Protein Composition and Pentosan Content in Relation to Dough Stickiness of 1B/1R Translocation Wheats

A. S. DHALIWAL,¹,² D. J. MARES,¹ D. R. MARSHALL,¹ and J. H. SKERRITT²

ABSTRACT

Wheat cultivars containing the 1B/1R translocation are characterized by a number of quality deficiencies, the most serious of which is "sticky dough." These defects presumably are related to differences in protein composition or some other flour constituent that result from the absence of the short arm of wheat chromosome 1B or the presence of the short arm of rye chromosome 1R. Flour proteins were extracted sequentially from two hard and one soft wheat cultivar and their 1B/1R derivatives with a range of solvents and examined by acidic buffer- and SDS-polyacrylamide gel electrophoresis. Wheat cultivars and their derivatives showed a similar quantitative protein distribution pattern except for an increase in the proportion of water-soluble protein in the 1B/1R lines. On the basis of electrophoretic and immunological properties, this difference was attributed to the presence of rye secalin proteins, which are more watersoluble than their wheat counterparts. Total and water-soluble pentosans also were determined. Water-soluble pentosan content was slightly higher in some 1B/1R lines but did not exceed the range normally found in bread wheats. These results are discussed in relation to the possible cause of sticky dough in 1B/1R wheat derivatives.

The 1B/1R translocation, in which the short arm of chromosome 1B of wheat is usually replaced by the short arm of the 1R chromosome of rye, carries linked genes for resistance to several major wheat diseases including powdery mildew and stem, leaf, and stripe rusts (Mettin et al. 1973, Zeller 1973, Moonen and Zeven 1984). Further, the fact that many high-yielding wheat cultivars now possess this translocation suggests that it may increase the yield of its carriers. As a consequence, it is being widely used in both winter and spring wheat breeding programs (e.g., Mettin and Bluhner 1984, CIMMYT 1985). In Australia, the 1B/1R translocation has been incorporated into a number of current commercial wheat cultivars by backcrossing, and resistance to stem, leaf, and stripe rusts confirm the presence of the 1B/1R

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translocation. Studies on advanced 1B/1R lines indicate that selected progeny have excellent yield characteristics and good resistance to all current Australian strains of stem and stripe rust.

Experience in Australia and elsewhere indicates that wheats carrying this rye chromosome segment usually have poor bread-making quality (Law and Payne 1983, Moonen and Zeven 1984, Martin and Stewart 1986) associated with reduced dough strength, intolerance to overmixing, and dough stickiness. The latter is of particular concern in modern mechanized bakeries. The 1B/1R translocation has no deleterious effects on several quality characteristics such as 1,000-grain weight, test weight, grain protein, flour protein, grain hardness, flour yield and color, and farinograph water absorption (Dhalwal et al 1987). However, it substantially and consistently reduces SDS-sedimentation volume and dough development time in hard wheats. However, in soft wheat derivatives, there is no evidence that the 1B/1R translocation significantly reduces SDS-sedimentation volume or dough development time. A tendency toward reduced extensibility resistance and extensibility was also noticed. Other side effects of the rye chromosome segment, such as increased water absorption of the dough, alterations in the outer layers of the grain, and damage to the starch during milling, have been suggested (Bolling and Meyer 1981, Zeller et al 1982). Zeller et al (1982) suggested that increased water absorption of the flours of the translocation wheats leads to formation of sticky doughs. The precise contribution of protein and other grain constituents towards dough stickiness remains to be established. As a step towards this end, quantitative differences in protein solubility and pentosan content of three Australian commercial wheat cultivars, their backcross derivatives containing the 1B/1R translocation, and several ryes were studied, and the electrophoretic, solubility, and immunological properties of these proteins further investigated.

