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Genetic diversity of French common wheat germplasm based on gliadin alleles

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Abstract Analysis of gliadin electrophoretic (APAGE) patterns made it possible to identify 79 alleles at six *Gli-1* and *Gli-2* loci (from 9 to 18 per locus) and 173 gliadin genotypes in the 187 French common wheat cultivars considered. Six new alleles were registered in the catalogue of gliadin alleles. The genetic diversity of French common wheats was found to be high ($H = 0.714$) and had not changed much during the last 25–50 years. Analysis of genetic distances showed some gradual changes in French wheat germplasm over the course of time. Genetic distances between French and several European wheat germplasm were analysed; genotypes of European wheats were found to relate very distantly to Canadian genotypes. The considerable differentiation of wheat genotypes from different countries and cereal companies might be caused by breeders' personal preferences and by hidden natural selection specific to each local environment. In French cultivars, genetic variation in earliness, and in the North/South habit of the cultivars studied, correlated significantly with allelic variation at *Gli-B1*, *Gli-A2* and *Gli-D2* for earliness, and at *Gli-D2* for the North/South habit. Early and late cultivars are grown mainly in Southern and Northern France, respectively ($r^2 = 0.30$). Cultivars having either the 1B/1R translocation or allele *Gli-D2g* are, on average, later and more resistant to cold; they hence are grown in the North of France. Alternatively, cultivars with the allele *Gli-D2m* are earlier and cold-sensitive, and are grown in the South of France.

Key words Wheat · Gliadin alleles · Genetic diversity · Genetic distances · Earliness

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Introduction

Genetic erosion, or the reduction of the genetic base of the common wheat germplasm caused by frequent use of the same parental genotypes for breeding activities, is becoming a serious problem (Porceddu et al. 1988). It restricts the genetic potential of wheat, complicates wheat improvement and could lead to problems. Few plant characteristics, however, serve as effective genetic markers to monitor and evaluate the changes occurring in wheat germplasm over the course of time.

Gliadins, which are alcohol-soluble seed storage proteins, show the highest level of intervarietal polymorphism when studied by a standard method of acid electrophoresis (APAGE) (Zillman and Bushuk 1979 b). The gliadin pattern of a cultivar is not affected by the area of plant growth (Zillman and Bushuk 1979 a). Most gliadins are controlled in common wheat by six main *Gli* loci located on the chromosomes of the first (*Gli-1*) and sixth (*Gli-2*) homoeological groups (Payne et al. 1982). A vast multiple allelism has been described at each of these loci; an allele encodes several gliadin APAGE bands inherited as a Mendelian unit (block) (Sozinov and Poperelya 1980). Alleles of a locus differ in the number and electrophoretic mobility of the encoded gliadins (Sozinov and Poperelya 1980; Metakovsky 1991). Combinations of different alleles at the six main loci ensure a great diversity of APAGE patterns and, therefore, makes it possible to distinguish a number of common wheat genotypes and to describe them in terms of gliadin allele composition.

There are also several "additional", or "minor", *Gli* loci (*Gli-3*, *Gli-5*, *Gli-6*) which each control a few minor gliadin bands (Sobko et al. 1986; Pogna et al. 1993; Metakovsky et al. 1997). Two genotypes identical at *Gli-1* and *Gli-2* may be distinguished by alleles at "additional" *Gli* loci using the same gliadin pattern (Metakovsky et al. 1994).

Common wheat cultivars bred in France are widely implemented in different scientific and breeding

programmes, but their genotypes are still not well described or classified using efficient genetic markers. Also, gliadin alleles have never been subjected to direct selection by breeders. Therefore, analysis of gliadin alleles in French wheats may adequately describe changes in wheat germplasm including genetic erosion caused by breeding activities.

It is known that most French common wheat cultivars registered in the period 1950–70 originated from a rather restricted group of parental genotypes (Jonard 1951; Joudrier 1974). Therefore, the risk of narrowing the genetic variation of new cultivars and advanced lines has been discussed (Branlard and Chevalet 1984); and wheat improvement by traditional methods of breeding has become less efficient (Joudrier 1974).

In the present study, gliadin alleles in more than 200 samples of common wheat cultivars registered in France over the last 50 years were identified. Genetic variation in different groups of French cultivars was compared, genetic distances between these groups and between French wheats and those from other countries were studied, and correlations between gliadin alleles and some agronomically important characteristics were revealed.

Materials and methods

Most French common wheat grain samples studied were taken from the INRA collection, but some were also obtained from PBI (Cambridge, England) and ISC (S. Angelo Lodigiano, Italy). About 30 cultivars were represented by more than one sample. In each sample, 3–15 (as a rule, 4–6) single grains were analysed.

Acid (pH 3.1) polyacrylamide-gel electrophoresis (APAGE) was performed as described by Metakovsky and Novoselskaya (1991), but using a LKB commercial apparatus. Alleles were identified by using a set of standard cultivars (Metakovsky 1991).

The genetic diversity at each locus was calculated according to Nei (1973) as $H = 1 - \Sigma p_i^2$, where H is Nei's genetic variation index, and p_i the frequency of a particular allele at that locus. The genetic distances between groups of cultivars according to Rogers (1972) and Nei (1972) were computed using BIOSYS-1 software. Pairwise comparisons of allelic frequencies were performed using a standard Fisher's test.

