Wheat Breeding for the Improved Bread-making Quality Using PCR Based Markers of Glutenins

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Abstract: The relation between high-molecular-weight (HMW) glutenin subunits and bread-making quality could enable selection for improved bread-making quality in early stages of breeding process. The composition of HMW glutenin subunits was investigated in F_2 and F_7 progenies derived from the cross between winter wheat varieties Sulamit and Clever. The presence of *Glu-A1* (*AxNull* and *Ax1*), *Glu-B1* (*Bx6+By8* and *Bx 17+By18*) and *Glu-D1* alleles (*Dx 5+Dy10* and *Dx 2+Dy12*) was monitored using a PCR based assay. Segregation of alleles corresponded with the theoretically assumed 1:2:1 Mendelian ratio in F_2 generation, however, the values of χ^2-test in F_7 generation indicated a strong affection of allelic frequencies by the breeding process. Significant variation was also observed in *Glu-1* score frequency between F_2 and F_7 generation. These changes were probably caused by deliberate phenotypic selection for important agronomical traits. SDS and Zeleny sedimentation tests, mixographic parameter breakdown and HMW glutenin composition were analyzed in F_7 to reveal the effects of different combinations of HMW glutenin alleles on the bread-making quality characters. The results showed statistically significant differences in the contribution of HMW glutenin alleles. In general, the alleles *Ax1*, *Bx17+By18* and *Dx5+Dy 10* can be considered as markers of good baking quality. The data presented in this paper suggest that heterozygous constitution may also have a positive effect on bread-making quality.

Keywords: bread-making quality; breeding; glutenin subunits; MAS; winter wheat

Breeding for improved bread-making quality is an important breeding goal. This character is associated to a large extent with the amount and composition of endosperm seed proteins in the wheat kernel. Especially, the high-molecular-weight (HMW) glutenin subunits are considered to be significantly related to the technological quality of common wheat (Payne & Corfield 1979; Ahmad 2000; Schwarz *et al.* 2004).

The HMW glutenin subunits are encoded by genes at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arms of homoeologous chromosomes 1A, 1B and 1D (Payne *et al.* 1981). Two tightly linked genes are present at each *Glu-1* locus, one encoding a subunit of higher and the other of lower molecular weight, designated as the *x* and *y* type subunit, respectively (Harberd *et al.* 1986). At the *Glu-1A* locus, the *x* subunit is occasionally expressed and the *y* subunit is always absent. The *x* and *y* subunits coded by the *Glu-1B* and *Glu-1D* loci are almost always expressed, that means the *Glu-B1 y*-subunit can be absent. The HMW glutenin alleles *Ax1* and *Ax2** at *Glu-1A* locus, *Bx17+By18* and *Bx7+By8* or *By9* at *Glu-1B* locus and *Dx5+Dy10* at *Glu-1D* locus are connected with stronger dough and better baking properties, whereas *AxNull*, *Bx6+By8* and *Dx2+Dy12* are associated with poor baking quality (Payne *et al.* 1987).

Wheat breeders have been selecting wheat varieties with superior bread-making quality using SDS and Zeleny sedimentation tests, mixographic evaluation, baking test and other methods. However, these methods are not useful in very early hybrid generations, because they require a large amount of kernels and tests and they are destructive to tested samples. It is advantageous that the developed PCR based markers of HMW glutenin subunits may give quick characteristics of bread-making quality already in the early hybrid generations (Gale 2005).

The aim of this study was to describe the HMW glutenin allelic composition in F_2 and F_7 generations of the progeny derived from the cross between winter wheat varieties Sulamit and Clever, using a PCR based assay, and to find out changes in allelic frequencies in these generations. It was also aimed to reveal the effects of different HMW glutenin composition on different bread-making quality characters.

