Effect of 1B/1R Translocation on Selected Grain Quality Parameters in a Set of Doubled Haploid Wheat Lines

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Abstract: A set of quality parameters (crude protein content, Zeleny sedimentation test, wet gluten content, gluten index, albumins + globulins content, gliadin content, sum of glutenins, proportion of albumins + globulins in crude protein and relative viscosity) was tested in 17 doubled haploid (DH) wheat lines differing in the presence or absence of 1B/1R translocation. The presence of 1B/1R translocation (allele Gli 1B3) affected the significantly higher content of albumins and globulins and significantly lower value of gluten index. Nevertheless, a certain translocation influence on the other parameters (Zeleny sedimentation test; relative viscosity; proportion of albumins + globulins in crude protein) was also registered. The deteriorative effect of the 1B/1R translocation on indirect technological grain parameters was confirmed more markedly in gluten index than in Zeleny sedimentation test. A high number of significant differences between lines was found in Zeleny sedimentation test, content of glutenin, content of albumins + globulins and their proportion in crude protein. In spite of lower variability between lines relative viscosity showed a high dependence on genotype and was indifferent to the other quality parameters.

Keywords: wheat; grain quality; Gli-/Glu-alleles; 1B/1R translocation

While only about 17% of annual wheat production is used for animal feed in the world (Rose 2003), in the Czech Republic the ratio is much higher (50–60%, e.g. 59.6% in 2004) (Ministry of Agriculture 2004). The effect of wheat genotypes on feeding value was confirmed by several authors (Fuller *et al.* 1989; Annison 1993 and others), nevertheless, no rapid indirect tests suitable for wheat breeding programmes are well known (Rose 2003).

Kasarda *et al.* (1976) classified wheat soluble proteins in dilute salt solutions (albumins and globulins) as cytoplasmatic proteins. These "soluble" proteins differ distinctly in their amino acid (AA) composition from the gluten storage proteins (gliadins and glutenins) and are nutritionally important because about 45% of total AAs are created by essential AAs. Gluten and its protein fractions (gliadins and glutenins) are nutritionally inferior to soluble proteins, primarily because of extremely low lysine scores. From this point of view, the breeding of feed cereals is mainly focused on modification of low-quality protein fractions (gliadins and glutenins), increasing of limited essential amino acids and total digestibility of fodder (Čermák 2002).

Water-soluble, non-starch polysaccharides (soluble arabinoxylans) of cereal grains are among the major anti-nutritive factors in poultry diets (Fischer & Stone 1986). Soluble arabinoxylans increase the viscosity of the contents of the rumen digestive tract and affect the efficiency of digestive enzymes and allow a greater proliferation of the bacterial flora (Chots *et al.* 1999). A negative correlation between the content of water-soluble

Supported by the Ministry of Agriculture of the Czech Republic, Project No. 1G57065.

arabinoxylans and the value of metabolised energy of wheat diets was also confirmed by Chots and Annison (1990, 1992).

Because of its agronomic benefits (yield increase, rusts and powdery mildew resistance) the chromosome 1R of rye (*Secale cereale* L.) has been widely used in wheat (*Triticum aestivum* L.) breeding, essentially in the form of the Robertsonian translocation 1BL/1RS. Several hundreds of wheat cultivars are known to possess this translocated chromosome and many others will carry it unrecognized. Gli – BII marker for 1BL/1RS translocation has also a high frequency in the set of Chinese cultivars (Rabinovich 1998; Gao *et al.* 2005).

On the other hand, the loss of the wheat loci Glu-3B (encoding low molecular weight – LMW glutenins) and the Gli-1B (encoding gliadins) clearly caused a reduction of bread making quality (Amiour *et al.* 2002). Nevertheless, the impact of translocation on a wider range of wheat grain parameters or nutritive value is not known very well yet. Rose (2003) mentioned that comparisons of the nutritive value of cultivars with and without the translocated chromosome gave variable results and there was no general evidence that the presence of 1B/1R chromosome was associated with nutrient availability.

Our research was aimed at a 1B/1R translocation effect on selected grain parameters of wheat DH lines. The emphasis was put on variability of protein fractions and relative viscosity as potential criteria of feeding value and characterising their relations to other technological parameters as well.

