Effects of HMW- & LMW-glutenins and grain hardness on size of gluten polymers

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Abstract

Bread quality depends on various traits of wheat flour including storage protein composition, grain hardness and size of gluten polymers. To better understand relationships between these traits, a statistical study was performed on a set of French varieties cultivated in 3 locations during 2 years. Variation in grain hardness was primarily associated with SNPs within the *PinB* gene and not in *PinA* (no *PinA* variants were detected), and also with the HMW-*Glu-D1* locus. The mass of storage protein polymers varied from 5 to 49 million Daltons, as evaluated by Asymmetric Flow Field Flow Fractionation. Polymer mass and its heterogeneity were mainly influenced by the LMW-*Glu-D3* locus. Percentages of storage protein fractions, evaluated by SE-HPLC, were significantly affected by the LMW-*Glu-B3*, *-A3* and HMW-*Glu-D1* loci. Interactions between glutenins and puroindoline-B alleles significantly influenced polymer characteristics and percentages of ω-gliadins. These effects were variable depending on glutenin and puroindoline alleles. Environmental effects on flour quality traits were also studied. The sum of mean June and July temperatures, the two last months of grain development, was significantly correlated with polymer characteristics but not with storage protein proportions. Moreover, temperatures during the last 2 months of grain development had variable effects on the characteristics of gluten polymers depending upon grain hardness. This study is the first report of interactions between glutenins and puroindoline alleles affecting storage protein polymer traits.

Introduction

Previous studies on wheat near-isogenic lines showed that kernel hardness affects the molecular mass of storage protein polymers (Lesage et al. 2011). The amount of unextracted polymeric proteins (UPP) is well known to have a major influence on dough properties. Many studies have developed approaches to assess the polymeric size of storage proteins using SE-HPLC (Dachkevitch and Autran 1989). The Asymmetrical Flow Field-Flow Fractionation (AFFFF) technique is a tool able to assess the size diversity of gluten polymers. Only a few studies have reported variation in wheat storage proteins polymers using AFFFF (Stevenson and Preston 1996) and their influence on rheological properties of dough (strength, elasticity and extensibility). To assess effects of both grain hardness and glutenin alleles on storage protein polymers, genetic diversity of various traits (grain hardness, protein content, glutenin alleles, polymer characteristics and percentages of five protein fractions) was investigated in a set of French cultivars.

Materials and methods

A total of 68 bread wheat varieties were cultivated two successive years (2009, 2010) in 3 locations in France. In each location, 40 varieties were grown in two replicates in conventional conditions with full fungicide protection.

Grain hardness (GH) was assessed by Near-Infrared Spectrometry (NIRS) on whole meal flour according to the AACC 39-70A method. Three classes of grain hardness were recorded according to NIRS scores: Soft varieties below 35, Medium varieties between 35 and 75, and Hard varieties above 75. Protein content (PC) was measured by NIRS according to the AACC 39-11.01 method. High molecular weight (HMW)- and low molecular weight (LMW)-glutenin alleles were determined by SDS-PAGE electrophoresis. Puroindoline A and B genes (*PinA* and *PinB*) were sequenced by the Sanger method. Storage protein polymers characteristics (mass (Mw2), polydispersity index (Mw/Mn2), polymer radius (Rw2)) were evaluated by

Asymmetrical Flow Field-Flow Fractionation (AFFFF) described by Chiaramonte and colleagues (Chiaramonte et al. 2012). Percentage of five protein fractions (%F1 to %F5) was obtained by Size Exclusion-HPLC (SE-HPLC) on a TSK G4000-SW column (Tosohaas). Statistical analyses (General Linear Model and Partial Least-Squares procedures) were performed using Statgraphics software (Statpoint Technologies, Inc., VA, USA). These analyses allowed assessment of the influence of glutenin loci and glutenin alleles, as well as puroindoline-B alleles, on polymer characteristics and on SE-HPLC fractions. Environmental effects on polymer size were also studied.

Results and discussion

1. Large variations in polymer characteristics were measured using AFFFF

Over the 240 samples of bread wheat, a very large variation of polymer characteristics was observed. For example, the mass (Mw2) varied from 5.4 million to 48.8 million Daltons. Polymer radius (Rw2) varied from 36.5 to 116.2 nm. On the same samples, polymer mass obtained by SE-HPLC varied from 0.75 million to 2 million Daltons. Taking into account that polymer mass measured by AFFFF and SE-HPLC F1 and F2 fractions were not correlated, these results indicate that SE-HPLC results do not represent the entire polymer mass variation in storage proteins.