Information about the origin of cultivars relative to cereal companies, about earliness, zone of growth and cold resistance were taken from the "Bulletin des variétés, Céréales" published annually by INRA/GEVES in France dating from 1968 to 1994.

Results and discussion

Authenticity of the grain samples studied and gliadin allelic compositions of French wheat cultivars

In total, 195 cultivars grown in France were studied. However, gliadin genotypes of eight cultivars were either not identical in different grain samples analysed (Alvina, Centurion, Festival, Tenor, Unic and Viking), or were very heterogeneous and carried heterozygous grains (cv Decibel and Rotonde). These eight cultivars were excluded from the study. Cultivars Aboukir, Corin, Marathon and Open each contained one or two admixed grains, but were used in the analysis.

The gliadin allelic compositions in 187 cultivars are shown in Table 1. In total, considering *Gli-1* and *Gli-2* loci, 173 gliadin allelic compositions were found: 160 French cultivars had unique gliadin genotypes, and 13 gliadin genotypes while not unique were found in 27 cultivars. In several cases, however, identical patterns occurred in pairs of cultivars which had nothing in common with their claimed pedigrees (for example, Berlioz and Cargidoc, Hamilcar and Joss, or Blason and Ducat). Moreover, Fluto and Jano, unrelated in their pedigrees, both carried, in addition to an identical allelic composition at the *Gli-1* and *Gli-2* loci, a very rare combination of alleles *Gli-A1f-Gli-A5b* which could occur only as a result of recombination between the tightly linked *Gli-A1* and *Gli-A5* genes (Pogna et al. 1993). An accidental coincidence of gliadin allelic compositions, especially of rare allelic combinations, in unrelated cultivars is quite unlikely taking into account the large number of gliadin alleles at each locus. Therefore, the coincidence of gliadin genotypes in two unrelated cultivars indicates either non-authenticity of their studied grain samples or mistakes in claimed pedigrees.

For 18 out of 187 cultivars, the gliadin allelic composition of all parental genotypes was also studied. The expected correspondence between parental and daughter cultivars was absent in five cases (cvs Berlioz, Garant, Hardi, Milpain, and Score). The absence of correspondence between parental and daughter cultivars in about 30% of all French wheats studied was noticed also when analyzing glutenin polymorphism in these wheats (Picard et al. 1992).

These discrepancies appear to be the consequences of mistakes: erroneous labelling, intermixing, breeder's error in a claimed pedigree, or an unadvertised cross-pollination between different samples in a collection. Similar difficulties were encountered during an analysis of Italian wheats (Metakovsky et al. 1994) and other wheat collections (Metakovsky, unpublished). These results demonstrate some uncertainty when using a wheat collection for analysis, and also emphasize a requirement for great care during the maintenance and handling of such collections. Among the 187 cultivars 121 (64%) were still registered in the French wheat catalogue in 1996. The same year these cultivars covered more than 95% of the bread wheat sites in the country. Although the 187 cultivars had not been selectively chosen they represented an appropriate and representative set of genotypes for the evaluation of French common wheat germplasm.

New and modified gliadin alleles in French cultivars

In total, 79 alleles were identified at six main loci, 11 of which were not previously registered in the catalogue of gliadin alleles.

Table 1 Gliadin allelic compositions in common wheat cultivars grown in France. Alleles designated by "?" were not definitely identified. "New" means a newly found but uncatalogued allele. Alleles designatted by *Gli-A2t** and *Gli-B2e** are slightly different from *Gli-A2t* and *Gli-B2e*, respectively; these variants were not considered as new alleles to avoid the presence of hardly differentiated alleles in the catalogue. Pernel is a heterogeneous cultivar having two biotypes differing at *Gil-D2* (Fig. 1, lanes 10, 11)

Abo	k	b	b	g	b	a
Aboukir	k	f	f	l	o	a
Admiral	o	l	l	r	g	g
Aiglon	f	e	b	j	o	g
Albatros	o	g	b	g	l	new
Alto	k	f	b	g	o	n
Amélio	f	f	b	l	m	h
Ami	o	b	b	g	o	n
Apexal	b	e	b	e	b	b
Apollo	o	l	b	h	l	g
Arbon	a	b	b	g	?	v
Arcane	a	f	f	g	g	j
Arche	o	f	b	l	o	n
Arcole	o	f	b	g	o	h
Arfort	k	b	a	t	c	m
Aristide	b	b	j	t	g	q
Armada	m	g	b	g	o	n
Arminda	f	f	b	r	r	n
Armur	k	f	b	g	g	n
Arpege	b	g	b	l	l	a
Arsenal	f	e	a	e	e	m?
Artaban	o	f	l	p	ac	a
Arum	b	g	b	l	l	g
Arval	f	g	b	g	g	a
Aubaine	f	f	j	t	c	n
Austerlitz	f	f	f	g	l	h
Avalon	f	b	b	j	l	g
Axel	o	f	b	p	o	n
Baroudeur	o	b	b	l	l	h
Beauchamp	o	f	b	p	l	h
Beaver	b	l	g	b	g	g
Belaviso	o	l	b	g	h	h
Berlioz	f	f	b	l	g	h
Bison	f	b	b	l	o	h
Blason	o	f	b	g	g	h
Boreal	o	f	a	l	c	h
Bosco	f	l	b	e	b	n
Brimstone	f	f	f	l	g	a
But	o	f	b	l	g	g
CampRemy	o	f	b	j	o	a
Capelle D.	o	f	b	g	g	g
Capitole	o	f	b	p	g	h
Carat	m	b	b	l	l	a
Cargidoc	f	f	b	l	g	h
Cargimarec	f	e	k	g	c	a
Cargo	b	b	b	g	o	a
Carlos	a	f	f	g	h	n
Castan	a	f	b	o	h	h
Caton	f	f	b	p	o	h
Champion	f	f	g	g	ad	h
Champlein	f	f	b	l	o	n
Champtal	f	g	b	l	p	a
Choisel	f	f	a	p	o	a
Chopin	f	d	b	g	ad	h
Clement	k	l	b	p	l	a
Cobra	b	b	b	e	b	b
Cocagne	f	f	b	j	o	g
Copain	o	b	b	l	b	h
Corin	o	f	l	j	g	g
Corot	o	f	b	l	g	g
Cosmos	f	g	b	l	o	n

