Inheritance of Gluten Protein Components of High-Protein Hard Red Spring Wheat Lines Derived from *Triticum turgidum* var. *dicoccoides*[1]

KHALIL KHAN,[2] RICHARD FROHBERG,[3] TRUMAN OLSON,[2] and LINDA HUCKLE[2]

ABSTRACT

Three hard red spring (HRS) wheat lines were derived from crosses with a high-protein donor, *Triticum turgidum* var. *dicoccoides*. The three experimental lines were higher in protein content (14.0% moisture basis) by up to 3.0 percentage points more than their HRS wheat parental genotypes. These lines also were higher in water absorption, wet gluten content, and loaf volume than the HRS parents. Polyacrylamide gel electrophoresis of gliadin proteins showed that all three experimental lines inherited protein components in the α- and β-gliadin regions from *T. t. dicoccoides*. All three experimental lines were known to inherit the first three protein components of lowest mobility in the ω-gliadin region from one of the parents, RL4352-1 (a selection of Columbus for rust resistance), because these three components do not occur in the other parents, Len, Coteau, and *T. t. dicoccoides*. Reversed-phase high-performance liquid chromatography showed that all three lines inherited gliadin components from *T. t. dicoccoides* that eluted in the most hydrophobic region of the elution profile. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the high molecular weight subunits of glutenin showed that two of the lines had the same subunit composition (2*, 7+9, 5+10) as the HRS wheat parents. One of the lines contained the subunit composition 1, 7+9, and 5+10, but subunit 1 is not present in the parent varieties. The possibility of contamination or genetic recombination with respect to the appearance of subunit 1 is discussed.

Cereal Chem. 66(5):397-401

Attempts to increase the protein content of cultivated wheats must aim to satisfy several criteria. The nutritional quality of the protein must be maintained at current levels or improved, especially by increasing lysine, the limiting amino acid in wheats. Functional properties of proteins, such as baking and bread-making qualities, need to be maintained or improved. Protein quantity should be increased to levels appreciably higher than those that now exist, especially in regions where higher protein levels can be of economic benefit to producers. Current yields should be maintained or improved by overcoming the inverse relationship between increased protein content and grain yield. Other agronomic factors, such as resistance to disease, sprouting, lodging, shattering, etc., need to be maintained or improved.

During the last decade many attempts have been made to increase the protein content of cultivated wheats. High protein contents, ranging from 17 to 27%, were found in wild tetraploid wheats (Avivi 1978). Of these wild species, *T. t. dicoccoides* was found to contain the higher protein contents. As a result attempts were made to introduce the high protein factor(s) from *T. t. dicoccoides* into bread and durum wheats through conventional plant breeding techniques.

In Israel, Avivi et al (1983) derived high-protein durum wheat lines containing protein contents of 18–23% from crosses of a durum variety of 15% protein with *T. t. dicoccoides*. However, under field conditions the durum lines showed severe lodging. In Australia, Kushnir and Halloran (1984) produced bread wheat lines with high kernel weight and high protein from crosses with *T. t. dicoccoides*. We are not aware, however, of such derived high-protein lines in commercial production in the United States or other countries.

At North Dakota State University, three lines of high-protein bread wheats were developed from crosses with *T. t. dicoccoides*. These lines were grown under field conditions at different locations in North Dakota in 1986 and 1987. In this paper we report some physicochemical and functional properties in an attempt to identify the high-protein factor(s) from *T. t. dicoccoides*, the high-protein donor parent.

MATERIALS AND METHODS

Wheat Samples

All wheat varieties and lines were obtained from the Department of Crop and Weed Sciences, North Dakota State University, Fargo. The high protein lines ND 643, ND 644, and ND 645 were derived from three-way crosses as shown in Table I. RL4352-1 is a selection of the Canadian variety Columbus for rust resistance. The accession of *T. t. dicoccoides* was FA15-3 (Israel A) (Avivi 1978).

Quality Evaluation

All wheat samples were tempered to 16% moisture and milled on a Buhler laboratory mill.

Falling number, protein content, wet gluten, and flour ash were determined according to AACC approved methods (1983), respectively.

The baking procedure was the three-hour fermentation procedure of D'Appolonia et al (1970).

