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Associations Between Electrophoretic Composition of Proteins, Quality Characteristics and Agronomic Attributes of Durum Wheats I. Protein–Protein Associations

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Polyacrylamide gel electrophoresis of gliadins and sodium dodecyl sulfate polyacrylamide gel electrophoresis of total reduced proteins were carried out for 95 durum wheat genotypes. The relative proportions of each component were determined by densitometric scanning of the stained gels. Statistical techniques (linear correlations and principal component analysis) were applied to the densitometer data in order to identify those components that are likely to belong to multigenic families (positive correlations) or to correspond to different allelic types (negative correlations). Strong associations between LMW-glutenin subunits, γ-gliadins, ω-gliadins and some β-gliadins were confirmed, and new presumed α- and β-gliadin allelic groups were described and related to chromosomal loci. These results provide a basis for further investigation of associations between specific durum wheat proteins and technological or agronomic attributes.

Introduction

Electrophoresis patterns of wheat storage proteins (gliadin and glutenin subunits) are essentially genotypic characteristics that are qualitatively unaffected by growing conditions of the plant. Electrophoretic analysis has become a widely accepted method for characterising genotypes, particularly in cultivar identification.

Because storage proteins also have a major influence on grain quality, many investigations have been carried out on relationships between electrophoresis patterns and the quality potential of the genotypes. It has been demonstrated that allelic variation at *Glu-1* loci encoding HMW glutenin subunits and, to a lesser extent, at other loci (*Gli-1* and *Gli-2*) encoding gliadins and LMW glutenin subunits, determines the baking strength of bread wheat as assessed by the SDS-sedimentation test[1–3], Alveographe characteristics[4,5] or gluten viscoelasticity[6].

Durum wheats in particular have been studied following the discovery of a very close relationship between specific γ-gliadins and gluten viscoelasticity, a major factor in pasta cooking quality[7,8]. In contrast to bread wheats, the gluten characteristics of durum wheats are primarily determined by allelic variation at the *Gli-B1* locus, which encodes

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γ-gliadins: the presence of the electrophoretic component γ-45 (according to the nomenclature of Zillman and Bushuk[9]) is associated with increased elasticity or firmness of gluten[10] or with dough strength[11,12], while the presence of another allele, γ-gliadin 42, is associated with poor viscoelastic properties or with dough weakness. This relationship has proved to be an extremely useful tool in the breeding of durum wheat genotypes[13] since gliadin composition can be determined on a single seed[14]. This unique association was also used as a valuable model for investigating functional properties of gluten proteins through different approaches based on physical chemistry[15,16] or molecular biology[17].

More recently, it was shown that the association involved not one, but a group of γ- and ω-gliadin proteins, that the genes coding for them were tightly linked to genes coding for LMW glutenin subunits[18], and that the latter, because of their strong aggregative properties, could be the direct causal agents of gluten viscoelasticity, γ-gliadins simply acting as genetic markers of the whole *Gli-B1* locus[19,20]. On the other hand, mapping of genes located on the short arm of chromosome 1B showed an association between the *Gli-B1* locus and other characters such as red-glume colour[21], ear colour and downiness[22] or resistance to certain races of yellow rust[23].

However, most of these reports have concerned one locus (*Gli-B1*) only, out of the six that encode the major storage proteins in durum wheats[18,24]. With the exception of a few reports on HMW-glutenin subunits[25–27] or on DSG durum wheat, sulfur-rich glutenins (DSG)[28], the remaining polypeptides, including α- or β-gliadins and many protein components observed in SDS–PAGE patterns, have not been investigated for possible relationships with technological or agronomic characteristics.

In a programme aimed at a further improvement of wheat breeding strategy, using durum wheat as a model, we have extended the investigations to all groups of PAGE bands (α-, β-, γ- and ω-gliadins) and to the main groups of SDS–PAGE bands, from a large number of durum wheat genotypes, in order to identify new markers of technological or eventually agronomic data. In this first part of the work, we have quantified each major component, and used statistical techniques such as linear correlation and principal component analysis to analyse electrophoretic data and to investigate new allelic variations and associations between protein components.

Experimental

Plant material

The 95 genotypes used for this study are representative of the main durum wheat cultivars grown in France and of the genetic material currently developed by breeders. They comprised (a) 34 genotypes, which were submitted for registration and grown between 1975 and 1985 to produce samples (200 kg) evaluated through pilot tests from four growing locations, (b) 37 officially, which were registered cultivars grown and evaluated under the same conditions, but for which more information was available from the wheat variety trial reports, and, (c) 24 F_6 to F_8 breeding lines, which were descended from different crosses and grown in 1985 (three growing locations) to produce samples (3 kg) submitted for laboratory tests only.

Electrophoretic analysis

Electrophoretic analyses were carried out for all the different growing locations of each genotype and were repeated on three different protein extracts. A preliminary screening of the samples was carried out in order to eliminate the lines that showed possible heterozygousity in the patterns, or some variation in the patterns between the different growing locations. The 95 genotypes analysed did not show the presence of any biotypes or apparent polymorphism within samples.

(1) Electrophoresis (PAGE) of gliadins. Gliadin proteins were extracted from semolina and electrophoresed in 6% polyacrylamide gels using an aluminium lactate buffer, (pH 3·2), according to Bushuk and Zillman[29]. Gliadin bands were identified according to the two-digit nomenclature of Zillman and Bushuk[9], but using the durum wheat γ-gliadin 51 as reference band[7].