**MATERIALS AND METHODS**

Pedigrees of translocation lines used in the present study were described in detail in an earlier paper (Dhalwal et al 1987). Cook and Oxley are Australian prime hard and northern hard quality bread wheats, respectively, whereas Egret is a soft wheat. The wheat cultivars Aurora, Skorospelka, and Kavkaz have been used as sources of the 1B/1R translocation (Rabonovich 1972, Mettin et al 1973, Zeven and Zeven-Hissink 1976). All the lines were sown in the field at the I. A. Watson Wheat Research Centre, Narrabri, NSW, Australia, during early June 1985 and harvested during late November 1985. Aurora was grown in winter wheat trials during 1984. The flour sample of 1B/1R German winter wheat, Perseus, was supplied by J. P. Ohms (Landwirts. Untersuchungs- und Forschungsanstalt der Landwirtschaftskammer, Hannover, Hameln).

**Milling**

Seeds were conditioned overnight to 15% moisture content and milled on a Quadramat Senior mill (C. W. Brabender). Flour protein content was determined by the semi-micro-Kjeldahl (protein N × 5.7) method. The samples of NSW, SA, and Imperial ryes were whole-meal flours.

**Extraction of Proteins**

Proteins were extracted sequentially following the procedure described by Chen and Bushuk (1970). This method gave five protein fractions: a) water-soluble proteins or albumins, b) salt-soluble proteins or globulins, c) alcohol-soluble proteins, mainly gliadins, d) acetic acid-soluble proteins, mainly glutenins, and e) insoluble residual protein. Flour samples (5 g) were extracted with 0.5M sodium chloride by vortex and end-over-end mixing for 2 hr. After centrifugation (15 min, 5,000 × g) the residue was extracted for another hour. The third extraction was carried out with distilled water for 30 min. The three supernatants were combined and dialyzed against purified water (5°C) for 48 hr and centrifuged to separate the precipitated salt-soluble proteins. The alcohol- and acid-soluble fractions were obtained following extraction of the residue with 70% (v/v) aqueous ethanol and 0.05M acetic acid, respectively. Unless otherwise indicated, all extractions were performed at 5°C. Ethanol was removed by rotary evaporation.

The semi-micro-Kjeldahl method was used for nitrogen estimations on all the protein extracts and the residue fraction. The residue and salt-soluble fractions were freeze-dried prior to nitrogen estimations. All the extractions were performed in duplicate, and the results are the average of the two replications.

**Electrophoretic Characterization of Extracted Proteins**

For polyacrylamide gradient-gel electrophoresis under acidic buffer conditions (GG-PAGE), milled flours and individual protein fractions from the various wheats, ryes, and translocation lines were solubilized with 1M urea (1 hr, 20°C, 6 µl/mg of flour or 100 µl/mg protein fraction) and 10 µl of each sample extract was applied for electrophoresis. The extractions were analyzed by electrophoresis in a sodium lactate buffer (4 mM Na+, pH 3.1) on 75-mm square, 3-mm thick gels containing a gradient of polyacrylamide from 2.5 to 27% (Gradiopore Ltd., Pyrmont, NSW, Australia), as earlier described (Skerritt et al 1984). Gels were run at 200 V, 20°C 100 times indicated in legend. Figures 1-3 and stained with 0.025%, (w/v) Coomassie Blue G-250 in 12% (w/v) trichloroacetic acid. Destaining was unnecessary. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on proteins extracted from the above samples (55°C, 4 hr) with a solution of 2% (w/v) SDS, 10% (v/v) 2-mercaptoethanol, 16% (v/v) N,N-dimethyl formamide, and 62 mM Tris-HCl, pH 6.8. They were fractionated by discontinuous buffer system SDS-PAGE on a 1.5-mm thick 15% T (T = acrylamide + bisacrylamide) separating gel, as described elsewhere (Skerritt et al 1984) for either 1,200 or 2,000 Vhr as indicated in legends to Figures 4 and 5. Gels were stained 20 hr in 0.2% (w/v) Coomassie Blue R-250 in methanol, acetic acid, and water, 40:10:50; and destained 6 hr in the same solvent minus dye.

