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ORIGINAL PAPER

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# Chromosomal location of genes for novel glutenin subunits and gliadins in wild emmer wheat (*Triticum turgidum* L. var. *dicoccoides*)

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**Abstract** The glutenin and gliadin proteins of wild emmer wheat, *Triticum turgidum* L. var. *dicoccoides*, have potential for improvement of durum wheat (*T. turgidum* L. var. *durum*) quality. The objective of this study was to determine the chromosomes controlling the high molecular weight (HMW) glutenin subunits and gliadin proteins present in three *T. turgidum* var. *dicoccoides* accessions (Israel-A, PI-481521, and PI-478742), which were used as chromosome donors in Langdon durum- *T. turgidum* var. *dicoccoides* (LDN-DIC) chromosome substitution lines. The three *T. turgidum* var. *dicoccoides* accessions, their respective LDN-DIC substitution lines, and a number of controls with known HMW glutenin subunits were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), urea/SDS-PAGE, and acid polyacrylamide gel electrophoresis (A-PAGE). The results revealed that all three *T. turgidum* var. *dicoccoides* accessions possess *Glu-A1* alleles that are the same as or similar to those reported previously. However, each *T. turgidum* var. *dicoccoides* accession had a unique *Glu-B1* allele. PI-478742 had an unusual 1Bx subunit, which had mobility slightly slower than the 1Ax subunit in 12% SDS-PAGE gels. The subunits controlled by chromosome 1B of PI-481521 were slightly faster in mobility than the subunits of the *Glu-B1n* allele, and the 1By subunit was identified as band 8. The 1B subunits of Israel-A had similar mobility to subunits 14 and 16. The new *Glu-B1* alleles were designated as *Glu-B1be* in Israel-A, *Glu-B1bf* in PI-481521, and *Glu-B1bg* in PI-478742. Results from A-PAGE revealed that PI-481521, PI-478742, and Israel-A had eight, 12, and nine unique gliadin bands, respectively, that were assigned to specific chromosomes. The identified glutenin subunits and gliadin proteins in the LDN-DIC substitution lines provide the basis for evaluating their effects on end-use quality, and they are also useful biochemical markers for identifying specific chromosomes or chromosome segments of *T. turgidum* var. *dicoccoides*.

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## Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) quality is a complex function of several components. The most important proteins are the glutenins and gliadins, each of which is subdivided into additional classes. The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits are controlled by orthologous gene sets located on the long and short arms of the group 1 chromosomes, respectively. The only known exception is an LMW glutenin subunit conditioned by the *Glu-D5* locus and located on chromosome 7D (Sreeramulu and Singh 1997).

There are four major classes of gliadins designated as $\alpha$-,$\beta$-,$\gamma$-, and $\omega$-gliadins. The $\omega$-gliadins and most of the $\gamma$-gliadins are controlled by genes on the short arms of the group 1 chromosomes and tightly linked to the genes controlling LMW glutenin subunits, and the $\alpha$-gliadins and most of the $\beta$-gliadins are controlled by genes on the short arms of the group 6 chromosomes (Payne et al. 1984). Gliadin bands observed in acid polyacrylamide gel electrophoresis (A-PAGE) gels were originally identified according to their relative mobilities (RM) (Bushuk and Zillman 1978). However, because RM values of gliadins can be affected by several factors (Metakovsky and Novoselskaya 1991), identification of gliadin alleles based on banding patterns was proposed by Metakovsky (1991).

Each HMW glutenin subunit locus encodes two genes, and hence produces x- and y-type subunits. The y-type subunits have faster electrophoretic mobility than the x-type. Because each locus controls two subunits, there are potentially six HMW glutenin subunits in cultivated wheat. However, in cultivated wheat, gene silencing results in expression of none or only one protein from the *Glu-A1* locus and either one or two proteins from the *Glu-B1* locus. Gene silencing also occurs in wild wheat, but wild tetraploid species with four subunits are not uncommon. Analysis of the genes controlling HMW glutenin subunits can be further complicated by faint bands described as 1Ax′ and 1By′ by Levy et al. (1988). Because these bands appeared to be coded by the same genes as the 1Ax and 1By subunits, they could be produced by proteolytic digestion, post-translation modification, premature transcriptional termination, incomplete reduction during glutenin separation, or re-oxidation during electrophoresis.

Wild wheats, including *T. turgidum* L. var. *dicoccoides* (abbreviated as DIC), are important sources of new genes for wheat quality improvement. There are presently 22 *Glu-A1* and 56 *Glu-B1* alleles listed in the catalogue of gene symbols, several of which were first identified in *T. turgidum* var. *dicoccoides* (McIntosh et al. 2003). The HMW glutenin subunit genes present in *T. turgidum* var. *dicoccoides* have been reported by at least four studies. In the first of these studies, Vallega and Waines (1987) reported three new *Glu-A1* alleles and six new *Glu-B1* alleles. Subsequent studies by Levy et al. (1988), Ciaffi et al. (1993), and Pflüger et al. (2001) reported several additional new subunit patterns. These alleles represent potential sources for improvement of quality characteristics.

In durum, substitution lines can facilitate genetic analysis of complexly inherited agronomic and quality traits. In the 1980s, USDA geneticist L.R. Joppa developed a set of Langdon durum-*T. turgidum* var. *dicoccoides* (LDN-DIC) substitution lines using *T. turgidum* var. *dicoccoides* accession Israel-A (FA-15-3) as the chromosome donor (Joppa and Cantrell 1990). Each substitution line carries 13 pairs of chromosomes from the recurrent parent (LDN) and one pair from the donor parent (Israel-A). When the substitution lines are compared to the recurrent and donor parents for agronomic or quality characteristics, any differences can be attributed to genes on the chromosome derived from the donor (*T. turgidum* var. *dicoccoides*) parent.

