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# A 1B-coded low-molecular-weight glutenin subunit associated with quality in durum wheats shows strong similarity to a subunit present in some bread wheat cultivars

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**Abstract** Good quality durum wheats usually present the LMW-2 type of SDS-PAGE pattern, whereas the LMW-1 type of pattern is usually associated with poor quality durum wheats. The two patterns are distinguished mainly by the presence of a strongly expressed protein band with molecular weight around 42,000 (42 K subunit) in the LMW-2-type pattern; this subunit is absent in the LMW-1-type pattern. Here we show that this particular low-molecular-weight glutenin subunit has strong similarity to a subunit present in some bread wheat cultivars. This correspondence has been demonstrated through SDS-PAGE, PCR analysis of the corresponding genes, a comparison of the deduced amino acid sequences, and RP-HPLC. This last approach showed a slight difference in retention time between the 42 K protein of bread and durum wheats that might be attributed to the eight amino acid differences found between the deduced amino acid sequences of the two corresponding genes.

**Key words** Durum wheat · Bread wheat · Low-molecular-weight glutenin subunits · Low-molecular-weight glutenin genes · Wheat quality

## Introduction

The low-molecular-weight glutenin subunits (LMW-GS) include multiple components both in durum and bread wheat cultivars. Because of their large number and considerable heterogeneity, their characterization has been to date incomplete. Pasta-making quality of the semolina of durum wheat cultivars is strongly correlated with the presence of specific SDS-PAGE patterns – the LMW-2 type in good quality cultivars and the LMW-1 type in poor quality cultivars (Payne et al. 1984; Pogna et al. 1988). A large number of subunits in bread wheat cultivars result from the presence of the D-genome proteins, and the LMW-GS show a smaller (although still significant) correlation with bread-making quality differences than the high-molecular-weight glutenin subunits (HMW-GS) (Payne et al. 1987; Gupta et al. 1989; 1994; Masci et al. 1993). The opposite is found for durum cultivars where the LMW-GS show the strongest correlation with quality.

The LMW-2 and LMW-1 groups of proteins differ mainly by the presence in the LMW-2 group SDS-PAGE pattern of a band with molecular weight 42,000 (42 K subunit). It is the largest protein (slowest moving) in the group and is absent from the LMW-1 type of pattern. Like all major LMW-2 and LMW-1 proteins, this 42 K subunit is coded on chromosome 1B (Ruiz and Carrillo 1995) at the *Glu-B3* locus. The LMW-1 group is linked to γ-gliadin 42 and to the three ω-gliadin subunits 33, 35, and 38, whereas the LMW-2 group is linked to γ-gliadin 45 and ω-gliadin 35 (gliadin components are defined by mobilities in acid-PAGE gels).

The LMW-2 and LMW-1 groups show quantitative differences, with the main components of the former more abundant than those of the latter (Autran et al. 1987). This difference in amount has been shown to be due mainly to the presence of the above mentioned slowest moving 42 K LMW-GS band in the LMW-2 type pattern (Masci et al. 1995). The larger amount of protein represented especially by the 42 K subunit is likely to be the main cause of the different quality characteristics between the two types (Autran et al. 1987; Masci et al. 1995; 1998). There is not enough information, however, to exclude with certainty the possibility of structural differences between the major components of the LMW-1 or LMW-2 groups. Neither LMW-1 nor LMW-2 has been detected in bread wheat cultivars so far. However, we have now found that a LMW-GS present in some bread wheat cultivars shows strong similarities to the 42 K subunit of the LMW-2 group and describe these similarities here.

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## Materials and methods

### Plant material

Cultivar 'Lira' biotype γ-42 (LMW-1 type) and 'Lira' biotype γ-45 (LMW-2 type) along with cv 'Langdon' (LMW-1 type) were used as durum wheat genotypes. Three additional durum wheat cultivars, namely 'Fortore', 'Salentino', and 'Valira', were used only for RP-HPLC analysis. The bread wheat cultivars analysed were: 'Arkan', 'Atlas', 'Benito', 'Centauro', 'Chinese Spring', 'Cheyenne', 'Ciano', 'Columbus', 'Conway', 'Darius', 'Fiocco', 'Frondoso', 'Liocorno', 'Maestra', 'Melchior', 'Nap Hal', 'Pegaso', 'Red River 68', 'Salmone', 'Scout 66', 'Solar', 'Spada', and 'Yecora Rojo'. The intervarietal 1B chromosome substitution line of bread wheat cv 'Ciano' in 'Chinese Spring' was used for chromosomal location analysis. Wheat cultivars and lines were obtained from many sources over a number of years, but were increased by us, and all have been characterized by us as appropriately designated according to their protein gel electrophoretic patterns.

### Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Insoluble glutenin subunits were prepared by twice extracting gliadins (and some glutenin) with 50% propanol at a 1:50 ratio (mg/ml), according to the procedure of Singh et al. (1991). The residue proteins were then solubilized with reducing sample buffer (0.07 *M* TRIS-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) pyronine Y, 1% dithiothreitol) in a 1:10 ratio (mg/µl). About 30 µg of the residue extracts were loaded into the slots of a 1-mm-thick acrylamide gel (T=11, C=2.67, 0.37*M* TRIS pH 8.8, 0.05% SDS in the separating gel and T=3.75, C=2.67, 0.125 *M* TRIS pH 6.8, 0.1% SDS in the stacking gel). The running buffer was 0.3 *M* glycine, 0.02 *M* TRIS, 0.1% SDS. Analyses were performed using an SE 600 apparatus (Hoefer, South San Francisco, Calif.) for about 18 h at 9 mA per gel at room temperature until the dye reached the bottom of the gel. Gels were stained overnight according to Neuhoff et al. (1988) and destained in tap water.

### Acid polyacrylamide gel electrophoresis (APAGE)

Gliadins were extracted from crushed seeds or flour for at least 2 h at room temperature with 1.5 *M* dimethylformamide in a 1:5 ratio (mg/µl). The extracts were then analysed by APAGE according to the method of Khan et al. (1985) with minor modifications.

### Reversed-phase high performance liquid chromatography (RP-HPLC)

Glutenin subunits were solubilized from the residue prepared as described for SDS-PAGE, but for HPLC the residue was instead extracted with 50% propanol buffered at pH 8.0 with 50 m*M* TRIS-HCl, containing 1% DTT (1:10 ratio) for 1 h at room temperature. After centrifugation (10,000 *g* at room temperature for 10 min), the supernatant was filtered through a PVDF membrane with 0.45-µm pores and 50 µl was injected onto a Supelco $C_8$ column (4.6 mm×25 cm) (Supelco, Bellefonte, Pa.). The column was eluted with a Beckman Solvent Delivery Module 126 and monitoring was by UV Detector Module 166 (Beckman, Palo Alto, Calif.) at 210 nm. The acetonitrile-water gradient used for separation was as follows (flow rate 1 ml/min): 5 min at 29% acetonitrile, up to 43% acetonitrile in 50 min, then up to 70% acetonitrile in 10 min, back to the initial conditions in 5 min and stop after 80 min. Gradients were formed from water (containing 0.07% trifluoroacetic acid) and acetonitrile (containing 0.05% trifluoroacetic acid). Fractions were collected, and those corresponding to detector peaks were checked by SDS-PAGE for protein bands.

### DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from leaves by the procedure reported in D'Ovidio et al. (1992). PCR analysis was carried out as previously reported (D'Ovidio 1993; D'Ovidio and Porceddu 1996).

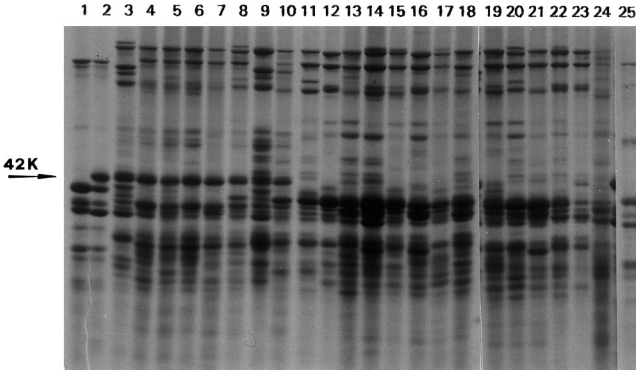


**Fig. 1** SDS-PAGE of glutenin subunits of the durum wheat cultivars [*lanes*: *1* 'Lira' biotype γ-42 (LMW-1 type); *2* 'Lira' biotype γ-45 (LMW-2 type), *25* 'Langdon' (LMW-1 type)] and the bread wheat genotypes [*3* 'Yecora Rojo, *4* 'Conway', *5* 'Solar', *6* 'Benito', *7* 'Melchior', *8* 'Red River', *9* 'Ciano', *10* 'Chinese Spring' (Ciano 1B), *11* 'Chinese Spring', *12* 'Cheyenne', *13* 'Atlas', *14* 'Salmone', *15* 'Arkan', *16* 'Columbus', *17* 'Liocorno', *18* 'Frondoso', *19* 'Scout 66', *20* 'Centauro', *21* 'Spada', *22* 'Fiocco', *23* 'Maestra', *24* 'Marquis']. The 42 K protein band is indicated

