Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2010 8(4), 1012-1023
ISSN: 1695-971-X
eISSN: 2171-9292

Allelic variation in HMW glutenins in Spanish wheat landraces and their relationship with bread quality

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Abstract

The allelic variation of high molecular weight glutenins as principal determinants of bread quality has been analyzed in 165 Spanish wheat (*Triticum aestivum* ssp. *vulgare* L.) landraces provided by the Plant Genetic Resources Centre. The identification by standard electrophoresis techniques has been supported by a new PCR screening method, allowing the identification of the 2•• glutenin subunit from the *Glu-A1* locus in some landraces. The relation of high molecular weight glutenins and bread quality has been evaluated by SDS-sedimentation tests and mixographs. A positive influence on quality has been found for the 2•• glutenin subunit from the *Glu-A1* locus, pairs 7 + 8 and 13 + 16 from the *Glu-B1* locus, and pair 5 + 10 from the *Glu-D1* locus. The presence of a wide range of values for quality traits in landraces with the same high molecular weight glutenin composition points to the possible influence of other prolamins such as the low molecular weight glutenins. Their influence on bread quality will be assessed in future studies. A complete description of the high molecular weight glutenin composition and quality values of all the landraces analyzed in this study is provided for use in wheat breeding programs.

Additional key words: mixograph; sedimentation volume; *Triticum aestivum*.

Resumen

Variación en gluteninas de alto peso molecular (HMW) en variedades tradicionales españolas de trigo y su relación con la calidad panadera

En este trabajo, se ha analizado la variación alélica para gluteninas de alto peso molecular (HMW) en una colección de 165 variedades tradicionales españolas de trigo blando (*Triticum aestivum* ssp. *vulgare* L.) provenientes del Centro Nacional de Recursos Fitogenéticos. La identificación mediante técnicas estándar de electroforesis se ha complementado con un método de análisis por PCR que ha permitido la identificación de la subunidad 2•• del locus *Glu-A1* en varios cultivares. La relación entre la composición en gluteninas de alto peso molecular y la calidad panadera se ha analizado mediante la prueba del volumen de sedimenatción con SDS y los datos del mixógrafo. Se ha encontrado una influencia positiva en la calidad por parte de la subunidad 2•• del locus *Glu-A1*, los pares de subunidades 7 + 8 y 13 + 16 del locus *Glu-B1*, y el par 5 + 10 del locus *Glu-D1.* Variedades con la misma composición en gluteninas de alto peso molecular han mostrado un amplio rango de valores en los parámetros de calidad, apuntando a la participación de otras prolaminas, como las gluteninas de bajo peso molecular, cuya influencia en la calidad será analizada en el futuro. En el presente trabajo, se incluye la descripción detallada de la composición en gluteninas de alto peso molecular y de parámetros de calidad de todos los cultivares analizados para su posible utilización en programas de mejora de trigo.

Palabras clave adicionales: mixógrafo; *Triticum aestivum;* volumen de sedimentación.

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Received: 24-03-10; Accepted: 21-10-10.

Abbreviations used: BDR (breakdown resistance), CRF-INIA (Centro de Recursos Fitogenéticos- Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria), HMW-GS (high molecular weight glutenin subunits), LMW-GS (low molecular weight glutenin subunits), MDT (mixograph development time), PCR (polymerase chain reaction), SDSS (sodium dodecyl sulphate sedimentation volume test).

Introduction

Many recent studies have looked at the high molecular weight glutenin subunit (HMW-GS) allelic variation among registered and old cultivars from different world regions. The lower commercial value of landraces (some of which are currently only cultivated in small regions of traditional farming and conserved in germplasm collections) has relegated them to second place. Nevertheless, wheat landraces are an important source of genetic variability for agronomic traits (resistance to abiotic and biotic stresses, adaptability, etc.) and they are essential in breeding programs to obtain new commercial cultivars of high quality and well adapted to different environments (Skovmand and Rajaram 1990). Moreover, landraces play an important role in low-input agriculture (Murphya *et al.*, 2005).

Bread quality depends on the viscoelastic properties of dough, and these are mainly determined by the quantity and quality of gluten proteins (Finney, 1943). Several studies have reported the relationship between the HMW-GS composition of wheat grain and the gluten strength of dough measured by different tests (sodium dodecyl sulphate sedimentation volume test, alveograph and mixograph) (Payne *et al.,* 1979, 1984, 1987; Pogna and Mellini, 1986; Branlard *et al.,* 1992). The allelic variation in HMW-GS has been reported to account for up to 60% of the variation in bread making quality (Payne *et al.,* 1987).

HMW-GS are encoded by the complex *Glu-1* loci located on the long arm of chromosomes from homeologous group 1 and called *Glu-A1*, *Glu-B1* and *Glu-D1* (Shewry *et al.,* 1992). In each chromosome, the locus contains two closely linked genes that encode for two polypeptides, x-type glutenin subunit and y-type glutenin subunit (Shewry *et al.,* 1992). Some of these genes are silent so most cultivars present from three to five different subunits. Thus, the *Glu-A1* locus encodes for zero or one glutenins (the y-type glutenin of this locus is not expressed) and the loci *Glu-B1* and *Glu-D1* encode for one or two glutenins each (Payne *et al.,* 1981).

The HMW-GS characterization in bread wheat breeding programs is essential to improve flour baking quality. They can be characterized easily by protein electrophoresis, however, in many cases it is difficult to distinguish small differences in the molecular weight of different glutenins. Fortunately, genome information is now available for the HMW-GS genes since most of the alleles have been cloned and sequenced, allowing the development of molecular markers based on DNA sequence and PCR techniques (Liu *et al.,* 2008).

In modern cultivars, the HMW-GS allelic variability is not very large and the cultivars used for high strength wheat breeding are very similar in their HMW glutenin composition. Wheat landraces have a higher allelic variability and could be very useful for broadening the currently narrow genetic basis of modern cultivars in breeding programs (Christiansen *et al.*, 2005).

The bread-making quality of Spanish landraces has only been previously evaluated in a reduced set of 27 landraces by comparison with the quality of modern commercial cultivars (Gómez *et al*., 2009). The relationship between quality and HMW-GS composition was not assessed in this study.

The overall objective of the present work was to characterize the HMW-GS composition and bread quality of a wide selection of Spanish wheat landraces (*Triticum aestivum* ssp. *vulgare* L.) conserved at the Plant Genetic Resources Centre (CRF-INIA) for their possible use in breeding programs.

