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Variation of High Molecular Weight Glutenin Subunits in Two Neglected Tetraploid Wheat Subspecies

Leonor CABALLERO, Luis M. MARTÍN and Juan B. ALVAREZ

Department de Genetics, School of Agricultural and Forestry Engineering, University of Cordoba, Spain

Abstract: The genetic diversity of 140 accessions of *Triticum turgidum* ssp. *carthlicum* Nevski em. A. Löve & D. Löve and 159 accessions of *T. turgidum* ssp. *polonicum* L. em. Thell. was evaluated by the analysis of HMW glutenin subunits. Seven allelic variants were found among the *carthlicum* accessions: three at the *Glu-A1* locus (two of them were novel alleles) and four at the *Glu-B1* locus (one of them novel). More variability was found among the *polonicum* accessions with 16 allelic variants: six at the *Glu-A1* locus (three of them novel), and ten at the *Glu-B1* locus (five of them novel). Totally, ten new alleles were found, one of which appeared in both subspecies. Out of 19 different combinations of alleles detected in both subspecies, 14 were novel. Based on the available passport data, the *carthlicum* accessions could be separated by origin into 18 groups, and the *polonicum* accessions into 33 such groups. The genetic diversity was lower among the *carthlicum* (*Ht* = 0.174) than among the *polonicum* accessions (*Ht* = 0.562). In both subspecies, most diversity was present between groups differing in origin, whereas diversity within the groups was very low. The detected variability offers possibilities for the improvement of bread making quality in durum wheat through introduction of newly detected alleles and for the broadening of genetic diversity in this wheat species.

Keywords: tetraploid wheat; seed storage proteins; genetic diversity; electrophoresis

The erosion of genetic variability in crops seriously affects their vulnerability and limits the space for crop improvement. Genetic erosion has various causes. Paradoxically, also the genetic improvement of crops contributed to the erosion of genetic variability among cultivars. The actual plant breeding programmes aimed at obtaining high yielding cultivars have very narrowed the genetic resources for the development of new cultivars, reducing progressively the possibilities of further significant improvements.

The endosperm storage proteins contain excellent markers for the genetic variability in gluten properties in durum and common wheat. Two main protein groups, gliadins and glutenins, can be distinguished according to their molecular characteristics (Payne 1987). Glutenins can be further subdivided into high-molecular-weight (HMWGs) and low-molecular-weight (LMWGs) subunits (Singh & Shepherd 1988; Pogna *et al.* 1990). Among these proteins, the best studied are the HMWGs, coded at the *Glu-1* loci on the long arm of the group-1 homoeologous chromosomes (Payne 1987), while the LMWGs are coded at the *Glu-3* loci on the short arm of the same chromosomes.

During the domestication process, only a part of the variability, present in the wild relatives, was introduced into cultivated wheats. Their wild relatives could be, therefore, a rich source of genes controlling glutenin properties (Feldman & Sears 1981; Jauhar 1993). Other possible sources are traditional wheat landraces and neglected wheat species, that have been cultivated in the past.

Such are two subspecies of the tetraploid *Triticum turgidum*: ssp. *carthlicum* Nevski em. A. Löve et D. Löve, commonly known as "Persian wheat" and ssp. *polonicum* L. em. Thell. commonly known as "Polish wheat". Both have been cultivated in earlier times in Spain, but now, most of their genetic resources survive only conserved in Germplasm Banks, with the risk of loosing germinating power due to the scarce demand for these wheats during the 20th Century (Hammer 2003).

The main goal of the current study was to evaluate the variability of the HMWGs in two collections of „Persian" and „Polish" wheat of worldwide origin and to examine, if this variability can be used to widen the genetic variability in durum wheat.

