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Linkage relationships between prolamin genes located on chromosome 1H^{ch} in *Hordeum chilense*

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Abstract The endosperm storage proteins of *Hordeum chilense* Roem. et Schult., a species used in the synthesis of the amphiploid tritordeum (×*Tritordeum* Ascherson et Graebner), have a great effect on the gluten strength of this amphiploid. We have analysed electrophoretically the heredity of these proteins, which are synthesised by genes located on chromosome 1H^{ch}, and detected up to five loci in a cross between two lines of *H. chilense*. These loci present a certain homology with loci synthesising the same proteins in wheat. The genetic distances between these loci were calculated.

Introduction

Hordeum chilense Roem. et Schult. (section *Anisolepis* Nevski) is a diploid wild barley species native to South America. It has been crossed with durum and bread wheat to obtain a new cereal, tritordeum (×*Tritordeum* Ascherson et Graebner), which has shown promising characteristics as a new man-made cereal (for review, Martín et al. 1999). Studies have indicated that this hexaploid tritordeum has some potential for bread making (Alvarez et al. 1995; Alvarez and Martín 1996), a trait that has been associated with the endosperm storage proteins synthesised by the H^{ch} genome (Alvarez et al. 1999a).

A high degree of variability has been detected for some endosperm storage proteins of *H. chilense*. Up to ten allelic variants of the gene coding for the high-molecular-weight (HMW) glutenin subunits synthesised by genes located in the Glu-H^{ch}1 locus (Alvarez et al. 2001) and 23 patterns of the D-low-molecular-weight (LMW) glutenin subunits were observed in a collection of lines used as the female parent of tritordeum (Alvarez et al. 1999b). Nevertheless, genetic data on these proteins are scarce, mainly due to the small size of the flower of *H. chilense*, which has made it difficult to perform artificial crosses between lines of this species. It has been suggested that most of the prolamins from *H. chilense* are synthesised by loci on chromosome 1H^{ch} (Payne et al. 1987; Tercero et al. 1991), although the same authors suggest that some α-gliadins are coded by loci on chromosomes 5H^{ch} and 7H^{ch}. Some of the loci described in these investigations are extremely complex, with a number of them comprising up to 11 different bands (Tercero et al. 1991).

We recently attempted to clarify this situation, although due to the absence of F_2 generations of *H. chilense*, we had to use advanced progenies from two crosses of hexaploid tritordeum (Alvarez et al. 1999a; Caballero et al. 2001). The data we obtained suggested the presence of several loci, although some of these were only identified by the presence of one band without an allelic alternative. Subsequent analysis of some of these proteins from the H^{ch} genome was difficult in tritordeum due to overlapping with proteins from the A and B genomes. The genetic distances between the loci detected were calculated despite the fact that this material was not optimal for such an analysis.

The aim of the investigation reported here was to determine the linkage relationships and genetic distances between the different prolamin genes. To accomplish this, we analysed the segregation of glutenin and gliadin genes located on chromosome 1H^{ch} in a cross between two lines of *H. chilense*.

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Materials and methods

Seeds of 95 F_2 plants from a cross between two lines of *Hordeum chilense* (H1 and H7), along with the parent lines, were analysed.

Because of the small size of the *H. chilense* grain (approx. 3 mg), about 80 mg of flour obtained from 25 crushed grains of each F_2 plant were used to extract the endosperm storage proteins. These proteins were extracted according to the sequential method described by Alvarez et al. (2001).

Gliadins were separated by acid-polyacrylamide gel electrophoresis (PAGE) at 8.5% (C: 2.67%) with low catalyst levels (ferrous sulfate and hydrogen peroxide) for increasing the gel firmness (Khan et al. 1985). Electrophoresis was performed at a constant current of 20 mA per gel at 18°C. Reduced and alkylated glutenins were analysed on vertical sodium dodecyl sulfate (SDS)-PAGE slabs in a discontinuous Tris-HCl-SDS buffer system (pH 6.8/8.8) at a 10% polyacrylamide concentration (w/v, C: 1.28%) both 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 per gel at 18°C until 30 min after the tracking dye had 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 tap water.

Recombination fractions were estimated by the method of maximum likelihood, and map distance (in centiMorgans) by the Kosambi function (Kosambi 1944).

