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ORIGINAL PAPER

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Genetic variability of the low-molecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta* L. em Thell.)

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Abstract The low-molecular-weight glutenin subunit composition of a collection of 403 accessions of spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell) was analyzed by SDS-PAGE. Extensive variation was found, including 46 different patterns for zone B and 16 for zone C. Patterns within zone B exhibited from two to six bands and patterns in zone C had between four and six bands in SDS-PAGE gels. A higher number of bands was observed when urea was added to the gels. Zone B exhibited between six and 11 bands, and we identified 14 new patterns in this zone. For zone C, up to ten new patterns that comprised between five and nine bands were detected. For both zones, 86 patterns were found. The variability detected in this material is greater than that detected in other hulled wheats.

Introduction

Spelt wheat (2n=6x=42; AABBDD; *Triticum aestivum* ssp. *spelta* L. em Thell.) is the hexaploid hulled wheat from which modern bread wheat (*T. aestivum* ssp. *aestivum* L. em. Thell.) is derived. This wheat was produced by spontaneous crossing between cultivated emmer wheat (*T. turgidum* ssp. *dicoccum* Schrank) and a wild grass (*Aegilops tauschii* ssp. *strangulata* Cross) (McFadden and Sears 1946; Kerber and Rowland 1974). This species was widely cultivated in the past, although now only a relict. In Spain, it is still possible to find it in marginal areas of Asturias (northern Spain), where traditional agriculture with archaic practices still exists. Today, spelt wheat is endangered (Peña-Chocarro and Zapata-Peña 1998).

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In recent years, interest in spelt wheat has increased, due to demand for ecologically grown foods, the low input, suitability for growth without the use of pesticides, underharsh ecological conditions and in marginal areas of cultivation and by its resistance to several diseases (D'Antuono 1989; Damania et al. 1990; Kema 1992). Furthermore, the great variation detected in the endosperm storage proteins in recent work suggests that this species could be used in wheat quality breeding programs (Caballero et al. 2001, 2003).

The endosperm storage proteins of wheat are divided into gliadins and glutenins. Gliadins are monomeric prolamins, controlled by the *Gli-1* and *Gli-2* loci, located on the short arms of chromosomes of the homoeologous groups 1 and 6, respectively (Payne et al. 1982; Payne 1987). Glutenins can be divided into high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits. The best-studied are the HMW glutenin subunits that are encoded by the *Glu-1* loci situated on the long arm of group 1 homoeologous chromosomes (Payne 1987). The genes coding for the LMW glutenin subunits, *Glu-3*, are located on the short arm of these same chromosomes, and are tightly linked at the *Gli-1* loci that encode ω- and γ-gliadins (Singh and Shepherd 1988; Pogna et al. 1990).

The LMW glutenin subunits are divided into B-LMWG (low-molecular-weight group), C-LMWG and D-LMWG groups based on their mobility in SDS-PAGE and their isoelectric points (Jackson et al. 1983). The D-LMWG subunits are the most acidic and have the lowest mobility of the LMW glutenin subunits. The C-LMWG has a wide range of isoelectric points and, due to their mobility in SDS-PAGE, overlap with α-, β- and γ-gliadins. The B-LMWG group comprises the greatest number of subunits, which are the most basic proteins, which have slightly lower mobility than α-, β- and γ-gliadins in SDS-PAGE. This group of proteins has not been studied in detail, due to their large number and considerable heterogeneity.

Biochemical and genetic aspects of wheat storage proteins have received great attention due to their

importance in determining the nutritional and technological properties of cultivated wheats. Gliadins are responsible for gluten extensibility and glutenins determine gluten strength and elasticity. Although the HMW glutenin subunits have a great importance in the bread-making quality of wheat, recent studies suggest that this importance could be lower than traditionally indicated (Graybosh et al. 1990). The LMW glutenin subunits are known to be essential contributors that determine dough properties in bread wheat (Gupta and Shepherd 1988; Gupta et al. 1989; Khelifi and Branlard 1992; Zhen et al. 1997). For this reason, the variation of the LMW glutenin subunits and gliadins and the interaction between all these components is of interest (Carrillo et al. 1990).

The main goal of this work was the analysis of the polymorphism of the LMW glutenin subunits in a wide collection of spelt accessions of Spanish origin.

Materials and methods

Plant material

Four hundred-three accessions of spelt wheat, obtained from the National Small Grain Collections (Aberdeen, Idaho, USA) and Centro Nacional de Recursos Fitogenéticos (Alcalá de Henares, Spain), were analyzed for the composition of the LMW glutenin subunits.