MATERIAL AND METHODS

Plant material

The analysed collection consisted of 140 *carthlicum* and 159 *polonicum* accessions from several Genebanks. Care was taken to avoid duplications. We used 83 *carthlicum* and 62 *polonicum* accessions from the National Small Grain Collection at Aberdeen, USA, 34 *carthlicum* and 67 *polonicum* accessions from the Gatersleben Genebank in Germany, 13 *carthlicum* and 13 *polonicum* accessions from the RICP Genebank at Prague, Czech Republic, and 10 *carthlicum* and 17 *polonicum* accessions from the Centre for Genetic Resources at Wageningen, Netherlands. The accessions were of worldwide origin and not associated with particular growing regions. Based on available passport data, the *polonicum* and *carthlicum* accessions could be separated by origin into 33 and 18 groups, respectively.

Protein extraction and electrophoretic analysis

Proteins were extracted from a single crushed seed per accession according to the protocol described by Alvarez *et al.* (2001). Reduced and alkylated glutenin subunits were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of T: 8% (C: 1.28%) with and without 4M urea. The Tris-HCl/glycine buffer system of Laemmli (1970) was used. Electrophoresis was performed

Table 1. Allelic frequencies for the *Glu-A1* and *Glu-B1* loci among the evaluated *carthlicum* and *polonicum* accessions

Locus	Allele	Subunits	*Carthlicum* accessions		*Polonicum* accessions	
			N	%	N	%
	a	1	–	–	5	3.2
	b	2*	–	–	1	0.6
	c	*null*	137	97.9	119	74.8
Glu-A1	VIII	VIII	2	1.4	10	6.3
	IX	IX	1	0.7	–	–
	X	X	–	–	23	14.5
	XI	XI	–	–	1	0.6
	a	7	2	1.4	70	44.0
	b	7+8	134	95.7	11	6.9
	d	6+8	3	2.2	13	8.1
	an	6	–	–	2	1.3
	am	18	–	–	3	1.9
Glu-B1	XIX	7+8'	1	0.7	–	–
	XX	13**+8	–	–	2	1.3
	XXI	17*+18*	–	–	9	5.7
	XXII	20*	–	–	47	29.6
	XXIII	7+20y'	–	–	1	0.6
	XXIV	20x'+17'	–	–	1	0.6

at 30 mA/gel at 18°C for 45 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. Tap water was used for de-staining.

Nomenclature

The nomenclature of known glutenin alleles followed the recommendations of McIntosh *et al.* (1998). Novel alleles were given Roman numerals to avoid confusion for the inclusion in the Wheat Gene Catalogue.

Statistical analysis

Allelic frequency at the *Glu-A1* and *Glu-B1* loci was calculated for each subspecies within and between accession groups. The allele frequency was classified according to Marshall and Brown (1975) as frequent, rare and very rare, respectively, at frequencies of ≥ 5%, ≤ 5%, and ≤ 1%, respectively.

The total genetic diversity within each subspecies (*Ht*) and the average genetic diversity within (*Hs*) and between (*Dst*) the accession groups were calculated according to Nei (1973). The relative magnitude of genetic differentiation between accession groups (*Gst)* was estimated as *Dst*/*Ht*.

RESULTS

The HMWGs composition, together with the frequency of each allele for each subspecies, is given in Table 1. A representative sample of the variability detected for the HMWGs in *carthlicum* and *polonicum* accessions is shown in Figures 1 and 2, respectively. Some tetraploid and hexaploid wheat lines were included to compare and classify the detected subunits in both analysed subspecies. Eighteen allelic variants (seven at the *Glu-A1* locus and eleven at the *Glu-B1* locus) were detected in the evaluated accessions (Table 1). Ten of the variants were novel (McIntosh *et al.* 1998), 4 at the *Glu-A1* locus and 6 at the *Glu-B1* locus. The new subunits have been named according to their proximity to other previously described subunits and asterisks or quotation marks were added, to indicate this difference. The distribution of these alleles in both subspecies was unequal, so for the *Glu-A1* locus, the new *Glu-A1-VIII* allele (subunit VIII; Figure 1, lane 8) was found in both subspecies, being rare in *carthlicum* and frequent in *polonicum* accessions. The *Glu-A1-X* allele (subunit X; Figure 2, lane 8) was frequent in *polonicum* accessions, but the *Glu-A1-XI* allele (subunit XI; Figure 2, lane 14) was very rare. It occurred only in one accession (Table 1). For the *Glu-B1* locus, the *Glu-B1a* allele (subunit 7; Figure 1, lane 4, and Figure 2, lane 2) was classified as rare in *carthlicum accessions* and

