Effects of Growing Location on Response of Protein Polymerization to Increased Nitrogen Fertilization for the Common Wheat Cultivar Soissons: Relationship with Some Aspects of the Breadmaking Quality

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ABSTRACT

A French soft wheat cultivar, Soissons, was grown in 20 locations and treated by the application of various nitrogen fertilizations at different stages. Phosphate sodium dodecyl sulfate (SDS) extraction and size-exclusion high-performance liquid chromatography (SE-HPLC) combined with nitrogen determination were used to quantify different protein pools according to SDS solubility and apparent molecular size: F_i, F_1, F_2, F_3, and F_4 (glutenin macropolymers, large SDS-soluble polymers [MW > 500 kDa], small SDS-soluble polymers [MW 100–500 kDa], gliadins, and albumins-globulins, respectively). The yields, the quality properties, and the amounts of all the protein fractions were affected more strongly by the nitrogen treatment than by the environmental factor, whereas the kernel weight, the protein proportion of three glutenin polymer fractions (F_i%, F_1%, and F_2%) and (F_1 + F_2) / F_i ratio appeared to be influenced only by the environmental factor. The differences in the distribution between SDS-soluble (F_1 + F_2) and SDS-insoluble glutenin polymers (F_i) resulted in a different contribution to the potential breadmaking quality properties. These results suggest that the baking strength of wheat flour is not only determined by the quantity of total glutenin polymers or macro-polymers, but also by the polymerization mode and the distribution of polymers between the SDS-extractable polymers and SDS-unextractable polymers. Environmental factors appear to be the main origin of the differences in the polymerization modes and in the polymer distribution.

High yield and good breadmaking quality are important features in today's wheat market. Both can be improved through nitrogen (N) fertilization strategies, such as the rates and timings of N fertilization (Martin et al 1992) and the source of N fertilization (Peltonen and Virtanen 1994). Many studies have shown that the increase of flour protein content resulting from N application can lead to changes in protein composition (Fullington et al 1983, Gupta et al 1992). The relationship between wheat flour proteins, especially the gluten protein (glutenins and gliadins) content, and the breadmaking quality has been recognized for many years (Wall 1979, Bietz and Huebner 1980, Pomeranz 1988).

Glutenins are a heterogeneous class of wheat endosperm proteins. They cannot be exhaustively solubilized with various solvents (Pomeranz 1965, Orth and Bushuk 1972, Danno et al 1974, Kruger et al 1988). The insoluble fraction (F_i) corresponds to highly polymerized glutenins that can only be completely solubilized by further extraction with sodium dodecyl sulfate (SDS) solution in combination with a reducing agent (Danno et al 1974) or by sonication (Singh et al 1990a). This insoluble fraction usually correlates positively with breadmaking quality properties (Dachkevitch and Autran 1989, Gupta et al 1993). The soluble glutenins can be fractionated into two parts by gel filtration or size-exclusion high-performance liquid chromatography (SE-HPLC): 1) an excluded fraction (F_1) corresponding to high molecular weight (HMW) glutenin polymers and 2) an unexcluded fraction (F_2) corresponding to low molecular weight (LMW) glutenin polymers (Huebner and Wall 1976, Bietz 1984). Depending on whether the highest molecular weight polymers were or were not largely extracted, the correlation between the quality properties and F_1% or F_1 / F_2 ratio was positive (Huebner and Wall 1976, Field et al 1983) or negative (Bietz 1984, Dachkevitch and Autran 1989), respectively.

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If we take into account the important contributions of these diverse types of glutenin polymers to the breadmaking properties, we must pay special attention to the influence of environmental factors on glutenin polymerization.

Dachkevitch and Autran (1989) reported that the F_1 / F_2 ratio and the F_1%, F_2%, and F_i% differed significantly from cultivar to cultivar. They also reported that only the F_2% and F_i% can be significantly affected by environmental conditions. Further studies showed that F_1, F_2, and F_i content in flour increased with increasing N fertilizer, whereas F_1 and F_2 proportions remained unchanged with increasing N supply (Scheromm et al 1992). Recently, our work (Jia et al 1995) has shown that the polymerization level can also be affected by maturation conditions resulting in a (F_1 + F_2) / F_i ratio change. The repartition between soluble polymers and insoluble polymers has different effects on the expression of the proteins and the total glutenin fraction on the dough strength. However, this result occurred for one cultivar grown on one location only and under the same climatic conditions.

The aim of the present work was to assess the effects of different growing locations on the responses of glutenin polymerization and dough rheological properties to increasing N fertilization at different development stages, for one specific common wheat cultivar, Soissons.

MATERIALS AND METHODS

Field Experiment

Only one cultivar, Soissons, was selected in this study. This cultivar was grown at 20 locations in the south of France. According to the quantities and the different timings of N fertilizer (NH_4NO_3) supply, three experiments were done: A, B, and C. There were four locations for experiment A, nine locations for experiment B, and seven locations for experiment C.

The N fertilizer was applied at different levels for a given yield target and at different stages of wheat plant development (Table I), defined by the balance RAMSES method (Justes 1993). A preventative spray program was used for disease. The seeds were harvested at maturity and milled for biochemical analysis in a laboratory mill.