SDS-PAGE analysis

Proteins were extracted from crushed endosperm. Before glutenin solubilization, the gliadins were extracted with an aqueous 1.5 M dimethylformamide solution following a double-wash with 50% (v/v) propan-1-ol at 60°C for 30 min with agitation every 10 min. Glutenin was solubilized with 250 µl of buffer containing 50% (v/v) propan-1-ol, 80 mM Tris-HCl pH 8.5, and 2% (w/v) dithiothreitol at 60°C for 30 min. After centrifugation, 200 µl of the supernatant was transferred to a new tube, mixed with 3 µl of 4-vinylpyridine and incubated for 30 min at 60°C. The samples were precipitated with 1 ml of cold acetone and the pellet air-dried. The dried pellet was solubilized in buffer containing 625 mM Tris-HCl pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, and 2% (w/v) dithiothreitol in a 1:5 ratio (mg/µl) to whole meal.

Reduced and alkylated proteins were fractionated by electrophoresis in vertical SDS-PAGE slab gels in a discontinuous Tris-HCl-SDS buffer system (pH 6.8/8.8) at a 10% polyacrylamide concentration (w/v, C=1.28%) with and without 4 M urea. The Tris-HCl/glycine buffer system of Laemmli was used (Laemmli 1970). Electrophoresis was performed at a constant current of 30 mA/gel at 18°C for 30 min after the tracking dye migrated off the gel. Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. De-staining was carried out with tap water.

Results

Some work has demonstrated that the LMW glutenin subunits are controlled by different loci (Gupta and Shepherd 1988; Ruiz and Carrillo 1993). Nevertheless, in this study we have considered each band group as a block because we detected great variation and the available information about the allelic segregation of LMW glutenin subunits in this species is very limited. This approximation, although it is not the most correct genetically, is most often used in variability studies.

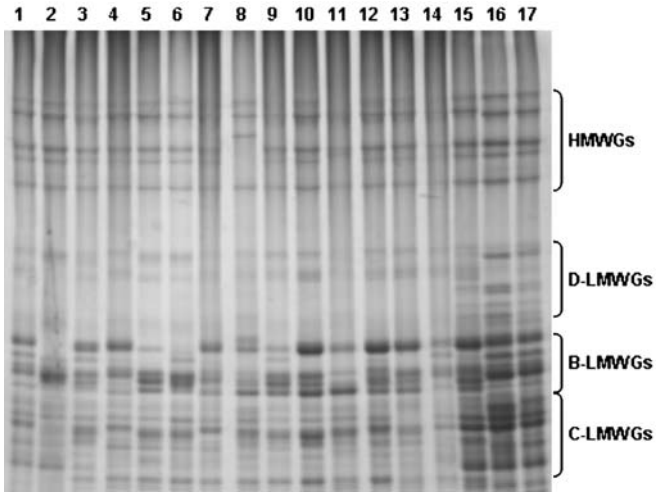

Fig. 1 Samples representative of the variation detected by SDS-PAGE of the variation found for the two zones from spelt wheat. Lanes as follows: *1* B12, C11; *2* B24, C12; *3* B2, C7; *4* B1, C2; *5* B38, C5; *6* B21, C5; *7* B1, C2; *8* B44, C7; *9* B13, C5; *10* B3, C7; *11* B39, C15; *12* B3, C7; *13* B3,C3; *14* B20, C1; *15* B3, C9; *16* B33, C8; *17* B27, C8

Gels were divided into two zones, B-LMWGs and C-LMWGs, according to the grouping used in hexaploid wheat where a clear gap was observed between these zones. A representative sample of the variation detected for both zones is shown in Fig. 1.

B-LMW glutenin subunits

For the B-LMWG zone, 24 bands were detected, which formed up to 46 different patterns. Sixteen of these patterns were found in only one accession. Diagrams of patterns in the B zone, with an indication of their frequencies, are shown in Fig. 2.

The patterns consisted of two to six bands, with patterns with three and four bands (17 and 15 patterns, respectively) being the most frequent. Patterns B1 and B2 were the most frequent, appearing in 48 and 36 accessions, followed by patterns B3 and B4, which were detected in 31 and 25 accessions, respectively. Up to 50.9% of the accessions are included in the first seven patterns (Fig. 2, B1–B7), while the other 39 patterns were detected in the rest of the accessions.

