Inheritance of Gluten Protein Components of a High-Protein Hard Red Spring Wheat Line Derived from *Triticum turgidum* var. *dicoccoides*—Semipreparative RP-HPLC, Gel Electrophoresis, and Amino Acid Composition Studies

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ABSTRACT

A high-protein hard red spring (HRS) wheat line (ND 643), derived from crosses with *Triticum turgidum* var. *dicoccoides* (a high-protein wild tetraploid) and the HRS wheats Len and RL 4352-1, was investigated for inheritance of its protein components. Protein fractions of a 70% ethanol extract (gliadin proteins) of meal samples were collected by semipreparative reversed-phase high-performance liquid chromatography (semiprep RP-HPLC). Polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate (SDS)-PAGE of the fractions from RP-HPLC showed that ND 643 inherited most of its gliadin components in the α- and β-gliadin regions from *T. t. dicoccoides*. PAGE and SDS-PAGE of the fractions from an Osborne solubility fractionation procedure showed that ND 643 inherited most of its glutenin and residue proteins from Len and RL 4352-1. Quantitative data from the Osborne procedure showed that ND 643 contained significantly more gliadin and residue proteins per unit weight of sample than Len or RL 4352-1. ND 643 also seemed to retain a ratio of gliadin to total glutenin proteins similar to those of its HRS parents of good bread-making quality. Amino acid composition analyses of the fractions from RP-HPLC showed that ND 643 possessed proteins having compositions similar to those of its HRS parents.

Many researchers have attempted to increase the protein contents of cultivated wheats. High protein contents, ranging from 17 to 27% have been found in wild tetraploid wheats (Avivi 1978). Of the wild species examined, *Triticum turgidum* var. *dicoccoides* had the highest protein contents. Attempts have been 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% by crossing a durum variety of 15% protein with *T. t. dicoccoides*. However, under field conditions the durum lines were prone to severe lodging. In Australia, Kushner and Halloran (1984) produced bread wheat lines with high kernel weights and high-protein contents from crosses with *T. t. dicoccoides*. At North Dakota State University, Joppa and Cantrell (1990) developed a high-protein durum line from crosses with *T. t. dicoccoides*. We are not aware, however, of any similarly derived high-protein lines in commercial production in the United States or other countries. At North Dakota State University, three high-protein bread wheat lines were developed from crosses with *T. t. dicoccoides*. A previous article (Khan et al. 1989) reported on the inheritance of some of the gliadin and the high-molecular-weight glutenin proteins of these three lines. The present study is a more detailed examination of the gluten proteins of one of these lines, ND 643, to determine why this high-protein line possessed the best rheological and bread-making properties (Khan et al. 1989). A combination of techniques such as semipreparative reversed-phase high-performance liquid chromatography (RP-HPLC), polyacrylamide gel electrophoresis (PAGE), protein solubility fractionation, and amino acid composition analyses provided additional information on inheritance and bread-making quality of the protein components of ND 643.

MATERIALS AND METHODS

Wheat Samples
The high-protein line ND 643 and its parents (RL 4352-1, Len, and *T. t. dicoccoides* [access. #FA15-3]) were obtained from R. Frohberg, Crop and Weed Sciences Department, North Dakota State University, Fargo. ND 643 was derived from a three-way cross as outlined in Table I of Khan et al. (1989). Samples were obtained from replicated plots grown in the same environment.

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PAGE and SDS-PAGE

PAGE was performed according to Khan et al. (1985, 1988). Sodium dodecyl sulfate (SDS)-PAGE was performed by a modified procedure of Laemmli (1970) as described by Khan et al. (1989). Fractions isolated by semipreparative RP-HPLC were freeze-dried and then subjected to gel electrophoresis.

RP-HPLC

RP-HPLC was performed according to a modification of the procedure of Huebner and Bietz (1987) and Lookhart et al. (1987) as described by Khan et al. (1989). For the semipreparatory (semiprep) procedure, the linear HPLC elution gradient began at 25% acetonitrile (solvent B) and increased to 35% B at 40 min, 40% B at 80 min, 45% B at 120 min, 50% B at 160 min, 100% B at 170 min, and returned to 25% B at 175 min. The column was reequilibrated at 25% B for 25 min before the next injection. A SynChropak RP column (C18, 300 Å pore size, 250 x 10-mm) (SynChrom Inc., Linden, NJ) was used. It was eluted at 55°C, with a flow rate of 1 ml/min; 100 µl of extract was injected (from a 500-µg/ml extracted sample—see Khan et al. [1989] for more details); absorbance of the eluate was monitored at 210 nm; and 1-ml fractions were collected.

Protein Solubility Fractionation

The modified Osborne protein solubility fractionation procedure of Chen and Bushuk (1970) was used to obtain albumin, globulin, gliadin, glutenin, and residue fractions.

