MULTIPLE APPROACH (IEF, SDS-PAGE AND A-PAGE) OF THE COMPOSITION OF LMW SUBUNITS OF GLUTENIN AND ITS EFFECT ON DOUGH PROPERTIES[1]

Marie-Hélène MOREL, Joëlle BONICEL, Valérie MÉLAS and Jean-Claude AUTRAN

Laboratoire de Technologie des Céréales, INRA, 2 Place Viala,
34060 Montpellier Cedex 1, France[2]

INTRODUCTION

Wheat has long been extensively studied in search of relationship of its proteins to flour baking quality. During the last decade, the presence of specific high-molecular-weight (HMW) subunits of glutenin has been correlated with baking quality in several countries [1-4]. In France, however, this system largely failed to satisfy breeders' expectation, making it necessary to investigate other fractions of the gluten complex [5,6].

Low-molecular-weight (LMW) subunits of glutenin are the least characterized protein fractions in bread wheats. They have proved much more difficult to analyse than gliadins or HMW subunits because of their unusual solubility, tendency to aggregate through various types of bonds and overlapping with some of the classical gliadins in 1-D SDS-PAGE systems. Consequently, with perhaps the exception of Australia [7-9], the description of LMW allelic types among bread wheats is still incomplete, and sometimes erroneous, and little information is available about the relative contributions of the various LMW variants to dough properties or baking quality.

Because of the difficulty to describe exhaustively LMW alleles with one single system, the aim of this study is to examine comparatively the potential of three different electrophoretic systems based on different principles of separation: SDS-PAGE [10], Acid-PAGE [11], and IEF [12].

EXPERIMENTAL

Plant materials

The genotypes examined included:

- Ten intervarietal chromosome substitution lines (that are part of the complete set of 36 lines) obtained from Station d'Amélioration des Plantes, INRA, Clermont-Ferrand (France) in which individual chromosome pairs of the homeologous groups 1 and 6 of the recipient variety Courtot have been replaced by their homologues from five donor varieties (Vilmorin 23, Cappelle, Magnif 27, Magdalena and Prinqual).
- Twenty one Italian cultivars that were assumed to contain a wide range of LMW alleles, on the basis of the allelic variation at the *Gli-1* (ω-gliadins) loci previously determined by N.E. Pogna (Istituto Sperimentale per la Cerealicoltura, S. Angelo Lodigiano) according to the nomenclature of Metakovsky [13]
- Fourty two French cultivars containing a well-balanced sampling of the most typical alleles of HMW subunits of glutenin and on which dough and baking scores are available at GEVES (Ministry of Agriculture).

Sequential extraction of proteins

Glutenin purification and solubilization were achieved according to Singh et al [14]. Flour (20 mg) was extracted three times with 50 % (v/v) propan-2-ol prior to solubilization of glutenin with 50 % (v/v) propan-2-ol, 0.08 *M* Tris-HCl (pH 8.0), 1% dithiothreitol and alkylation with 4-vinyl-pyridine. Glutenin was precipitated from 50 µl with 200 µl of acetone and the dried pellet was solubilized in 25 µl of 20 % glycerol, 6 *M* urea, 25 *mM* acetic acid.

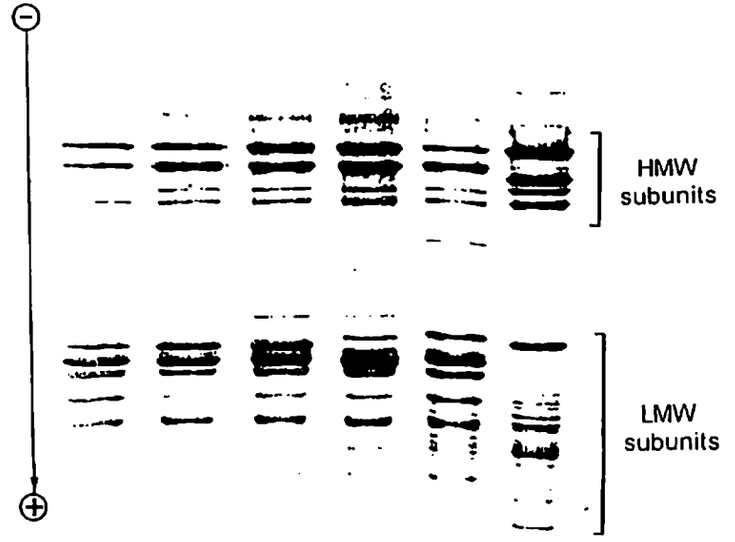

Figure 1. SDS-PAGE patterns of reduced and alkylated glutenin extracts from chromosome-1A substitution lines of cv. Courtot. 1, Courtot; 2, Vilmorin 23-1A; 3, Magnif 27-1A; 4, Prinqual-1A; 5, Magdalena-1A; 6, Gabo-1A