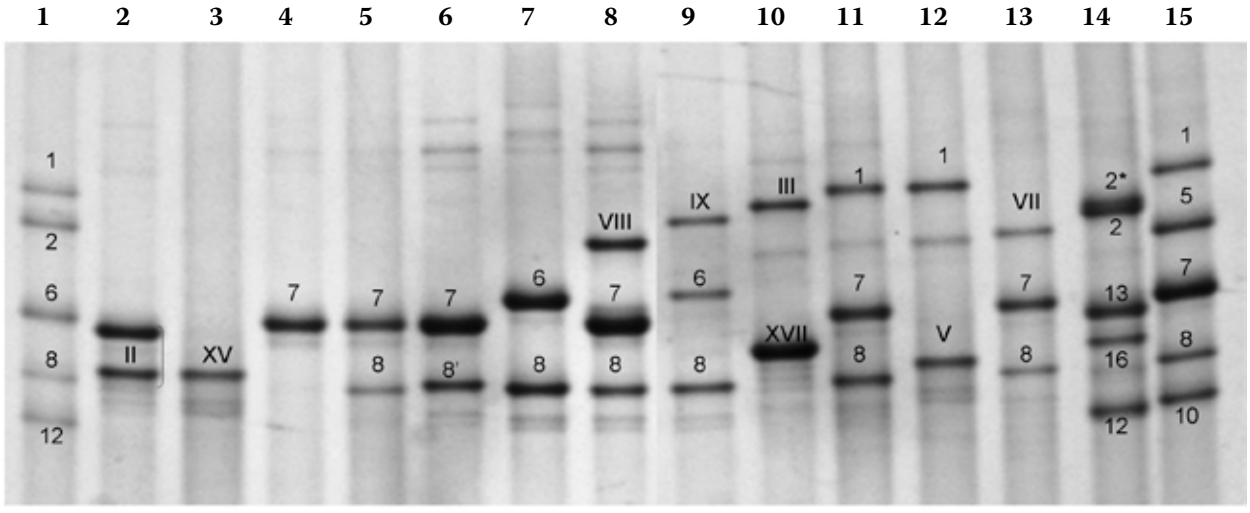

Figure 1. SDS-PAGE of some allelic variants of HMW glutenin subunits from some *carthlicum* wheat accessions at the *Glu-A1* and *Glu-B1* loci; lanes as follow: 1 – bread wheat (cv. Lancota); 2 – emmer (DIC-001); 3 – emmer (DIC-003); 4 – PI-349041; 5 – PI-78812; 6 – PI-70738; 7 – Citr-7665; 8 – PI-168672; 9 – C0204376; 10 – emmer (DIC-004); 11 – emmer (DIC-005); 12 – emmer (DIC-018); 13 – emmer (DIC-022); 14 – bread wheat (cv. Opata); 15 – bread wheat (cv. Rinconada)