Table 1 Continued

Courtot	k	b	b	t	c	a
Créneau	k	b	b	g	l	f
Damier	f	l	b	p	l	a
Darius	o	f	null	l	g	g
David	f	f	b	g	g	a
Declic	o	b	a	g	v	h
Delfi	k	b	b	g	b	b
Diam	o	f	b	j	o	n
Divio	o	f	b	g	g	v
Ducat	o	f	b	g	g	h
E. de Choisy	o	m	b	p	ae	a
Ecrin	b	g	b	t	l	a
Epiroux	o	b	b	g	g	v
Estica	f	f	b	k	r	a
Eureka	f	b	b	k	l	a
Faust	o	m	b	p	g	n
Favori	o	f	b	l	o	n
Festin	b	b	b	g	h	a
Feuvert	f	g	b	g	l	a
Fidel	o	f	g	g	o	h
Fiel	f	f	l	l	g	g
Fleurus	o	f	b	g	o	n
Floreal	a	h	l	j	e*	a
Florin	f	f	b	t	g	a
Fluto	f	f	b?	j	o	a
Fortin	b	f	h	g	g	g
Fournil	b	e	g	g	h	q
Frandoc	b	b	b	p	g	m
Friedland	f	f	b	l	d	h
Futur	f	f	b	r	r	g
Gaillard	o	m	b	g	ae	g
Gala	o	f	b	f	g	n
Galahad	b	g	b	l	g	g
Galaxie	a	f	b	l	l	a
Garant	k	b	b	g	v	h
Gavroche	f	f	b	g	c	a
Genial	k	f	b	r	r	n
Gerbier	o	f	a	g	g	v
Glanor	f	f	b	g	o	n
Goelent	f	q	a	f	o	h
Goya	o	q	a	f	c	a
Halmicar	o	f	b	p	l	n
Hardi	o	f	b	p	o	a
Hereward	b	f	b	h	g	a
Heurtebise	m	b	m	l	g	g
Hickling	o	f	b	p	ac	n
Hobbit	o	f	b	l	g	g
Horace	f	f	b	p	l	a
Huntsman	f	g	b	l	l	a
Iena	f	f	b	t	o	a
Jano	f	f	b?	j	o	a
Joss	o	f	b	p	l	n
Lodi	k	f	f	l	o	e
Louvre	f	f	b	l	l	e
Lutin	k	b	b	g	o	n
Magali	m	e	g	l	o	n
Magdalena	o	b	a	j	b	a
Magister	f	l	b	j	c	n
Magnif 27	b	k	b	b	new	a
Marathon	f	f	b	l	g	g
Marignan	o	f	b	p	ac	a
Marius	o	f	b	p	g	h
Martial	o	f	a	g	v	u
Master	f	g	b	l	f	a
Match	f	b	b	t	c	n
Merit	o	b	b	p	g	h
Messidor	f	f	b	t	o	a
Milpain	b	f	b	g	g	a