Quality data of a set of LDN-DIC (Israel-A) substitution lines has been reported (Joppa and Cantrell 1990; Joppa et al. 1991). Unique quality traits of these lines included a gene for high grain-protein content on chromosome 6B (Joppa and Cantrell 1990), and improved mixing characteristics of the 1B chromosome substitution (Joppa et al. 1991). However, the HMW glutenin subunit and gliadin banding patterns of the LDN-DIC (Israel-A) substitution lines have not been reported.

Because mixing characteristics are associated with gluten proteins, knowledge of the proteins present in Israel-A, which contributes to improved mixing characteristics, would be useful in facilitating the introgression of improved quality from *T. turgidum* var. *dicoccoides* to local durum cultivars. In addition, substitution lines based on two other *T. turgidum* var. *dicoccoides* accessions (PI-481521 and PI-478742) have been recently developed by L.R. Joppa. The objective of the present study was to determine the HMW glutenin subunit and gliadin banding patterns, and identify the chromosomal locations of the corresponding genes in the three sets of LDN-DIC substitution lines.

## Materials and methods

### Plant materials

Three sets of LDN-DIC chromosome substitution lines developed by L. R. Joppa and their respective *T. turgidum* var. *dicoccoides* chromosome donor parents Israel-A, PI-481521, and PI-478742, as well as durum wheat LDN, were used in this study. The abbreviated designations for the 38 available substitution lines are listed in Table 1. For the set based on Israel-A, the LDN(ISA-2B) was not available. The set based on PI-481521 included all 14 chromosome substitutions. The set derived from PI-478742 was missing substitutions for chromosomes 2A, 3A, and 3B.

The three sets of LDN-DIC lines were produced by crossing each LDN durum D-genome disomic substitution line (LDN D-genome DS) (Joppa and Williams 1988) as female to each *T. turgidum* var. *dicoccoides* accession. Five to seven backcrosses were made to the LDN D-genome DS to restore the LDN genetic background. The LDN-DIC substitution lines were selected after one generation of self-pollination of $BC_5$, $BC_6$, and $BC_7$ plants. The seed samples of LDN-DIC (Israel-A) substitutions used in this study were progenies of $BC_5F_5$ plants and the seeds of LDN-DIC (PI-481521) and LDN-DIC (PI-478742) substitutions were harvested from $BC_6F_2$ or $BC_7F_2$ plants.

Sixteen additional *T. turgidum* var. *dicoccoides* accessions and two durums having unique, known HMW glutenin subunits (Vallega and Waines 1987; Pflüger et al. 2001) were obtained from Dr. Harold Bockelman, National Plant Germplasm System, USDA-ARS, Aberdeen, Idaho (Table 2). The HMW glutenin subunits present in those accessions are described in Table 2. The accessions of *T. turgidum* var. *dicoccoides* from the studies of Levy et al. (1988) and Ciaffi et al. (1993) were not obtained because

**Table 1** Designations for the 38 LDN-DIC disomic chromosome substitution lines used in this study

| Chromosome | Abbreviated designations of LDN-DIC substitution lines for: | | |
|---|---|---|---|
| | Israel-A | PI-481521 | PI-478742 |
| 1A | LDN(ISA-1A) | LDN(521-1A) | LDN(742-1A) |
| 2A | LDN(ISA-2A) | LDN(521-2A) | – |
| 3A | LDN(ISA-3A) | LDN(521-3A) | – |
| 4A | LDN(ISA-4A) | LDN(521-4A) | LDN(742-4A) |
| 5A | LDN(ISA-5A) | LDN(521-5A) | LDN(742-5A) |
| 6A | LDN(ISA-6A) | LDN(521-6A) | LDN(742-6A) |
| 7A | LDN(ISA-7A) | LDN(521-7A) | LDN(742-7A) |
| 1B | LDN(ISA-1B) | LDN(521-1B) | LDN(742-1B) |
| 2B | – | LDN(521-2B) | LDN(742-2B) |
| 3B | LDN(ISA-3B) | LDN(521-3B) | – |
| 4B | LDN(ISA-4B) | LDN(521-4B) | LDN(742-4B) |
| 5B | LDN(ISA-5B) | LDN(521-5B) | LDN(742-5B) |
| 6B | LDN(ISA-6B) | LDN(521-6B) | LDN(742-6B) |
| 7B | LDN(ISA-7B) | LDN(521-7B) | LDN(742-7B) |

**Table 2** Alleles and subunit compositions of 16 *Triticum turgidum* var. *dicoccoides* accessions and two durum wheat selections used for comparison to the subunits identified in this study

| Accession no. | Unique allele[a] | Subunit composition for chromosome | |
|---|---|---|---|
| | | 1A | 1B |
| PI-94683 | *Glu-A1h* | I | – |
| CI-12213 | *Glu-A1i* | II | – |
| PI-190924 | *Glu-A1-III* | III | 6+8 |
| PI-352359 | *Glu-A1j* | III | – |
| PI-192855 (Escuro)[b] | *Glu-A1-IV* | IV | – |
| PI-308879 | *Glu-A1v* | VII | 7+8 |
| PI-94640 | *Glu-B1m* | – | Null |
| PI-277674 | *Glu-B1-II* | Null | II |
| PI-355505 | *Glu-B1n* | – | II |
| PI-352354 | *Glu-B1o* | – | III |
| PI-94665 | *Glu-B1p* | – | IV |
| PI-275996 | *Glu-B1-IV* | 1 | IV |
| PI-94633 | *Glu-B1q* | – | V |
| PI-94669 | *Glu-B1r* | – | VI |
| PI-190931 | *Glu-B1-VI* | 1 | VI |
| PI-433757 (Lambro)[b] | *Glu-B1-VI* | – | VI |
| PI-277681 | *Glu-B1ay* | Null | XVI |
| PI-348620 | *Glu-B1az* | III | XVII |