2. Glutenin loci affect differently the polymer characteristics and SE-HPLC fractions

Polymer characteristics (molecular mass, Mw2; polydispersity index, Mw/Mn2; and polymer radius, Rw2) were mainly influenced by the *Glu-D3* locus, and to a lesser extent by *Glu-B1* (Table 1).

As shown by R^2 values, the percentages of SE-HPLC protein fractions reflected the diversity of glutenin loci much more than polymer characteristics. It is known that fractions F1 to F5 contain different protein classes (F1: HMW glutenin polymers, F2: LMW glutenin polymers, F3: ω-gliadins, F4: γ and β gliadins, F5: α-gliadins and albumins-globulins). The *Glu-B3* locus had a significant effect on SE-HPLC percentages of all protein fractions. The *Glu-A3* locus impacted %F1, %F3 and %F4, whereas the *Glu-D1* locus significantly influenced %F1, %F2 and, to a lesser extent, %F3.

Glu-D1 also had a significant effect on grain hardness (Table 1), which was significantly higher in varieties carrying Glu-D1 5+10 alleles than in those carrying Glu-D1 2+12 (Fig. 1).

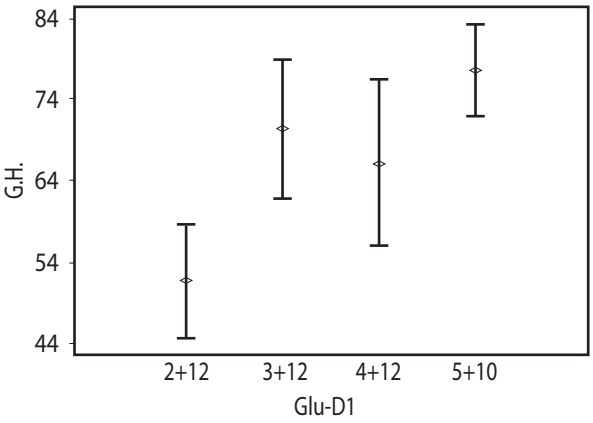

Fig. 1 Relationship between *Glu-D1* alleles and grain hardness.

Table 1. Effects of glutenin loci on some quality traits.

		Protein content	Grain hardness	Mw2	Mw/Mr2	Rw2	%F1	&F2	&F3	&F4	%F5
	Glu-A1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
HMW-GS	Glu-b1	ns	ns	*	**	*	*	*	ns	ns	ns
	Glu-D1	ns	***	ns	ns	ns	***	***	*	ns	ns
	Glu-A3	ns	*	ns	**	ns	***	ns	**	***	ns
LMW-GS	Glu-B3	ns	ns	ns	ns	ns	***	***	***	***	**
	Glu-D3	ns	ns	***	***	***	ns	ns	ns	ns	*
R^2 %		0.2	19.9	9	15.9	11.2	47.1	58.7	16.9	45.3	10.5

HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits; ns = not significant; MW2 = molecular mass; Mw/Mn2 = polydispersity index; Rw2 = polymer radius.

This observation indicated a strong relationship between Glu-D1 alleles and grain hardness, both of which are important characteristics for wheat end use quality.

3. Effects of glutenin alleles on polymer mass

Partial Least Square analysis revealed that glutenin alleles had different effects on polymer mass, some being positive, and others negative (Fig. 2). *Glu-B1-7*, usually associated with poor bread quality, had a much more positive effect on final mass of polymer than other alleles. The two *Glu-D3* alleles showed opposite effects on polymer mass, *Glu-D3-b* being positive and *Glu-D3-c* negative. *Glu-B3-b* also had a negative effect on polymer mass Mw2.