Amino Acid Composition Determination

Amino acid compositions were determined on protein samples hydrolyzed at 155°C for 55 min. Amino acids of the hydrolysates were derived with phenylisothiocyanate (PTC), and the PTC derivatives were separated by HPLC and quantified (Jones and Poule 1990).

RESULTS AND DISCUSSION

Figure 1 is a representative RP-HPLC profile obtained with an extract of ND 643 using the semiprep column. Twelve fractions were collected and analyzed by PAGE, SDS-PAGE, and amino acid composition analyses. Elution times were used as criteria for matching peaks among the four samples (T. t. dicoccoides, ND 643, Len, and RL 4352-1).

PAGE and SDS-PAGE of RP-HPLC Fractions

Figure 2A and B shows the PAGE patterns of the fractions (collected from semiprep HPLC) of ND 643 and its parents (Len, RL 4352-1, and T. t. dicoccoides). Comparison of the patterns revealed that ND 643 inherited most of its α- and β-gliadin components from T. t. dicoccoides. For example, the components in fractions F3, F5, F7, F8, and F9 of ND 643 (Fig. 2A and B) were inherited from T. t. dicoccoides. The gliadin components in the ω region were inherited from RL 4352-1 and Len. The inheritance of other components such as F4, F6, F10, and F11 was not quite clear from the PAGE patterns, but it seems they were inherited from Len and RL 4352-1. The PAGE patterns of the proteins of the RP-HPLC fractions also revealed that the ω-gliadins eluted first from the semiprep column, followed by β- and γ-gliadins, then by α-gliadins, and finally by other β- and γ-gliadins. The sequence of the gliadin elutions of this study agrees with that of Lookhart et al. (1988). The RP-HPLC gliadin protein fractions contained a mixture of components of similar PAGE mobilities but of different surface hydrophobicities.

SDS-PAGE (Fig. 3A and B) of the fractions from semiprep RP-HPLC clarified uncertainties in the patterns in Figure 2A.

![Fig. 1. Semipreparative reversed-phase high-performance liquid chromatography profile of a 70% ethanol extract of ND 643. F1 (fraction 1) to F12 indicates fractions that were collected and analyzed.](image)

![Fig. 2. Polyacrylamide gel electrophoresis (PAGE) patterns of gliadin protein fractions (F) isolated by semipreparative reversed-phase high-performance liquid chromatography (RP-HPLC). F1-F6 (A) and F7-F12 (B) = fractions collected by RP-HPLC and analyzed by PAGE. The sequence of sample application is the same as in patterns 1-4: 1, Triticum turgidum var. dicoccoides (TD); 2, ND 643 (643); 3, Len; 4, RL 4352-1 (RL). 643, Len, and RL always follow TD in the same order. When only two patterns are indicated, as in F6, the first is TD and the second is 643, following the same sequence.](image)
and B and confirmed the inheritance of the gliadin protein components of ND 643. Judging from the number and intensity of the protein components of the fractions (Fig. 3), it appears that ND 643 inherited most of its α- and β-gliadin components from *T. t. dicocoides*, the high-protein donor parent.

The PAGE patterns (Fig. 2) also revealed that F1, F2, and F3 each contained a number of components, mainly α-gliadins. However, SDS-PAGE (Fig. 3A) revealed components with molecular weights of around 50,000–70,000 in these fractions, and in addition F2 and F3 contained intensely stained components with molecular weights of around 14,000. The latter components are most likely albumin-globulin components that did not appear in the F2 and F3 PAGE patterns because these components eluted off the gel in the extended gliadin electrophoresis procedure (Khan 1982). Some albumins and globulins were presumably extracted together with the gliadins in the 70% ethanol extracts of meal or flour used for the RP-HPLC separations. The SDS-PAGE patterns (Fig. 3A and 3B) also revealed that most fractions contained approximately six major bands and that even though PAGE indicated that the α-, β-, and γ-gliadins differed greatly in electrostatic charge (Fig. 2A and 2B), their molecular weights all fell within a narrow range (30,000–45,000).

The PAGE (Fig. 2) and SDS-PAGE (Fig. 3) patterns also revealed that some gliadin components that ND 643 inherited from its parents had different surface hydrophobic properties. For example, F6, F7, F8, and F10 of ND 643 inherited from Len (F5), *T. t. dicocoides* (F6, F7), and Len (F10), respectively, eluted with longer retention times (were more hydrophobic) than the equivalent components from its parents. Only F4, the fraction that ND 643 inherited from RL 4352-1 (F4), eluted with a shorter retention time than the equivalent gliadin components of the parent.