Material and methods

We selected 165 bread wheat landraces from the Plant Genetic Resources Centre (CRF, INIA, Alcalá de Henares, Madrid, Spain, see appendix). Each landrace was sown in a single row (3 m long, 1.12 m apart) at the *ETSI Agrónomos* Agricultural Experimental Station (Madrid, Spain) in 2006-2007.

Isolation of genomic DNA from leaves was performed as previously described (Saghai-Maroof *et al.,* 1984). The primers G2F1 (5'-TTTCATACTATCCAGG CCAAGC-3') and G2R1 (5'-GGTTGTTGCCATTGT CCTGA-3') were designed with the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from the sequences available in GenBank for the *Glu-A1x* 2* and *Glu-A1x* 2•• alleles (M22208 and DQ533690, respectively). Sequence alignment was performed with CLC Free Workbench (http://www.clcbio.com). PCR amplification was carried out in a 25 µL volume containing 100 ng of genomic DNA, 1x buffer (Biotools), 2 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM primers and 0.75 U Taq DNA polymerase (Biotools). PCR was conducted in a MyCycler thermocycler (BioRad) as follows: heat denaturation at 94°C for 2 min, followed by 5 cycles of touchdown PCR (94°C for 30 s, an annealing step starting at 61°C for 1 min

and decreasing 1°C per cycle, and 72°C for 45 s), then 35 more cycles of PCR (94°C for 30 s, 56°C for 1 min, and 72°C for 45 s) and a final extension step 72°C for 5 min. Amplification products were analyzed in 3% NuSieve agarose gels (Cambrex) stained with GelRed (Biotium).

Protein extraction from the wholemeal flour was performed according to Singh *et al.* (1991). Extracted proteins were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrilamide gels as described by Payne *et al.* (1980).

Grain protein content (14% moisture basis) was measured by near-infrared reflectance analysis using the Technichon Infranalyzer 300. The gluten strength was evaluated by SDS sedimentation volume test (SDSS) and mixograph. SDSS volume was measured according to Dick and Quick (1983). The mixograph variables were: mixing development time, MDT (time to maximum peak height in seconds), and breakdown resistance, BDR (difference in percentage between maximum peak height and height at 3 min after the peak). All measurements were run in duplicate using 10 g of flour in a National Manufacturing Co. Mixograph (Lincoln, NE), as described by Finney and Shogren (1972).

The statistical analysis of the data was performed with the SAS software (SAS Institute, Cary, NC, USA).

Results

Combining SDS-PAGE and PCR analysis we identified four different HMW-GS from the *Glu-A1* locus, nine different combinations from the *Glu-B1* locus and three different combinations from the *Glu-D1* locus in the 165 Spanish landraces analyzed. The 2* subunit and the 20x + 20y and 2 + 12 combinations were the most abundant in each locus (Table 1).

In the *Glu-A1* locus, the 1, 2* and Null glutenin subunits were easily identified by SDS-PAGE. This analysis showed the possible presence of the *Glu-A1x* 2•• glutenin subunit in some landraces. The correct assignment of this allele is difficult due to a similar electrophoretic mobility as the allelic 2* glutenin subunit. For unambiguous identification of the 2•• glutenin subunit, a screening method by PCR was designed. The alignment of 2•• and 2* nucleotide sequences showed a 18 bp deletion present in the 2* sequence and not in the 2•• sequence (Gobaa *et al.,* 2007). Two primers (G2F1 and G2R1) were designed to target this polymorphism. The G2F1 and G2R1 primers efficiently amplified a 100 bp fragment in the 2* glutenin subunit and a 118 bp fragment in the 2•• glutenin subunit of the *Glu-A1* locus (Fig. 1).

The gluten strength was evaluated by protein content, sedimentation test (SDSS) and two mixograph variables, MDT and BDR. The results are presented in Table 1.

Table 1. Mean values ± standard deviation (SD) for protein content, SDSS sedimentation volume, mixograph variables MDT (mixograph development time) and BDR (breakdown resistance) for the 165 landraces analyzed. Samples are grouped by high molecular weight glutenin subunit (HMW-GS) composition in each *Glu-1* locus

Locus	Allele	HMW-GS	N	%	Protein (%) Mean ± SD	SDSS (mm) Mean ± SD	MDT (s) Mean ± SD	BDR (%) Mean ± SD
Glu-A1	*a*	1	22	13.3	14.1 ± 0.8	63.6 ± 19.5	50.7 ± 27.4	42.0 ± 6.1
	b	2*	89	53.9	14.1 ± 1.1	59.9 ± 20.9	53.3 ± 22.4	42.6 ± 8.3
	c	Null	28	17.0	14.0 ± 1.2	68.6 ± 24.4	68.1 ± 30.2	38.4 ± 9.3
		2••	26	15.8	13.3 ± 0.9	72.2 ± 11.2	71.8 ± 26.7	37.8 ± 7.1
Glu-B1	*a*	7	11	6.7	14.3 ± 0.1	75.5 ± 16.9	65.7 ± 29.4	39.1 ± 6.7
	b	7 + 8	7	4.2	13.4 ± 0.7	84.6 ± 9.7	82.4 ± 17.4	32.1 ± 7.3
	d	6 + 8	7	4.2	13.8 ± 0.6	70.1 ± 17.0	56.1 ± 20.4	39.6 ± 4.8
	e	20x + 20y	110	66.7	14.0 ± 1.1	57.5 ± 20.1	50.0 ± 20.1	43.3 ± 8.2
	f	13 + 16	24	14.5	13.3 ± 1.0	76.0 ± 13.8	84.6 ± 29.7	35.8 ± 5.9
	i	17 + 18	1	0.6	16.8 ± 0.4	100.5 ± 2.0	114.0 ± 3.2	35.4 ± 0.7
	as	13	1	0.6	13.3 ± 0.2	89.5 ± 1.7	84.0 ± 2.7	32.4 ± 0.6
	u	7* + 8	2	1.2	15.6 ± 1.1	56.8 ± 8.8	31.0 ± 7.3	44.7 ± 5.3
		7* + 9	2	1.2	14.1 ± 1.2	83.0 ± 19.8	75.0 ± 15.6	30.9 ± 1.2
Glu-D1	*a*	2 + 12	122	73.9	13.9 ± 1.1	65.6 ± 19.8	60.4 ± 27.5	40.1 ± 8.5
	c	4 + 12	40	24.2	13.9 ± 1.1	56.6 ± 21.1	50.4 ± 20.4	41.2 ± 7.6
	d	5 + 10	3	1.8	15.4 ± 0.7	86.8 ± 16.4	80.7 ± 21.0	42.4 ± 5.2

