Identification of Wheat Lines Containing the 1BL/1RS Translocation by High-Performance Liquid Chromatography[1]

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ABSTRACT

Analysis by high-performance liquid chromatography (HPLC) of 70% ethanol-solubilized grain proteins was used to identify 1BL/1RS in a sample of 63 experimental wheat lines and six released cultivars. The veracity of this technique was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separation of reduced grain proteins, Southern blotting of genomic DNA followed by hybridization with a rye-specific DNA probe, and direct karyotyping of chromosomes of each tested line. Simultaneous application of multiple techniques for identifying 1RS in wheat genetic backgrounds is suggested as a way to isolate new translocation lines that lack undesirable gene products. Of the methods studied, only HPLC and SDS-PAGE can be used to determine the presence of lines carrying the rye translocation in flours; the other techniques require viable grain samples. Presently, only direct karyotypic analyses can differentiate a 1BL/1RS translocation from a 1AL/1RS or a 1DL/1RS translocation.

Wheat-rye translocations are ideal vehicles for the transfer of desirable genes from rye to wheat (Zeller 1973). The most common translocations in North American wheat breeding programs are the 1BL/1RS translocation, which is found in derivatives of the German lines Salzmunder Bartweizen and Neuzucht, and the 1AL/1RS translocation, which is derived from the wheat cultivar Amigo (Zeller and Hsam 1983). The 1BL/1RS translocation chromosome carries genes for stem, stripe, and leaf rust resistance and for powdery mildew resistance; the 1AL/2RS translocation chromosome carries genes for greenbug and powdery mildew resistance. In addition, a perceived grain yield and protein advantage is associated with each chromosome. The 1BL/1RS translocation lines Kavkaz and Aurora (two of the earliest derived Soviet 1BL/1RS wheat lines) have contributed to the pedigrees of several eastern European lines (Burgas 2, Amika, Istra, Odessa 3, and others), the CIT Veery releases, and the Nebraska variety Siouxland. The 1AL/1RS translocation is present in the Texas varieties TAM 107 and 200 and in the Oklahoma variety Century. In the Twenty-First International Winter Wheat Performance Nursery (1989), 12 of the 29 wheat lines entered contain 1RS (Lukaszewski, *unpublished data*). Thus, it is apparent that lines carrying 1RS are common in wheat production and breeding programs throughout the world.

Despite the numerous beneficial effects associated with 1BL/1RS, deleterious influences on wheat end-use quality have been identified (Zeller and Hsam 1983; Moonen and Zeven 1984; Dhaliwal et al 1987, 1988). The most commonly cited difficulties encountered with 1BL/1RS are reduced gluten strength (diminished mixing tolerance) and dough "stickiness." No objective test of dough stickiness is available, but poor mixing tolerance is easily observed in a number of 1BL/1RS lines. These problems must be overcome if 1BL/1RS wheats are to gain acceptance in the hard red winter and spring wheat growing regions. Future use of 1BL/1RS will no doubt depend on the identification of genetic backgrounds in which the effects are diminished. Thus, simple, rapid means of identifying lines carrying 1BL/1RS wheats must be developed.

Howes et al (1989) reported the use of a monoclonal antibody to a wheat γ-gliadin as a way to identify 1BL/1RS wheat lines. However, this method is based on the absence of a wheat gene product; this absence could result from mutations or chromosomal deletions independent of 1BL/1RS. A positive test for the presence of 1BL/1RS would be more reliable. Koebner and Shepherd (1986) analyzed unreduced total endosperm protein extracts with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to identify lines carrying either 1DL/1RS or 1BL/1RS translocations. Dhaliwal et al (1988) identified 1BL/1RS lines through electrophoretic separation at pH 3.0 of flour proteins extracted with 1 *M* urea. Both methods are based on the differential migration of rye versus wheat proteins in the respective electrophoretic systems. However, genetic variation for gliadin and glutenin composition in hexaploid wheats is extensive (Metakovsky and Sozinov 1987), and comigration of wheat and rye proteins might occur in some genetic backgrounds. Thus, alternative methods of identifying 1RS in wheat backgrounds may be required.

In the present study, grain proteins were analyzed by high-

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performance liquid chromatography (HPLC) to confirm the presence of 1RS in several experimental wheat lines. The utility of this method was confirmed by SDS-PAGE of reduced grain proteins, Southern blotting of genomic DNA followed by hybridization with a rye-specific DNA probe, and direct karyotyping of chromosomes from each tested line.