(2) Electrophoresis (SDS–PAGE) of total reduced proteins. Reduced proteins were extracted and reduced from semolina by treatment with Tris–SDS–mercaptoethanol buffer[30]. The procedure was expected to extract all the different protein species in the grain (soluble proteins, gliadins, LMW and HMW glutenin subunits).

Reduced proteins were electrophoresed in vertical SDS–PAGE slabs in a discontinuous, pH 6·8/8·8 Tris–HCl–SDS buffer system[30] at a gel concentration of 13%[19] using a Desaga apparatus. Gels were fixed in 12% trichloroacetic acid and stained overnight with Coomassie Blue.

Since the most commonly accepted nomenclature of SDS–PAGE components is restricted to the HMW region[31], we used a three-digit band nomenclature system derived from that of Berger and Le Brun[32], which covered all the groups of components, and in which reference band 1000 corresponded to the major α-gliadin group.

(3) Densitometry. Black and white prints of the gels were scanned with a soft laser Ultroscan densitometer (Pharmacia-LKB, France). The densitometer curves were processed (baseline subtraction, peak identification, integration) either with Gelscan software (Pharmacia-LKB, France) on an Apple IIe microcomputer or with Nelson software (Stang Instruments, France) on an IBM PC-XT microcomputer. Experiments indicated that the reproducibility of the densitometer analyses was ±2% when scanning the same electrophoretic pattern and only ±10% when scanning different patterns of the same sample. This is consistent with previous reports[33,34], and determinations of band areas in this report correspond to the means of three scans, from three different protein extracts of each semolina sample; this was assumed to decrease the effect of densitometric error on the overall analysis.

Alternatively, cultivar arrays (relative mobility and relative intensity of the bands) were printed using a simplified numerical expression (intensities: 1 to 5) as described previously[35].

Statistical analysis

Because the three above mentioned sets of genotypes were not submitted to the same analysis, the information available was computed in three different files that were considered independently for the statistical analyses. Each file comprised the relative amounts of all gliadin bands and reduced protein components, and the various technological and agronomic data. Except where mentioned, only the correlations that have been confirmed in at least two sets of genotypes have been considered.

The classical calculation of linear correlations was complemented by principal component analysis (PCA) as previously described[36].

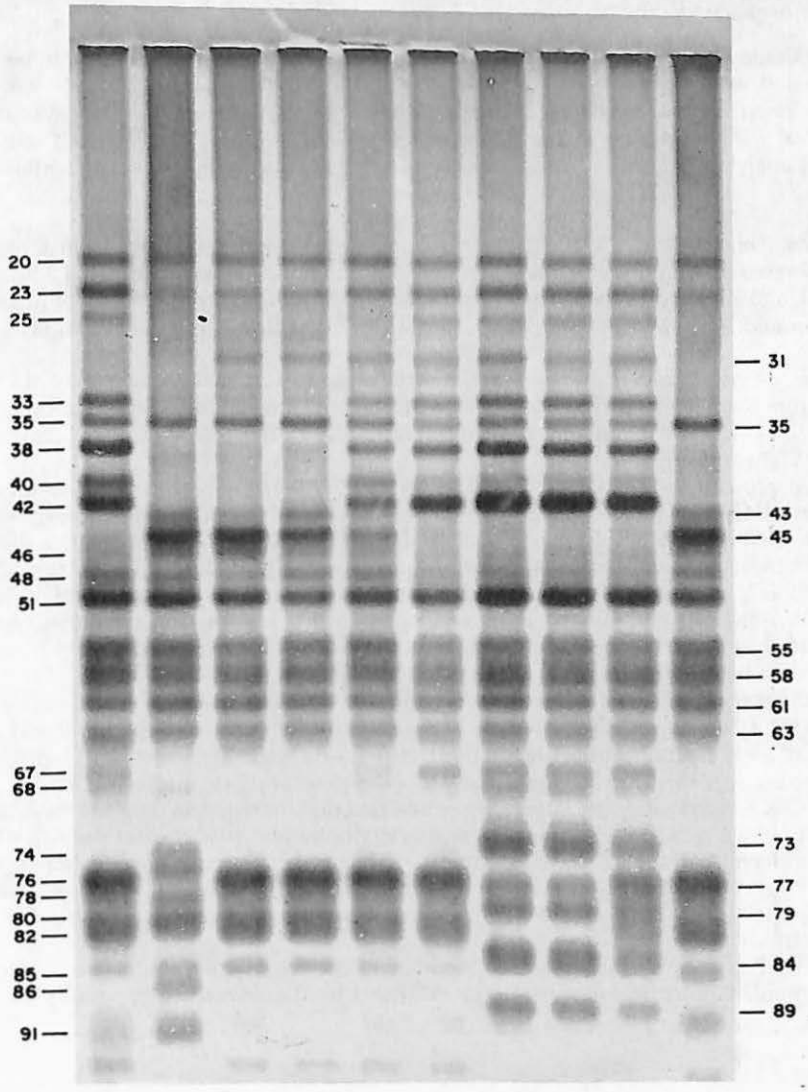


FIGURE 1. PAGE patterns of gliadin proteins separated in a pH 3·2 aluminium lactate gel. The bands are numbered according to the band numbering system of Zillman and Bushuk[9].