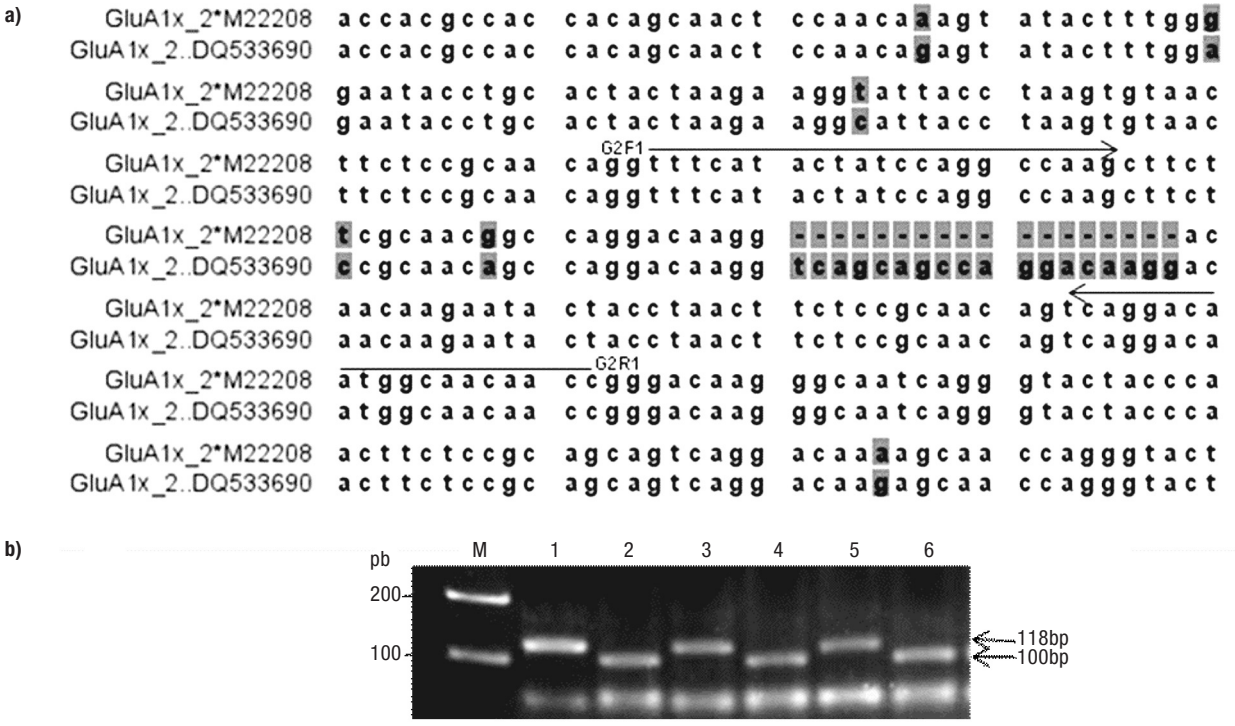

Figure 1. PCR screening for *GluA1x*2•• glutenin. a) Nucleotide alignment of a region of around 280 nucleotides from HMW glutenin subunit 1 (locus *Glu-A1*) glutenin gene coding for 2* and 2•• subunits. Alleles are labeled on the left side of sequences; the GeneBank accession number is also shown. This fragment corresponds to the region from nucleotides 2435 to 2714 of sequence M22208, and to the region from nucleotides 2638 to 2899 of sequence DQ533690. The polymorphism between both sequences is highlighted by the darker background and the 18 bp deletion is shown with dashes. b) PCR products amplified with G2F1 and G2R1 primers. Lanes 1, 3 and 5 are samples carrying the 2•• glutenin subunit; lanes 2, 4 and 6 are samples carrying the 2* glutenin subunit.

Mean protein content was around 14% in all the cases, with the widest ranges in the presence of the 7* + 8 pair of the *Gu-B1* locus and the 5 + 10 pair of the *Glu-D1* locus.

Landraces with the same HMW-GS composition showed a wide range of SDSS and mixograph values (Table 1, Fig. 2). The 2•• glutenin subunit had the highest mean SDSS value (72.2 mm) of all landraces with *Glu-A1* glutenins. In the case of the *Glu-B1* locus, the highest mean values were for the landraces carrying the 17 + 18, 13, and 7 + 8 pairs of glutenins (100.5, 89.5 and 84.6 mm, respectively) and the lowest value was for landraces with the 7* + 8 and 20x + 20y combination (56.8 and 57.5 mm, respectively). In the case of the *Glu-D1* locus, the highest and lowest mean SDSS values were for the 5 + 10 and 4 + 12 combinations respectively (Table 1).

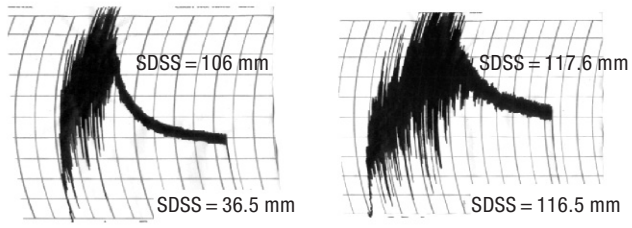

Figure 2. Mixograms of two landraces with the same high molecular weight glutenin composition (2*, 20x + 20y, 2 + 12). The SDS-Sedimentation test value is shown at the bottom.

The Null and 2•• subunits of the *Glu-A1* locus, the 17 + 18, 13, 13 + 16 and 7 + 8 pairs of *Glu-B1*, and the 5 + 10 pair of subunits of the *Glu-D1* locus had the highest mean values for the MDT and lowest for BDR (Table 1).

Pearson's correlation analysis showed a high and positive correlation between the SDSS volume and the MDT value ($R^2 = 0.82$; $p < 0.01$) and a high and negative correlation between the SDSS volume and the BDR value ($R^2 = –0.63$; $p < 0.01$). This means that a sample with a high sedimentation volume will also have a high mixing time value, which is a characteristic of dough with high stability, elasticity and mixing tolerance. Negative correlations with the BDR value will give a better resistance to over-kneading. Landraces with a high SDSS value also had a high MDT value and a low

BDR value, all of which are indicators of good dough development. Negative correlations were also found between the protein content and SDSS volume ($R^2 = –0.15$; $p < 0.05$) and MDT value ($R^2 = 0.36$; $p < 0.05$) and positive correlations were found between the protein content and the BDR value ($R^2 = 0.44$; $p < 0.01$).