[a] From Vallega and Waines (1987), Pflüger et al. (2001), and McIntosh et al. (2003)
[b] PI-192855 (Escuro) and PI-433757 (Lambro) are durum wheat and all other accessions are *T. turgidum* var. *dicoccoides*

those studies did not identify the *T. turgidum* var. *dicoccoides* accessions that carried the unique HMW glutenin subunits. In addition, common wheat (*T. aestivum* L.) varieties 'Chinese Spring', 'Glupro', 'Len', 'Norquay', 'Sappo', and the durum variety 'Renville' were used as controls.

### Protein extraction and electrophoresis

A-PAGE of gliadin proteins was performed according to the procedure of Khan et al. (1989) using a Hoefer SE600 vertical gel apparatus (Amersham Biosciences, Piscataway, N.J.). Briefly, the procedure involved electrophoresis on 1.5 mm gels (7.0% acrylamide, 0.25% bis-acrylamide, 0.024% ascorbic acid, and 0.0004% ferrous sulfate, polymerized by the addition of hydrogen peroxide). Electrode running (tank) buffer (0.25% aluminum lactate, pH adjusted to 3.1 with lactic acid) was used to prepare the gel solution. Gliadin proteins were extracted without reducing agent from ground grain with 70% ethanol, and then an aliquot of the supernatant was combined with an equal volume of sample buffer (10 g sucrose, 0.2 g methyl green tracking dye, and 18 ml tank buffer) before loading of 5 $\mu$l on the gels.

Sodium dodecyl sulfate (SDS)-PAGE was carried out according to the Laemmli (1970) procedure as modified by Khan et al. (1989). Gels were prepared 0.75-mm thick, with the separating gel consisting of either 8% or 12% total acrylamide, and the stacking gel layer at 5%. Samples were extracted with reducing sample buffer [1% Dithiothreitol (DTT) in 0.0625 M Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, and 0.02% Pyronin-Y tracking dye], and each gel lane was loaded with a 5-$\mu$l aliquot. After electrophoresis, the A-PAGE and SDS-PAGE gels were stained with Coomassie Brilliant Blue G-250 according to the procedure of Neuhoff et al. (1988). The HMW glutenin subunits were identified according to the HMW glutenin subunit numbering system of Payne et al. (1979).

Additional experiments were conducted after review of the initial results. The HMW banding patterns for the group 1 in LDN-DIC substitution lines were compared to banding patterns observed in 16 *T. turgidum* var. *dicoccoides* accessions that have unique subunits (Table 2). To search for possible matches, the substitution lines were then re-run in lanes adjacent to lines that had a similar banding pattern. Urea/SDS-PAGE was prepared as described by Goldsbrough et al. (1989). This procedure differed from SDS-PAGE only by the addition of 4 M urea to the gels. Gliadin proteins were described in terms of RM. Procedures and nomenclature followed Bushuk and Zillman (1978), except that our band 50 was described as band 51 by Bushuk and Zillman (1978).

The gel images were captured using Kodak Logic 100 system and were analyzed using Kodak 1-D Image Analysis Software, version 3.6.1 (Eastman Kodak, Rochester, N.Y.).

## Results and discussion

### HMW glutenin subunits in three *T. turgidum* var. *dicoccoides* accessions and LDN-DIC substitutions

Chromosomal locations of HMW glutenin subunit genes were determined by analyzing results from SDS-PAGE of the LDN-DIC substitution lines. The presence/absence of a band indicated the chromosomal location of the gene controlling that subunit. Results from SDS-PAGE of the three sets of LDN-DIC substitutions indicated that, for most HMW glutenin subunit bands, the three *T. turgidum* var. *dicoccoides* accessions differed from LDN, which has band pattern 6+8 (Branlard et al. 1989). The three *T. turgidum* var. *dicoccoides* accessions all had five bands located in the HMW range (Figs. 1, 2, 3), although some of these were faint bands similar to the 1Ax′ and 1By′ bands described by Levy et al. (1988). For example, Israel-A had a 1By′ band appearing below the 1By band and with slightly faster migration than band 8 (Fig. 1, lanes 1, 10). Also, LDN produced a 1By′ band that

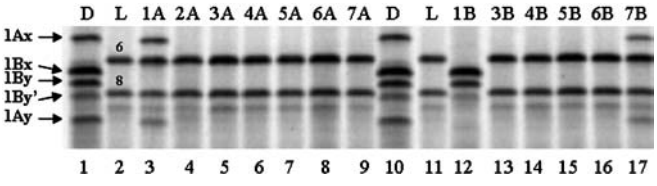


**Fig. 1** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel of LDN-DIC (Israel-A) substitution lines. *D* is Israel-A, *L* is Langdon, and the remaining lines *1A* to *7B* are substitution lines LDN (ISA-1A) to LDN(ISA-7B). The numbers at