Results and discussion

H. chilense is highly polymorphic at both the morphological and biochemical levels. Over 200 populations have been collected in their natural habitats (Chile and a small area in Argentina) and evaluated under field conditions in Cordoba (Spain) for several morphological, physiological and molecular characters. Two main groups have been identified, and accessions H1 and H7 are considered to be representative of these two groups (Vaz Patto et al. 2001). Lines H1 and H7 have been used for the synthesis of amphiploids and for the development of bread wheat addition lines (Miller et al. 1982). Both of these *H. chilense* lines (H1 and H7) show distinct differences with respect to their pattern of gliadins and glutenins and, therefore, are the appropriate material for a genetic analysis.

Gliadins and glutenins analysis

In previous investigations we have indicated the convenience of use the term gliadins for the monomeric prolamins found in *H. chilense* (Alvarez et al. 1999a) because of the high homoeology between the H^{ch} genome of this species and the D genome of *Aegilops tauschii* Coss. (Cabrera et al. 1995). In Fig. 1A, it is possible to observe that this *H. chilense* does not have proteins in the γ-gliadin zone according to wheat classification.

The gliadins were divided in three groups: ω-, β- and α-gliadins. Earlier results indicated that only the ω-gliadins are synthesised by genes located on chromosome $1H^{ch}$. For this group, up to five bands were detected, three from the H1 parent (ω1, ω2 and ω5) and two from the H7

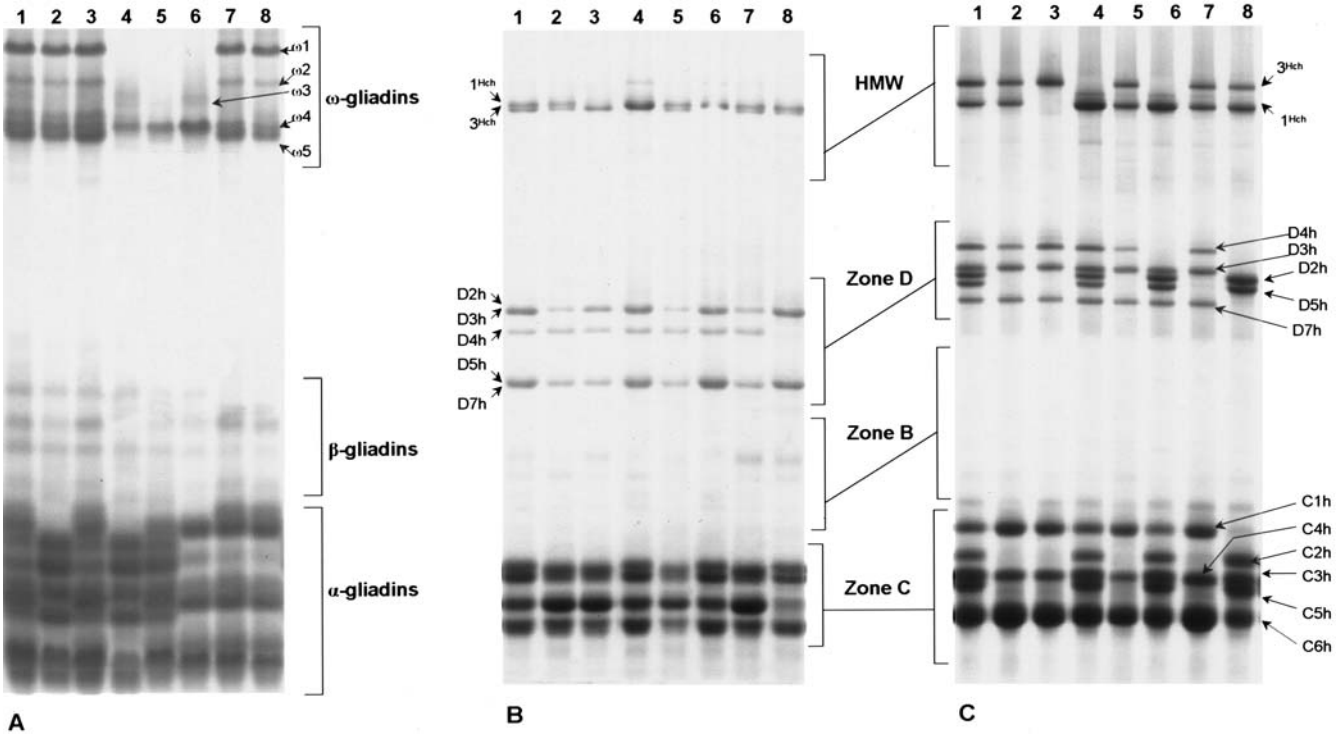

Fig. 1 **A** Acid-PAGE separation of the gliadin fraction of several lines (*lanes 1–8*) from the cross tested. **B**, **C** Separation of the HMW and D-LMW glutenin subunits of several lines (*lanes 1–8*) from the cross tested on SDS-PAGE gels without (**B**) and with (**C**) 4 *M* urea. The same lines are tested on each gel, with the same lane *number* indicating the same line on each gel