The *t*-test statistical analysis for SDSS mean values according to HMW-GS composition is shown in Table 2. The 2•• glutenin subunit from the *Glu-A1* locus produced a high SDSS and low BDR value compared to 2* glutenin subunits, and higher mixograph values compared to Null and 1 subunits. The Null glutenin subunit produced higher mixograph values than 2* and 1 subunits. Similarly, the glutenin pairs 13 + 16 and 7 + 8 from the *Glu-B1* locus gave higher values than pairs 20x + 20y and 6 + 8. In the *Glu-D1* locus the 5 + 10 and 2 + 12 pairs gave higher SDSS and MDT values than the 4 + 12 pair. Moreover, the MDT value of the 5 + 10 pair was significantly higher than that of the 2 + 12 pair.

Discussion

HMW-GS variation

In a previous study, for the *Glu-A1* locus in 308 Spanish landraces (53 of them also present in this work) Rodríguez-Quijano *et al. (*1990) found only the HMW-GS Null, 1, and 2* (with a frequency of 44%, 24% and 32% respectively); the same subunits that had been previously described by Payne and Lawrence (1983) in a set of 300 cultivars from all over the world. In a study of 1,380 landraces from Europe (Finland, France,

Table 2. Statistical *t*-test analysis of the influence on quality traits of high molecular weight glutenin subunit composition for each *Glu-1* locus

Allelic composition compared	Trait[1]	*t*-test[2]
Glu-A1 2•• *vs Glu-A1* 1	MDT	2.7**
	BDR	–2.1*
Glu-A1 2•• *vs Glu-A1* 2*	SDSS	2.9**
	MDT	3.6**
	BDR	–2.6**
Glu-A1 Null *vs Glu-A1* 2*	MDT	2.9*
	BDR	–2.3*
Glu-A1 Null *vs Glu-A1* 1	MDT	2.2*
Glu-B1 7 + 8 *vs Glu-B1* 20x + 20y	SDSS	3.5**
	MDT	4.1**
	BDR	–3.5**
Glu-B1 7 + 8 *vs Glu-B1* 6 + 8	MDT	2.6*
	BDR	–2.3**
Glu-B1 13 + 16 *vs Glu-B1* 20x + 20y	SDSS	4.3**
	MDT	6.9**
	BDR	–4.2**
Glu-B1 7 *vs Glu-B1* 20x + 20y	SDSS	2.9*
	MDT	2.4*
Glu-B1 13 + 16 *vs Glu-B1* 6 + 8	MDT	2.4*
Glu-D1 2 + 12 *vs Glu-D1* 4 + 12	SDSS	2.5*
	MDT	2.1*
Glu-D1 5 + 10 *vs Glu-D1* 4 + 12	SDSS	2.4*
	MDT	2.5*
Glu-D1 5 + 10 *vs Glu-D1* 2 + 12	MDT	1.3*

[1] SDSS: SDS-Sedimentation volume. MDT: mixograph development time. BDR: breakdown resistance. [2] *,**: $p < 0.05$ and $p < 0.01$, respectively.

Italy, UK), Asia (India, China, Pakistan), Australia and the Americas (Argentine, Brazil, Canada, México, USA) the same three HMW-GS were described with similar frequencies (33%, 32% and 36%) (Morgunov *et al.,* 1993). In four more recent works (Tohver, 2007; Singh *et al.,* 2007; Fang *et al.,* 2009; Xu *et al.,* 2009) with landraces from North and Central Europe, India and China, the allelic variation for the *Glu-A1* locus is also restricted to the Null, 1, and 2* glutenins. The absence of the 2•• glutenin subunit in all of these studies could be due to the difficult assignment of this glutenin subunit using SDS-PAGE electrophoresis, and some of the samples classified as 2* could, in fact, have been 2••. The 2•• glutenin subunit has been found, in the landraces analyzed in this work, at a similar frequency to 1 and Null glutenins. This glutenin subunit was first described in a work with 155 wheat lines derived from a Portuguese landrace called Barbela (Igrejas *et al.,* 1997). Subsequently, this glutenin subunit was described in a Portuguese cultivar called 'Ribeiro' (Brites *et al.,* 2000), in a North Spain *Triticum aestivum* ssp. s*pelta* landrace (Elía, 2007) and in the breeding line '211.12014' from Switzerland (Gobaa *et al.,* 2007).

The development of a PCR screening method for the identification of this glutenin subunit has allowed easy and accurate identification. However, in the region amplified by the G2F1 and G2R1 primers there is no size polymorphism among 1, Null and 2•• glutenin subunits encoded by the *Glu-A1* locus, so, this method does not replace the SDS-PAGE analysis necessary for the other HMW glutenins. Additionally, some primers have been described to specifically detect the 2* subunit and discriminate it from Null and 1 glutenin subunits (De Bustos *et al*., 2000; Liu *et al.,* 2008).

In the present work, the allelic variation for the *Glu-B1* locus is similar to that found in a previous study on Spanish landraces (Rodríguez-Quijano *et al.,* 1990). The high frequency of the pairs of subunits 20x + 20y and 13 + 16 is a strong difference from landraces from other world regions. In other previous works these two combinations have been found with a very low frequency, between 1 and 3% (Payne and Lawrence, 1983; Morgunov *et al.,* 1993; Tohver, 2007) or have not been found at all (Fang *et al.,* 2009; Li *et al.,* 2009). However, in studies with Portuguese landraces, the 20x + 20y and the 13 + 16 pairs have been found at a higher frequency: 50% and 17.2% (Rodríguez-Quijano *et al.,* 1990) and 5.8% and 23.1%, respectively (Brites *et al.,* 2000). The 13 + 16 pair has been also described at a high frequency (88%) in *Triticum aestivum* ssp. *spelta* from North Spain (Rodríguez-Quijano *et al.,* 1990; Caballero *et al.,* 2004). These data confirm the conservation of these alleles in the Iberian Peninsula.