**Protein Solubility Fractionation**

The modified Osborne protein solubility fractionation procedure of Chen and Bushuk (1970) was used to compare the protein distribution and amount of each protein in each protein class (details of the protein content of samples were reported by Khan et al. [1989]). The proportion of protein in each solubility class, as a ratio of total protein (Table I), was not significantly different among the albumin fractions of the lines. *T. t. dicocoides* contained a significantly lower proportion of globulin than the other samples. ND 643 contained globulin proportions similar to those of Len and RL 4352-1. There was no difference in the proportion of gliadin among the samples. *T. t. dicocoides* contained significantly higher proportions of acid-soluble glutenin than the other samples, but the proportion of glutenin in ND 643 was similar to that in Len and RL 4352-1. The proportion of residue proteins followed the general trend observed by Orth and Bushuk (1972)—that is, when the proportion of gliadin or acid-soluble glutenin was high or low, then the proportion of residue proteins was low or high, respectively. For example, *T. t. dicocoides* has low residue protein amounts and high glutenin compared to Len, RL 4352-1, and ND 643, which all have high residue and low glutenin amounts.

In Table II, the amount of each class of protein per gram of sample reveals a trend for albumin and globulin similar to that seen in Table I. The amount of gliadin, however, is significantly higher in *T. t. dicocoides* and ND 643 than in Len and RL 4352-1. ND 643 had a gliadin content similar to that of the high-protein parent *T. t. dicocoides*. The acetic acid-soluble glutenin had a trend similar to that seen in Table I, but the residue fraction of ND 643 was significantly greater than in the other samples.

The protein fractionation results may partly explain the good bread-making characteristics of ND 643. For example, ND 643 had a gliadin content similar to that of *T. t. dicocoides*, but its glutenin and residue contents were similar to those of Len and RL 4352-1. ND 643 thus retained a protein solubility profile.

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**TABLE I**

Quantitative Comparison of Duncan's Test of the Protein Fractions of the Osborne Procedure from ND 643 and Its Parental Lines

<table>
<thead>
<tr>
<th>Lines</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Gliadin</th>
<th>Glutelin</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. t. dicocoides</em></td>
<td>11.6 a</td>
<td>4.8 c</td>
<td>28.1 a</td>
<td>33.1 a</td>
<td>16.6 c</td>
</tr>
<tr>
<td>ND 643</td>
<td>11.2 a</td>
<td>9.5 b</td>
<td>31.5 a</td>
<td>10.9 b</td>
<td>32.9 b</td>
</tr>
<tr>
<td>Len</td>
<td>10.5 a</td>
<td>10.9 ab</td>
<td>27.4 a</td>
<td>6.9 b</td>
<td>35.8 a</td>
</tr>
<tr>
<td>RL 4352-1</td>
<td>10.8 a</td>
<td>11.9 a</td>
<td>30.9 a</td>
<td>9.5 b</td>
<td>32.5 b</td>
</tr>
</tbody>
</table>

*Means with the same letter in columns are not significantly different at the 5% probability level.

**TABLE II**

Quantitative Comparison of Duncan's Test of the Protein Fractions of the Osborne Procedure from ND 643 and Its Parental Lines

<table>
<thead>
<tr>
<th>Lines</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Gliadin</th>
<th>Glutelin</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. t. dicocoides</em></td>
<td>30.0 a</td>
<td>12.4 b</td>
<td>73.1 a</td>
<td>86.1 a</td>
<td>43.2 c</td>
</tr>
<tr>
<td>ND 643</td>
<td>24.1 a</td>
<td>20.5 a</td>
<td>67.8 ab</td>
<td>23.5 b</td>
<td>71.0 a</td>
</tr>
<tr>
<td>Len</td>
<td>20.2 b</td>
<td>18.8 a</td>
<td>46.9 c</td>
<td>11.8 b</td>
<td>61.4 b</td>
</tr>
<tr>
<td>RL 4352-1</td>
<td>19.8 b</td>
<td>21.8 a</td>
<td>56.7 bc</td>
<td>17.3 b</td>
<td>59.6 b</td>
</tr>
</tbody>
</table>

*Means with the same letter in columns are not significantly different at 5% probability level.
similar to that of a typical good bread-making quality HRS wheat and did not contain disproportionate amounts of fractions as did \( T. \ t. \ dicoccoides \). For example, the glutenin and residue protein fractions of \( T. \ t. \ dicoccoides \) showed an opposite trend (that is, more glutenin, less residue) compared to the proportion of these fractions in the other three wheat samples used in this study. Even though ND 643 had a higher gliadin content—an indicator of poor bread-making quality (Orth and Bushuk 1972)—than Len and RL 4352-1, ND 643 also had a higher residue content—an indicator of good bread-making quality (Orth and Bushuk 1972)—than Len and RL 4352-1. The relative proportions of the various gluten proteins necessary for good bread-making quality seem to have been maintained in ND 643.

