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Allelic variation of the D-prolamin subunits encoded at the H^{ch} genome in a collection of primary hexaploid tritordeums

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Abstract Hexaploid tritordeum is the amphiploid derived from the cross between *Hordeum chilense* and durum wheat. The storage proteins synthesised by the H^{ch} genome have an influence on the gluten strength of this amphiploid. The D-prolamins of *H. chilense* are glutenin-like proteins. The variability has been analysed electrophoretically and up to 20 different patterns have been detected in a world collection of this species. This genetic variability of *H. chilense* could be a source of additional variation in tritordeum and in breeding wheat for quality.

Keys words *Hordeum chilense* · Prolamins · Tritordeum · SDS-PAGE · Genetic variability · Bread-making quality · Storage proteins

Introduction

Hexaploid tritordeum (× *Tritordeum* Ascherson et Graebner) is the amphiploid derived from the chromosome-doubling of the hybrid between *Hordeum chilense* Roem. et Schult. and durum wheat, *Triticum turgidum* conv. *durum* Desf. em. M.K. (Martín and Sanchez-Monge 1982). Some favourable agronomic traits such as biomass, number of spikelets/spike, seed size, or protein content, indicate the potential of this amphiploid as a possible new crop (Martín and Cubero 1981; Cubero et al. 1986; Martín et al. 1996).

Alvarez et al. (1992) tested some lines of hexaploid tritordeum, and found that its flour exhibited viscoelastic properties that were similar to those of medium-quality bread wheat. These results have been confirmed in later studies (Alvarez et al. 1994; Alvarez and Martín 1996). When the baking quality of hexaploid tritordeum was examined, it was found to be similar to that of bread wheat (Alvarez et al. 1995 a).

The polypeptide composition of the endosperm storage protein of hexaploid tritordeums has been studied by SDS-PAGE (Alvarez et al. 1993) and proved to involve the addition of polypeptides from both parents. The storage proteins synthesised by the H^{ch} genome have an influence on the bread-making quality of hexaploid tritordeum (Alvarez et al. 1995 b; Campos 1997).

In wheat, the glutenin fraction is usually separated by SDS-PAGE gels, which are devided into four zones: A, B, C and D. This last zone has been located between zone A (high-M_r glutenin subunits) and zone B. Some studies have suggested that, probably, the proteins present in this zone may be considered as contaminants and classified as ω-gliadins (Branlard et al. 1992; Khelifi and Branlard 1991). Payne et al. (1987), who worked with wheat-*H. chilense* addition lines, indicated that the endosperm proteins of *H. chilense* detected between the high-M_r and B-low-M_r prolamin subunits (D-zone) are prolamins encoded by chromosome $1H^{ch}$. However, these authors did not indicate whether these proteins should be considered as hordein-gliadins or glutenins.

The genetic basis of tritordeum has been expanded by the synthesis of new amphiploids using additional accessions of *H. chilense* collected in Chile and Argentina (Tobes et al. 1995; Gimenez et al. 1997). In the present report, lines of primary hexaploid tritordeum have been analysed by the electrophoretic separation of endosperm protein components, with the aim of establishing the variability level in the D-zone of the low-M_r

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glutenin subunits encoded by the H^{ch} genome in hexaploid tritordeum.

Materials and methods

Grain samples

Fifty one lines of primary hexaploid tritordeum, obtained from the chromosome-doubling of hybrids between 37 accessions of *H. chilense* Roem. et Schult. and 28 lines of durum wheat, were analysed (see Table 1). The genomic composition of all the tritordeum lines evaluated in this work was $H^{ch}H^{ch}$ AABB, where H^{ch} is the *H. chilense* genome.

Protein extraction

Embryo-less seeds crushed into a fine powder were used to extract the endosperm storage proteins. Before glutenin solubilization, the monomeric prolamins 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 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 were transferred to a new tube, mixed with 2.8 µl of 4-vinylpyridine, and incubated for 30 min at 60°C. Glutenin was 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, 4 M urea, and 2% (w/v) dithiothreitol in a 1 : 5 ratio (mg/µl).

SDS-PAGE separation

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 a polyacrylamide concentration of 10% (w/v, C = 1.28%) with 4 M urea. The Tris-HCl/glycine buffer system of Laemmli (1970) was employed. 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. De-staining was carried out with distilled water.

Results

In Fig. 1, the SDS-PAGE separation of the glutenin fraction of tritordeum reveals that the ω-gliadins synthesised by the A and B genomes are not found in durum wheat (lanes 2–4). Likewise, no band is detected at this position in *H. vulgare* (lane 5). Nevertheless, in hexaploid tritordeum (lane 1), some proteins synthesised by the H^{ch} genome were present in this region. Since these proteins remain after extraction with 1.5 M dimethylformadide and a double-wash with 50% propan-1-ol at 60°C, it is suggested that they represent glutenin subunits.

