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Allelic diversity of HMW and LMW glutenin subunits and omega-gliadins in French bread wheat (*Triticum aestivum* L.)

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Abstract

Wheat endosperm storage proteins, namely gliadins and glutenins, are the major components of gluten. They play an important role in dough properties and in bread making quality in various wheat varieties. In the present study, the different alleles encoded at the 6 glutenin loci and at 3 ω-gliadin loci were identified from a set of 200 hexaploid wheat cultivars grown primarily in France using SDS PAGE. At *Glu-A1*, *Glu-B1* and *Glu-D1*, encoding high molecular weight glutenin subunits (HMW-GS), 3, 8 and 5 alleles were observed respectively. Low molecular weight glutenin subunits (LMW-GS) displayed similar polymorphism, as 5 and 11 alleles were identified at loci *Glu-A3* and *Glu-B3* respectively. Four alleles were observed at *Glu-D3* loci. Omega-gliadin diversity was also very high, as 7, 13 and 9 alleles were found at *Gli-A1*, *Gli-B1* and *Gli-D1*, respectively. A total of 147 (or 149) patterns resulted from the genetic combination of the alleles encoding at the six glutenin loci (or *Glu-1* and *Gli-1* loci). Although *Glu-1* and *Glu-3* loci were located on different chromosome arms and were theoretically independent, some associations were revealed due to pedigree relatedness between some French wheat cultivars. The usefulness of allelic identification of LMW-GS together with HMW-GS and gliadins for future genetic and technological wheat improvement is discussed.

Abbreviations: HMW-GS – high molecular weight glutenin subunits, LMW-GS – low molecular weight glutenin subunits, SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis

Introduction

The endosperm of bread wheat is mainly comprised of starch (approximately 70%) and proteins (approximately 10–15% dw). In the latter component, the storage proteins (80%) are comprised of gliadins (40%), high molecular weight glutenin subunits (HMW-GS, 10%) and low molecular weight glutenin subunits (LMW-GS, 30%). These proteins are the major components of the gluten and their importance in dough properties and bread-making quality has long been recognised (MacRitchie 1992).

Most gliadins are controlled by six main *Gli* loci located in the homoeologous chromosomes of group 1 (*Gli-1*) and 6 (*Gli-2*) (Payne et al. 1982) Several additional loci encoding a few minor gliadin bands have been identified (Pogna et al. 1993; Ruiz and Carrillo 1993; Metakovsky et al. 1997). Sozinov and Poperelya have shown that several bands of gliadins as revealed by acid electrophoresis (A-PAGE), were inherited as a Mendelian unit (block) and corresponded to an allele. Marked multiple allelism has been described in bread wheats at each of these loci (Metakovsky 1991). The genetic polymorphism of the

gliadins has been used to analyse genetic diversity within several germplasms, for example from Australia (Metakovsky et al. 1990), from Yugoslavia (Metakovsky et al. 1991) and from France (Metakovsky and Branlard 1998).

Using a reducing agent, the glutenins were divided into two groups: high molecular weigh (HMW-GS, 80–120 kDa) and low molecular weight (LMW-GS, 30–50 kDa) glutenin subunits (Payne and Corfield 1979). The HMW-GS are encoded at genes named *Glu-A1*, *Glu-B1* and *Glu-D1* where numerous alleles were identified (Payne and Lawrence 1983) and updated (McIntosh et al. 1994). Many scientists have described the allelic diversity of the HMW-GS found in bread wheat and durum wheat grown in different countries. This is particularly true for French bread and durum wheat (Branlard and Le Blanc 1985; Branlard et al. 1989). The main LMW-GS (named B-subunits) are controlled by genes called *Glu-A3*, *Glu-B3* and *Glu-D3*, located on the short arms of group 1 chromosomes (Payne et al. 1984). The allelic diversity found at these loci was first described by Jackson et al. (1983) and Gupta and Shepherd (1990a, 1990b). LMW-GS genes are linked to genes (*Gli-1* loci) coding for ω and γ gliadins. Sreeramulu and Singh (1997) found two new LMW-GS loci, named *Glu-4* and *Glu-5*, and suggested that *Glu-5* is located on chromosome 7. Due to the linkage between *Gli-1* and *Glu-3* loci, Cornish and Lukow (1996), proposed to identify the *Glu-3* allele through analysis of *Gli-1* encoded ω-gliadins using SDS-PAGE. A correspondence between *Gli-1* and *Glu-3* was used to describe Australian cultivars (Gupta et al. 1994) and a new system of nomenclature has been proposed for the alleles of genes encoding some gliadins and LMW-GS in bread wheat (Jackson et al. 1996). The allelic diversity of LMW-GS in durum wheat has been studied (Nieto-Taladriz et al. 1997) and their effect on dough properties linked with pasta quality demonstrated (Pogna et al. 1988). Due to the complexity of the LMW-GS patterns and the fact that some of the bands overlap, only a few collections of bread wheat have been analysed (Gupta and Shepherd 1990a; Jackson et al. 1996; Igrejas et al. 1999; Flæte 2000).