Table 1 Continued

Mission	o	g	b	g	g	n
Monitor	o	b	l	j	g	g
Moulin	b	f	b	l	g	g
Nabucco	f	f	b	l	o	h
Nautica	f	l	b	p	h?	g
Nectar	o	f	a	l	g	j
Nord D.	o	f	b	j	g	g
Nougat	f	f	b	l	l	h
Open	e	b	g	r	m	e
Orepi	f	f	b	r	ab	a
Ouest	o	f	b	b	g	g
Pactole	o	f	b	g	g	v
Pepital	b	h	b	r	o	a?
Pernel	c	f	a	g	g	v+h
Petrel	o	d	j	p	o	h
Pistou	f	f	b	o	o	n
Poncheau	f	f	b	g	o	n
Priam	o	f	b	g	ae	h
Prinqual	f	c	a	c	c	h
Promentin	f	l	b	p	o	h
Proqual	i	f	b	new	b	n
Protinal	f	b	b	g	o	n
Qualital	o	f	b	g	m	b
Radja	m	b	b	l	c	m
Real	o	f	b	g	l	a
Recital	o	f	b	j	p	n
Rempart	c	m	g	g	b	f
Renan	f	b	g	k	m	e
Rescler	f	b	b	t*	g	g
Rex	f	b	b	m	c	m
Riol	f	b	b	p	ac	n
Rivoli	f	b	b	b	o	n
Roazon	o	b	b	g	?	v
Rossini	b	b	b	l	l	g
Royal	o	b	b	t	g	g
Rudi	o	h	b	g	g	a
Rurik	f	f	b	l	l	g
Sabre	f	e	b	l	h	a
Scipion	o	b	a	t*	ac	h
Score	f	f	b	g	l	a
Sensor	f	l	b	r	c	h
Sideral	o	f	b	r	b	n
Soissons	k	b	b	t	o	q
Storch	o	f	b	g	h	g
Talent	f	f	b	l	o	h
Tango	o	f	b	l	g	h
Tarasque	o	b	b	c	b	m
Tarquin	f	l	b	p	o	a
Thésée	f	f	b	l	l	a
Titien	o	m	b	u	ae	a
Top	o	f	b	g	g	a?
Touzelle	e	null	null	o	o	q
Tracy	b	g	b	l	l	a
Tremie	b	b	b	l	l	g
Ulm	k	f	b	l	g	n
Vasco	o	l	l	p	c	n
Vizir	b	f	b	g	h	m
Voyage	m	l	b	g	o	n

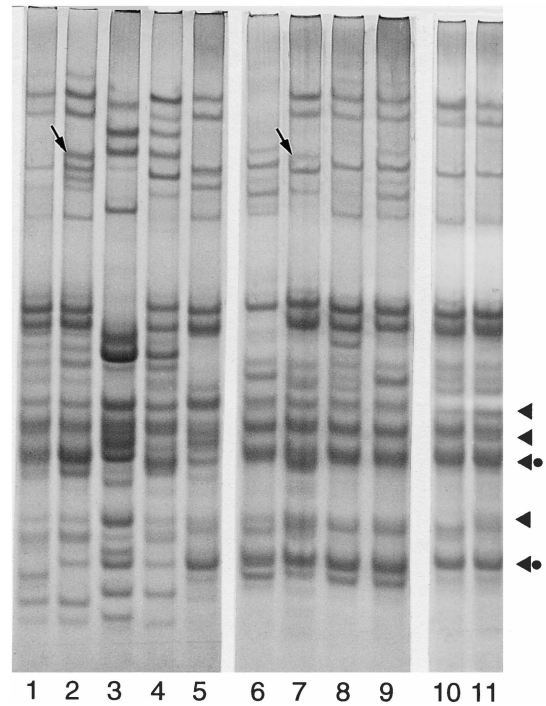

Fig. 1 APAGE patterns of the international standard cultivar Marquis (*lane 4*) and some French common wheat cultivars: Futur (*1*), Genial (*2*), Touzelle (*3*), Choisel (*5*), Darius (*6*), Arfort (*7*), Fortin (*8*), Cappelle Desprez (*9*), Pernel, biotype *Gli-D2h* (*10*), Pernel, biotype *Gli-D2v* (*11*). Bands controlled by *Gli-D2v* are marked by *arrowheads*; those of them which strongly overlap other gliadins are marked by dots. The *Gli-A6c*-controlled ω-gliadin is shown by an *arrow*

It was found that cv Touzelle carried null alleles (no controlled gliadin bands) at both *Gli-B1* and *Gli-D1* (Fig. 1, lane 3). Null-alleles at *Gli-D1* and *Gli-B1* in common wheat are well-known since the first work of Sozinov and Kopus (1983) and Payne et al. (1984), respectively. A null allele at *Gli-D1* in cv Darius (Fig. 1, lane 6) was found earlier and its effect on bread-making quality was studied by Branlard and Dardevet (1994). Null-alleles at all six main *Gli* loci have been described elsewhere (Metakovsky et al. 1993 a). It is suggested for gliadins that null-alleles should be placed in the catalogue under the term "null allele", which is clear and convenient, rather than under specific letters.

Nine new allelic variants were found in French wheats, six of them were catalogued. New alleles *Gli-B2ab*, *Gli-B2ac* and *Gli-B2ad* showed a considerable similarity in their controlled gliadin blocks with alleles *Gli-B2m*, *Gli-B2c* and *Gli-B2p*, respectively (Fig. 2). The similarity of encoded blocks of APAGE components obviously indicates a related origin of the alleles. Two other newly catalogued alleles, *Gli-B2ae* and *Gli-D2v*, are also shown, with an appropriate standard cultivar Bezostaya 1 (Metakovsky 1991), in Fig. 2. These five new alleles occurred in more than one cultivar each. The sixth newly catalogued allele, *Gli-D1m*, which is present in the cultivar Heurtebise differed from *Gli-D1d* only by the fact it does not contain the slowest gliadin minor band in the ω-region of the APAGE pattern (data not shown).

