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Diversity of Seven Glutenin and Secalin Loci within Triticale Cultivars Grown in France

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Abstract

Although the endosperm storage protein of hexaploid triticale have already been analysed, the allelic diversity of glutenins and secalins remains to be described. Analysis by SDS-PAGE of the majority of hexaploid triticales (69 cultivars) grown in France allowed to identify 36 alleles at seven loci: *Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-B2, Glu-A3* and *Glu-B3*. *Glu-B1* and *Glu-B3* loci were the most polymorphic with 9 alleles each. On the basis of allelic frequencies at the seven loci, genetic distances between hexaploid triticales grouped according to their origins revealed two groups: winter triticales mostly originating from European germplasm and spring triticales essentially of CIMMYT origin. Comparison of allele frequencies between hexaploid triticale cultivars and a world collection of bread (*Triticum aestivum*) and durum (*Triticum durum*) wheat was investigated at *Glu-A1* and *Glu-B1*: only a significant association was found for *Glu-A1* alleles ($\chi^2 = 2.26$, $p = 0.36$) between triticale and bread wheat.

Keywords: polymorphism, rye, storage proteins, triticale, wheat

Introduction

Genetic characterization of storage proteins in the parental species of triticale: hexaploid wheat, tetraploid wheat and rye has been carried out and structural genes of high molecular weight (HMW) glutenins and HMW secalins have been assigned to loci on the long arms of chromosomes 1A (*Glu-A1*) (Lawrence and Shepherd, 1980), 1B (*Glu-B1*) (Bietz *et al.,* 1975), 1D (*Glu-D1*) (Orth and Bushuk, 1974) and 1R (Glu-*R1* or *Sec-3*) (Lawrence and Shepherd, 1981). The gliadins are known to be controlled by genes *Gli-1* and *Gli-2* located on the short arms of groups 1and 6 of chromosomes A, B and D (Shepherd, 1988) respectively, whereas one omega-secalin and two 40K gamma-secalins are coded at loci *Gli-R1* (or *Sec-1*) (Shepherd, 1968), two omega-secalins at *Gli-R3* (or *Sec-4*) (Carrillo *et al.,* 1992) on chromosome 1RS and 75K gamma-secalins at *Gli-R2* (or *Sec-2*) on chromosome 2RS, (Shewry *et al.,* 1984). The low molecular weight (LMW) glutenins are encoded at *Glu-3* loci (Singh, 1985) and closely linked to *Gli-1* loci (Jackson *et al.,* 1983). In contrast of these two parental species, wheat and rye, their combining product, triticale hybrid, has yet to be investigated sufficiently in this way. A study of unreduced prolamin fractions of eu-and alloplasmatic octoploid triticale forms was published using acid-polyacrylamid gel electrophoresis (A-PAGE) and a nomenclature of the prolamin band patterns of the triticale forms was suggested (Rozinek *et al.,* 1998). Another study was undertaken by Brzezinski and Lukaszewski (1998) who screened a set of 139 lines of hexaploid winter triticale from breeding programs in Europe and North America for their allelic composition at the *Glu-A1, Glu-B1, Glu-R1* and *Gli-R2* loci by sodium dodecyl sulphate-polyacrylamid gel electrophoresis (SDS-PAGE) following Laemmli (1970). They found a high heterogeneity with up to seven different electrophoretic variants per line. Among uniform lines, they were able to localize some secalin bands but their allelic forms were not defined. In response to this lack of information, Amiour *et al.* (2001b) analysed offsprings of ten crosses of hexaploid triticale and identified allelic forms at *Glu-1* and *Glu-3* loci encoding for high molecular weight (HMW) of glutenin and secalin subunits and low molecular weight (LMW) of glutenin subunits respectively.

Storage proteins are important in wheat; they play a major role in breadmaking quality due to their ability to form gluten. Many studies have dealt with this topic (Payne, 1981; Branlard and Dardevet, 1985; Payne, 1987 and for a review see MacRitchie, 1999). Storage proteins are also considered as useful genetic markers and have been widely used for cultivar identification in wheat (Autran and Bourdet, 1975; Pogna *et al.,* 1985; Branlard and Le Blanc, 1985; Vallega and Waines, 1987; Branlard *et al.,* 1989; Metakovsky and Branlard, 1998; Igrejas *et al.,* 1999a). Rye storage proteins are unable to develop a cohesive viscoelastic gluten (Kipp *et al.,* 1996). In addition, its allogamic system makes the identification of cultivars using storage proteins difficult. Consequently, prolamins

of this species have not been investigated to the same extent as those of wheat. Triticale has two forms: primary and secondary one. Primary triticale is the amphiploid derived from the hybridisation of wheat and rye. Secondary triticale is mostly derived from crosses between primary triticales. The secondary hexaploid triticale with the three genomes: A and B from wheat and R from rye is most frequently cultivated. Triticale occupies an intermediate position with a breadmaking quality inferior of that of wheat. Studies have mainly focused on cytogenetics, yield potential and agronomic traits. Nevertheless, some experiments have been undertaken trying to improve its breadmaking quality (Lukaszewski *et al.,* 1987; Hohmann, 1988; Kazman and Lelley, 1996), some others dealt with protein variability (Rubio *et al.,* 1996; Brzenski *et al.,* 1998; Igrejas *et al.,* 1999b).

