Characterization of glutenin genes in cereals and their contribution to the gluten properties

Jouve N., Soler C., Rubio P., Bernardo A., Cuadrado A., Peña E., De Bustos A.

in

Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

Zaragoza : CIHEAM / IRTA
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81

2008
pages 271-275

Characterization of glutenin genes in cereals and their contribution to the gluten properties[1]

N. Jouve*, C. Soler, P. Rubio*, A. Bernardo*, A. Cuadrado*, E. Peña* and A. De Bustos***
*Department of Cell Biology and Genetics, University of Alcalá, Campus, 28871,
Alcalá de Henares (Madrid), Spain
**Department of Biotechnology, INIA La Canaleja (Apd. 1045)
28800 Alcalá de Henares (Madrid), Spain
E-mail: nicolas.jouve@uah.es

SUMMARY – It is analyzed the primary structure of some of the high molecular weight (HMW) glutenin genes and their degree of conservation in the genera *Triticum*, *Secale* and *Aegilops*. The primary structure presented information on the allelic composition and the quantities of the endosperm protein fractions (HMW, LMW and gliadins) in the doughs of different common wheat cultivars and lines in process of selection. Dough W is correlated with the quantity of HMW subunits present in the gluten and in particular with the quantity of type x HMW subunits. The gliadins and the LMW subunits appear to act as a "solvent" which modifies the rheological properties of the dough by interfering with the polymerization of the HMW subunits, or by altering the relative amounts of the different types of subunits available. The contribution of each type of subunit in the organization of the intermolecular links, the formation of the multiproteic aggregates, and the properties of the gluten, is discussed.

Glutenins are the major storage protein fraction in the endosperm of common wheat, *Triticum aestivum* L*,* and its related species. In wheat, glutenins are composed of two types of subunits, one of high molecular weight (HMW) the other of low molecular weight (LMW), coded for by *Glu-1* and *Glu-3* genes, respectively. Each *Glu-1* locus contains two paralogous tightly linked genes (x and y) coding for protein subunits of different molecular weight. Comprehensive studies have been performed to characterise the HMW glutenin subunits and their influence in flour quality and rheological properties (Payne, 1987; Shewry *et al*., 2001). Different authors undertook the molecular characterisation of the *Glu-1* loci of wheat (Forde *et al*., 1985, Thompson *et al*., 1985, Halford *et al*., 1992, Anderson and Greene 1989, De Bustos *et al*., 2000) and rye (De Bustos *et al*., 2001a, 2003).

The present communication reports: (i) the allelic composition of the proteins of the gluten and the analysis of the correlation of their absolute and relative quantities with the main parameters determining the rheological properties; (ii) the analysis of the HMW glutenin genes of *Secale cereale* (2n=14; genome R) and the diploid species *Aegilops comosa* (2n=14; genome M), *Ae. uniaristata* (2n=14; genome Un) and *Ae. speltoides* (2n=14; genome S); and (iii) the study of the phylogenetic relationships between the genes coding HMW glutenin subunits of the *Triticeae* including the orthologous sequences of *Secale* and *Aegilops,* herein described.

Types of alleles, quantities of the HMW and LMW glutenin proteins and their relationships with dough rheological properties

The rheological properties of the common wheat cultivars, their allelic composition with respect to HMW and LMW glutenin subunits, and the quantities of the total and individual HMW and LMW protein fractions examined by RP-HPLC were previously reported (Peña *et al*., 2005). The plant material was grown in a randomised complete block design with three replications on the experimental field of "La Canaleja", of the I.N.I.A. at Alcalá de Henares (Madrid). The grains were milled using the mill of Chopin. The rheological properties (strength: W; tenacity: P; extensibility: L and the ratio P/L) were determined following the method of Faridi and Rasper (1987) using the

[1] Financial support of this work grant No. AGL2003-08128-C02. A. de Bustos is supported by the *Ramón y Cajal* Programme of the MCYT.

alveograph of Chopin. The method of Melas *et al*. (1994) was used to obtain the HMW glutenins. RP-HPLC was used to quantify the different protein fractions in each type of flour, and to establish the alleles of the main genes involved in glutenin synthesis.