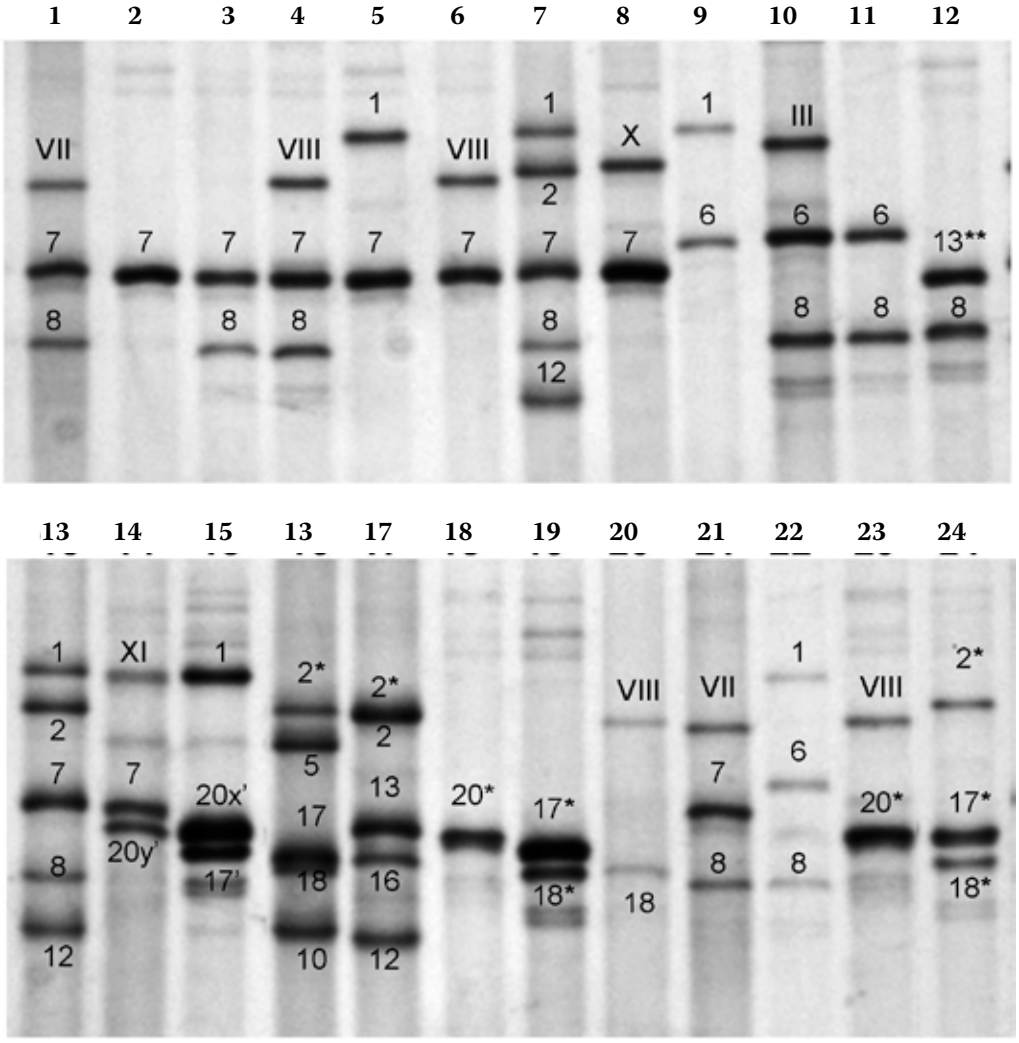

Figure 2. SDS-PAGE of some *polonicum* wheat accessions representative of the different allelic variants detected at the *Glu-A1* and *Glu-B1* loci; lanes are as follow: 1 – emmer (DIC-22); 2 – CItr-14139; 3 – PI-191810; 4 – CItr-14803; 5 – C0201267; 6 – PI-191881; 7 – bread wheat (cv. Pitic); 8 – PI-191903; 9 – PI-272570; 10 – emmer (DIC-20); 11 – CItr-13919; 12 – PI-191823; 13 – bread wheat (cv. Pitic); 14 – PI-352487; 15 – CGN13143; 16 – bread wheat (cv. Pavon); 17 – bread wheat (cv. Opata); 18 – CItr-14869; 19 – TRI2997; 20 – PI-272565; 21 – emmer (DIC-22); 22 – PI-286547; 23 – PI-387479; 24 – CGN12289

very frequent in *polonicum* accessions (Table 1). Another notable case is the new allele *Glu-B1-XXII* (subunit 20*, Figure 2, lane 18) that was frequent in *polonicum* accessions, but it was not detected among *carthlicum* accessions.