MATERIAL AND METHODS

A set of 80 doubled haploid (DH) wheat lines was developed by Ing. Ladislav Kučera, from the crossing of wheat cultivar Šárka with advanced line UH 410 (donor of 1B/1R translocation) in the Department of Molecular Biology of RICP Prague. A set of selected 17 DH wheat lines with a higher agronomical potential and according to the presence or absence of allele Gli 1B3 characterising 1B/1R translocation was subsequently divided into two numerically comparable sub-sets (8 lines with 1B/1R translocation and 9 lines without 1B/1R translocation).

Each selected DH line was genetically evaluated by means of gliadin and HMW glutenin alleles. Electrophoresis of HMW glutenin subunits was carried out in conditions of SDS-PAGE according to Laemmli (1970). Glutenin alleles were identified according to Payne *et al.* (1983). Gliadin blocks were identified according to Metakovsky (1991) and Šašek and Černý (2000) in conditions of Acid-PAGE (ČSN 46 1085-2, 1998).

The lines together with two check cultivars Šárka and Nela were multiplied in large plot experiments in two localities for 3 years (2003–2005) (Table 1). Consequent effects of specific locality and year on parameter variability were described for statistical evaluation as three independent environments (E1; E2; E3).

The following 9 grain parameters were tested: content of crude protein – Kjeldahl method (ČSN 56 0512-12); Zeleny sedimentation test (ČSN ISO 5529); wet gluten content (WG) and gluten index (GI) – Glutomatic 2200 (AACC 38-12); protein fractions content (albumins + globulins and their proportion in crude protein; gliadins) – modified Osborne method according to Dvořáček *et al.* (2001).

Content of sum glutenins was calculated as a difference between the content of crude protein and sum of albumins + globulins and gliadins. Relative viscosity was measured with a microviscosimeter Anton Paar according to Saulnier *et al.* (1994)

The software "Statistica 7.0 CZ" was used to test significant differences by ANOVA/MANOVA, Tukey HSD test and correlation matrix.

Table 1. Characterization of plot experiments

Env.	Locality	Foregoing crop	Fertiliser rate (kg)	Year	Plot area (ha)
E1	Prague-Ruzyně	pea	70 N* + 60 P_2O_5	2003	0.2
E2	Kralovice	oil seed rape	126 N + 54 P_2O_5	2004	0.3
E3	Kralovice	oil seed rape	134 N + 54 P_2O_5	2005	0.5

*according to the analysis of N content in soil

Table 2. Technological characteristics of parental genotypes (three-year averages)

Line	Presence of 1B/1R translocation	Crude protein content in dry matter (%)	Wet gluten content (%)	Zeleny sedimentation test (ml)	Falling number (s)
UH 410	+	13.47	34.1	26	296
Šárka	–	12.83	31.5	25	324

RESULTS AND DISCUSSION

Three-year average values of 4 important technological parameters of both parental genotypes (UH 410 and cultivar Šárka) were relatively comparable (Table 2). The line UH 410 showed a slightly higher content of crude protein (13.47%), wet gluten (34.1%) and a lower value of falling number (296 s). Both genotypes do not practically differ in Zeleny sedimentation test.

Characterisation of DH lines and their parents by Gli-/HMW Glu-alleles

The DH wheat lines and their parents were characterised by allelic compositions of gliadins and HMW glutenins on polyacrylamide gel.

Parental components mutually differed in gliadin loci 1-1A (Šárka 1-1A 2 and UH 410 1-1A 4) and 1B (Šárka 1B 4 and UH410 1B 3) (Table 3) and were homologous in gliadin loci 2-1A 0 and 1D 1. The line UH 410 was a bearer of secalin allele Gli 1B 3 confirming according to Černý and Šašek (1998) the 1B/1R translocation. HMW glutenin alleles on chromosome 1B were different (Šárka 1B 7+8 and UH 410 1B 7+9) but homogeneous at other two detected loci (1A 0 and 1D 5+10).

All DH lines were logically homologous in the same gliadin and HMW glutenin loci as their parents. On the other hand, the six different allelic combinations of parental Gli-/HMW Glu-alleles were identified (Table 3) in the set of seventeen lines. Slight admixtures of complementary parental alleles Gli 1-1A 2 or 4 and Glu 1B 7+8 or 7+9 (less than 20%) were observed in 4 lines (line No. 146, 159, 167, 171).