SDS-PAGE

The buffer system of Laemmli [15] was used with polyacrylamide gels (10.3% T - 3,45% C). The gels (160 x 180 x 0.75 mm) were run at 40 mA/gel for 4 hr 30 min at 18° C. The alkylated glutenin extract (see above) was mixed with an equal volume of a solution

[1] Research supported in part by a grant from the Commission of the European Communities, ECLAIR Programme, Contract AGRE 0052.

[2] With grateful acknowledgement of:
- Station d'Amélioration des Plantes, INRA, Clermont Ferrand (France) for providing us with chromosome substitution lines, and
- Istituto Sperimentale per la Cerealicoltura, S. Angelo Lodigiano (Italy) for providing us with Italian cultivars and with identification of their *Gli-1* alleles.

containing 2 % SDS, 40 % glycerol, and 20 µl of each sample were loaded. Typical results obtained by SDS-PAGE of reduced and alkylated glutenin extracts are presented in Fig. 1.

Acid-PAGE

Composition of gels and electrode buffers were as described by Clements [16]. Polyacrylamide gels (12% T - 3.1% C) contained 2*M* urea, 0.1 % ascorbic acid, 0.014 % ferrous sulfate 7 H_2O, and 0.75% glacial acid acetic, pH 3.1. The gels (160 x 180 x 1.5 mm) were cast one day before use and stored at ambient temperature. To cast one gel, 40 ml of the above gel solution were deaerated 5 min under vacuum at ambient temperature and then 55 µl of 0.6 % (v/v) H_2O_2 catalyst were added. Seven µl of protein extract were loaded and electrophoresis was carried out for 3 hr 45 min at 500 volts at 18 °C. Typical results obtained by A-PAGE of reduced and alkylated glutenin extracts are presented in Fig. 2.

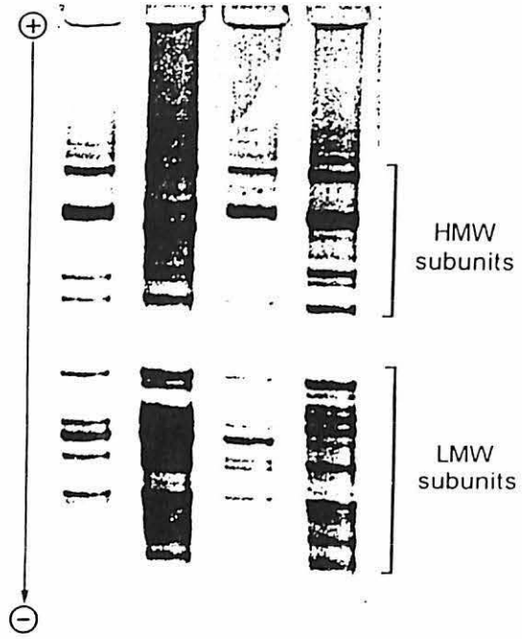

Figure 2. Acid-PAGE patterns of reduced and alkylated glutenin extracts from chromosome-1B substitution lines of cv. Courtot. 1, Magdalena-1B; 2. Prinqual-1B; 3, Cappelle-1B; 4, Magnif 27-1B

Isoelectric focusing

Isoelectric focusing (IEF) was carried according to Morel and Autran [12] in ultra-thin (0.2 mm) polyacrylamide gels (5 % T, 2.8 % C, in 45 mM Tris-HCl, pH 8.8, containing 10 % glycerol). The polymerization of gel solutions (20 ml) was catalyzed with 60 µl of TEMED and 60 µl of a 15% (w/v) ammonium persulfate solution. After polymerization for 20 min, the gels were washed 3 x 10 min with water, 1 x 30 min with glycerol 10 % (v/v), then

dried overnight at room temperature. Before use, the gels were simply rehydrated by spreading 15 ml of a 8 *M* urea solution containing 2.6 % w/v of BDH ampholytes pH 6.0-9.5 and 50 *mM* DTE) on the gel surface. Isoelectric focusing was performed at 17° C with glutamic acid and sodium hydroxide as anolyte and catholyte, respectively. After a 400 V x h prerun at constant power (7 W), 5 µl samples were loaded at the anodic side of the gel. Focusing conditions were set to 2000 V x h at 7 W (maximum power) and then to 1500 V x h at 2800 V (constant voltage). Typical results obtained by IEF of reduced and alkylated glutenin extracts are presented in Fig. 3.