The aim of the present study is to describe the allelic diversity of the *Glu-3* loci in French bread wheats. In addition, in order to determine the polymorphism of the major genes controlling the gluten protein in French bread wheats, the diversity found at *Glu-3* loci was combined with those already reported at *Glu-1* and *Gli-1*.

Materials and Methods

Plant Material

200 hexaploid cultivars - mostly registered in the French catalogue - were used to analyse the allelic diversity of glutenins. All these cultivars, which were grown at the Plant Breeding Station in Clermont-Ferrand (France), were obtained and used by the majority of private and public wheat breeders between 1949 and 1994. Among the cultivars listed in Table 1, several have been used as European standards for allelic identification of LMW-GS and HMW-GS, as previously mentioned (Jackson et al. 1996).

Electrophoresis

Proteins were extracted from individual grains using the sequential procedure of Singh et al. (1991). Up to ten grains were analysed per variety. To increase the density of the extracting solution, the concentration of glycerol was raised to 24% (w/v). Electrophoresis of HMW-GS and LMW-GS was performed on vertical gel (180×160×1 mm) according to the SDS-PAGE protocol described by Singh et al. (1991) with two modifications: constant gel concentration was preferred to gradient gel, T (acrylamide + bis-acrylamide), and the cross linker C was chosen as follows: T = 12.8% and C = 0.99%.

Nomenclature

The nomenclature of Payne and Lawrence (1983) was used for HMW-GS. The nomenclature of Gupta and Shepherd (1990a) and Jackson et al. (1996) was used for both LMW-GS and ω-gliadins.

Statistics

The genetic diversity at each locus was calculated according to Nei (1973) as follows: $H = 1-\Sigma pi^2$, where H is Nei's genetic variation index and pi the frequency of a particular allele at that locus. The Chi-square test was used to test independence of alleles at different loci. All the statistical analyses were performed using Statgraphics® software.

Results and Discussion

Allelic diversity of HMW-GS and LMW-GS

A total of 16 HMW-GS alleles were found among the

Table 1. Allelic composition at loci *Glu-A1*, *Glu-B1*, *Glu-D1* (encoding HMW-GS), *Glu-A3*, *Glu-B3*, *Glu-D3* (LMW-GS), *Gli-A1*, *Gli-B1*, *Gli-D1* (ω-gliadins) for 200 varieties.

Cultivars	HMW-GS			LMW-GS			ω-Gliadins		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*
'Abo'	c	b	d	a	b	c	k	b	b
'Aboukir'	c	b	d	a	g	b	k	f	f
'Aiglon'	c	a	d	ef	g	c	f	e	b
'Albatros'	c	b	a	d	f	c	o	e	b
'Alpe'	b	b	d	a	b	c	b	b	b
'Alto'	c	b	d	a	g	c	k	f	b
'Alvina'	c	b	d	a	c	c	k	b	b
'Ami'	c	b	d	a	c′	c	o	b	b
'Apexal'	c	c	d	a	g	c	b	e	b
'Apollo'	c	d	a	d	j	c	o	l	b
'Apostole'	c	i	a	ef	g	b	b	f	g
'Arbon'	c	d	a	a	b	c	a	b	b
'Arcane'	c	b	a	a	g	b	a	f	f
'Arche'	c	d	a	d	g	c	o	f	b
'Arcole'	c	b	c	d	g	c	o	f	b
'Arfort'	b	i	d	a	b	a	b	b	b
'Aristide'	b	a	d	a	c′	c	b	b	j
'Armada'	c	d	a	ef	f	c	m	g	b
'Arminda'	c	a	a	a	g	c	f	f	b
'Armur'	c	c	d	a	g	c	k	f	b
'Arsenal'	a	d	a	a	g	a	f	e	b
'Arum'	c	d	a	ef	f	c	b	g	b
'Arval'	c	d	d	a	f	c	f	g	b
'Aubaine'	b	b	d	ef	g	c	f	f	j
'Austerlitz'	b	b	a	ef	g	a	f	f	b
'Avalon'	a	d	b	a	b	c	f	b	b
'Axel'	c	b	a	d	g	c	o	f	b
'Baroudeur'	c	c	a	d	b	c	o	b	b
'Bastian'	b	c	d	a	i	a	a	h	b
'Beauchamp'	c	d	a	d	g	c	o	f	b
'Beaver'	c	d	a	ef	j	c	b	l	b
'Belaviso'	c	b	a	d	j	c	o	l	b
'Berlioz'	c	a	a	a	g	c	f	f	b
'Bison'	c	c	a	a	b	c	f	b	b
'Blason'	c	b	a	d	g	c	o	f	b
'Boreal'	c	d	d	d	g	a	o	f	l
'Brimstone'	c	d	a	a	g	d	f	f	b
'But'	c	a	a	d	g	c	o	f	b
'Campremy'	b	a	b	d	g	c	o	f	b
'Cappelle'	c	a	a	d	g	c	o	f	b
'Capitole'	c	c	a	d	g	c	o	f	b
'Carat'	c	d	d	ef	c	c	m	b	b
'Cargidoc'	c	a	a	a	g	c	f	f	b
'Cargimarec'	a	a	e	a	g	a	x?	e	k
'Cargo'	b	b	d	a	c	c	k	b	b
'Carlos'	c	a	a	a	g	c	a	f	b
'Castan'	c	a	d	a	g	c	a	f	b
'Caton'	c	c	a	a	g	c	f	f	b
'Champion'	c	d	a	ef	g	c	f	f	j
'Champlein'	c	b	c	a	g	c	f	f	b
'Champtal'	b	b	d	a	f	c	f	g	b
'Choisel'	c	b	d	a	g	a	f	f	b
'Chopin'	a	c	d	a	d	c	f	d	b
'Clement'	c	d	a	ef	j	c	k	l	b
'Copain'	a	a	a	d	b	c	o	b	b

