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## Genetic variation for grain protein components and industrial quality of durum wheat cultivars sown in Argentina

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### Abstract

Eleven cultivars of durum wheat from Argentina and Chile were analysed for their endosperm storage protein allelic composition and for industrial quality characteristics. For the HMW glutenin subunits, all cultivars carried the null allele *Glu-A1c*, and four variants were observed at *Glu-B1*: *Glu-B1b* (encoding subunits 7 + 8), *Glu-B1d* (subunits 6 + 8), *Glu-B1e* (subunits 20 + 20y) and *Glu-B1z* (subunits 7 + 15). For the *Glu-3* encoded B-LMW glutenin subunits, alleles *Glu-A3a* (subunit 6), *Glu-A3b* (subunit 5), *Glu-B3a* (subunits 2 + 4 + 15 + 19), *Glu-B3b* (8 + 9 + 13 + 16) and *Glu-B3c* (2 + 4 + 14 + 15 + 19) were observed, and for the *Glu-B2* encoded B-LMW glutenin subunits, *Glu-B2a* (subunit 12) and *Glu-B2b* (null allele). A B-LMW glutenin subunit not previously reported was observed in the cultivar Buck Cristal and provisionally designated subunit 6.1. A wide range of values for the characters gluten index, SDS-sedimentation volume and the Farinograph parameters energy level and tolerance was obtained from grain harvested from field trials sown in Argentina. There were strong correlations between these characters, and less strong correlations with grain protein and gluten content. While some cultivars varied for quality performance between years, others were relatively consistent for both extremes of performance. The analyses suggested that the gene pool represented by the cultivars investigated comprises useful variation for future breeding programmes, and provides pointers for directions that such programmes could take.

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### 1. Introduction

In Argentina, few cultivars of durum wheat (*Triticum turgidum* var. *durum*) have been developed, thus may cause problems in meeting future demands for dry pasta as local consumption increases, and for exploiting opportunities in export markets. Our studies are aimed at providing information needed to plan breeding programmes to avoid this situation, placing special emphasis on industrial quality. We also hope to provide information that might demonstrate the value of utilising germplasm from this region in breeding programmes elsewhere. Experience with

bread wheat in Argentina has shown that opportunities can be exploited when sufficient investment is placed in cultivar production: approximately, 6.5 million hectares of bread wheat are sown in this country, compared to less than a 100,000 of durum wheat (SAGPyA, 2004). A further aim was to assess whether Chilean cultivars offered interesting alternatives to those currently sown in Argentina.

In durum wheat, quality differences between cultivars are strongly dependent upon their allelic composition for endosperm storage proteins (Carrillo et al., 1990, 2000). One of the most important categories of proteins are the *Glu-3* encoded, B-low-molecular-weight (LMW) glutenin subunits, which have been shown to exert a more pronounced effect upon cultivar differences than variation in the *Glu-1* encoded, high-molecular-weight (HMW) glutenin subunits (Carrillo et al., 1990; Ruiz and Carrillo, 1995). This is in contrast to the situation in bread wheat (Payne et al., 1984a). Nonetheless, the latter category must also be taken into account (Carrillo et al., 2000; Ruiz

**Abbreviations:** EL, energy level in farinograph; GI, gluten index; HMW, high-molecular-weight; LMW, low-molecular-weight; SDS-S, sodium dodecyl sulphate-sedimentation test; TOL, tolerance in farinograph.

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and Carrillo, 1995). Additionally, allelic variation for the gliadins can provide useful indicators of quality, principally as indirect markers, due to the tight linkage between the loci *Gli-1* and *Glu-3* (Payne et al., 1984b; Pogna and Mellini, 1988; Pogna et al., 1988). Therefore, we determined the allelic status of all three classes of proteins in cultivars available in the region. Additionally, we determined the status of the locus *Glu-B2* encoding further B-LMW glutenin subunits, even though the only two alleles currently known at this locus do not appear to differ in their effect on quality (Ruiz and Carrillo, 1996).

