

## Genetic variation for glutenin and gliadins associated with quality in durum wheat (*Triticum turgidum* L. ssp. *turgidum*) landraces from Spain

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### Abstract

The allelic variation at seven prolamin loci involved in quality has been studied in a set of durum wheat landraces from all the Spanish regions where this crop has been traditionally cultivated. The genetic variability was higher than that found in other germplasm collections. All the loci, except *Glu-B2*, displayed a genetic variability higher than 0.62, with *Glu-3* the most polymorphic. In total, five alleles were studied at *Glu-A1*, nine at *Glu-B1*, 15 at *Glu-A3*, 18 at *Glu-B3*, two at *Glu-B2*, and eight at *Gli-A1* and *Gli-B1*. New allelic variants not previously identified in durum wheat were detected. The 30 different genotypes of B low-molecular-weight (B-LMW) glutenin subunits analysed, of which 25 are novel, provide an important source of genetic variability for quality breeding. Protein patterns for convars. *durum* and *turgidum*, and for the North and South of Spain were identified for the loci with significant influence on quality. Higher variability was observed in convar. *turgidum* and in the North zone than in convar. *durum* and the South, respectively, mainly for the *Glu-B1* and *Glu-B3*. Also, convar. *turgidum* appeared to be a valuable source for new alleles for the LMW glutenin subunits. Wheats from the South were, however, more diverse for prolamins encoded at *Glu-A3*.

**Additional key words:** convar. *durum*, convar. *turgidum*, germplasm, LMW patterns, prolamin alleles, quality breeding.

### Resumen

**Variación genética de las gluteninas y gliadinas asociadas con calidad en variedades locales españolas de trigo duro (*Triticum turgidum* L. ssp. *turgidum*)**

Se ha estudiado la variación alélica en siete loci de prolaminas relacionados con la calidad en un grupo de variedades locales de trigo duro procedentes de todas las provincias españolas donde se cultivaba tradicionalmente. La variabilidad genética encontrada fue mayor que la observada en otras colecciones de germoplasma. Todos los loci, excepto el *Glu-B2*, mostraron una variabilidad genética mayor que 0,62, siendo los *Glu-3* los más polimórficos. En total se estudiaron cinco alelos en el *Glu-A1*, nueve en el *Glu-B1*, 15 en el *Glu-A3*, 18 en el *Glu-B3*, dos en el *Glu-B2* y ocho en el *Gli-A1* y *Gli-B1*. Se han detectado variantes alélicas nuevas que no se habían identificado antes. Los 30 genotipos diferentes analizados de subunidades B de gluteninas de bajo peso molecular (B-LMW), de los cuales 25 son nuevos, representan una fuente importante de variabilidad genética para la mejora de la calidad. Se identificaron patrones de proteínas para las convars. *durum* y *turgidum*, y para el norte y sur de España para los loci con influencia significativa en calidad. En la convar. *turgidum* y en el norte se observó mayor variabilidad que en la convar. *durum* y en el sur, respectivamente, principalmente en el *Glu-B1* y *Glu-B3*. Además, la convar. *turgidum* parece ser una fuente valiosa de nuevos alelos para las subunidades LMW de gluteninas. Sin embargo, los trigos del sur son más diversos para las prolaminas codificadas en el *Glu-A3*.

**Palabras clave adicionales:** alelos de prolaminas, convar. *durum*, convar. *turgidum*, germoplasma, mejora de la calidad, patrones LMW.

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## Introduction

In durum wheat, quality differences between cultivars are strongly dependent upon their allelic composition for endosperm storage proteins, gliadins and glutenins (Carrillo *et al.*, 1990, 1991; Kaan *et al.*, 1993; Turchetta *et al.*, 1995; Porceddu *et al.*, 1998). Genetic studies have revealed that these proteins (prolamins) are encoded at several, complex and highly polymorphic loci. Gliadins are controlled by six *Gli* loci (*Gli-1* and *Gli-2*) mapped on the short arms of the group 1 and 6 chromosomes, respectively (Wrigley and Shepherd, 1973; Payne *et al.*, 1982). Glutenin subunits are classified as high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits on the basis of their mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The LMW glutenin subunits are subdivided into B, C and D (Jackson *et al.*, 1983), with the B subunits playing a major role in gluten quality (Pogna *et al.*, 1990; Ruiz and Carrillo, 1995; Turchetta *et al.*, 1995). HMW subunits are encoded at the *Glu-1* loci on the long arm of the group 1 chromosomes (Payne *et al.*, 1980). B-LMW glutenin subunits are inherited as groups (designated LMW-) controlled at *Glu-A3*, *Glu-B3* and *Glu-B2* (Singh and Shepherd, 1988; Ruiz and Carrillo, 1993). *Glu-A3* and *Glu-B3* are tightly linked to *Gli-A1* and *Gli-B1*, respectively (Singh and Shepherd, 1988). The natural variation found at all these prolamin loci is very important to improve durum wheat quality. However, the narrow genetic background of modern wheat cultivars may lead to a loss of the exploitable diversity (genetic erosion) and a decrease in the efficiency of breeding. In this context, the diversity of old varieties and forms preserved in gene banks represents an important source of genetic variability and consequently, of valuable traits for wheat improvement. In fact, landrace varieties have proven especially useful to supply new alleles at the protein loci involved in gluten strength (Carrillo *et al.*, 1991; Porceddu *et al.*, 1998; Raciti *et al.*, 2003). On the other hand, prolamins are valuable molecular markers for genetic analysis and for the quantification of the genetic diversity present in wheat collections (Kudryavtsev *et al.*, 1996; Aguiriano *et al.*, 2006; Moragues *et al.*, 2006).

In this paper, the allelic variation at seven prolamin loci has been studied in a set of durum wheat landraces from Spain. Allelic frequencies were used to analyse genetic diversity and the presence of protein patterns for convars. *durum* and *turgidum*, and for the North and South of Spain.