**Immunological Studies**

In some experiments, proteins were transferred from acidic buffer GG-PAGE to nitrocellulose membranes (Schleicher and Schuell, Dassel, FRG, 0.45 µm pore size) by placing membranes on the cathodic side of the gel in 0.7% (v/v) acetic acid, at 36 V for 180 min using a GelMan (Cromwell) transblotting unit. Following transfer, membranes were washed in 5% (w/v) KOH and nonspecific antibody binding blocked by incubation in 3% (v/v) bovine serum albumin (BSA) in phosphate-buffered saline (Skerritt et al 1984). The preparation and antibodies of the monoclonal antibodies used in this study have been described elsewhere (Skerritt et al 1984, Skerritt and Underwood 1986). Antibody 122/24 (IgG1) and 246/21 (IgM) bound to certain γ- and α-gliadins from wheat (and homologous proteins from rye (Skerritt et al, unpublished) but did not bind significantly to albumin and globulin proteins (Skerritt and Underwood 1986).

The monoclonal antibodies (diluted in blocking agent) were incubated for 90 min at 37°C; nitrocellulose squares were washed 4 × 5 min with 10 mM Tris-HCl, 140 mM NaCl, pH 8.0, containing 0.05% (v/v) Tween 20, then incubated with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Promega, Madison, WI) diluted 1:5,000 in the wash buffer containing 2% BSA for 60 min at 20°C. Following four further washes, specific antibody binding was revealed as blue-purple zones on the nitrocellulose membrane by addition of substrate for 5 min at 20°C (nitroblue tetrazolium, 330 µg/ml; 5-bromo-chloro-indolyl phosphate, 165 µg/ml in 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl2).

**Pentosans**

Pentosans in the flour and the water extracts were determined with phloroglucinol according to the method of Douglas (1981). Ten milliliters of a solution of glacial acetic acid (16M final concentration), HCl (0.17M), phloroglucinol (0.85%, w/w), ethanol (4.2%, v/v), and glucose (0.015%, w/v) was added to 5 mg of flour in 2 ml of water in a stoppered boiling tube. Tubes were
placed in a boiling water bath for 25 min, and the absorbance was measured at 552 and 510 nm. Pentosans were estimated by subtracting the absorbance at 510 nm from that at 552 nm, and xylose was used as the standard.

RESULTS

The acidic buffer GG-PAGE pattern of proteins extracted with 1M urea from flour of several wheats, ryes, and 1B/1R translocation lines is shown in Figure 1. The 1B/1R translocation wheats (b,d,f,h,i) were characterized by the presence of two clusters of protein bands (indicated by arrows) essentially absent in parent wheats (a,c,e,g). The three ryes (j,k,l) also had these protein bands, except for Petkus R5 (k), where one band in the lower mobility cluster is missing. Petkus R5 also differs from other ryes and 1B/1R lines in the composition of certain protein bands running in the γ-gliadin region of the gel. All the wheats had two additional protein bands, a fast α-gliadin band (marked by the upper asterisk), and the band that ran with γ-gliadin (marked by the lower asterisk), not found in the translocation lines. These bands are also seen clearly on analysis of alcohol-soluble extracts (marked by the upper and lower asterisks, Fig. 2B). GG-PAGE of water-soluble and alcohol-soluble proteins solubilized with 1M urea are shown in Figure 2A and B, respectively. Both extracts reveal the 1B/1R translocation wheats and three ryes possess two clusters of protein bands (indicated by arrows) absent in parent wheats. The three wheats had two additional protein bands (marked by the asterisks, Fig. 2A) absent in 1B/1R lines and ryes. However, no difference was noticed in the GG-PAGE pattern of salt-soluble proteins (which precipitated upon dialysis against water) of wheats and 1B/1R lines.

Water- and alcohol-soluble proteins of wheats, ryes, and 1B/1R lines were transferred from acidic buffer polyacrylamide gels (Fig. 3A) to nitrocellulose membranes. Two monoclonal antibodies, 122/24 (IgG1) and 246/21 (IgM) were used in the present study. These antibodies bind to certain γ- and α-gliadins from wheat (and homologous proteins from rye) but do not bind significantly to albumin and globulin proteins. Specific antibody binding to prolamin was revealed by the appearance of blue-purple zones on the nitrocellulose membranes (Fig. 3B and C). Both antibodies bound strongly to the protein bands characteristic of 1B/1R lines and ryes.