The aim of the present work was to analyse the genetic diversity of these proteins in a set of hexaploid triticale cultivars grown in France. The allelic diversity observed at the *Glu-1*, *Gli-2* and *Glu-3* loci allowed us to compare our hexaploid triticale cultivars grown in France with other European triticale cultivars according to their country of breeding. On the basis of allelic frequencies at the *Glu-1* loci described in the three species triticale, bread and durum wheat, the involvement of these two latter species in the origin of French triticale is discussed

Material and methods

Material

It has been analysed 69 cultivars of secondary hexaploid triticale grown in France and registered in the official French Catalogue (Tab. 1). Most of the cultivars registered were kindly provided by GEVES (Groupement Expérimentation Variétale et Etude des Semences), France. The cultivars were obtained with kind permission from INRA of Clermont Ferrand, France.

Methods

Protein extraction and SDS-PAGE

Proteins were extracted from a single half seed using the sequential procedure of Singh *et al.* (1991). Sodium Dodecyl Sulfate Polyacrylamid gel Electrophoresis (SDS-PAGE) was performed according to Singh *et al.* (1991).

Protein nomenclature

The nomenclatures of Payne and Lawrence (1983) and Vallega and Waines (1987) were adopted for HMW glutenin subunits. Gupta and Shepherd (1990) and Jackson *et al.* (1996) bread wheat nomenclatures were used for LMW glutenin subunits. For *Glu-R1* and *Gli-R2* loci and the allelic forms at the other *Glu-* loci, the nomenclature proposed by Amiour *et al.* (2001b) was used.

Tab. 1. Allelic composition of the seven following loci *Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-A3, Glu-B3,Glu-B2* respectively, found in 69 triticale grown in France

1) a, a, a, c, d, h, b	23) b, b, c, a, e, b, b	45) c, a, a, c, e, b, b
'Fleurus'	'Alimac'	'Vivero'
2) a, b, a, a, a, i, b	24) b, b, c, c, d, d, b	46) c, a, a, c, d', k, b
'Partout'	'Agrilac'	'Amarillo'
3) a, b, c, c, a, h', b	25) b, b, c, c, a, k, b	47) c, a, a, c, e, d, b
'Auriac'	'Triveder'	'Ragtac'
4) a, b, c, c, a, i, b	26) b, b, d, c, a, h', b	48) c, a, b, c, a, i, b
'Sw Morado'	'Rhino'	'Osorno'
5) a, d, d, a, a, i, b	27) b, b, d, c, e, k, b	49) c, a, c, c, d, d, b
'Grandual'	'California'	'Beauval'
6) a, k, b, d, d, h, b	28) b, b, e, c, d, d, b	50) c, a, e, c, a, d, b
'Tridoc'	'Trigone'	'Maximal'
7) a, r, b, c, e, k, b	29) b, d, b, c, a, h', b	51) c, b, b, c, a, d, b
'Lupus'	'Balzac'	'Automnal'
8) a, s, c, c, d, d, b	30) b, d, c, a, d-f, j, b	52) c, b, c, c, d, k, b
'Espoir'	'Trouvere'	'Magnat'
9) b, a, c, c, d, k, b	31) b, d, c, c, d, b, b	53) c, b, c, a, a, h, b
'Kortego, Passo'	'Aprim'	'Precocius'
10) b, a, c, c, a, d, b	32) b, d, c, c, d, i', b	54) c, b, c, c, a, h', b
'Cedro, Sw Midelo'	'Trignac'	'Collegial'
11) b, a, c, a, a, d, b	33) b, d, d, c, a, b, b	55) c, b, c, c, a, d, b
'Trilogie, Triptic'	'Stimulus'	'Gwendal'
12) b, a, c, c, a, h', b	34) b, f, c, c, d', d, b	56) c, b, c, c, d', h', b
'Triplus'	'Constant'	'Seconzac'
13) b, a, c, d, a, d, b	35) b, k, c, c, e, k, b	57) c, b, c, c, f, d, b
'Trivial'	'Lamberto'	'Innoval'
14) b, b, a, c, e, b, b	36) b, p, b, c, a, i, b	58) c, b, e, c, a, d, b
'Puerto'	'Delgado'	'Pivot'
15) b, b, a, c, d, k, b	37) b, r, c, c, d, k, b	59) c, c, b, c, a, h', b
'Triskell'	'Bienvenu'	'Kansas'
16) b, b, b, c, a, h', b	38) b, r, c, c, a, h, b	60) c, d, c, b, e, i, b
'Bellac'	'Bilbao'	'Matinal'
17) b, b, c, c, d, b, b	39) b, r, c, c, a, h', b	61) c, d, c, c, a, h, b
'Triade, Protignac'	'Blenio'	'Tanguy'
18) b, b, c, a, f, j, b	40) b, r, c, c, a, d, b	62) c, d, d, c, a, d, b
'Tremplin'	'Sw Talentro'	'Diwann'
19) b, b, c, c, d', j, b	41) b, s, a, c, d, k, b	63) c, k, e, c, d', d, b
'Wilfried'	'Beneficio'	'Tritikon'
20) b, b, c, c, a, i, b	42) b, s, c, a, d, h', b	64) c, p, d, c, a, i, b
'Sw Falmoro'	'Trimour'	'Bedretto'
21) b, b, c, a, a, h', b	43) b, s, c, c, a, i', b	65) c, r, c, d, d, k, b
'Floirac'	'Ticalo'	'Raboliot'
22) b, b, c, c, a, b, b	44) b, s, c, c, e, b', b	
'Grenado'	'Benetto'	