## Material and methods

### Material

A collection of the Plant Genetic Resources Centre at the Spanish National Institute for Agricultural and Food Research and Technology (CRF-INIA) was analysed for allelic variation at seven prolamin loci. The sample included 52 accessions of Spanish durum wheat (*Triticum turgidum* L. ssp. *turgidum*) landraces. Table 1 shows the Bank number, local name, and geographical region of the landraces analysed. Additional passport and characterisation data are available in <http://wwwx.inia.es/crf>. About 50 seeds per accession were sown in a single row (2 m long, 1.12 m apart) during the 2001-2002 season at Alcalá de Henares, Madrid. One plant representative of the accession was bagged. To select this plant it was previously verified that its agromorphological characters matched the descriptions published for these varieties (Gadea, 1954). The variety identity was also confirmed using the spike collection held since the 1950s at the CRF-INIA. The rest of the plants of the same variety plot were harvested and milled as a whole. The grain of the bagged selected plant was sown in one row with a 1.12 m inter-row spacing during 2003-2004. Each row was harvested by hand and the flour used for prolamin analysis. These gliadin profiles were compared with those from the flour obtained with the plants of the same variety harvested as a whole, and, in some cases, with those from a grain of the spike collection. The results of these comparisons allowed confirming that the selected genotype represented the variety.

### Prolamin analysis

Glutenins were extracted following a sequential procedure (Singh *et al.*, 1991). Electrophoresis of glutenin subunits was performed on SDS-PAGE according to Payne *et al.* (1980). HMW glutenin alleles at *Glu-A1* and *Glu-B1* loci were identified using the nomenclature of Payne and Lawrence (1983). B-LMW glutenin alleles at *Glu-A3*, *Glu-B3* and *Glu-B2* loci were designated following Nieto-Taladriz *et al.* (1997). B-LMW subunits were numbered according their relative electrophoretic mobility. New bands between two subunits previously catalogued (Nieto-Taladriz *et al.*, 1997) were designated with the same number as the higher subunit plus "\*" in agreement with Martínez *et al.* (2004). The locus to which a novel allele belonged was predicted on

**Table 1.** Genebank number, local name and geographical region of the landraces analysed

| Genebank number | Local name                  | Region      |
|-----------------|-----------------------------|-------------|
| BGE 2869        | Caravaca 7                  | Murcia      |
| BGE 2881        | Morisco de Tenerife         | Tenerife    |
| BGE 2882        | Raspinegro de Alcolea       | Córdoba     |
| BGE 2883        | Recio de Aranjuez           | Madrid      |
| BGE 2887        | Rubial de Liebana           | Asturias    |
| BGE 4165        | -                           | Burgos      |
| BGE 8366        | Trigo                       | Sevilla     |
| BGE 12346       | Raspinegro canario          | Las Palmas  |
| BGE 12383       | Caravaca 4                  | Murcia      |
| BGE 12537       | Cascalvo                    | Córdoba     |
| BGE 12555       | Rubion de Higuera           | Albacete    |
| BGE 13065       | Rubio de Madrigalejo        | Cáceres     |
| BGE 13066       | Espiga negra                | Jaén        |
| BGE 13068       | Negro vellosa               | Jaén        |
| BGE 13076       | Chile                       | Palencia    |
| BGE 13077       | Trigo alto                  | Avila       |
| BGE 13080       | Blat mort                   | Baleares    |
| BGE 13089       | Gigante lampiño de Najera   | La Rioja    |
| BGE 13090       | Cañivano                    | Almería     |
| BGE 13093       | Bizarzari                   | Vizcaya     |
| BGE 13100       | Radondell blanco            | Gerona      |
| BGE 13103       | Heraldo del Rhin            | Barcelona   |
| BGE 13590       | Trigo                       | Toledo      |
| BGE 13603       | Claro de Balazote           | Albacete    |
| BGE 13622       | Las Mesas                   | Cuenca      |
| BGE 13645       | Mondragon de Castronuevo    | Valladolid  |
| BGE 13651       | Obispado de Lebrija         | Sevilla     |
| BGE 13683       | Molla temprano              | Baleares    |
| BGE 13688       | Recion                      | Málaga      |
| BGE 17170       | -                           | Granada     |
| BGE 18266       | Obispado                    | Cádiz       |
| BGE 18267       | Tremen                      | Sevilla     |
| BGE 18268       | Rubio de Espiel             | Córdoba     |
| BGE 18271       | Macho                       | Ciudad Real |
| BGE 18304       | Colorado de Jerez           | Cádiz       |
| BGE 18602       | Rojo de Lebrija             | Sevilla     |
| BGE 18615       | Caravaca colorado cañiguero | Murcia      |
| BGE 18619       | Griego de Baleares          | Baleares    |
| BGE 18646       | Asturias L7                 | Asturias    |
| BGE 18648       | Blat obeia                  | Tarragona   |
| BGE 18653       | Redondillo de Fuentesauco   | Zamora      |
| BGE 18654       | Redondillo                  | Navarra     |
| BGE 19293       | Duro mocho                  | -           |
| BGE 20940       | -                           | -           |
| BGE 20942       | -                           | -           |
| BGE 20946       | Trigo                       | Huelva      |
| BGE 20948       | -                           | Badajoz     |
| BGE 21774       | Fino                        | Badajoz     |
| BGE 21783       | Blanquillon de Boñar        | León        |
| BGE 21787       | Carita de raton             | Cádiz       |
| BGE 26954       | Rubion                      | Almería     |
| BGE 30923       | Asturias H1                 | Asturias    |

the basis of the electrophoretic mobility of the gene products. Thus, for example, new subunits 21 and 22 were provisionally assigned to *Glu-A3* because subunit 20 in the zone of highest mobility was encoded at that locus (Nieto-Taladriz *et al.*, 1997; Martinez *et al.*, 2004). In the same way, the rest of new subunits were assigned to *Glu-B3*. The provisional location of some subunits was confirmed with the analysis of linkage relations to gliadin alleles. All these alleles were designated following the international nomenclature system by McIntosh *et al.* (2003) Supplement 2006. Gliadins were extracted from flour and fractionated in acid (pH 3.1) polyacrylamide gel electrophoresis (A-PAGE) according to Lafiandra and Kasarda (1985). The identification of *Gli-A1* and *Gli-B1* alleles was performed following the catalogue and nomenclature proposed by Kudryavtsev *et al.* (1996). The new alleles detected in the present work were termed as 'new-'. Genetic diversity (Ht) was calculated according to Nei (1973).