4. Characterization of puroindoline-B alleles

PinA and *PinB* are major genes of the *Ha* locus involved in grain hardness. They were sequenced in all varieties. The *PinA-D1a* allele was present in all varieties and no SNP or deletions were found within its sequence. *PinB* sequences comprised five alleles, the first one, *Pinb-D1a*, being the *PinB* allele of Soft varieties. Three alleles, *PinB-D1b*, *-D1d* and *-D1b+d*, carried SNPs at positions T217A, G223A, T217A + G223A from the start codon, respectively. These SNPs led to the following modifications in the sequence of puroindoline B: W73R, G75S and W73R + G75S from the starting methionine (Table 2). The fifth allele, *PinB-D1c*, contained a SNP (T266C) leading to a L89P substitution from the starting methionine. *PinB-D1b*, *-D1d* and *-D1c* were already described in the literature, whereas the *PinB-D1b+d* allele is new.

5. Interaction between glutenin and puroindoline B loci

Interaction effects were calculated for glutenin loci and 3 alleles of *PinB* (*PinB-D1a, -D1b and –D1d*); the frequencies of alleles *PinB-D1c* and *-D1b+d* were too low for meaningful comparisons.

Grain hardness was obviously highly explained by the interaction of the glutenin and puroindoline-B loci, except, strikingly, for the interaction between *Glu-A3* and *PinB* (Table 3). Nevertheless, explanation of the trait by the

Table 2. Amino acid sequence variation of puroindoline-B in 68 French bread wheat varieties.

Puroindoline variant	Partial amino acid sequence Position: 73-75----------89		
PINB-D1a	WPTKWWKGGC	----------	L
PINB-D1b	WPTKWWKSGC	----------	L
PINB-D1d	WPTKWRKGGC	----------	L
PINB-D1c	WPTKWWKGGC	----------	P
PINB-D1b + d	WPTKWRKSGC	----------	L

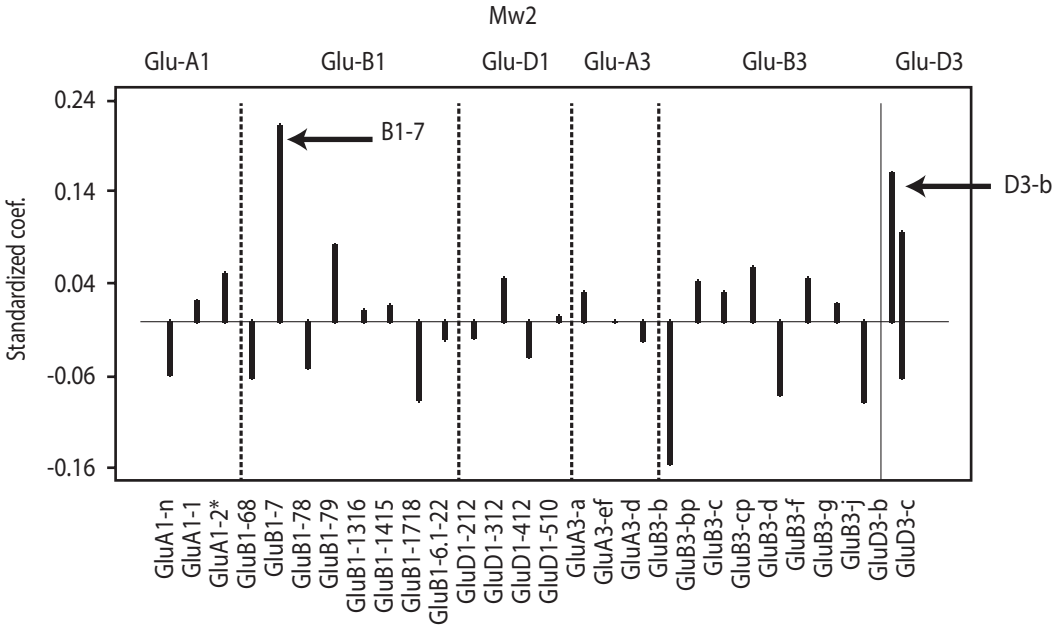

Fig. 2 Effects of individual glutenin alleles on polymer mass Mw2.

interaction was always lower or at least equal (R^2 = 69.4% for *Glu-B3* x *PinB*) than that of all loci *Glu* + *PinB* without interaction.