Polyacrylamide Gel Electrophoresis (PAGE) and Sodium Dodecyl Sulfate-PAGE

PAGE was carried out according to the procedure of Khan et al (1985, 1988) as follows: the gel solution (100 ml) contained 7.0 g of acrylamide, 0.25 g of bisacrylamide, 0.0004 g of ferrous sulfate-heptahydrate, and 0.10 ml of a 3% hydrogen peroxide solution. The buffer solution (gel and electrode) contained 0.25 g/100 ml aluminum lactate adjusted to pH 3.1 with lactic acid.

Sodium dodecyl sulfate (SDS)-PAGE was carried out on total extracts (40 mg/ml) of ground grain (multiple kernels) according to a modified Laemmli (1970) procedure on 12% (w/v) acrylamide (0.1%, w/v, bisacrylamide) gels. Extraction conditions were the same as described by Ng and Bushuk (1987). A Hoefer SE600 vertical gel apparatus (Hoefer Scientific, San Francisco, CA) was used to make 1.5-mm thick gels and 5 μl of sample extract was applied. Gels were electrophoresed overnight at 7.5 mA per gel for 18 hr. The tracking dye, Pyronine Y, migrated off the gel during this time. Gels were then stained for 6 hr in 0.1% (w/v) Coomassie Brilliant Blue R250 containing 10% trichloroacetic acid, 50% methanol, and 10% glacial acetic acid. Gels were destained in 40% methanol/7.5% (v/v) glacial acetic acid until the gel background was clear for photography.

Reversed-Phase High-Performance Liquid Chromatography

Reversed-phase high-performance (pressure) liquid chromatography (RP-HPLC) was carried out according to a modified procedure of Huebner and Bietz (1987) and Lookhart et al (1987).

[1]Published with the approval of the Director of the Agricultural Experiment Station, North Dakota State University, Fargo, as Journal Series no. 1,764.
[2]Associate professor, food technologist, and chemist, respectively, Cereal Science and Food Technology, North Dakota State University, Fargo 58105.
[3]Professor, Department of Crop and Weed Sciences, North Dakota State University.

This manuscript was prepared for electronic processing.

Wheat proteins were extracted from whole meal with 70% aqueous ethanol (50 mg/ml) for 1 hr with a 30-sec vortex every 10 min. Extracts were centrifuged at 20,000 × *g* for 20 min, and the supernatant was filtered using Gelman LC13 0.45-μm filters (Ann Arbor, MI). HPLC was performed with a Hewlett Packard model 1090 chromatograph (Hewlett-Packard, Minneapolis, MN) which consisted of a PV5 solvent delivery system, a filter photometric detector, and an HP 3393A computing integrator. Analyses were carried out with a SynChropak RP column (C18, 300 Å pore size, 250 × 4.1 mm) preceded by a guard column of the same packing material (5 × 4.6 mm) (SynChrom Inc., Linden, IN).

Gradients were generated with water containing 0.06% trifluoracetic acid (TFA) (solvent A) and acetonitrile containing 0.05% TFA (solvent B). All solvents were filtered (0.45 μm), degassed, and sparged continuously with helium. The gradient began with 25% B, increased to 30% B at 5 min, and to 50% B at 55 min with a 5-min hold. Solvent B was then increased to 100% to wash the column and held there for 5 min before returning to 25% at 75 min. There was a 15-min column equilibration at initial conditions between manual injections. Samples of 50 μl were analyzed at a flow rate of 1 ml/min at 55°C and a wavelength of 210 nm.

Statistical Analyses

The experimental design used was a completely random design and the GLM procedure of the Statistical Analysis System (SAS Institute 1983) was used to obtain the least significant difference values reported in Table II.

TABLE I
***T. t. dicoccoides*-Derived High-Protein Hard Red Spring Wheat Lines**

Genotype	Parent or Pedigree[a]
Len	Parent
Coteau	Parent
RL4352-1 (Columbus)	Parent
T. t. dicoccoides	Parent
ND 643	RL4352-1/*T. t. dicoccoides*//Len
ND 644	RL4352-1/*T. t. dicoccoides*//Len
ND 645	RL4352-1/*T. t. dicoccoides*//Coteau

[a]The cross RL4352-1/*T. t. dicoccoides* was male sterile.