## Results

### Prolamin analysis

Duplicate profiles were produced for seven of the 52 entries, so these putative duplicates were removed from the statistical analysis. Table 2 shows the alleles identified at the prolamins loci and their frequencies. The value of Ht across the collection is 0.721, ranging from 0.458 to 0.856 for the individual loci. Five and nine alleles were detected at *Glu-A1* and *Glu-B1*, respectively (Table 2). Six of these alleles were rare (frequency <5%). One allele not previously described in wheat was identified at *Glu-B1* (*new-1*). This allele controlled two subunits with mobilities lower and higher than that of subunit 8\* (Fig. 1 lane 3). A total of 15, 18 and two alleles were detected at *Glu-A3*, *Glu-B3* and *Glu-B2*, respectively. Twenty-four of these alleles were rare. Eight new alleles were studied at *Glu-A3* and 12 at *Glu-B3* (Table 2). LMW-subunits encoded by these new alleles are shown in Table 3. In general, most of *Glu-A3* new alleles encoded subunits 5\*, 21 or 22 in addition to other subunits (Fig. 1 lanes 7 and 9). In contrast, most of the new alleles at *Glu-B3* produced combinations of previously reported subunits (e.g., *new-1* and *-2*, see Fig. 1 lanes 5 and 11; and *-4*, *-9*, *-10*, *-12* and *-13*, see Table 3). Some new subunits were also identified at this locus (specifically, 1\*, 1\*\*, 7\*, 7\*\*, 7\*\*\*, 8\* and 14\*, see Fig. 1). Subunits 1\* and 1\*\* were not present with subunit 1, subunits 7\*, 7\*\*, 7\*\*\* and 8\* with 7, 8 or 9, and subunit 14\* with 14 or 13. This observation is consistent with the suggestion that these

**Table 2.** Allelic frequencies at each loci and gene diversity (Ht) for the whole sample, convars. *durum* and *turgidum*, and the North and South geographical groups

| Locus         | Allele        | Total |       | convar. <i>durum</i> |       | convar. <i>turgidum</i> |       | North |       | South |       |
|---------------|---------------|-------|-------|----------------------|-------|-------------------------|-------|-------|-------|-------|-------|
|               |               | Freq  | %     | Freq                 | %     | Freq                    | %     | Freq  | %     | Freq  | %     |
| <i>Glu-A1</i> | <i>a</i>      | 15    | 33.3  | 7                    | 25.9  | 8                       | 44.4  | 6     | 46.2  | 7     | 29.2  |
|               | <i>b</i>      | 11    | 24.4  | 2                    | 7.4   | 9                       | 50.0  | 6     | 46.2  | 3     | 12.5  |
|               | <i>c</i>      | 17    | 37.8  | 17                   | 63.0  | -                       | -     | 1     | 7.7   | 13    | 54.2  |
|               | <i>f</i>      | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | -     | -     |
|               | <i>o</i>      | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | Ht            |       | 0.685 |                      | 0.529 |                         | 0.549 |       | 0.567 |       | 0.603 |
| <i>Glu-B1</i> | <i>a</i>      | 3     | 6.7   | 1                    | 3.7   | 2                       | 11.1  | 2     | 15.4  | -     | -     |
|               | <i>an</i>     | 2     | 4.4   | 1                    | 3.7   | 1                       | 5.6   | 1     | 7.7   | -     | -     |
|               | <i>b</i>      | 2     | 4.4   | 1                    | 3.7   | 1                       | 5.6   | -     | -     | 2     | 8.3   |
|               | <i>d</i>      | 24    | 53.3  | 17                   | 63.0  | 7                       | 38.9  | 4     | 30.8  | 16    | 66.7  |
|               | <i>e</i>      | 5     | 11.1  | 5                    | 18.5  | -                       | -     | 1     | 7.7   | 3     | 12.5  |
|               | <i>f</i>      | 3     | 6.7   | 2                    | 7.4   | 1                       | 5.6   | 1     | 7.7   | 1     | 4.2   |
|               | <i>q</i>      | 3     | 6.7   | -                    | -     | 3                       | 16.7  | 3     | 23.1  | -     | -     |
|               | <i>y</i>      | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>new-1</i>  | 2     | 4.4   | -                    | -     | 2                       | 11.1  | 1     | 7.7   | 1     | 4.2   |
|               | Ht            |       | 0.683 |                      | 0.559 |                         | 0.783 |       | 0.804 |       | 0.527 |
| <i>Glu-A3</i> | <i>a</i>      | 16    | 35.6  | 12                   | 44.4  | 4                       | 22.2  | 6     | 46.2  | 7     | 29.2  |
|               | <i>b</i>      | 4     | 8.9   | 1                    | 3.7   | 3                       | 16.7  | 1     | 7.7   | 1     | 4.2   |
|               | <i>c</i>      | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>d</i>      | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | <i>e</i>      | 9     | 20.0  | 6                    | 22.2  | 3                       | 16.7  | 2     | 15.4  | 5     | 20.8  |
|               | <i>f</i>      | 2     | 4.4   | 2                    | 7.4   | -                       | -     | -     | -     | 2     | 8.3   |
|               | <i>h</i>      | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | <i>new-1</i>  | 3     | 6.7   | 1                    | 3.7   | 2                       | 11.1  | 1     | 7.7   | 1     | 4.2   |
|               | <i>new-2</i>  | 2     | 4.4   | 1                    | 3.7   | 1                       | 5.6   | 1     | 7.7   | 1     | 4.2   |
|               | <i>new-3</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>new-4</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>new-5</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | 1     | 7.7   | -     | -     |
|               | <i>new-6</i>  | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | <i>new-7</i>  | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
| <i>new-8</i>  | 1             | 2.2   | -     | -                    | 1     | 5.6                     | 1     | 7.7   | -     | -     |       |
| Ht            |               | 0.812 |       | 0.738                |       | 0.863                   |       | 0.733 |       | 0.846 |       |
| <i>Glu-B3</i> | <i>a</i>      | 15    | 33.3  | 14                   | 51.9  | 1                       | 5.6   | 1     | 7.7   | 11    | 45.8  |
|               | <i>b</i>      | 2     | 4.4   | 1                    | 3.7   | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>d</i>      | 3     | 6.7   | 3                    | 11.1  | -                       | -     | -     | -     | 3     | 12.5  |
|               | <i>f</i>      | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>h</i>      | 4     | 8.9   | 3                    | 11.1  | 1                       | 5.6   | 2     | 15.4  | 2     | 8.3   |
|               | <i>i</i>      | 2     | 4.4   | 1                    | 3.7   | 1                       | 5.6   | -     | -     | 1     | 4.2   |
|               | <i>new-1</i>  | 3     | 6.7   | -                    | -     | 3                       | 16.7  | 3     | 23.1  | -     | -     |
|               | <i>new-2</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | 1     | 7.7   | -     | -     |
|               | <i>new-3</i>  | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | <i>new-4</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | -     | -     | -     | -     |
|               | <i>new-5</i>  | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | -     | -     |
|               | <i>new-6</i>  | 3     | 6.7   | -                    | -     | 3                       | 16.7  | 2     | 15.4  | 1     | 4.2   |
|               | <i>new-7</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | 1     | 7.7   | -     | -     |
|               | <i>new-8</i>  | 1     | 2.2   | -                    | -     | 1                       | 5.6   | 1     | 7.7   | -     | -     |
|               | <i>new-9</i>  | 2     | 4.4   | -                    | -     | 2                       | 11.1  | 1     | 7.7   | -     | -     |
|               | <i>new-10</i> | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
|               | <i>new-11</i> | 1     | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2   |
| <i>new-12</i> | 1             | 2.2   | -     | -                    | 1     | 5.6                     | 1     | 7.7   | -     | -     |       |
| heterozygous  | 1             | 2.2   | 1     | 3.7                  | -     | -                       | -     | -     | 1     | 4.2   |       |
| Ht            |               | 0.856 |       | 0.696                |       | 0.900                   |       | 0.863 |       | 0.753 |       |