The glutenins are the most influential components of strength (W) and the ratio tenacity/extensibility (P/L), especially the high molecular weight (HMW) glutenins and in particular the type x form. The gliadins and the LMW glutenin subunits appear to act as a "solvent" which modifies the rheological properties of the dough by either interfering with the polymerization of the HMW glutenin subunits, or by altering the relative amounts of the different types of glutenin subunits available. HMW glutenin subunits are tyrosine-rich proteins, and these amino acids might mediate in the formation of covalent unions that influence the special conformation of the gluten network. The role of disulfide bonds formed between cysteine residues and crosslinks between tyrosines is a matter of discussion (Peña *et al*., 2006). RP-HPLC was used to determine the quantity of dityrosine and isodityrosine derivatives produced under different treatments during gluten formation. The addition of oxidizing agents ($KBrO_3$) to the hydrated flour stimulated the formation of crosslinks between the tyrosines. Our results indicate that, although there is a significant increase in the formation of dityrosine derivatives, this does not influence the rheological properties (W and P/L) of the gluten, which seem to be more dependent on the quantity and types of proteins present. Thus, the number of crosslinks between tyrosine appears to be small and of little importance in the structure of the gluten network compared to the disulfide bonds that form between cysteine residues.

Identification of new HMW glutenin genes in rye and diploid species of the genus *Aegilops*

In view of the results on the influence of the glutenins in the flour behavior, we were interested in knowing the characteristics of these proteins in the related species, which could be used as potential donors in future experiments of transformation. The analysis of sequences flanking the coding region of the genes *Glu-A1* and *Glu-D1*, permitted to design primers for the heterologous amplification of the orthologous genes in related species, as described by De Bustos *et al*. (2000 and 2001a) and De Bustos and Jouve (2003).

We used the primers with template DNA from the following lines and wild materials of *Triticum, Secale* and *Aegilops*: a selected line of common wheat, 'S-149', having the 1R(1D) chromosome substitution, the 1R addition line of 'Imperial' rye into *T. aestivum* 'Chinese Spring', the rye cultivars 'Smith', 'Petkus' and 'Imperial' and the wild subspecies *ancestrale, afghanicum, dighoricum* and *segetale* of *Secale cereale,* and different accessions of *Ae. comosa*, *Ae. uniaristata* and *Ae. speltoides*. Wild material was kindly supplied by Dr. A. Börner of the Germplasm Bank, at Gatersleben (Germany).

The primers designed amplified two products in wheat lines carrying the chromosome 1R, *Secale cereale* and its wild relatives, *Ae. uniaristata* and *Ae. comosa*, but only one in *Ae. speltoides.* Subcloning and sequencing of the amplification products was performed as described by De Bustos *et al*. (2001b). The coding sequences of x-type genes ranged from 2211 bp to 2439 bp and for the y-type from 1854 bp to 2212 bp (Table 1). For *Ae. speltoides*, only the y-type was identified and characterized. The homology of the new genes to subunits encoding HMW glutenins in wheat was confirmed by shoutern blotting and sequencing. The new glutenin genes were named *Glu-R1x, Glu-R1y* (*Secale cereale*)*, Glu-Mx, Glu-My* (*Aegilops comosa*)*, Glu-Unx, Glu-Uny* (*Ae. Uniaristata*) and *Glu-Sy* (*Ae. speltoides*). The complete nucleotide sequence of the coding region of each subunit was deposited in the EMBL GeneBank and DDBJ (last colum Table 1).

The complete nucleotide sequence of the amplification products of rye and *Aegilops* species, showed the same general structure as the HMW glutenin genes of wheat. All showed the 'TATA' box 89-91 bp upstream of the ATG starting codon and lacked introns. All have a signal peptide of 21 residues almost identical to that of wheat and rye glutenins (De Bustos and Jouve 2003). The coding sequence of the mature polypeptide is similar to that of other HMW glutenins of wheat in that it can be divided into a number of distinct segments on the basis of amino-acid composition. The size agreed with the migration pattern of the proteins in SDS-PAGE (see details in Table 1). The central repetitive region of the HMW glutenin subunits was composed of the same basic repeat motifs described for all the other glutenin genes (Halford *et al*., 1992).

The amino acid composition of the proteins is quite similar to that of the wheat glutenins, especially with respect to the number and position of cysteine residues, to which dough quality has been related (Anderson and Greene 1989). The glutenin genes of the *Aegilops* species characterized in this study would be of little interest in improvement programs compared to those of other, previously characterized species (De Bustos and Jouve, 2003). However, as the SDS-PAGE results showed, more protein were comparatively extracted from the endosperm of the *Aegilops* seeds than from wheat seeds, which could have importance if final properties of wheat flour are influenced by the quantity of gluten proteins available, especially glutenins and gliadins (Peña *et al*., 2005).