Electrophoretic patterns

Some of the diversity of the gliadin proteins revealed by PAGE from the semolina extracts of the 95 genotypes is illustrated in Fig. 1. Each pattern comprises approximately 20–25 major bands, which have been numbered according to Zillman and Bushuk[9] among a set of 50 possible bands that could be unambiguously identified in the whole

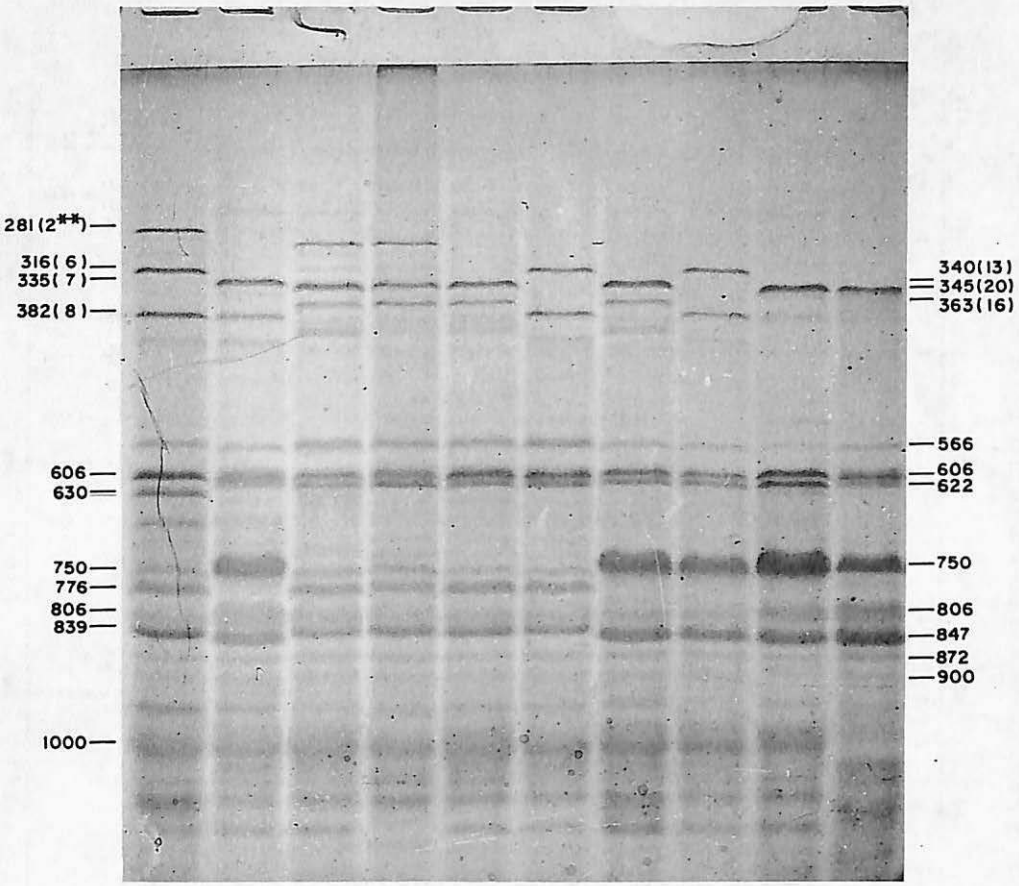
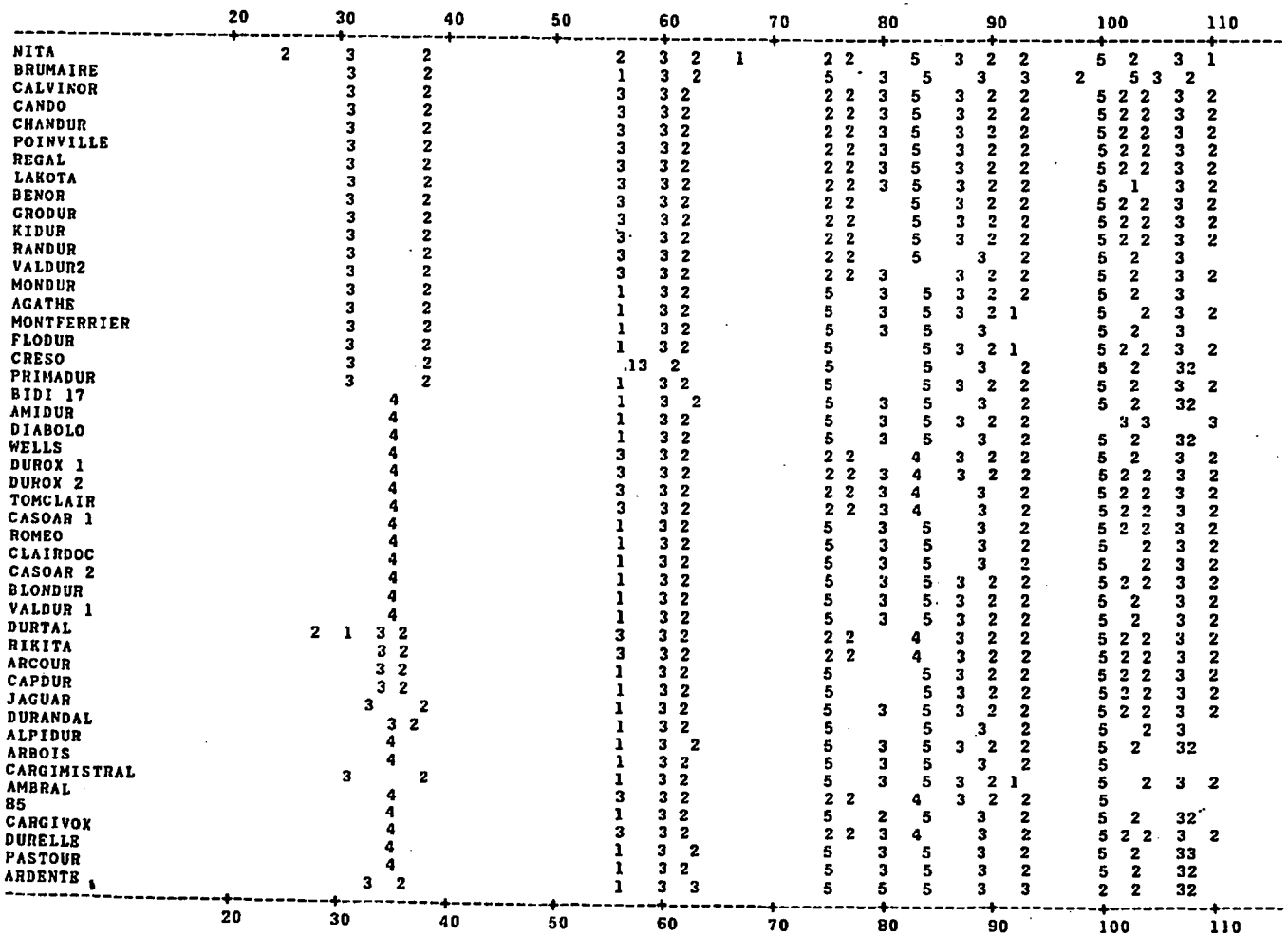