Statistics

Genetic distances between groups of cultivars were computed using Statistica-6 software. Comparison of allelic frequencies was performed using the χ^2 test.

Results and discussion

Diversity of glutenin subunits and secalin patterns

Most of triticale cultivars were homogenous, only 'Trouvere' showed two diagrams in LMW glutenin subunits. Extensive variability was observed for the HMW glutenins and secalins (Fig. 1).

A total of 17 HMW subunits and four of 75K gamma secalins were revealed from the analysis of different triticales. The majority of the sixty-nine cultivars analysed possessed four to five bands and 42 types of patterns were determined (Tab. 2).

Each diagram was encountered in one to seven cultivars. Concerning the B-LMW subunits, a large variability in patterns was detected (Fig. 1) and 25 diagrams were listed (Tab. 3). The number of cultivars for each diagram of B-LMW subunits varied from one to eleven.

The hexaploid triticale, which is composed from three genomes, has glutenins and secalins which had not as yet been analysed. Although some progenies had rather complex polymorphism, we were able to screen the variability of glutenin subunits and particularly the LMW glutenins encoded on *Glu-A3* and *Glu-B3*.

On the other hand, HMW glutenins and HMW secalins were easily screened. 75K gamma-secalins, encoded at *Gli-R2*, were composed by two or three bands: t1 corresponded to the triplet bands and d1 and d2 corresponded to two double bands. The majority of cultivars (79.7%) possessed the triplet t1 (*Gli-R2c*). Only one cultivar, 'Matinal', possessed the two double bands d2 (*Gli-R2b*) and three cultivars, 'Tridoc', 'Trivial' and 'Raboliot', show absence of secalins (*Gli-R2d*).

The results showed that the greatest polymorphism of storage proteins in hexaploid triticale was on chromosome

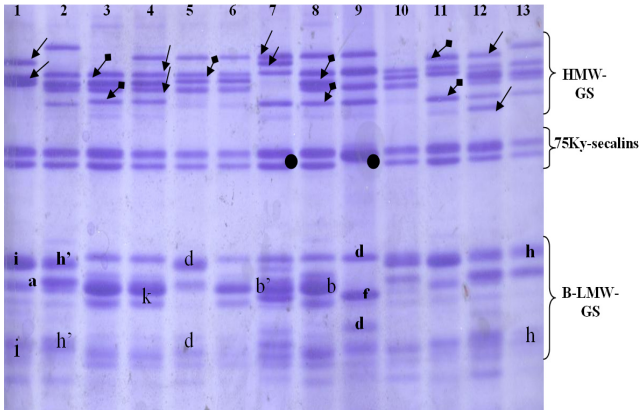

Fig.1. Diversity of storage proteins of triticales grown in France revealed by SDS-PAGE. 1. 'Osorno'; 2. 'Auriac'; 3. Dagro, 4. 'Bienvenu'; 5. Cedro; 6. Passo; 7. Domital; 8. Triade; 9. 'Trouvere' (heterogeneous); 10. 'Beauval'; 11. Spatial; 12. 'Bedretto'; 13. 'Bilbao'. (lanes 3, 7 and 11 triticale cheks).← *Glu-R1,* ↔*Glu-B1,* ●*Gli-R2*