Table 2. (Cont.)

| Locus         | Allele        | Total    |       | convar. <i>durum</i> |       | convar. <i>turgidum</i> |       | North |       | South |      |
|---------------|---------------|----------|-------|----------------------|-------|-------------------------|-------|-------|-------|-------|------|
|               |               | Freq     | %     | Freq                 | %     | Freq                    | %     | Freq  | %     | Freq  | %    |
| <i>Glu-B2</i> | <i>a</i>      | 16       | 35.6  | 9                    | 33.3  | 7                       | 38.9  | 6     | 46.2  | 8     | 33.3 |
|               | <i>b</i>      | 29       | 64.4  | 18                   | 66.7  | 11                      | 61.1  | 7     | 53.8  | 16    | 66.7 |
|               | Ht            | 0.458    |       | 0.444                |       | 0.475                   |       | 0.497 |       | 0.444 |      |
| <i>Gli-A1</i> | <i>b</i>      | 16       | 35.6  | 9                    | 33.3  | 7                       | 38.9  | 4     | 30.8  | 10    | 41.7 |
|               | <i>c</i>      | 10       | 22.2  | 7                    | 25.9  | 3                       | 16.7  | 3     | 23.1  | 3     | 12.5 |
|               | <i>e</i>      | 7        | 15.6  | 5                    | 18.5  | 2                       | 11.1  | 1     | 7.7   | 5     | 20.8 |
|               | <i>f</i>      | 2        | 4.4   | 2                    | 7.4   | -                       | -     | -     | -     | 2     | 8.3  |
|               | <i>g</i>      | 6        | 13.3  | 3                    | 11.1  | 3                       | 16.7  | 2     | 15.4  | 3     | 12.5 |
|               | <i>k</i>      | 1        | 2.2   | -                    | -     | 1                       | 5.6   | 1     | 7.7   | -     | -    |
|               | <i>new-2</i>  | 1        | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2  |
|               | <i>new-3</i>  | 2        | 4.4   | -                    | -     | 2                       | 11.1  | 2     | 15.4  | -     | -    |
|               | Ht            | 0.777    |       | 0.768                |       | 0.765                   |       | 0.792 |       | 0.742 |      |
|               | <i>Gli-B1</i> | <i>a</i> | 2     | 4.4                  | 1     | 3.7                     | 1     | 5.6   | -     | -     | 1    |
| <i>b</i>      |               | 4        | 8.9   | 4                    | 14.8  | -                       | -     | -     | -     | 4     | 16.7 |
| <i>c</i>      |               | 18       | 40.0  | 15                   | 55.6  | 3                       | 16.7  | 2     | 15.4  | 13    | 54.2 |
| <i>new-1</i>  |               | 9        | 20.0  | 3                    | 11.1  | 6                       | 33.3  | 6     | 46.2  | 3     | 12.5 |
| <i>new-6</i>  |               | 3        | 6.7   | 1                    | 3.7   | 2                       | 11.1  | -     | -     | 1     | 4.2  |
| <i>new-7</i>  |               | 4        | 8.9   | 1                    | 3.7   | 3                       | 16.7  | 2     | 15.4  | 1     | 4.2  |
| <i>new-8</i>  |               | 3        | 6.7   | -                    | -     | 3                       | 16.7  | 3     | 23.1  | -     | -    |
| <i>new-9</i>  |               | 1        | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | -     | -    |
| heterozygous  |               | 1        | 2.2   | 1                    | 3.7   | -                       | -     | -     | -     | 1     | 4.2  |
| Ht            |               | 0.772    |       | 0.649                |       | 0.789                   |       | 0.685 |       | 0.655 |      |
| Ht            | 0.721         |          | 0.625 |                      | 0.732 |                         | 0.706 |       | 0.653 |       |      |