Staining

The gels were stained with Coomassie Brilliant Blue R 250 (0.05 % w/v) in 12,5% trichloroacetic acid and destained with 10 % (w/v) TCA solution.

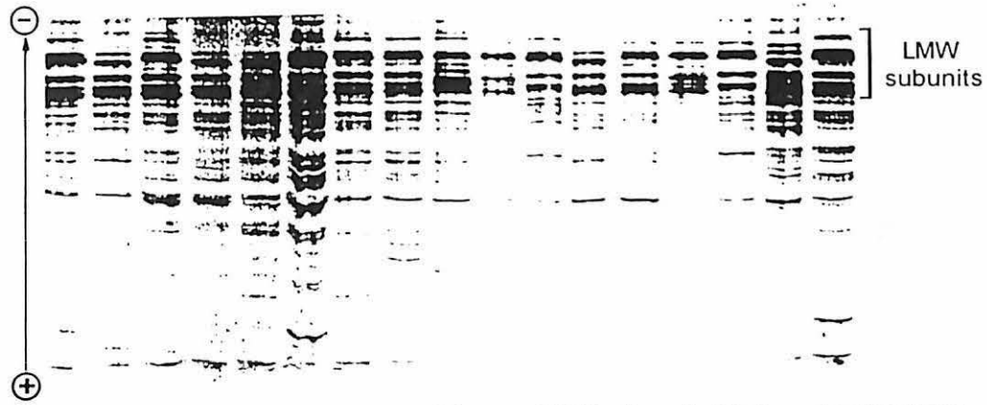

Figure 3. Isoelectric focusing patterns (pI range 6-9.5) of a set of Italian wheat cultivars.

RESULTS AND DISCUSSION

Intervarietal chromosome substitution lines

This set of lines afforded an accurate and unambiguous identification of several LMW allelic types from each of the A, B and D genome.

For instance, the analyses of chromosome-1A substituted lines by the three techniques allowed identification of four allelic types at the *Glu-A3* locus. The allelic variant observed in Vilmorin-1A differs from that of Courtot (or Magnif 27-1A) by a single minor band in A-PAGE or SDS-PAGE whereas both Prinqual-1A and Magdalena-1A can be distinguished from Courtot by a major LMW band in the three electrophoretic systems. At the *Glu-B3* locus (Fig. 4), a first allelic type referred to as 'I' could be clearly identified in Magnif 27-1B line in the three systems. In contrast, the discrimination between the two other variants referred to as 'II' in Courtot, and in Magdalena-1B, and 'III', in Prinqual-1B and in Cappelle-1B, was much easier from A-PAGE or IEF patterns than from that of SDS-PAGE.

For the *Glu-D3* alleles, no difference was observed from IEF patterns, but there are strong differences between the variants observed in Prinqual-1D and Magdalena-1D, Magnif 27-1D, and the standard Courtot in both A-PAGE and SDS-PAGE.