Sample Preparation

Flour samples (240 mg) were stirred for 2 hr at room temperature in presence of 30 ml of 0.1*M* sodium phosphate buffer (pH 6.90) containing 2% (w/w) SDS. These extractions were followed by a denaturation of the proteases (5 min at 90°C) and by centrifugation for 30 min at 12,500 × *g* at 20°C in a Jouan centrifuge (model MR 1822). Clear supernatants (soluble fraction, F_s) were immediately frozen at –18°C. Pellets (insoluble fraction, F_i) were freeze-dried, and the protein insoluble fraction was determined by nitrogen analysis. Three or four replicates were done and combined for analysis.

Nitrogen Determination

The Dumas method (AOAC 7 024) was used to determine the N concentration of the dry samples (flour, lyophilized F_i). Protein concentration was determined on a Perkin Elmer apparatus (model PE 2410 series II) by multiplying N values by 5.7.

SE-HPLC

The SE-HPLC apparatus was a ABI model that includes a pump model 400 and a diode array detector model 1000S. A TSK 4000-SW size-exclusion analytical column (7.5 × 300 mm, 450 Å, TOSOHAAS, Stuttgart, Germany) protected by a guard column (7.5 × 75 mm, 250 Å) was used. A 0.1*M* sodium phosphate buffer (pH 6.90) containing 0.1% (w/w) SDS was used as eluent with a flow rate of 0.7 ml/min as previously described (Bietz 1984, Dachkevitch and Autran 1989). During the fractionation, the column thermostat was at 25°C. The frozen supernatants were heated at room temperature (22°C) and then filtered through a 0.45 µm nylon membrane (C.I.L.). Clear supernatant (20 µl) was applied to the column using a loop injector. The column effluent was monitored at 214 nm and 0.1 Absorbance units full scale, and chromatograms were analyzed using Labcalc software (Galactic Industries Corp.). The major peaks (F_1 to F_4) were eluted between 9 and 20 min as described previously (Dachkevitch and Autran 1989). The first fraction (F_1) should correspond to highly aggregated material (mainly glutenins) and eluted at the void volume of the column. Fraction 2 (F_2) eluted as proteins between 100 and 520 kDa and should consist of aggregates smaller than those of F_1. Fractions 3 and 4 (F_3 and F_4) corresponded essentially to monomeric proteins, gliadins and salt-soluble proteins, respectively. Apparent molecular weight of major peaks were estimated by calibrating the column with four unreduced protein standards: thyroglobulin (669,000), bovine serum albumin (66,000), chymotrypsinogen (25,700), and cytochrome C (12,400). All quantitative data are expressed both on a relative basis (i.e., in percentages of total area of chromatograms) and on an absolute basis (i.e., in amount of proteins).

TABLE I
Quantity and Timing of Nitrogen Fertilization

Experiment (no. of locations)	Type of Nitrogen Fertilization[a]	Mean Quantity (and Range) of Total Nitrogen Supply (kg/ha)	Timing of Nitrogen Supply (tillering : 1-cm head : booting : head emergence)
A	NB	56 (54-64)	56 : 0 : 0: 0
(*n* = 4)	NB + 1	156 (100-188)	56 : 100 : 0 : 0
	NR	194 (160-221)	56 : 100 : 0 : 38
B	NB	46 (36-65)	46 : 0 : 0 : 0
(*n* = 9)	NB + 2	165 (148-192)	46 : 65 : 55: 0
	NR	237 (211-257)	46 : 65 : 70 : 56
C	NB	65 (47-96)	65 : 0 : 0 : 0
(*n* = 7)	NB + 1	153 (134-188)	65 : 82 : 0 : 0
	NR	196 (168-236)	65 : 82 : 50 : 0
	NRR	229 (208-248)	65 : 82 : 50 : 30

[a] NB = basal fertilization; NB + 1 = basal fertilization + one application; NB + 2 = basal fertilization + two applications; NR = late N fertilizer supply defined by RAMSES test; NRR = supplemental N fertilizer supply in addition to the NR.

Technological Tests

Baking strength determination was based on the *W* index (Chopin alveograph, Tripette et Renaud) according to the standard 5530/4 of the International Organization for Standardization.

Statistical Analysis

The Stat-ITCF (ITCF, France) computer package was used for correlation analyses based on linear regression. The symbols *, **, and *** represent significant correlations at 5, 1, and 0.1% probability, respectively.

RESULTS AND DISCUSSION

Variation Origin for Yield, Quality Parameters and Protein Characteristics

Table II shows that the diversity of growth location and nitrogen fertilization levels are reflected in very wide ranges for yield, breadmaking quality attributes (i.e., *W, P, G, P/L*), and several protein fractions, such as F_1 and F_3 flour content and $(F_1 + F_2) / F_i$ ratio (CV > 13%). By contrast, the kernel weight, the F_3% and total glutenins ($F_1 + F_2$ for + F_i)% have a much lower variability (CV ≤ 6%).