Some bands of the B-LMWG zone seem to be common to several patterns; this is the case for the bands that form pattern B9 that were observed in 15 patterns (Fig. 2). Other patterns were very similar, with small differences such as as one additional band (e.g., patterns B1 and B3 or B2 and B4) or changes in the mobility of one band (e.g., patterns B1, B5 and B7).

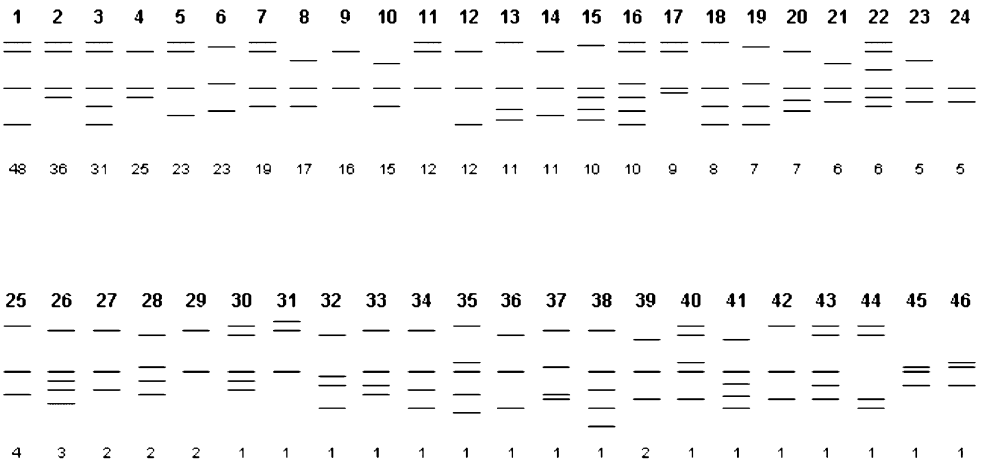

Fig. 2 Diagrammatic representation of the patterns of the B-LMWG (low-molecular-weight group) zone. The bands with low intensity are shown with *discontinuous line*. The patterns are numbered from 1 to 46, in decreasing order of presence among all accessions (*first number below patterns*)

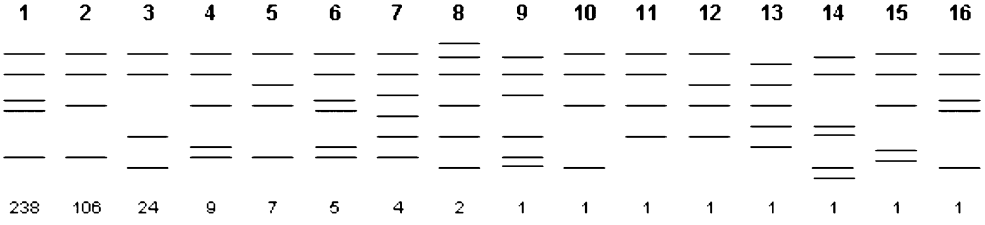

Fig. 3 Diagrammatic representation of 16 patterns of the C-LMWG zone. The patterns are numbered as in Figure 2

C-LMW glutenin subunits

For the C-LMWG zone, 23 different bands were detected, which formed 16 different patterns and represented a lower variation than that observed in the B-LMWG zone. Diagrams of these C patterns with their frequencies are given in Fig. 3. The patterns consisted of four to six bands. Eight out of 16 patterns only appeared in one accession. Pattern C1 was the most frequent and was detected in 238 accessions, followed by patterns C2 and C3, which were found in 106 and 24 accessions, respectively. Patterns C1, C2 and C3 accounted for about 91.3% of all accessions (pattern C1: 59.1%; pattern C2: 26.3%; and pattern C3: 6.0%).

For both zones, 43 different bands were found, with a maximum of 12 bands per accession, which formed a total of 62 different patterns. The combination of these patterns formed 100 different combinations, with the combination formed by patterns B1 and C1 (40 accessions) being the most frequent, followed by combinations B2-C2 and B3-C2 which appeared in 22 and 21 accessions, respectively. In addition, 49 combinations were found in only one accession. Generally, accessions with the same pattern for the B-LMWG zone had a different pattern for the C-LMWG zone.

Characterization of B-LMW and C-LMW glutenin subunits in SDS-PAGE with urea

Previous investigations carried out in our group have shown that the variability detected by normal SDS-PAGE could be an underestimate of the actual variability (Alvarez et al. 2001; Caballero et al. 2001; Pflüger et al. 2001). It is possible that these proteins present anomalous mobility due to conformational differences that cause some subunits to appear to migrate in similar positions such that they cannot be identified. This anomalous mobility is eliminated by the addition of a strong denaturant, such as 4 M urea (Goldsbrough et al. 1989; Lafiandra et al. 1993). In the present work, this technique has been used to distinguish some subunits.