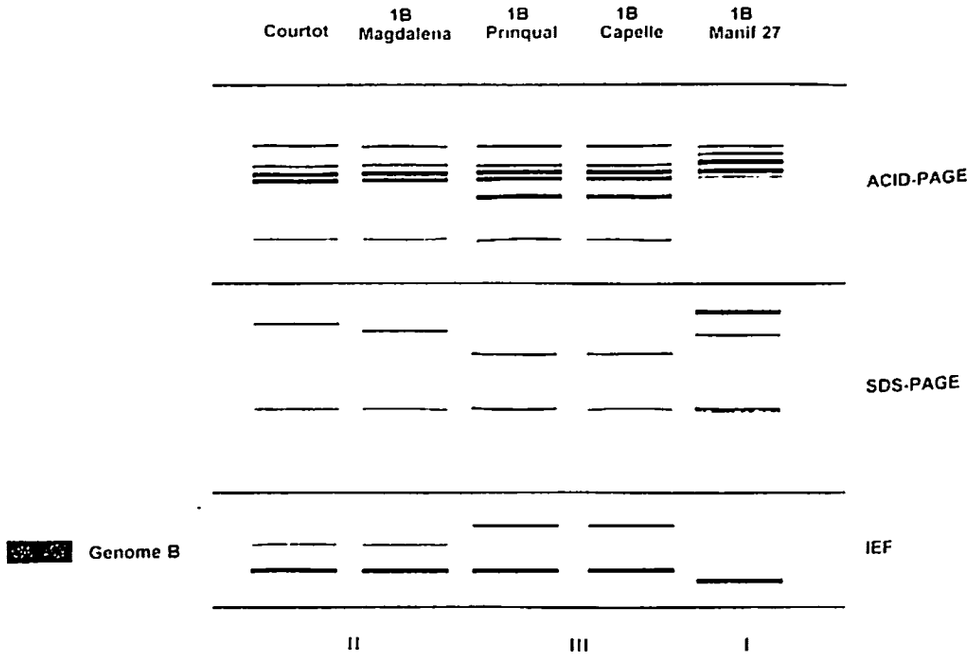

Figure 4. Schematic summary of the allelic variation observed at the *Glu-B3* locus among chromosome-1B substitution lines of cv. Courtot on the basis of A-PAGE, SDS-PAGE, and IEF patterns.

As a whole, the three electrophoretic systems do not have the same discriminating power for describing the various LMW allelic types. For instance, IEF separations (in fact, the most basic region of the IEF patterns) were helpful for the rapid identification of the allelic variants 'I', 'II' and 'III' of the *Glu-B3* locus, but they have a limited interest for locating the other types of alleles. On the other hand, whereas all the 16 bands of Courtot identified in A-PAGE could be assigned to a specific chromosome, about 50 % of the bands observed in SDS-PAGE could not be assigned, because of a too great number of LMW components having very similar molecular sizes. In the following, we will therefore present by priority the A-PAGE separations, using SDS-PAGE mainly as a checking tool.

Description of LMW alleles among Italian cultivars

Among the set of 21 Italian cultivars the above mentioned alleles were identified, and some new others. This task was made easier by taking advantage of the classification proposed by N.E. Pogna on the basis of the allelic variation at the *Gli-1* locus derived from the study of ω-gliadins and based on the nomenclature of Metakovsky [13].

For instance, according to the identification of Pogna, allelic types at the *Glu-A3* locus were classified into five groups as 'b/f', 'a', 'm', 'o', and a null allele. At the *Glu-B3* locus, six types were found: 'k/m', 'd', 'g', 'b', 'e', and 'f' that correspond to subgroups of the three

alleles 'I', 'II' and 'III' previously defined from chromosome substitution lines. At the *Glu-D3* locus, the patterns were classified into 5 types as 'k', 'd', 'a', 'f', and 'b'.

In fact, the description of all these alleles has been obtained step by step, by putting together cultivars having similar patterns at the two first loci, e.g. *Glu-A3* and *Glu-B3*, and seeking for a variation thus attributable to the third locus *Glu-D3*, and so on (Fig. 5).