Table 1. (*continued*)

Cultivars	HMW-GS			LMW-GS			ω-Gliadins		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*
'Corin'	c	d	b	d	g	c	o	f	l
'Corot'	c	a	a	d	g	c	o	f	b
'Cosmos'	c	d	c	a	f	c	f	g	b
'Courtot'	b	b	a	a	c′	c	k	b	b
'Creneau'	a	b	a	a	c′	c	k	b	b
'C. Spring'	c	b	a	a	a	a	a	a	b
'Damier'	c	d	a	ef	j	c	f	l	b
'Darius'	c	a	a	d	g	b	o	f	null
'David'	a	b	a	a	g	c	f	f	b
'Delfi'	c	b	d	a	c′	c	k	b	b
'Democrat'	b	c	a	a	h	a	b	d	k
'Diam'	c	d	b	d	g	c	o	f	b
'Divio'	c	a	d	d	g	c	o	f	b
'Ducat'	a	a	d	d	g	c	o	f	b
'E. de Choisy'	c	b	a	d	i	c	o	m	b
'Ecrin'	c	i	a	ef	f	b	b	g	b
'Epiroux'	c	a	a	d	b	c	o	b	b
'Estica'	c	d	a	a	g	c	f	f	b
'Eureka'	c	d	a	a	c′	c	f	b	b
'Faust'	c	a	d	d	i	c	o	m	b
'Favori'	c	b	d	d	g	c	o	f	b
'Festin'	c	a	a	ef	b′	c	b	b	b
'Festival'	c	c	a	d	c	c	o	f	b
'Feuvert'	c	b	a	a	f	c	f	g	b
'Fidel'	c	b	d	ef	g	b	m	f	g
'Fleurus'	c	b	c	d	g	c	o	f	b
'Floreal'	a	c	d	a	d	c	a	h	l
'Florin'	a	b	c	a	g	c	f	f	b
'Fluto'	c	b	c	ef	g	c	f	f	b
'Fortin'	c	a	d	a	g	a	b	f	b
'Fournil'	c	b	d	a	g	c	b	e	g
'Frandoc'	c	c	a	ef	b	b	b	b	b
'Friedland'	c	d	d	a	g	c	f	f	b
'Futur'	c	b	a	a	g	c	f	f	b
'Gabo'	b	i	a	b	b	b	f	b	f
'Gaillard'	c	b	a	d	i	c	o	m	b
'Gala'	c	b	c	d	g	c	o	f	b
'Galahad'	c	a	a	ef	f	c	b	g	b
'Galaxie'	c	d	d	a	g	c	a	f	b
'Garant'	c	a	d	a	c′	c	k	b	b
'Gavroche'	a	b	d	ef	g	c	f	f	b
'Genial'	c	a	d	a	g	c	k	f	b
'Gerbier'	a	b	d	d	g	a	o	f	b
'Glanor'	c	b	d	a	g	c	f	f	b
'Goelent'	c	d	d	a	b	a	f	q	b
'Goya'	c	d	d	d	c	a	o	q	b
'Hamilcar'	c	d	a	d	g	c	o	f	b
'Hardi'	b	a	a	d	g	c	o	f	b
'Hereward'	c	c	b	ef	g	c	b	f	b
'Heurtebise'	c	a	a	a	c	b	m	b	b
'Hickling'	c	d	a	d	g	c	o	f	b
'Hobbit'	c	a	b	d	g	c	o	f	b
'Horace'	c	d	a	a	g	c	f	f	b
'Huntsman'	c	d	a	a	f	c	f	g	b
'Iena'	c	b	c	a	g	c	f	f	b
'Insignia'	a	e	d	b	c	c	f	i	i