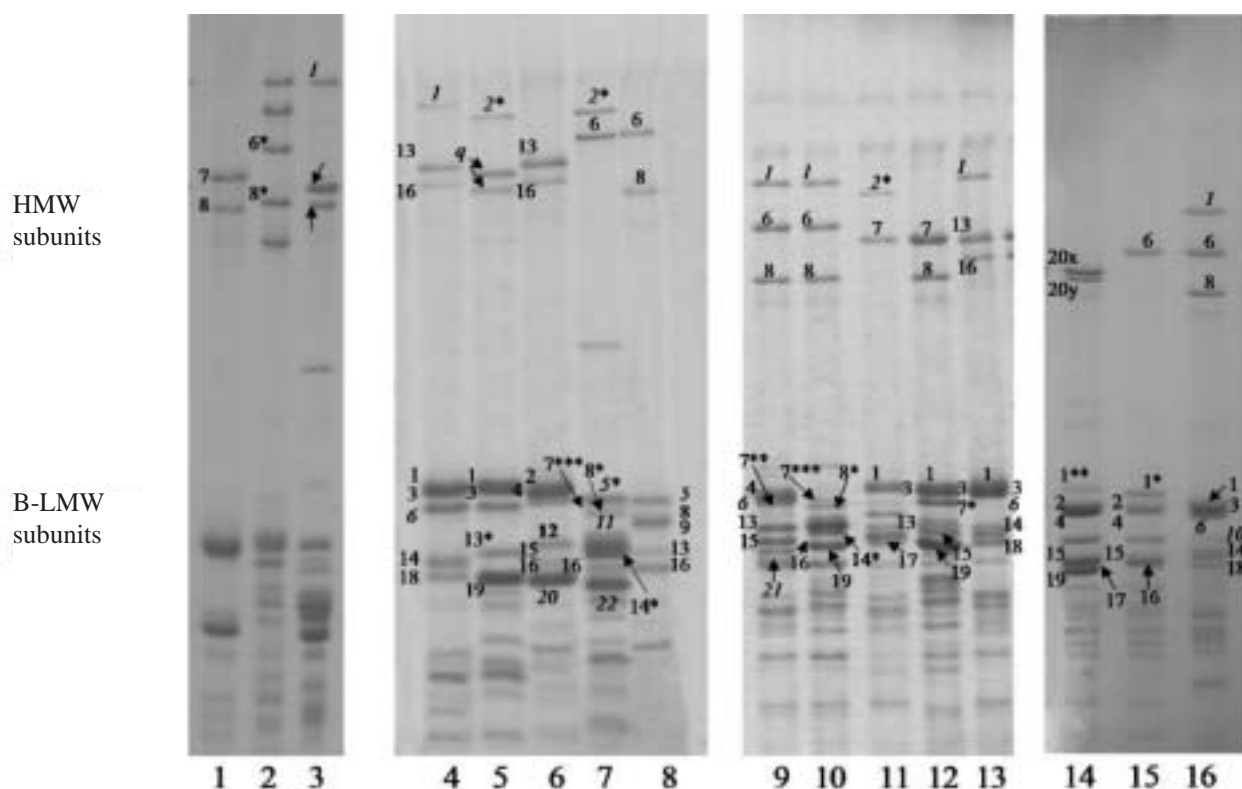
subunits are allelic variants of established subunits. For gliadins, eight alleles were detected at *Gli-A1* and *Gli-B1*. Six of these alleles were rare. One new allele was

identified at *Gli-A1* (*new-3*) and four at *Gli-B1* (*new-6* to *new-9*). Gliadins controlled by these alleles are described in Table 3 and shown in Fig. 2.

**Table 3.** B low-molecular-weight (B-LMW) glutenin subunits and gliadins encoded by the new alleles found in the durum wheat varieties analysed. New B-LMW glutenin subunits previously not identified (Nieto-Taladriz *et al.*, 1997; Martinez *et al.*, 2004) are in bold

|              | <i>Glu-A3</i>             | <i>Glu-B3</i>                                 | <i>Gli-A1</i>     | <i>Gli-B1</i>             |
|--------------|---------------------------|---|-------------------|---------------------------|
| <i>New1</i>  | <b>5*</b> , 11, 20        | 1, 3, 13*, 19                                 | -                 | ω-34-37 γ-44 <sup>a</sup> |
| <i>New2</i>  | 10                        | 1, 3, 13, 17                                  | γ-53 <sup>a</sup> |                           |
| <i>New3</i>  | 5, 11, <b>21</b>          | <b>1**</b> , 2, 4, 15, 17, 19                 | γ-48-50-51        |                           |
| <i>New4</i>  | 11, <b>21</b>             | 7, 8, 15, 17                                  |                   |                           |
| <i>New5</i>  | 6, <b>21</b>              | <b>1*</b> , 2, 4, 15, 16                      |                   |                           |
| <i>New6</i>  | <b>5*</b> , 20            | <b>7***</b> , <b>8*</b> , <b>14*</b> , 16     |                   | γ-40                      |
| <i>New7</i>  | <b>5*</b>                 | <b>7***</b> , <b>8*</b> , <b>14*</b> , 16, 19 |                   | γ-43                      |
| <i>New8</i>  | <b>5*</b> , 11, <b>22</b> | 4, <b>7**</b> , 13, 15                        |                   | ω-37 γ-44                 |
| <i>New9</i>  |                           | 13, 15, 19                                    |                   | ω-35 γ-41                 |
| <i>New10</i> |                           | 13, 17, 19                                    |                   |                           |
| <i>New11</i> |                           | 1, 3, <b>7*</b> , 15, 19                      |                   |                           |
| <i>New12</i> |                           | 15, 17, 19                                    |                   |                           |

<sup>a</sup>Described in Aguiriano *et al.* (2006)



**Figure 1.** High-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits of some entries with new alleles. Lane 1: ‘Camacho’ (*Glu-B1b*); lane 2: *Triticum aestivum* L. cv. Dawbull (*Glu-B1w*); lane 3: BGE 18646 (*Glu-B1new1*); 4: ‘Alaga’ (*Glu-B3h*); 5: BGE 13077 (*Glu-B3new-1*); 6: ‘Claro de Balazote’ (*Glu-B3g*); 7: BGE 13089 (*Glu-A3new-8*, *Glu-B3new-6*); 8: ‘Langdon’ (*Glu-A3b*, *Glu-B3b*); 9: BGE 13093 (*Glu-A3new-5*, *Glu-B3new-8*); 10: BGE 13100 (*Glu-B3new-7*); 11: BGE 13103 (*Glu-B3new-2*); 12: BGE 13590 (*Glu-B3new-11*); 13: ‘Alaga’; 14: BGE 8366 (*Glu-B3new-3*); 15: BGE 12537 (*Glu-B3new-5*); 16: BGE 12555 (*Glu-A3c*, *Glu-B3h*). Subunits encoded at *Glu-A1* and *Glu-A3* are in italic, at *Glu-B1* and *Glu-B3* in regular, and at *Glu-B2* in bold. ‘Camacho’, ‘Dawbull’, ‘Alaga’, ‘Claro de Balazote’ and ‘Langdon’ are test varieties for the alleles indicated.