FIGURE 2. SDS–PAGE patterns of reduced proteins separated in a pH 8·8 Tris buffer gel. The bands are numbered according to the band numbering system of Berger and Le Brun[32]. Assignments according to the more commonly used nomenclature for HMW-glutenin subunits[31] is given in brackets.

durum wheat collection[37]. Because many of these bands were found infrequently, only those present in more than three genotypes have been taken into consideration in the statistical analyses; this restricted the number of bands to 38.

Similarly, patterns of total reduced proteins revealed by SDS–PAGE (Fig. 2) comprised 15–17 major components among a set of 30 possible components[32]. As for gliadin bands, only those components that were present in more than three genotypes and those belonging to clearly resolved regions have been considered. There was little point in calculating correlations for bands (e.g. with mobilities greater than 900) that, from previous reports based on two-dimensional analysis, obviously included many overlapping components. On the other hand, most of the proteins belonging to albumin or globulin types, or to other very low molecular weight components were not considered in quantitative estimations; this restricted the catalogue of components to 17.

TABLE I. Printout of cultivar formulae of french durum wheats based on SDS–PAGE electrophoregrams



We cannot rule out, however, the possibility that some salt-soluble fractions, such as β-amylase, may contribute in part to some of the intermediate molecular weight components[39]. The diversity of the patterns in SDS–PAGE can be illustrated by using a simplified computerized system in which band proportions were converted into intensities (from 1: traces, to 5: very strong)[35,38] in the case of 34 durum wheat cultivars (Table I).

An example of a typical densitometer curve is given in Fig. 3. Each major band was assigned a number representing the densitometric estimate of its proportion in the initial protein fraction; this analysis was restricted to the classical storage type polypeptides. For instance, gliadin bands were reported as a proportion (%) of the total fractions that migrate in acid PAGE with mobilities lower than 95 units[9] and SDS–PAGE components were reported as a proportion (%) of the total reduced proteins with M_r greater than 25000[39]. All the patterns were stored in files in which both relative mobility and the proportions of the different components (38 gliadins and 17 reduced proteins) were noted.

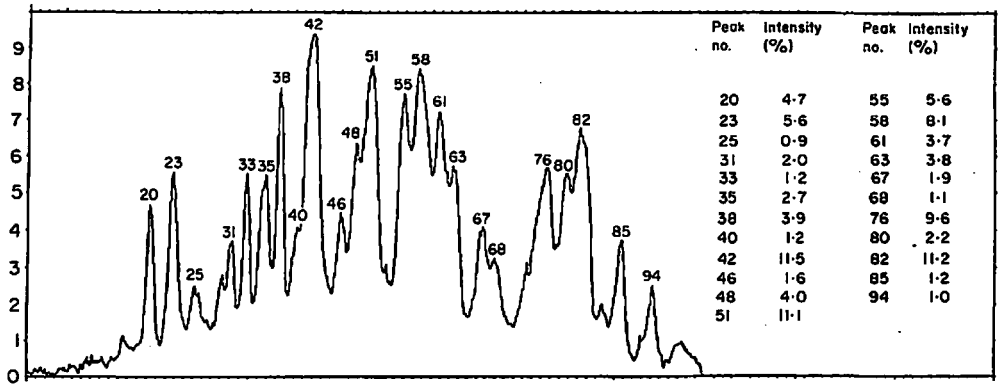


FIGURE 3. Densitometer trace of a PAGE electrophoresis pattern of cv. Calvinor. Designations for components are given according to Zillman and Bushuk[9].

Associations between protein components

The 38 gliadin bands (PAGE) included seven ω- (mobility:20–40), six γ- (mobility:42–55), 10 β- (mobility:58–68) and 15 α- (mobility:73–94) components, and the 17 reduced protein components included six HMW- (mobility:281–382), four intermediate (mobility: 566–630), five LMW- (mobility:750–847) and three fast-moving (mobility: 872–900) components. Simple correlation coefficients were calculated between the relative intensities of each gliadin component and of each protein component measured by densitometry, in a way similar to that previously used by Branlard[40] on gliadins for bread wheat gliadins.