**SDS-PAGE of Osborne Fractions**

SDS-PAGE of the albumins and globulins of the Osborne fractionation procedure showed that ND 643 inherited the majority of its components from Len and RL 4352-1 (Fig. 4). One minor component (arrow in Fig. 4) in the albumin fraction seemed to be inherited from \( T. \ t. \ dicoccoides \).

SDS-PAGE of the gliadin fractions (Fig. 5) showed that ND 643 inherited a number of protein components (arrows in pattern 2) from \( T. \ t. \ dicoccoides \). These have molecular weights in the 31,000—45,000 region. SDS-PAGE of the glutenin fraction (Fig. 5, patterns 5—8) showed that ND 643 inherited a few components (arrows in pattern 6) from \( T. \ t. \ dicoccoides \) in the 14,000—50,000 mol wt region. Components with molecular weights of 45,000 and above were inherited from Len and RL 4352-1. ND 643 did not inherit any of the high-molecular-weight (HMW) subunits of glutenin from \( T. \ t. \ dicoccoides \). This confirms the results of our previous study (Khan et al. 1989), in which we discussed the appearance of the slowest HMW subunit in the patterns of ND 643 (but absent in its parents), possibly due to biotypes of its parents. The patterns of the residue fraction (Fig. 5, patterns 9—12) showed that the majority of residue proteins were inherited from Len and RL 4352-1.

**Amino Acid Composition**

Table III compares the amino acid compositions of the proteins in three protein peaks (F4, F7, and F9, in Fig. 1) separated by semipreparative RP-HPLC from 70% ethanol extracts of ND 643 and its parent lines. These represent early (less hydrophobic), medium, and later (more hydrophobic) eluting peaks. Comparison of the amino acid composition of the three peaks revealed that the early eluting peak (F4) contained greater amounts of proline and phenylalanine than F7 and F9. The later eluting peak (F9) showed higher contents of valine, methionine, lysine, and cysteine. The mid-eluting peak (F7) had a lower content of phenylalanine.

Comparison of the amino acid compositions of fractions from ND 643 with those from its HRS wheat parent lines revealed a general similarity—that is, all F4, F7, and F9 compositions were similar. This may explain how ND 643 maintained its good bread-making quality characteristics (Khan et al. 1989). \( T. \ t. \ dicoccoides \) was generally similar in amino acid composition to at least one of the other lines except for lower glutamic acid and lysine contents and higher methionine contents in fraction 9.

**CONCLUSION**

Through the use of a combination of biochemical techniques such as semipreparative RP-HPLC and protein solubility fractionation, combined with PAGE and SDS-PAGE of protein fractions obtained by these techniques, this study provides additional information on the inheritance of the protein components of ND 643, a high-protein HRS wheat, previously reported by Khan et al (1989). ND 643 inherited most of its more intensely stained \( \alpha \)- and \( \beta \)-gliadin components from \( T. \ t. \ dicoccoides \), a wild tetraploid species, but it inherited most of its glutenin components from the two HRS wheats, Len and RL 4352-1. That ND 643 inherited gliadins from \( T. \ t. \ dicoccoides \) is not surprising because \( T. \ t. \ dicoccoides \) does not contain the D genome and would be expected to contribute protein components from the A and B genomes, where a number of gliadin...
components are located (Payne 1987). Joppa and Cantrell (1990) also demonstrated that the genes for a high-protein durum line derived from chromosomal substitutions with T. t. dicoccoides were predominantly located on chromosome 6B. In the present study the glutenin components were inherited from the HRS wheat parents. T. t. dicoccoides does not possess the D genome that contains the genes coding for most of the highly aggregated glutenin proteins. Differences in aggregation of the glutenins was seen in the results of the Osborne solubility procedure: T. t. dicoccoides contained a very large amount of the easily-extractable soluble glutenin (less aggregated), and Len and RL 4352-1 contained much higher amounts of residue (highly aggregated) proteins (insoluble glutenins). The ND 643 line had the largest amount of highly aggregated insoluble glutenin, which is indicative of good bread-making quality. These results seem to indicate that the unique interactive properties of the glutenin proteins may be the important factor or factors that determine the final expression of bread-making quality. For example, reports by Payne et al. (1987), Gupta et al. (1989, 1991), and Pogna et al. (1990) have indicated that, in addition to the HMW glutenin subunits, gluten strength is also influenced by the presence of certain low molecular weight glutenin subunits. Therefore, it seems that ND 643 inherited a proper balance of proteins from its parents to maintain the good bread-making quality (Khan et al 1989) characteristic of its HRS wheat parents.

ACKNOWLEDGMENT

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