Tab. 2. Allelic composition of HMW glutenin subunits, HMW secalin subunits and 75 gamma-secalins of hexaploid triticale

Cultivar	loci			
	Glu-A1	*Glu-B1*	*Glu-R1*	*Gli-R2*
1) 'Fleurus'	1	7	1r-4r	t1
2) 'Partout'	1	(7-8)	1r-4r	d1
3) 'Auriac', 'Sw Morado'	1	(7-8)	6r-13r	t1
4) 'Grandual'	1	(6-8)	2r-9r	d1
5) 'Tridoc'	1	22	2r-6.5r	null
6) 'Lupus'	1	(7-18)	2r-6.5r	t1
7) 'Espoir'	1	6.8-20y	6r-13r	t1
8) Kortego, Cedro, Passo, Sw Midelo, 'Triplus'	2*	7	6r-13r	t1
9) 'Trilogie, Triptic'	2*	7	6r-13r	d1
10) 'Trivial'	2*	7	6r-13r	null
11) 'Puerto', 'Triskell'	2*	(7-8)	1r-4r	t1
12) 'Bellac'	2*	(7-8)	2r-6.5r	t1
13) Triade, 'Wilfried', 'Sw Falmoro', 'Grenado', 'Agrilac', Protignac, 'Triveder'	2*	(7-8)	6r-13r	t1
14) 'Tremplin', 'Floirac', 'Alimac'	2*	(7-8)	6r-13r	d1
15) 'Rhino', 'California'	2*	(7-8)	2r-9r	t1
16) 'Trigone'	2*	(7-8)	6.5r	t1
17) 'Balzac'	2*	(6-8)	2r-6.5r	t1
18) 'Trouvere'	2*	(6-8)	6r-13r	d1
19) 'Aprim', 'Trignac'	2*	(6-8)	6r-13r	t1
20) 'Stimulus'	2*	(6-8)	2r-9r	t1
21) 'Constant'	2*	(13-16)	6r-13r	t1
22) 'Lamberto'	2*	22	6r-13r	t1
23) 'Delgado'	2*	(7-8-9)	2r-6.5r	t1
24) 'Bienvenu', 'Bilbao', 'Blenio' Sw Talentro	2*	(7-18)	6r-13r	t1
25) 'Beneficio'	2*	6.8-20y	1r-4r	t1
26) 'Trimour'	2*	6.8-20y	6r-13r	d1
27) 'Ticalo', 'Benetto'	2*	6.8-20y	6r-13r	t1
28) 'Vivero', 'Amarillo', 'Ragtac'	null	7	1r-4r	t1
29) 'Osorno'	null	7	2r-6.5r	t1
30) 'Beauval'	null	7	6r-13r	t1
31) 'Maximal'	null	7	6.5r	t1
32) 'Automnal'	null	(7-8)	2r-6.5r	t1
33) 'Magnat', 'Collegial', 'Gwendal', 'Seconzac', 'Innoval'	null	(7-8)	6r-13r	t1
34) 'Precocius'	null	(7-8)	6r-13r	d1
35) 'Pivot'	null	(7-8)	6.5r	t1
36) 'Kansas'	null	(7-9)	2r-6.5r	t1
37) 'Matinal'	null	(6-8)	6r-13r	d2
38) 'Tanguy'	null	(6-8)	6r-13r	t1
39) 'Diwann'	null	(6-8)	2r-9r	t1
40) 'Tritikon'	null	22	6.5r	t1
41) 'Bedretto'	null	(7-8-9)	2r-9r	t1
42) 'Raboliot'	null	(7-18)	6r-13r	null