Table 1. (*continued*)

Cultivars	HMW-GS			LMW-GS			ω-Gliadins		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*
'Jano'	c	b	c	ef	g	c	f	f	b
'Joss'	c	d	a	d	g	c	o	f	b
'Kadett'	a	c	d	a	b	c	a	b	b
'Lodi'	c	b	d	a	g	b	k	f	f
'Longbow'	c	a	a	d	g	c	o	f	l
'Louvre'	c	c	a	ef	g	c	f	f	b
'Luja'	b	b	d	d	b′	a	o	b	b
'Lutin'	c	a	d	a	c′	c	k	b	b
'Magali'	c	b	a	ef	f	b	m	e	g
'Magdalena'	c	c	d	d	b	a	o	b	b
'Magister'	c	d	a	ef	j	c	f	l	b
'Magnif 27′	b	f	a	a	i	b	b	m	b
'Manital'	b	i	a	a	b′	a	a	b	k
'Marignan'	a	a	a	d	g	c	o	f	b
'Marius'	c	c	a	d	g	c	o	f	b
'Martial'	c	b	a	d	g	a	o	f	b
'Master'	c	d	a	a	f	c	f	g	b
'Match'	a	b	a	ef	c′	c	f	b	b
'Merit'	c	c	d	d	c	c	o	b	b
'Messidor'	b	b	a	ef	g	c	f	f	b
'Milpain'	a	c	a	a	g	c	b	f	b
'Mission'	c	d	a	d	f	c	o	e	b
'Monitor'	c	a	a	d	b′	c	o	b	l
'Moulin'	c	i	a	ef	g	c	b	f	b
'Nabucco'	c	c	a	a	g	c	f	f	b
'Nautica'	a	d	a	ef	j	c	f	l	b
'Nectar'	b	a	d	d	g	a	o	f	b
'Nisu'	b	c	d	ef	d	b	f	h	b
'Nougat'	c	c	a	a	g	c	f	f	b
'Open'	a	i	a	a	c	b	m	b	g
'Orca'	a	a	a	d	d	c	o	h	l
'Orepi'	a	a	c	a	g	c	f	f	b
'Ouest'	c	c	a	d	g	c	o	f	b
'Pactole'	c	b	a	d	g	c	o	f	b
'Pandas'	a	c	a	a	i	c	a	m	b
'Pepital'	c	d	d	d	d	c	b	h	l
'Pernel'	a	b	d	a	g	a	b	f	a
'Petrel'	c	a	d	d	h	c	o	d	j
'Pistou'	c	c	a	a	g	c	f	f	b
'Poncheau'	c	a	d	a	g	c	f	f	b
'Priam'	c	b	a	d	g	c	o	f	b
'Prinqual'	b	i	a	ef	g	a	f	c	b
'Promentin'	c	d	c	a	j	c	f	l	b
'Proqual'	c	b	c	b	g	c	x?	f	b
'Protinal'	c	b	d	a	c′	c	f	b	b
'Qualital'	b	c	d	d	g	c	o	f	b
'Radja'	b	b	a	e	b′	b	m	b	b
'Real'	c	b	a	d	g	c	o	f	b
'Recital'	b	d	d	d	g	c	o	f	b
'Rempart'	a	b	b	a	i	b	k	m	g
'Renan'	b	b	d	a	c	b	f	b	g
'Rescler'	b	c	d	ef	c′	c	f	b	b
'Resistente'	c	s	a	a	c	c	a	m	b
'Rex'	a	c	d	a	b′	c	f	b	b
'Riband'	c	d	a	d	f	c	o	g	b
'Riol'	c	d	a	a	c	c	x?	b	b

Table 1. (*continued*)