MATERIAL AND METHODS

Plant material

Hybrid material obtained from the cross between winter wheat varieties Sulamit and Clever, differing in the composition of HMW glutenin subunits, was subjected to these analyses. The elite (E) parental variety Sulamit is characterized by the following composition of glutenins: *AxNull*/*Bx17+By18*/*Dx5+Dy10*. The variety Clever of "A" baking quality class had the following composition: *Ax1*/*Bx6+By8*/*Dx2+Dy12* (Bradová & Šašek 2005). Varieties Sulamit and Clever were crossed in 1999 at the breeding station in Stupice, Selgen a.s. The obtained plant progenies were gradually reproduced to the F_7 generation in 2006. Plants were selected according to their phenotypical traits mainly for resistance to rusts and also for high grain yield and winter hardiness since F_2 generation. Randomly selected F_2 individuals were obtained from the seeds stored in the gene bank of the breeding station in 2006. The PCR analyses of F_2 and F_7 individuals and the quality tests of F_7 individuals were carried out in 2006. The number of PCR investigated individuals in F_2 and F_7 generation was 120 and 88, respectively. Bread-making evaluation was carried out on 69 F_7 lines.

DNA extraction and PCR conditions

Genomic DNA was isolated from 100 mg of leaf tissues of single plants by an isolation kit GenElute Plant Genomic DNA Kit (Sigma).

PCR analyses were performed in a Biometra T-Gradient thermal cycler with heated lid. 25 µl of PCR reaction mixture contained: 50–100 ng of template DNA, 1 × PCR buffer, 2.0 mmol/l $MgCl_2$, 0.4 µmol/l of each primer, 0.2 mmol/l of dNTP, 1U *Taq* polymerase (Fermentas). Temperature conditions for the PCR reactions were 94°C for 5 min followed by 35 cycles of 94°C for 1 min, annealing temperature (see Table 1) for 45 s and 72°C for 1 min, followed by a final extension of 72°C for 5 min.

The horizontal electrophoresis from Bio-Rad with a 2% agarose gel in 1 × TBE buffer was used

Table 1. The set of allele specific primers for HMW glutenin genes

Gene at *Glu 1*	References	Type	Forward and reverse primers (5' → 3')	Annealing temperature (°C)
AxNull	Lafiandra *et al.* (1997)	dominant	ACGTTCCCCTACAGGTACTA TATCACTGGCTAGCCGACAA	60
Ax1 and *Ax2**	Lafiandra *et al.* (1997)	dominant	CCATCGAAATGGCTAAGCGG GTCCAGAAGTTGGGAAGTGC	60
Dx5	D'Ovidio and Anderson (1994)	dominant	GCC TAG CAA CCT TCA CAA TC GAA ACC TGC TGC GGA CAA G	63
Dy10 or *Dy 12*	Smith *et al.* (1994)	codominant	GTT GGC CGG TCG GCT GCC ATG TGG AGA AGT TGG ATA GTA CC	63
Bx6 or *Bx7* or *Bx17*	Ma *et al.* (2003)	codominant	CGCAACAGCCAGGACAATT AGAGTTCTATCACTGCCTGGT	58

for the confirmation of amplified DNA. DNA was visualized according to the standard methodology using ethidium bromide and documented by the Gel Doc XR documentation system (Bio-Rad). The presence of the specific part of a gene was verified using standard O'GeneRuler DNA Ladder Mix (Fermentas). Table 1 shows sets of allele specific primers used for the identification of *Glu-1* genes encoding individual HMW glutenin subunits.

Bread-making quality evaluation

SDS sedimentation test: 5 g of whole wheat flour was analyzed for sedimentation in SDS (sodium dodecyl sulphate). After specified shaking and rest time the sedimentation volume was measured. The test was performed using the procedure described by standard ČSN 461021 (1998).

Zeleny sedimentation test: 3.2 g of flour was analyzed for sedimentation in a lactic acid solution with isopropanol in the presence of bromophenol blue. After specified shaking and rest time the sedimentation volume was determined according to standard ISO 5529 (2000).

Mixographic parameter breakdown, which is associated with the weakening of the dough, was obtained from mixographic evaluation using a Reomixer instrument (ReoLogica Instruments AB). Mixing properties of dough according to standard AACC 54-40A (1995) were obtained from 10 g of flour.

Each analysis was carried out at least twice in seed samples of F_7 generation (harvest in 2006).