RESULTS AND DISCUSSION

Quality Data

Table II shows the quality data of the high-protein lines and the controls, Len and Waldron, from different locations and different years. Falling number of the high-protein lines is slightly lower than the controls, however, the values are above 400 sec, which is indicative of sound wheats. Flour extraction of ND 644 and ND 645 are lower while that of ND 643 is higher than the controls. The high-protein lines showed higher values than the controls for all other quality factors. The higher protein content of about three percentage points in the high-protein lines is not lost after milling, indicating that the increase in protein content is in endosperm proteins. Wet gluten content is about 10% higher in ND 643 and ND 644 and about 15% higher in ND 645 compared with the controls. It should be pointed out that the glutens of ND 644 and ND 645 were stickier than that of ND 643 or the controls. Flour ash, absorption, and loaf volume show higher values for the high-protein lines than the controls. The quality data, therefore, indicate that the three high-protein lines performed equally well, and in many cases were superior to the controls Len and Waldron.

Inheritance of Gliadin Proteins

Figure 1 shows the PAGE patterns of the 70% ethanol extracts of the high-protein lines and their parents (arrowheads numbered 1–18, square brackets, and curved brackets on the patterns in Fig. 1 are used to identify specific bands beginning at the anodic end). The first three arrowheads indicate that ND 643, ND 644, and ND 645 inherited the first three bands from RL4352-1 (Columbus). These three bands are quite different in mobility and intensity from the two most slowly moving bands of Len and Coteau. The fourth arrowhead indicates bands also inherited from RL4352-1. The fifth arrowhead in ND 645 indicates a band inherited from *T. t. dicoccoides*. This band is absent in ND 643 and ND 644. The sixth arrowhead in ND 643 and ND 644 indicates bands inherited from Len, whereas in ND 645 it indicates a band inherited from *T. t. dicoccoides* or Coteau. The seventh arrowhead in ND 643 and ND 644 indicates bands inherited from Len, whereas in ND 645 it indicates a band inherited from Coteau (patterns 8 and 9). The eighth arrowhead indicates a band in ND 643, absent in ND 644 and ND 645, inherited from *T. t.*

TABLE II
Data of *T. t. dicoccoides*-Derived High-Protein Hard Red Spring Wheat Lines and Their Parents

Sample	Wheat Falling No. (sec)	Protein Content[a]		Flour Extract (%)	Wet Gluten (%)	Flour Ash (%)	Absorption (%)	Loaf Volume (cm^3)
		Wheat (%)	Flour (%)					
1986[b]								
Controls								
Len	444	15.5	14.8	70.0	37.1	0.49	67.9	863
Waldron	457	15.5	14.7	71.2	39.4	0.50	66.5	835
High-protein lines								
ND 643 WB[c]	424	18.4	17.9	72.2	45.9	0.54	71.6	1,200
ND 644 WB	419	18.1	17.6	68.8	48.0	0.55	72.4	1,125
ND 645 WB	425	19.0	18.4	65.3	51.1	0.59	72.5	1,140
LSD (0.05)[d]	33	1.8	1.4	NS	3.4	NS	4.9	186
1987[e]								
Controls								
Len	500	15.6	14.9	70.5	39.4	0.49	66.2	913
Waldron	500	15.9	15.1	70.3	42.2	0.49	65.7	947
High-protein lines								
ND 643 WB[c]	439	19.3	18.4	72.7	49.3	0.55	69.0	1,088
ND 644 WB	486	18.8	17.9	67.6	49.7	0.56	71.3	1,043
ND 645 WB	468	19.3	18.4	66.1	55.4	0.57	70.7	1,072
LSD (0.05)[d]	NS	1.0	1.0	2.9	3.7	NS	2.1	109

[a]14.0% moisture basis.
[b]Averages of two locations.
[c]WB = with bromate.
[d]LSD = least significant difference at 0.05 level of significance; NS = not significantly different.
[e]Averages of three locations.

dicoccoides. The ninth arrowhead (patterns 5, 8, and 9) indicates two bands inherited by ND 645 from Coteau. Arrowheads 10 and 11 (in patterns 8 and 9) indicate two bands in ND 645 also inherited from Coteau.