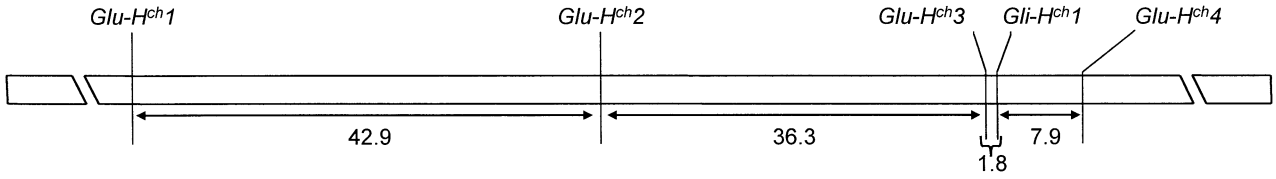

Fig. 2 Genetic map of chromosome $1H^{ch}$

Table 1 F_2 segregation data for progeny from the cross H1/H7 as deduced by electrophoretic analysis

Locus	Prolamin bands		Number of progeny				χ^2 (1:2:1)	χ^2 (3:1)	Conclusion
	A	B	A-	A B	-B	- -			
Glu-H^ch 1	1^{Hch}	3^{Hch}	19	48	28	-	1.79 ns	-	Allelism
Glu-H^ch 2	-	D4h	-	-	75	20	-	0.89 ns	Allelism
Glu-H^ch 3	D2h+D5h	D3h+D7h	20	49	26	-	0.92 ns	-	Allelism
Glu-H^ch 4	C1h+C4h	C2h+C3h+C5h	20	49	26	-	0.92 ns	-	Allelism
Gli-H^ch 1	ω1+ω2+ω5	ω3+ω4	23	45	27	-	0.60 ns	-	Allelism

ns not significant

parent (ω3 and ω4). The β-gliadins show a distinctive decrease in intensity compared to the other gliadins analysed.

SDS-PAGE separation of the glutenin fraction of several F_2 plants of the lines tested is shown in Fig 1B, C. In this species, the glutenins appeared in only three out of four zones described for wheat. No band was found in the B-LMW zone (Fig1B, C), which could be related to the absence of γ-gliadins in *H. chilense*. (Fig. 1A). This is in discrepancy with the findings of Atienza et al. (2002), who found bands in this zone using other methodology. Changes in the extraction method could be the cause of this: the method used in the present work is based on a sequential extraction method (Marchylo et al. 1989) and provides a glutenin preparation with very little contamination from other classes of seed proteins. In the light of our results, the bands detected in the B zone of *H. chilense* by other authors may be considered as contaminants.

The *H. chilense* lines used here were previously analysed for their HMW-glutenin subunit composition (Alvarez et al. 2001). Line H1 carries band 1^{Hch} (*Glu-H^ch 1a*), and line H7 carries band 3^{Hch} (*Glu-H^ch 1c*). Because the differences in mobility between these two bands is small when a normal gel is used, (Fig. 1B), we added urea to the gel. This resulted in band 3^{Hch} showing a lower mobility than band 1^{Hch}, which enabled us to clearly identify the homozygous and heterozygous genotypes (Fig. 1C).

This procedure was also useful for the D-LMW zone. On the normal gel, four of the five bands appeared to overlap two on two (Fig. 1B), whereas all five bands were unmistakably identifiable on the urea gel (Fig. 1C). Only two bands were detectable in line H1, while the other three appeared in the line H7. Caballero et al. (2001), who studied this zone in lines of hexaploid tritordeum derived from these two *H. chilense* lines, labelled these bands as D2h and D5h (H1) and D3h, D4h and D7h (H7) based on their position on the urea gel. The fast bands of this zone appeared in a position that could suggest inclusion in the B zone. However, the addition of urea produced such a change in their mobility that they appeared to be clearly separate from the slow bands of the B zone of the wheat control (Fig. 1C).