Table 1. Size of coding sequence, putative proteins and accession numbers of the HMW subunits characterized

Species	Gene type	New genes	Size of coding sequence (bp)	Size of putative protein (residues)	GenBank accession
T. aestivum 'S149' 1R(1D)	x	*Glu-R1x*	2304	745	AF216868
T. aestivum 'S149' 1R(1D)	y	*Glu-R1y*	2145	692	AF216869
T. aestivum add 1R	x	*Glu-R1x*	2433	811	AJ314784
T. aestivum add 1R	y	*Glu-R1y*	2160	720	AJ314785
S, cereale 'Smith'	x	*Glu-R1x*	2262	754	AJ314779
S, cereale 'Smith'	y	*Glu-R1y*	2121	707	AJ314767
S, cereale 'Petkus'	x	*Glu-R1x*	2262	754	AJ314778
S, cereale 'Petkus'	y	*Glu-R1y*	2139	713	AJ314780
S, cereale 'Imperial'	x	*Glu-R1x*	2343	781	AJ314782
S, cereale 'Imperial'	y	*Glu-R1y*	2139	713	AJ314781
S. ce. subsp. ancestrale	x	*Glu-R1x*	2262	754	AJ314773
S. ce. subsp. ancestrale	y	*Glu-R1y*	2139	713	AJ314774
S. ce. subsp. segetale	x	*Glu-R1x*	2262	754	AJ314768
S. ce. subsp. segetale	y	*Glu-R1y*	2139	713	AJ314777
S. ce. subsp. dighoricum	x	*Glu-R1x*	2229	743	AJ314776
S. ce. subsp. dighoricum	y	*Glu-R1y*	2139	713	AJ314775
S. ce. subsp. afghanicum	x	*Glu-R1x*	2433	811	AJ314769
S. ce. subsp. afghanicum	y	*Glu-R1y*	2211	737	AJ314770
Ae. comosa	x	*Glu-Mx*	2439	813	AY455789
Ae. comosa	y	*Glu-My*	1854	618	AY455788
Ae. speltoides	y	*Glu-Sy*	2112	704	AF513640
Ae. uniaristata	x	*Glu-Unx*	2406	802	AY455786
Ae. uniaristata	y	*Glu-Uny*	1917	639	AY455787

Phylogenetic relationships between the orthologous genes of the HMW glutenin subunits in the *Triticeae*

To perform the analysis of phylogenetic relationships trees were constructed using both the distance and parsimony methods of the PHYLIP (Phylogeny Inference Package) Version 3.6 (Felsenstein, 2004) (Fig. 1). The protein sequence of a HMW hordein of *H. vulgare* was used as outgroup. Thirty nine HMW glutenin sequences (18 y-type and 21 x-type) belonging to the species of the genera *Aegilops, Triticum* and *Secale* were used in the phylogenetic analysis of this important group of genes, were distance or parsimony methods found similar trees. The deepest dichotomy is

between x- and y-types of HMW glutenin genes. Overall, both subunits grouped the taxa in the same way. The x-type and y-type sequences of the genus *Secale* fell into subgroups within separate clades, whereas the sequences of *Triticum* and *Aegilops* appeared in a common group. The trees were supported in both analyses by high bootstrap values. Moreover, more similitude was found between y-type sequences of *Triticum* and *Aegilops* than those of x-type in the distance analysis.