These calculations are expected to show the groups of bands that are correlated with any given band. Significant positive correlations between the intensities of different bands are likely to indicate that these bands appear together either always or often. This approach, therefore, represents a way of investigating the existence of blocks of proteins

TABLE II. Correlation coefficients relating the intensities of some gliadin bands for 37 durum wheat varieties[a] (all coefficients are multiplied by 1000)

Gliadin band[b]	23	38	40	42	43	45	58	60	61	65	73	75	76	79	80	81
20	−165	−153	−93	−85	221	83	−535**	222	−495**	407*	231	−283	−101	207	−74	−307
23	1000	844***	774***	782***	−743***	−778***	35	−117	59	−177	−263	−42	264	−290	162	−3
33	131	229	124	159	−133	−104	403*	−424**	338*	−480*	−78	−43	211	−46	159	18
35	−184	−440**	−329*	−419**	370*	291	−74	154	−17	179	−3	231	−261	−88	−266	167
38	844***	1000	877***	965***	−890***	−919***	152	−211	131	−348*	−344*	−64	383*	−338*	256	−17
40	774***	877***	1000	905***	−855***	−898***	182	−192	102	−282	−290	−46	303	−289	134	3
42	782***	965***	905***	1000	−912***	−957***	130	−204	100	−301	−309	−46	336*	−308	191	6
43	−743	−890***	−855***	−912***	1000	888***	−196	63	−174	316*	355*	−26	−300	343*	−176	−38
45	−778***	−919***	−898***	957***	888***	1000	−72	107	−34	237	309	−12	−256	310	−133	−42
51	−381*	−234	−376*	−275	251	400*	225	−193	314	−254	−20	57	52	18	61	94
55	160	341*	380*	353*	−388*	−290	786***	−553**	719***	−740***	−322	232	287	−287	207	308
58	35	152	182	130	−196	−72	1000	−634***	924***	−882***	−190	226	155	−167	112	321
59	−179	−336*	−272	−290	309	246	−902***	675***	−912***	979***	277	−112	−283	244	−223	−210
60	−117	−211	−192	−204	63	107	−634***	1000	−641***	662**	−173	−36	1	−172	−69	−147
61	59	131	102	100	−174	−34	924***	−641**	1000	−898***	−174	183	170	176	140	266
62	−153	−320*	−265	−282	265	214	−877***	797***	−887***	967***	189	−102	−218	164	−153	−204
63	−156	−66	−61	−121	−11	75	539**	−256	538**	−532**	−171	128	−44	−104	−80	55
65	−177	−348*	−282	−301	316*	237	−882***	662**	−898***	1000	267	−54	−308	232	−248	−157
73	−263	−344*	−290	−309	355*	309	−190	−173	−174	267	1000	−247	−580***	973***	−552**	−223
75	−42	−64	−46	−46	−26	−12	226	−36	183	−54	−247	1000	−540**	−247	−523**	961**
76	264	383*	303	336*	−300	−256	155	1	170	−308	−580***	−549**	1000	−578**	946***	−495**
77	−227	−388*	−308*	−329*	348*	291	−537**	130	−515**	609***	715***	−134	−617***	691***	−588**	−237
78	293	382*	329*	364*	−372*	−329*	484**	−151	451**	−462**	−780***	465**	442**	−778***	414*	542**
79	−290	−338*	−289	−308	343*	310	−167	−172	−176	232	973***	−247	−578***	1000	−551**	−222
80	162	256	134	191	−176	−133	112	69	140	−248	−552**	−523**	946***	−551***	1000	−471**
81	−3	−17	3	6	−38	−42	321	−147	266	−157	−223	961***	−495**	−222	−471**	1000
82	219	318*	255	273	−245	−205	146	37	165	−284	−577***	−547***	980***	−576***	959***	−493**
84	−264	−402*	−323*	−345*	343*	285	−52**	138	−483**	593***	763***	−146	−647***	729***	−616***	−249
94	−190	−112	−156	−174	216	155	276	−125	263	−336*	−519**	2	540**	−518**	536**	15

[a] Correlation coefficients above 575 are very highly significant (*** $P < 0.001$), those over 418 are highly significant (** $P < 0.01$), and those over 325 are significant (* $P < 0.05$).

[b] Band nomenclature is according to Zillman and Bushuk[9].

belonging to a given multigenic family (closely linked genes), either within gliadin or within glutenin groups, or eventually between both groups. Conversely, negative correlations are likely to indicate mutually exclusive bands, that could correspond to different allelic types.

(a) Associations between gliadin bands (PAGE). Some of the most significant correlations observed between gliadin bands in a set of 37 cultivars are reported in Table II. Similar, highly significant correlations between the same bands have been found from the other sets of genotypes, indicating that such band groupings are consistent and general in durums, and that they are not due to a restricted choice of samples.

The most significant associations involved the γ and ω regions of the patterns. As previously reported[7,39], the γ-gliadin 42 was always present along with the γ-gliadins 40, 38, 33, while γ-gliadin 45 was associated with bands 43 and 35 (less significantly for the latter, which can be seen in both blocks). Strong negative correlations were also observed between the intensities of one or other of the bands 33, 38, 40, or 42 and those of either the bands 43 or 45. Interestingly, an association between the intensities of bands 33, 38, 40 or 42 and those of ω-band 23 and fast β-bands 67 or 68 was also noted.