Relations of genotypes and 1B/1R translocation to grain quality parameters

The three years evaluation of nine grain quality parameters in the set of 17 DH lines (with and without 1B/1R translocation) is described in Tables 4–6.

Except the parameters such as content of gliadins (non-significant differences between lines) and relative viscosity (non-significant differences between three environments) the other parameters were significantly influenced by lines and interactions of both environmental components (years and localities).

The highest number of significant differences between DH lines was found in the content of albumins + globulins, their proportion in crude

Table 3. Parent cultivars and groups of DH lines with identical Gli-/HMW Glu-allelic blocks

Gli-/HMW Glu-allelic blocks	Name of parent/Number of line
GLI 1-1A2, 2-1A0, 1B4, 1D1; GLU 1A0, 1B7+8, 1D5+10	Šárka
GLI 1-1A4, 2-1A0, 1B3, 1D1; GLU 1A0, 1B7+9, 1D5+10	UH 410
GLI 1-1A4, 2-1A0, 1B4; 1D1; GLU 1A0, 1B7+8, 1D5+10	136,163, 159*, 171*
GLI 1-1A4, 2-1A0, 1B3; 1D1; GLU 1A0, 1B7+9, 1D5+10	110,112,119
GLI 1-1A2, 2-1A0, 1B4; 1D1; GLU 1A0, 1B7+8, 1D5+10	131,144, 146*, 167*
GLI 1-1A2, 2-1A0, 1B3; 1D1; GLU 1A0, 1B7+8, 1D5+10	157,174
GLI 1-1A2, 2-1A0, 1B4; 1D1; GLU 1A0, 1B7+9, 1D5+10	121
GLI 1-1A2, 2-1A0, 1B3; 1D1; GLU 1A0, 1B7+9, 1D5+10	126,139,164

* lines containing less than 20% of complementary parental allelic combination

Table 4. Grain quality parameters in DH wheat lines with and without 1B/1R translocation (means of studied years 2003–2005)

