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Allelic variation of the HMW glutenin subunits in Spanish accessions of spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.)

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Abstract Spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.) is a hulled wheat of Germanic origin that survives at marginal areas in Asturias (Spain). The HMW glutenin subunit composition of 403 accessions of spelt wheat from Spain has been analysed by SDS-PAGE. Three allelic variants were detected for *Glu-A1*. For the *Glu-B1* locus, two of seven alleles detected have not been found before; while four of nine alleles detected for the *Glu-D1* are not previously described. Considering the three loci, twenty five combinations were found among all the evaluated lines. This wide polymorphism could be used to transfer new quality genes to wheat, and widen the genetic basis of them.

Keywords Genetic diversity · Hulled wheats · Glutenins

Introduction

Modern agronomic practices have reduced the genetic variability of cultivated wheats, which has given great importance in the search for species that could be useful in contributing genes for wheat improvement (Jauhar 1993). Between these species, the hulled wheats such as einkorn (2n=2*x*=14, **AA**; *Triticum monococcum* ssp. *monococcum* A. et D. Löve), emmer (2n=4*x*=28, **AABB**; *T. turgidum* ssp. *dicoccum* L. em. Thell.) or spelt (2n=6*x*=42, **AABBDD**; *T. aestivum* ssp. *spelta* L. em. Thell.) have proved to be rich-sources of useful genes (Srivastava and Damania 1989). Unfortunately, because of the progressive neglect of these crops by other more-economic profit, most of the genetic resources for these species are only present in germplasm banks. The increasing demand for unconventional foods, together with the search for low-input agriculture, has led to a revival of traditional food where hulled wheats could play an important role.

In Spain, the hulled wheats, mainly emmer and spelt, were widely cultivated during the first part of the 20th Century, but decreased towards the late 1960s when agricultural mechanisation in many areas of Spain began. In the case of spelt, this species survives in marginal farming areas of Asturias (North of Spain), where it is named as escanda (this term has been applied to both emmer and spelt) and is endangered (Peña-Chocarro and Zapata-Peña 1998).

The analysis of the seed storage proteins (glutenins and gliadins) has been a useful tool for plant breeding, due to their relationship with the technological properties of wheat. Among these proteins, the best studied are the high-molecular-weight (HMW) glutenin subunits which are coded at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arm of group-1 homoeologous chromosomes in hexaploid wheat (Payne 1987). Each locus consists of two linked genes which code for two types of HMW glutenin subunits with different mobility in SDS-PAGE, and named as the *x*- and *y*-type (Harberd et al. 1986).

Investigations on seed storage protein composition have been scarce between the hulled wheats from Spain. Recently, the seed storage protein composition of a collection of Spanish emmer wheat has been analysed by our group (Pflüger et al 2001). We believe that the high degree of variation detected could be used for widening the genetic basis of wheat. For spelt wheat, Rodriguez-Quijano et al (1990) analysed a collection of 118 accessions, which are part of the material used in the present work, and found a low variability for the HMW glutenin subunits.

The main goal of the present study was to analyse the HMW glutenin subunit composition of an extensive collection of spelt accessions collected in the North of Spain at the first half of the 20th Century.

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Material and methods

Four hundred and three accessions of spelt wheat, obtained from the National Small Grain Collections (Aberdeen, USA) and Centro de Recursos Fitogenéticos INIA (Alcalá de Henares, Spain), were analysed in this study.

Proteins were extracted from crushed endosperm. Before glutenin solubilisation, the gliadins were extracted with a 1.5 M dimethylformamide aqueous 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 solubilised with 250 µl of buffer containing 50% (v/v) propan-1-ol, 80 mM of Tris-HCl pH 8.5, and 2% (w/v) dithiothreitol at 60°C for 30 min. After centrifugation, 200 µl of the supernatant were 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. The dried pellet was solubilised 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 wholemeal.