Table 4 shows the different LMW genotypes identified and the gliadin alleles linked to them. Some accessions having new alleles are included in the Table 4 and Figs. 1 and 2. Eight different genotypes of the LMW-2 model, five of them not previously identified, were studied. All these LMW-2 variants had the *Glu-B3* alleles *a* or *new-8* (Fig. 1 lane 9), most of them linked to the gliadin alleles *Gli-B1b* or *c* (both coding for gliadin  $\gamma$ -45). Five LMW-2\* genotypes, all of them new, were identified. These patterns possessed the *Glu-A3* alleles *e* or *new-4*, linked to *Gli-A1e* or *f* (both coding for  $\gamma$ -49 instead of the most common  $\gamma$ -51). Five LMW-2\* variants, all of them new, were observed. These patterns contained *Glu-B3h* (Fig. 1 lane 4) or new alleles, linked to *Gli-B1new-1* ( $\gamma$ -44), with one exception. The two LMW-1 genotypes detected had been previously described, while the two LMW-1\* were new. All of them were linked to *Gli-B1a* ( $\gamma$ -42) or *new-6* ( $\gamma$ -40). Eight patterns were identified in the “others”

group, most of them with new alleles at both *Glu-3* loci. Four genotypes were linked to *Gli-B1new-1* or *new-8* (both coding for  $\gamma$ -44) and the other four to *Gli-B1new-7* ( $\gamma$ -43).

#### Analysis of convar. *durum* and convar. *turgidum*

Table 2 also shows the allelic frequencies and gene diversity separately for convars. *durum* and *turgidum*. At *Glu-A1* locus, allele *c* (subunit Null) was the most frequent in convar. *durum* but it was absent in convar. *turgidum*, while alleles *a* and *b* (subunits 1 and 2\*) were very common in the latter group. At *Glu-B1*, allele *d* (subunits 6+8) was the most frequent in both groups. In contrast to convar. *durum*, none variety of convar. *turgidum* had the allele *e* (20x+20y). Also, convar. *turgidum* possessed larger variability at this locus. Considering LMW subunits, the *Glu-A3* alleles *a* and *e* were the

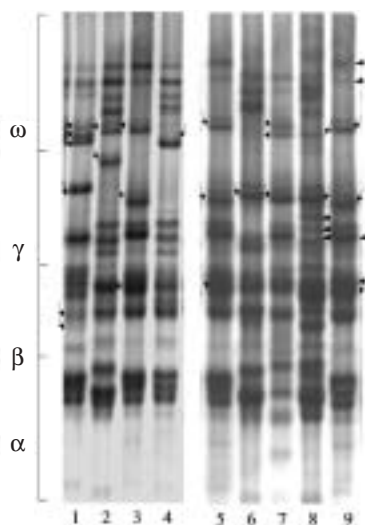
**Table 4.** Glutenin genotype of the low-molecular-weight (LMW) models found and the gliadin alleles linked to them. For the new patterns the alternative *Glu-3* or *Glu-B2* allele previously found in Nieto-Taladriz *et al.* (1997) or Martinez *et al.* (2004) is within parenthesis. A bank accession for the new alleles is also included

| Model  | <i>Glu-A3</i> | <i>Glu-B3</i>    | <i>Glu-B2</i> | <i>Gli-A1</i> | <i>Gli-B1</i>   | Bank accession |
|--------|---------------|------------------|---------------|---------------|-----------------|----------------|
| LMW-2  | <i>a</i>      | <i>a</i>         | <i>a</i>      | <i>c/b</i>    | <i>c</i>        |                |
|        | <i>d</i>      | <i>a</i>         | <i>b</i>      | <i>b</i>      | <i>b</i>        |                |
|        | <i>f</i>      | <i>a</i>         | <i>b</i>      | <i>b</i>      | <i>c/b</i>      |                |
| *      | <i>a</i>      | <i>a</i>         | <i>b (a)</i>  | <i>c</i>      | <i>c/b</i>      |                |
| *      | <i>a</i>      | <i>a</i>         | <i>b a)</i>   | <i>b</i>      | <i>c/ new-1</i> |                |
| *      | <i>new-6</i>  | <i>a</i>         | <i>a</i>      | <i>g</i>      | <i>c</i>        | BGE 18268      |
| *      | <i>new-7</i>  | <i>a</i>         | <i>a</i>      | <i>g</i>      | <i>c</i>        | BGE 13603      |
| *      | <i>new-5</i>  | <i>new-8</i>     | <i>b</i>      | <i>k</i>      | <i>c</i>        | BGE 13093      |
| LMW-2- |               |                  |               |               |                 |                |
| *      | <i>e</i>      | <i>d</i>         | <i>b (a)</i>  | <i>e</i>      | <i>c</i>        |                |
| *      | <i>e</i>      | <i>d</i>         | <i>b (a)</i>  | <i>f</i>      | <i>b</i>        |                |
| *      | <i>e</i>      | <i>new-3</i>     | <i>b</i>      | <i>e</i>      | <i>c</i>        | BGE 8366       |
| *      | <i>e</i>      | <i>new-5</i>     | <i>b</i>      | <i>e</i>      | <i>new-9</i>    | BGE 12537      |
| *      | <i>new4</i>   | <i>f</i>         | <i>b</i>      | <i>e</i>      | <i>c</i>        | BGE 13090      |
| LMW-2* |               |                  |               |               |                 |                |
| *      | <i>a (d)</i>  | <i>h</i>         | <i>b</i>      | <i>b/c</i>    | <i>new-1</i>    |                |
| *      | <i>c (d)</i>  | <i>h</i>         | <i>b</i>      | <i>b</i>      | <i>new-1</i>    |                |
| *      | <i>a</i>      | <i>new-1 (1)</i> | <i>b</i>      | <i>b</i>      | <i>new-1</i>    | BGE 13077      |
| *      | <i>e</i>      | <i>new-2</i>     | <i>b</i>      | <i>e</i>      | <i>new-1</i>    | BGE 13103      |
| *      | <i>e</i>      | <i>new-11</i>    | <i>a</i>      | <i>new-2</i>  | <i>c</i>        | BGE 13590      |
| LMW-1  |               |                  |               |               |                 |                |
|        | <i>b</i>      | <i>b</i>         | <i>b</i>      | <i>b</i>      | <i>a</i>        |                |
|        | <i>b</i>      | <i>i</i>         | <i>b</i>      | <i>b</i>      | <i>new-6</i>    |                |
| LMW-1- |               |                  |               |               |                 |                |
| *      | <i>h</i>      | <i>b</i>         | <i>a (b)</i>  | <i>f</i>      | <i>a</i>        |                |
| *      | <i>e</i>      | <i>new-4</i>     | <i>b</i>      | <i>c</i>      | <i>new-6</i>    | BGE 13080      |
| Others |               |                  |               |               |                 |                |
| *      | <i>e</i>      | <i>new-7</i>     | <i>a</i>      | <i>new-3</i>  | <i>new-8</i>    | BGE 13100      |
| *      | <i>new-1</i>  | <i>new-6</i>     | <i>a</i>      | <i>g</i>      | <i>new-8</i>    | BGE 18646      |
| *      | <i>new-8</i>  | <i>new-6</i>     | <i>a</i>      | <i>g</i>      | <i>new-8</i>    | BGE 13089      |
| *      | <i>new-1</i>  | <i>new-9</i>     | <i>a</i>      | <i>g</i>      | <i>new-7</i>    | BGE 20942      |
| *      | <i>new-2</i>  | <i>new-9</i>     | <i>a</i>      | <i>new-3</i>  | <i>new-7</i>    | BGE 30923      |
| *      | <i>new-2</i>  | <i>new-10</i>    | <i>b</i>      | <i>e</i>      | <i>new-7</i>    | BGE 13622      |
| *      | <i>b</i>      | <i>new-12</i>    | <i>a</i>      | <i>c</i>      | <i>new-7</i>    | BGE 2887       |
| *      | <i>new-3</i>  | <i>new-6</i>     | <i>a</i>      | <i>b</i>      | <i>new-1</i>    | BGE 20948      |