We also evaluated cultivars for industrial quality in field trials sown in Argentina. While recognising that there are too few cultivars available in our region to make meaningful associations between individual alleles and quality parameters, we hoped to evaluate the range of quality types available, to characterise a potentially useful source of quality variation for future breeding programmes.

## 2. Experimental

### 2.1. Cultivars

The cultivars for which endosperm storage proteins were characterised were: the Argentinean durum wheat cultivars Bonaerense Valverde, Bonaerense Quilacó, Bonaerense INTA Cumenay, Bonaerense INTA Facón, Buck Ambar, Buck Topacio, Buck Cristal and Buck Esmeralda, and the Chilean cultivars Chagual INIA, Llaretá INIA and Guayacán INIA. All analyses used original seed kindly provided by the relevant breeder.

### 2.2. Field trials

Table 1 shows the cultivars sown in the different years of this study. The field trials were sown in a typical Argiudol soil type in three replicate blocks in randomised complete block designs on the Experimental Farm of the Faculty of Agronomy, Universidad Nacional del Centro de la Provincia de Buenos Aires, Azul, Province of Buenos Aires, Argentina, map coordinates 36° 49' 53" South, 59° 53' 23"

Table 1  
Years in which each cultivar was grown

Cultivars	1998	1999	2000
Cumenay	x	x	x
Valverde	x	x	
Quilacó	x	x	
Ambar	x	x	
Facón	x	x	x
Topacio	x	x	x
Guayacán			x
Llaretá			x
Esmeralda			x
Chagual	x	x	x

West. In the trials 14 rows were sown in an area of 8 m × 2.8 m. Fertilizer was applied according to the model (kg/ha): applied N–NO<sub>3</sub> = 150 kg/ha N–NO<sub>3</sub> – available soil N–NO<sub>3</sub> at sowing, in two applications: 30% at sowing and 70% at the end of tillering (Zadoks 31; Zadocks et al., 1979). Disease was controlled by fungicide application, particularly for fusarium at the heading stage (Zadoks 59). Weeds were controlled by early application of herbicides at the four/five leaf stage (Zadoks 14/15). The primary objective of the field trials from which the grain for the analysis of industrial quality originated was to evaluate the quality performance of cultivars currently on the market. Buck Cristal was not included in these trials since it was not on the market during the period of the trials.

### 2.3. Electrophoresis

The HMW and LMW subunits of glutenin, reduced with 2-mercaptoethanol and alkylated with 4-vinylpyridine, were separated by SDS-PAGE gels (at 10% acrylamide concentration) using the extraction method of Nieto-Taladriz et al. (1994). Subunits were identified using the 11 varietal standards of Nieto-Taladriz et al. (1997) plus two further cultivars described by Brites and Carrillo (2000). Gliadins were separated by A-PAGE at pH 3.1 according to Bushuk and Zillman (1978).

### 2.4. Quality tests

For the analysis of industrial quality, the samples were conditioned to 15% humidity for 20 h and a Brabender Quadrumat Junior mill with a 335 µm sieve used to obtain semolina. The analyses of industrial quality carried out were: protein content (%P, using NIR with a Infralizer 400 apparatus), gluten content (%G, using ICC method 137), gluten index (GI, using ICC method 155), energy level (EL) and tolerance (TOL) from the Farinograph (at constant water absorption and fixed mixing time). SDS-sedimentation (SDS-S) was performed according to Dick and Quick (1983), with modifications described in Carrillo et al. (1990). The units shown in the current study correspond, in agreement with this method, to mm of sediment.

All quality characters were scored on each plot from the field trials, and the data analysed by ANOVA, correlation and regression.

## 3. Results

### 3.1. Electrophoretic analyses

Electrophoretic separation of the grain proteins of some of the cultivars obtained by SDS-PAGE are shown in Fig. 1a. The allelic composition of each variety is given in Table 2.