RESULTS AND DISCUSSION

The five pairs of primers in AS-PCR (Allele Specific PCR) were used for the detection of alleles encoding the HMW glutenin subunits in 120 F_2 and 88 F_7 individuals derived from the cross between the cultivars Sulamit and Clever. Two primer sets designated by Lafiandra *et al.* (1997) identified the allelic composition at the *Glu-1A* locus. A 920 bp amplified fragment (Figure 1a) is characteristic of *AxNull* allele, the marker of poor bread-making quality. The absence of this fragment in the evaluated genotype showed the presence of one of the two alleles *Ax1* or *Ax2**, which are considered to be the markers of good bread-making quality. A PCR fragment of 1.5 kb proved the presence of *Ax1* allele from the parent Clever (Figure 1b). The pair of primers according to Ma *et al.* (2003) was used to verify alleles for HMW glutenin subunits at the *Glu-B1* locus (Figure 1c). The presence of the high baking quality marker, allele *Bx17* (Payne *et al.* 1987) from the variety Sulamit, was verified with the fragment of 669 bp after visualisation on the agarose gel. A strong association was described

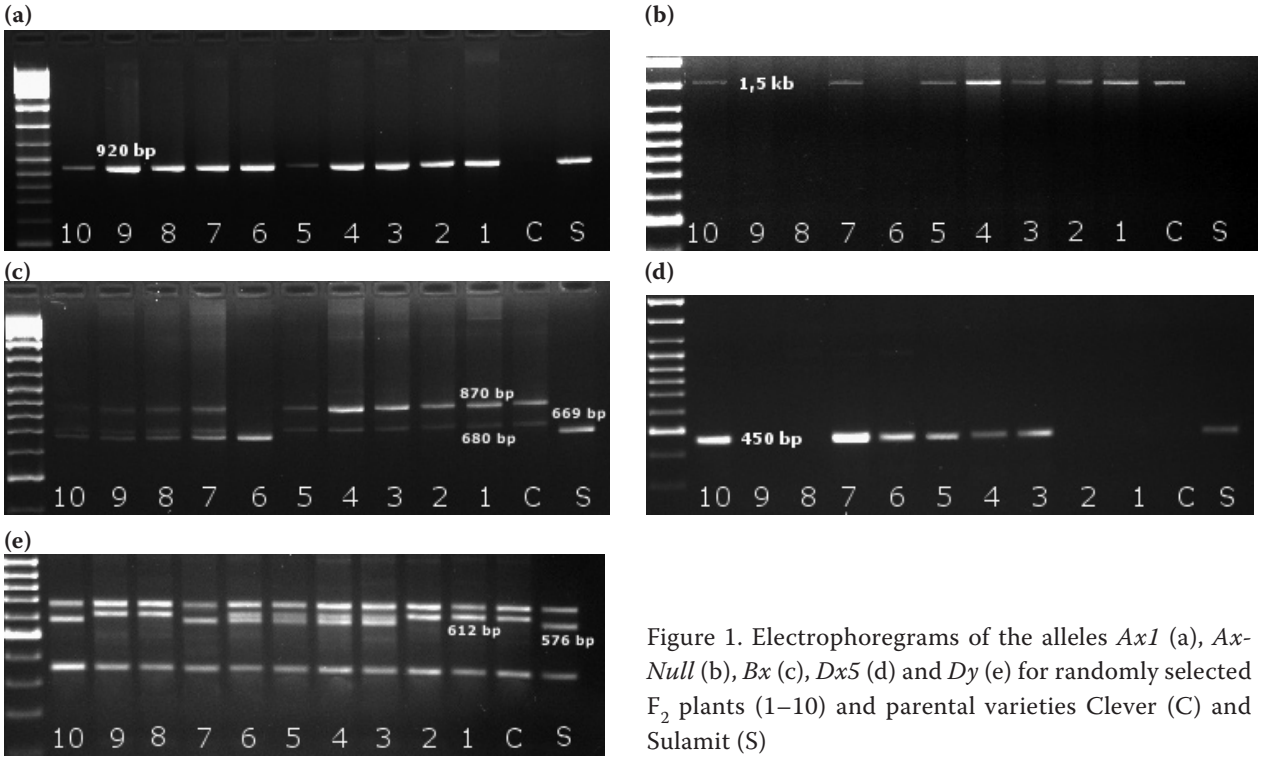

Figure 1. Electrophoregrams of the alleles *Ax1* (a), *AxNull* (b), *Bx* (c), *Dx5* (d) and *Dy* (e) for randomly selected F_2 plants (1–10) and parental varieties Clever (C) and Sulamit (S)