\*Pattern not previously described

most frequent in both groups. Allele *b*, very common in convar. *turgidum*, was rare in the *durum* set. The two groups had identical number of alleles but convar. *turgidum* included more new alleles, more equally distributed and larger gene diversity (Table 2). At *Glu-B3*, more than the 50% of *durum* accessions had the allele *a* (pla-

ced within LMW-2 model), while the most frequent alleles in convar. *turgidum* were *new-1* and *new-6* (within LMW-2\* or "others"). This latter group exhibited greater variability being the highest allele frequency of 16.7%. The two *Glu-B2* alleles presented similar frequencies in both convarieties. Respect to the gliadin



**Figure 2.** Gliadin electrophoretic spectra of some entries with new gliadin alleles. Lane 1: 'Langdon' (*Gli-B1a*); 2: BGE 12537 (*Gli-B1new-9*); 3: 'Mexicali 75' (*Gli-B1c*); 4: BGE 13080 (*Gli-B1new-6*); 5: 'Mexicali 75' (*Gli-B1c*); 6: BGE 20942 (*Gli-B1new-7*); 7: 'Alaga' (*Gli-B1new-1*); 8: BGE 13100 (*Gli-A1new-3*, *Gli-B1new-8*); 9: 'Mexicali 75' (*Gli-A1c*, *Gli-B1c*). Arrowheads and arrows refer to bands encoded at the *Gli-A1* and *Gli-B1* loci, respectively. 'Langdon', 'Mexicali 75' and 'Alaga' are test varieties for the alleles indicated.

alleles, *Gli-A1b* and *c* (both coding for  $\gamma$ -51) were widely distributed in the two groups. At *Gli-B1*, alleles *c* ( $\gamma$ -45) and *new-1* ( $\gamma$ -44) were the most common in convars. *durum* and *turgidum*, respectively. In the latter group the gliadin alleles coding for  $\gamma$ -44 had a frequency of 50%, whereas alleles controlling  $\gamma$ -45 were present in the 70% of the *durum* germplasm. Gene diversity was larger in the *turgidum* group although more alleles were identified in convar. *durum*.

### Analysis of the North and South geographical areas

The accessions were classified into two groups according to their geographical region of origin: the North (latitude  $> 41^{\circ} 5' 16''$  N) and the South of Spain (latitude  $\leq 41^{\circ} 5' 16''$  N). These two wide geographical areas had significant environmental differences mainly in solar radiation and temperature. Of the 52 accessions, three had no geographical origin known, five came from the islands and seven were duplicates. We finally studied 37 accessions. Some differences in the allele frequencies were detected between both geographical groups (Table 2). Among HMW glutenin alleles, *Glu-A1c* was more fre-

quent in the South, whereas *a* and *b* were more common in the North. At *Glu-B1*, some alleles were present in one set but not in the other, showing the North accessions higher diversity. For LMW glutenins, the *Glu-A3* locus presented higher variability in the wheats from the South and *Glu-B3* in those from the North. The allele *Glu-B3a* was the most common in the South (45.8%) and *new-1* in the North. At *Glu-B2*, allele *b* was more frequent than *a* in the South, whereas they appeared almost equally distributed in the North accessions. Regarding gliadin alleles, *Gli-A1e* and *Gli-B1c* were more frequent in the South, and *Gli-A1c* and *Gli-B1new-1* in the North. The latter group displayed higher variability at *Gli-B1*.

### Discussion

The sample analysed included landraces from all the Spanish regions where durum wheat has been traditionally cultivated. The genetic variability of 0.72 was higher than the 0.36 which was obtained in cultivars from Portugal (Igrejas *et al.*, 1999) and the 0.67 obtained in Mediterranean landraces (Moragues *et al.*, 2006). All the loci, except *Glu-B2*, displayed a genetic variability higher than 0.62, being *Glu-3* the most polymorphic (Table 2). The number of alleles detected at *Glu-1*, *Glu-3* and *Gli-1* was higher than those from other collections such as a world and ICARDA (International Centre for Agricultural Research in the Dry Areas) collection (Kaan *et al.*, 1993; Raciti *et al.*, 2003), landraces from Turkey, Portugal and Mediterranean basin (Turchetta *et al.*, 1995; Igrejas *et al.*, 1999; Moragues *et al.*, 2006) and cultivars grown in Portugal and Spain (Brites *et al.*, 1996; Nieto-Taladriz *et al.*, 1997).