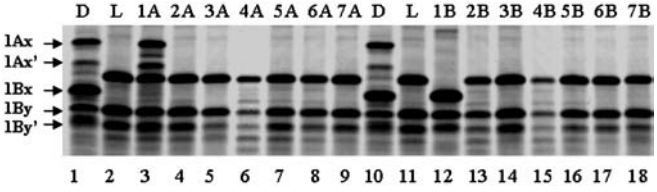


**Fig. 2** SDS-PAGE gel of LDN-DIC (PI-481521) substitution lines. *D* is PI-481521, *L* is Langdon, and the remaining lines *1A* to *7B* are substitution lines LDN(521–1A) to LDN(521–7B). The numbers at the *bottom* of the gels are lane numbers

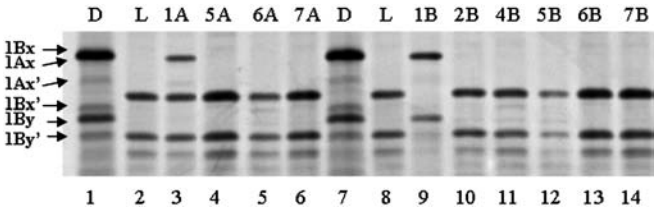


**Fig. 3** SDS-PAGE gel of LDN-DIC (PI-478742) substitution lines. *D* is PI-478742, *L* is Langdon, and the remaining lines *1A* to *7B* are substitution lines LDN (742–1A) to LDN(742–7B). The numbers at the *bottom* of the gels are lane numbers

migrated similar to band 10 (Fig. 1, lane 2). Because these faint bands are considered byproducts of 1A and 1B subunits, they will not be discussed further.

Israel-A had four HMW bands (Fig. 1, lane 1). The 1Ax subunit had a mobility that appeared similar to subunit 2*, and the 1Ay subunit produced a weak band that had faster mobility than subunit 12. The *Glu-A1* allele in Israel-A appeared to be identical to one of the alleles reported by Ciaffi et al. (1993). Both the 1Bx and 1By subunits had mobility intermediate to subunits 6 and 8 (Fig. 1, lane 12). This appeared similar to the *Glu-B1-IV* allele reported by Vallega and Waines (1987), which has subsequently been designated as *Glu-B1p*.

PI-481521 produced three HMW subunits (Fig. 2, lane 1). The 1Ax subunit had mobility similar to subunit 1. The 1Bx subunit had mobility intermediate to subunits 6 and 8, while the 1By subunit had mobility equal to subunit 8 (Fig. 2, lanes 1, 10, 12). The *Glu-B1* allele in PI-481521 appeared to be similar to the *Glu-B1n* allele (Pflüger et al. 2001).

Only two HMW bands were observed in PI-478742 (Fig. 3, lane 1). The lower band was a 1By subunit having intermediate mobility to bands 6 and 8 (Fig. 3, lanes 1, 7, 9). The upper band was intensely stained and had an electrophoretic mobility similar to subunit 1. When the substitution lines were analyzed, it was found that this band was composed of both a 1Ax and a 1Bx subunit (Fig. 3, lanes 3, 9). In order to increase resolution of bands in the HMW glutenin subunit region, we repeated the electrophoresis of some samples while extending the gel running time for several additional hours after the marker dye was off the bottom of the gel. In those gels, two bands were resolved in the upper region (Fig. 4, lane 3).

Addition of 4 M urea to the SDS-PAGE gels resulted in a change in RM of some HMW subunits (Fig. 5). Consistent with the results of Gianibelli et al. (2001) and Mackie et al. (1996), observations of Glupro and Len indicated that HMW band 5 had slower mobility than band 1 or band 2* (Fig. 5, lanes 1, 5). Urea/SDS-PAGE also clearly indicated the presence of two upper bands in PI-478742, with the 1Bx subunit having a higher molecular weight than the 1Ax subunit (Fig. 5, lanes 2–4). The 1Bx subunit had mobility similar to subunit 5, and the 1Ax subunit had mobility intermediate to subunits 1 and 2* (Fig. 5, lanes 1–5).

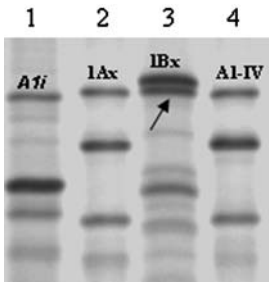


**Fig. 4** SDS-PAGE on 12% separating gel showing two upper bands in PI-478742. *1* CI-12213 (*Glu-A1i*), *2* LDN(742–1A), *3* PI-478742, *4* PI-192855 (*Glu-A1-IV*). Notations are band names or alleles (*italic*). An *arrow* indicates 1Ax at *lane 3*

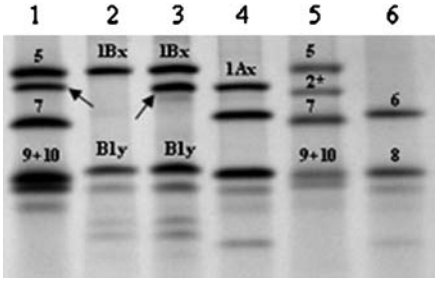


**Fig. 5** Urea/SDS-PAGE of PI-478742, LDN(742–1A), and LDN(742–1B) substitution lines. *1* Glupro (1, 7+9, 5+10), *2* LDN (742-1B), *3* PI-478742, *4* LDN(742-1A), *5* Len (2*, 7+9, 5+10), *6* Langdon (6+8). *Notations* are band names. Two *arrows* indicate band 1 of Glupro at *lane 1* and 1Ax of PI-478742 at *lane 3*, respectively