The HMW glutenins from the *Glu-D1* locus showed an allelic distribution similar to that found in other previous studies on Iberian landraces (Rodríguez-Quijano *et al.,* 1990; Brites *et al.,* 2000) and Chinese landraces, where the 2 + 12 pair was also the most frequent (Fang *et al.,* 2009; Li *et al.,* 2009) and different from the distribution found in studies on landraces from other world regions (Payne and Lawrence, 1983; Morgunov *et al.,* 1993; Tohver, 2007).

Quality analysis

Mean protein content was around 14% in all the landraces studied. Some authors (Branlard and Dardevet, 1985; Brunori *et al.,* 1989) have shown that dough strength is independent of this variable but the expression of a good quality genotype requires sufficient protein content. Although the protein content has no relationship with the HMW glutenin composition, it has some influence in mixograph variables. This influence has been analyzed in other studies, with contradictory results. Some authors have found a significant and positive correlation between SDSS values and protein content (Graybosch *et al.,* 1996), but others have not found any correlation (Dhaliwal *et al.,* 1987). We have found a significant, but low and negative, correlation between these variables, showing that, in general terms, wheat landraces with low protein content (12-13%) have a high SDSS value.

The 2•• glutenin subunit from the *Glu-A1* locus produced a high sedimentation volume compared to 1 and 2* glutenin subunits. The influence of the 2•• glutenin subunit on bread quality has been previously reported by Gobaa *et al.* (2007) who analyzed a population of double haploids. Nevertheless, this is the first time that its influence on quality has been analyzed in landraces.

To date, 1 and 2* glutenins subunits have been considered «good quality glutenins», both having a larger influence than the Null glutenin subunit (Cornish *et al.,* 2006). In this work, the mixograph values of landraces carrying the Null subunit have been significantly higher than the values of landraces with the 1 or 2* subunits. This discrepancy can be explained by the influence of HMW-GS from other loci. Landraces with the 1 or 2* glutenin subunits in the *Glu-A1* locus also

showed a high proportion of 20x + 20y glutenin subunits, a «bad quality» pair, in the *Glu-B1* locus. Conversely, in landraces with the Null glutenin subunit in the *Glu-A1* locus, the pair 20x + 20y is poorly represented.

The pairs of glutenins 13 + 16 and 7 + 8 from the *Glu-B1* locus have shown a high sedimentation volume compared to pairs 20x + 20y and 6 + 8. This result confirms previous studies that have considered both glutenin pairs of «bad quality» (Cornish *et al.,* 2006). The positive influence of the *Glu-D1* 5 + 10 pair compared to the 2 + 12 and 4 + 12 has also previously been shown (Cornish *et al.,* 2006). Most authors consider the 4 + 12 pair the one with the lowest influence on the sedimentation volume.

The large influence of HMW-GS on bread quality is widely known, but some influence of LMW-GS has also been reported (Payne *et al.,* 1987; Gupta *et al.,* 1989). This influence could explain the wide differences observed in quality traits (SDSS and mixograph) among landraces with the same HMW composition (Fig. 2). To address this issue, we have made a preliminary SDS-PAGE analysis and we have found that landraces with the same HMW glutenin subunit composition and different sedimentation volume also have different LMW composition, and landraces with similar LMW glutenins show similar sedimentation values (differences less than 15 mm, data not shown). To obtain an insight into the influence of the different LMW glutenins on bread quality we are generating advanced lines from crosses between landraces with the same HMW glutenin subunit composition and very different quality values.

Acknowledgements

We thank the Plant Genetic Resources Centre (Centro de Recursos Fitogenéticos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Alcalá de Henares, Madrid, Spain), for providing the samples, and Jose Rodriguez for technical assistance. This work was supported by Grant No. AGL 2006-8025 from Comisión Interministerial de Ciencia y Tecnología (CICYT) of Spain.

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Appendix. List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

