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Variation in the high-molecular-weight glutenin subunits coded at the *Glu-H^{ch}1* locus in *Hordeum chilense*

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Abstract *Hordeum chilense* Roem. et Schult. is a native South American diploid wild barley included in the section *Anisolepis* Nevski. *H. chilense* occurs exclusively in Chile and Argentina and has been used in the synthesis of a new amphiploid named tritordeum (×*Tritordeum* Ascherson et Graebner). The HMW glutenin subunits of *H. chilense* have a great influence on gluten strength of tritordeum. The variability of these proteins has been analysed electrophoretically, and up to ten allelic variants have been detected in a world collection of this species. This genetic variability has been included in 121 lines of tritordeum and could be used for widening the genetic basis of tritordeum and wheat.

Keywords *Hordeum chilense* · Prolamins · SDS-PAGE · Genetic variability · Tritordeum

Introduction

Interspecific hybridisation with wild species is a useful tool in breeding *Triticeae* crops. A direct approach for exploiting wild species genetic variability is the synthesis of amphiploids or artificial polyploids by means of duplicating the chromosomes of hybrids with colchicine. Polyploidy is in actual fact a natural mechanism in the evolution of cereals, as in durum and bread wheats. Although there have been many attempts (Jauhar 1993), only the genome of rye (*Secale cereale* L.) has been successfully integrated with the wheat genomes to produce one man-made crop, triticale (×*Triticosecale* Wittmack), which has already proven useful in several regions (Varughese 1996). Recently, a new cereal, named tritordeum (×*Tritordeum* Ascherson et Graebner), has been synthesised by using a native South American diploid wild barley (*Hordeum chilense* Roem. et Schult.). This new crop has shown promising characteristics as a new man-made cereal (see Martín et al. 1999 for review).

The storage protein polypeptide composition of the endosperm of hexaploid tritordeums has been studied by SDS-PAGE (Alvarez et al. 1993) and A-PAGE (Alvarez et al. 1999a). At this level, the polypeptide composition of the amphiploids consisted of the addition of polypeptides from both parents. The storage prolamins synthesised by the **H^{ch}** genome, derived from *H. chilense*, influence the gluten strength of hexaploid tritordeum, mainly the high-molecular-weight (HMW) glutenin subunits coded at the *Glu-H^{ch}1* locus (Alvarez et al.1999a). This locus has been identified as being homeologous to the *Glu-1* loci of wheat and is located on long arm of the chromosome 1H^{ch} (Payne et al. 1987; Tercero et al. 1991).

The genetic basis of tritordeum has been expanded with the synthesis of new amphiploids using new accessions of *H. chilense*. Most of these accessions are derived from the natural populations collected in expeditions carried out by our group in Chile and Argentina (Tobes et al 1995; Gimenez et al 1997).

In the investigation reported here these new lines of *H. chilense*, which have been used as the maternal parents of 121 lines of primary tritordeum, were analysed. Our aim was to evaluate the variability of the HMW glutenin subunits coded at the *Glu-H^{ch}1* locus in *H. chilense* included in tritordeum.

Materials and methods

Seeds of 38 lines of *H. chilense* derived from natural populations collected in Chile between 29° 55’ and 41° 40’ latitude south were

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used in this study. These lines have been self-pollinated during two generations. Seeds of bread wheat, cvs. Chinese Spring (null, 7+8, 2+12) and Yecora (1, 17+18, 5+10), were used as references.

Proteins were extracted from crushed endosperm. Before glutenin solubilisation, the monomeric prolamins were extracted with a 1.5 *M* dimethylformamide aqueous solution followed by 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 m*M* TRIS-HCl pH 8.5, 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 divided into two aliquots of 100 µl and precipitated with 1 ml of cold-acetone. The dried pellet was solubilised in buffer containing 625 m*M* 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 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. Destaining was carried out with distilled water.

Results and discussion

The analysis of endosperm protein has proven to be a useful tool in the evaluation of variability in cereals and quality improvement in breeding programmes. We studied variation in the HMW glutenin subunits in 38 lines of *H. chilense* collected in Chile and representative of the distribution area of the species (Table 1). The H1, H11 and H12 accessions were obtained from germplasm banks where geographical data were unfortunately missing.

All of the prolamins (monomerics and polymerics) of *H. vulgare* L. are usually named hordeins. Consequently, some authors have applied the name hordeins to the prolamins of *H. chilense* (Payne et al. 1987; Tercero et al. 1991), even though no evidence has been found that identified these proteins to the prolamins of *H. vulgare*. In fact, a biochemical comparison between *H. chilense* and *H. vulgare* showed great differences between these species (Fernández et al. 1987). Some results, such as the similarity of the chromosome banding pattern after *in situ* hybridisation with probe pAs1 between *H. chilense* and *Aegilops tauschii* Coss. (Cabrera et al. 1995) or cytoplasm compatibility (Millán and Martín 1992) have suggested that the *H. chilense* genome could be more similar to wheat than to barley. On basis of these results, the H^{ch} prolamins observed after the extraction procedure used in this report have been considered to be glutenin-like proteins.

