 sister lines derived from the cross TX81V6610 (1AL/1RS) × Siouxland (1BL/1RS), were examined by ELISA. Four homogeneous lines were selected from each of four classes: 1AL/1RS, 1BL/1RS, DT (both translocations present), and normal (neither translocation present). Mean response (averaged over two locations [Lincoln, NE and Vernon, TX]) of all eight single translocation lines (1AL/1RS or 1BL/1RS present) and the four DT lines was significantly higher than that of all normal lines (Fig. 7). One 1BL/1RS line was significantly higher than the remaining single translocation lines. DT lines had significantly higher mean response than did all 1AL/1RS lines and all but one of the 1BL/1RS lines. Thus, when tested in a common genetic background, little differential response was detected among 1RS wheats. The presence of both translocations elevated the concentration of bound antigen above that of nearly all lines carrying single translocations.

Although diverse methods for the identification of 1RS in wheat have been developed (Lookhart et al 1991), none of them offer the potential rapidity and potential for screening large numbers of samples that ELISA does. Thus, the antisecalin MAB provides a system capable of separating 1RS wheats from normal wheats in breeding programs and in the milling and baking industry, and it allows assessment of the influence of genotype and environment on the expression of a foreign gene in wheat.

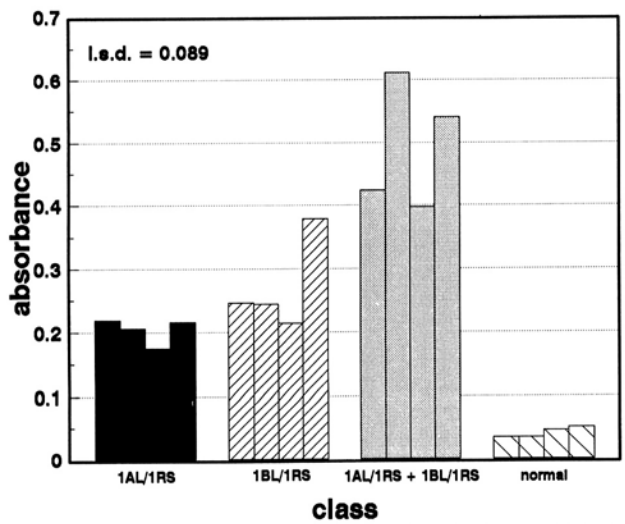

Fig. 7. Binding of the antisecalin monoclonal antibody to 0.04*M* NaCl-soluble flour proteins from sister lines of TX86V6610 × Siouxland grown at two locations.

ACKNOWLEDGMENTS

Monoclonal antibodies were produced in the Monoclonal Antibody Facility, Center for Biotechnology, University of Nebraska, Lincoln. We wish to thank Sandie Franks, Vern Hansen, and Dana Grant for technical assistance, and Ken Shepherd for seed of the Gabo translocation lines and Dave "Cowboy" Worrall for seed of progeny of TX81V6610 × Siouxland. Financial support of ConAgra Grain Processing Company and the Nebraska Wheat Marketing and Utilization Board is gratefully acknowledged.

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[Received August 3, 1992. Accepted January 25, 1993.]