Cultivars	HMW-GS			LMW-GS			ω-Gliadins		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*
'Rita'	b	c	d	a	b	b	f	b	g
'Rivoli'	c	c	a	a	b	c	f	b	b
'Roazon'	c	a	a	d	b	c	o	b	b
'Rossini'	a	b	a	ef	b	c	b	b	b
'Rotonde'	c	a	a	ef	c	c	x?	f	b
'Royal'	c	a	d	d	b	c	o	b	b
'Rudi'	c	a	a	d	d	c	o	h	b
'Runar'	b	b	d	d	b′	b	o	b	b
'Rurik'	c	d	d	a	g	c	f	f	b
'Ruso'	a	c	d	e	i	a	m	h	b
'Sabre'	c	b	a	a	g	c	f	e	b
'Salmone'	a	c	a	a	c	c	a	s	b
'Scipion'	c	b	a	d	c	a	o	b	b
'Score'	c	d	a	a	g	c	f	f	b
'Sensor'	b	d	d	ef	j	c	f	l	b
'Sideral'	c	c	a	d	g	c	o	f	b
'Soissons'	b	b	d	a	b′	c	k	b	b
'Storch'	a	b	a	d	g	c	o	f	b
'Talent'	c	c	c	a	g	c	f	f	b
'Tango'	c	c	a	d	g	c	o	f	b
'Tapio'	c	c	d	a	b	a	a	b	b
'Tarasque'	b	c	d	d	b	b	o	b	b
'Tarquin'	c	d	a	a	j	c	f	l	b
'Thesee'	c	d	a	a	g	c	f	f	b
'Titien'	c	c	d	d	i	c	o	m	b
'Top'	c	b	b	d	g	c	o	f	b
'Tremie'	c	d	b	ef	c	c	b	b	b
'Ulm'	b	d	d	a	g	c	k	f	b
'Unic'	c	d	a	ef	j	c	b	l	b
'Vasco'	a	a	a	d	j	c	o	l	b
'Vicking'	c	d	c	d	d	c	o	h	b
'Vizir'	c	a	a	ef	g	c	b	f	b
'Voyage'	c	d	b	ef	j	c	m	l	b

set of 200 wheat cultivars studied. 3, 8 and 5 alleles were identified at *Glu-A1*, *Glu-B1* and *Glu-D1* loci respectively. This diversity is slightly different from that registered in 1985, when alleles *Glu-B1* e and l (subunits 20 and 14–19 respectively) were described in some French cultivars (Branlard and Le Blanc 1985). A total of twenty alleles encoding LMW-GS were found in the collection, 5, 11 and 4 alleles corresponded to *Glu-A3*, *Glu-B3* and *Glu-D3* loci respectively, and are shown in Figure 1. Some alleles were particularly hard to identify. This was the case at *Glu-A3f*, which encodes a subunit often hidden by LMW-GS encoded at *Glu-B3*. As *Glu-A3f* was difficult to distinguish from *Glu-A3e* (''null'' allele), the two were combined. Most of the LMW-GS alleles found in the French material have been described previously. The new *Glu-B3b′* allele, first detected by Jackson et al. (1996), was also found in French wheats. In addition, a new allele named *Glu-B3c′* (Figure 1 lane 6) was identified in 10 out of 183 wheat cultivars. The schematic presentation of the subunits characterising the different alleles encoded at *GluA3*, *Glu-B3* and *Glu-D3* is shown in Figure 2. Although we used several standard cultivars previously described by other groups, two of them exhibited differences: Brimstone, which was reported to have the Glu-D3c allele (Jackson et al. 1996), was seen to contain the Glu-D3d allele in our sample; and Insignia, which was reported to carry the Glu-A3e allele (Gupta and Shepherd 1990a), had Glu-A3b in our sample.

The wheat collection analysed exhibited a wide diversity of ω-gliadins as evidenced by the fact that 7, 13 and 9 alleles were found at *Gli-A1*, *Gli-B1* and *Gli-D1* respectively. The gliadin bands that make up the different alleles revealed in SDS PAGE are shown