Reduced and alkylated proteins were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH 6.8/8.8) at an 8% polyacrylamide concentration (w/v, C=1.28%) with and without 4 M urea. The Tris-HCl/glycine buffer system of Laemmli (1970) was used. 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 and discussion

One of the principal problems of the germplasm banks is that many accessions have not been sown for many years, due to the scarce interest that the same species had in the second half of the 20th Century. This could condition their seedling production and the future of these collections. Between these species stands of the hulled wheats (einkorn, emmer and spelt) have been neglected in the greater part of their traditional land area. Because of the increased interest for these species in recent years, we have initiated a programme of evaluation of the variability of hulled Spanish wheats for regaining, mainly, the lines with an interest for quality.

In Fig. 1, the HMW glutenin subunit composition of several spelt accessions representative of the variation

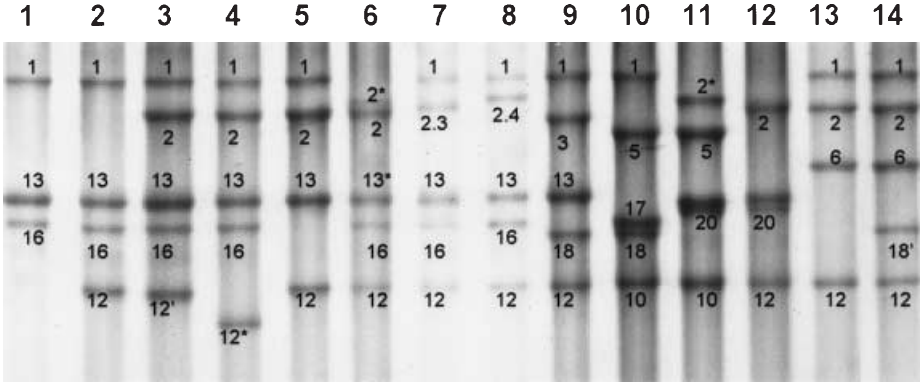

Fig. 1 SDS-PAGE (8%) patterns of HMW-Gs from some Spanish accessions of spelt wheat, representative of the different allelic variants detected at the *Glu-A1, Glu-B1* and *Glu-D1* loci. *Lanes*: *1* Alaga, *2* PI-348680, *3* PI-348495, *4* PI-348672, *5* PI-348676, *6* PI-348767, *7* PI-348465, *8* PI-348473, *9* PI-348752, *10* Yecora, *11* BG-020900, *12* PI-190963, *13* PI-591900, and *14* PI-190960

Table 1 Allelic frequencies at three glutenin loci in 403 Spanish spelt wheat landraces

Locus	Allele[a]	HMWG subunits	Accessions		Previous work[b]	
			No.	%	No.	%
Glu-A1	a	1	349	86.60	100	84.70
	b	2*	50	12.41	3	2.60
	c	*null*	4	0.99	15	12.70
Glu-B1	e	20	5	1.24	–	–
	f	13+16	354	87.84	103	87.30
	an	6	1	0.25	–	–
	at	13+18	23	5.71	14	11.90
	as	13	4	0.99	1	0.80
	XVIII	13*+16	13	3.23	–	–
	XIX	6+18´	3	0.74	–	–
Glu–D1	a	2+12	359	89.08	106	89.90
	b	3+12	35	8.68	4	3.30
	d	5+10	1	0.25	–	–
	r	2.3+12	2	0.50	3	2.60
	l	*null*+12	2	0.50	5	4.20
	I	2+12´	1	0.25	–	–
	II	2+12*	1	0.25	–	–
	III	2.4+12	1	0.25	–	–
	IV	2.5+12	1	0.25	–	–

[a] According to McIntosh et al. 1998. The new alleles appear indicated with a Roman numberal

[b] Rodriguez-Quijano et al. 1990

detected is shown. Up to 19 allelic variants (three alleles at the *Glu-A1* locus, seven at the *Glu-B1* locus, and nine at the *Glu-D1* locus) were found in the evaluated accessions (Table 1). These results showed a sharp discrepancy with the findings of Rodriguez-Quijano et al. (1990), who reported only three allelic variants at the *Glu-B1* locus and four at the *Glu-D1* locus. Six novel allelic variants were identified by us, two at the *Glu-B1* locus and four at the *Glu-D1* locus. These new alleles were named according the progressive Roman numeral nomenclature of Vallega and Waines (1987) and Branlard et al. (1989), which we used for the classified new allelic variants in emmer wheat (Pflüger et al. 2001). The International nomenclature indicated by McIntosh et al. (1998) has been employed to name the alleles previously described.