Line	Presence of translocation	Crude protein content in dry matter (%)	Zeleny sedimentation (ml)	Wet gluten content (%)	Gluten index (GI) (%)	Content of albumins + globulins in dry matter (%)	Content of gliadins in dry matter (%)	Sum of glutenins in dry matter (%)	Proportion of albumin + globulins in crude protein (%)	Relative viscosity
L110	Y	13.38^{ab}	31.67^{ab}	26.98^{ab}	42.28^{ab}	4.03^{g}	4.34^{a}	5.01^{a}	30.23^{fg}	2.17^{b}
L112	Y	12.82^{ab}	29.33^{a}	20.98^{a}	46.38^{ab}	3.69^{cdefg}	4.00^{a}	5.13^{abc}	29.05^{cdefg}	2.03^{ab}
L119	Y	14.61^{b}	42.00^{abcd}	32.38^{b}	29.56^{a}	3.64^{cdefg}	4.88^{a}	6.09^{abcd}	24.95^{abcd}	1.99^{ab}
L126	Y	13.42^{ab}	35.00^{ab}	25.42^{ab}	59.78^{ab}	3.82^{efg}	4.07^{a}	5.53^{abcd}	28.58^{cdefg}	2.03^{ab}
L139	Y	13.61^{ab}	39.00^{abcd}	26.22^{ab}	56.53^{ab}	3.58^{bcdefg}	4.19^{a}	5.84^{abcd}	26.44^{abcdefg}	1.97^{ab}
L157	Y	13.08^{ab}	41.67^{abcd}	25.88^{ab}	55.08^{ab}	3.55^{abcdefg}	4.11^{a}	5.42^{abcd}	27.31^{abcdefg}	2.04^{ab}
L164	Y	14.52^{b}	41.00^{abcd}	30.13^{ab}	57.51^{ab}	3.75^{defg}	4.30^{a}	6.47^{d}	25.87^{abcdef}	2.16^{b}
L174	Y	13.17^{ab}	31.67^{ab}	22.80^{ab}	63.27^{ab}	4.01^{fg}	3.72^{a}	5.44^{abcd}	30.68^{g}	1.98^{ab}
L121	N	12.96^{ab}	47.00^{bcd}	25.85^{ab}	82.15^{ab}	3.32^{abcde}	3.80^{a}	5.85^{abcd}	25.70^{abcdef}	1.91^{ab}
L131	N	12.14^{ab}	37.00^{abc}	21.37^{ab}	89.25^{b}	3.21^{abc}	3.39^{a}	5.54^{abcd}	26.59^{abcdefg}	2.01^{ab}
L136	N	12.55^{ab}	43.00^{abcd}	24.17^{ab}	85.92^{b}	3.29^{abcd}	3.73^{a}	5.54^{abcd}	26.21^{abcdefg}	1.99^{ab}
L144	N	14.06^{ab}	52.67^{cd}	30.58^{ab}	66.37^{ab}	3.43^{abcde}	4.24^{a}	6.39^{cd}	24.32^{abc}	1.97^{ab}
L146	N	13.72^{ab}	54.33^{d}	26.67^{ab}	84.47^{b}	3.22^{abc}	4.28^{a}	6.22^{abcd}	23.57^{ab}	2.07^{ab}
L159	N	12.38^{ab}	35.33^{ab}	25.72^{ab}	81.83^{ab}	3.12^{ab}	3.60^{a}	5.66^{abcd}	25.29^{abcde}	1.62^{a}
L163	N	13.36^{ab}	39.67^{abcd}	26.75^{ab}	82.61^{ab}	3.05^{a}	3.96^{a}	6.35^{bcd}	22.89^{a}	1.85^{ab}
L167	N	12.64^{ab}	36.33^{ab}	23.40^{ab}	76.18^{ab}	3.52^{abcdef}	3.59^{a}	5.54^{abcd}	27.79^{bcdefg}	2.02^{ab}
L171	N	13.69^{ab}	46.00^{bcd}	26.02^{ab}	87.78^{b}	3.39^{abcde}	4.18^{a}	6.12^{abcd}	24.78^{abcd}	2.10^{b}
Nela – check		11.74^{a}	33.33^{ab}	22.38^{ab}	71.34^{ab}	3.09^{ab}	3.62^{a}	5.02^{a}	26.35^{abcdefg}	2.20^{b}
Šárka– check		12,61^{ab}	38.33^{abcd}	22.27^{ab}	77.60^{ab}	3.51^{abcdef}	3.63^{a}	5.47^{abcd}	28.01^{bcdefg}	2.01^{ab}
Influence of line (%)		29.61	47.57	34.64	51.90	56.18	Ns.	45.43	56.81	53.40
E1: (Prague 2003)		14.11^{a}	40.26^{a}	27.53^{a}	62.42^{a}	3.48^{a}	4.42^{a}	6.20^{a}	24.71^{a}	2.01^{a}
E2: (Kralovice 2004)		11.95^{b}	32.74^{b}	21.36^{b}	82.36^{b}	3.22^{b}	3.39^{b}	5.33^{b}	27.06^{b}	2.05^{a}
E3: (Kralovice 2005)		13.49^{a}	46.11^{c}	27.84^{a}	59.83^{a}	3.75^{c}	4.13^{a}	5.62^{b}	27.90^{b}	1.95^{a}
Influence of environment (%)		44.44	32.36	34.06	18.79	31.99	41.85	30.78	24.17	Ns.

Values of parameters marked by the same letters are not significantly different at $P \leq 0.05$

Table 5. Relationship of identified Gli-/HMW Glu-allelic blocks with higher frequency to grain quality parameters in the set of DH lines (studied years 2003–2005)

Gli-/HMW Glu-allelic blocks		Crude protein content in dry matter (%)	Zeleny sedimentation (ml)	Wet gluten content (%)	Gluten index (GI) (%)	Content of albumins + globulins in dry matter (%)	Content of gliadins (%)	Sum of glutenins (%)	Proportion of albumin + globulins in crude protein (%)	Relative viscosity
Gli 1-1A	2	13.29^{a}	40.15^{a}	25.74^{a}	67.35^{abc}	3.58^{abc}	3.93^{a}	5.78^{a}	27.03^{a}	2.01^{a}
	4	13.24^{a}	36.10^{a}	25.66^{a}	57.44^{ab}	3.62^{abc}	4.12^{a}	5.50^{a}	27.50^{a}	2.01^{a}
Gli 1B	3	13.48^{a}	36.30^{a}	26.16^{a}	51.68^{a}	3.75^{c}	4.17^{a}	5.56^{a}	28.03^{a}	2.06^{a}
	4	12.97^{a}	42.13^{a}	25.48^{a}	81.51^{c}	3.29^{a}	3.81^{a}	5.87^{a}	25.45^{a}	1.93^{a}
Glu 1B	7+8	13.13^{a}	42.37^{a}	25.55^{a}	77.40^{bc}	3.36^{ab}	3.91^{a}	5.85^{a}	25.74^{a}	1.96^{a}
	7+9	13.62^{a}	37.86^{a}	26.85^{a}	53.46^{a}	3.69^{bc}	4.23^{a}	5.70^{a}	27.26^{a}	2.04^{a}