Genebank number	Local name	Region	HMW-GS			SDSS (mm)	Protein (%)	MDT (s)	BDR (%)
			Glu-A1	*Glu-B1*	*Glu-D1*				
BGE 1982	Parrón	Asturias	2*	6 + 8	2 + 12	81.0	13.6	66	39.5
BGE 2834	Cañivano de Málaga	—	2*	20x + 20y	2 + 12	65.0	15.6	48	46.3
BGE 2836	Caspino	—	Null	20x + 20y	2 + 12	34.5	14.6	38	49.4
BGE 2838	Catalán Rojo	—	1	20x + 20y	2 + 12	70.0	14.3	33	38.9
BGE 2849	Hembrilla de Jaca	Huesca	1	20x + 20y	2 + 12	52.0	12.3	33	36.4
BGE 3540	Trigo de Campo del País	Lugo	2••	13 + 16	2 + 12	65.0	13.6	50	32.7
BGE 3610	Serodio	Lugo	2*	20x + 20y	2 + 12	50.5	13.8	42	39.7
BGE 3612	Trigo Tremesino	Lugo	2*	20x + 20y	2 + 12	74.5	14.7	45	40.5
BGE 4789	Trigo de Monte	Lugo	2••	20x + 20y	2 + 12	61.5	13.9	57	35.4
BGE 5443	Cascón	Palencia	Null	7	2 + 12	110.0	12.4	144	21.0
BGE 8707	Barbilla de Sevilla	—	2*	20x + 20y	5 + 10	84.0	16.2	80	38.5
BGE 10003	Caña Gruesa	Palencia	2*	7* + 9	2 + 12	97.0	14.9	96	31.2
BGE 11826	Perdigón	Cáceres	2••	13 + 16	2 + 12	60.0	13.7	45	38.5
BGE 11869	Blanco de Segarra	Lérida	2*	20x + 20y	2 + 12	64.5	13.3	50	34.4
BGE 11870	Chamorro de Hellín	Albacete	2*	7* + 9	2 + 12	69.0	13.2	54	30.7
BGE 11872	Nápoles de Alcaraz	Albacete	2••	13 + 16	2 + 12	57.5	12.9	72	36.6
BGE 11874	Jeja Blanca	Tarragona	2*	20x + 20y	2 + 12	55.5	13.8	60	42.9
BGE 11887	Escandín de Salas	Asturias	Null	7* + 8	2 + 12	50.5	14.8	30	38.6
BGE 11935	Portugués	Cáceres	2••	7	2 + 12	73.5	14.4	50	34.8
BGE 11947	Jeja Mocha	Barcelona	2*	20x + 20y	2 + 12	54.5	14.9	57	44.2
BGE 11950	Cabezorro	Cáceres	2••	13 + 16	2 + 12	86.5	12.8	69	41.5
BGE 11955	Chamorro de Viveros	Albacete	2*	20x + 20y	4 + 12	59.0	12.9	32	32.2
BGE 11959	Mocho Velloso de Garay	Vizcaya	2*	20x + 20y	5 + 10	72.0	14.8	60	48.4
BGE 11985	Montnegre	Barcelona	Null	7 + 8	2 + 12	84.0	13.9	86	40.0
BGE 11995	Negrete de Priego	Cuenca	2*	20x + 20y	2 + 12	57.0	13.3	33	33.9
BGE 11998	Cerrudo	Huesca	Null	6 + 8	2 + 12	83.0	12.9	60	38.7
BGE 11999	Cerrudo	Huesca	2*	20x + 20y	4 + 12	35.0	12.5	46	36.4
BGE 12011	Miranda	Asturias	2*	20x + 20y	4 + 12	69.5	12.8	42	34.5
BGE 12012	Somiedo	Asturias	2*	6 + 8	2 + 12	92.0	13.9	60	38.8
BGE 12019	Negrete de Cuenca	Cuenca	2*	20x + 20y	4 + 12	45.5	13.3	42	48.5
BGE 12025	Negrete de Priego	Cuenca	2*	20x + 20y	4 + 12	25.0	13.5	48	45.4
BGE 12039	Negrillo de Alcolea	Guadalajara	2*	20x + 20y	2 + 12	48.0	13.9	12	42.1
BGE 12048	Blanquillo de Bonillo	Albacete	2*	20x + 20y	4 + 12	60.5	15.1	33	48.7
BGE 12056	Negrete de Altarejos	Cuenca	2*	20x + 20y	4 + 12	35.5	12.9	24	41.1
BGE 12061	Negrete de Cuenca	Cuenca	2*	20x + 20y	4 + 12	30.5	13.1	37	46.1
BGE 12067	Blanquillo	Soria	2*	20x + 20y	4 + 12	25.0	12.6	38	43.5
BGE 12083	Negrete de Huelves	Cuenca	2*	20x + 20y	4 + 12	53.5	13.8	30	47.3
BGE 12092	Negro de Salas	Asturias	2*	20x + 20y	4 + 12	41.0	12.8	33	40.3
BGE 12112	Blanco Mocho	León	2••	20x + 20y	4 + 12	73.0	12.5	48	36.1
BGE 12114	Pelado	Cáceres	2••	20x + 20y	4 + 12	79.5	12.9	60	45.7
BGE 12122	Xexa Candeal	Baleares	2*	20x + 20y	2 + 12	74.0	13.0	42	38.8
BGE 12124	Richela Blanca	Lérida	2*	20x + 20y	2 + 12	95.0	12.8	83	93.4
BGE 12125	Mocho	Zamora	Null	7 + 8	4 + 12	85.5	12.5	96	21.2
BGE 12127	Candeal de Sierra Nevada	Granada	2*	20x + 20y	2 + 12	67.5	13.1	42	40.3
BGE 12128	Chamorro de Humanes	Guadalajara	2*	20x + 20y	2 + 12	81.0	13.2	87	39.9
BGE 12129	Chamorro de Madrigueras	Albacete	2*	7	2 + 12	81.0	13.1	87	39.0
BGE 12130	Mocho Blanco	Salamanca	Null	13 + 16	2 + 12	76.0	14.5	48	42.9
BGE 12193	Mocho de las Regueras	Asturias	2*	20x + 20y	2 + 12	53.5	13.2	33	47.5
BGE 12194	Hernani	Guipuzcoa	2*	20x + 20y	4 + 12	54.0	14.5	30	50.0