When urea was added to the gel, the mobility of all subunits showed changes and permitted us to detect new patterns in the B-LMWG zone which have not been possible to identify in normal gels. In these gels we were able to detect 79 different bands, 44 for the B-LMWG zone and 35 for the C-LMWG zone, a much larger number than detected in normal gels.

For the B-LMWG zone (Fig. 4), the number of bands in these the patterns varied from six to 11 bands, with patterns consisting of seven bands being the most frequent. The change in the mobility of some bands has permitted us to identify up to 14 new patterns. Therefore, accessions PI-348455, PI-348465, PI-348519 and PI-348572, which present pattern B2 in normal gels, showed four different patterns when urea was added to the gel (Fig. 4A, B, lanes 1–4). The same phenomenon was seen with accessions PI-348439, PI-348478 and PI-348640, with pattern B21 in gels without urea (Fig. 4A, B, lanes 7–9). For the C-LMWG zone (Fig. 5), the patterns consisted of five to nine bands, with patterns with six bands being the most frequent, and ten new patterns were detected for this zone. For example, all the accessions

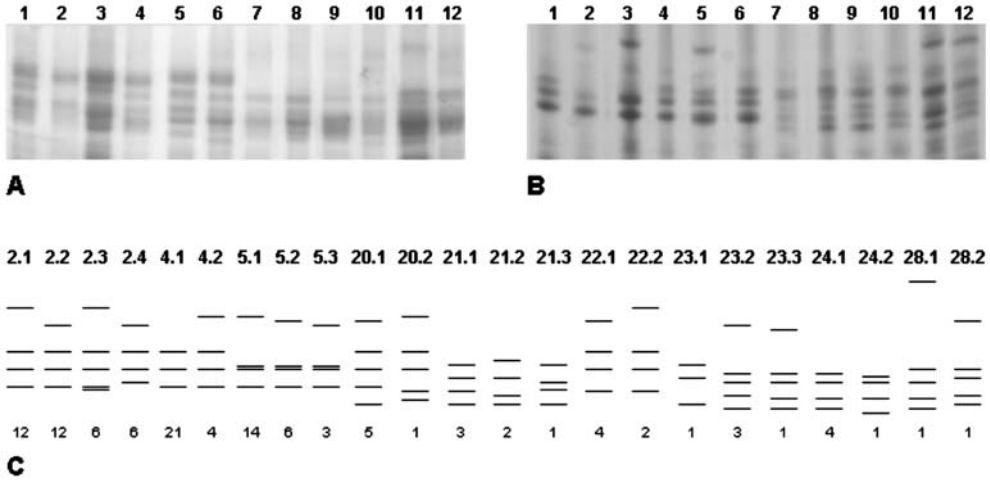

Fig. 4 SDS-PAGE without (**A**) and with (**B**) 4 M urea of the same representative sample of the variation detected for the B-LMWG zone. Lanes: *1* PI-348455, B2.1; *2* PI-348465, B2.2; *3* PI-348519, B2.3; *4* PI-348572, B2.4; *5* PI-348442, B20.1; *6* PI-469039, B20.2; *7* PI-348439, B21.1; *8* PI-348478, B21.2; *9* PI-348640, B21.3; *10* PI-348702, B21.1; *11* PI-348570, B23.1; *12* PI-348592, B23.2. **C** Diagrammatic representation of the new B-LMWG patterns detected in 4 M urea-SDS-PAGE gels. The patterns are numbered as in Fig. 2