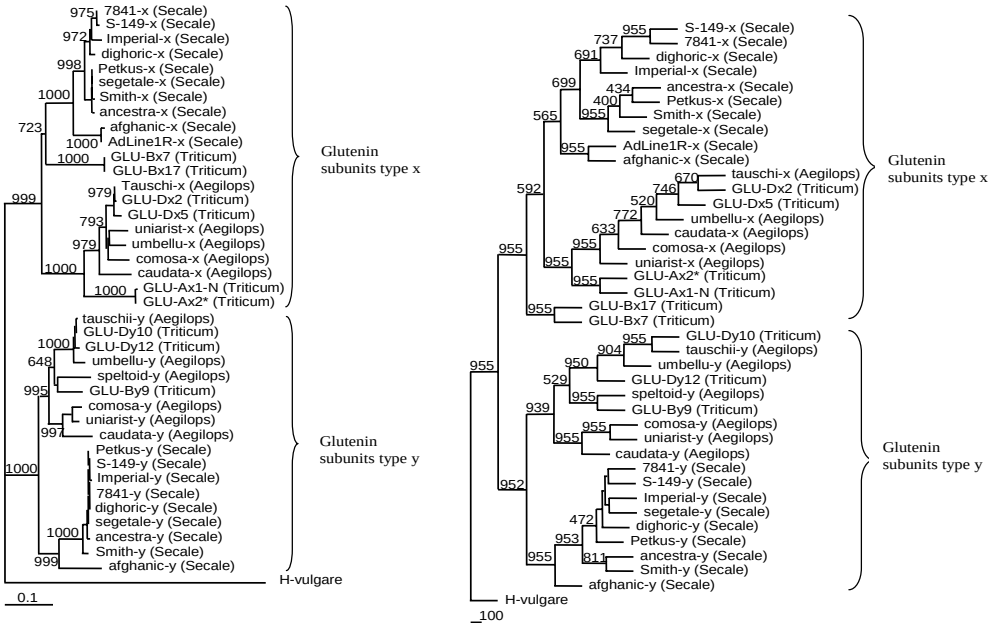

Fig. 1. Phylogenetic relationships between species of the Tribe Triticeae belonging to the genera *Aegilops*, *Triticum* and *Secale* performed using distance (left) and parsimony (right) methods. *Hordeum vulgare* was taken as outgroup. Bootstrap values are also indicated.

Acknowledgements

The authors would like to thank the MCYT (*Ministerio de Ciencia y Tecnología*) of Spain for its financial support of this work (Grant No. AGL2003-08128-C02). Alfredo De Bustos is supported by the *Ramón y Cajal* Programme of the MCYT.

References

Anderson, O.D. and Greene, F.D (1989). The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. *Theor. Appl. Genet.,* 77: 689-700.

De Bustos, A. and Jouve, N. (2003). Characterization and analysis of new HMW-glutenin alleles encoded by *Glu-R1* locus of *Secale cereale. Theor. Appl. Genet.,* 107: 74-83.

De Bustos, A., Rubio, P. and Jouve, N. (2000). Molecular characterisation of the inactive allele of the gene Glu-A1 and the development of a set of AS-PCR markers for HMW glutenins of wheat. *Theor. Appl. Genet.,* 100: 1085-1094.

De Bustos, A., Rubio, P.,Soler, C., Garcia, P. and Jouve, N (2001a). Marker assisted selection to improve HMW-glutenins in wheat. *Euphytica*, 119: 69-73.

De Bustos, A., Rubio, P. and Jouve, N. (2001b). Characterisation of two gene subunits on the 1R chromosome of rye as orthologs of each the *Glu*-1 genes of hexaploid wheat. *Theor. Appl. Genet.,* 103: 733-742.

Faridi, H. and Rasper, V.F. (1987). The alveograph procedure. In American Association of Cereal Chemists (Ed.). *The Alveograph Handbook*. Inc. Minnesota, USA, pp. 17-22.

Felsenstein, J. (2004). *PHYLIP (Phylogeny Inference Package, version 3.6).* Distributed by the author. Department of Genetics, University of Washington, Seattle.

Forde, J., Malpica, J.M., Halford, N.G., Shewry, R.P., Anderson, O.D., Greene, F.D. and Miflin, B.J. (1985). The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1 A of wheat *(Triticum aestivum* L.). *Nucleic Acid Res.,* 13: 6817-6832.

Halford, N.G., Field, J.M., Blair, H., Urwin, P., Moore, K., Robert, L., Thompson, R., Flavell, R.B., Thatam, A.R. and Shewry, P.R. (1992). Analysis of the HMW glutenin subunit encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality. *Theor. Appl. Genet.,* 83: 373-378.

Melas, V., Morel, M.H., Autran, J.C. and Feillet, P. (1994). Simple and rapid method for purifying low molecular weight subunit of glutenin from wheat. *Cereal Chemistry,* 71: 234-237.

Payne, P.I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Ann. Rev. Plant Physiol.*, 38: 141-153.

Peña, E., Bernardo, A., Soler, C. and Jouve, N. (2005). Relationship between common wheat (*Triticum aestivum L.*) gluten proteins and dough rheological properties. *Euphytica,* 143: 169-177.

Peña, E., Bernardo, A., Soler, C. and Jouve, N. (2006). Do tyrosine crosslinks contribute to formation network in common wheat (*Triticum aestivum* L.) dough? *Journal Cereal Sciences* (in press).

Shewry,, P.R., Pompineau, Y., Lafiandra, D. and Belton, P. (2001). Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. *Trends Food Sci. Tech.,* 11: 433-441.

Thompson, R.D., Bartels, D., Harberd, N.P. and Flavell, R.B- (1985). Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW glutenin subunit. *Nucleic Acid Res.*, 13: 6833-6846.

Varghese, J.P., Struss, D. and Kazman, M.E. (1996). Rapid screening of selected European winter wheat varieties and segregating populations for the Glu-D1d allele using PCR. *Plant Breeding,* 115: 451-454.