Similar to other cultivated wheats (Shewry et al. 1992), only one active component was found for the *Glu-A1* locus. All three allelic variants detected were previously described in spelt wheat by Rodriguez-Quijano et al. (1990); however, the frequencies of the subunits 2* and *null* were different from those found by these authors. In our material, the *null* allele (*Glu-A1c*) appeared in only four accessions (PI-190963, PI-348626, PI-348750 and BG-002014), whereas it was found in 15 accessions by Rodriguez-Quijano et al. (1990). The subunit 2* coded by the *Glu-A1b* allele (Fig. 1, lanes 6 and 12) appeared in 51 accessions, which is clearly different from the findings of Rodriguez-Quijano et al. (1990) where this subunit was present in only three accessions. In both studies, the most-frequent allele was *Glu-A1a* (subunit 1) which was found in 348 of the 403 accessions evaluated in the present work.

For the *Glu-B1* locus, the allele *Glu-B1f* (subunits 13+16) was the most frequent among the evaluated lines of spelt wheat (87.59%). By contrast, this allele, associated with good bread-making quality, is rare or appears with a low frequency in bread wheat (Payne and Lawrence 1983; Branlard and Le Blanc 1985; Lawrence 1986; Lukow et al 1989). The other two alleles previously described by Rodriguez-Quijano et al. (1990) were found in four accessions for the *Glu-B1as* allele (subunit 13; Fig. 1, lane 9) and in 23 accessions for the *Glu-B1at* allele (subunits 13+18; Fig. 1, lane 10). Furthermore, five alleles which were not found by Rodriguez-Quijano et al. (1990) were detected in our material. Some of these alleles have been described in durum and bread wheat, while other ones have not been previously found. In the latter cases, we have named the subunits according to their proximity to the subunits previously described. For example, subunit 13* that appears with subunit 16 (Fig. 1, lane 6) was slightly faster than subunit 13. This novel allele (*Glu-B1-XVIII*) was found in 13 accessions.

The allele *Glu-B1an* (subunit 6) was rare and was only found in the PI-591900 accession (Fig. 1, lane 13); it was detected in two landraces of bread wheat by Rodriguez-Quijano et al. (1990) but not in spelt wheat. Other alleles were not detected in spelt wheat, but the allele *Glu-B1e* (subunit 20; Fig. 1, lanes 11 and 12) was found in five accessions (Table 1). The new allele *Glu-B1-XIX* presents two major components, subunit *x* with a mobility similar to subunit 6, and subunit *y* similar to subunit 18 (Fig. 1, lane 14), which was found in only one accession (PI-190960).

The lines evaluated present a large homogeneity for the *Glu-D1* locus, in fact 89.08% of them showed the *Glu-D1a* allele (subunits 2+12). Although these subunits have been associated with poor quality in bread wheat, it is important to emphasize that the species evaluated here is not bread wheat and, consequently, the characteristics of the different alleles detected may not be similar to bread wheat. Another allele found with a relatively high frequency was *Glu-B1b* (subunits 3+12; Fig. 1, lane 9) which appears in 35 accessions. The rest of the alleles found appear in one or two accessions (Table 1).This is the case for subunits 5+10 (allele *Glu-D1d*), associated with good quality in bread wheat, and which appear in only one accession (BG-020900). The subunits 12′ (PI-348495) and 12* (PI-348672), that appear with subunit 2 (alleles *Glu-D1-I* and *Glu-D1-II*, respectively), showed differences in mobility with respect to subunit 12. Subunit 12′ was slightly faster than subunit 12, while subunit 12* showed a clear difference to both subunits (Fig. 1, lanes 3, 4 and 5, respectively). Another novel allele, named *Glu-D1-III* (subunits 2.4+12), was detected in only one accession (PI-348473). Subunit 2.4 was slower than subunit 2.3 (Fig. 1, lanes 8 and 7, respectively). This last allele (*Glu-D1r*, subunits 2.3+12) was detected in two accessions (PI-348465 and PI-348720), like the allele *Glu-D1l*, subunit 12 (PI-348680 and PI-348778).