Analysis of variance was used to determine the respective influences of growing location and nitrogen fertilization factors on agronomic characteristics, quality attributes, and protein composition characteristics. These results for the three experiments are presented in Table III. For experiment A, the quality properties, the total grain protein content, the amount of protein fractions (i.e., F_1, F_2, F_3 and F_4), F_3%, and $(F_i + F_1 + F_2) / F_3$ ratio are essentially N fertilizer dependent. However, F_1 content, F_1 %, and F_1 / F_2 ratio are significantly influenced by the environmental factor. For experiments B and C, Table III shows that the effects of N and growth location are significant for almost all the characteristics (the quality attributes, the yield, the amounts of protein fractions). The variances for N fertilizer levels are generally much larger than those for the growing locations. In particular, the F_3%,

TABLE II
General Data of Wheat Samples and Mean and Ranges of Technological and Biochemical Parameters

Characteristic	Range	Mean	CV%
General			
1,000 kernel weight	34.6–45.9	39.9	6.3
Yield (q/ha)	32.1–98.2	71.6	21.8
Quality property[a]			
Grain protein content	8.7–14.1	11.1	11.4
W (×10^{-4} J)	56.0–324.0	171.5	35.4
P	42.7–88.0	61.2	16.7
G	10.3–24.4	19.2	16.4
P/L	0.35–2.62	0.90	40.2
Protein %dm[b]			
F_i	2.10–3.72	2.84	13.5
F_1	0.29–0.59	0.42	16.0
F_2	0.95–1.64	1.25	13.8
F_3	2.59–5.33	3.70	15.8
F_4	1.97–2.69	2.31	7.1
$F_1 + F_2 + F_i$	3.43–5.76	4.50	12.1
% Total proteins			
F_i	21.5–29.5	25.7	7.8
F_1	2.76–5.21	3.77	11.3
F_2	9.77–12.9	11.3	5.8
F_3	29.5–37.8	33.4	5.0
F_4	18.0–25.7	21.1	8.0
$F_1 + F_2 + F_i$	37.6–44.6	40.7	3.7
Ratio			
F_1 / F_2	0.28–0.41	0.33	6.6
$(F_1 + F_2) / F_i$	0.47–0.81	0.59	14.1
$(F_1 + F_2 + F_i) / F_3$	1.05–1.40	1.22	6.8

[a] *W* = flour strength; *P* = dough tenacity; *G* = dough swelling (=2.2 $L^{1/2}$); *L* = dough extensibility.
[b] dm = dry material.

as in experiment A, is influenced only by the N fertilizer level, whereas the kernel weight, the $(F_1 + F_2) / F_i$ ratio (except for experiment C), the F_1%, F_2%, and Ut% (Ut = $F_1 + F_2 + F_i$) (except for experiment C) are significantly influenced only by the environmental factor. In the case of experiment B, the F_1 / F_2 ratio is affected by both the N level and the environmental factor, but the variance for the N fertilization level is larger than that for growing location. These results demonstrate that the N nutrition level is a predominant factor for the variation of wheat yields, total protein content, each protein fraction content, as well as the quality properties. However, the kernel weight, the glutenin polymerization level and the distribution among the different glutenin polymers are also highly sensitive to the environment.

Effects of N Fertilizer Level and Its Timing on the Yield, Quality Properties, and Protein Characteristics

As the responses of yield, grain protein content, and breadmaking quality properties to increased N fertilization are similar for all locations of the same experiment, only the mean values of yield, grain protein content, and quality properties for the different N treatments are presented in Figure 1. When N supply is divided into two applications (NB + 1) (Table I, experiments A and C), the second N application at 1-cm head stage results in a significant increase both in yield and in grain protein content compared to the basal N supply (NB) for experiment A, whereas it results only in an increase in yield for experiment C.

When N supply is divided into three applications (NB + 2) (experiment B), the two later N applications (at the boot stage and the head emergence stage) result not only in a significant increase in yield and in grain protein content but also in a significant improvement in the breadmaking quality properties.

Compared with NB + X, the late N application (at the head emergence stage for experiments A and B or at the flag leaf stage for experiment C) significantly increased yield, grain protein, and