MATERIALS AND METHODS

Plant Samples

Sixty-three experimental lines and six released cultivars were used in the study. The experimental lines were developed as part of a joint USDA-ARS/University of Nebraska program to produce hard red winter wheat germ plasm with improved endosperm protein quality and quantity. Several of the experimental lines had pedigrees that contained a 1BL/1RS parent. The released varieties included in the study were Brule, Colt, Lancota, Plainsman V, Redland, and Siouxland. Of the released varieties, only Siouxland carries 1BL/1RS.

HPLC

The HPLC procedure of Lookhart et al (1986) was used with the exceptions noted below. Ground rye, wheat, or flour samples (250 mg) were extracted with 0.750 ml of 70% ethanol for 30 min at room temperature and centrifuged at 4,000 × *g* (Beckman J-6B) for 10 min at 25°C. The supernatants were decanted and stored at room temperature until analysis by reversed-phase HPLC. Extracts (10 μl) were analyzed on a Hewlett-Packard 1090 chromatography data system by elution from a reversed-phase column (Vydac, No. 218TP54) at 1 ml/min.

The elution conditions consisted of a multistep linear gradient containing a starting solvent of 25% acetonitrile and 75% water, each of which contained 0.1% trifluoroacetic acid. The acetonitrile concentration increased to 35% at 5 min, to 50% at 10 min, to 75% at 17 min, to 85% at 18 min, and returned to the initial conditions (25% acetonitrile) at 19 min. The total time from injection to injection was 30 min, which included an 11-min reequilibration step between runs. A diode-array detector (Hewlett-Packard 1040A) was used to detect the eluted components at 210 nm (0.500 absorbance units full scale). A data point was stored every 640 msec on a computer (Hewlett-Packard 9000-300) for subsequent integration, replotting, and comparison. All samples were analyzed within four days of extraction.

SDS-PAGE

Rye secalins were purified from flour of the cultivar Rymin according to Shewry et al (1982). Wheat grain proteins were solubilized from 1.5 mg of ground wheat with 1 ml of 70% ethanol or 1 ml of 0.04*M* NaCl. Material was extracted at room temperature with constant agitation for 30 min. After centrifugation at 14,000 × *g* for 5 min, supernatants were collected and dried under vacuum. Dry pellets containing protein were solubilized and then separated on polyacrylamide gels (0.75-mm gradient, 11–17.5%) and silver stained according to Graybosch and Morris (1990).

Repetitive DNA Probes

Restriction endonucleases were obtained from commercial suppliers and used according to manufacturers' specifications. Plasmid pSC74 (obtained from B. Gill, Kansas State University) carries a 480 base pairs repetitive DNA sequence cloned from the rye genome (Bedbrook et al 1980, Lapitan et al 1988). The pSC74 was digested to completion with ECORI and end-labeled with digoxigenin-UTP. Genomic DNA was purified from pooled samples of lyophilized leaf tissue from five seedlings by the method of Saghai-Maroof et al (1984). Genomic DNA digested by TAQI was separated on 1% agarose gels using standard procedures (Maniatis et al 1982). DNA then was transferred to nylon membranes (Nytran, Schleicher, and Schuell, Keene, NH) with a Sartorius Sartoblot dry-transfer apparatus; DNA was affixed to the membranes by baking at 80°C for 1 hr. The membranes then were probed with labeled pSC74. A Genius nonradioactive DNA labeling and detection kit (Boehringer-Mannheim, Indianapolis, IN) was used to label probe with digoxigenin-UTP and detect hybridization.

Karyotypic Analysis

Root tips from three or four germinating seeds per line were harvested and C-banded (Lukaszewski and Gustafson 1983) to identify lines carrying wheat-rye translocations. Two lines were heterogeneous for the presence of 1BL/1RS; in these cases, additional seeds were tested.

RESULTS AND DISCUSSION

HPLC

Graybosch et al (1990) used SDS-PAGE to analyze the proteins solubilized by 0.04*M* NaCl or by 70% ethanol from each of the 63 lines and six cultivars included in the present study. Eleven lines contained putative rye proteins and carried a protein with a relative molecular weight of 43,000, which comigrated with a rye omega secalin of 43,000 mol wt. The ω-secalins were mapped by Shewry et al (1986) to genes on 1RS. The protein of 43,000 mol wt could be extracted from wheats with either 0.04*M* NaCl or 70% ethanol. In addition, the protein of 43,000 mol wt was absent in wheat varieties known to lack 1RS.