between allele *Bx6* and poor bread-making quality (Schwarz *et al.* 2004). This allelic composition, after detection by the AS-PCR method, was determined on agarose gel by the presence of two fragments of approximately 680 bp and 870 bp. Allele *Bx7* was confirmed on the agarose gel by two fragments of 630 bp and 766 bp. The presence of a 450 bp band showed the occurrence of *Dx5* HMW subunit allele that is associated with improved baking quality (D'Ovidio & Anderson 1994). No amplified fragment of 450 bp (Figure 1d) may be considered as a marker of the presence of other alleles at *Glu-D1* locus (e. g. *Dx2, Dx3* or *Dx4*) that are associated with poor bread-making quality. This marker turned out to be useless because of its dominant character. Dominant markers cannot discriminate between heterozygous and homozygous individuals. Early hybrid generations of wheat are still heterozygous. Hence, it was essential to use codominant markers or combination of more than one dominant marker for the differentiation between alleles during segregating generations. Using the primer combination designed by Smith *et al.* (1994), a difference between alleles *Dy10* and *Dy12* was confirmed. PCR reaction provided a fragment of 576 bp for *Dy10* allele and of 612 bp for *Dy12* allele (Figure 1e). Witkowski *et al.* (2005) used the sodium the method of dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) to study the inheritance of HMW glutenin subunits coded by the *Glu-1A* locus. Because of the lack of specific band for the *AxNull* allele, this method was not successful in distinguishing heterozygous genotypes. The primer sets used in this study reliably distinguished the complete HMW glutenin allelic composition at all studied loci and determined heterozygous genotypes. Furthermore, this approach can be applied before harvest, because analyses are done from leaves. The SDS PAGE method is limited to be applied to grain, hence the analysis cannot be performed before harvest.

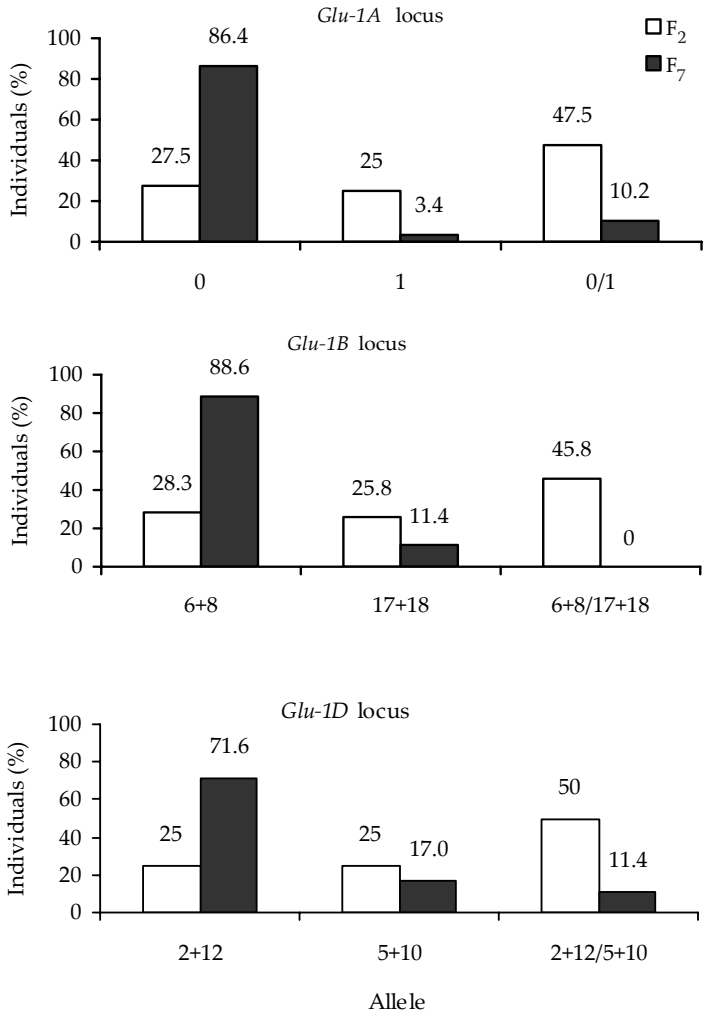

Figure 2. Comparison of the frequency distribution of HMW glutenin alleles at *Glu-1* loci in F_2 and F_7 generation