Tab. 3. Allelic composition of LMW glutenin subunits of hexaploid triticale

Cultivars	Loci		
	Glu-A3	*Glu-B3*	*Glu-B2*
1) 'Stimulus', 'Grenado'	a	b	b
2) 'Cedro', Trilogie, 'Automnal', 'Sw Midelo', 'Sw Talentro', 'Triptic', 'Trivial', 'Diwann', 'Maximal', 'Pivot', 'Gwendal'	a	d	b
3) 'Bilbao', 'Precocius', 'Tanguy'	a	h	b
4) 'Kansas', 'Rhino', 'Bellac', 'Auriac', 'Blenio', 'Balzac', 'Floirac', 'Triplus', 'Collegial'	a	h'	b
5) 'Partout', 'Osorno', 'Bedretto', 'Sw Falmoro', 'Sw Morado', 'Grandual', 'Delgado'	a	i	b
6) 'Ticalo'	a	i'	b
7) 'Triveder'	a	k	b
8) 'Triade', 'Aprim', 'Protignac'	d	b	b
9) 'Beauval', 'Agrilac', 'Espoir', 'Trigone'	d	d	b
10) 'Trouvere'	d-f	j	b
11) 'Tridoc', 'Fleurus'	d	h	b
12) 'Trimour'	d	h'	b
13) 'Trignac'	d	i'	b
14) 'Raboliot', Kortego, 'Magnat', 'Bienvenu', 'Passo', 'Triskell', 'Beneficio'	d	k	b
15) 'Tritikon', 'Constant'	d'	d	b
16) 'Seconzac'	d'	h'	b
17) 'Wilfried'	d'	j	b
18) 'Amarillo'	d'	k	b
19) 'Alimac', 'Puerto', 'Vivero'	e	b	b
20) 'Beneto'	e	b'	b
21) 'Ragtac'	e	d	b
22) 'Matinal'	e	i	b
23) 'Lamberto', 'Lupus', 'California'	e	k	b
24) 'Innoval'	f	d	b
25) 'Tremplin'	f	j	b

1B with 9 allelic forms at the loci *Glu-B1* and *Glu-B3*. The most frequent alleles at *Glu-B1* locus were '*b*', coding subunits 7-8 (39.1%), and the allele '*a*' coding subunit 7 (21.7%). At *Glu-B3* locus the predominant alleles were '*d*' and '*k*' with 27.5% and 17.4% respectively. Variability was also important at *Glu-R1* with five allelic forms. The most frequent alleles at *Glu-R1* were '*c*' (62.3%), '*a*' *and* '*b*' (11.6%). At *Glu-A3* five alleles were observed with the predominance of '*a*' (49.3%). *Glu-A1* remains the least polymorphic locus with three alleles and '*b*' (subunit 2*) was the most frequent (58.0%).

Analysis of B-LMW zone was based on the nomenclature of Gupta and Shepherd (1990) and Jackson *et al.* (1996) who identified many allelic groups of B-LMW glutenins in hexaploid wheat. However, correspondence could be done between *Glu-B3d* according to Gupta and Shepherd (1990) and *Glu-B3a* encoding subunits 2-4-15-19 and identified in durum wheats by Nieto-Taladriz *et al.* (1997). Studies carried out on some Portugal triticales had revealed that B-LMW subunits of glutenin can be of *T. durum* type (Igrejas *et al.,* 1999).

Allelic diversity of glutenins and secalins

To study the genetic diversity of glutenin and secalin subunits, the proteins encoded at the following seven loci were considered: *Glu-A1, Glu-B1, Glu-R1, Gli-R2, Glu-A3* and *Glu-B2*. They are located on chromosome arms 1AL, 1BL, 1RL, 2RS, 1AS, 1BS respectively. Considering that individual seeds were analysed for each cultivar to determine the corresponding diagrams, only 1 out of 69 cultivars was heterogeneous (1%).

A total of 36 alleles were identified at the seven loci above. On the basis of the genetic diversity found at the seven loci, 65 patterns were established among the 69 triticale cultivars analysed (Tab. 1). 61 patterns were specific of one cultivar each.

At the *Glu-A1* locus, allelic distribution among the 69 cultivars was 58.0% and 30.4% for the two alleles '*b*' and '*c*' respectively and lower for allele '*a*' with 11.6%.

At the *Glu-B1* locus, alleles '*c*' encoding for subunits 7-9, '*f*' encoding for subunits 13-16 and '*p*' for subunits 7-8-9 were rare with respectively 1.4% and 2.9%. Otherwise, the most frequent alleles were *Glu-B1b* and *Glu-B1a* encoding for the 7-8 and 7 subunits with 39.1% and 21.7% respectively.

At *Glu-R1* locus, subunits 6^r-13^r encoded by *Glu-R1c* were predominant (62.3%). The less frequent alleles were *Glu-R1d* (2^r-9^r) observed only in six triticale cultivars (8.7%) and *Glu-R1e (6.5^r)* found only in four lines (5.8%).

At the *Gli-R2* locus we observed with low frequency one allele: *Gli-R2b* encoded only in one triticale cultivar (1.4%). The most frequently observed allele was *Gli-R2c* (t1) (79.7%). Moreover *Gli-R2a* was found in ten cultivars (14.5%) (Tab. 2). The 75K gamma-secalins encoded at the Gli-R2 locus were absent in 3 triticale cultivars (4.3%). This absence could be due to a possible *2D/2R* substitution in triticales. To answer this question, the triticale cultivars showing no 75K gamma-secalins will be checked using wheat microsatellite markers located on the chromosome 2D from wheat. The results will allow us to conclude that absence of 75K gamma-secalins in some triticales of the collection was due to the substitution *SD/2R* previously named *Gli-R2d*. Amplification of the microsatellites mapped on chromosome 2D also will indicate that no recombinant occurred between chromosomes 2D and 2R.