### Nucleotide sequence comparison

A comparison between the deduced amino acid sequences of the gene encoding the 42 K protein from the bread wheat cv 'Yecora Rojo' (Masci et al. 1998) and that of a *lmw-gs* gene present in a durum wheat line (D'Ovidio et al. 1999) was performed using the PC/GENE software (IntelliGenetics, Mountain View, Calif.). The charge at pH 2 (the pH of the RP-HPLC separation) and the grand average of hydropathicity (GRAVY) of these two polypeptides were calculated. GRAVY was based on the Klein et al. (1985) method and computed with an assigned interval of 15 amino acids.

## Results

The 42 K LMW-GS typical of LMW-2 patterns was found in the patterns of 7 of the 21 bread wheat cultivars analysed by SDS-PAGE and also in that of the 'Lira' biotype γ-45. The cultivars containing the 42 K subunit were 'Yecora Rojo', 'Red River 68', 'Conway', 'Solar', 'Benito', 'Melchior', and 'Ciano' (Fig. 1, lanes 3–9).

PCR analysis with primers UTV7F/UTV7R (D'Ovidio 1993) specific for the genes encoding the glutenin subunits of the LMW-2 group, performed on the same cultivars analysed by SDS-PAGE, showed that the 1.5-kb amplification band, characteristic of cultivars possessing the LMW-2 group (D'Ovidio 1993) is also present in the bread wheat cultivars expressing the 42 K protein (Fig. 2, lanes 3–9) and absent from those genotypes not showing this polypeptide, including durum wheat lines and cultivars possessing LMW-1 (Fig. 2, lanes 1 and 11–25). The similarity between the genes encoding the 42 K protein both in durum and bread wheats was further confirmed by PCR analysis with primers UTV7F/UTV10R specific for the LMW-2 group (D'Ovidio and Porceddu 1996) (data not shown).

The chromosomal location of the gene coding for the 42 K protein in bread wheat was deduced from the patterns shown in Figs. 1 and 2. The 42 K protein subunit and the 1.15 kb amplification product are present in the