Our previous investigations carried out with other species, such as *Hordeum chilense* Roem. et Schult. (Alvarez et al. 2001) and emmer wheat (Pflüger et al. 2001), have shown that the variability detected by normal SDS-PAGE gels could be lower than real. This seems to be due to conformational differences between the proteins (Goldsbrough et al. 1989), which cause the anomalous mobility of some subunits that appear in similar positions to the other subunits described. This is eliminated by the addition of a strong denaturant, such as 4 M urea (Goldsbrough et al. 1989; Lafiandra et al. 1993) used in the present work.

When urea was added to the gel, the mobility of all subunits showed changes which differentiate some subunits better. For example, subunit 2* appears clearly separate from subunit 2, which is not always possible in normal SDS-PAGE gels (Fig. 2, lanes 3 and 5). Another subunit that showed more clear differences than in normal gels was subunit 18 present in the alleles *Glu-B1at* (13+18) and *Glu-B1-XIX* (6+18). These subunits have similar mobility in normal gels (Fig. 2A, lanes 3 and 5), while in the urea gel, subunit 18 of allele *Glu-B1-XIX* was faster than that of the allele *Glu-B1at* (Fig. 2B, lanes 3 and 5). Consequently, subunit *y* of the first allele has been named as 18′. Furthermore, the position of some subunits in urea-SDS-PAGE gels suggested the presence of new alleles. The allele present in the PI-348572 accession that was catalogued as *Glu-D1-III* (2.4+12) showed a different mobility to the component *x* in the urea gel. This new sub-

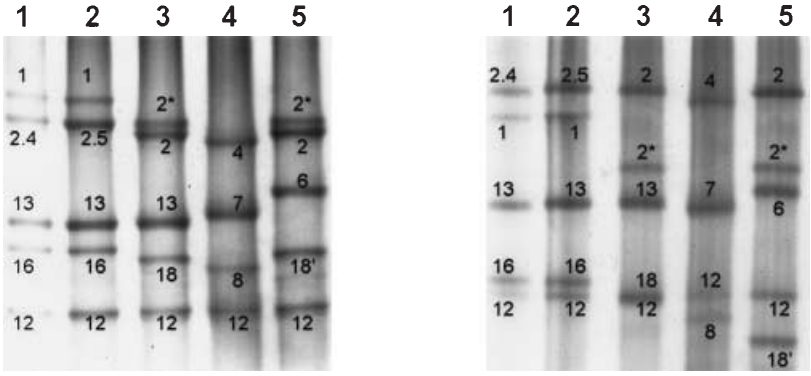

Fig. 2 SDS-PAGE without (**A**) and with (**B**) 4 M urea of some allelic variants detected at the *Glu-B1* and *Glu-D1* loci. *Lanes*: *1* PI-348473, *2* PI-348572, *3* PI-348627, *4* Champlein, and *5* PI-348631

Table 2 Frequencies of the HMW glutenin subunits compositions found among 403 accessions analysed