Using the bread wheat nomenclatures proposed by Gupta and Shepherd (1990) and Jackson *et al.* (1996) for LMW glutenin subunits, we were able to identify 15 LMW subunits of the 69 cultivars.

The *Glu-B3d* allele was frequent in the collection (27.5%). Otherwise, the less frequent alleles were the null

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Tab. 4. Allelic frequencies per locus per country where triticale cultivars originated

Locus	Origin and number of cultivars									
	FR	POL	GE	CH	SW	IT	PT	UK	SP	MX
	69	24	15	10	7	8	5	3	21	4
GLUA1										
a (1)	11.6	20.8	06.7	30.0	/	25.0	40.0	66.7	47.6	75.0
b (2*)	58.0	58.3	60.0	30.0	71.4	25.0	/	33.3	09.5	/
c (null)	30.4	20.8	33.3	40.0	28.6	50.0	60.0	/	42.9	25.0
GLUB1										
a (7)	21.7	/	/	/	/	/	/	/	/	/
b (7-8)	39.1	16.7	06.7	10.0	/	/	/	33.3	04.8	/
c (7-9)	1.4	04.2	/	/	/	/	/	/	04.8	/
d (6-8)	13.0	20.8	33.3	20.0	14.3	12.5	20.0	33.3	/	/
e (20)	/	/	/	/	/	/	/	/	04.8	/
f (13-16)	1.4	/	20.0	10.0	/	25.0	/	33.3	19.1	50.0
IV (23-18)	/	/	/	/	12.5	/	/	14.3	/	/
k (22)	4.3	/	/	/	/	/	/	/	/	/
p (7-8-9)	2.9	/	/	/	/	/	/	/	/	/
r (7-18)	8.7	50.0	06.7	30.0	42.8	37.5	/	/	19.1	25.0
s (6.8-20y)	7.2	08.3	33.3	30.0	42.8	12.5	80.0	/	33.3	25.0
GLUR1										
a (1r-4r)	11.6	12.5	06.7	10.0	28.6	/	/	/	/	/
b (2r-6.5r)	11.6	16.7	33.3	/	/	12.5	20.0	/	/	/
c (6r-13r)	62.3	66.7	40.0	80.0	71.4	75.0	80.0	66.7	76.2	50.0
d (2r-9r)	8.7	04.2	20.0	/	/	/	/	33.3	/	/
e (6.5r)	5.8	/	/	/	/	/	/	/	/	/
f (0.8r-6r)	/	/	/	10.0	/	12.5	/	/	19.1	50.0
g (5.8r)	/	04.8	/	/	/	/	/	/	/	/
GLIR2										
a (d1)	14.5	/	/	60.0	14.3	50.0	100.0	/	52.4	25.0
b (d2)	1.4	/	/	/	/	/	/	/	/	/
c (t1)	79.7	95.8	93.3	40.0	85.7	/	/	100.0	04.8	/
d (null)	4.3	/	06.7	/	/	50.0	/	/	42.9	75.0
e (t2)	/	04.2	/	/	/	/	/	/	/	/
GLUA3										
a	49.3	12.5	33.3	40.0	14.3	25.0	60.0	33.3	42.8	25.0
d	27.5	62.5	40.0	50.0	42.8	50.0	40.0	33.3	42.8	25.0
d'	7.2	/	/	10.0	/	25.0	/	/	14.4	/
e	13.0	20.8	26.7	/	28.6	/	/	/	/	50.0
f	2.9	04.2	/	/	14.3	/	/	33.3	/	/
GLUB3										
b	11.6	04.2	/	/	/	25.0	20.0	/	/	25.0
b'	1.4	16.7	20.0	/	14.3	12.5	20.0	/	28.6	/
d	27.5	08.3	33.3	80.0	/	25.0	60.0	33.3	47.6	75.0
h	7.2	4.2	/	/	/	25.0	/	33.3	14.3	/
h'	15.9	/	/	/	/	/	/	/	04.8	/
i	11.6	37.5	46.7	/	28.6	/	/	/	04.8	/
i'	2.9	/	/	/	/	/	/	33.3	/	/
j	4.3	/	/	/	/	/	/	/	/	/
k	17.4	29.1	/	20.0	57.1	12.5	/	/	/	/
GLUB2										
a	/	/	/	10.0	/	/	/	/	/	/
b	100.0	100.0	100.0	90.0	100.0	100.0	100.0	100.0	100.0	100.0