When both loci were taken into consideration, six and seventeen allelic combinations were found in the *carthlicum* and *polonicum* accessions, respectively. Their frequencies are shown in Table 2. In the *carthlicum* accessions the combination *Glu-A1c* and *Glu-B1b* (subunits *null* and 7+8, respectively), dominated, being present in 94.3% of the accessions; while the five remaining combinations were rare or very rare (Table 2). The two new alleles at the *Glu-A1* locus were found in accessions of different origin. The *Glu-A1-VIII* allele appeared in two accessions, one from Canada and another from China, while the *Glu-A1-IX* allele was found in one accession from the former Soviet Union. The *Glu-B1-XIX* allele was detected only in one accession from Iraq. Among the *polonicum* accessions, eleven allelic combinations were rare or very rare. In contrast, the new allele *Glu-B1-XXII* (subunit 20*) was frequent (in 47 accessions) and mostly in combination with *Glu-A1c* (subunits *null*, 20*). This combination or the combinations

Table 2. Frequencies of the HMW glutenin subunit compositions among the *carthlicum* and *polonicum* wheat accessions

Species	Composition	N	Accession standards
T. turgidum ssp. *carthlicum*	*null*, 7+8	132	PI-78812
	null, 6+8	2	CItr 7665, CGN6596
	null, 7+8'	1	PI-70738
	null, 7	2	PI-349041, TRI-9535
	VIII, 7+8	2	PI-168672, PI-532510
	IX, 6+8	1	CGN12284
T. turgidum ssp. *polonicum*	*null*, 7	44	PI-191852, TRI891, C0200962, CGN8388
	null, 7+8	8	PI-191810, TRI17453, CGN12292
	null, 6+8	12	PI-272564, TRI3248, CGN8386
	null, 13**+8	2	PI-191823, TRI15114
	null, 17*+18*	8	TRI2997, C0200088, CGN8385
	null, 20*	45	PI-134945, TRI642, C0201058, CGN8394
	1, 7	1	C0201267
	1, 6+8	1	PI-286547
	1, 6	2	PI-272570, CGN12291
	1, 20x'+17'	1	CGN13143
	2*, 17*+18*	1	CGN12289
	VIII, 7	2	PI-191881, PI-352488
	VIII, 7+8	3	CItr-14803, PI-191808, TRI18279
	VIII, 18	3	PI-272565, TRI17459, C0201091
	VIII, 20*	2	PI-387479, TRI15654
	X, 7	23	PI-191903, TRI768, C0201092, CGN10477
	XI, 7+20y'	1	PI-352487

Glu-A1c+Glu-B1a (subunits *null*, 7) and *Glu-A1-X+Glu-B1a* (subunits X, 7) were present in 70.4% of the evaluated *polonicum* accessions.

The genetic diversity at the *Glu-1* and *Glu-B1* loci in both subspecies is shown in Table 3. The total genetic diversity (*Ht*) of the *carthlicum* accessions was 0.140 for *Glu-A1* and 0.208 for *Glu-B1,* in average 0.174. Among the *polonicum* accessions *Ht* was higher: 0.451 for *Glu-A1* and 0.673 for *Glu-B1*, in average 0.562. When this diversity was split into diversity within (*Hs*) and between (*Dst*) origins (Nei 1973), then the diversity between origins, relative to the total (*Gst*), accounted for 69.0% of the *carthlicum* accessions and 71.6% of the *polonicum* accessions. This indicated, that in both subspecies the genetic diversity was much higher among accessions of different origin than among accessions of the same origin.