New statistical associations were also discovered among α- or β-gliadins. The major parts of these regions clearly comprised groups of closely associated components, which were mutually exclusive. For instance, strong β-gliadin bands 55, 58, 61 and 63 typically made up a group present in cvs. Agathe or Mondur, while β-gliadin bands 59, 60, 62 and 65 made up a second group present in cvs. Bidi, Clairdoc or Tomclair. In the same way, four different associations were identified in the α-gliadin region (the major bands are italicized): *73*, 77, *79*, 84 and *89* (Mondur, Montferrier), 74, *75*, 78, *81*, 85 and 86 (Lakota, Primadur), *76*, 78, 80, *82*, 85 and 94 (Capdur, Régal), 75, 77, *84*, 86 and *91* (Arbois). In general, very few significant correlations could be observed either between major components belonging to α-gliadins and to β-gliadins respectively, or between these last groups and the γ plus ω- types, although 'γ-45' types seem more often to contain β-gliadins 59, 60, 62 and 65 and α-gliadins 73, 77, 79, 84 and 89 than 'γ-42' types.

Such associations can be interpreted on the basis of previous genetic studies, especially on the basis of Sozinov's earlier theory of gliadin blocks[41,42]. For instance, the typical band groupings in the ω-, and γ-gliadin regions and the negative correlations between block 23, 33, 38, 40 and 42 and block 35, 43 and 45 are in total agreement with the control of these proteins by tightly linked groups of allelic genes, located on the short arm of chromosome 1B[18,24,43]. The observations also confirm the occurrence in these new sets of cultivars or breeding lines of two major allelic types only for this family of ω- plus, γ-gliadins. It can be assumed from the work of Payne *et al.*[18] and of Du Cros *et al.*[24], however, that the components that have been assigned here to α- and β-gliadin blocks, and that seem to be inherited together in a monofactorial manner[44], correspond to closely linked families of genes encoded by the short arms of chromosomes 6A (locus *Gli-A2*) and 6B (locus *Gli-B2*). This would explain why no highly significant correlation between the different allelic compositions of α-, β- and γ- plus ω-gliadin bands was observed, except between the γ-42 block and the fast β-gliadin bands 67 and 68, an association previously identified by Lafiandra[43] and Du Cros *et al.*[24]. For the different

TABLE III. Correlation coefficients relating the intensities of some reduced protein subunits for 34 durum wheat cultivars (all coefficients are multiplied by 1000)[a]

SDS–PAGE subunits[b]	316	340	345	363	382	566	622	750	776	839	847	872	893	900
316 (6)	1000													
340 (13)	−255	1000												
345 (20)	−628***	−331	1000											
363 (16)	−218	742***	−302	1000										
382 (8)	887***	−9	−594***	−250	1000									
566	349*	68	−252	208	314	1000								
622	199	298	−277	333	244	479**	1000							
750	−386*	−21	173	−131	−323	−869***	−395*	1000						
776	−142	192	39	269	−167	535**	378*	−606***	1000					
839	227	−16	−123	73	206	833***	380*	−889***	701***	1000				
847	−291	−30	133	−168	−243	−852***	−496**	928***	−712***	−921***	1000			
872	300	139	−378*	136	273	448**	245	−476**	391*	447**	−504**	1000		
893	−431**	−272	531**	−248	−403*	−250	−211	262	−158	−162	228	−703***	1000	
900	256	94	−377*	46	294	460**	100	−387*	377*	455**	−440**	809***	−653***	1000

[a] Correlation coefficients above 587 are very highly significant (*** $P < 0{\cdot}001$), those over 430 are highly significant (** $P < 0{\cdot}01$), and those over 335 are significant (* $P < 0{\cdot}05$).

[b] Subunit nomenclature is from Berger and le Brun[32]. Assignment of bands according to the more commonly used system of nomenclature for HMW-glutenin subunits[31] is given in brackets.

cultivars and lines used in this study, it can also be deduced that locus *Gli-A2* comprises four major allelic types and locus *Gli-B2* two major allelic types on the basis of the major gliadin components.

(b) Associations between protein components (SDS–PAGE). Similar associations have been shown amongst the different sets of genotypes on the basis of SDS–PAGE patterns (Table III). Highly significant correlations between HMW-glutenin subunits (mobilities between 281 and 382) indicate the occurrence of five main allelic types (316 plus 382, 335 plus 382, 345, 340 plus 363 and 281 plus 340 plus 363) in the different sets of cultivars and lines. These correspond to the allelic types 6 plus 8, 7 plus 8, 20, 13 plus 16 and 2** plus 13 plus 16 according to the usual nomenclature for HMW subunits encoded by the long arm of 1A or 1B chromosome[26,27,31,45–49].

Other strong associations were observed among components of intermediate mobility (from 556 to 847) that have been shown to contain most of the ω- and γ-gliadins and LMW-glutenin subunits[18,39]. For instance, there was a close linkage between two major subunits (750 and 847), which are part of the strong triplet (called LMW2) occurring in type-45 durum wheats, whilst another association was observed between the subunits 776 and 839, which are part of the weaker quadruplet (called LMW1) in type-42 durum wheats. The latter subunits were also correlated with component 556, which occurs in either ω-gliadin 35 group or ω-gliadin group 33 plus 35 plus 38[39]. This is in agreement with previous reports that demonstrated the close linkage on the short arm of 1B chromosome between genes coding for all these ω- and γ-gliadin and LMW-glutenin components[18,20]. Another association was observed in the fast moving region, where two mutually exclusive patterns occur: either a doublet 872 plus 900, or a single band 893.