HPLC analysis of 16 selected lines (the 11 experimental lines suspected of carrying 1RS plus five lines lacking the putative rye proteins) indicated the presence of unique peaks in lines carrying the putative rye proteins. HPLC patterns of ethanol-soluble proteins from wheat sister lines with (N86L090, N86L096) and without (N86L085) the rye protein are compared with those of Rymin rye in Figure 1. Common peaks were found in extracts of samples from rye and from wheats suspected of carrying 1RS (Fig. 1 shows only typical data). When the pattern of the line without 1BL/1RS (N86L085) was subtracted from that of its sister lines (N86L090 and N86L096) with 1BL/1RS (Fig. 2), two unique rye protein peaks, eluting at 6.8 min (one peak with a shoulder) and at 11.6 min (the front side peak of a doublet) were more evident. Those peaks were collected separately from the extract of N86L090 and pooled from 10 consecutive injections. Fraction 1 was collected from 6.63 to 6.83 min, fraction 2 from 6.95 to 7.02 min, and fraction 3 from 11.41 to 11.61 min. Fractions 1 and 2 were the front shoulder and main peak, respectively, of the 6.8-min peak, whereas fraction 3 was the first peak of the doublet at 11.6 and 11.8 min.

SDS-PAGE of HPLC-Purified Proteins

The putative rye proteins, collected by HPLC, were lyophilized and then analyzed by SDS-PAGE. The SDS-PAGE patterns of

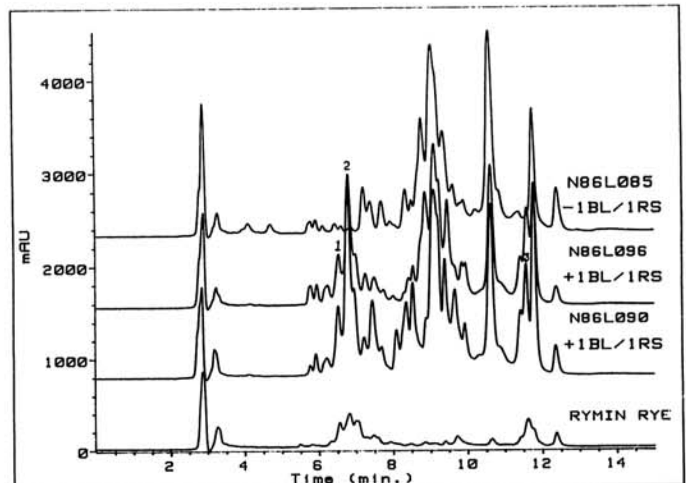

Fig. 1. High-performance liquid chromatography prolamin patterns of sister lines N86L085, N86L096, and N86L090 and of Rymin rye. The N86L085 line did not contain the 1BL/1RS translocation. 1–3 = possible rye peaks.

the collected peaks were compared with the patterns of the wheat proteins solubilized by 0.04*M* NaCl from N86L085, N86L090, and to the patterns of the secalins purified from Rymin rye (Fig. 3). The SDS-PAGE pattern of the HPLC fraction 1 (collected from 6.63 to 6.83 min) contains two groups of proteins (lane c): a cluster with a relative molecular weight of 54,000 and a second group with a relative molecular weight of 43,000. The same protein bands were present in fraction 2 (collected from 6.95 to 7.02 min, lane d); however, the abundance of the cluster of 54,000 mol wt was decreased, while the fraction was enriched for proteins in the cluster of 43,000 mol wt. The major protein of 43,000 mol wt found in fractions 1 and 2 also occurred in 0.04*M* NaCl extracts of N86L090 and of Rymin rye (lanes b and f) but did not occur in N86L085. The proteins of 54,000 mol wt were absent in the SDS-PAGE patterns from N86L085 and from rye. The SDS-PAGE patterns of fraction 3 (collected from 11.41 to 11.61 min) exhibited proteins of 36,000 mol wt (lane e). These proteins comigrated with secalins (36,000 mol wt) of Rymin rye. Proteins of equivalent molecular weight occurred in both N86L085 and N86L090. However, the abundance of proteins in the vicinity of 36,000 mol wt was five to ten times greater in N86L090 than in the non-1BL/1RS sister line N86L085.