Table 1 Origin of 35 of the 38 lines of *H. chilense* used in this study

Population	Geographical locations			Accessions
	Latitude (S)	Longitude (W)	Altitude (m)	
PH671	29° 55’	71° 14’	150	H286
PH676	30° 32’	70° 41’	50	H293
PH677	30° 33’	71° 29’	150	H297
PH681	30° 37’	71° 14’	250	H309
PH682	30° 41’	71° 22’	150	H295
PH692	31° 47’	70° 35’	1150	H308
PH693	31° 48’	71° 21’	650	H304
PH695	31° 53’	71° 29’	25	H290
PH701	31° 54’	72° 22’	500	H17
PH702	31° 56’	71° 31’	0	H7
PH703	32° 15’	71° 32’	0	H203
PH708	32° 18’	71° 31’	1750	H16, H255
PH709	32° 25’	70° 55’	1100	H213
PH714	32° 58’	71° 10’	350	H205
PH715	33° 01’	70° 54’	750	H202
PH716	33° 00’	70° 57’	1200	H204
PH720	34° 04’	70° 56’	300	H8, H35, H217, H228
PH722	33° 06’	71° 28’	300	H209
PH728	33° 39’	70° 21’	1060	H210
PH729	34° 03’	71° 38’	200	H39
PH731	34° 45’	70° 34’	800	H59, H60, H200
PH732	34° 51’	70° 34’	1000	H55, H56
PH736	36° 45’	73° 09’	0	H51, H52
PH737	36° 45’	72° 18’	83	H47, H220
PH751	38° 41’	73° 24’	0	H252
PH752	41° 40’	73° 35’	0	H255

Novel HMW glutenin subunits

Previous data have shown that *H. chilense* has one HMW glutenin subunit coded at the *Glu-H^{ch}1* locus on chromosome $1H^{ch}$ (Payne et al 1987; Tercero et al 1991), that is related with gluten strength in hexaploid tritordeum (Alvarez et al. 1999a). Until now, only three allelic variants, H^{ch}a, H^{ch}b and H^{ch}c, have been identified, with subunit H^{ch}b being associated with good quality in tritordeum (Alvarez et al. 1999a). Our results revealed a wide range of variation for the *Glu-H^{ch}1* locus; in fact, although this locus presents only one gene, ten allelic variants were found using two types of gels. The relative frequencies of each allelic variant are given in Table 2. The SDS-PAGE electrophoregrams in normal and urea gels of all the subunits are presented in Fig. 1 A and 1B, respectively. The nomenclature of the HMW glutenin subunits of *H. chilense* has been changed following international recommendations (McIntosh et al 1998). The subunits H^{ch}a, H^{ch}b and H^{ch}c have been renamed 1^{Hch}, 2^{Hch} and 3^{Hch}, while the alleles have been named *Glu-H^{ch}1a*, *Glu-H^{ch}1b* and *Glu-H^{ch}1c*, respectively.

In the normal gel (Fig. 1 A), all the subunits found, except subunit 10^{Hch} (*Glu-H^{ch}1j*) present in line H210, showed faster mobility than subunit 1^{Hch} (*Glu-H^{ch}1a*). This last subunit was the most frequent among the evaluated lines (28.9%) and corresponded with the component

Table 2 Relative frequencies of alleles of the locus *Glu-H^{ch}1* amongst 38 lines of *H. chilense*

Alleles	Subunits	*H. chilense*		Tritordeums derived (n = 121)
		Percentage	Accessions	
Glu-H^{ch}1a	1^{Hch}	28.9	H1, H8, H12, H59, H60, H200, H204, H209, H213, H217, H225	46
Glu-H^{ch}1b	2^{Hch}	2.6	H11	2
Glu-H^{ch}1c	3^{Hch}	13.2	H7, H17, H39, H55, H56	26
Glu-H^{ch}1d	4^{Hch}	18.4	H16, H35, H202, H203, H228, H304, H308	17
Glu-H^{ch}1e	5^{Hch}	10.5	H47, H51, H52, H205	10
Glu-H^{ch}1f	6^{Hch}	7.9	H220, H286, H290	4
Glu-H^{ch}1g	7^{Hch}	5.3	H293, H295	4
Glu-H^{ch}1h	8^{Hch}	5.3	H297, H309	7
Glu-H^{ch}1i	9^{Hch}	5.3	H252, H255	4
Glu-H^{ch}1j	10^{Hch}	2.6	H210	1