SDS-PAGE patterns of flour proteins from various wheats, ryes, and 1B/1R wheats are shown in Figure 4. There did not appear to be any difference in the high molecular weight glutenin subunits between Cook and its 1B/1R derivatives, SUN 89D and SUN 89E. However, M3344 and WW345 were different from their recurrent parents Oxley and Egret, respectively, in having an additional band (marked by arrow) and missing the other one (approximately Mr. 94,000). This band is also seen clearly on analysis of acid-soluble extracts (Fig. 5B, marked by arrow). Aurora, another 1B/1R translocation wheat, had a pattern similar to M3344 and WW345. Unfortunately, parent cultivars of Aurora

![Image](https://example.com/image1.png)

**Fig. 1.** Electrophograms obtained by gradient polyacrylamide gel electrophoretic analysis of proteins (extracted with 1M urea) from Cook (a) and its 1B/1R derivative SUN 89D (b); Oxley (c) and its 1B/1R derivative M3344 (d); Egret (e) and its 1B/1R derivative WW345 (f); Timgalen (g), one of the recurrent parents of SUN 89D and M3344; Aurora (h), donor of rye chromosome segment; (i) Perseus, a 1B/1R German winter wheat; and three ryes, Petkus R1 (j), Petkus R5 (k), and Black Winter rye (l) (gel running time, 4 hr [800 Vhr]).

![Image](https://example.com/image2.png)

**Fig. 2.** Gradient polyacrylamide gel electrophoretic pattern of, A, water-soluble proteins (gel running time 1.5 hr [300 Vhr]) and B, alcohol-soluble proteins (gel running time 3 hr [600 Vhr]) resolved with 1M urea from wheats, ryes, and 1B/1R lines. Cook (a), SUN 89D (b), Oxley (c), M3344 (d), M3345 (e), Egret (f), WW345 (g), Aurora (h), Imperial rye (i), New South Wales rye (j), and South Australian rye (k).
(MacRitchie et al. 1986) were not available for analysis. Wheats such as Cook, Oxley, Egret, and Timigal had two additional protein bands (marked by an asterisk, $M_r$ approximately 43,000) missing in 1B/1R lines and ryes. Although the differences in composition between water extracts of wheats, 1B/1R translocation lines, and ryes were marked on acidic buffer GG-PAGE, they were much less marked on SDS-PAGE (Fig. 5A). However, proteins of apparent $M_r$ 45,000 and 50,000 (marked by arrows) were seen in the water extracts of 1B/1R lines examined by SDS-PAGE and were absent in their recurrent parents. The protein band marked by an asterisk (at $M_r$ approximately 67,000) was essentially absent in all the 1B/1R lines (Fig. 5B).

The quantitative differences in protein solubilized by the different solvents are illustrated in Table I. SUN 89D, a 1B/1R translocation line, and Aurora, donor of the short arm of the 1R chromosome, had higher water-soluble protein contents than Cook. There was no significant difference in amounts of other fractions between Cook and SUN 89D. Aurora, however, had a higher proportion of proteins in the acetone-soluble fraction and less residue protein than Cook and SUN 89D. Similarly, two other translocation wheats, M3344 and M3345, where Oxley was a major recurrent parent, contained a higher proportion of proteins in the water-soluble fractions than their parent. There was no significant difference in other fractions except the alcohol-soluble fraction, which was reduced in one translocation line, M3345. Furthermore, in the soft wheat lines, the only significant difference between Egret and its translocation derivatives, WW345 and M4174, was an increased proportion of protein in the water-

**Fig. 3.** A–C, Fractionation of 1M urea-solubilized water-soluble (a–l) and alcohol-soluble (p–l) proteins from wheats, ryes, and 1B/1R lines by gradient gel (3–27% acrylamide) electrophoresis. Proteins from identical gels run in parallel were transferred to nitrocellulose and were reacted with antibody 122/24 (IgG1), and C, with 246/21 (IgM). Samples are (a) Cook, (b) SUN 89D, (c) Oxley, (d) M3344, (e) Egret, (f) WW345, (g) Aurora, (h) Imperial rye, (i) New South Wales rye, (j) Cook, (k) SUN 89D, and (l) Imperial rye (gel running time 3 hr [600 Vhr]).