We further investigated the identity of the proteins of 54,000 mol wt found in peaks 1 and 2. Wheat proteins were solubilized from ground grain samples of several 1BL/1RS lines of Bezostaya 1 (a non-1BL/1RS variety that contributed to the pedigrees of Aurora and Kavkaz), and of N86L085 (a non-1BL/1RS line). Extracted proteins were compared with secalins extracted from Rymin rye (Fig. 4). The proteins of 54,000 mol wt in the samples obtained from Bezostaya 1, Aurora, N86L090, and N86L096 (lanes b, c, e, and f, respectively) did not occur in the samples from Siouxland (lane a), N86L085 (lane d), N86L238 (lane g), N86L250 (lane h), or Rymin (lane i). Since these proteins occurred in Bezostaya 1 (a non-1BL/1RS line), were absent in Rymin rye, and were not ubiquitous in 1BL/1RS lines, they probably are wheat proteins that coelute with secalins of 43,000 mol wt during HPLC separation. Based on the molecular weight estimate, these proteins are omega-gliadins (Shewry et al 1984). All 1BL/1RS lines shown in Figure 4 contained a major protein that comigrated with the Rymin rye secalin of 43,000 mol wt and were enriched by a factor of 5–10 for proteins that comigrated with the secalins of 36,000 mol wt.

HPLC elution characteristics are determined by the amino acid composition of the external surface of the protein (Regnier 1987). The coelution of the secalins of 43,000 mol wt with the wheat omega-gliadins of 54,000 mol wt thus indicates some similarity between these proteins. Based on amino acid compositions, Shewry et al (1984) considered omega-gliadins and omega-secalins to be homologous. Comparison of our electrophoretic separations of secalins of Rymin rye with those of Shewry et al (1986) confirms the identity of the proteins of 43,000 mol wt as omega-secalins. Shewry et al (1986) also mapped γ-secalins of 40,000 mol wt to 1RS; in our system these proteins migrated as secalins of 36,000 mol wt and were components of the late-eluting HPLC peaks. The 75,000 mol wt secalins of Shewry et al were found to have a relative molecular weight of 66,000 in our system; these proteins arise from genes on chromosome 2RS. The secalins of 66,000 mol wt were not observed in any of the wheat lines tested in this study.

The identical HPLC retention characteristics of rye omega-secalins and omega-gliadins of certain wheat genotypes might obscure the identification with HPLC of wheat lines carrying 1RS. However, no wheat proteins coeluted on HPLC at fraction 3 with the γ-secalins of 36,000 mol wt. Thus, 1RS can be identified

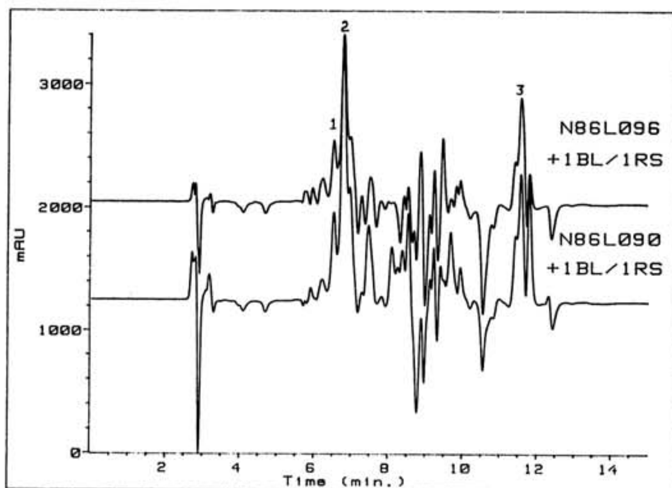

Fig. 2. High-performance liquid chromatography prolamin patterns of lines N86L096 and N86L090 containing 1BL/1RS after subtracting from them the prolamin pattern of their sister line N86L085, which does not contain 1BL/1RS. The most positive peaks (1–3) show material in these lines that was not present in the sister line without the rye translocation.