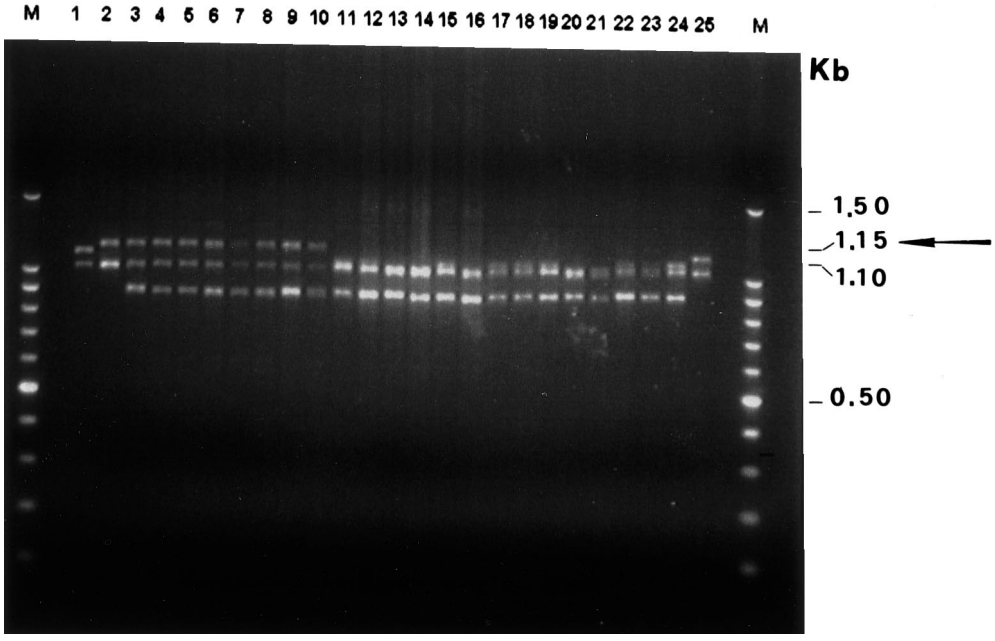


**Fig. 2** PCR amplification pattern of the same genotypes shown in **Fig. 1**, in the same order. *M* 100 bp ladder. The PCR amplification band of 1.15 kb (arrowed) corresponds to the 42 K protein gene

**Table 1** Bread wheat cultivars showing the 42 K protein (A), retention time (min) shown by the 42 K protein in RP-HPLC (B)

| A | B |
|---|---|
| Bread wheats | |
| Melchior | 41.7 |
| Red River | 42.1 |
| Benito | 42.3 |
| Conway | 42.6 |
| Yecora Rojo | 42.6 |
| Solar | 42.7 |
| Ciano | 42.8 |
| Durum wheats | |
| Valira | 40.7 |
| Lira biotype γ-45 | 40.9 |
| Salentino | 41.0 |
| Fortore | 41.1 |

patterns of bread wheat cv 'Ciano' (lane 9 in both figures) but absent in those of cv. 'Chinese Spring' (lane 11 in both figures). The intervarietal chromosome 1B substitution line of the bread wheat cv 'Ciano' in 'Chinese Spring' (lane 10 in both figures) shows the 42 K band and the 1.15 kb amplification product, confirming that they are both determined at loci located on chromosome 1B, presumably the *Glu-B3* locus, the same as for the 42 K protein in the LMW-2 types of durum cultivars (Ruiz and Carrillo 1995).

RP-HPLC analysis of glutenin subunits from the reduced residue fractions was carried out in order to determine the surface hydrophobicity characteristics of the 42 K protein subunits of durum and bread wheat lines and cultivars. Identification of the 42 K protein was based on SDS-PAGE analysis of the collected peaks. In order to compare a larger sample of the 42 K protein subunits from durum wheat, we included 3 additional LMW-2 type durum wheat cultivars, namely 'Fortore', 'Salentino' and 'Valira', in the analysis. The elution times of the peak corresponding to the protein of interest are reported in Table 1; in durum wheats they ranged from 40.7 to 41.1 min, whereas in bread wheat they ranged from 41.7 to 42.8 min. Elution times were reproducible to plus or minus 0.1 min or better. These results indicate that the 42 K protein of bread wheat has a slightly higher surface hydrophobicity than its durum wheat equivalent.

A comparison (Fig. 3) was made between the deduced amino acid sequences of the gene encoding the 42 K protein from bread wheat cv 'Yecora Rojo' (Masci et al. 1998) and that of a *lmw-gs* gene very likely corresponding to the 42 K band present in a durum wheat line (D'Ovidio et al. 1999). The first 7 amino acids of the N-terminal region (SHIPGLE) and the last 7 (GTGVGAY) in the bread wheat cultivar, or 8 (GTGVGAYL) in the durum wheat cultivar, were not considered in the comparison because they correspond to the sequence of primers used for PCR amplification and cloning of the genes, although such regions are characteristically conserved in *lmw-gs* genes. The comparison of the remaining 355 amino acid residues showed a very high degree of similarity, with only 7 amino acid substitutions and a deletion of a single amino acid in the durum wheat gene.

The charge at pH 2 was 15.6 for the 42 K protein present in 'Yecora Rojo' and 17.6 for the one from the durum wheat line. The GRAVY index was –9.01 for the 'Yecora Rojo' 42 K protein and –9.49 in the durum wheat line. In both cases, the values found are in accordance with the observed differences among retention times because a higher charge is associated with a lower retention time and a higher GRAVY value is correlated with a higher surface hydrophobicity, at least on the basis of the primary structure alone.