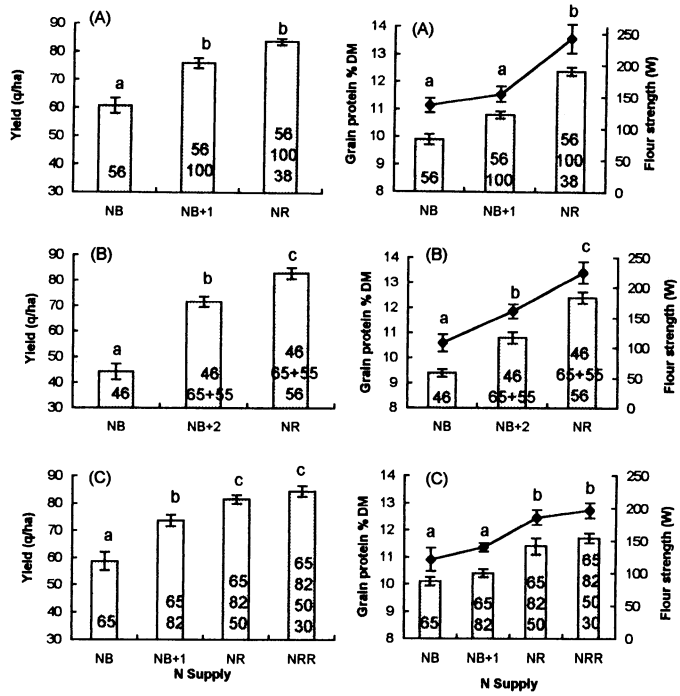

Fig. 1. Main effect of nitrogen nutrition as determined by the Newman-Keuls homogeneity test for: yield, grain protein content, and breadmaking quality (index *W*) for the three experiments (A, B, and C). NB = basal fertilization; + 1, + 2 = plus one or two applications; NR = late N fertilizer supply defined by RAMSES test; NRR = supplemental N fertilizer supply in addition to the NR. Mean values with the same letter are not significantly different for each characteristic. Values at the bottom of vertical bars represent the mean N fertilizer amounts applied at the different wheat development stages. Vertical bars represent grain protein content. Lines represent *W* index.

TABLE III
Influence of Nitrogen Fertilization Level (N) and Growth Location (L) Determined by Analysis of Variance (*F*-test)[a] on Quality and Protein Characteristics for Three Experiments

	Experiment A ($n = 4 \times 3$)		Experiment B ($n = 9 \times 3$)		Experiment C ($n = 7 \times 4$)	
Characteristic	$Ó^2_N / Ó^2_R$	$Ó^2_L / Ó^2_R$	$Ó^2_N / Ó^2_R$	$Ó^2_L / Ó^2_R$	$Ó^2_N / Ó^2_R$	$Ó^2_L / Ó^2_R$
1,000 kernel weight	8.6*	4.8*	ns[b]	9.9**	ns	122**
Yield (Q_x/ha)	22.0**	13.0**	97.8**	5.3**	31.4**	9.2**
Quality[c]						
Grain protein (%)	44.6**	ns	76.6**	4.2**	18.0**	5.8**
W ($\times 10^{-4}$ J)	8.1*	ns	23.0**	ns	11.3**	5.9**
P	15.7**	ns	4.5*	8.2**	4.5*	10.6**
G	6.9*	ns	46.6**	4.1**	8.2**	9.6**
P/L	ns	ns	26.3**	9.9**	ns	3.7*
Protein (% dm)[d]						
F_i	ns	ns	20.6**	2.8*	11.9**	5.4**
F_1	15.8**	5.1*	16.9**	8.2**	ns	11.2**
F_2	27.6**	ns	44.4**	5.2**	6.8**	5.3**
F_3	43.7**	ns	86.8**	3.7*	14.4**	ns
F_4	5.8*	ns	8.3**	ns	ns	ns
$F_1 + F_2 + F_i$	11.9**	ns	46.9**	3.9**	21.4**	12.1**
% Total proteins						
F_i	ns	ns	ns	ns	ns	ns
F_1	ns	5.0*	ns	9.2**	ns	7.8**
F_2	ns	ns	ns	10.6**	ns	3.7*
F_3	28.4**	ns	49.6**	ns	7.9**	ns
F_4	ns	ns	22.2**	ns	8.8**	4.5**
$F_1 + F_2 + F_i$	ns	ns	ns	ns	ns	2.7*
Ratio						
F_1 / F_2	ns	9.9*	12.6**	5.3**	ns	8.3**
$(F_1 + F_2) / F_i$	ns	ns	ns	4.3**	ns	ns
$(F_1 + F_2 + F_i) / F_3$	5.8*	ns	11.5**	ns	ns	ns

[a] * and ** = *F*-test significance at the 5 and 1% level of propability, respectively.
[b] Not significant.
[c] *W* = flour strength; *P* = dough tenacity; *G* = dough swelling (=2.2 $L^{1/2}$); *L* = dough extensibility.
[d] Dry material.

breadmaking quality. However, the additional N supply at the head emergence stage, in addition to the late N supply (NR) for the experiment C, had no significant effect on yield, grain protein content, or baking quality.

The influence of N fertilizer level on protein fraction accumulation is shown in Table IV. When N supply is divided into two applications (NB + 1), as in experiments A and C, the second N application at 1-cm head stage results only in an increase in F_3 content for experiment A, whereas it has no significant effect on the increase in the amount of all the protein fractions for experiment C.

When N supply is divided into three applications (NB + 2), as in experiment B, the last two N applications (at the 1-cm head stage and the boot stage) result in a significant increase in F_i, F_1, F_2, and F_3 content and F_3%, whereas F_4%, F_1 / F_2, and (F_1 + F_2 + F_i) / F_3 ratios decrease significantly. These results indicate that N supply at the flag leaf stage favors the accumulation of all the storage protein fractions. The decrease in (F_1 + F_2+ F_i) / F_3 ratio with increasing N supply indicates that the accumulation of gliadins (F_3) is more responsive than the accumulation of total glutenins (F_1 + F_2+ F_i). This finding is in agreement with previous reports (Peltonen and Virtanen 1994, Jia et al 1995). The decrease in F_1 / F_2 ratio with increasing N supply indicates also that the F_2 accumulation is more responsive than is the F_1. This is in agreement with Timms et al (1981), who found that the F_1 / F_2 ratio markedly decreased with high protein levels.