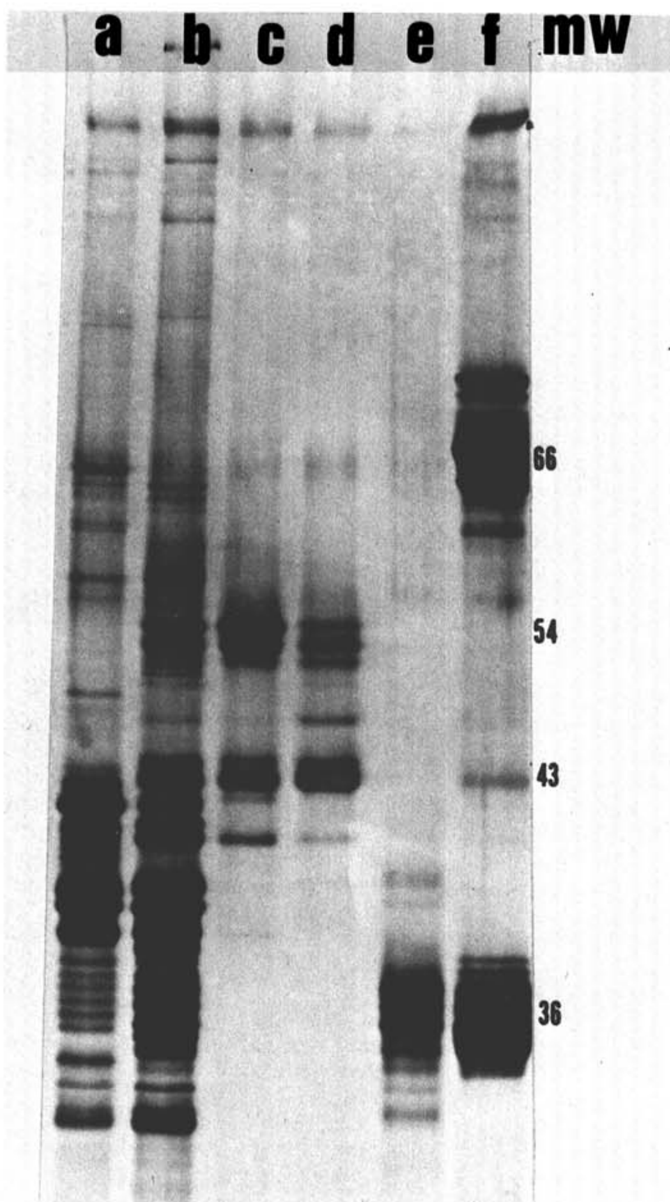

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of protein fractions purified by high-performance liquid chromatography (HPLC). a = 0.04*M* NaCl soluble grain proteins of N86L085, b = 0.04*M* NaCl soluble grain proteins of N86L090, c = proteins collected from HPLC peak 1 of N86L090, d = proteins collected from HPLC peak 2 of N86L090, e = proteins collected from HPLC peak 3 of N86L090, f = secalins from Rymin rye. Molecular weights (mw) are expressed in kilodaltons.

in wheat backgrounds by the cooccurrence of fraction 3 with fractions 1 and 2. Likewise, it is possible that electrophoretic comigration of rye and wheat proteins might occur in certain genetic backgrounds. Using HPLC, along with the electrophoretic methods used in this study or those previously published (Koebner and Shepherd 1986, Dhaliwal et al 1988) should resolve any such conflicts.

Repetitive DNA Probes

TAQI-digested genomic DNA was separated on 1% agarose gels and probed with labeled pSC74 digested by ECORI (Fig. 5, lanes a and b). The rye-specific repetitive DNA sequence in pSC74 contains two internal TAQI sites approximately 480 bp apart. Thus, digestion of genomic DNA with TAQI should release a fragment of 480 bp. Lines carrying rye proteins (Fig. 5, N86L028, N86L031, and N86L040, lanes d, e, and f, respectively) carried a 480-bp TAQI fragment that hybridizes strongly with this probe. TAQI-digested DNA from lines lacking rye proteins (Fig. 5, lanes a–c) showed no hybridization with this probe. The intensity of hybridization of DNA from N86L040 was approximately half that of the DNA from N86L028 and N86L031. Karyotypic analysis showed that N86L040 was heterogeneous for 1BL/1RS (discussed later). N86L031 also was heterogeneous, but this line had a much lower frequency of non-1BL/1RS individuals.

Lapitan et al (1988), using in situ hybridization, found no sequence homology in wheat chromosomes to the repetitive DNA sequence in pSC74. Hybridization was detected only with rye chromosomes. When we probed undigested genomic DNA with labeled pSC74 (not shown), we detected some hybridization with DNA from wheat lines that lacked 1BL/1RS; however, the intensity of the hybridization was reduced. Digestion with TAQI resulted in specific hybridizations only with DNA from 1BL/1RS lines. Thus, some sequence homology to pSC74 exists in the wheat genome that was not detected by in situ hybridization.