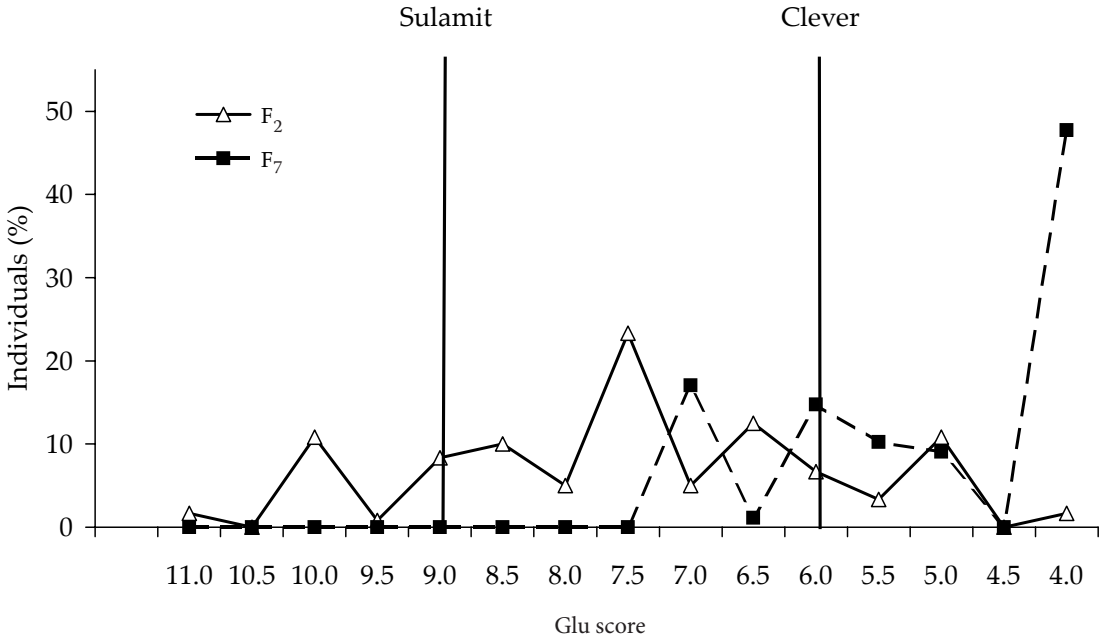

Figure 3. Relationship between the *Glu-1* score (Payne *et al.* 1987) value for groups of HMW glutenin lines and frequencies of plants in F_2 and F_7 generation

According to the Mendelian inheritance, 25% of the F_2 plants were expected to be homozygous for each *Glu-1* allele and 50% were expected to be heterozygous. With the increased proportion of homozygous plants due to the self-pollination only 1.56% of the F_7 plants were expected to be heterozygous and 49.22% homozygous for each allele. Sample frequency distributions in F_2 and F_7 generations were compared with these theoretical frequency distributions using χ^2-test. The results of χ^2-test confirmed the hypothesis of supposed theoretical distribution in F_2 generation and disproved this hypothesis in F_7 generation. χ^2-test values and significance probabilities for 2 degrees of freedom are shown in Table 2. The ratio 1:2:1 gained in F_2 generation can be considered as verification of well performed cross. The values of χ^2-test for F_7 generation indicate a strong affecting of Mendelian inheritance for the evaluated genes by the breeding process.

The observed allelic composition is summarized in Figure 2. It has been shown that allelic variation in HMW glutenins tended to the higher frequencies of alleles with a negative effect on bread-making quality in F_7 generation. It might be explained by targeted selection for resistance to the rust diseases, including leaf rust *Puccinia recondita*. The *Lr 37* gene, conferring resistance to leaf rust, is present in a small translocation from *Aegilops ventricosa* on a short arm of 2A chromosome (Błasczyk *et al.* 2004). Kohutová (unpublished) detected the *Lr37* using CAPS marker in both F_2 and F_7 populations of progeny derived from Sulamit and Clever parents. Variety Clever is the donor of gene *Lr37*. The observed ratio of F_2 generation agreed with the 1:2:1 theoretically expected ratio ($\chi^2 = 1.11$, $P = 0.57$). However, 94.5% of F_7 genotypes were found to carry gene *Lr37* in dominant homozygous constitution, which implies improvement in resistance to the leaf rust as a consequence of deliberate