## Novel HMW glutenin subunits

Because of the similarity of most of the *Glu-A1* and *Glu-B1* alleles to other alleles previously described in *T. turgidum* var. *dicoccoides*, we prepared experiments where the substitution lines were electrophoresed next to their possible matches. Samples were electrophoresed using 12% separating gels, but to increase resolution, samples were also electrophoresed using 8% separating gels with current applied for approximately 2 h after the tracking dye had migrated off the gel. The results indicated that PI-481521 had band 1 (Figs. 6, 7, lanes 2–4). The 1Ax subunit of PI-478742 consistently had slightly faster mobility than band 1. On 8% gels, the 1Ax of PI-478742 migrated equal to the subunit controlled by *Glu-A1i* (Fig. 6, lanes 6, 7); however, on 12% gels, this same subunit migrated slower than the subunit controlled by *Glu-A1i* (Fig. 7, lanes 6, 7) and equal to the subunit controlled by *Glu-A1-IV* (Fig. 7, lanes 7, 9). This result suggests that the 1Ax of PI-478742 might be a novel variant of glutenin subunits. Further gel separations will be carried out to verify this band.

The LDN (742-1B) line was electrophoresed on the gel testing *Glu-A1* alleles so that we could more closely compare the mobility of the 1Ax and 1Bx subunits in PI-478742. Consistent with the urea/SDS-PAGE gels (Fig. 5,

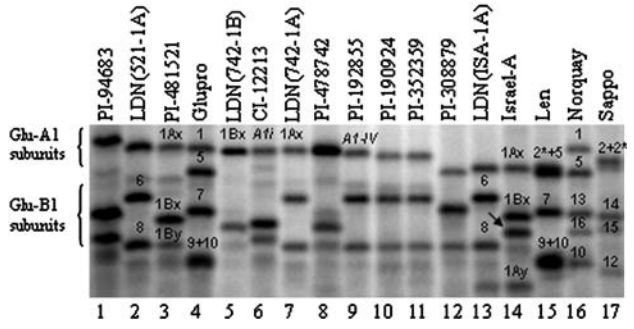


**Fig. 6** SDS-PAGE on 8% separating gel of *T. turgidum* var. *dicoccoides* accessions having unique *Glu-A1* alleles. Notations are band names or alleles (*italic*). An *arrow* indicates 1By subunit of Israel-A at *lane 14*

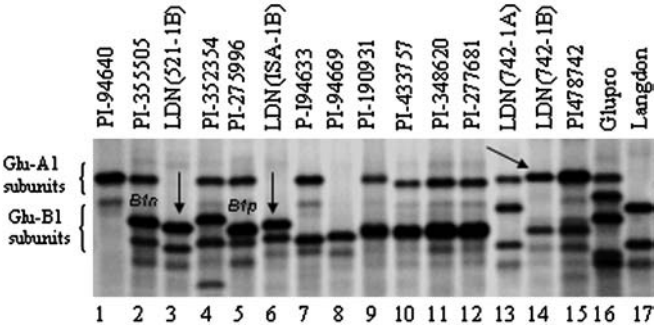


**Fig. 8** SDS-PAGE on 12% separating gel of *T. turgidum* var. *dicoccoides* accessions having unique *Glu-B1* alleles, indicated by *arrows*

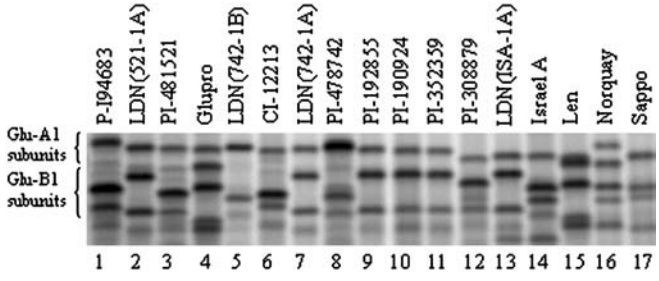


**Fig. 7** SDS-PAGE on 12% separating gel of *T. turgidum* var. *dicoccoides* accessions having unique *Glu-A1* alleles. Notations are the same as those in Fig. 6

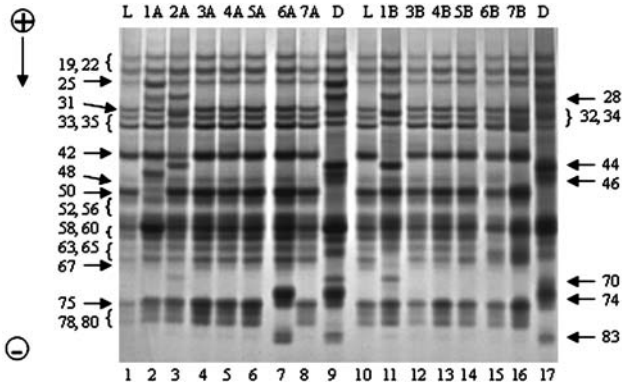


**Fig. 9** A-PAGE of gliadins in LDN-DIC (Israel-A) substitution lines. *D* is Israel-A, *L* is Langdon, and the remaining lines *1A* to *7B* are substitution lines LDN (ISA-1A) to LDN(ISA-7B). The numbers at the *bottom* of the gels are lane numbers. The numbers in the *left side* are the RM values of the bands in Langdon (calculated based on lane 10) and the *right* are unique bands in Israel-A (calculated based on lane 9)

lanes 2–4), the 1Bx subunit had slightly slower mobility than the 1Ax subunit (Fig. 7, lanes 5, 7). The 1Ax subunit from Israel-A consistently migrated slightly slower than the 2* subunit, and the 1Ay subunit migrated faster than subunits 10 and 12 (Figs. 6, 7, lanes 14 –17). The *Glu-A1* allele present in Israel-A may be identical to one of the alleles described by Levy et al. (1987) or Ciaffi et al. (1993).