Total glutenins and gliadins content and the percentage of gliadins increased significantly with a late N fertilizer supply (NR at head emergence stage for experiments A and B or at the boot stage for experiment C), compared to with a basic N supply (NB). For instance, the increases in F_i, F_1, and F_2 content are significant for experiment B, but the increases in F_i and F_1 content are not significant for experiments A and C, respectively.

These results tend to demonstrate that N fertilization level and its timing have a very strong influence on the accumulation of both gliadins and glutenins, and on the gliadins-to-glutenin ratio, whereas the glutenin polymerization appears to be more strongly affected by the environmental conditions (F_1 / F_2 ratio).

Variation of Protein Polymerization and Contribution to the Potential Breadmaking Quality Properties

Figure 2 shows the effects of growing locations on the response to increasing N level for different glutenin polymer fractions. With an increase in the total protein accumulation in the grain, the SDS-soluble (F_1 and F_2) and SDS-insoluble glutenin polymers (F_i) content increases. Although the F_1 and F_2 contents are relatively important, the F_i content was relatively poor in five locations where the samples (n = 16) present a relatively high (F_1 + F_2) / F_i ratio (0.69 ± 0.07). In contrast, it shows an opposite behavior at eight other locations where the samples (n = 27) present a relatively lower (F_1 + F_2) / F_i ratio (0.55 ± 0.05). The remaining seven locations (n = 24) are in the intermediate position (0.58 ± 0.06). To clarify the presentation of the effects of the growing locations on the repartition between the SDS-soluble and SDS-insoluble glutenin polymers and the effects on the breadmaking quality properties, the intermediate case is not shown here. However, whatever the growing location, the response of total glutenin amount to the total N accumulation in the grain is highly linear (Fig. 3): r = 0.95 (n = 43, P<0.001). It is the same for gliadins r = 0.98 (n = 43, P < 0.001). These results indicate that the distribution between the glutenin polymers (F_1 + F_2) and F_i are different mainly according to the growing locations.

Considering the effects of the growing conditions on the glutenin polymers distribution, the question is raised whether this distribution can affect the baking quality properties. Figure 4 shows that the slopes of the regression line of F_1, F_2, and F_i versus W are steeper for the growing locations where the samples present a lower (F_1 + F_2) / F_i ratio (0.55 ± 0.05) than the ones with a higher (F_1 + F_2) / F_i ratio (0.69 ± 0.07). The same is true for the correlation slopes between W and gliadins or total glutenins (Fig. 5). These results indicate that the different glutenin polymer distributions caused by growing environment have different effects on dough strength.

Furthermore, Figures 6 and 7 show that the F_i, F_1, F_2, and F_3 are also strongly correlated with both dough tenacity (as measured by P index) and dough extensibility (as measured by G index) for the samples with low (F_1 + F_2) / F_i ratio (0.55 ± 0.05) on eight locations. However, for the samples with a high (F_1 + F_2) / F_i ratio (0.69 ± 0.07) on five other locations, these four proteins groups are correlated with G index, whereas the P index remains constant (≈50) whatever the variation of these four proteins groups might be.

Overall, these results tend to confirm that the potential breadmaking properties of wheat flour are not only determined by the quantity of total glutenin (Singh et al 1990b, Gupta et al 1992) or the amount of GMP (Dachkevitch and Autran 1989, Gupta et al 1993), but also by the distribution of polymers between the SDS-extractable and SDS-unextractable ones (Jia et al 1995).

The different functional properties of the glutenin polymers may result from the difference in the glutenin polymerization mode, more or less reticulate, which leads to different interactions between the gliadins and glutenins. The growing environmental conditions are the origin of the differences in the polymerization modes and the distribution among the glutenin polymers.

TABLE IV
Effect of Nitrogen Fertilization Level on Protein Content (% dm), Protein Proportion (% total protein), and Protein Ratio for Three Experiments

	Protein Content (% dm)						Protein Proportion (% total protein)		Ratio	
Experiment[a]	F_i	F_1	F_2	F_3	F_4	$F_1+F_2+F_i$	F_3	F_4	F_1/F_2	$(F_1+F_2+F_i)/F_3$
A										
NB	2.75	0.35a[b]	1.05a	3.11a	2.12a	4.15a	31.5a	21.5	0.33	1.33a
NB+1	2.94	0.37a	1.15a	3.50b	2.25ab	4.47a	32.5a	20.8	0.33	1.28ab
NR	3.25	0.44b	1.37b	4.27c	2.46b	5.06b	34.4b	19.8	0.32	1.19b
B										
NB	2.36a	0.39a	1.08a	2.95a	2.19a	3.83a	31.2a	23.2a	0.36a	1.30a
NB+2	2.69b	0.43b	1.24b	3.59b	2.27a	4.35b	33.3b	21.2b	0.34b	1.21b
NR	3.07c	0.47c	1.43c	4.35c	2.44b	4.98c	35.2c	19.8c	0.33c	1.15b
C										
NB	2.60a	0.38	1.13a	3.22a	2.28	4.12a	31.9a	22.7a	0.34	1.28
NB+1	2.64a	0.38	1.16a	3.38a	2.27	4.19a	32.6a	21.9a	0.33	1.24
NR	2.99b	0.41	1.27b	3.84b	2.33	4.67b	33.8b	20.5b	0.32	1.22
NRR	3.10b	0.42	1.30b	3.99b	2.34	4.81b	34.2b	20.2b	0.32	1.21

[a] NB = basal fertilization; NB+1 = basal fertilization + one application; NB+2 = basal fertilization + two applications; NR = late N fertilizer supply defined by RAMSES test; NRR = supplemental N fertilizer supply in addition to the NR.