**Fig. 4.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins from various wheats, ryes, and 1B/1R translocation lines. Samples are (a) Cook, (b) SUN 89D, (c) SUN 89E, (d) Oxley, (e) M3344, (f) M3345, (g) Egret, (h) WW345, (i) M4174, (j) Timigal, (k) Aurora, (l) Perseus, (m) Petkus R1, (n) Petkus R5, (o) Black Winter rye, and (p) molecular weight markers (electrophoresis 2,000 Vhr).
soluble fraction in the latter. From Table 1, it is evident that all the translocation lines in hard and soft wheat categories contained higher proportions of water-soluble proteins than their recurrent parents. However, different classes of wheats do vary in the amount of protein that can be extracted by the solvents used in this study. Hard quality wheats (Cook and Oxley and their derivatives) had, in general, higher contents of acetic acid solubles and residue fraction than the soft wheats (Eget and derivatives), whereas soft wheats contained higher proportions of alcohol-soluble proteins. All three ryes had higher amounts of water- and salt-soluble proteins and lower contents of alcohol-soluble and acetic acid soluble fraction as compared to wheats and 1B/1R lines. The higher content of residue protein in ryes could be because of the use of whole meal flour for extraction of different proteins.

Table II shows the amounts of total and water-soluble pentosans. SUN 89E had a higher total pentosan content than its parent Cook. Similarly, only one line, M3346, contained higher amounts of flour pentosans than Oxley. In the soft wheat category, M3342-2 and M3342-12 exhibited greater amounts of flour pentosans than Egeter. There were no significant differences in water-soluble pentosans between Egeter and its 1B/1R derivatives. Three 1B/1R lines (M3344, M3345, M3346) where Oxley was a major recurrent parent and one line (SUN 89F) where Cook was a major recurrent parent had higher water-soluble pentosans than their respective parents. All the ryes exhibited greater amounts of total and water-soluble pentosans as compared to wheats and 1B/1R lines.

**DISCUSSION**

The present study describes the protein composition and pentosan content of various wheats, ryes, and 1B/1R translocation lines.

Wheat proteins, which have been shown to largely determine the breadmaking properties of the dough, can be separated into albumins, globulins, gliadins, and glutenins. The high molecular weight (HMW) subunit of glutenin, coded for by the genes on the long arm of the homoeologous chromosomes 1, are considered to be the most important component with respect to breadmaking quality (Payne et al., 1979, 1981, 1984; Burnouf and Bourriquet, 1980; Moonen et al., 1983). There were no differences in the SDS-PAGE pattern of HMW subunits of glutenin of Cook and its 1B/1R lines, SUN 89D and SUN 89E. M3344, WW345, and Aurora had an additional band in this region and were missing one present in other 1B/1R lines. This missing band is termed "band 8" in the terminology of Payne and Lawrence (1983) and has been shown by these workers to be a product of a gene on chromosome 1BL (Payne et al., 1982). Although these wheats are not true substitution lines, there appears to be a transfer of a small part of the long arm of the chromosome in some lines. Quality defects such as dough stickiness of translocation wheats cannot therefore be attributed to changes in HMW glutenin subunit composition, as some of the 1B/1R lines producing sticky dough had a pattern of HMW subunits similar to that of their recurrent parents. Moonen and Zeven (1984) reached a similar conclusion by analyzing 1B/1R lines that produced sticky doughs and other wheats that also produced sticky doughs but are not 1R(1B) substitution or 1BL/1RS translocation lines. Differences in HMW glutenin subunits and in the proportion of protein in acetic acid-soluble and residue fraction were observed as expected in different wheat cultivars and particularly in wheats of different quality classes. The hard quality lines contained a higher proportion of the gluten proteins and residue fraction than soft wheats.