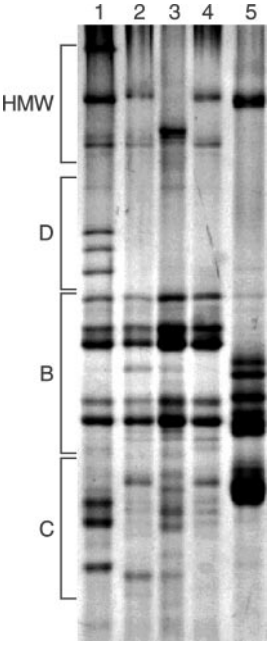

Fig. 1 One-dimensional separation of prolamins on SDS-PAGE with 4 M urea of hexaploid tritordeum (*1*), three lines of durum wheat (*2–4*), and *H. vulgare* (*5*)

Although Campos (1997) detected a certain degree of variation at this zone in hexaploid tritordeum, these bands cannot be clearly studied since they appear to be mixed with bands synthesised by the A and B genomes of tritordeum. By contrast, the method used for extraction protein in this work, together with the presence of urea in the sample and gel, has led to larger differences in mobility between the bands, which has allowed us to detect a higher degree of variability in the D-zone than with a SDS-PAGE gel without urea. In fact, 20 different patterns (a–t) were detected (Fig. 2). Most frequent were the b, d and f patterns, each one of them appearing in four accessions; but nine of the patterns were unique for a single *H. chilense* accession (Table 1). These patterns are made up by one, two or three bands, the pattern with two bands being the most frequent (55%). In some cases, as in patterns d and e (Fig. 2), one fast common band and one slow variable band are distinguishable. In pattern f, the slow overlaps with the fast band. Likewise, this fast band was detected in 11 of the 20 patterns. Other patterns appeared as a consequence of the gain or loss of bands, e.g. pattern b results from the gain of a fast band to pattern d (Fig. 2).

When the lines of tritordeum were classified on basis of the *H. chilense* accessions used in its synthesis, it was observed that 11 of these 20 patterns were present in two or more accessions of *H. chilense*. In two cases the patterns observed on different tritordeums synthesised with the same line of *H. chilense* have been different. Tritordeum lines derived from H39 present patterns d and p, and the tritordeum lines from H16 present patterns e and f. It is noteworthy that all these patterns display one fast common band and the variation was detected in the mobility of the slow band. Because the primary tritordeum lines are homozygotic for all

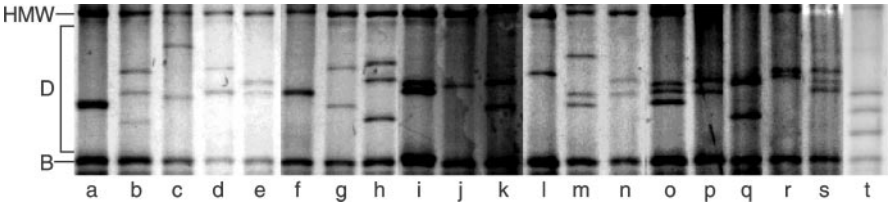

Fig. 2 SDS-PAGE patterns of D-prolamins in hexaploid tritordeum. The last band of the HMW zone and the first one of the B zone have been included for comparison

Table 1 D-prolamin subunit composition of the genotypes analysed

Genotypes	Parents		Pattern
	H. chilense	Durum wheat	
HT7	H12	cv 'Sacaba 81'-dwarf mutant	a
HT22	H1	cv 'Cocorit 71'	a
HT27	H7	cv 'Cocorit 71'	b
HT48	H11	CBDW 173-CIMMYT 86	c
HT51	H60	CBDW 60-CIMMYT 86	d
HT52	H7	cv 'Buck Candisur'	b
HT54	H13	CBDW 136-CIMMYT 86	a
HT60	H16	cv 'Sacaba 81'-dwarf mutant	e
HT61	H8	CBDW 181-CIMMYT 86	d
HT75	H61	CBDW 176-CIMMYT 86	f
HT78	H17	CBDW 173-CIMMYT 86	b
HT79	H61	CBDW 156-CIMMYT 86	f
HT85	H12	CBDW 176-CIMMYT 86	a
HT86	H8	CBDW 71-CIMMYT 86	d
HT94	H52	CBDW 82-CIMMYT 86	g
HT102	H57	CBDW 58-CIMMYT 86	d
HT114	H55	CBDW 73-CIMMYT 86	e
HT119	H61	CBDW 58-CIMMYT 86	f
HT122	H56	CBDW 82-ICARDA	g
HT126	H47	CBDW 206-CIMMYT 86	h
HT127	H35	CBDW 82-CIMMYT 86	f
HT129	H60	CBDW 73-CIMMYT 86	d
HT131	H39	cv 'Sacaba 81′-dwarf mutant	p
HT133	H16	CBDW 212-CIMMYT 86	f
HT137	H12	CBDW 211-CIMMYT 86	a
HT138	H47	CBDW 83-CIMMYT 86	h
HT141	H59	CBDW 212-CIMMYT 86	f
HT145	H39	CBDW 211-CIMMYT 86	d
HT146	H17	CBDW 206-CIMMYT 86	b
HT147	H51	CBDW 83-CIMMYT 86	i
HT160	H204	cv 'Aronas'	j
HT164	H220	cv 'Yavars 79'	k
HT165	H225	cv 'Cocorit 71'	l
HT171	H217	cv 'Yavaros 79'	m
HT178	H202	cv 'Lloyd'	n
HT181	H220	cv 'Mexicali 75'	k
HT182	H213	cv 'Aronas'	o
HT183	H209	cv 'Gediz 75'	p
HT187	H225	cv 'Chagual'	l
HT191	H228	cv 'Jori C69'	o
HT198	H203	cv 'Lloyd'	k
HT202	H255	cv 'Yavaros 79'	q
HT206	H205	cv 'Cocorit 71'	i
HT208	H204	cv 'Arcola'	j
HT224	H295	CBDW 73-CIMMYT 86	r
HT231	H304	cv 'Sacaba 81'-dwarf mutant	j
HT237	H308	CBDW 95-ICARDA	s
HT244	H297	cv 'Gediz 75'	b
HT256	H286	CBDW 95-ICARDA	b
HT257	H303	cv 'Cappelli'-dwarf mutant	n
HT288	H252	CBDW 181-CIMMYT 86	t