[b] Comparison of mean values by the Newman-Keuls homogeneity test at the level of 5%. Within the columns, means followed by the same letter are not significantly different.

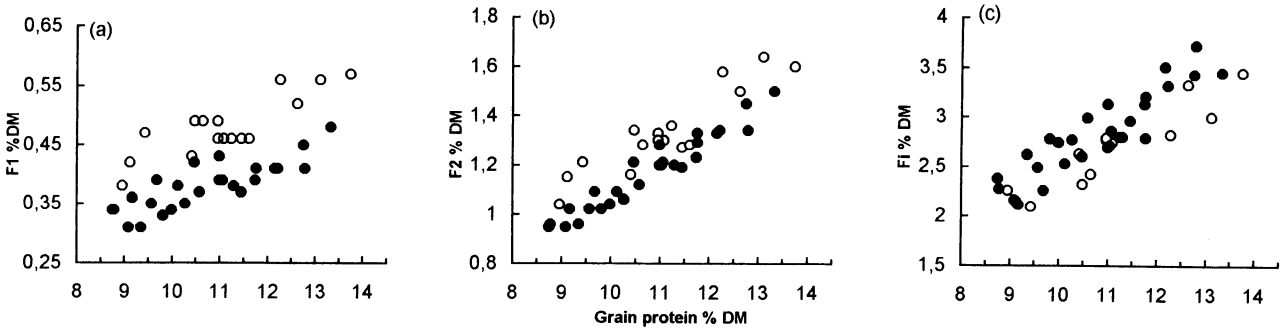

Fig. 2. Effects of growing locations on the relationship between the grain protein content and the amount of different glutenin polymer fractions. Sodium dodecyl sulfate (SDS) extractable polymers: F_1 **(a)** and F_2 **(b)**; SDS-unextractable polymers: F_i **(c)**; (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown on five locations.

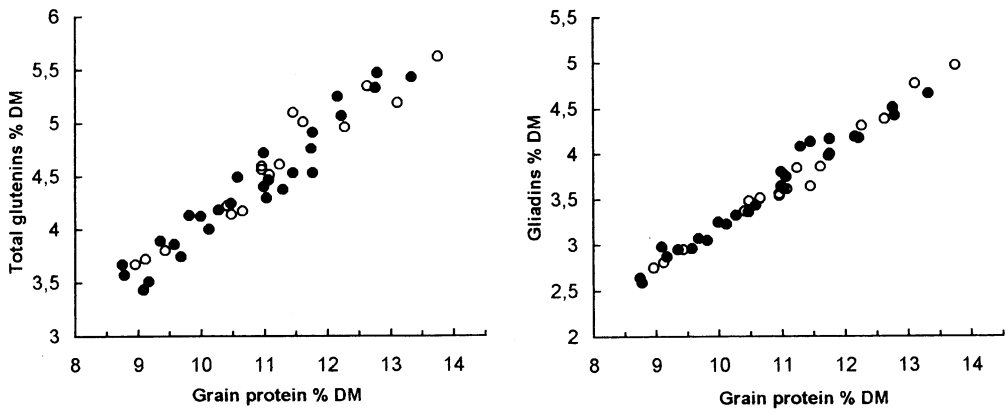

Fig. 3. Effects of growing locations on the relationship between the grain protein content and the amount of wheat storage proteins. Gliadins (left) and total glutenins (right); (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown at five locations.

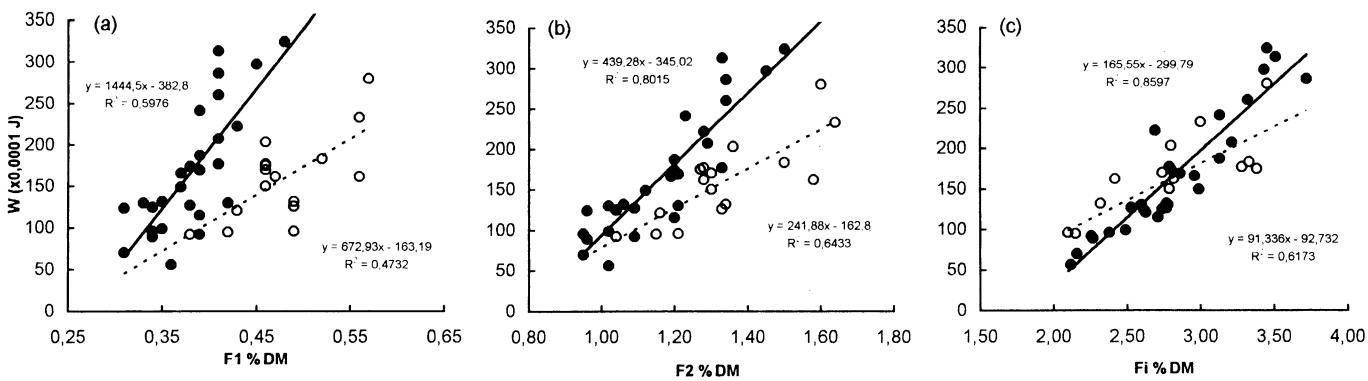

Fig. 4. Effects of growing locations on the relationship between the Chopin Alveographic index W and the amount of different glutenin polymer fractions. Sodium dodecyl sulfate (SDS) extractable polymers F_1 **(a)** and F_2 **(b)**; and SDS-unextractable polymers F_i **(c)**; (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown at five locations.