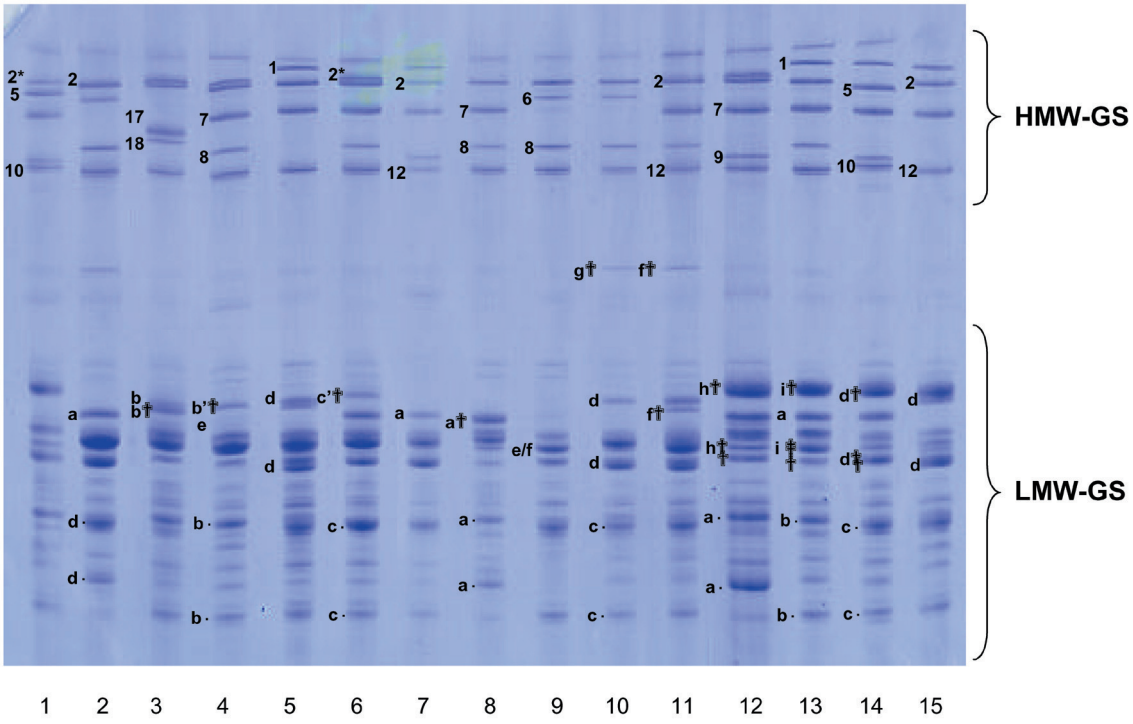

Figure 1. SDS-PAGE separation of the glutenin subunits found in some bread wheat varieties. 1- 'Nisu'; 2- 'Brimstone'; 3- 'Gabo'; 4- 'Radja'; 5- 'Copain'; 6- 'Courtot'; 7- 'Salmone'; 8- 'Chinese Spring'; 9- 'Clément'; 10- 'Arche'; 11- 'Albatros'; 12- 'Democrat'; 13- 'Rempart'; 14- 'Chopin'; 15- 'Orca'

in Figure 3. The *Gli-A1a,b,c,d* alleles, which were not identified using SDS PAGE, were identified using Acid PAGE. We gave the name *Gli-A1b* to the only type we found using SDS PAGE. In four cultivars it was impossible to attribute the *Gli-A1* allele named x? with certainty (Table 1). Acid PAGE showed three of the cultivars had either alleles *Gli-A1f* or *GliA1i* (Metakovsky and Branlard 1998). Alleles *Gli-B1k* and *Gli-B1m* could not be distinguished using SDS-PAGE and were named *Gli-B1m*.

The allelic composition of HMW-GS, LMW-GS and ω-gliadin loci in each of the 200 wheats grown primarily in France is shown inTable 1. The diversity of French cultivars evaluated with gliadin alleles encoded at six loci has been reported to be high (Metakovsky and Branlard 1998). In our study, the

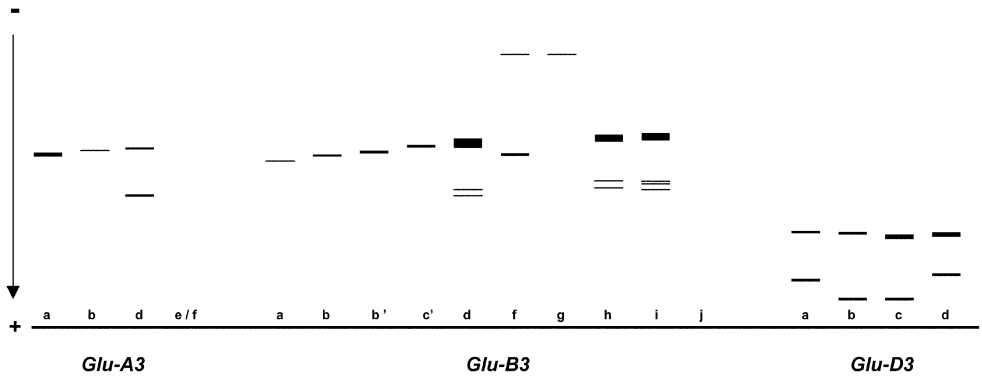

Figure 2. Schematic representation of the mobility on SDS PAGE of the different LMW-GS alleles encoded at *Glu-3* loci found in the 200 wheat cultivars studied.