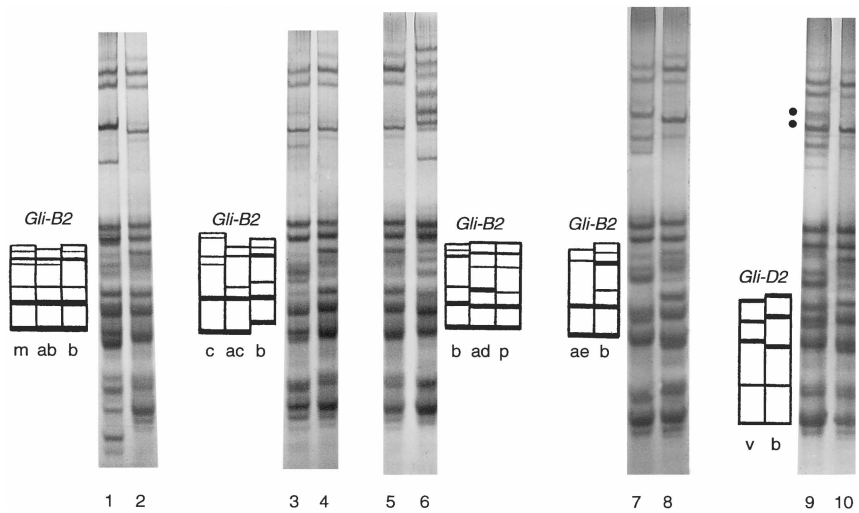

Fig. 2 Newly catalogued gliadin alleles found in French wheats. Cultivars Orepi (*lane 1*), Scipion (*3*), Champion (*6*), Priam (*7*), Epiroux (*9*), and standard Bezostaya 1 (*2,4,5,8,10*) are shown. The schemas demonstrate blocks of gliadin components controlled by alleles in Bezostaya 1 (designated "b"), by new alleles (*Gli-B2ab*, *Gli-B2ac*, *Gli-B2ad*, *Gli-B2ae*, *Gli-D2v*) and catalogued blocks which are similar to new ones. The *Gli-B5b*-controlled minor ω-gliadins are marked by *dots*

The origin of newly catalogued alleles can be identified. For example, all four French cultivars with the allele *Gli-B2ae* are, in fact, the cultivar Etoile de Choisy and its close descendants. Obviously, the donor of this allele is one of parents of Etoile de Choisy. The allele *Gli-B2ad* probably came into French germplasm from US 43, the only common parent of both French cultivars having this allele. Allele *Gli-B2ac* is present in the old cultivar Hickling and therefore its origin could be traced to some land races.

Allele *Gli-D2v* occurred in six French cultivars having only one common progenitor, VPM, in their pedigrees and in three related cultivars. Indeed, a sample of VPM studied earlier (Metakovsky, unpublished) was very heterogeneous and had, among others, an allele identical to *Gli-D2v*. The allele *Gli-D2v* encoded gliadin bands which were similar in their APAGE positions to bands controlled by other common wheat *Gli-D2* alleles and therefore probably originated from cv Marne, the only common wheat parent of VPM.

Three other new alleles which occurred in cultivars Albatros, Magnif 27 and Proqual were not catalogued because their gliadin compositions were not precisely established.

Allelic frequencies and genetic variation of French common wheat germplasm

The distribution of the 79 gliadin alleles found in French common wheats was clearly uneven: upwards of nine alleles were found at *Gli-A1* and up to 18 alleles at *Gli-B2*; 16 alleles were present in one cultivar, 50 alleles occured in three or more cultivars, and 24 alleles occured in at least ten cultivars each. The frequency of the most common allele at a *Gli* locus ranged from 76% to 26%. The uneven distribution of gliadin alleles has also been found in groups of cultivars from other countries (Metakovsky et al. 1991, 1993 b, 1994; Chernakov and Metakovsky 1994).

Table 2 Genetic diversity (H) in groups of common wheat cultivars difffering in their year of release

Number of cultivars	Year of release	H
17	1945–70	0.662
18	1974–76	0.588
15	1977–78	0.606
15	1980	0.638
17	1984	0.634
17	1985	0.674
15	1986	0.641
15	1987–89	0.679
14	1990–92	0.709

Generally, genetic diversity in the set of French wheats studied was rather high ($H = 0.714$), and only a small decrease of the parameter H in the groups of cultivars released in 1974–78 is evident (Table 2). At that time, French common wheats apparantly lost their genetic variation for several characters, including the gliadin APAGE pattern (Branlard and Chevalet 1984).

About 15 catalogued gliadin alleles, which had been absent in the oldest cultivars studied (registered in 1945–76), appeared in French germplasm over the last 10 years (data not shown). On the other hand, only six alleles, which were rare in the oldest cultivars, *Gli-B1k*, *Gli-B1m*, *Gli-D1null* , *Gli-A2m*, *Gli-A2b* and *Gli-A2o*, did not reappear in the more recent ones. Moreover, genetic variation in the group of the newest cultivars (1989–92) was 20% higher than in the cultivars released 15–20 years ago, and even slightly higher than that in the oldest cultivars (Table 2). Therefore, the data obtained using gliadin alleles as genetic markers did not indicate a decay in the genetic variation of French common wheat germplasm over the last 25–50 years.