The open brackets in the γ-gliadin region indicate three bands, characteristic of the Len gliadin pattern, inherited by ND 643 and ND 644. Arrowhead 12 indicates a faint band inherited from RL4352-1 by ND 643, ND 644, and ND 645. Arrowhead 13 indicates a band in ND 643 and ND 645, absent in ND 644, inherited most likely from RL4352-1. Arrowheads 14, 15, and 16 indicate bands in ND 643 and ND 644 inherited from either Len or *T. t. dicoccoides*, whereas in ND 645 these arrowheads indicate bands inherited from either Coteau (compare patterns 8 and 9) or *T. t. dicoccoides*.

The curved brackets in the patterns of ND 643 and ND 645 indicate bands in the α-gliadin region inherited from *T. t. dicoccoides*, and the square brackets in ND 644 indicate two bands inherited from Len. The last two arrowheads (17 and 18) in the pattern of ND 644 indicate bands inherited from either Len or RL4352-1.

All gliadin bands appearing in the high-protein lines were inherited from the three parents. However, it is not obvious in the pattern of ND 644 which bands were inherited from *T. t. dicoccoides*, since many bands of the parent varieties have the same mobility. It also seems that certain bands were inherited in groups such as those bands indicated by the first five arrowheads inherited from RL4352-1 (Columbus), those three bands indicated by the square bracket in the γ-gliadin region inherited from Len, and those bands indicated by the curved brackets in the α-gliadin region inherited from *T. t. dicoccoides*. These results would seem to be in accord with the observations of Sozinov and Poperelya (1980) that the expression of various gliadin components is controlled by different gene clusters or blocks so that the first five bands of RL4352-1, for example, correspond to the Gli-1 locus of chromosome 1D.

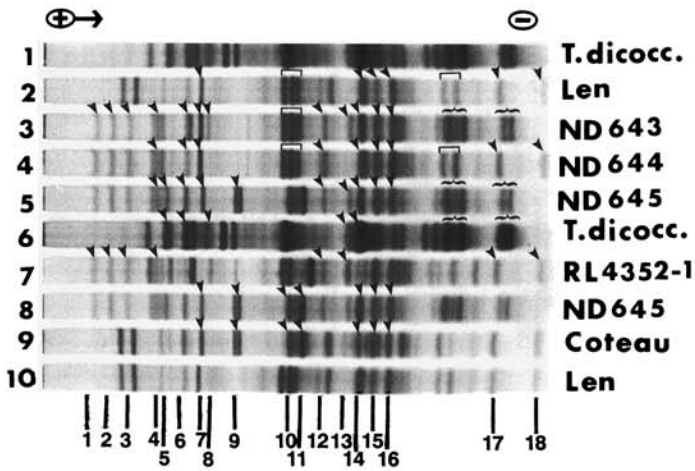

Fig. 1. Polyacrylamide gel electrophoretic patterns at pH 3.1 in aluminum lactate buffer of the gliadin proteins from the high protein cultivars: ND 643, ND 644, and ND 645, and their parents.

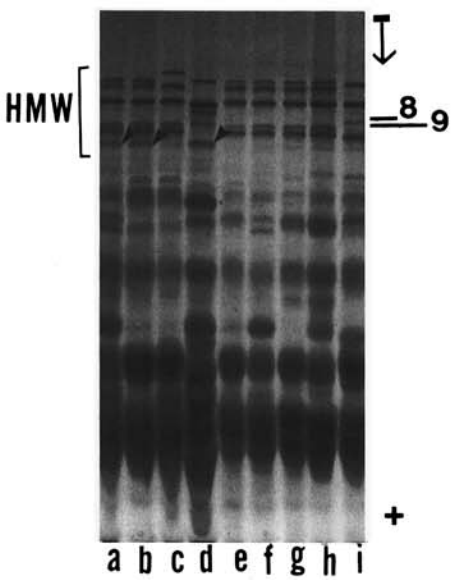

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of the high protein cultivars and their parents: ND 645 (**a**), ND 644 (**b**), ND 643 (**c**), *T. t. dicoccoides* (**d**), Len (**e**), Coteau (**f**), RL-4352-1 (**g**), and Thatcher (**h**) and Chinese Spring (**i**) as reference cultivars. Subunit designations are given in Table III.