The repetitive DNA sequences that hybridize to pSC74 occur in the telomeric regions of all rye chromosomes (Bedbrook et al 1980, Lapitan et al 1988). Thus, when used alone to analyze a wheat line containing rye DNA of unknown origin, pSC74 cannot detect specific rye chromosomes. Positive hybridization with probe pSC74 confirms the presence of telomeric DNA of a rye chromosome but reveals no information on the identity of the rye chromosome. However, if used in conjunction with analysis of grain proteins by HPLC or SDS-PAGE, or with knowledge of the pedigree of the line in question, probing of TAQI-digested DNA with pSC74 can confirm the presence of 1BL/1RS. Appels et al (1986) used 1RS-specific rDNA probes to verify the presence of this chromosome arm in wheat lines. Obviously, a chromosome-specific probe is more desirable than a probe such as pSC74 (which hybridizes to DNA sequences from all rye chromosomes). However, our intent in this study was to confirm our identification of rye proteins through their cooccurrence with rye DNA sequences. Based on analysis of the pedigrees of our experimental lines, the only rye chromosome arm expected to occur was 1RS. For our purposes, pSC74 was suitable; however, it is not an efficient probe for use in experiments designed to identify rye-containing wheat lines of unknown parentage.

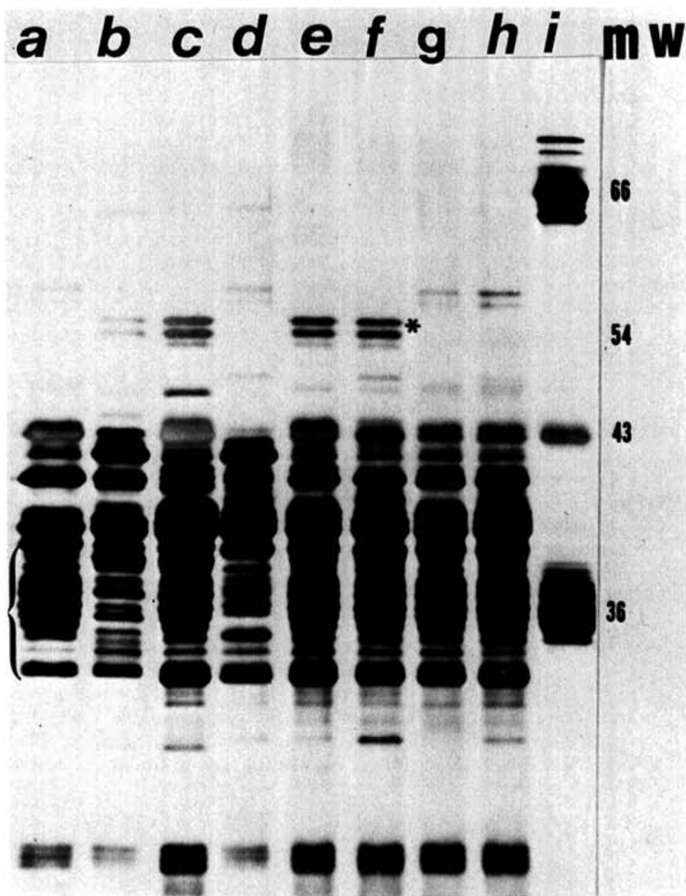

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of ethanol-soluble grain proteins from selected wheat lines compared with secalins from Rymin rye. a = Siouxland, b = Bezostaya 1, c = Aurora, d = N86L085, e = N86L090, f = N86L096, g = N86L238, h, = N86L250, i, = secalins from Rymin rye, * = wheat proteins of 54,000 mol wt in high-performance liquid chromatography peaks 1 and 2, bracket = region of secalins of 36,000 mol wt enriched in 1B/1R lines. Molecular weights (mw) are expressed in kilodaltons.