Some HMW-GS loci and LMW-GS loci also exhibited significant interaction with the *PinB* locus for polymer characteristics and %F3 (Table 3). *Glu-B1* and *Glu-A3* loci effects on polymer mass (Mw2) increased when combined with effects of *PinB* alleles from 10% to 13.6% and 13.9%, respectively. The effect of interaction on polymer dispersity index (Mw/Mn2) increased from 16.8% to 17.8% and 20.5% for *PinB* x *Glu-B1* and *PinB* x *Glu-A3*, respectively. In regard to the polymer radius trait (Rw2), the interaction was not significant for LMW-Glutenin loci x *PinB*, whereas a marginally significant interaction effect was revealed for *PinB* x HMW-*Glu-B1*, increasing the R^2 from 12.7% to 14.4%. Concerning percentages of protein fractions, *Glu-B1* and *Glu-B3* had significant interactions with the puroindoline B locus, increasing the explained effect on the %F3 fraction from 19.4% to 22.8% and 20.2%, respectively. No effects on F1 and F2 percentages were evident.

6. Effects of interaction between glutenin alleles and puroindoline-B alleles

In regard to interactions between glutenins and puroindoline-B alleles, we observed different effects depending on alleles. For instance, *Glu-B1-b* encoding subunits 7+8 showed a favorable effect on polymer mass (Mw2) in Soft varieties carrying *PinB-D1a*, whereas their effect was unfavorable in Medium and Hard genotypes carrying *PinB-D1b* and *-D1d* (Fig. 3A). In contrast, the interaction between *Glu-A3* alleles and the puroindoline B soft allele *-D1a* resulted in a much lower polymer mass than the mass observed for the interaction between *Glu-A3* alleles and *PinB-D1b* alleles (Fig. 3B).

Table 3. Effects of interaction between the glutenin and puroindoline-B loci.

		Grain hardness			Mw2			Mw/Mn2			Rw2			%F3		
		Glu effect	Glu x PinB effect	R^2%	Glu effect	Glu x PinB effect	R^2%	Glu effect	Glu x PinB effect	R^2%	Glu effect	Glu x PinB effect	R^2%	Glu effect	Glu x PinB effect	R^2%
HMW-GS	A1	ns	***	25.8	ns	ns	11.3	ns	ns	16.9	ns	ns	14.3	ns	ns	19
	b1	ns	***	56.6	*	**	13.6	**	**	17.8	*	**	14.4	ns	*	22.8
	D1	***	***	27.7	ns	ns	10.9	ns	ns	14.6	ns	ns	12.4	*	ns	16.4
LMW-GS	A3	*	ns	17.7	ns	**	13.9	**	***	20.5	ns	ns	15.2	**	ns	15.6
	B3	ns	***	69.4	ns	ns	10.1	ns	ns	17	ns	ns	13.2	***	***	20.2
	D3	ns	***	45.4	***	ns	4.9	***	ns	7.4	***	ns	6.8	ns	ns	21.7
	R^2 %	19.9			9			15.9			11.2			16.8		
Glu + PinB	R^2 %	69.4			10			16.8			12.7			19.4		

HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits; ns = not significant; MW2 = molecular mass; Mw/Mn2 = polydispersity index; R2 = polymer radius.

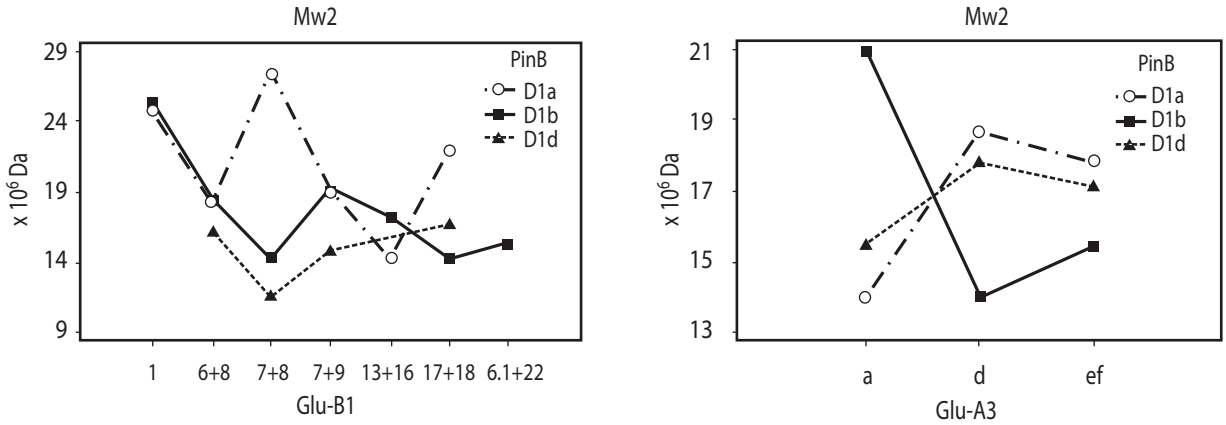

Fig. 3 Interaction values found for *PinB* x *Glu-B1* alleles (A) and *PinB* x *Glu-A3* alleles (B). MW2 = polymer mass.