Gladiol proteins are controlled by genes on the short arms of the homoeologous chromosome 1 and 6. The 1B/1R translocation wheats were characterized by the presence of the rye secalins. This was confirmed with the help of two monoclonal antibodies that bond to certain K- and ω-gliadins from wheat (and homologous proteins from rye) but did not bind significantly to albumin and globulin proteins. Both antibodies had a strong reaction with proteins from water-soluble and alcohol-soluble fractions of 1B/1R lines and wheats but a smaller weaker reaction with wheat water-soluble protein extracts. Gradient gel electrophoretic and immunological studies of water-soluble extracts indicated that the increased content of protein in the water-soluble fraction of 1B/1R wheats was due to the rye secalins. All the ryes contained a higher proportion of water-soluble proteins. In a comparative study of the various protein fractions, Chen and Bushuk (1970) showed that rye or triticale endosperm contained large quantities of water-soluble proteins.

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<th>1B/1R Line/</th>
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<th>Water-Soluble Pentosan (as D(+)-xylose)</th>
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*1B/1R translocation derivatives where Cook was a major recurrent parent and Aurora was a donor of the rye chromosome segment.

*Oxley 1B/1R derivatives where Kavkaz was a donor of the rye chromosome segment.

*Egeter 1B/1R derivatives where Skorospelka and Kavkaz were rye chromosome segment donors.

*1B/1R translocation wheat.

*Australian northern hard wheat.

*The three ryes, Imperial, South Australian, and NSW, are not included in the statistical analysis.
Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of A, (a–h) water-soluble and (i–p) salt-soluble proteins (electrophoresis 1,200 V/hr) and B, (a–h) alcohol- and (i–p) acid-soluble proteins (electrophoresis 2,000 V/hr). Samples are (a) Cook, (b) SUN 89D, (c) Oxley, (d) M3344, (e) M3345, (f) Egret, (g) WW345, (h) Aurora, (i) Cook, (j) SUN 89D, (k) Oxley, (l) M3344, (m) M3345, (n) Egret, (o) WW345, and (p) Aurora.
Dough stickiness of the translocation or substitution lines has been related causally to water-soluble proteins and soluble pentosans by Zeller et al. (1982) and Bolling and Meyer (1981). The studies of Zeller et al. (1982) indicate that increased water content of the flour, which may be a result of the increased proportion of the soluble pentosans and water-soluble protein, leads to the formation of “damp flagging” doughs. Water-soluble pentosans are considered to be important in breadmaking because of their effect on water-binding capacity (Bushuk 1966, Tao and Pomeranz 1967, Jelaca and Hlylnka 1971, Patil et al. 1976), effects on the consistency of the dough (Pence et al. 1950), and involvement in the oxidation of flour (Neukom et al. 1962, Hoseney et al. 1972). Pentosans extracted from various classes of wheat increased water absorption and decreased dough development time and dough stability (Tao and Pomeranz 1967, Zeller et al. 1982). No significant correlation was found between water-soluble pentosans and water absorption level of the flour or dough development time. Water absorption and dough development time of 1B/1R lines has been reported in an earlier study (Dhaulilal et al. 1987). At first sight, the data for water-soluble proteins and soluble pentosans appear to support the suggestions made by Zeller et al. (1982). However, the high pentosan content of both hard and soft wheats that have no history of stickiness together with the low pentosan content of Auxor and a 1B/1R translocation wheat with a high degree of dough stickiness, indicated that water-soluble pentosans as such are probably not the primary factors which caused dough stickiness in 1B/1R wheats. Further studies of the involvement of rye secalins in dough stickiness using flour fractionation and reconstitution techniques are in progress.

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LITERATURE CITED