TABLE I
Experimental Wheat Lines Found To Carry 1BL/1RS

Line	Pedigree
N86L028	CI13447/NE R 3899//NB69457/3/NapHal/CI13449//Burgas 2[a]
N86L031[b]	CI13447/NE R 3899//NB69457/3/NapHal/CI13449//Burgas 2
N86L040[b]	CI13447/NE R 3899//NB69457/3/NapHal/CI13449//Burgas 2
N86L090	NE75414/3/Atlas66//NE701152/Aurora
N86L096	NE75414/3/Atlas66//NE701152/Aurora
N86L238	Atlas66/NapHal//NE701152/Aurora/3/NE75424
N86L250	Atlas66/NapHal//NE701152/Aurora/3/NE75424
N86L265	Atlas66/NapHal//NE701152/Aurora/3/NE75424
N86L266	Atlas66/NapHal//NE701152/Aurora/3/NE75424
N87U112	Sel. 14-53/3/Lancer/2/Atlas66/Cmn/4/Odessa 3
N87U113	Aurora/NE701154/3/NE7060/2/Rannayal2/Bezostaya4

[a] 1BL/1RS parents are underlined.
[b] Lines heterogeneous for 1BL/1RS.

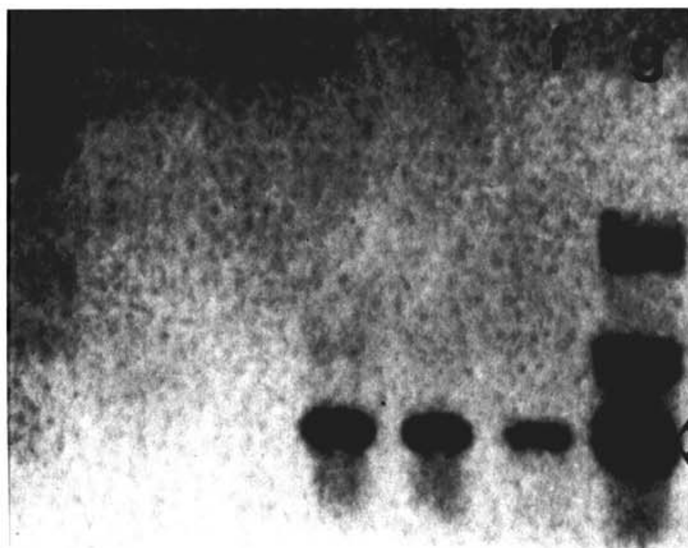

Fig. 5. Southern blotting of genomic DNAs digested by TAQI and hybridization with labeled pSC74. a = N86L011, b = N86L016, c = N86L022 d, = N86L028, e = N86L031, f = N86L040, g = TAQI-digested pSC74.

Karyotypic Analysis

Karyotypic analysis showed that the 11 experimental lines carrying the putative rye proteins and the repetitive rye DNA carried 1BL/1RS. No additional lines containing rye chromosomes were found among the 69 samples. Two lines, N86L031 and N86L040, were heterogeneous for the presence of 1BL/1RS (Table I). All experimental lines carrying 1BL/1RS have a known 1BL/1RS parent in their pedigrees. Twelve lines in the study had a 1BL/1RS parent in their pedigrees; 11 of these were found to carry 1BL/1RS. All lines were selected on the basis of agronomic performance, yield, and endosperm protein content; no conscious selection for 1BL/1RS was exercised. The high frequency of occurrence of 1BL/1RS in these lines suggests that the advantages conferred by this chromosome are significant.

Potential Impact of Using Multiple Methods To Identify 1RS

Genes encoding the omega- and γ-secalins are tightly clustered on the telomeric satellite region of chromosome 1RS (Sybenga et al 1990). The differential solubility characteristics of rye secalins in relation to wheat gliadins and glutenin subunits might be related to the poor end-use quality of 1RS-carrying wheats (Dhaliwal et al 1988). Identification of 1RS-carrying wheat lines that lack some or all of the secalins but retain the desirable disease resistance genes would be of great benefit to wheat breeding programs throughout the world. Analysis of storage protein compositions of 1RS-carrying wheats by HPLC or one of the various electrophoretic methods available could prove useful in identifying translocation lines that lack one or more rye storage proteins. Screening secalin-minus lines derived from 1RS-carrying parents with rye-specific DNA probes might reveal the presence of wheats that totally lack secalins but carry the desirable traits of 1RS.

CONCLUSIONS

Any of the four methods presented in this article can confirm the presence of 1RS in wheat lines of known pedigree. However, of these methods, only karyotypic analysis can differentiate between a 1BL/1RS translocation and 1AL/1RS or 1DL/1RS translocations. Only HPLC or electrophoretic analysis can verify the presence of 1RS-carrying wheat lines in flour samples. Both karyotypic and DNA analyses require living plant tissue. For determining 1RS in one or two particular lines, C-banding may be best, but for screening nurseries with known pedigrees, HPLC or SDS-PAGE may be the appropriate method.

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