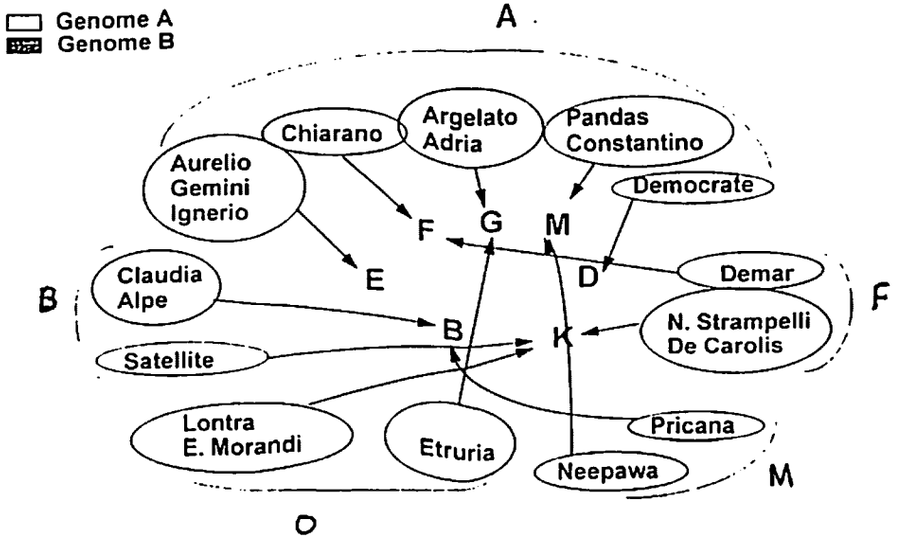

Figure 5. Discrimination between the main allelic types encoded by the B genome among Italian cultivars

Such an identification can be achieved, however, if the sampling of genotypes affords various combinations of each allele with alleles of the other loci. For instance, it has not been possible to assign the *Glu-3* bands of the rare allele classified as 'j' because only one combination ('m'-'d'-'j' in cv. Neepawa) was available.

As for the previous set of samples, the A-PAGE method has been found as the most adapted for discriminating between LMW variants. As an example, some LMW alleles such as the 'm' at the *Glu-A3* locus could even be identified through A-PAGE only, while it cannot be easily distinguished from the null allele by SDS-PAGE.

Effect of allelic variation of LMW glutenin subunits on dough properties of French bread wheats

Several previous studies suggested that a more effective predictive model of dough properties should include the composition of both the high and low molecular weight studies of glutenin [5, 6, 9]. On the basis of the above-mentioned description of the main LMW allelic types, 42 French cultivars were therefore surveyed in the aim of finding how

much variability of LMW glutenin existed and to what extent these different binding patterns influenced the dough properties, in conjunction with the variation of HMW subunits.

This population of French wheats, however, showed relatively few variants, some of these occurring with extremely high frequency (Table 1).

Table 1
The HMW and LMW banding patterns among a set of 42 French bread wheat cultivars and their occurrence.

	Glu-1 loci	Count	*Glu-3* loci	Count
Genome A	null	29	b/f	14
	2*	8	o	13
	1	5	m	8
			a	4
			n	3
Genome B	7-8	14	III	21
	7	10	II	18
	7-9	9	n	3
	6-8	9		
Genome D	2-12	23	b	27
	5-10	18	f	9
	4-12	1	d	3
			a	3

For instance, at the *Glu-B3* locus, two major groups predominate, viz. 'II' (18) and 'III' (21), whereas the type I occurred very infrequently, in contrast with the Italian set that we analyzed. Three cultivars with the null allele (that were in fact 1B/1R translocated) were also identified. Moreover, about 65 % of the total cultivars contain only two variants ('b/f' and 'o') at the *Glu-A3* locus and one variant 'b' at the *Glu-D3* locus.

Despite the fact that the composition of our population of cultivars was much better balanced for HMW subunits than for LMW subunits, a statistical evaluation was attempted to investigate relationships with dough properties estimated by the various parameters of Chopin Alveograph (*W*, baking strength; *P*, dough tenacity; *G*, dough extensibility).

A first analysis of variance carried out from HMW subunits showed that the variation at the *Glu-1* loci explained only 15-20 % of the variation of both *W* index, (with a slight positive effect of the *Glu-B1* subunits 7+8 or 7+9 alleles) and *G* index (Table 2) (with a positive effect of the *Glu-D1* subunits 2+12, opposed to that of the subunits 5+10).

In contrast, in agreement with an earlier report from Gupta *et al.* [9], as much as 35 % of the variation of *W* and 25 % of the variation of *G* were explained by the allelic

composition at the *Glu-3* loci, with special effects of the *Glu-B3* and *Glu-A3*. For instance, the *Glu-B3* 'II' and 'III' alleles have positive effects on *W* index, but the latter only seems to determine significantly higher *G* values. At the *Glu-A3* locus, alleles 'a' and 'm' show significant positive effects on *W* index, whereas *G* index seems primarily determined by alleles 'o' and 'n'.