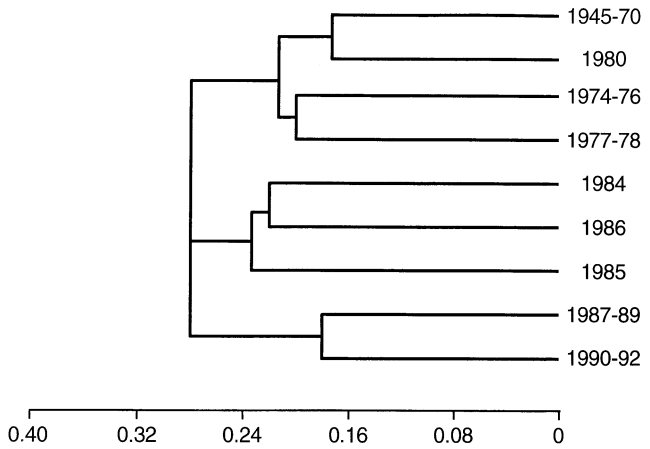

Fig. 3 Dendrogram based on Rogers' (1972) genetic distances between groups of cultivars with different years of release grown in France

Analysis of genetic distances between groups of cultivars registered in different years showed three clusters which fully accorded with the year of cultivar release: before 1984, after 1986, and during 1984–1986, so that the genotypes of recent cultivars appeared different from the oldest ones studied (Fig. 3). This means that gradual changes in French common wheat germplasm had effectively taken place. Therefore, old cultivars should be preserved in collections because their genotypes may contain unique genes and allele combinations that are absent from recent cultivars.

Alleles at minor *Gli* loci in French wheats

An interesting peculiarity of French wheats (Fig. 1, lanes 2 and 7) is the frequency of an allele, *Gli-A6c*, which controls a rather strong ω-gliadin (Metakovsky et al. 1997). The allele *Gli-A6c* was present in the 26 cultivars studied, but only accompanied alleles *Gli-A1k* (always) and *Gli-A1f* (sometimes), and never occurred with any other allele at *Gli-A1*. The presence of *Gli-A6c* may positively relate to dough quality: it occurred in 5 out of 20 French cultivars having the highest dough strength (W), but in none of the 20 having the lowest strength (data not shown).

Allele *Gli-B5b* controlling a pair of slow-moving ω-gliadins occurs in cv Epiroux together with allele *Gli-B1b* (Fig. 2, lane 9). (We suggest that the allele at *Gli-B5* which does not code for this pair of gliadins should be called *Gli-B5a* because these gliadins are absent from the cultivar Chinese Spring in which all alleles should be designated by the letter "*a*".) A similar rare combination of alleles (*Gli-B1g-Gli-B5b*) which was a result of recombination between *Gli-B1* and *Gli-B5* (Pogna et al. 1993) has been described in the Italian cultivar Este (Metakovsky et al. 1994). In one case, two cultivars with identical allelic compositions at *Gli-1* and *Gli-2* (Capitole and Marius) could be distinguished by analysis of minor gliadin loci (data not shown).

Relation between common wheat germplasms from different cereal companies and from France and other countries

Analysis of genetic distances between groups of cultivars from different countries has shown that common Italian wheat germplasm is much closer to Bulgarian and Yugoslavian types than to English, or more especially to Canadian, wheat germplasms (Metakovsky et al. 1994). In contrast, genotypes of French wheats were found to be more similar to English than to Italian wheats (Fig. 4). Similar results were obtained (either from Rogers', or Nei's distances; data not shown) when using only five *Gli* loci (without *Gli-B2* because, as a rule, there are some doubtful and unidentified alleles at this locus in the different groups of cultivars studied), or only four of the most common alleles at each of the six loci (to avoid a contribution of rare alleles).

The same relationships between wheat genotypes from these countries were observed when only one gliadin locus was used for calculations of genetic distances if either *Gli-A1*, or *Gli-B1*, or *Gli-A2* had been considered. However, when only *Gli-D1* was used, Bulgaria came into the first cluster; and when only *Gli-B2* or *Gli-D2* were considered, Italy moved to the first cluster. These results indicated that not only do the differences in breeders' partialities for parental wheat genotypes used for hybridization cause the allelic differentiation existing between countries (Fig. 4), but also that natural selection may specifically affect allelic frequencies at certain loci (chromosome regions marked by these loci) in different environments (Nevo et al. 1988; Allard 1996).

Wheat cultivars grown and studied in France originated from breeders working all over France and neighbouring countries and were the property of more than 25 cereal breeding companies and units. The differentiation of genotypes of wheat cultivars from the

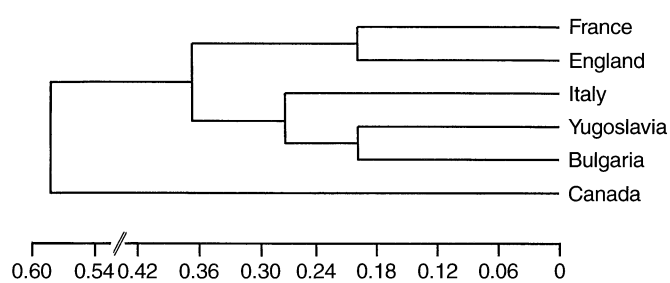

Fig. 4 Dendrogram based on Rogers' (1972) genetic distances between groups of cultivars grown in different countries