In order to determine if the 42 K protein is associated with γ-45 and ω-35 in bread wheats as in the LMW-2 type durum wheats (Payne et al. 1984), we analysed the gliadin patterns of all the bread wheat cultivars containing the 42 K protein by APAGE (Fig. 4). None of the

**Fig. 3** Comparison between the deduced amino acid sequences of the 42 K protein from bread wheat cv 'Yecora Rojo' (EMBL Data library accession no. Y17845) and a durum wheat line (EMBL Data library accession no. AJ007746). The amino acid sequences corresponding to the primers used for PCR amplification that have not been considered in the comparison are *underlined*. Differences between the two sequences in *bold*

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BREAD WHEAT - SHIPGLERPSQQQPLPPQQTLSHHHQQQPIQQQPQQFPQQQPCSQQQQQP -50
              |||||||||||||||||||||||||||||||||||||| ||||||||||||||
DURUM WHEAT - SHIPGLERPSQQQPLPPQQTLSHHHQQQPIQQQPHQFPQQQPCSQQQQQP -50

BREAD WHEAT - PLSQQQQPPFSQQQQPPFSQQQQPVLPQQPSFSQQQLPPFSQQQQPPFSQ -100
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
DURUM WHEAT - PLSQQQQPPFSQQQQPPFSQQQQPVLPQQPSFSQQQLPPFSQQQQPPFSQ -100

BREAD WHEAT - QQQPVLPQQPSFSQQQLPPFSQQLPPFSQQQQPVLPQQPPFSQQQLPPFS -150
              |||||||||||||||||||||||||||||||| |||||||||||| ||||
DURUM WHEAT - QQQPVLPQQPSFSQQQLPPFSQQLPPFSQQQ-PVLPQQPPFSQQQPPPFS -149

BREAD WHEAT - QQLPPFSQQQQPVLPQQPPFSQQQQQPILPQQPPFSQQQQPVLLQQQIPF -200
              |||||||||||||||||||||||||||| |||||||||||||||||||||
DURUM WHEAT - QQLPPFSQQQQPVLPQQPPFSQQQQQPIPPQQPPFSQQQQPVLLQQQIPF -199

BREAD WHEAT - VHPSILQQLNPCKVFLQQQCSPVAMPQSLARSQMLQQSSCHVMQQQCCQQ -250
              |||||||||||||||||||| ||||||||||||||||||||||||||||
DURUM WHEAT - VHPSILQQLNPCKVFLQQQCSPWAMPQSLARSQMLQQSSCHVMQQQCCQQ -249

BREAD WHEAT - LPQIPQQSRYEAIRAIVYSIILQEQQQVQGSIQTQQQQPQQLGQCVSQPQ -300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
DURUM WHEAT - LPQIPQQSRYEAIRAIVYSIILQEQQQVQGSIQTQQQQPQQLGQCVSQPQ -299

BREAD WHEAT - QQSQQQLGQQPQQQQLAQGTFLQPHQIAQLELMTSIALRTLPTMCNVNVP -350
              |||||||||||||||| |||||||||||| |||||||||||||| |||
DURUM WHEAT - QQSQQQLGQQPQQQQLAHGTFLQPHQIAQLEVMTSIALRTLPTMCNWNVP -349

BREAD WHEAT - LYRTTTRVPFGVGTGVGAY  -369
              ||||||||||||||||||
DURUM WHEAT - LYRTTTRVPFGVGTGVGAYL -369
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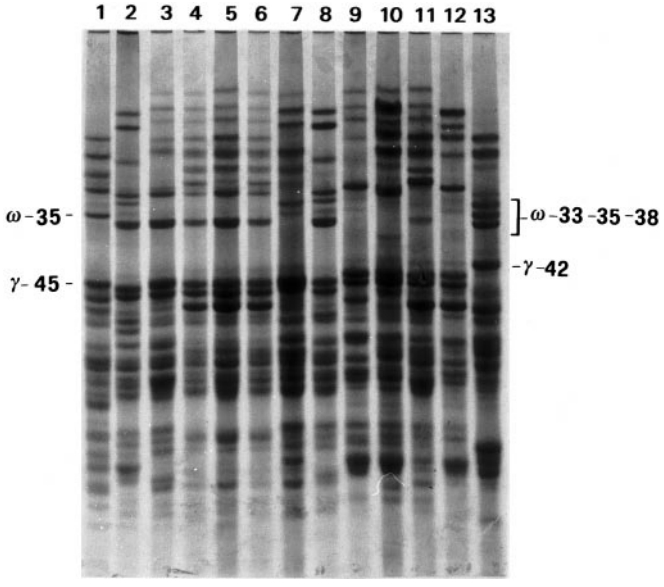