Table 2. Verification of Mendelian segregation at a 1:2:1 ratio in F_2 and F_7 generations using χ^2-test (df = 2)

Value	*Glu-1A* locus		*Glu-1B* locus		*Glu-1D* locus	
	F_2	F_7	F_2	F_7	F_2	F_7
χ^2	0.38	118.72	0.82	62.25	0	92.68
P	0.90–0.75	0.005–0	0.75–0.50	0.005–0	1.00–0.995	0.005–0

df – degrees of freedom

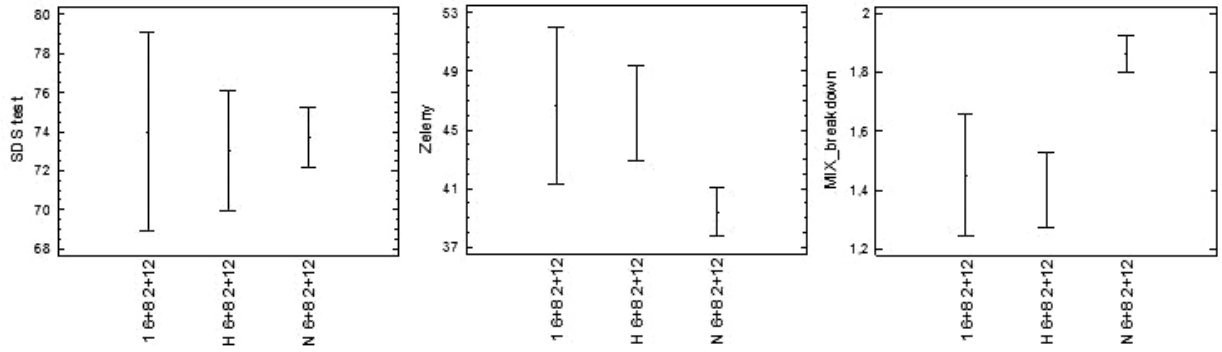

Figure 4. Means and 95.0 percent confidence intervals for three bread-making quality characters and groups of lines differing in the composition of alleles encoding HMW glutenins at *Glu-1A* locus

phenotypic selection. A high level of this resistance seems to be associated with the presence of alleles responsible for deterioration of baking quality. A similar negative correlation between the leaf rust resistance and baking quality was reported by Drijepondt *et al.* (1990).

The frequency distribution of HMW glutenin alleles could be also influenced by the selection for winter hardiness. Witkowski *et al.* (2008) have found the lines containing *AxNull* allele to be more frost tolerant than the lines containing *Ax1* or *Ax2** alleles, which suggests a linkage between winter hardiness and *Glu-1A* alleles responsible for deterioration of bread-making quality. Moreover, the parental variety Sulamit possessing *AxNull* allele is known to be more frost tolerant than the parental variety Clever with *Ax1* allele.

Glu-1 score value for all allelic groups was calculated according to Payne *et al.* (1987). The value for heterozygous loci was calculated as an average of both alleles present at the locus. Significant differences were observed in *Glu-1* score frequency between F_2 and F_7 generation (Figure 3). It was found that F_2 *Glu-1* score was in 13.3% of plants higher than and in 8.3% of plants the same as in the parental variety Sulamit of superior grain quality. 55.8% of plants had *Glu-1* score higher than and 6.7% the same as the parental variety Clever (6). Only 15.8% of F_2 plants had a lower value than both parental varieties. By contrast, in F_7 generation, no plant with higher *Glu-1* score value than Sulamit was found; 18.2% of plants reached a higher value than and 14.8% the same value as Clever in F_7 generation. 67.1% of evaluated individuals from the F_7 generation had assigned *Glu-1* score below 6 and of it 47.7% of plants had the lowest obtained score (4).