Table 2
Analysis of variance for *G* index of Chopin Alveograph

Source of variation	Sum of squares	d.f.	F ratio
Main effects			
Glu-A3	36.025	4	1.098*
Glu-B3	50.951	2	3.105***
Glu-D3	8.004	3	0.325
Residual	262.528	32	
Total	375 423	41	

A multiple range analysis for *G* allowed to specify the ranking of the various alleles with regard to dough parameters. Considering *W* index, the strong positive effect of the *Glu-B3* 'II' and 'III' alleles was confirmed (mean *W*: 165 and 180, respectively), by comparison with the null allele 'n' (mean *W*: 102), as well as a positive effect of the *Glu-A3* 'm' and 'a' alleles. Considering *G* index (Table 3), it was also confirmed that the *Glu-B3* 'III' allele tended to determine higher values than alleles 'II' or 'n'. In addition, some *Glu-A3* alleles such as 'o' and 'n' seem to positively influence dough extensibility, as opposed to 'm' or 'b/f'.

Table 3
Multiple range analysis for *G* index of Chopin Alveograph.

Locus	Mean *G*	Count	Homogeneous groups
Glu-B3 alleles			
n	17.9	3	A
II	19.7	18	A
III	21.6	21	B
Glu-A3 alleles			
m	18.6	8	A
b/f	18.8	14	A
a	19.5	4	AB
o	20.7	13	B
n	21.2	3	B

On the other hand, to the extent that our approach and sample set permit, the statistical analysis stands against a pure cumulative effect of LMW and HMW subunits. For instance whereas cvs with LMW 'III' have a mean value of *G* greater than those with LMW 'II', an interaction with the effect of HMW subunits is clearly apparent, as shown in Fig. 6. For instance, when associated with subunits 5+10, the presence of the 'III' allele does not impart higher average values of *G* index compared with the 'II' allele, whereas a very significant increase in *G* is observed (from 19.6 to 22.1) when the allele 'III' is associated with subunits 2+12. The improving effect of 'III' is apparent only in the presence of the 2+12 type of HMW subunits, as if the '5+10' subunits, that determine a higher tenacity of the dough, was blocking the orderly slipping of molecules that characterizes the extensible behaviour. So far, we have no clear explanation of this interaction. Whether it results from specific interactions between *x* or *y* HMW subunits with certain chain terminator LMW types warrants further investigation and should be addressed in a future work.

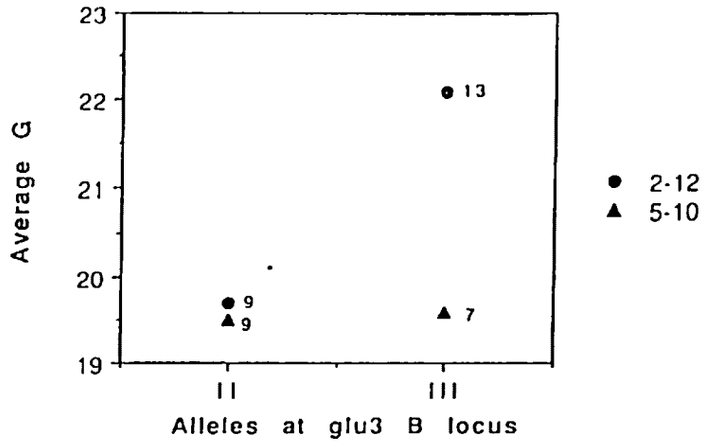

Figure 6. Interaction between glutenins encoded at the *Glu-D1* and *Glu-B3* loci.

The results of this investigation can be helpful in wheat breeding programmes aimed at improving baking quality. For instance, associating on the same genotype the *Glu-D1* alleles that seem to impart a high tenacity (*e.g.* subunits 5+10) with the *Glu-3* alleles that are found associated with the highest values of dough extensibility (e.g. 'o' or 'n' allele at the *Glu-A3* and 'III' allele at the *Glu-B3*) should result in the development of new wheats adapted to the modern French baking processes (recommended *W* index > 200, with $P/L \leq 0.7$). In a similar way, associating the same LMW alleles that seem to determine high extensibility with HMW subunits 2+12 at the *Glu-D1* locus should allow breeding for biscuit-quality wheats (recommended *W* index: 100-150 with *P/L*: 0.3-0.5).