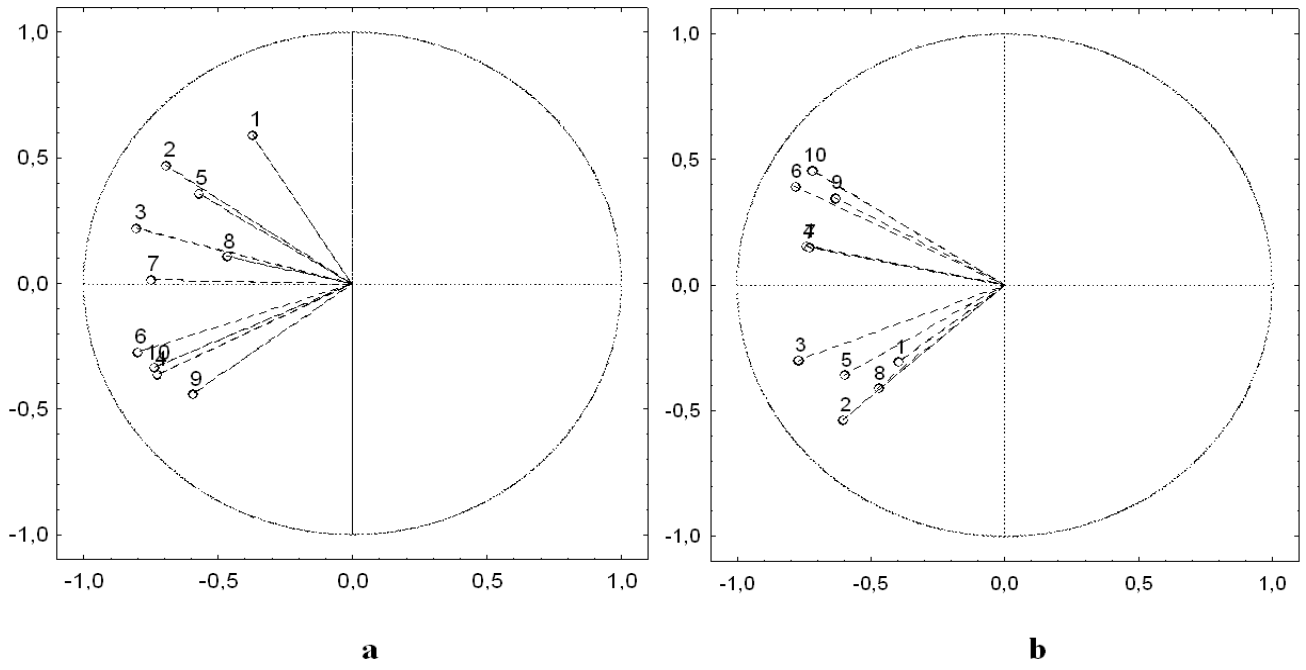

Fig. 2. Genetic distances between groups of cultivars originating from different countries including: a) The six following loci: *Glu-A1, Glu-B1, Glu-R1, Glu-A3, Glu-B3* and *Glu-B2*; b) the same loci plus *Gli-R2* locus. 1- France; 2- Poland; 3- Germany; 4- Switzerland; 5- Sweden; 6- Italy; 7- Portugal; 8- Great Britain; 9- Spain; 10-Mexico

allele '*j*' and the allele 'i'' which were observed only in three and two cultivars (4.3% and 2.9%) respectively.

Concerning *Glu-A3*, 76.8% of the collection analysed can be characterized by only 2 alleles: *Glu-A3a* and *Glu-A3d* found in 49.3% and 27.5% of the cultivars respectively. The three other alleles'*d*', *'e'* and '*f'* were less common. The *Glu-B2* locus was less polymorphic with one allele: the *Glu-B2b* which was predominantly present.

Genetic distances

Frequencies of the alleles encoded at seven loci were calculated for our triticale cultivars grown in France and for each country of origin of triticale cultivars tested by Amiour *et al.* (2001a) (Tab. 4).