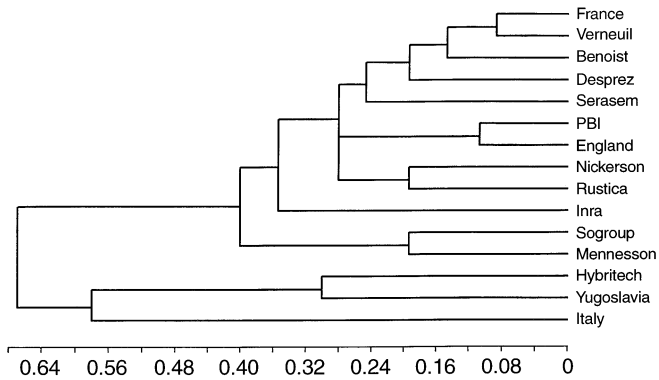

Fig. 5 Dendrogram based on Nei's (1972) genetic distances between groups of cultivars from different cereal companies and from some countries. 'Verneuil', 'Benoist', 'Desprez', 'Serasem', 'PBI', 'Nickerson', 'Rustica', 'INRA', 'Sogroup', 'Mennesson', and 'Hybritech' are cereal breeding companies and units

main companies (Fig. 5) indicated some narrowing of the wheat germplasm used by breeders working with different companies. For example, three and four cultivars out of six studied from "Hybritech" had alleles *Gli-A2e* and *Gli-B2b*, respectively, although the frequency of these alleles in all cultivars grown in France was only 2.1% and 5.9%, respectively. Therefore, the genotypes of cultivars from the "Hybritech" related more closely to Italian and Yugoslavian than to French genotypes. (Fig. 5). In contrast, cultivars from "Verneuil", "Benoist" and "Desprez" were very similar to the "all-France" group of wheats (Fig. 5). The highest intra-group genetic variation was found in the "Rustica" ($H = 0.724$) and "INRA" ($H = 0.720$) cultivars, while cultivars from "Mennesson and RAGT" were the most uniform ($H = 0.391$). Again, the differences between groups of cultivars might be caused by breeders' personal preferences as well as by hidden natural selection specific for each location where a breeder works. A strong genetic differentiation and association of genotypes to environmental factors has been shown in wheats by Nevo et al. (1988, 1995).

A considerable differentiation of common wheat germplasms from different countries and breeding centres was discovered earlier using gliadin alleles as wheat genotype markers (Metakovsky et al. 1991, 1994; Chernakov and Metakovsky 1994). Allelic frequences differed strongly between groups of cultivars; in extreme cases, an allele frequent in one country may have been completely absent from others. For example, *Gli-A1m* occured in 73% of the Canadian cultivars studied (Metakovsky et al. 1993 b), but in few cultivars from Europe and Australia; alleles *Gli-A1h* and *Gli-D1h*, and *Gli-B1k* and *Gli-A2o*, occurred in Bulgaria and Italy, respectively, with a frequency about 10–20% each but only occasionally elsewhere, and so on.

The absolute value of Nei's (1972) genetic distances obtained in our study was rather high: it was 0.13 even between the closest groups of cultivars from France and England (data not shown), 0.34 between cultivars from "INRA" and several other groups of cultivars grown in France (Fig. 5), and was as high as 1.94 between wheats from Canada and other countries (data not shown). For comparison, the highest value of Nei's distance obtained in the study of 42 allozyme loci in 33 populations of wild emmer wheat from Israel and Turkey was only 0.68 (Nevo and Beiles 1989). Obviously, this difference may be explained by the unusually high levels of polymorphism of gliadins as compared with allozymes.

Association of some gliadin alleles with agronomically important characters

For European winter wheats, time of flowering and subsequent maturity is determined by a complex assemblage of genes which strongly influence yield potential and therefore is one of the most important selection characters (reviewed in Worland et al. 1994). In our work, we found that in French cultivars, genetic variation in plant earliness might be affected, to some extent, by chromosome segments marked by *Gli-B1*, *Gli-A2* and *Gli-D2* because allelic variation at these loci significantly correlated with earliness (SAS general linear-model procedure, significance probability value < 0.0001, 0.0005, and 0.0007, respectively). This result was confirmed when "PBI" cultivars, which were all late flowering due to the presence of the *ppd1* allele (Worland et al. 1994) located on chromosome 2D, were excluded from the analysis. It was found that alleles *Gli-B1b*, *Gli-A2f*, *Gli-A2t* and *Gli-D2m* were significantly more frequent in the group of the earliest 39 cultivars selected from the set of cultivars studied, and alleles *Gli-B1g*, *Gli-B1l* (1B/1R translocation) and *Gli-D2g* were characteristic of the latest 37 cultivars.

Two other extreme groups of cultivars were analysed: one of them included 39 cultivars grown in the South, and the other 61 cultivars grown in the North of France ("PBI" cultivars were excluded). The occurrence of cultivars in these groups correlated ($r^2 = 0.30$) with their earliness, so that early and late cultivars were grown mainly in Southern and Northern France, respectively. These data confirm a well known observation that early cultivars are more advantageous in Southern Europe (see Worland et al. 1994). It was found that allelic variation at *Gli-D2* correlated significantly ($P < 0.05$) with the North/South habit of the cultivars studied (Table 3). In addition, all cultivars with *Gli-B1l* were grown only in the North where the frequency of this allele was therefore significantly higher than in the South.