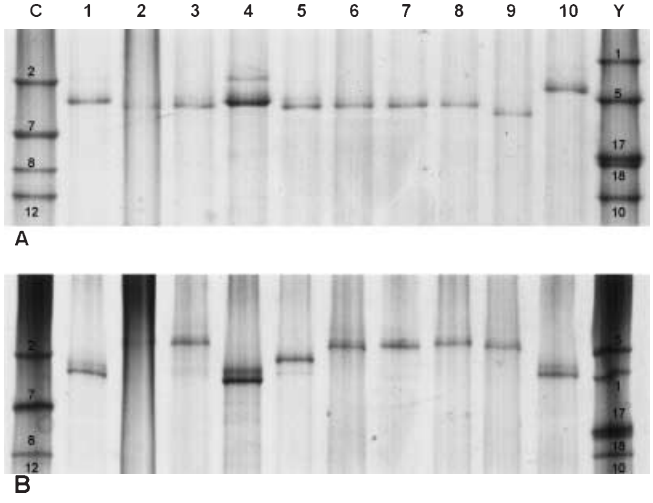

Fig. 1A, B SDS-PAGE separation of HMW glutenin subunits of *H. chilense* (lanes 1–10) on 8% polyacryamide gels (**A**) and 8% concentration gels containing 4 *M* urea (**B**). The HMW glutenin subunits of bread wheat cultivars Chinese Spring (*C*) and Yecora (Y), are numbered according to Payne and Lawrence (1983)

1 identified by Payne et al. (1987). Subunit 9^{Hch} (*Glu-H^{ch}1i*) showed the fastest mobility, being found in 2 lines (H252 and H255) collected in the extreme south of the distribution area (38° 41' - 41° 40' LS). For the subunits 3^{Hch} (*Glu-H^{ch}1c*), 5^{Hch} (*Glu-H^{ch}1e*), 6^{Hch} (*Glu-H^{ch}1f*), 7^{Hch} (*Glu-H^{ch}1g*) and 8^{Hch} (*Glu-H^{ch}1h*), slight differences in relative mobility between them were apparent. These differences were only detectable with gels of a low polyacrylamide concentration (T= 8%, C= 1.28%), which could explain why its presence was overlooked in previous investigations (Villegas 1998).

Some investigations have indicated that the HMW glutenin subunits may present anomalous mobility in SDS-PAGE gels, which could be eliminated by the addition of a strong denaturant to the gel, such as 4 *M* urea (Goldsbrough et al. 1989; Lafiandra et al. 1993). Since the former could result in real variability of the HMW glutenin subunits of *H. chilense* being under-evaluated, the same samples shown in Fig. 1 A were loaded in gels with 4 *M* urea. The results are shown in Fig. 1B. The mobility of the bands was notably changed, which enabled us to see the differences between the subunits more clearly.

Contrary to observations in previous works (Alvarez et al. 1999a), we did not find differences between subunits 2^{Hch} and 3^{Hch}. Only in the normal gel (Fig. 1 A) was a very slight difference in mobility detected. Nevertheless, because of the distinct differences shown in other investigations, we have to consider that the subunit 2^{Hch}, present only in H11, may be different from 3^{Hch}.

Because of the *H. chilense* lines evaluated in this work have been used in the synthesis of tritordeums, we studied the distribution of the allelic variants detected in the 121 lines of tritordeum derived. The frequencies of each subunit inside this collection of tritordeum appear in Table 2. Again, subunit 1^{Hch} was the most frequent, appearing in 46 of 121 lines, followed by subunit 3^{Hch} (26 of 121) and 4^{Hch} (17 of 121). Subunits 2^{Hch} and 10^{Hch} were the least frequent, being present in 2 and 1 lines of tritordeum, respectively.

Distribution of the HMW glutenin subunits inside the collected area

It is important to emphasise that all of the lines evaluated have been grown for several generations under self-pollination conditions. Consequently, only part of the variability of the original populations has been evaluated. In any case, when the lines were classified on the basis of geographic origin, it was observed that, overall, the allelic variants were randomly distributed inside the collection area. Subunits 1^{Hch} and 4^{Hch} showed the widest distribution, with subunit 1^{Hch} found in lines collected between 32° 18' and 34° 45' LS and subunit 4^{Hch} appearing between 31° 47' and 34° 04' LS. On the contrary, other subunits such as 3^{Hch}, 5^{Hch} and 6^{Hch} were found in several zones separated by large distances. Subunit 5^{Hch} appeared in two zones separated by more than 4000 km. Other subunits (7^{Hch}, 8^{Hch} and 10^{Hch}) appeared only in conspicuous zones. Subunit 8^{Hch} was only found in the north, inside the distribution area of subunit 7^{Hch} (30°

32’ and 30° 41’ LS), while the subunit 10^{Hch} appeared in only 1 population. Subunit 9^{Hch} was only found in the south inside 2 populations separated by 3300 km.

On the other hand, some of the lines evaluated derived from the same population but had different subunits. For example, inside the PH720 population, lines H8 and H217 presented subunit 1^{Hch} while the lines H35 and H228 had subunit 4^{Hch}.

Alvarez et al. (1999b), using the same lines of *H. chilense*, found a high degree of variation in the D-prolamins. This, together with the variation at the *Glu-$H^{ch}1$* locus detected in this report, suggests that *H. chilense* is a very polymorphic species at the level of endosperm storage proteins. Our knowledge of their effect on bread making quality is still slight, although some of these new allelic variants are at present being studied. Therefore, we believe that this species could contribute to widening the genetic basis with respect to the quality of bread and durum wheats because of **H^{ch}** genome promotes a similar effect on gluten strength as the **D** genome from *Ae. tauschii*. In this respect, tritordeum could also be used as a bridge species for the transfer of these useful traits to wheat, independent of the development of tritordeum as a new man-made crop.

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