As shown in Table III, few highly significant correlations were found between bands belonging to different regions of the SDS–PAGE patterns, since the corresponding proteins are likely to be encoded by genes located on different chromosome arms[50] and have a high recombination frequency[51]. On the other hand, a major group of components with mobilities ranging from 606 to 630 has not been found to be systematically associated with other bands in the different sets of genotypes; this observation can be explained by the homology between these proteins and those identified in both salt extracts and glutenin extracts[39] or those identified as HMW-albumins in bread wheats[50], which are encoded by group 4 chromosomes.

(c) Associations between gliadins (PAGE) and reduced protein components (SDS–PAGE). A comparison of all protein groupings, within gliadin groups, within reduced component groups and also between both groups is presented in Fig. 4, based on the printout of the principal component analysis (PCA) of these components, for 34 varieties submitted for registration.

The occurrence of closely associated components belonging to gliadin and reduced protein component groups is clearly indicated on PCA printouts, for instance, between ω- plus γ-gliadin bands 23, 33, 38, 40 and 42 and subunits 566, 776 and 839, between γ-gliadin bands 43 and 45 and subunits 750 and 847, between β-gliadin type 55, 58, 61 and 63 and components 872 and 900, between β-gliadin type 59, 60, 62 and 65 and component 893. On the other hand, no significant association was observed between

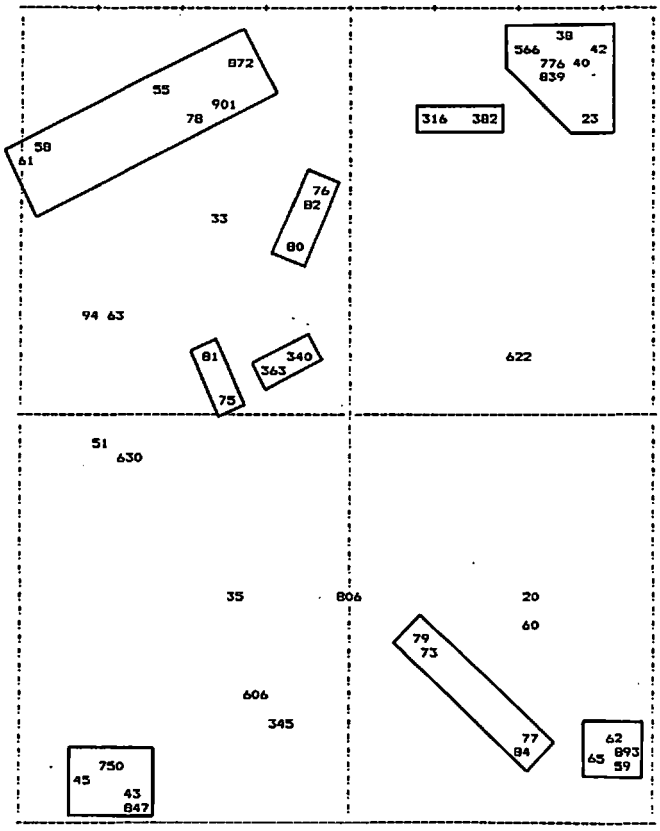


FIGURE 4. Principal component analysis distribution in the first principal plan of the proportions of 38 gliadin bands (numbered from 20 to 94) and 17 reduced protein subunits (numbered from 316 to 900) from 37 varieties submitted for registration.

fast-moving (α-type) gliadins and any of the SDS PAGE components that have been considered. This was to be expected since most of the α-gliadins correspond to SDS–PAGE bands with mobilities greater than 900, which have been excluded from this study because each band comprises several different proteins[39]. The same is true for most of the β-gliadin components, with the exception of components 872 and 900 or 893, which are likely to belong to this group. In general, few significant associations were observed between one allelic group and another, due to a high recombination frequency between groups that are encoded on different arms of the 1A, 1B and 6B chromosomes.

General Discussion

Many conventional genetic analyses of progenies have afforded evidence that wheat storage protein genes are clustered in complex loci and are inherited as linked groups (blocks), codominantly and in proportion to gene dosage in the triploid endosperm[1,2,43,52]. Because recombinations within a block occur very rarely, and because the

protein pattern of a homozygous genotype is simply represented by the sum of allelic variants, genetic analyses have permitted qualitative identification of a number of gliadin bands or glutenin subunits inherited together or, in some cases, of silent genetic loci.

Different approaches based on statistical analysis of protein components have been attempted previously either by looking for correlations between the proportions of different bands[4,5,40,53,54] or by agglomerative classification and generation of a minimum spanning tree[55–59], the proportions of different bands being determined either by densitometric scanning of electrophoretic patterns[4,5] or subjectively[53,54]. Although statistical correlation does not provide direct and indisputable evidence of genetic relationships, it is a simple and powerful tool, which can complement genetic analysis of progenies in identifying those protein components that occur either together and that are likely to belong to multigenic families, or that are mutually exclusive and that are likely to correspond to allelic types.

Care must be taken, however, when dealing with statistical results based on the scanning of one-dimensional electrophoretic patterns: besides problems inherent in all densitometric measurement (the amount of dye in a band is not always linearly associated with an actual amount of protein), it must be borne in mind that some bands are not pure molecular species and that variations may occur when scanning different samples of the same genotype because of experimental differences and because of possible effects of protein content and growing conditions. To avoid misleading conclusions, we have based our calculations on patterns obtained from several growing locations and from scans averaged from several repeats in order to obtain a better indication of the electrophoretic array of the genotype. We also excluded from the analysis a number of components that had been reported to contain several protein species.