Inheritance of the High Molecular Weight Glutenin Subunits

SDS-PAGE was used to trace the inheritance of the high molecular weight (HMW) subunits of glutenin. Figure 2 shows the HMW subunits of glutenin separated on a 12% gel. A total of eight HMW subunits were found in the parents and progenies. Table III shows the HMW subunit composition according to the nomenclature of Payne et al (1980, 1981). All the hard red spring wheat parent varieties have the 2*, 7+9, 5+10 subunit composition. *T. t. dicoccoides*, however, has quite a different subunit composition: 2*, 17+18, and a subunit between 8 and 9 designated "8+," and one slightly faster than 12 designated "12+." The high-protein lines ND 644 and ND 645 have the 2*, 7+9, 5+10 combination plus the 12+ subunit, (see arrowheads in Fig. 2) the latter inherited from *T. t. dicoccoides*. However, ND 643 is unique in that it contains the subunit 1, 7+9, 5+10 combination but does not have the 2* or the 12+ subunit.

It is surprising that ND 643 should contain subunit 1 since none of the parents possesses this 1A subunit. There are two, and perhaps three, possibilities to explain the appearance of subunit 1 in ND 643. Fist, it is possible that Len may not be one of the parents of ND 643 but instead a contaminant variety that contained subunit 1. Protein extracts of possible contaminant varieties that contained HMW glutenin subunit 1 were electrophoresed by PAGE to examine their gliadin protein patterns and compared to the Len gliadin pattern. The variety Shield showed the closest pattern to Len. Shield was, therefore, electrophoresed side by side with Len, ND 643, RL4352-1, and *T. t. dicoccoides* to compare gliadin PAGE patterns. The pattern of Shield, however, did not have the three bands characteristic of Len in the γ-gliadin region (square brackets in γ-region of Len pattern in Fig. 1). These three bands also appeared in ND 643. Therefore, contamination by another variety does not seem to be the answer for the appearance of HMW subunit 1 in ND 643. Len, therefore, seems to be one of the parents of ND 643.

A second possibility, and perhaps a more plausible one, is that the original seeds of the Len parent may have contained some heterozygosity or a biotype possessing subunit 1 instead of the 2* subunit. Alternatively, the *T. t. dicoccoides* parent may have contained a mixture of different accessions in which subunit 1 was present. Cole et al (1981) showed that some accessions of *T. t. dicoccoides* contained an HMW glutenin subunit similar to subunit 1. Even though single-kernel analyses (original seed source used for crossing) of both Len (80 kernels) and *T. t.*

TABLE III
High Molecular Weight Subunit Composition of Glutenin of *T. t. dicoccoides*-Derived High-Protein Hard Red Spring Lines and Their Parents

Sample	Chromosome 1A	Chromosome 1B	Chromosome 1D	Other High Molecular Weight Subunits
RL4352-1	2*	7,9	5,10	···
Len	2*	7,9	5,10	···
Coteau	2*	7,9	5,10	···
T. t. dicoccoides	2*	17,18	···	8+,12+
ND 643	1	7,9	5,10	···
ND 644	2*	7,9	5,10	12+
ND 645	2*	7,9	5,10	12+