Subunit composition			No.	%	Accession standards
Glu–A1	*Glu–B1*	*Glu–D1*			
null	20	2+12	2	0.50	–
null	13+16	2+12	2	0.50	–
1	13	2+12	4	0.99	–
1	20	2+12	1	0.25	PI–348687
1	6	2+12	1	0.25	PI–591900
1	6+18′	2+12	2	0.50	–
1	13+16	12	2	0.50	–
1	13*+16	2+12	8	1.99	–
1	13+16	2+12′	1	0.25	PI–348495
1	13+16	2+12*	1	0.25	PI–348672
1	13+16	2+12	273	67.74	–
1	13*+16	3+12	3	0.74	–
1	13+16	3+12	27	6.70	–
1	13+16	2.3+12	2	0.50	–
1	13+16	2.4+12	1	0.25	PI–348473
1	13+16	2.5+12	1	0.25	PI–348572
1	13+18	2+12	21	5.21	–
1	13+18	3+12	1	0.25	PI–348752
2*	20	2+12	1	0.25	PI–469037
2*	20	5+10	1	0.25	BG–020900
2*	6+18′	2+12	1	0.25	PI–348631
2*	13*+16	2+12	2	0.50	–
2*	13+16	2+12	40	9.93	–
2*	13+16	3+12	4	0.99	–
2*	13+18	2+12	1	0.25	PI–348627

unit, named 2.5, is slightly slower that subunit 2.4 (Fig. 2, lanes 1 and 2, respectively). This new allele formed by subunits 2.5 and 12 was named *Glu-D1-IV*.

For the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, 25 combinations were detected. Their frequencies are shown in Table 2, where there is a clear dominance of the combination 1,13+16,2+12 that appears in 67.74% of the accessions. This was also the most-frequent combination in the study of Rodriguez-Quijano et al. (1990), where it appeared in 75 of the 118 accessions evaluated. Other combinations found for these authors as *null*,13+16,2+12 and 1,13+18,2+12 showed a sharp difference with our results (Table 2). The first combination was found in only two accessions, whereas 13 accessions were identified by Rodriguez-Quijano et al. (1990). This notable difference is related with the scarce presence of the *null* allele (*Glu-A1c*) in our material as opposed to those analysed by these authors.

The rest may be made up of two combinations, which appeared with a relatively high frequency; the combination 1,13+16,3+12 appears in 27 accessions while the 2*,13+16,2+12 combinatory was found in 41. Eleven of the combinations appeared in only one accession. Between them these make up the 2*, 20,5+10 (BG-020900) combination, which shows good alleles for the *Glu-A1* and *Glu-D1* loci according to those indicated in bread wheat. By contrast, nine combinations present the six novel alleles, whose effects on bread making quality have not been evaluated at yet.

Conclusions

The variability detected in the present work showed sensitive differences with the findings of Rodriguez-Quijano et al. (1990), who used part of the present material. The

cause of these differences could be due to two facts, the different number of accessions analysed (118 of the 403) and the use of different types of gel. In fact, we have used a low-polyacrylamide-concentration gel that permits best discrimination at the level of the HMW glutenin subunits. Consequently, certain variants present here might have been overlooked by these authors and classified with a different number. This variability has been confirmed with the use of the urea-SDS-PAGE gel, which showed the presence of some subunits more clearly than did the normal SDS-PAGE gel.

Diverse studies carried out on the ethnobotanical aspects of the hulled wheats (Peña-Chocarro 1996; Peña-Chocarro and Zapata-Peña 1998), have shown that the land area for spelt wheat has been reduced to Asturias (North of Spain) during the 20th Century. This could be the cause for the presence of the combination 1,13+16,2+12 between the lines evaluated, which were mostly collected in this zone by personnel of the Swiss Federal Research Station for Agroecology and Agriculture during 1939 (Dr. F. Weilenmann, personal communication).

On the other hand, besides new alleles not previously described, the information may also be of interest to plant breeders for choosing parents to obtain recombinant lines with good bread-making quality. The wide polymorphism detected should be evaluated for its effects on technological properties through the transfer to bread wheat of new allelic variants or the analysis of spelt wheat itself. Consequently, we think that this species could be used as a source of genes for quality improvement in bread wheat, independent of the development of spelt as a crop in modern agriculture, which demands new products.

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