The data on variation and average performance of 69 F7 lines in three grain quality characters, in comparison with parental varieties, are presented in Table 3. A comparison of the groups of lines differing in the composition of HMW weight subunits is provided in Table 4. The analysis of the results of SDS test, Zeleny test and the mixographic parameter breakdown showed the unequal contribution of alleles encoding HMW glutenin subunits to the examined parameters of bread-

Table 3. Results of SDS and Zeleny sedimentation tests (in ml) and mixographic evaluation (in mixograph units) of 69 lines of F_7 generation in comparison with the parental varieties Sulamit and Clever

	SDS test	Zeleny test	Mixograph breakdown
Average	74.97	42.67	1.55
SD	6.34	6.68	0.39
CV (%)	8.45	15.66	25.04
Minimum value	64	31	0.78
Maximum value	89	60	2.34
Sulamit	93	53	0.11
Clever	81	38	1.47

SD – standard deviation, *CV* – coefficient of variation

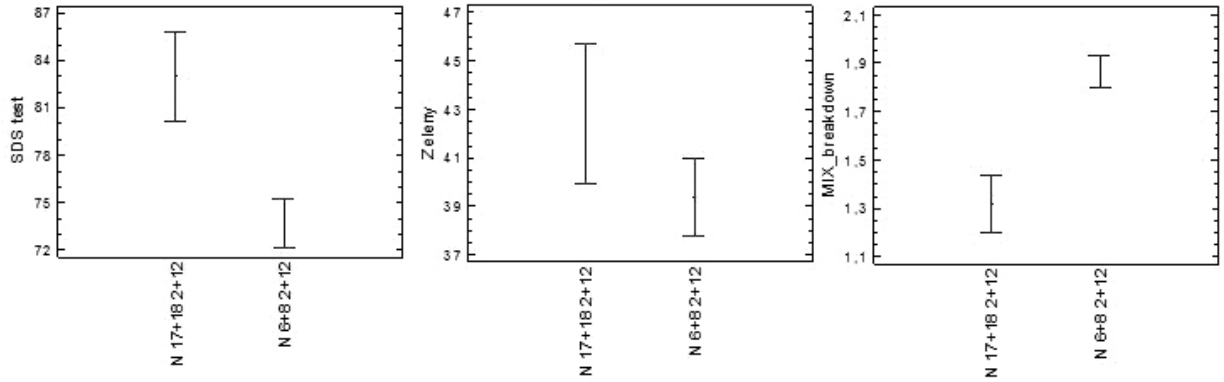

Figure 5. Means and 95.0% confidence intervals for three bread-making quality characters and groups of lines differing in the composition of alleles encoding HMW glutenins at *Glu-1B* locus

making quality. Luo *et al.* (2001) reported a high variation in the quality parameters associated with the possession of a certain allele. Three groups of genotypes were compared. The first group of genotypes differed in alleles encoding HMW glutenin subunits at the *Glu-1A* locus. The second group had various alleles at the *Glu-1B* locus. No heterozygous genotype was found in F_7 generation for this group. The third group differed in HMW alleles at the *Glu-1D* locus.

Differences in the genotypic group variant in *Glu-1A* alleles were statistically significant ($P < 0.05$) for Zeleny test and mixographic parameter breakdown. Heterozygous allelic composition and *Ax1* allele at this locus showed a positive effect on Zeleny test. A positive effect on the mixographic parameter breakdown, which is in negative correlation with bread-making quality, was found for heterozygous allelic composition (not significant) and *Ax1* allele ($P < 0.05$). No statistically significant difference was however found in the SDS sedimentation volume. These results correspond with the results of Payne *et al.* (1987) or Nieto-Taladriz *et al.* (1994). The data presented in this study suggest that heterozygous constitution may also have a positive effect on bread-making quality (Table 4, Figure 4). Significant differences ($P < 0.05$) between *Null/17+18/2+12* and *Null/6+8/2+12* allelic com-

Table 4. Means of bread-making quality parameters for groups of HMW glutenin lines in F_7 generation in comparison with parental varieties

Composition of HMW glutenin subunits	No. of lines	Payne score value	SDS test (ml)	Zeleny test (ml)	Mixograph breakdown (MU)
H 6+8 2+12	8	5.0	73.0a	46.1a	1.4a
N 6+8 2+12	32	4.0	73.7a	39.4b	1.9b
1 6+8 2+12	3	6.0	74.0a	46.7ab	1.5a
N 6+8 2+12	32	4.0	73.7a	39.4a	1.9a
N 17+18 2+12	10	6.0	83.0b	42.8a	1.3b
N 6+8 H	6	5.5	72.5a	45.5ab	1.2a
N 6+8 2+12	32	4.0	73.7a	39.4a	1.9b
N 6+8 5+10	10	7.0	73.9a	47.4b	1.1a
Sulamit (N 17+18 5+10)		9.0	93.0	53.0	0.1
Clever (1 6+8 2+12)		6.0	81.0	38.0	1.5