These frequencies permit us to compute genetic distances between groups of cultivars according to their origins including our collection of triticale cultivars grown in France. The clustering was particularly influenced by two loci: *Gli-R2* and *Glu-B3*. Allelic variation at *Gli-R2* allowed us to distinguish 2 groups: group'1' characterized by allele *Gli-R2c* coding for triplet t1 and mainly present in British, Polish, German, Swedish and French cultivars (percentage between 79 and 100%) and group '2' where two forms were predominant: *Gli-R2a* coding for doublet d1 very common in Portuguese, Swiss, Italian and Spanish samples (between 50 and 100%) and the null form (absence of secalins named *Gli-R2d*) present only in five countries, particularly in Italian, Spanish and Mexican cultivars with high frequency (Tab. 4). A *Gli-R2* effect was evidenced in the comparison of the two graphics presented in (Fig. 2). Concerning *Glu-B3*, the effect was less obvious: we observed allele *'d'* more frequent in Swiss, Mexican, Portuguese and Spanish cultivars.

Our division into two groups agreed with the classification based on morphological and agronomic traits made on triticales by Royo (1995) and agreed with the classification made by Amiour *et al.* (2001a) based on the comparison between hexaploid triticale cultivars according to their country of breeding. Group '1' (Great Britain, Poland, Germany, Sweden and France) was developed from European germplasm, which is mostly of winter type. Group '2' characteristic to the south countries of Europe has two origins: Spanish cultivars derived from Iberian material using tetraploid wheat (Sanchez-Monge, 1996) and CIMMYT germplasm containing essentially spring type with two distinct pools: 'complete' triticale with complete rye genome and 'substituted' triticale with *2D/2R* substitution.

Comparison of allele frequencies

Although they are of a complex origin, most triticale cultivars have been developed from hexaploid and/or tetraploid (durum) wheat crossed with rye. In order to improve our understanding of triticale origin, we compared allelic frequencies at loci having the same allelic nomenclature for the two parental species of triticale: bread and durum wheat (Branlard *et al.,* 1989) (Tab. 5).

At the *Glu-A1* locus the three alleles were observed in the three species with a significant relationship between triticale and bread wheat (χ^2= 2.26, p=0.36). At *Glu-B1*

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Tab. 5. Comparison of allele frequencies at the *Glu-A1* and *Glu-B1* loci between triticale cultivars registered in the French Catalogue and a world collection of bread and durum wheats

Origin	Loci													
	GLU-A1			*GLU-B1*										
	a	b	c	a	b	c	d	e	f	k	p	r	s	IV
	1	2*	nul	7	7-8	7-9	6-8	20	13-16	22	7-8-9	7-18	6.8-20y	23-18
Triticale cultivars	11.6	58.0	30.4	18.2	36.4	1.1	15.9	/	2.3	3.4	2.3	10.2	10.2	/
Bread wheat cultivars[1]	28	28	44	19	20	27	19	5	1.5	/	/	/	/	/
Durum wheat cultivars[1]	7	4.6	83.5	0.8	25.9	/	23.6	33.5	5.5	/	/	/	/	3.4

1 From Branlard *et al.,* 1989

locus, 4 alleles out of 11 were common to the three species. Nevertheless, some particularities were observed: the very common allele of bread wheat *Glu-B1c* (7-9) which is absent in durum wheat and the allele *Glu-B1IV* (23-18) observed in durum wheat but not in bread wheat, were both present and absent in triticale respectively. In spite of this, there were no significant statistical relationships between the three species for *Glu-B1* locus.

As expected, the absence of any significant association between the three species for *Glu-B1* reflects the complex origin of this man-made cereal. However, the allelic identity outlined above could only be used to trace the origin of some triticale cultivars.

Conclusions

Analysis of hexaploid triticale has been a useful means of investigating the genetic diversity of these cereal storage proteins. In spite of its being a new species and therefore with a short existence, triticale shows a high polymorphism for its storage proteins. This is due to the genetic variability introduced by breeders via various routes in order to increase the agronomic performance of secondary triticale. Genetic diversity of HMW and LMW subunits of glutenins and secalins, as revealed by SDS-PAGE, enabled us to describe a large set of triticale cultivars grown in France. This allelic identification could be a powerful tool in studying the respective effects of glutenin and secalin subunits dealing with variations in the technological characteristics of triticale.

The frequency of alleles which correlate with good bread-making quality, *Glu-A1a, Glu-A1b* and *Glu-B1b* was high in our triticale cultivars. However, factors like low gluten content, inferior gluten strength mainly due to the lack of D genome, presence of R genome and high level of α-amylase make triticale often unsuitable for breadmaking quality (Varughese *et al.,* 1996). Efforts to solve these problems are undertaken in many triticale breeding programs. Genetic diversity of triticale storage proteins could be of a high interest in the breadmaking quality improvement. In addition, the numerous possible allelic combinations within the extent of variation observed would be useful tool for the identification of pure lines and hybrids. Other molecular markers would be very useful for tracing the genomic structure, particularly in triticales carrying substitutions and/or translocations.

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