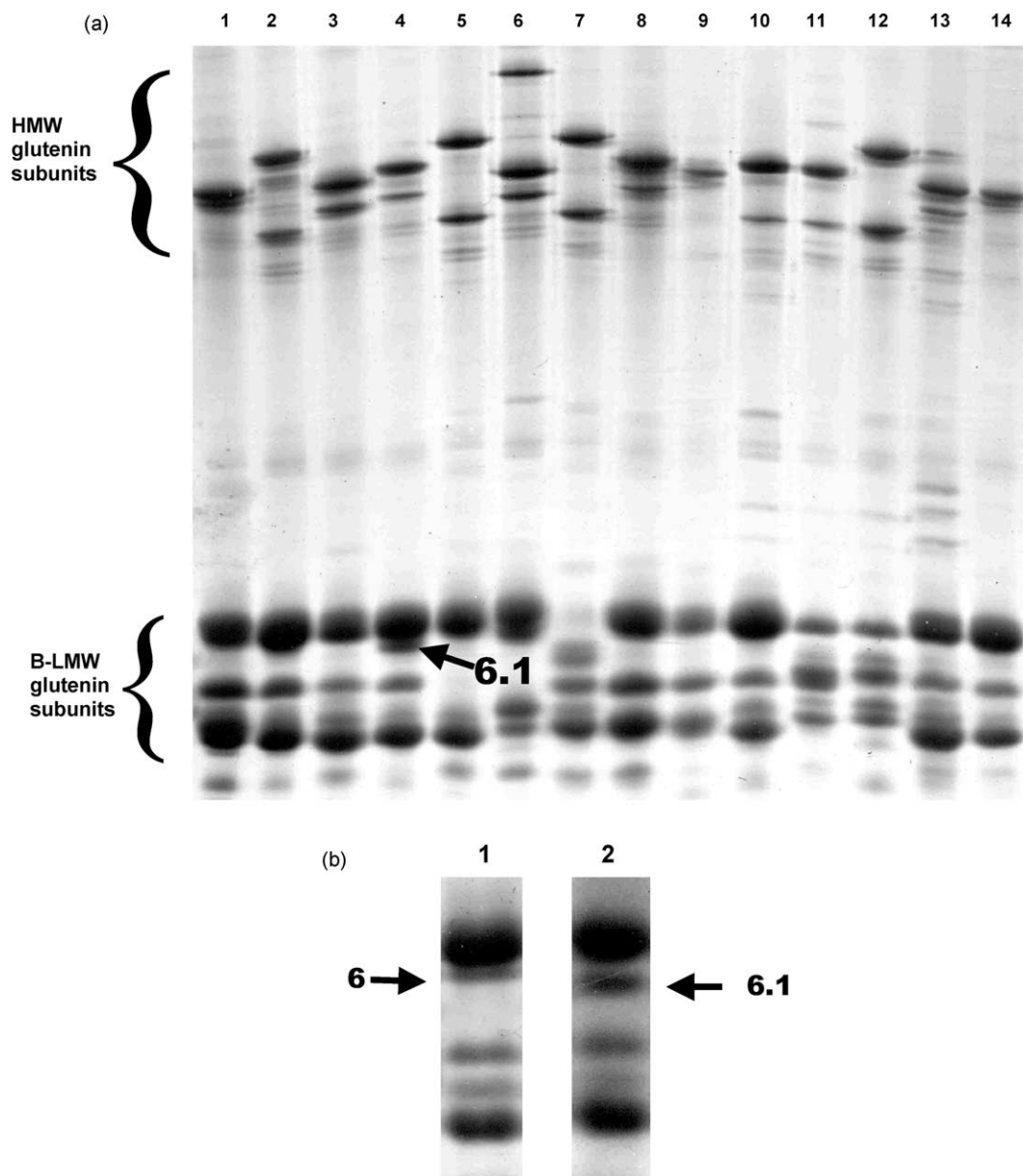


Fig. 1. Electrophoretic separations by SDS-PAGE of reduced HMW and LMW glutenin subunits (a) a sample of the cultivars under study (lanes 2, 4, 11 and 14) and standard cultivars (lanes 1, 3, 5, 6, 7, 8, 9, 10, 12 and 13): 1-Mundial; 2-Quilacó; 3-Claro de Balazote; 4-Cristal; 5-Jiloca; 6-Alaga; 7-Blatford; 8-Ardente; 9-Granja Badajoz; 10-Cocorit; 11-Chagual; 12-Langdon; 13-Clarofino; 14-Esmeralda. (b) Detail comparing Mexicali (lane 1) with Cristal (lane 2).