7. Temperature during grain development was a major factor influencing polymer characteristics

Variance analysis revealed that both year and location significantly influenced polymer characteristics (R^2: 44.9%, 51.0%, and 56.4% for Mw2, Mw/Mn2 and Rw2, respectively) confirming that environment had a major influence on gluten polymers.

We further analyzed this environmental effect, using the sum of mean temperatures for June and July, the two last months of grain development to ripeness. As revealed through variance analysis, the summed mean temperatures for June and July had a similar statistical effect to year and location effects on polymer characteristics only. The other traits were not significantly influenced.

Moreover, the summed mean temperatures for June and July differentially affected the molecular weights of polymers in the three hardness classes (Fig. 4). A difference of one hundred degrees (recorded between 6 experimental locations) increased the final mass of polymers by an order of 2 in Hard varieties (from 10 to 20 million Da), and by an order of nearly 3 in Soft varieties (from 13 to 33 million Da). Considering these huge variations, it becomes clear that they may account for the strong impact of genotype x environment interactions on the dough quality often observed and rarely explained.

Interestingly, no significant effect was observed on percentages of protein fractions, indicating that storage protein composition was not affected by temperature during the two last months of grain development. The fact that even the F1 fraction was not impacted by temperature clearly indicates that the F1 fraction does not contain the largest polymers. These results provide evidence that the largest polymers were not retrieved by SE-HPLC and that AFFFF gives more reliable data for native polymer characterization than SE-HPLC.

It should be noted that the present results, indicating that Hard varieties resulting from SNPs of *PinB* had lower polymer mass than Soft varieties, are contrary to those obtained for Hard and Soft Falcon NILs, where the Hard phenotype resulted from deletion of the *PinA* gene (Lesage et al. 2012). In this previous study, we observed that 1) stress-related and folding proteins were more abundant; 2) endosperm development reached completion earlier; and 3) polymer mass was higher in the developing Hard kernels than in the Soft ones. This discrepancy between effects of *PinA* and *PinB* variants on polymer mass could be due to different roles played by these two proteins in the endosperm, and particularly in the ER compartment that controls cellular stress response. Study of the interactions between storage proteins, puroindolines and environment could be important for understanding protein matrix formation in wheat grain.

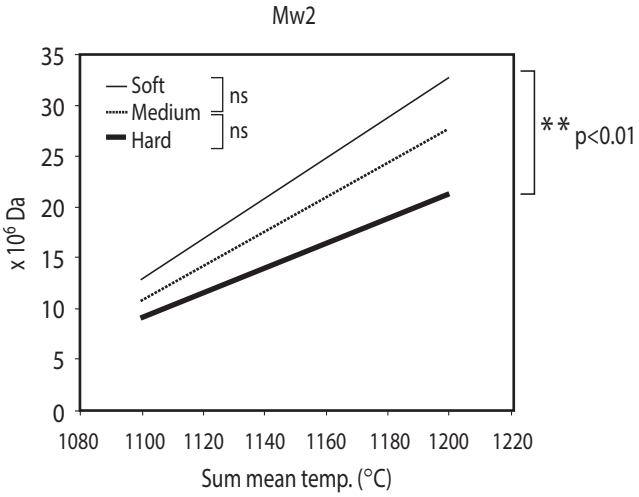

Fig. 4 Differential effect of sum of mean temperatures on polymer mass (MW2) depending on grain hardness classes.

Conclusions

We highlight that AFFFF provides more reliable data than SE-HPLC on polymer size. SE-HPLC reflects more clearly the composition of storage proteins. Interactions between glutenins and puroindoline-B alleles had significant effects on polymer characteristics. These effects varied depending on the particular glutenin allele. In addition, temperatures during the last two months of grain development differentially impacted polymer characteristics according to grain hardness. Further molecular analysis of these results needs to be carried out on grain produced under controlled environmental conditions.

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