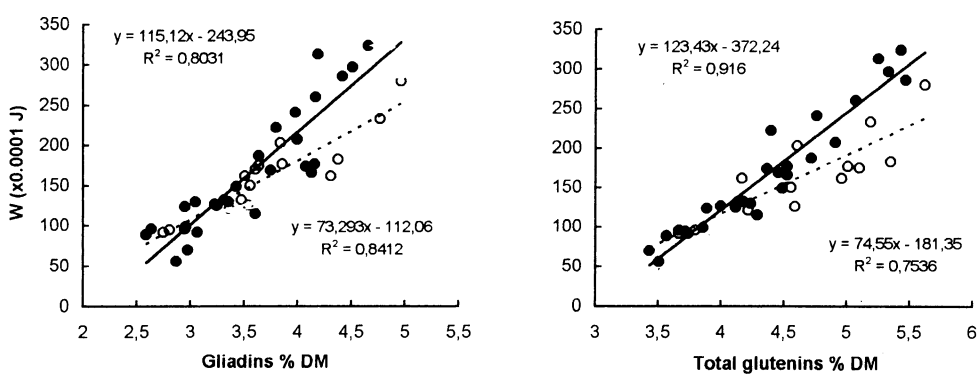

Fig. 5. Effects of growing locations on the relationship between the Chopin Alveographic index W and the amount of wheat storage proteins. Gliadins (left) and total glutenins (right); (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown at five locations.

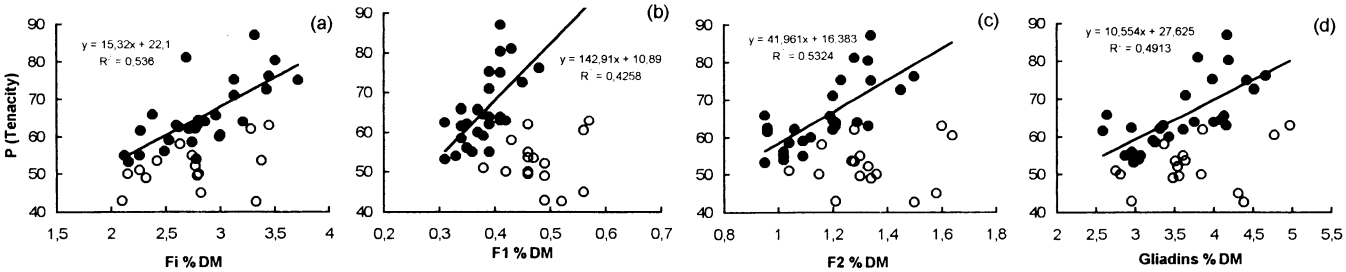

Fig. 6. Effects of growing locations on the relationship between the Chopin Alveographic index P (dough tenacity) and the amount of different storage protein fractions. Sodium dodecyl sulfate (SDS) unextractable polymers F_i **(a)**; SDS-extractable extractable polymers F_1 **(b)** and F_2 **(c)** and gliadins **(d)**; (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown at five locations.

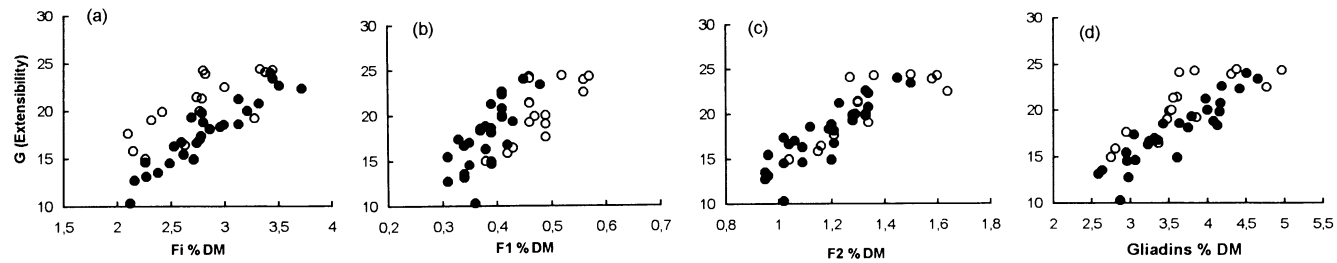

Fig. 7. Effects of growing locations on the relationship between the Chopin Alveographic index G (dough extensibility or swelling) and the amount of different storage protein fractions. Sodium dodecyl sulfate (SDS) unextractable polymers F_i **(a)**; SDS-extractable polymers F_1 **(b)** and F_2 **(c)** and gliadins **(d)**; (●) samples (n = 27) with low ($F_1 + F_2$) / F_i ratio (0.55 ± 0.05) grown at eight locations; (○) samples (n = 16) with high ($F_1 + F_2$) / F_i ratio (0.69 ± 0.07) grown at five locations.