their genes, due to their origin (chromosome-doubling with colchicine), the results suggest that these two *H. chilense* lines, which were derived from natural populations, were polymorphic for these proteins.

Discussion

For bread-making quality traits, the results have indicated a pronounced influence of the prolamins synthesised at the H^{ch} genome on the quality of tritordeum, showing characteristics which up to now have only been found in *T. tauschii* (Alvarez et al. 1995 a, b). This genome was derived from the wild parent of tritordeum, *H. chilense*, which had been collected between 28° and 39° Latitude South in Chile (Tobes et al. 1995; Giménez et al. 1997).

Some authors have identified hordeins as the prolamins of *H. chilense* (Payne et al. 1987; Tercero et al. 1991); however, *H. chilense* presents only slight similarity with *H. vulgare* at the isoenzymatic and prolamin levels (Fernández et al. 1987). Some results, such as the similarity in chromosome banding pattern after in situ hybridization with probe pAsl between *H. chilense* and *T. tauschii* (Cabrera et al. 1995) or cytoplasm compatibility (Millán and Martín 1992), suggested that the *H. chilense* genome could be more similar to the D genome of wheat than that of barley. All these results, however, support the opinion of Löve (1982) who proposed, the division of the genus *Hordeum* into two: the genus *Hordeum* which includes *H. vulgare* (genome I) and the genus *Critesion* in which *H. chilense* (genome H) has been included (Dewey 1984). The H^{ch} proteins observed after the extraction procedure used in the present report must be considered as glutenin-like proteins, which could explain the effect of these proteins on gluten strength in tritordeum (Campos 1997). On the basis of these results, *H. chilense* could be used for the development of tritodeum both as a new crop and as a source of quality genes for wheat breeding.

Among the more frequent patterns, b, d and f, the patterns d and f were present in accessions collected in the same geographical zone (approximately 35° LS), while pattern b appeared in more septentrional accessions (30°LS). These results suggested that the variability of the prolamins in *H. chilense* is related to the geographical

distribution of this species, which is in accordance with the findings of Villegas (1998).

The results obtained by Campos (1997) suggested that the presence of the slow band of pattern c found in HT48 (Fig. 2) could be related with the good bread-making quality of hexaploid tritordeum. Nevertheless, the extraction protocol used in that work gave rise to a profile in which the c pattern was intermixed with proteins synthesised by the A and B genomes of hexaploid tritordeum. The present report, involving the use of new extraction procedure which deletes the ω-gliadins from the A and B genomes, has permitted a more accurate analysis of this zone.

Some glutenin subunits of wheat, mainly high-M_r glutenin subunits, show anomalous mobility on SDS-PAGE (Goldsbrough et al. 1989). The cause of this behaviour seems to be due to conformational differences between the proteins; this is because this anomalous mobility is eliminated by the addition of a strong denaturant (Goldsbrough et al. 1989), such as the 4 M urea used in the present work and which has been useful to detect a higher degree of variation.

At present, these proteins from *H. chilense* are being analysed with the same method used in this report for establishing the relationships between them (allelism or linkage) and their importance in the bread-making quality of hexaploid tritordeum.

The high variation detected for the D-prolamin subunits, together with the knowledge of their effect on bread-making quality, will provide a useful tool in breeding for quality and for the selection in early generations of lines which represent the best combinations of D-prolamins subunits.

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