For the C-LMW zone, up to six bands were detected. These were named C1h (the slowest) to C6h (the fastest) (Fig. 2). Band 6 appeared in both parents, which suggests that it is probably a double band, although it has not been possible to resolve both components. For this reason it has been included in both parents. Bands C2h, C3h, C5h and C6h were detected in line H1, while bands C1h, C4h and C6h derived from the other parent (H7).

Inheritance of glutenin and gliadin genes

One locus was detected for the ω-gliadins (Table 1), which was named *Gli-H^ch 1* because of its homology with the *Gli-1* locus (ω- and γ-gliadins) of wheat. It included the genes coding for bands ω1+ω2+ω5 (*Gli-H^ch 1a*) and its alternative allelic, *Gli-H^ch 1b*, bands ω3+ω4. This locus was identified in our previous investigation, although due to the use of tritordeum and not *H. chilense* we only associated one band to this locus (Alvarez et al. 1999a).

Our analysis of the glutenin subunits confirmed that the HMW glutenin subunits detected in this cross were inherited according to a 1:2:1 segregation by that showing that the corresponding genes located on the *Glu-H^ch 1* locus were allelic (Table 1). Similar results were observed for the D-LMW glutenin subunits, where bands D3h+D7h were allelic to those of D2h+D5h. We found that the band D3h appeared in all samples containing band D7h, a correlation coefficient of 1 that is associated with absolute linkage, which suggests that this hypothesis would be correct. Similar results were found for subunits D2h and

Table 2 Linkage relationships between prolamins in chromosome $1H^{ch}$ in the cross studied

Prolamin subunits		χ^{2a}	R (%)	Distance (cM)
Locus 1	Locus 2			
Glu-H^{ch} 1	*Glu-H^{ch} 2*	18.9***	34.74	42.9
Glu-H^{ch} 2	*Glu-H^{ch} 3*	37.6***	31.05	36.3
Glu-H^{ch} 3	*Glu-H^{ch} 4*	47.6***	8.83	9.7
Gli-H^{ch} 1	*Glu-H^{ch} 3*	78.3***	2.13	1.8
Gli-H^{ch} 1	*Glu-H^{ch} 4*	46.3***	8.81	7.9

*** Significant at the 0.1% level
[a] For joint segregation

D5h. This complex locus has been named *Glu-H^{ch} 3*, and the alleles detected were named *Glu-H^{ch} 3a* (subunits D3h+D7h) and *Glu-H^{ch} 3b* (subunits D2h+D5h). Likewise, the results showed that the alternative allele to the D4h gene could be a *null* gene because the product of this gene did not appear on any of the gels used (Table 1). This conclusion agrees with our earlier findings on D-LMW glutenin subunit variability in *H. chilense* (Alvarez et al. 1999b), where we found that there are one to three bands in this zone. This new locus was named *Glu-H^{ch} 2*, and it has two alleles: *Glu-H^{ch} 2a* (subunit D4h) and *Glu-H^{ch} 2b* (null).

Two allelic variants of the one new locus, named *Glu-H^{ch} 4*, were detected for the C-LMW glutenin subunits. The first one includes bands C2h+C3h+C5h (*Glu-H^{ch} 4a*), while the second includes bands C1h+C4h (*Glu-H^{ch} 4b*). Band C6h has not been analysed due to the absence of segregation—it is present in both parents and in all F_2 lines.

Table 2 shows the linkage relationships between all loci found on the chromosome $1H^{ch}$. Four were located on the short arm of this chromosome, while the *Glu-H^{ch} 1* locus is on the long arm (Payne et al. 1987). Cabrera et al. (2002) showed that this locus is homoeologous of the *Glu-1* loci of wheat and appears in a similar physical position (0.6±0.01 fraction lengths) with respect to the centromere on the long arm of chromosome $1H^{ch}$. It is important to emphasise the great proximity (1.8 cM) between the *Gli-H^{ch} 1* and *Glu-H^{ch} 3* loci, which could be homologous to the wheat *Gli-1* and *Glu-3* loci. Both loci from *H. chilense* have been implicated in the characteristic of gluten strength in tritordeum (Alvarez et al. 1999a). On the other hand, the presence of proteins with a homologous sequence to the γ-gliadins in *H. chilense* (Barro et al., unpublished results) suggests that this species has γ-gliadins that are larger than those of wheat that could appear in the ω-gliadin zone. Nevertheless, because classification of these proteins has been performed on the basis of the wheat proteins, we prefer the names ω-gliadins and D-LMWGs, even though these proteins are probably homoeologous to the γ-gliadins and B-LMWGs of wheat. A representation of the genetic map that indicates the positions of these loci on chromosome $1H^{ch}$ is shown in Fig. 2.