A great part of the variability found was due to the presence of new alleles at all the loci, except for *Glu-B2*. At *Glu-A1*, allele *f* has not been previously reported in durum wheat. This allele identified in bread wheat (Igrejas *et al.*, 1997) conferred better quality than the "Null" allele *c*, usually found at this locus (Brites *et al.*, 2000). Most of new alleles at *Glu-A3* controlled new subunits. Subunit 5\* present in cv. 'Claro de Balazote' (Fig. 1 lane 6) probably corresponds to subunit 3 encoded at *Glu-A3* analysed by Ruiz and Carrillo (1993). Only six subunits encoded at *Glu-A3* have been identified so far (Nieto-Taladriz *et al.*, 1997; Lerner *et al.*, 2004). Although the assignment of new subunits is provisional, the three new subunits reported in the present work would increase considerably the variability at this locus. Subunit 13\* has been identified previously by Martinez *et al.* (2004), where it occurred in conjunction with subunits 1, 3 and 16 by *Glu-B3aa* (McIntosh *et al.*, 2003, Supplement 2006).



The gliadin alleles *Gli-A1new-2* and *Gli-B1new-1* were previously studied in Aguiriano *et al.* (2006). Furthermore, all new alleles at *Gli-B1* encoded other gliadins different from the most common  $\gamma$ -42 and  $\gamma$ -45. A total of 30 LMW patterns were described in the present work, whereas 11 and 18 were reported by Turchetta *et al.* (1995) and Moragues *et al.* (2006), respectively. LMW model variation was also higher in the landraces analysed than in the cultivars grown in Spain (Nieto-Taladriz *et al.*, 1997). The most common LMW-pattern in both collections had allele *a* at *Glu-3* (LMW-2), but differed in *Glu-B2* alleles (*a* in cultivars and *b* in landraces) and in their frequency (40% in cultivars vs. 13% in landraces). In the present work, this model was typical of convar. *durum*, which is consistent with its high frequency in cultivars. On the other hand, five different variants for LMW-2\* models were found in the landraces in contrast to the one found in the cultivars.

The frequencies of alleles associated with quality were compared with those from other collections. At *Glu-A1*, allele *c* was the most frequent (subunit Null) in other genepools (Brites *et al.*, 1996; Igrejas *et al.*, 1999; Raciti *et al.*, 2003; Moragues *et al.*, 2006). In the present study, similar frequencies were observed for alleles *a* (subunit 1), *b* (2\*) and *c* (Null), probably due to the inclusion of convar. *turgidum* in our germplasm (Table 2). Several studies (Ruiz and Carrillo, 1995; Turchetta *et al.*, 1995; Brites and Carrillo, 2001) have shown the better quality associated with the presence of an encoded subunit at this locus. At *Glu-B1*, the most frequent alleles were *d* (6+8) and *e* (20x+20y) in agreement with Brites *et al.* (1996), Igrejas *et al.* (1999) and Raciti *et al.* (2003). Other studies have shown negative effects of *Glu-B1e* on quality (Ruiz and Carrillo, 1995; Turchetta *et al.*, 1995; Brites and Carrillo, 2001). At *Glu-A3*, alleles *a* and *e* were the most frequent in our collection, while *b* and *h* were more widespread in germplasm from other Mediterranean countries (Igrejas *et al.*, 1999; Moragues *et al.*, 2006). Alleles *a* and *h* have been associated with good gluten quality and *e* and *b* with poor quality (Ruiz and Carrillo, 1995; Carrillo *et al.*, 2000; Martinez *et al.*, 2005). Similar to other collections the *Glu-B3a* allele was the most common (Igrejas *et al.*, 1999; Moragues *et al.*, 2006). This allele related to good quality (Ruiz and Carrillo, 1995; Brites and Carrillo, 2001; Martinez *et al.*, 2005) was very frequent in convar. *durum*. Gliadin alleles *Gli-B1c* ( $\gamma$ -45) and *new-1* ( $\gamma$ -44) were widely distributed in the Spanish germplasm, whereas *Gli-B1a* ( $\gamma$ -42) and *c* ( $\gamma$ -45) were very common in landraces from Portugal (Brites *et al.*, 1996). Several studies have shown that  $\gamma$ -45 and  $\gamma$ -44 were associated with better quality than  $\gamma$ -42 (Carrillo *et al.*, 1990;

Pogna *et al.*, 1990; Ruiz and Carrillo, 1995). All these results indicate that the sample analysed in the present work could be an important source of valuable alleles for quality.

The linkage relationships between *Glu-3* and *Gli-1* (Table 4) have shown that gliadins can be useful to detect new alleles coding for LMW glutenin subunits (not linked to the most common  $\gamma$ -45 or  $\gamma$ -51) and to easily and quickly discard materials (presence of  $\gamma$ -42 or  $\gamma$ -40) for quality improvement.

Relationships between prolamins composition and the botanical and geographical classification were detected. Differences between convars. *durum* and *turgidum* often coincided with those between North and South zones. While convar *turgidum*, more resistant to low temperatures, has been traditionally grown in the North, convar *durum*, more resistant to drought, has been grown in the South (Gadea, 1954). As a result, 77% of varieties from the North belonged to convar. *turgidum*, and 83% of varieties from the South belonged to convar. *durum*. Both convars. differ in some agromorphological traits related to the spikes and grain. Convar. *durum* is more commonly grown and associated with better quality. The most important allelic differences were at *Glu-A1*, *Glu-B1*, *Gli-B1* and *Glu-B3*. Alleles *Glu-A1c* and *Glu-B1e* only present in convar. *durum* have shown negative effects on quality, while *Glu-A1b* and *a*, very frequent in convar. *turgidum* have shown positive effects (Ruiz and Carrillo, 1995; Turchetta *et al.* 1995; Brites and Carrillo, 2001). In the present study, the genotype *Glu-B3new-1* (LMW -2\*) - *Gli-B1new-1* ( $\gamma$ -44) was very common in convar. *turgidum* and the North, and *Glu-B3a* (LMW-2)- *Gli-B1c* ( $\gamma$ -45) in convar. *durum* and the South. This result is consistent with the better quality, in general, of convar. *durum* than *turgidum* considering that *Glu-B3* alleles show the most significant effect on gluten quality (Ruiz and Carrillo, 1995; Porceddu *et al.*, 1998; Carrillo *et al.*, 2000). On the other hand, Kudryavtsev *et al.* (1996) pointed out that the *Gli-B1* block (*new-1*) of cultivar 'Lambro' was probably inherited from *Triticum turgidum* L. ssp. *dicoccoides* (Körn. ex Asch. et Graebn.) Thell or *Triticum carthlicum* (Nevski) Mackey. So, convar. *turgidum* could be more related to these species than convar. *durum*.

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