Values of parameters marked by the same letters are not significantly different at $P \leq 0.05$

Table 6. Correlations between grain quality parameters in the set of DH lines (studied years 2003–2005)

Quality parameters	Crude protein content in dry matter (%)	Zeleny sedimentation (ml)	Wet gluten content (%)	Gluten index (GI) (%)	Content of albumins + globulins in dry matter (%)	Content of gliadins in dry matter (%)	Sum of glutenins in dry matter (%)	Proportion of albumin + globulins in crude protein (%)	Relative viscosity
Crude protein content	1.00								
Zeleny sedimentation	0.57	1.00							
Wet gluten	0.87	***0.61***	1.00						
Gluten index	–0.59	–0.07	–0.63	1.00					
Content of albumins + globulins	0.50	0.11	0.38	***–0.66***	1.00				
Content of gliadins	0.96	0.52	0.86	–0.65	***0.48***	1.00			
Sum of glutenins	0.80	0.58	***0.69***	–0.16	–0.05	0.66	1.00		
Proportion of albumins + globulins.	-0.47	–0.45	***–0.47***	–0.10	0.53	–0.43	***–0.83***	1.00	
Relative viscosity	0.08	–0.06	–0.05	–0.08	0.15	0.11	–0.04	0.09	1.00

Statistical significant correlations at $P \leq 0.05$ are written in ***italics*** protein and also in Zeleny sedimentation test, which was not different in parental genotypes. All three parameters confirmed their high linkage to wheat genotype. On the other hand, according to Prugar and Hraška (1986) the biosynthesis of storage proteins (gliadins and glutenins) is more influenced by other external factors in comparison with proteoplasmatic proteins (albumins + globulins). The content of this protein fraction is more stable and relatively low dependent on environment. In our case, a higher impact of lines on the calculated sum of glutenins was caused by relatively high variability of albumins and globulins content.

Zeleny sedimentation value ranged from 29.33 ml (line 112) to 54.33 ml (line 146) in the particular lines. The most contrast values of GI were found out in line 131 (89.25%) and line 119 (29.56%). The content of albumins + globulins in dry matter varied from 3.05% (line 163) to 4.03% (line 110) and their proportion in crude protein reflected the trend of this protein fraction content in dry matter. The highest values of their proportion were found in lines 110 (30.23%) and 174 (30.68%), on the other hand, the lowest value was observed in line 163 (22.89%) (Table 4).

As for relative viscosity, the statistically significantly lower value was obtained in line 159 (1.62). Differences in this parameter for the rest of lines were not statistically significant. In spite of the lower number of statistical differences between lines it is obvious that relative viscosity had a strong linkage to genotypes. The strong genotypic relationship was also confirmed by Martinant *et al.* (1998) for relative viscosity, who found a major QTL on 1BL chromosome responsible for arabinoxylan synthesis.

The homologous DH lines in tested Gli-/HMW Glu-allelic combinations generally showed analogous values of these more genetically controlled parameters. Nevertheless, there were also observed slightly higher non-significant differences between some parameters (e.g. proportions of albumins + globulins: lines 163 (22.89%) and 136 (26.21%) or Zeleny sedimentation test: lines 112 (29.33 ml) and 119 (42.00 ml)). Such variability of parameters in the homologous DH lines may probably be explained by the presence of other (among lines) different allelic combinations having specific impacts on these characteristics. Therefore further detailed genetic analysis of homologous lines could be useful in these cases.

The statistically significant influence of 1B/1R translocation (presence of allele Gli 1B 3) was identified for a higher content of albumins + globulins and lower value of gluten index (Table 5). Nevertheless, insignificant trends of a higher proportion of albumins + globulins in crude protein, higher value of relative viscosity, higher content of crude protein and lower values of both technological parameters (Zeleny sedimentation and GI) were also observed in wheat lines with 1B/1R translocation. The 1B/1R translocation effect on a significantly higher content of crude protein and slightly higher value of relative viscosity were confirmed by Amiour *et al.* (2002).