For the HMW glutenin subunits, all cultivars carried the null allele *Glu-A1c*, and four allelic variants at *Glu-B1* were identified: *Glu-B1b* encoding subunits 7 + 8, *Glu-B1d* encoding subunits 6 + 8, *Glu-B1e* encoding subunit 20 and *Glu-B1z* encoding subunits 7 + 15.

For the B-LMW glutenin subunits encoded by the *Glu-A3* locus, the cultivars showed two variants, *Glu-A3b* encoding subunit 5 and *Glu-A3a* encoding subunit 6, with the exception of the cultivar Cristal, which carried neither band; instead, it showed a band in a zone of higher mobility than subunit 6. This band does not appear to correspond to any previously published band. We are currently studying its characteristics and provisionally designate it subunit 6.1.

Detail from a gel run for longer than the standard time is given in Fig. 1b, where the difference in relative mobility between subunit 6 carried by the standard cultivar Mexicali and subunit 6.1 of Cristal can be more clearly discerned.

For locus *Glu-B3*, the cultivars included three variants: *Glu-B3a* encoding subunits 2 + 4 + 15 + 19, *Glu-B3b* encoding subunits 8 + 9 + 13 + 16 and *Glu-B3c* encoding subunits 2 + 4 + 14 + 15 + 19.

For the remaining glutenin locus analysed, *Glu-B2*, two previously described B-LMW variants were observed: the null allele *Glu-B2b*, and *Glu-B2a* encoding subunit 12.

Two gliadin variants (Table 2) were observed for *Gli-B1*:  $\gamma$ -gliadin 45 and  $\omega$ -gliadin 35 in ten of the cultivars and

Table 2  
Protein and allelic (in parentheses) composition of each cultivar (N = null allele)

Cultivars	HMW glutenin		LMW glutenin			Gliadins	
	Glu-A1	Glu-B1	Glu-A3	Glu-B3	Glu-B2	$\gamma$	$\omega$
Cumenay	N (c)	7 + 8 (b)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Valverde	N (c)	7 + 8 (b)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Quilacó	N (c)	6 + 8 (d)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Ambar	N (c)	6 + 8 (d)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Facón	N (c)	6 + 8 (d)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Topacio	N (c)	6 + 8 (d)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Guayacán	N (c)	7 + 8 (b)	6 (a)	2 + 4 + 15 + 19 (a)	N (b)	45	35
Llaretá	N (c)	6 + 8 (d)	6 (a)	2 + 4 + 15 + 19 (a)	N (b)	45	35
Esmeralda	N (c)	20 + 20y (e)	6 (a)	2 + 4 + 15 + 19 (a)	12 (a)	45	35
Chagual	N (c)	7 + 8 (b)	5 (b)	8 + 9 + 13 + 16 (b)	N (b)	42	33,35,38
Cristal	N (c)	7 + 15 (z)	6.1 <sup>a</sup>	2 + 4 + 14 + 15 + 19(c)	12 (a)	45	35

<sup>a</sup> Chromosomal location is provisional only.

$\gamma$ -gliadin 42 and  $\omega$ -gliadins 33, 35 and 38 in the cultivar Chagual.