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

Genebank number	Local name	Region	HMW-GS			SDSS (mm)	Protein (%)	MDT (s)	BDR (%)
			Glu-A1	*Glu-B1*	*Glu-D1*				
BGE 12205	Chamorro de Villadiego	Burgos	Null	20x + 20y	2 + 12	108.0	14.1	89	28.1
BGE 12206	Toseta de Soria	Soria	2*	20x + 20y	2 + 12	84.5	14.4	57	40.0
BGE 12210	Mocho Rojo	Ávila	Null	7 + 8	2 + 12	102.0	13.8	105	25.1
BGE 12211	Blanquillo de Matamorosa	Cantabria	Null	7 + 8	4 + 12	90.0	14.3	94	27.8
BGE 12212	Mocho de Arróniz	Navarra	2*	20x + 20y	4 + 12	60.0	14.1	54	37.3
BGE 12214	Mocho Rioja	Álava	2*	20x + 20y	4 + 12	65.5	13.5	45	45.1
BGE 12238	Involcable	Álava	2*	7	2 + 12	95.5	15.2	75	39.1
BGE 12239	Richela Blanca	Lérida	2*	17 + 18	2 + 12	100.5	16.8	114	35.3
BGE 12575	Candeal de Muñogrande	Ávila	Null	20x + 20y	2 + 12	61.0	13.4	60	39.7
BGE 12576	Candeal Fino de Minaya	Albacete	1	20x + 20y	4 + 12	65.5	13.5	42	41.5
BGE 12577	Candeal de Arévalo	—	2*	20x + 20y	2 + 12	92.0	13.3	99	33.0
BGE 12580	Pichi	Badajoz	2*	20x + 20y	5 + 10	104.5	15.1	102	40.2
BGE 12582	Blanquillo de Toledo	Toledo	2••	13 + 16	2 + 12	68.5	12.3	72	44.9
BGE 12586	Candeal de Alcalá	Madrid	Null	20x + 20y	2 + 12	70.5	13.4	60	38.5
BGE 12591	Candeal de Vellisca	Cuenca	2*	20x + 20y	2 + 12	116.5	14.2	128	33.3
BGE 12595	Gabatx	Gerona	2*	20x + 20y	2 + 12	52.5	13.5	51	42.8
BGE 12596	Hembrilla de Alfaro	La Rioja	2*	20x + 20y	2 + 12	70.0	12.8	45	46.0
BGE 12596	Rojo de Campos	Palencia	2••	20x + 20y	4 + 12	90.5	12.7	86	36.2
BGE 12603	Caravaca	Murcia	1	20x + 20y	2 + 12	62.5	14.3	45	41.0
BGE 12207	Toseta de Jaca	Huesca	2*	20x + 20y	2 + 12	72.0	14.6	60	45.5
BGE 12744	Xexa de Mar	Barcelona	1	6 + 8	2 + 12	57.5	14.2	39	39.4
BGE 12746	Rojo de Eslava	Navarra	1	20x + 20y	2 + 12	83.0	13.9	53	43.7
BGE 12752	Negro	Madrid	2*	20x + 20y	2 + 12	60.5	16.2	27	42.3
BGE 12761	Catalán de Monte	Zaragoza	2*	20x + 20y	4 + 12	38.5	15.7	60	29.6
BGE 12785	Jeja Manchega	—	1	6 + 8	2 + 12	52.5	14.0	33	33.9
BGE 12861	Jeja Manchega	Ciudad Real	2••	20x + 20y	4 + 12	66.0	14.40	63	49.5
BGE 12875	Trigo del País	Asturias	1	6 + 8	2 + 12	76.0	13.50	93	38.5
BGE 12888	Trigo de Riego	Pontevedra	2••	13 + 16	2 + 12	71.0	12.80	81	35.8
BGE 12889	Trigo del País	Cantabria	2*	20x + 20y	2 + 12	73.0	14.8	45	37.3
BGE 12893	Trigo de Riego	Pontevedra	2*	20x + 20y	4 + 12	45.0	15.4	33	46.1
BGE 12894	Rojo del País	Lérida	Null	13 + 16	2 + 12	70.0	11.7	93	37.2
BGE 12896	Alcaraz Negro	Albacete	Null	7* + 8	2 + 12	63.0	16.3	42	50.9
BGE 13123	Blanco Entrefino de Oropesa	Toledo	2*	20x + 20y	2 + 12	62.5	16.2	57	42.6
BGE 13125	Hembrilla de Rueda	Valladolid	2*	20x + 20y	2 + 12	65.0	13.0	39	52.0
BGE 13128	Candeal de Tierra de Campos	Palencia	2••	20x + 20y	2 + 12	69.5	13.5	48	42.7
BGE 13131	Candeal de Salamanca	Salamanca	2••	13 + 16	2 + 12	68.5	13.0	57	33.3
BGE 13133	Candeal de La Sagra	Toledo	2••	20x + 20y	2 + 12	80.0	12.3	90	31.4
BGE 13134	Candeal de Aranda	Burgos	Null	20x + 20y	2 + 12	65.5	13.1	57	39.5
BGE 13137	Mahón	Baleares	2*	20x + 20y	2 + 12	71.0	14.6	69	38.2
BGE 13149	Blanquillo	Toledo	2••	13 + 16	2 + 12	80.5	13.8	129	28.0
BGE 13151	Trigo del País	Orense	2••	13 + 16	2 + 12	87.0	14.7	123	24.0
BGE 13152	Canet	Gerona	1	20x + 20y	2 + 12	43.0	15.2	17	45.8
BGE 13153	Jeja Colorada de Albacete	Albacete	1	20x + 20y	2 + 12	61.0	13.6	54	47.0
BGE 13155	Tremesino	Cáceres	Null	20x + 20y	2 + 12	65.0	14.3	66	35.1
BGE 13158	Tremesino de Gredos	Ávila	2*	20x + 20y	2 + 12	78.5	12.7	78	36.2
BGE 13162	Grandal	Lugo	2••	13 + 16	2 + 12	74.0	13.8	51	40.0
BGE 13164	Jeja Mallorquina	Baleares	2*	20x + 20y	2 + 12	81.0	14.7	75	37.5
BGE 13166	Menudillo	Zaragoza	Null	20x + 20y	2 + 12	35.5	16.3	33	56.8
BGE 13179	Colorado de Alfaro	La Rioja	Null	13 + 16	2 + 12	71.0	14.0	94	31.9