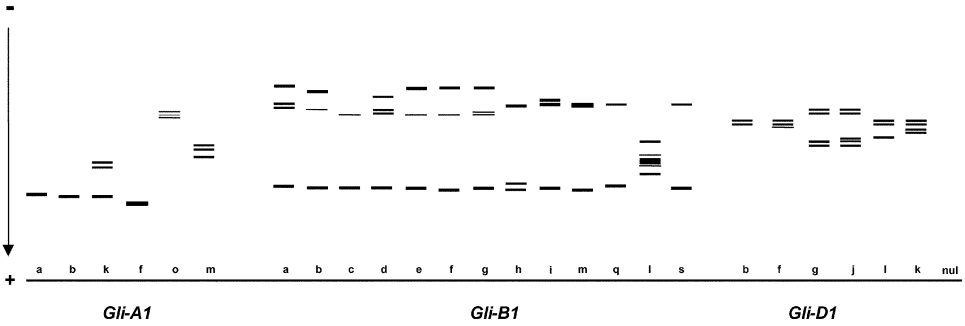

Figure 3. Schematic representation of the mobility on SDS PAGE of the different ω-gliadin alleles, encoded at Gli-1 loci found in the 200 wheat cultivars studied

genetic index for the six glutenin loci was H = 0.569. This figure, which is lower than the genetic index calculated for the six gliadin loci (H = 0.714), is still high considering that only part of the genetic diversity is revealed by SDS-PAGE. It is well known that in gliadin analysis, Acid PAGE is a better tool than SDS-PAGE to reveal mutations that affect the size and/or charge of these proteins. However SDS PAGE of glutenins and gliadins provides an easy tool for the allelic identification of storage proteins encoded at loci on group 1 chromosomes. And in fact, many genotypes can be distinguished using SDS PAGE. A total of 147 patterns were obtained in our collection with the alleles encoded at *Glu-1* and *Glu-3* loci. The maximum number of patterns that could be obtained from the all combinations of the alleles encountered at these six independent loci would be 3×8×5×5×11×4 = 24600. A total of 149 different patterns were obtained at the *Glu-1* and *Gli-1* loci in our collection of 200 cultivars. This figure is lower than that reported for gliadin analysis (loci *Gli-1* and *Gli-2*), where 173 genotypes out of 187 were distinguished using Acid PAGE (Metakovsky and Branlard 1998).

Allele frequencies

Several peculiarities can be mentioned in connection with HMW-GS alleles. The ''null'' *Glu-A1c* allele is very frequently encountered in French wheat (Table 2). This is almost certainly due to the French baking requirement for medium-elastic dough rather than strong gluten that results in less extensible dough. Some HMW-GS were rare, i.e. subunits 13–16, (allele *Glu-B1f*); subunits 20x–20y, (*GluB1e*); subunits 18*, (*GluB1s*); subunits 2–11, (*GluD1e*) each of which was found in only one cultivar. In comparison with HMW-GS frequencies previously found in 195 French bread wheats (Branlard and Le Blanc 1985), several alleles are now encountered more frequently, and is particularly true in the case of alleles known to have a favourable effect on dough properties: *Glu-B1b* (subunits 7–8), *Glu-B1c* (7–9) and *Glu-B1d* (5–10). The frequency of other alleles that have a negative effect on dough strength has decreased since the previous analysis, i.e. *Glu-B1a* (subunit 7) and *Glu-D1b* (3–12). However alleles *Glu-B1d* (6–8) and *Glu-D1a* (2–12) were present in 24.5 % and 53 % of the cultivars respectively. The low tensile strength and high extensibility found in the two last alleles are characteristics that are preferred not only for biscuit making but also for some wheats that are blended before milling for use in bread making. Although no extensive analysis has been reported on LMW-GS alleles in the French cultivars, some characteristics are worth mentioning. Alleles *Glu-A3a*, *Glu-A3d*, *Glu-B3g* and *Glu-D3c* were found in 44.5 %, 34.5 %, 50.5 % and 78 % of the cultivars respectively. These high frequencies may be the result of pedigree relatedness between cultivars. However some of these alleles were significantly correlated with the rheological properties of dough (Branlard et al. 2001). Although none were directly selected using SDS PAGE, but rather using conventional technological tests, these high frequencies may be due to their effect on bread-making quality. This is particularly true in the case of alleles *Glu-A3a* and *Glu-A3d*, which have a positive effect on dough properties (strength and extensibility) as well as to the Zeleny value. Allele *Glu-B3g* was also reported to be favourable for dough extensibility. The high frequency of *Glu-D3c*, which has been reported to have an unfavourable effect on dough properties, merits further investigation but could be the result of its frequent occurrence in the progenitors of existing French wheats.

Since the *Glu-3* and *Gli-1* loci are tightly linked on the short arm of chromosomes 1A, 1B and 1D, as

Table 2. Allele frequencies of HMW-GS, LMW-GS and ω-gliadins studied by SDS-PAGE in French bread wheat cultivars.