Generally, the lower impact of 1B/1R translocation on tested parameters was probably caused by higher similarity of both parents (especially in technological parameters). Nevertheless, this translocation effect was more significant than the impact of other different parental alleles. Only parental alleles in locus Glu 1B influenced one parameter (GI value) statistically significantly (Table 5).

A higher content of albumin fractions was obtained by Subda *et al.* (1997) in lines with 1B/1R translocation as well. The deterioration of breadmaking quality of many 1RS-bearing wheat cultivars (reflecting the lower value of GI) is caused by the presence of Sec-1 locus on chromosome 1RS. This locus produces a massive amount of secalins with a high water binding capacity, which is probably one of the causes of so-called 'sticky dough syndrome' (Martin & Stewart 1990; Zeller *et al.* 1982). On the contrary, the lower technological quality of lines carrying this translocation was not statistically confirmed by other important technological parameters such as Zeleny sedimentation test, the values of which were only non-significantly lower.

A similar result was reported by Fenn *et al.* (1994). However, other authors confirmed a decrease in Zeleny or SDS sedimentation value in lines with 1B/1R translocation as statistically significant (Bullrich *et al.* 1998; Martín & Carrillo 1999; Amiour *et al.* 2002). The non-significant decrease in Zeleny sedimentation value in lines bearing 1B/1R translocation could also be caused by the presence of HMW Glu- subunit 5+10, which generally contributes to improvement of rheological parameters (Chen *et al.* 2005). It seems that 1B/1R translocation was expressed more strongly in GI value than in Zeleny sedimentation test and its impact could be stronger on rheological properties of flour.

Correlations between grain quality parameters

Relations between all tested parameters were calculated by means of correlation matrix (Table 6). The highest positive correlation coefficients were determined for content of wet gluten, gliadins and crude protein (from 0.87 to 0.96). Their close relationships logically result from their mutual common composition: that main part of grain crude protein is incorporated into wheat gluten in the form of both storage protein fractions – gliadins and glutenins (Pomeranz *et al.* 1988).

Similarly, a high but negative correlation was calculated for the content of glutenins and proportion of albumins + globulins in crude protein. It is possible to emphasize the positive correlation between content of crude protein and sum of glutenins (0.80) and negative correlation between GI and albumins + globulins content. It may be expected that the improvement of feeding value based on higher albumins + globulins content will lead to lower bread-making quality. This theory is indirectly confirmed by results of feeding tests where the wheat varieties with lower bread-making quality were generally more effective as fodder (Bobková & Hromádko 2004). Nevertheless, their relationship to a higher content of albumins + globulins has to be still confirmed.

Relative viscosity was fully indifferent in our set of DH lines to all other tested parameters. Similar results were published by Amiour *et al.* (2002). He confirmed a negligible relative viscosity correlation to Zeleny sedimentation value, content of crude protein and grain hardness.

CONCLUSION

Relative viscosity, gluten index, Zeleny sedimentation test, content of albumins + globulins and their proportion in crude protein confirmed the higher relation to wheat genotype in the set of 17 DH lines.

The statistically significant influence of 1B/1R translocation (presence of allele Gli 1B 3) was only identified for a higher content of albumins + globulins and lower value of gluten index. The significantly lower gluten index confirmed a deteriorative effect of 1B/1R translocation more markedly than in the case of Zeleny sedimentation test. The effect of other parental alleles on tested parameters was negligible. A higher number of significant differences in Zeleny sedimentation test between DH lines confirmed the influence of other (non-tested) allelic combinations on this parameter. In consequence of the higher content of albumins + globulins in the presence of allele Gli 1B 3 it would be possible to expect a positive effect of the translocation on feeding value. On the contrary, the possible impact of this allele on an increase in relative viscosity could affect the feeding value of grain for poultry negatively. However, these hypotheses must be directly verified by a feeding test.

High positive correlations between contents of crude protein, wet gluten, and gliadins confirmed their close causal relation. In spite of lower variability between lines, relative viscosity showed a high dependence on genotype and was indifferent to the other quality parameters.

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Received for publication May 3, 2006
Accepted after corrections May 24, 2006

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