DISCUSSION

The bread making quality of durum wheat is generally considered poor, compared with bread wheat (reviewed by Boyacioglu & D'Appolonia 1994), although approximately one quarter of durum wheat is consumed in the form of bread (Quaglia 1988). This can be mainly ascribed to the presence of HMW glutenin subunits in bread wheat encoded at the *Glu-D1* locus (derived from *Aegilops tauschii* Coss.), which are obviously absent in durum wheats. The role of the other two loci (*Glu-A1* and *Glu-B1*) on bread-making

Table 3. Seed storage protein diversity within and between the *carthlicum* and *polonicum* wheat accessions on the basis of passport data

Species	Origins	Accessions	Locus	Alleles	*Ht*	*Hs*	*Dst*	*Gst* (%)
T. turgidum, ssp. *carthlicum*	18	128	*Glu-A1*	3	0.140	0.031	0.109	77.9
			Glu-B1	4	0.208	0.077	0.131	63.0
Mean					0.174	0.054	0.120	69.0
T. turgidum, ssp. *polonicum*	33	138	*Glu-A1*	5	0.451	0.108	0.344	76.2
			Glu-B1	10	0.673	0.212	0.461	68.5
Mean					0.562	0.160	0.403	71.6

Ht – total gene diversity; *Hs* – average gene diversity within populations; *Dst* – average gene diversity between populations; *Gst* – gene diversity between populations, relative to *Ht*

quality has been scarcely studied in durum wheat (Kovacs *et al.* 1993; Peña *et al.* 1994). However, the importance of these proteins for the baking quality of durum wheat could be undervalued due to a low variability at these loci in modern cultivars of durum wheat. Most cultivars contain only one or two HMW glutenin subunits encoded on chromosome 1B, mainly the subunits 6+8 or 7+8, since more than 80% of the cultivars have the *null* allele at the *Glu-A1* locus (Branlard *et al.* 1989). Consequently, a higher variability in these proteins could open new possibilities for the use of durum wheat in the baking industry.

Unfortunately, in the *carthlicum* and *polonicum* accessions, the *null* allele was the most frequent one (97.9% and 74.8%, respectively), which suggests a strong erosion of the genetic basis of these wheats. Especially in the *carthlicum* accessions, where 95.7% of the accessions carried also the allele *Glu-B1b* (subunits 7+8), while the remaining alleles were rare or very rare.

The evaluated accessions were of very diverse origin. Some of the accessions had no or imprecise passport data, complicating so the analysis of genetic diversity. Therefore, only accessions with available passport data were used for the analysis, i.e. 128 out of 140 *carthlicum* and 138 out of 159 *polonicum* accessions. Only in the case of *polonicum* accessions, one of the detected alleles (*Glu-A1b*, subunit 2*) was not found among the accessions with passport data. This allele appeared, together with the new allele *Glu-B1-XXI* (subunits 17*+18*), only in the accession CGN12289 from the CGR at Wageningen. In contrast to the mentioned low genetic diversity (*Ht*) among the *carthlicum* accessions, the variability among the *polonicum* accessions was high, with totally 16 allelic variants at the two loci. The observed low genetic diversity between accessions of same origin might be possibly connected with the practice of seed exchange between close farmers in the past. Such practice over several generations inevitably leads to lower genetic variability within a growing region and a differentiation between distant regions. A low frequency of the new alleles stresses the necessity of the protection and conservation of such accessions, since it is not very likely to find these alleles in other materials. A loss of these materials would result in the definitive loss of these alleles. Although the revival of these old crops in the new agricultural systems is possible, more can be expected from the newly found variation in these subspecies as new genetic resources of durum wheat for plant breeders.

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Corresponding author:

Dr. Juan B. Alvarez, University of Cordoba, Department of Genetics, School of Agricultural and Forestry Engineering, Gregor Mendel Building, Rabanales Campus, ES-14071 Cordoba, Spain
tel.: + 34 957 218 505, fax: + 34 957 218 503, e-mail: jb.alvarez@uco.es