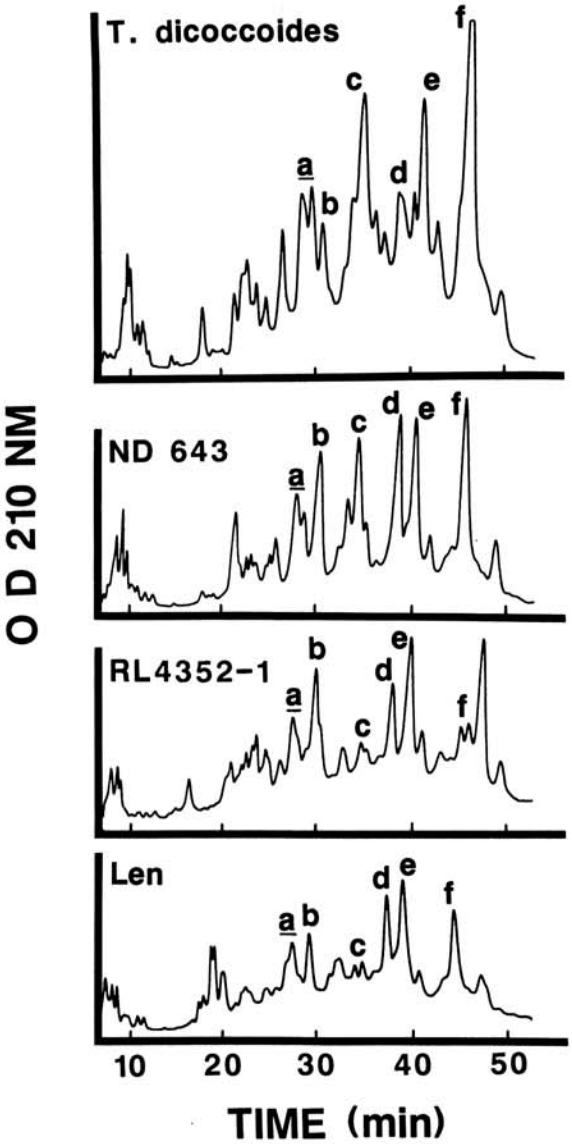

Fig. 3. Reversed-phase high-performance liquid chromatographic profiles of the high protein line ND 643 and its parents.

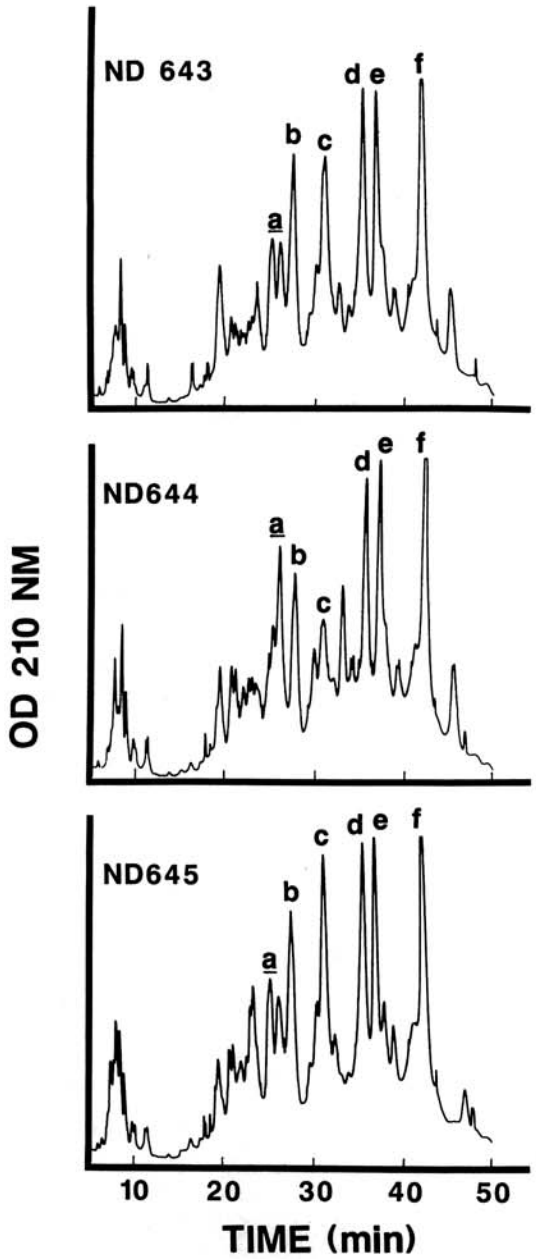

Fig. 4. Reversed-phase high-performance liquid chromatographic profiles of the three high protein lines: ND 643, ND 644, and ND 645.

dicoccoides (40 kernels) did not show heterozygosity or different biotypes, it still does not rule out this possibility. A third explanation would be a gene reactivation event. This type of occurrence, however, is very rare and has not as yet been documented.

RP-HPLC of 70% Aqueous Ethanol Extract

RP-HPLC was used to characterize the gliadin fraction of the three lines and to compare them with their parents (Figs. 3 and 4). Comparisons are based on similarities in elution times and peak height of the RP-HPLC profiles. The peak labeled b in Figure 3 would seem to be inherited from RL4352-1. Peaks labelled c and f are quite similar in elution time and peak intensity in *T. t. dicoccoides* and ND 643. These peaks are much smaller in Len and especially in RL4352-1. Therefore, the protein components in these peaks in ND 643 would seem to be inherited from *T. t. dicoccoides*. Peaks d and e of ND 643 seem to be more similar to peaks d and e of RL4352-1 and Len.