Different letters in a column denote a statistically significant difference of LSD test ($P < 0.05$); N – *AxNull* allele; H – heterozygous composition at the locus; MU – mixograph unit

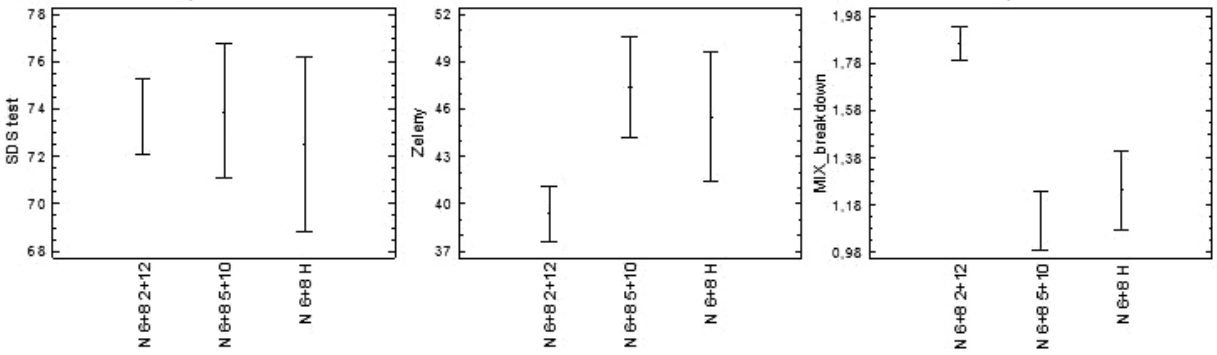

Figure 6. Means and 95.0% confidence intervals for three bread-making quality characters and groups of lines differing in the composition of alleles encoding HMW glutenins at *Glu-1D* locus

position were found in SDS sedimentation volume and mixographic parameter breakdown. A positive influence of the lines with 17+18 subunits on both quality tests was confirmed. This result confirms previous observations of Peltonen *et al.* (1993). There was no significant difference in the means of Zeleny test between the genotypes differing in *Bx6+By8* and *Bx17+By18* alleles (Table 4, Figure 5). *Dx5+Dy10* and heterozygous constitution at the *Glu-1D* locus had a positive effect ($P < 0.05$) on Zeleny test and mixographic parameter breakdown. A negative effect of *Dx2+Dy12* was published previously (Shewry *et al.* 1992; Ahmad 2000; Campos *et al.* 2004; Tabiki *et al*. 2006 and others) and our results confirm the results of these studies. There was no significant difference in the means of SDS test between the genotypes carrying *Dx5+Dy10* and *Dx2+Dy12* alleles. Heterozygous constitution at this locus showed a statistically significant ($P < 0.05$) positive influence on the breakdown parameter (Table 4, Figure 6).

Payne *et al.* (1987) developed a predictive scoring system for individual HMW glutenin subunits based on the SDS sedimentation volume. However, only the allelic pair *Bx17+By18* showed a difference in the SDS volume (Table 4). It could probably be ascribed to differences in the genetic background or to a reduced variability of lines in F_7 generation for this trait as a consequence of phenotypic selection for resistance to the rusts. As shown in Table 3, the coefficient of variation in the SDS sedimentation test was the lowest (8.5%), when compared with Zeleny test (15.7%) and mixographic parameter breakdown (25.0%).

In general, the obtained results demonstrate the usefulness of application of PCR based markers of glutenins in wheat breeding programs. It can be expected that the determination of HMW glutenin subunits composition can significantly contribute to the improvement of bread-making quality already in early hybrid generations. However, it should be taken into consideration that a deliberate selection for e.g. *Ax1* at *Glu-A1* may result in lower winter hardiness (Witkowski *et al*. 2008). With early strong selection for winter hardiness, or rust resistance in our case, the alleles with a negative impact on the end use quality can be fixed in advanced breeding materials.

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