**Fig. 4** APAGE pattern of gliadin subunits of bread wheat cvs 'Yecora Rojo' (*2*), 'Red River' (*3*), 'Conway' (*4*), 'Solar' (*5*), 'Benito' (*6*), 'Melchior' (*7*), 'Ciano' (*8*), 'Cheyenne' (*9*), 'Salmone' (*10*), 'Columbus' (*11*), 'Liocorno' (*12*). Bread wheat cultivars shown in *lanes 2–8* possess the 42 K protein, but do not show either γ-45 or γ-35, typical of the LMW-2-type durum wheats (*lane 1*, cv 'Lira' biotype γ-45). γ-42 and ω–33, ω–35, and ω–38, present in the LMW-1-type durum wheat cv 'Langdon' (*lane 13*), are also indicated

bread wheats showed proteins with mobilities matching those of either ω-35 and γ-45. However, because 'Red River 68' showed a protein band with a mobility similar to that of γ-45 (Fig. 4, lane 3), further analyses were performed on this cultivar. Two-dimensional (two-pH) analysis (Lafiandra and Kasarda 1985) and PCR analysis with primers specific for γ-45 and γ-42 (D'Ovidio et al. 1990) demonstrated that the gliadin subunit in question does not correspond to γ-45 (data not shown).

## Discussion and conclusions

High- and low-molecular-weight glutenin subunits exert the main influence in determining wheat quality through their participation in formation of the glutenin polymer system. The HMW-GS are most important in bread wheat flour and the LMW-GS the most important in durum wheat semolina. At present, most of the breeding programmes on durum wheat use LMW-2-type lines because of their superior performance. Durum wheat cultivars with LMW-2 have a greater amount of LMW-GS than LMW-1 type durum wheats (Autran et al. 1987; Masci et al. 1995), and the 42 K band found in LMW-2 contributes strongly to this quantitative difference (Masci et al. 1995). Because the 42 K protein is very likely to be a chain extender, having two cysteine residues available for forming intermolecular disulfide bonds (Masci et al. 1998), its greater quantity in durum wheats might explain its positive influence on quality through an increase in the ratio of chain extenders to chain terminators (Masci et al. 1995).

Structural differerences between the 42 K subunit characteristic of LMW-2 types and the allelic proteins of LMW-1 might, however, be responsible for the quality differences. The availability of the nucleotide sequences corresponding to the 42 K protein both in bread wheat cv 'Yecora Rojo' (Masci et al. 1998) and in a LMW-2-type durum line (D'Ovidio et al. 1999) has allowed us to

compare in detail their primary structures with those of other LMW-GS. This 42 K subunit has a rather large and regular repeated sequence domain that includes a high proportion of glutamine residues in the repeats. The repeats have the consensus sequence PPFSQQQQ. This repeated sequence domain might be expected to increase the viscosity and elasticity of doughs through intermolecular interactions of the large numbers of glutamine side chains, which are both good hydrogen bond donors and acceptors. This would be the case whether or not the repeated sequence domain has a flexible random structure or has some ordered extended conformational structure, such as a β-spiral, but we have suggested that the flexible random structure is more likely (Masci et al. 1998). It has not yet been determined if the positive effect on strength is a consequence of the larger quantity associated with the 42 K protein or of some unique structural characteristics of this component. We speculate that it is most likely a combination of the two factors.

Our results show that the 42 K protein is very similar between bread and durum wheats. Comparison between the above-mentioned deduced amino acid sequences show that both genes encode a polypeptide of 369 amino acids, with differences at only 8 amino acid positions. Such slight discrepancies, especially the single charge difference, are very likely the cause of the different retention times for the proteins from bread wheat as compared with the proteins from durum wheat in RP-HPLC.

As in durum wheat, the 42 K protein is coded by genes present on chromosome 1B, most likely at locus *Glu-B3*, as we have shown by using intervarietal substitution lines. Even though all the analysed bread wheat cultivars possessing the 42 K protein also had the *Glu-D1*-coded HMW-GS 5+10, a pair that is strongly associated with good bread-making characteristics, the 42 K protein may also contribute significantly to the quality characteristics of these cultivars. Furthermore, the effects of the 42 K LMW-GS proteins in bread wheat on quality might be different from those contributed by HMW-GS, perhaps, for example, increasing extensibility without loss of desired viscosity and elasticity. This possibility remains to be explored in future research.

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