Interestingly, as it was suggested among HMW subunits (*Glu-A1* and *Glu-B1*), some allelic types of LMW subunits seem associated with greater amounts of the protein components. For instance, at the *Glu-B3* locus the 'III' allele is encoding additional LMW bands compared with the 'II' or with the null allele. Whereas a greater amount of HMW

subunits at the *Glu-A1* or *Glu-B1* loci seems to impart higher tenacity of the dough, a greater amount of proteins encoded by the *Glu-3* loci seem, in contrast, to impart greater extensibility suggesting strong differences in the functional properties and contribution to viscoelasticity between LMW and HMW types of glutenin subunits.

CONCLUSIONS

1) The complete description of the *Glu-3* alleles is a very complex task that cannot be derived from a single technique *e.g.* SDS-PAGE.
2) Acid-PAGE separation of alkylated LMW subunits is adapted to the identification of the most typical *Glu-B3* variants, at least among French genotypes.
3) The specific effect of LMW subunits as well as the interactions between glutenin components encoded at the *Glu-1* and *Glu-3* loci might explain some major discrepancies previously observed in the relation between HMW composition and dough properties.
4) In the aim of breeding, for instance, biscuit-type wheats, it could be recommended to screen lines containing the *Glu-B3* 'III' allele associated with the *Glu-D1* allele coding for subunits 2+12.
5) The amount of protein expressed by the various *Glu-B3* alleles might be related to their effect on dough extensibility.

REFERENCES

1 Payne, P.I., Corfield, K.G., Holt, L.M. and Blakman, J.A. Correlations between the inheritance of certain high-molecular-weigh subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. 32 (1981), 51-60.

2 Payne, P.I., Holt, L.M., Krattiger, A F. and Carrillo, J.M. Relationships between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. J. Cereal Sci. 7 (1988), 229-235.

3 Lukow, O.M., Payne, P.I. and Tkachuk, R. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. J. Sci. Food Agric. 46 (1989), 451-460.

4 Rogers, W.J., Payne, P.I. and Harinder, K. The HMW glutenin subunit and gliadin compositions of German-grown wheat varieties and their relationship with bread-making quality. Plant Breed. 103 (1989), 89-100.

5 Gazanhes, V., Morel, M.H., and Autran J.C. The low-molecular-weight glutenin composition of French bread wheats and its effect on dough properties. Cereal Foods World, 36 (1991), 723.

6 Khelifi, D. and Branlard, G. The effects of HMW and LMW subunits of glutenin and of gliadin on the technological quality of progeny from four crosses between poor breadmaking quality and strong wheat cultivars. J. Cereal Sci. 16 (1992), 195-209.

7 Gupta, R.B. and Shepherd, K.W. Genetic control of LMW glutenin subunits in breac wheat and association with physical dough properties. In: Proc. 3rd Internationa Workshop on Gluten Proteins (R. Lásztity and F. Békés, Ed.), pp. 13-19, Work Scientific, Budapest (Hungary) 1987.

8 Gupta, R.B. and Shepherd, K.W. Two-step one-dimensional SDS-PAGE analysis o LMW subunits of glutelin. 1. Variation and genetic control of the subunits in hexaploic wheats. Theor. Appl. Genet. 80 (1990), 65-74.

9 Gupta, R.B., Békés, F. and Wrigley, C.W. Prediction of physical dough properties from glutenin subunit composition in bread wheats : correlation studies. Cereal Chem. 68 (1991), 328-333.

10 Payne, P.I. and Lawrence, G.J. Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheats. Cereal Res. Commun. 11 (1983), 29-35.

11 Morel, M.H. Acid-PAGE of wheat glutenins: a new tool for the separation of high- and low-molecular weight subunits. Cereal Chem. (in press).

12 Morel, M.H. and Autran, J.C. Separation of durum wheat proteins by ultra-thin IEF: a new tool for the characterization and quantification of low-molecular-weight glutenins. Electrophoresis 11 (1990), 392-399.

13 Metakovsky E.V. Gliadin allele identification in common wheat. II. Catalogue of gliadin alleles in common wheat. J. Genet. Breed. 45 (1991), 325-344.

14 Singh, N.K., Shepherd, K.W. and Cornish, G.B. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J. Cereal Sci. 14 (1991), 203-208.

15 Laemmli, UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (1970), 680-685.

16 Clements, R.L. 1988. A continuous acetic acid system for polyacrylamide gel electrophoresis of gliadins and other prolamines. Electrophoresis 9 (1988), 90-93.

Gluten Proteins

1993

Association of Cereal Research