Tests of the *Glu-B1* alleles indicated that the three *T. turgidum* var. *dicoccoides* accessions had alleles that had not been described (Fig. 8). The *Glu-B1* subunits in PI-481521 migrated faster than the subunits in *Glu-B1n* (Fig. 8, lanes 2, 3). The 1By subunit migrated similar to band 8 in all gels (Figs. 6, 7, lanes 2, 3), while the 1Bx subunit migrated intermediate to bands 7 and 17. Therefore, this allele combined subunit 8 with a new 1Bx subunit. With its 1Bx subunit that migrated similar to subunit 1 on SDS-PAGE gels and similar to subunit 5 in urea/SDS-PAGE, the allele in PI-478742 did not resemble any *Glu-B1* allele in this study or in the literature. The 1B subunits from Israel-A migrated slower than the subunits from *Glu-B1p* (Fig. 8, lanes 5, 6). However, the 1Bx and 1By subunits did migrate similarly to the subunit 14 from Sappo and 16 from Norquay, respectively (Figs. 6, 7, lanes 14, 16, 17). The three new *Glu-B1* alleles are designated as *Glu-B1be* in Israel-A, *Glu-B1bf* in PI-481521, and *Glu-B1bg* in PI-478742 based on the precedence in the catalogue of gene symbols (McIntosh et al. 2003).

Apparently, the subunit controlled by *Glu-B1* of PI-478742 is unusual. None of the prior studies (Ciaffi et al. 1993; Levy et al, 1988; Vallega and Waines 1987; Pflüger et al. 2001) of *T. turgidum* var. *dicoccoides* had identified a 1Bx subunit with mobility similar to 1Ax. Those studies assigned subunits to chromosomes based on RM in SDS-PAGE gels using the rule that 1Ax subunits have slower mobility than 1Bx subunits, and 1Ay subunits have mobility faster than 1By subunits. In the present study, the 1Bx subunit of PI-478742 was an exception to the rule as it had slightly slower mobility in SDS-PAGE gels than the 1Ax subunit (Fig. 4, lanes 2, 3).

## Chromosomal locations of gliadin genes

Results from A-PAGE of the three sets of substitution lines are shown in Figs. 9, 10, and 11. Similar to the HMW glutenin subunits, chromosomal location of gliadin

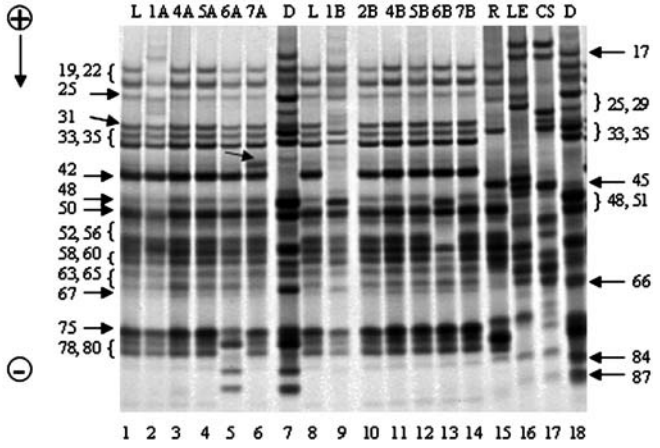


**Fig. 10** A-PAGE of gliadins in LDN-DIC (PI-478742) substitution lines. *D* is PI-478742, *L* is Langdon, *R* is Renville, *LE* is Len, *CS* is Chinese Spring, and the remaining lines *1A* through *7B* are substitution lines. The numbers in the *left side* are the RM values of the bands in Langdon (calculated based on lane 8) and the *right* are unique bands in PI-478742 (calculated based on lane 7). An *arrow* indicates an over-expressed band in LDN(472–7A)

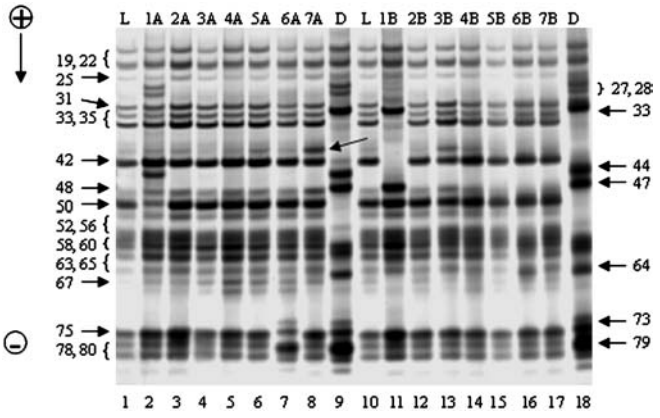


**Fig. 11** A-PAGE of gliadins in LDN-DIC (PI-481521) substitution lines. *D* is PI-481521, *L* is Langdon, and the remaining lines *1A* to *7B* are chromosome substitutions. The numbers in the *left side* are the RM values of the bands in Langdon (calculated based on lane 10) and the *right* are unique bands in PI-481521 (calculated based on lane 9). An *arrow* indicates an over-expressed band in LDN(521-7A)

genes were determined by observing the presence/absence of gliadin proteins by A-PAGE of LDN-DIC substitution lines. We identified bands by RM value because, although not always reproducible, RM values are convenient for initial description of bands until the allele can be identified. Relative mobilities were determined by comparison to band 1A-50 found in LDN and in all of the LDN-DIC substitution lines except the LDN (DIC-1A) substitutions.