Figure 4 compares the surface hydrophobicity of the three high-protein lines. The peaks labelled d, e, and f are similar in elution time in all three lines. Peaks c and f of ND 645 would seem to be inherited from *T. t. dicoccoides* as shown in Figure 3 for ND 643. It should be noted that all three high-protein lines inherited a large peak f, the most hydrophobic major peak, from *T. t. dicoccoides*..

Summary and Conclusions

Three high-protein hard red spring wheat lines were developed from three-way crosses with *T. t. dicoccoides*, a high-protein donor parent. These three lines were equal, and in some instances better, in breadmaking quality characteristics than control varieties. Protein characterization by PAGE, SDS-PAGE, and RP-HPLC showed that a number of components were inherited from *T. t. dicoccoides*. The gliadin components of ND 643 and 645 in the α-region were inherited from *T. t. dicoccoides* and were similar in staining intensity to the components of *T. t. dicoccoides*. The RP-HPLC profiles showed that all three high-protein lines contained a major peak in the most hydrophobic region of the profile similar in retention time to a major peak of *T. t. dicoccoides*.

Studies are continuing to identify and characterize the protein fraction(s) that contribute the high protein content in the three high-protein lines. This information will help in understanding the relationship between wheat protein composition and breadmaking quality.

ACKNOWLEDGMENTS

The financial support of the North Dakota Wheat Commission and the Minnesota Wheat Research and Promotion Council is gratefully acknowledged. The authors thank Juan Figueroa for technical assistance.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method numbers, approval dates. The Association: St. Paul, MN.

AVIVI, L. 1978. High grain protein content in wild tetraploid wheat *Triticum dicoccoides* Korn. Proc. Int. Wheat Genetics Symp., 5th, New Delhi, India, p. 372.

AVIVI, L., LEVY, A. A., and FELDMAN, M. 1983. Studies on high protein durum wheat derived from crosses with the wild tetraploid wheat, *Triticum turgidum* var. *dicoccoides*. Proc. Int. Wheat Genetics Symp., 6th, Kyoto, Japan, p. 199.

COLE, E. W., FULLINGTON, J. G., and KASARDA, D. D. 1981. Grain protein variability among species of *Triticum* and *Aegilops*: Quantitative SDS-PAGE studies. Theor. Appl. Genet. 60:17.

D'APPOLONIA, B. L., GILLES, K. A., and MEDCALF, D. G. 1970. Effect of water-soluble pentosans on gluten-starch loaves. Cereal Chem. 47:194.

HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high performance liquid chromatography. Cereal Chem. 64:25.

KHAN, K., HAMADA, A. S., and PATEK, J. 1985. Polyacrylamide gel electrophoresis for wheat variety identification: Effect of variables on gel properties. Cereal Chem. 62:310.

KHAN, K., JACOBSEN, A., and HUCKLE, L. 1988. Improvements in Polyacrylamide Gel Electrophoresis of Gliadin Proteins for Wheat Variety Identification: Effect of gel dimensions, apparatus design, buffer systems and other variables on resolution and separation. Manual. Department of Cereal Science and Food Technology, North Dakota State University: Fargo, ND.

KUSHNIR, U., and HALLORAN, G. M. 1984. Transfer of high kernel weight and high protein from wild tetraploid wheat (*Triticum turgidum dicoccoides*) to bread wheat (*T. aestivum*) using homologous and homoeologous recombination. Euphytica 33:249.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680.

LOOKHART, G. L., ALBERS, L. E., POMERANZ, Y., and WEBB, B. D. 1987. Identification of U.S. rice cultivars by high-performance liquid chromatography. Cereal Chem. 84:199.

NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324.

PAYNE, P. I. 1987. The genetical basis of baking quality in wheat. Aspects Appl. Biol. 15:79.

PAYNE, P. I., LAW, C. N., and MUDD, E. E. 1980. Control by homoeologous group 1 chromsomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor. Appl. Genet. 58:113.

PAYNE, P. I., CORFIELD, K. G., HOLT, L. M., and BLACKMAN, J. A. 1981. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheats. J. Sci. Food Agric. 32:51.

SAS INSTITUTE. 1983. SAS Users Guide: Statistics. The Institute: Cary, NC.

SOZINOV, A. A., and POPERELYA, F. A. 1980. Genetic classification of prolamines and its use for plant breeding. Ann. Technol. Agric. 29:229.

[Received December 9, 1988. Accepted April 20, 1989.]