### 3.2. Quality characters

Table 3 gives the values for the quality characters of each cultivar in each year. To facilitate comparisons, the cultivars are ranked according to their mean performance for GI over years (not given). The ranking for GI showed differences when individual years were compared, although some broadly stable relationships can be observed. For example, of the four cultivars grown in all three years, the cultivar Cumenay consistently gave superior values, the cultivar Chagual consistently inferior values, and the cultivar Facón fairly consistently intermediate to high values. These relationships held for the characters EL, TOL and SDS-S. The consistently inferior performance of cultivar Chagual was found in spite of its highest %G values among all the cultivars grown in years 1998 and 2000, and the lowest value in year 1999. The remaining cultivar grown in the three years, Topacio, showed a somewhat greater variation in performance.

Of the cultivars grown over two years, 1998 and 1999, Valverde and Ambar varied in GI performance, whereas Quilacó was fairly consistent at a level slightly lower than that of Cumenay. The cultivars grown in one year, Guayacán, Llaretá and Esmeralda, showed intermediate to low performance for GI.

It was found that the characters EL, TOL and SDS-S were highly correlated with GI when all cultivars were considered ( $r$  between GI and EL was 0.86 ( $P < 0.05$ );  $r$  between GI and TOL was  $-0.93$  ( $P < 0.05$ );  $r$  between GI and SDS-S was 0.81 ( $P < 0.05$ ) (see Fig. 2). GI was also correlated with %P and %G, though less strongly so:  $r$  between GI and %P was 0.61 ( $0.05 < P < 0.10$ ), and between GI and %G was 0.55 ( $0.05 < P < 0.10$ ).

Table 3  
Mean values of quality parameters for each cultivar in each year

Cultivars	GI	SDS-S	%P	%G	EL	TOL
<i>1998</i>						
Cumenay	99.5 a	47.3 a	13.8 a	28.9 bc	58.2 a	13 c
Valverde	96.8 a	41.3 b	13.1 bc	26.8 de	50.2 bc	13 c
Quilacó	87.7 bc	37.2 c	13.5 ab	27.7 cd	51.1 b	15 c
Ambar	94.1 ab	33.7 d	11.9 d	25.7 e	40.7 e	19 b
Facón	77.4 d	40.0 b	12.6 c	27.5 cd	44.9 d	13 c
Topacio	83.2 cd	34.0 d	13.1 bc	30.6 ab	47.6 cd	22 b
Guayacán						
Llaretá						
Esmeralda						
Chagual	24.8 e	20.7 e	12.1 d	31.8 a	38.9 e	32 a
I.s.d.	7.115	2.339	0.4746	1.749	3.438	3.386
<i>1999</i>						
Cumenay	94.0 a	61.8 a	16.4 a	38.3 b	67.1 a	21 e
Valverde	78.2 bc	65.2 a	16.5 a	41.3 a	67.6 a	24 e
Quilacó	84.0 b	39.2 d	16.0 ab	40.5 a	59.9 c	37 b
Ambar	73.0 cd	49.5 b	14.9 cd	36.9 b	58.2 c	31 c
Facón	77.6 c	43.3 c	15.4 bc	37.9 b	62.5 b	27 d
Topacio	67.2 d	40.5 cd	15.2 cd	38.7 b	55.5 d	34 bc
Guayacán						
Llaretá						
Esmeralda						
Chagual	4.00 e	19.0 e	14.7 d	34.3 c	40.5 e	45 a
I.s.d.	6.244	3.790	0.5752	1.847	2.445	3.251
<i>2000</i>						
Cumenay	94.0 a	49.7 a	12.9	21 e	51.9 a	23 c
Valverde						
Quilacó						
Ambar						
Facón	88.5 ab	33.2 b	12.1	24 e	43.2 b	28 bc
Topacio	79.5 b	23.8 d	12.3	37 b	39.9 c	27 bc
Guayacán	67.2 c	28.3 c	12.3	31 c	44.5 b	29 b
Llaretá	56.3 c	23.2 d	12.8	27 d	43.5 b	31 ab
Esmeralda	40.3 d	22.7 d	12.1	34 bc	40.0 c	35 a
Chagual	2.17 e	13.8 e	12.7	45 a	37.7 c	36 a
I.s.d.	10.91	3.407	n.d	3.251	2.923	5.146

Values followed by the same letter are not significantly different (0.05 level).