Although RM values were useful for describing each band, the problems described by Metakovsky and Novoselskaya (1991) in accurately reproducing RM values were present in this study. As a result, the band

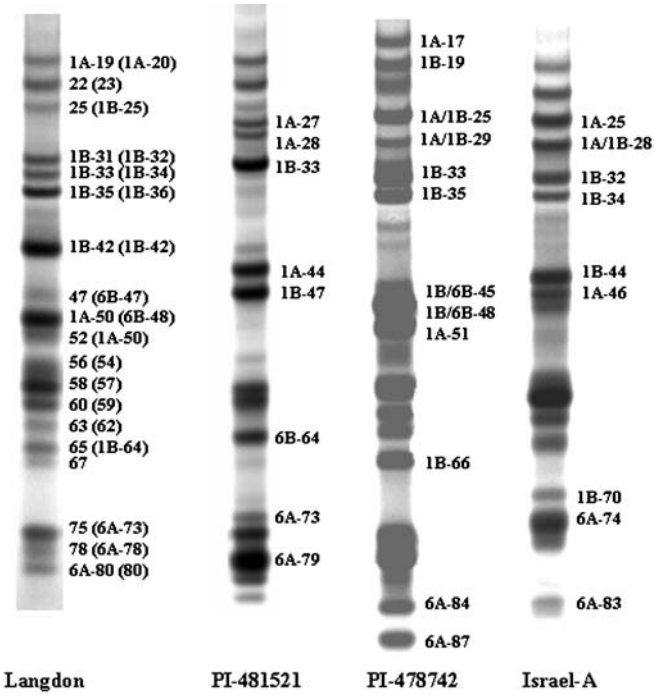


**Fig. 12** Chromosomal location and RM values of gliadins in Langdon and three *T. turgidum* var. *dicoccoides* accessions. In Langdon, the data in *parenthesis* are the chromosomal locations and RM values for the bands reported by Joppa et al. (1983)

described as band 20 by Joppa et al. (1983) was identified as 19 in this study (Fig. 12). Also, rounding RM values to the nearest whole number sometimes masked differences. For example, a band described as 1B-33 occurred in LDN, PI-481521, and PI-478742 (Fig. 12). The two bands in PI-481521and PI-478742 appeared similar to each other in mobility, but clearly had slower mobility than band 1B-33 of LDN (Fig. 10, lanes 7, 8; Fig. 11, lanes 9–11).

RM and chromosomal locations of the genes controlling these gliadins are summarized in Fig. 12. LDN had 19 gliadin bands. Except for the bands 50, 52, and 67 (Fig. 12), the gliadin bands in LDN from this study matched well with the corresponding bands from the study by Joppa et al. (1983). The band 50 in this study exhibited similar intensity and the same chromosomal location (1A) as band 50 in the study by Joppa et al. (1983). But its RM corresponded with the band 48, which was located on chromosome 6B in the study by Joppa et al. (1983). The band 67 was faint and was not identified by Joppa et al. (1983). Three faint bands (40, 43, and 86) reported by Joppa et al. (1983) were not identified in this study. The difference might be caused by different A-PAGE procedures and different apparatuses (horizontal vs vertical) used in different studies. The A-PAGE gels in this study were stained with Coomassie Brilliant Blue G-250 according to the procedure of Neuhoff et al. (1988), while the gels in the study by Joppa et al. (1983) were stained with Coomassie Brilliant Blue R-250. Joppa et al. (1983) located 14 of 22 gliadin bands in LDN to specific chromosomes using LDN D-genome disomic substitution

lines. The chromosomal location for band 80 was not previously determined by Joppa et al. (1983). We located this band to 6A (Fig. 9, lane 7; Fig. 10, lane 5).

PI-481521, PI-478742, and Israel-A had 18, 22, and 17 gliadin bands, respectively (Fig. 12). Except for the gliadin bands with similar mobility to the bands in LDN, eight (PI-481521), 12 (PI-478742), and nine (Israel-A) unique bands were located on specific chromosomes (Fig. 12). In cases where bands occurred in identical positions in both *T. turgidum* var. *dicoccoides* and LDN, it was not possible to determine which chromosome controlled the bands. For example, a band with RM 19 was observed in LDN and in all three *T. turgidum* var. *dicoccoides* accessions. The chromosome controlling this band could only be determined for PI-478742 (band 1B-19). This band was assigned to chromosome 1B because it was not located on chromosome 1A (Fig. 10, lane 2), and because gliadins from the Group 6 chromosomes do not normally occur in this region of an A-PAGE gel. A band with mobility slightly faster than band 25 of LDN was present in both the 1A and 1B substitutions of PI-478742 (Fig. 10, lanes 2, 7, 9). This suggested it was controlled by both chromosome 1A and 1B, and the band was designated 1A/1B-25. Band 29 of PI-478742 was present in both the 1A and 1B substitution lines as faint bands (Fig. 10, lanes 2, 7, 9, 18).