In conclusion, as many as five loci have been found for the prolamin genes on chromosome $1H^{ch}$ in *H. chilense*, one on the long arm and four on the short arm. Four appear among the glutenin fraction, one among the HMW glutenin subunits (*Glu-H^{ch} 1* locus), two among the D-LMW glutenin subunits (*Glu-H^{ch} 2* and *Glu-H^{ch} 3* loci) and one among the C-LMW glutenin subunits (*Glu-H^{ch} 4* locus). The other locus (*Gli-H^{ch} 1*) appears in the ω-gliadins.

Data suggest that *H. chilense* could be a useful source of valuable traits for wheat breeding. In addition to contributing to resistance to biotic and abiotic stresses, *H. chilense* could widen the genetic basis for the quality traits of bread and durum wheats because the H^{ch} genome promotes a similar effect on gluten strength as the D genome of *Ae. tauschii*. In this respect, tritordeum could also be used as bridge species for the transfer of these useful traits to wheat, independently of the development of tritordeum as a new man-made crop.

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References

Alvarez JB, Martín LM (1996) Breadmaking quality in tritordeum: the use-possibilities of a new cereal. In: Güedes-Pinto H, Darvey N, Carnide VP (eds) Triticale: today and tomorrow. Kluwer, Dordrecht, pp 799–805

Alvarez JB, Martín LM, Rogers WJ, Martín A (1995) Relationship between the endosperm storage proteins composition and the breadmaking quality in hexaploid tritordeum. Cereal Res Commun 23:167–172

Alvarez JB, Campos LAC, Martín A, Sillero JA, Martín LM (1999a) Genetic analysis of prolamins synthesised by the H^{ch} genome and their effects on gluten strength in hexaploid tritordeum. Euphytica 107:177–184

Alvarez JB, Martín A, Martín LM (1999b) Allelic variation of the D-prolamin subunits encoded at the H^{ch} genome in a collection of primary hexaploid tritordeums. Theor Appl Genet 99:296-299

Alvarez JB, Martín A, Martín LM (2001) Variation in the high molecular weight glutenin subunits coded at the *Glu-H^{ch}1* locus in *Hordeum chilense*. Theor Appl Genet 102:134–137

Atienza SG, Alvarez JB, Villegas AM, Giménez MJ, Ramírez MC, Martín A, Martín LM (2002) Variation for the low-molecular-weight glutenin subunits in a collection of *Hordeum chilense*. Euphytica 128:269–277

Caballero L, Alvarez JB, Martín LM (2001) Analysis of D-prolamins synthesized by the *Hordeum chilense* genome and their effects on gluten strength in hexaploid tritordeum. Plant Breed 120:185–187

Cabrera A, Friebe B, Jiang J, Gill BS (1995) Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. Genome 38:435–442

Cabrera A, Martín A, Barro F (2002) In situ comparative mapping (ISCM) of *Glu-1* loci in *Triticum* and *Hordeum*. Chromosome Res 10:49–54

Khan K, Hamada AS, Patek J (1985) Polyacrylamide gel electrophoresis for wheat variety identification: effect of variables on gel properties. Cereal Chem 62:310–313

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Marchylo BA, Kruger JE, Hatcher DW (1989) Quantitative reversed-phase high-performance liquid chromatography analysis off wheat storage proteins as a potential quality prediction tool. J Cereal Sci 9:113–130

Martín A, Alvarez JB, Martín LM, Barro F, Ballesteros J (1999) The development of tritordeum: a novel cereal for food processing. J Cereal Sci 30:85–95

Miller TE, Reader SM, Chapman V (1982) The addition of *Hordeum chilense* chromosomes to wheat. In: Broetjes C (ed) Induced variability in plant breeding. EUCARPIA Int Symp. PUDOC, Wageningen, pp 79–81

Payne PI, Holt LM, Reader SM, Miller TE (1987) Chromosomal location of genes coding for endosperm proteins of *Hordeum chilense*, determined by two-dimensional electrophoresis of wheat-*H. chilense* chromosome addition lines. Biochem Genet 25:53-65

Tercero JA, Bernardo A, Jouve N (1991) Encoding genes for endosperm proteins in *Hordeum chilense*. Theor Appl Genet 81:127-132

Vaz Patto MC, Aardse A, Buntjer J, Rubiales D, Martín A, Niks RE (2001) Morphology and AFLP markers suggest three *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. Can J Bot 79:204–213