CONCLUSION

Unlike the studies on monomers or protein subunits by SDS-PAGE or RP-HPLC that give fingerprints of genotypes, investigating the profile of protein polymers through SE-HPLC combined with N determination in F_i provides an effective means of evaluating the effects of nitrogen fertilization and growing location.

The effects of various timings of nitrogen fertilization show that the application at the early stage (1-cm head stage) increases yield, but the N supply at late stage (boot or head-emergence stage) increases significantly the amounts of all the protein fractions, and thus improves the baking-quality properties. However, the responses to increasing N fertilization of diverse types of glutenin polymers are very different according to the growing environmental conditions. The growing location and maturation conditions are the origin of the differences in distribution among glutenin polymers and in the mode of glutenin polymerization. The latter results in different contributions to the potential breadmaking quality properties.

LITERATURE CITED

BIETZ, J. A. 1984. Analysis of wheat gluten proteins by high-performance liquid chromatography Baker's Dig. 58(1):15-21.

BIETZ, J. A., and HUEBNER, F. R. 1980. Structure of glutenin: Achievements at the Northern Regional Research Center. Ann. Technol. Agric. 29:249-277.

DACHKEVITCH, T., and AUTRAN, J.-C. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chem. 66:448-456.

DANNO, G., KANAZAWA, K., and NATAKE, M. 1974. Extraction of wheat flour proteins with sodium dodecyl sulfate and their molecular weight distribution. Agric. Biol. Chem. 38:1947-1953.

FIELD, J. M., SHEWRY, P. R., and MIFLIN, B. J. 1983. Solubilisation and characterisation of wheat gluten proteins: Correlations between the amount of aggregated proteins and baking quality. J. Sci. Food Agric. 34:370-377.

FULLINGTON, J. G., COLE, E. W., and KASARDA, D. D. 1983. Quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total proteins extracted from different wheat varieties: Effect of protein content. Cereal Chem. 60:65-71.

GUPTA, R. B., BATEY, I. L., and MacRITCHIE, F. 1992. Relationship between protein composition and functional properties of wheat flours. Cereal Chem. 69:125-131.

GUPTA, R. B., KHAN, K., and MacRITCHIE, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. J. Cereal Sci. 18:23-41.

HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258-268.

JUSTES, E., 1993. Diagnostic de la nutrition azotée du blé, à partir de la teneur en nitrate de la base de la tige. Application au raisonnement de la fertilisation. Thèse de l'Institut National Agronomisue: Paris-Grignon.

KRUGER, J. E., MARCHYLO, B. A., and HATCHER, D. 1988. Preliminary assessment of a sequential extraction scheme for evaluating quality by reversed-phase high-performance liquid chromatography and electrophoretic analysis of gliadins and glutenins. Cereal Chem. 65:208-214.

JIA, Y. Q., MASBOU, V., AUSSENAC, T., FABRE, J.-L., and DEBAEKE, P. 1995. Effects of nitrogen fertilization and maturation conditions on protein aggregates and on the breadmaking quality of Soissons, a common wheat cultivar. Cereal Chem. 73:123-130.

MARTIN, R. J., SUTTON, K. H., MOYLE, T. N., HAY, R. L., and GILLESPIE, R. N. 1992. Effect of nitrogen fertilizer on the yield and quality of six cultivars of autumn-sown wheat. N.Z. J. Crop Hort. Sci. 20:273-282.

ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49:268-275.

PELTONEN, J., and VIRTANEN, A. 1994. Effect of nitrogen fertilizers differing in release characteristics on the quantity of storage proteins in wheat. Cereal Chem. 71:1-5.

PROMERANZ, Y. 1965. Dispersibility of wheat proteins in aqueous urea solutions. A new parameter to evaluate bread-making potentialities of wheat flours. J. Sci. Food Agric. 6:586-593.

PROMERANZ, Y. 1988. Composition and functionality of wheat flour components. Pages: 291-370 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

SCHEROMM, P., MARTIN, G., BERGION, A., and AUTRAN, J.-C. 1992. Influence of nitrogen fertilization on the potential bread-baking quality of two wheat cultivars differing in their responses to increasing

nitrogen supplies. Cereal Chem. 69:664-670.
SINGH, N. K., DONOVAN, R., BATEY, I. L., and MacRITCHIE, F. 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chem. 67:150-161.
SINGH, N. K., DONOVAN, R., and MacRITCHIE, F. 1990b. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chem. 67:161-170.
TIMMS, M. F., BOTTOMLY, R. C., ELLIS, J. R. S., and SCHOFIELD, J. D. 1981. The baking quality and protein characteristics of a winter wheat grown at different levels of nitrogen fertilization. J. Sci. Food Agric. 32:684-698.
WALL, J. S. 1979. The role of wheat proteins in determining baking quality. Pages. 275-311 in: Recent Advance in the Biochemistry of Cereals. D. L. Laidan and R. G. Wyn Jones, eds. Academic Press: New York.

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