Over-expression of a gliadin band, indicating increased protein quantity, was observed in the A-PAGE (noted by arrows) of both LDN (742-7A) (Fig. 10, lane 6) and LDN (521-7A) (Fig. 11, lane 8) where, in both lines, the same gliadin band from LDN was more intensely stained. There were also instances of gliadin bands being under-expressed. In the SDS-PAGE gels of LDN (742–4B) and LDN (742-5B) substitutions, two bands controlled by LDN chromosome 6A were absent (data not shown). However, the A-PAGE of LDN-DIC (PI-478742) indicated that these two substitutions were identical to LDN (Fig. 10, lanes 8, 11, 12). Wanous et al. (2003) observed that specific chromosome arms had positive or negative effects on protein expression level of some HMW glutenin subunit genes in CS. The results in this study suggested that chromosome 7A from PI-481521 and PI-478742 might have positive effect on expression of a gliadin protein from LDN or 7A in LDN might have negative effect on expression of the protein.

There are about 140 gliadin alleles located on A- or B-genome chromosomes of group 1 and group 6 in the catalogue of gene symbols. The catalogue refers only to alleles from common wheat (McIntosh et al. 2003). High variations of gliadins have been reported in *T. monococcum* (Metakovsky and Baboev 1992a), *T. boeoticum* (Metakovsky and Baboev 1992b), and durum wheat (Nieto-Taladriz et al. 1994). However, the gliadins of *T. turgidum* var. *dicoccoides* have not been reported in the literature. Therefore, the gliadin bands identified in this study are potential sources for novel alleles of the *Gli* loci located on A- or B-genome chromosomes.

## Unexpected glutenin subunit and gliadin banding profiles in LDN-DIC substitutions

Because genes for glutenin subunits are located on group 1 chromosomes, all substitutions involving chromosomes other than group 1 should have the HMW banding pattern of LDN. Likewise, because genes for gliadins are located on group 1 and group 6 chromosomes, all other chromosomes should be identical to LDN for gliadin bands. There were two exceptions to these assumptions, both involving the Israel-A substitutions. The SDS-PAGE revealed that LDN(ISA-7B) had a HMW glutenin subunit pattern identical to LDN(ISA-1A), indicating the presence of *Glu-A1* from Israel-A (Fig. 1, lane 17). Similarly, A-PAGE gels of LDN(ISA-2A) had a gliadin banding pattern similar to LDN(ISA-1B) (Fig. 9, lanes 3, 11). The possibilities of incorrect identification on LDN(ISA-2A) and LDN(ISA-7B) were excluded because of different glutenin subunit band pattern of LDN(ISA-2A) from LDN(ISA-1B) (Fig. 1, lanes 4, 12) and different gliadin band pattern of LDN(ISA-7B) from LDN(ISA-1A) (Fig. 9, lanes 2, 16).

We tested progeny from additional sources of the LDN(ISA-7B) for the presence of the *Glu-A1* allele and found that the lines were heterogeneous for *Glu-A1* from Israel-A. Among 17 LDN(ISA-7B) lines, only four had *Glu-A1* from Israel-A. We concluded that the unexpected banding profiles may be caused either by failure to eliminate a segment of *T. turgidum* var. *dicoccoides* during backcrossing or possibly a translocation. The LDN-DIC (Israel-A) substitution lines were developed by crossing the LDN D-genomic disomic substitutions to Israel-A followed by five backcrosses (Joppa and Cantrell 1990). Theoretically, in addition to a pair of chromosomes from Israel-A, each of the substitution lines still posses 1.56% of the genetic background of Israel-A.

Chromosome translocations are common in *T. turgidum* var. *dicoccoides* (Joppa et al. 1995). It is unlikely that the accessions of *T. turgidum* var. *dicoccoides* used in this study had reciprocal translocations involving whole chromosome arms, because quadrivalents and trivalents should have been observed during the breeding process of non-critical and critical crosses, respectively. This leaves the possibility that the substitution lines carry translocations that were induced late in the breeding process, or carry small, interstitial translocations. Chromosome pairing, C-banding, or molecular data are needed to confirm the presence of a translocation. If results indicate that translocations are not present in Israel-A, then it would be appropriate to re-select the LDN(ISA-2A) and LDN(ISA-7B) substitution lines.

In conclusion, the HMW glutenin subunits and gliadins present in three *T. turgidum* var. *dicoccoides* accessions were identified and the chromosomes controlling these proteins have been determined. These results will help in further investigating the effects of identified glutenin subunits and gliadins on end-use quality. Because substitution lines contain only a single pair of chromosomes from the donor parent, studies on complexly inherited

traits can be simplified. The LDN-DIC substitutions will be useful for studying mixing and end-use qualities related to glutenin and gliadin proteins. In the case of Israel-A, enhanced gluten strength and high protein content have already been associated with the 1B (Joppa et al. 1991) and 6B substitutions (Joppa and Cantrell 1990), respectively. The quality traits in the two sets of LDN-DIC substitution lines based on PI-481521and PI-478742 are currently being evaluated.

In addition, the identified, HMW glutenin subunits and gliadins are useful chromosome-specific biochemical markers for identifying specific chromosomes or chromosome segments of *T. turgidum* var. *dicoccoides*. Similar to the chromosome-specific molecular markers developed in LDN-DIC (PI-481521) in a recent study (Xu et al. 2003), the unique, HMW glutenin subunits and gliadins can be used for determining the authenticity of the LDN-DIC substitutions and might be useful for marker-assisted selection during introgression of quality genes from *T. turgidum* var. *dicoccoides* to durum wheat.

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