Some gliadin alleles were probably associated with cold resistance: the frequency of alleles *Gli-B1l*, *Gli-A2r* and *Gli-D2g* was significantly higher, and alleles *Gli-A1a*, *Gli-B2c* and *Gli-D2m* significantly lower, in the

Table 3 Frequencies (%) of some *Gli* alleles in cultivars grown in the South and North of France

Allele	Number of cultivars		Difference (*$P < 0.05$; **$P < 0.01$)
	South 39	North 61	
Gli-D2a	25.6	29.5	
Gli-D2f	–	3.3	
Gli-D2g	2.6	16.4	**
Gli-D2h	33.3	16.4	
Gli-D2j	5.1	–	*
Gli-D2m	12.8	–	**
Gli-D2n	7.7	24.6	*
Gli-D2v	5.1	4.9	
Others	7.8	4.9	

group of 42 cultivars with the highest cold resistance as compared with the group of 42 cultivars with the lowest resistance.

The association of gliadin alleles to different agronomically important characters were first noticed by Sozinov and Poperelya (1980). The most interesting finding on these relations reported here was the significant association between the known 1B/1R translocation [designated as allele *Gli-B1l* by Metakovsky (1991)] and each of the three physiological characters considered: French cultivars with this allele were later, more cold resistant, and therefore mainly grown in the North of France. Cultivars having the allele *Gli-D2g* conformed more or less to the same pattern. In contrast, cultivars with the allele *Gli-D2m* were, on average, earlier, cold sensitive and grown in the South of France. It is plausible to suggest that chromosomal segments marked by these alleles may be involved in multilocus combinations affecting the degree of plant adaptation to local environments (Allard 1996). Natural selection may recognize the adaptive properties of individual alleles of any locus, or the chromosome segments in which this locus reside (Nevo et al. 1988, 1989, 1995; Allard 1996).

Conclusion

Our study has confirmed that gliadin markers are an easy, cheap, and powerful tool for the evaluation of wheat genetic resources. Common wheat, being an inbreeding species and having a large genome size, is characterised by a low level of intervarietal polymorphism of DNA detectable by a conventional RFLP approach (Sharp et al. 1989) or by PCR molecular markers (Talbert et al. 1994). For example, to distinguish only 61 European common wheat cultivars using RFLP, 58 probes were required (Siedler et al. 1994). To differentiate 56 cultivars of different origin using moderately repeated, dispersed, and highly variable (MRDHV) genomic sequences as markers, at least three different restrictases were necessary (Liu et al. 1992). Wheat microsatellites have been shown to be randomly distributed over the entire genome but appeared less polymorphic (4.6 alleles per microsatellites, Röder et al. 1995) than wheat storage-protein loci. In fact, only DNA sequences coding for wheat storage-proteins are known to give an appropriate level of intervarietal polymorphism for wheat genotype identification (Vaccino et al. 1993; Devos et al. 1995; Pagnotta et al. 1995). Obviously, to detect the same polymorphism in the storage-protein genes, protein electrophoresis is much more convenient: it is cheaper and less labour-consuming, although less fashionable. In addition, DNA (PCR) markers based on storage-protein sequences may differentiate fewer variants of these genes than protein electrophoresis (Campenhout et al. 1995), although many *Gli-1* alleles can be distinguished in RFLP patterns when using a γ-gliadin probe (Vaccino and Metakovsky 1995). Therefore, for the limited purposes of evaluating common wheat germplasm, protein electrophoresis of gliadins and other highly polymorphic storage proteins, offers more advantages in comparison to DNA molecular markers. In contrast, DNA markers demonstrate interspecific polymorphism and can, theoretically, cover all wheat genomes and therefore may be more successfully used for tracing valuable genes that are transferred from wheat relatives into common wheat (Jia et al. 1996; Somers et al. 1996; Talbert et al. 1996).

The use of gliadin protein markers allowed us to reveal a high level of genetic diversity in French common wheat germplasm as well as its differentiation between different cereal companies and regions of France. Generally, the genetic diversity in the world common wheat collection which includes all specific alleles occurring in any country is even higher: rather strong differentiation exists between wheat germplasms from different countries (Fig. 4; Metakovsky et al. 1991, 1993 b, 1994; Chernakov and Metakovsky 1994). This differentiation, which can be considered as narrowing the genetic base of common wheat germplasm in a country, may be caused by traditions in breeding activities in a particular country (frequent use of the same parental forms for crosses), but also by specific environmental conditions (climate, soils) which means that only some wheat genotypes are suitable for that country. It is known that inbreeding plant species, including wheat, show very intense geographic and microgeographic differentiation (Brown 1979; Nevo et al. 1988, 1995).

A decrease in the genetic base of common wheat germplasm in a country is conditioned both by breeders' activities and natural selection. Obviously, only the first of these may be controlled and reduced by breeding genotypes from other countries as well as land races and old cultivars from the same country. Local genotypes would be an especially valuable source of

alleles and multilocus combinations already suitable for specific environments of the country concerned (Allard 1996). Genetic diversity in breeding material may be monitored by means of an analysis of polymorphic markers.

These arguments highlight the importance of the correct maintenance, evaluation and use in breeding of the world wheat collections (Porceddu et al. 1988). To preserve the common wheat germplasm of a country and fight erosion it would be well worth developing and maintaining local wheat collections which would both include both old cultivars and land races.

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