Locus	Allele	HMW-GS	Frequency	%
Glu-A1	a	1	30	15.0
	b	2*	31	15.5
	c	Null	139	69.5
Glu-B1	a	7	41	20.5
	b	7 + 8	61	30.5
	c	7 + 9	38	19.0
	d	6 + 8	49	24.5
	e	20	1	0.5
	f	13 + 16	1	0.5
	i	17 + 18	8	4.0
	s	18*	1	0.5
Glu-D1	a	2 + 12	106	53.0
	b	3 + 12	10	5.0
	c	4 + 12	14	7.0
	d	5 + 10	69	34.5
	e	2 + 11	1	0.5

Locus	Allele	Frequency	%
Glu-A3	a	89	44.5
	b	3	1.5
	d	69	34.5
	e	2	1.0
	e/f	37	18.5
Glu-B3	a	1	0.5
	b	21	10.5
	b′	8	4.0
	c	16	8.0
	c′	11	5.5
	d	7	3.5
	f	14	7.0
	g	98	49.0
	h	2	1.0
	i	9	4.50
	j	13	6.50
Glu-D3	a	23	11.50
	b	20	10.0
	c	156	78.0
	d	1	0.5
Gli-A1	a	14	7.0.
	b	24	12.0
	f	63	31.5
	k	17	8.5
	m	9	4.5
	o	69	34.5
	x ?	4	2.0
Gli-B1	a	1	0.5
	b	49	24.5
	c	1	0.5
	d	3	1.5
	e	9	4.5
	f	93	46.5
	g	11	5.5
	h	8	4.0
	i	1	0.5
	l	13	6.5

Table 2. (*continued*)

Locus	Allele	Frequency	%
	m	8	4.0
	q	2	1.0
	s	1	0.5
Gli-D1	a	1	0.5
	b	171	85.5
	f	4	2.0
	g	8	4.0
	i	1	0.5
	j	4	2.0
	k	3	1.5
	l	7	3.5
	null	1	0.5

Table 3. Probability associated to the Chi-square test of independence between alleles found at the nine loci encoding HMW-GS (*Glu-A1*, *Glu-B1*, *Glu-D1*), LMW-GS (*Glu-A3*, *GluB3*, *Glu-D3*) and ω-gliadins (*Gli-A1*, *Gli-B1*, *Gli-D1*).

Glu-B1	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-A1*	*Gli-B1*	*Gli-D1*	Locus
0.007	0.005	0.207	0.007	0.001	0.782	0.009	0.028	*Glu-A1*
	0.511	0.000	0.001	0.002	0.002	0.000	0.000	*Glu-B1*
		0.436	0.881	0.050	0.000	0.243	0.000	*Glu-D1*
			0.014	0.184	0.000	0.000	0.000	*Glu-A3*
				0.177	0.001	0.000	0.000	*Glu-B3*
					0.004	0.058	0.000	*Glu-D3*
						0.093	0.003	*Gli-A1*
							0.000	*Gli-B1*

expected, strong associations were observed between each locus (Table 3). However at the *Glu-1* and *Glu-3* (*or Gli-1*) loci, which are located on the long and short arms of chromosome 1 respectively and are considered to be genetically independent, some allelic associations were highly significant in French wheat cultivars. This was particularly true in the case of associations between the alleles encoded at *Glu-B1* and the alleles at most of the other loci studied as well as between the majority of ω-gliadin loci and other loci (Table 3). These associations are mainly the consequence of pedigree relatedness between cultivars. The absence of genetic recombinations could also be the result of selection pressure for specific agronomic and environmental conditions where favourable linkages are prevalent.

Conclusion

The allelic identification of LMW-GS in French wheat cultivars revealed considerable diversity. It would be possible to create at least 220 cultivars with different LMW-GS composition using the alleles found in our study material. Even greater diversity was obtained when combining only the alleles at *Gli-1* loci. Analysis of glutenins and gliadins alleles is known to be a powerful tool for genotyping genetic resources. Due to the difficulty in identifying some LMW-GS and gliadin alleles, this particular tool has not been widely used. However in addition to genetic resources, allelic identification of glutenins and gliadins is very important for improving wheat quality. Many favourable alleles encoded at *Glu-1 Glu-3* and *Gli-1* loci are worth analysing before crossing selected parents and cumulating them by pyramidal breeding. Analysis of allelic variants of storage proteins appears to be of prime importance to obtain molecular probes and make micro-arrays for use in high-density genotyping. In addition to these future developments, the characterisation of allelic variants will be very helpful in increasing our understanding of: (1) the protein interactions that take place in the formation of gluten; (2) the influence of the environment on the quantitative expression of storage proteins, particularly using a proteomic approach; (3) the contrast between dough properties (as well as bread-making quality), that sometime occurs in different wheat varieties.

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