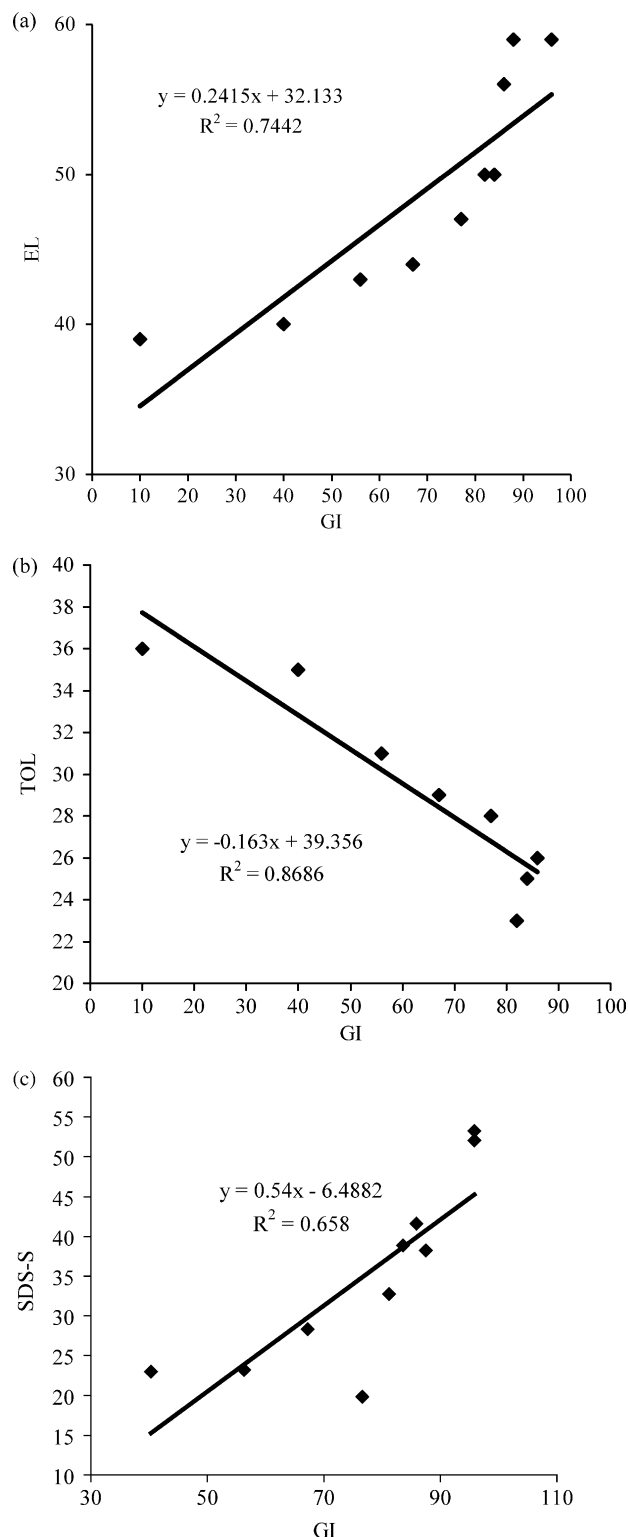


Fig. 2. Relationships between quality characters: regressions of (a) Energy Level (EL), (b) Tolerance (TOL) and (c) SDS-sedimentation test (SDS-S) on Gluten Index (GI).