On the other hand, it is essential to know whether the associations observed in the study are general for durum wheats or if they are restricted to a particular set of genotypes and derive from similarities in pedigree. To be valid, the conclusions have to be based on analysis of a large number of genotypes of diverse origins. Accordingly, three different sets of genotypes having a diverse background were sought, and only the associations that proved highly significant and reproducible from the different sets were retained in drawing conclusions.

To the extent that this experimental approach permits, several clear results have been obtained. For instance, correlation or PCA analysis based on band proportions have confirmed the occurrence in all cultivars and lines of strong associations between ω-gliadins, γ-gliadins, and LMW-glutenin subunits. Although the protein blocks generally comprise neighbouring bands, linkage of certain β-gliadins with γ- and ω-gliadins has also been found. Also, a catalogue of the main allelic types of the α and β regions of the durum wheat gliadin patterns has been established.

A summary of the main protein associations and presumed allelic variants that have been identified from the different sets of genotypes, is given in Fig. 5, with tentative chromosomal relationships on the basis of previous reports[18,24,43] and designations based on the most intense component of the block.

A relatively limited diversity was observed compared with previous reports based on

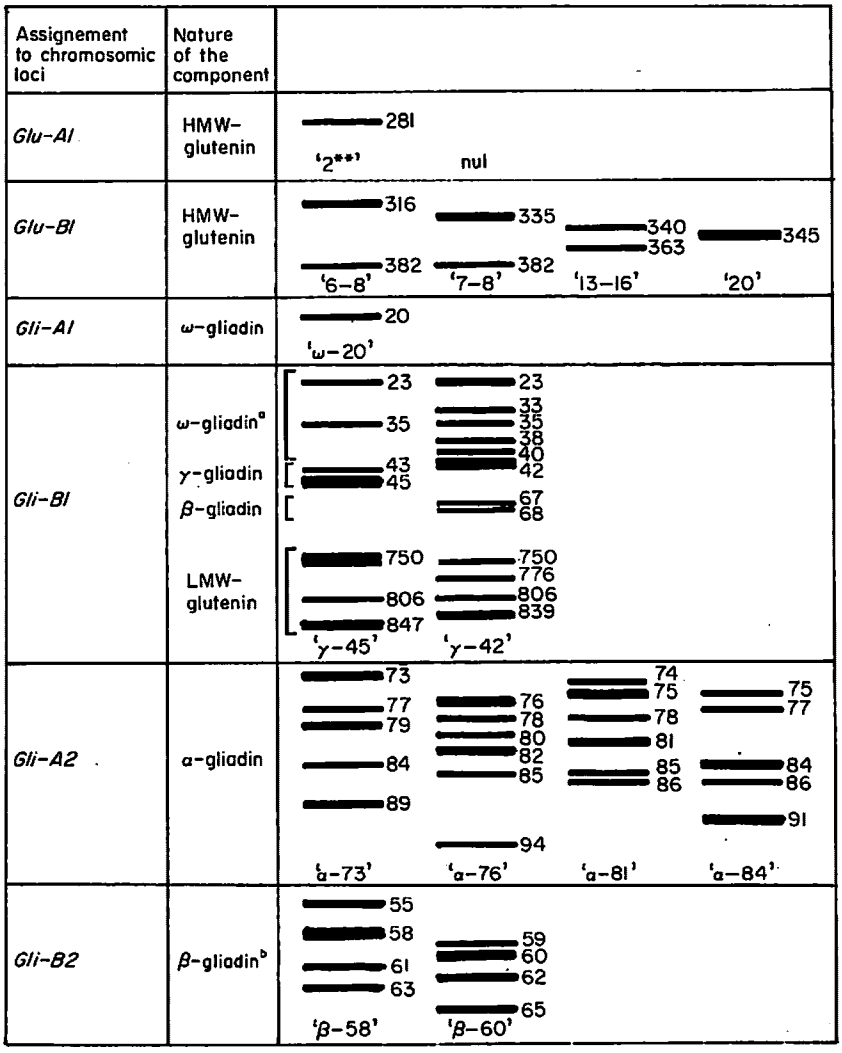


FIGURE 5. Band pattern variants of the major gliadin bands or glutenin subunits (identified in 1-dimensional PAGE or SDS–PAGE respectively) encoded by the different gene loci *Glu-A1*, *Glu-B1*, *Gli-A1*, *Gli-B1*, *Gli-A2* and *Gli-B2* in French durum wheat cultivars and breeding lines. The bands are numbered according to Zillman and Bushuk[9] for gliadins and according to Berger and Le Brun[32] for protein subunits. Chromosomal assignment has been assumed on the basis of the reports of Du Cros *et al.*[24], Lafiandra *et al.*[43] and Payne and Lawrence[31].

the study of bread wheat cultivars[46] or of durum wheat progenies[44]. Figure 5 does not include, however, minor variants of the main allelic types that could be observed from visual observation, because such variants may not appear from correlations or PCA analysis if they contain bands that apparently occur in different blocks.

Conclusions

Densitometric scanning of electrophoresis patterns of durum wheat proteins and statistical evaluation of quantitative data have been used to identify those components that are likely to belong to multigenic families or to correspond to different allelic types. Extending investigations to all groups of PAGE and SDS–PAGE bands has confirmed the strong associations between γ-gliadins, ω-gliadins and LMW-glutenin subunits and has identified new presumed allelic groups including α- and β-gliadins. These results provide a basis for further investigations of associations between specific durum wheat proteins and technological or agronomic attributes.

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