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

Genebank number	Local name	Region	HMW-GS			SDSS (mm)	Protein (%)	MDT (s)	BDR (%)
			Glu-A1	*Glu-B1*	*Glu-D1*				
BGE 13181	Jeja Cañifina	Tarragona	2*	20x + 20y	4 + 12	73.5	13.7	53	43.5
BGE 13182	Jeja	Castellón	2*	20x + 20y	4 + 12	75.0	13.4	57	39.0
BGE 13183	Blanquillo de Albacete	Albacete	1	20x + 20y	2 + 12	62.0	14.4	42	49.3
BGE 13184	Corriente de Jaca	Huesca	2*	20x + 20y	2 + 12	40.5	15.3	30	47.7
BGE 13185	Valle de Arán	Lérida	2*	20x + 20y	4 + 12	52.5	14.0	44	46.2
BGE 13186	Bergantiños	La Coruña	2••	13 + 16	2 + 12	92.0	11.8	112	34.4
BGE 13192	Manzanares	Ciudad Real	2*	20x + 20y	2 + 12	41.0	13.9	45	49.1
BGE 13195	Barbilla de Carbajales de Alba	Zamora	2••	13 + 16	2 + 12	81.5	12.1	135	21.7
BGE 13197	Rojo de Paredes	Cuenca	2*	20x + 20y	2 + 12	48.5	15.3	33	55.9
BGE 13198	Valenciano	Murcia	2*	20x + 20y	2 + 12	44.0	14.3	30	48.2
BGE 13199	Tremesino de Olivenza	Badajoz	1	20x + 20y	2 + 12	38.5	14.0	21	46.4
BGE 13203	Cañamaciza	Palencia	2*	20x + 20y	2 + 12	32.0	14.9	27	48.2
BGE 13216	Santa Marta	Badajoz	1	7	2 + 12	78.8	14.5	57	43.5
BGE 13218	Labradío	La Coruña	Null	13 + 16	2 + 12	96.5	14.4	126	36.5
BGE 13760	Isla de Fuerteventura	Las Palmas	Null	13 + 16	2 + 12	74.0	14.3	80	38.8
BGE 13762	Blanquillo de Cáceres	Cáceres	2*	13 + 16	2 + 12	62.0	12.7	77	34.9
BGE 13764	Gabatx de Fornes	Gerona	2••	20x + 20y	4 + 12	81.5	13.5	73	35.0
BGE 13767	Montjuich	Barcelona	Null	20x + 20y	2 + 12	38.5	16.6	27	61.7
BGE 13769	Casanya de Olot	Gerona	2*	20x + 20y	2 + 12	41.0	15.8	36	50.0
BGE 13771	Pirineos	Huesca	2••	20x + 20y	2 + 12	46.5	15.4	39	51.5
BGE 13773	Montearagón	Teruel	Null	20x + 20y	2 + 12	37.5	12.7	45	42.2
BGE 13776	Monte	Cantabria	1	20x + 20y	2 + 12	47.5	14.0	57	40.0
BGE 13795	Gamonal	Toledo	2*	13	2 + 12	89.5	13.3	84	32.4
BGE 13798	Mentana	Soria	1	7	2 + 12	69.0	14.3	66	41.0
BGE 13800	Hembrilla de Blecua	Huesca	1	6 + 8	2 + 12	48.5	14.8	42	48.8
BGE 13802	Vigo Cañifino	Pontevedra	2••	13 + 16	2 + 12	68.5	12.3	66	40.6
BGE 13811	Royo de Pamplona	Navarra	2*	20x + 20y	4 + 12	63.0	14.7	69	45.7
BGE 14261	Vimbodi	Tarragona	2*	20x + 20y	2 + 12	78.5	13.4	78	40.2
BGE 14281	Xexa de Alicante	Alicante	2*	20x + 20y	2 + 12	49.0	13.3	63	35.6
BGE 14285	Candeal de Vellisca	Cuenca	1	20x + 20y	4 + 12	82.3	13.8	76	36.6
BGE 15396	Jeja Colorada	Cuenca	2*	20x + 20y	2 + 12	34.5	12.7	30	51.3
BGE 15404	Trigo de Aruga	Cáceres	2••	13 + 16	2 + 12	55.5	13.3	81	43.2
BGE 18195	Candeal fino de Minaya	Albacete	2*	20x + 20y	2 + 12	69.0	13.6	65	41.0
BGE 18196	Jeja Candeal	Valencia	2*	20x + 20y	2 + 12	50.0	15.7	51	42.2
BGE 18200	Bonito	Cáceres	2••	13 + 16	2 + 12	75.5	13.4	57	42.8
BGE 18202	Jeja Pardilla	Cuenca	2*	20x + 20y	2 + 12	41.0	14.0	30	47.9
BGE 18207	Rojo de Caravaca	Murcia	2*	20x + 20y	2 + 12	36.0	13.6	43	41.9
BGE 18208	Coaña Blanco	Asturias	Null	13 + 16	2 + 12	77.5	14.6	77	35.4
BGE 18211	Lebaniego	Palencia	2*	20x + 20y	2 + 12	53.5	12.3	39	46.1
BGE 18214	Rojo de Villaseca	Toledo	Null	20x + 20y	4 + 12	14.5	14.5	30	39.5
BGE 18215	Rendía	Murcia	Null	7 + 8	2 + 12	71.0	13.6	73	36.6
BGE 18217	Rojo de Humanes	Guadalajara	Null	7 + 8	4 + 12	81.5	13.3	63	38.2
BGE 18219	Almendral Rojo Oscuro	Badajoz	2*	7	2 + 12	67.0	15.2	60	39.9
BGE 18222	Jeja de Cieza	Murcia	2*	20x + 20y	2 + 12	54.0	14.2	46	51.7
BGE 18225	Colorado de Soria	Soria	2*	7	2 + 12	44.5	14.3	46	43.0
BGE 18226	Colorado	Tenerife	1	7	2 + 12	70.0	14.5	51	43.4
BGE 18227	Jeja de Puerto Lápice	Ciudad Real	2*	20x + 20y	2 + 12	27.0	13.0	60	37.9
BGE 18228	Barbilla de León	León	1	13 + 16	2 + 12	117.5	12.0	146	27.8
BGE 18229	Cangrejero	Cangrejero	2*	20x + 20y	2 + 12	32.5	11.9	48	39.0

Appendix (cont.). List of the 165 bread wheat landraces (*Triticum aestivum* ssp. *vulgare* L.), provided by the Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain), analyzed in this study. The high molecular weight glutenin subunits (HMW-GS) composition, the sodium dodecyl sulphate sedimentation test value (SDSS), the grain protein content (Protein) and mixograph variables: peak mixing development time (MDT) and resistance to breakdown (BDR) are presented

Genebank number	Local name	Region	HMW-GS			SDSS (mm)	Protein (%)	MDT (s)	BDR (%)
			Glu-A1	*Glu-B1*	*Glu-D1*				
BGE 18230	Hembrilla de Soria	Soria	2*	20x + 20y	4 + 12	77.5	13.5	90	40.0
BGE 18231	Grosal	Teruel	2*	20x + 20y	4 + 12	70.0	13.5	84	25.3
BGE 18233	Vergara Temprano	Guipuzcoa	1	7	2 + 12	76.3	15.2	45	46.3
BGE 18234	Barbilla Roja	Huelva	2*	13 + 16	2 + 12	87.0	15.2	90	35.2
BGE 18239	Castrojeriz	Burgos	2*	20x + 20y	4 + 12	40.0	15.9	30	50.0
BGE 18242	Xeixa	Lérida	2*	20x + 20y	4 + 12	31.5	15.0	30	51.9
BGE 18244	Cabeza Negra	Huesca	2*	20x + 20y	4 + 12	38.5	15.4	33	51.0
BGE 18248	Jeja Fina	Teruel	2*	20x + 20y	4 + 12	21.5	17.2	22	55.7
BGE 18249	Blat Petit de Olot	Gerona	Null	20x + 20y	2 + 12	26.0	15.5	45	47.9
BGE 18253	Radondell de Las Llosas	Gerona	2••	20x + 20y	4 + 12	65.0	14.5	53	46.7
BGE 18254	Sierra Nevada	Granada	1	7	2 + 12	64.5	14.7	42	38.7
BGE 18355	Ruso	Ávila	Null	7 + 8	2 + 12	78.5	12.5	60	35.7
BGE 18671	Blat Mort	Baleares	2*	20x + 20y	2 + 12	48.5	15.4	60	52.3
BGE 18922	Raspinegro	Madrid	2*	20x + 20y	4 + 12	30.0	13.0	33	40.3
BGE 19317	Gejar	—	1	20x + 20y	2 + 12	21.0	14.2	28	56.7
BGE 19324	Villaverde de Trucios	Cantabria	2*	20x + 20y	2 + 12	36.5	13.7	36	56.6
BGE 19329	Blando Granja Melilla	—	2*	20x + 20y	2 + 12	38.0	12.9	47	39.8
BGE 38604	Trigo Medina	Badajoz	2*	20x + 20y	4 + 12	75.0	15.1	60	34.5