#### 4. Discussion

The presence of a suspected novel B-LMW glutenin subunit and the wide range of variation shown by the

cultivars studied demonstrates that the germplasm assessed has at least potential value for future national and international breeding programmes. We have tentatively assigned a number to the new subunit in spite of the current lack of information about its chromosomal location, and have followed the criteria of Nieto-Taladriz et al. (1997) and Brites and Carrillo (2000) of assigning subunit numbers that retain a certain amount of information about their relative mobility. The cultivar carrying this subunit, Buck Cristal, also carried the relatively unusual allele, *Glu-B3c*, encoding subunits 2 + 4 + 14 + 15 + 19, as well as the relatively unusual HMW glutenin subunits 7 + 15 encoded by *Glu-B1z*. These latter subunits have not yet been evaluated for their association with quality. We have made provisional quality assessment of this cultivar via SDS-S (unpublished data), and have found it to give values comparable to the cultivar Cumenay. Hence we are pursuing further studies with this cultivar, including crosses with other cultivars designed to map the potentially novel glutenin subunit and to determine its effect and that of the HMW subunits 7 + 15 on quality.

Although, as pointed out earlier, our study includes too few cultivars to make meaningful associations between individual alleles and quality parameters, we noted that the consistently best cultivar, Cumenay, and the consistently worst, Chagual, carried the same HMW glutenin subunits while varying considerably in B-LMW subunits. At least a part of the difference in performance between them may be due to the presence in Chagual of LMW subunits 8 + 9 + 13 + 16 encoded by *Glu-B3b*, and of subunit 5 encoded by *Glu-A3b*. Both these alleles have been shown to be associated with poor quality: the former by Payne et al. (1984b), Pogna and Mellini (1988) and Pogna et al. (1988), and the latter by Carrillo et al. (2000). The difference in quality between the two cultivars does not, from our results, appear to be due to differences in %G, since, in two of the 3 years, Chagual gave the highest value for this character compared with the other cultivars, including Cumenay.

Comparisons between the SDS-S values obtained in this study and those obtained by Carrillo et al. (1990) suggest that, while a substantial proportion of the genetic variation for quality is accounted for by the loci studied, there must be other factors involved in the genetic control of quality. We compared the SDS-S values of cultivars in the two studies of similar allelic composition, to find broad similarities but differences in detail.

From the point of view of breeding, the cultivar Cumenay appears to be a useful source of high quality for future programmes. The climatic conditions in each of the 3 years differed considerably (for example, 1999 was particularly hot and dry, where grain yields were low and grain protein content high), and yet this cultivar maintained its quality level over the three years. From the point of view of breeding within this gene pool and for basic genetic studies, a particularly interesting initial cross would be Cumenay × Chagual, since, apart from their obvious



differences in quality, the cultivar Chagual shows high yield performance under our conditions (Lerner, unpublished data), providing the opportunity to extract lines combining these complementary aspects.

From the point of view of the introduction of the Chilean cultivars into Argentina, one of the objectives of this study, there is no strong support for this in the context of their performance for quality. Two of the Chilean cultivars, Guayacán and Llaraeta gave intermediate quality values (1 year's data), and the other cultivar was the consistently poor Chagual. This does not imply that these cultivars are necessarily of poor to intermediate quality in their country of origin, since, not having been bred for Argentinean conditions, they may not have performed to their full potential in our trials. This is in spite of the fact that the region in Argentina where these trials were conducted was in general adequate for the expression of high quality. Data is available on their performance in Chile itself (INIA, 2004).

Some of the Argentinean cultivars included in the trials have been previously studied for storage protein composition, though not for quality characteristics, by Bullrich et al. (1998), but using a different nomenclature system for the LMW subunits, making direct comparison difficult. Some differences were noted between the HMW subunit composition reported and those obtained in the current study.

The South American cultivars studied here, although relatively few in number, showed an enormous range in quality, an important finding considering that the end-use of this crop is for pasta production, where high gluten strength is required. Although it is evident that, compared with bread wheat, little effort has been dedicated towards breeding durum wheat for this region, there appears to be an interesting genetic pool available, albeit that the introduction of novel variants from other germplasm may bring benefits. It seems vital for the future of the Argentinian industry that greater control is exercised over gluten protein composition in cultivars released, to avoid further release of cultivars of inadequate quality.

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