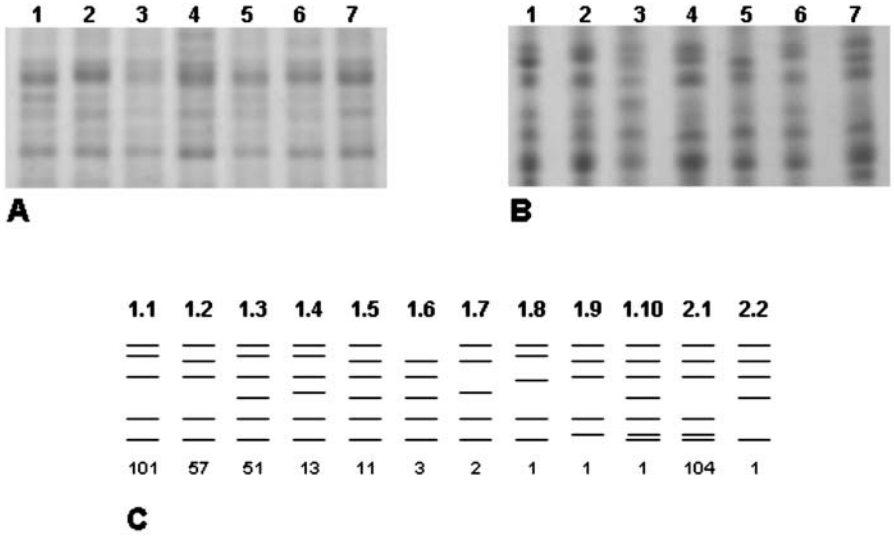

Fig. 5 SDS-PAGE without (**A**) and with (**B**) 4 M urea of the same representative sample of the variation detected for the C-LMWG zone. Lanes: *1* PI-348696, C1.5; *2* PI-348698, C1.3; *3* PI-348702, C1.4; *4* PI-348715, C1.1; *5* PI-348727, C1.6; *6* PI-348728, C1.3; *7* PI-348731, C1.2. **C** Diagrammatic representation of the new C-LMWG patterns detected in 4 M urea-SDS-PAGE gels. The patterns are numbered as in Fig. 2

presented pattern C1 in normal gels (Fig. 5A), but when urea was added, the gel showed six different patterns (Fig. 5B).

When both zones were analyzed together, the combination formed by patterns B8 and C13 had the maximum number of bands, 20, which was higher than in normal gels.

Discussion

The D-LMWG zone has not been analyzed previously because it was considered to be a contamination with ω-gliadins (Masci et al. 1993, 1999). This zone has not been very well described, although there are some studies, such as that of of Masci et al. (1991), in which the authors compared the B and D zones of the LMW glutenin subunits in two biotypes of bread wheat and found that only one of these cultivars possessed D-LMWG subunits. The work of Gianibelli et al. (2002) analyzed the polymorphism of the LMW glutenin subunits in *T. tauschii* (syn. *Ae. tauschii*) and identified one D band, which had been previously described in cv. Chinese Spring by Masci et al.(1993) and Jackson et al.(1993).

The variability detected in the B-LMWG zone for spelt wheat was slightly higher than that found in *T. tauschii* for the same zone by Gianibelli et al. (2002), who found 30 different patterns, and much higher than that found by Nieto-Taladriz et al. (1997), who detected 18 different patterns in durum wheat. Ruiz et al. (1998) found 24 different patterns in the analysis of a collection of local Spanish cultivars of durum wheat. Gupta and Shepherd

(1988) detected 28 patterns for this zone when analyzing 222 lines of hexaploid wheats, lower than the variability detected in spelt wheat. Some of the patterns described by these authors are very similar to those detected in our study, but we cannot confirm that they are the same because the techniques used in both studies are different.

For the C-LMWG zone, Lee et al. (1999) detected 20 different bands among 91 accessions of five different diploid wheat species studied, a number higher than detected in this work. Forty-three different patterns in this zone were observed by Gianibelli et al. (2002) in *T. tauschii*. The variation detected by these authors in diploid species was higher than the variability we found in spelt wheat. In contrast to this study, Gianibelli et al. (2002) found more variation for the C-LMWG zone than the B-LMWG zone.

Pflüger et al. (2001) analyzed the composition of the B-LMWG subunits in a collection of cultivated emmer wheat using gels with 4 M urea and found 23 different patterns formed by two to six bands; in our study, the variation of the LMW glutenins with 4 M urea was high, with 46 different patterns for this zone formed by six to 11 bands. The C-LMWG zone has not been analyzed in gels with urea in these wheat species.

Unfortunately, the area where spelt wheat is still cultivated in Spain has been drastically reduced, including some areas where the materials analyzed in this work were collected. Consequently, a great part of this variability could soon be lost. The low frequency of some patterns confirms the necessity of protection and conservation of these accessions because the possibility of finding the same alleles in other materials is very low. For this reason, these materials are being recuperated and multiplied to maintain this variability. Later, analysis of the other agronomic characteristics, including their quality, must be undertaken.

In conclusion, this study has shown that the variability for the LMW glutenin subunits in spelt wheat is higher than in other species. This variability could be used to improve spelt wheat per se, or to increase the genetic base of the durum and bread wheats by introgression.

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