Effect of Genotype, Environment and Their Interaction on Quality Parameters of Wheat Breeding Lines of Diverse Grain Hardness

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Abstract: Understanding the contribution of genotype, environment and genotype-by-environment interaction to wheat grain quality facilitates the selection for quality in breeding programs. Stability of grain quality characteristics is an important requirement in the baking industry. We assessed 24 winter wheat genotypes with different grain hardness in multi-environment trials at four locations and two levels of fertilization in each location. Grain samples were analyzed for hardness, protein and starch content, and wet gluten content, Zeleny sedimentation value, alveograph parameter (W) and hectoliter weight. All parameters were evaluated on whole grains using the near infrared transmittance technique. Differences between hard and soft genotypes appeared to be significant, apart from grain hardness, for protein content, Zeleny test and alveograph parameter. Genotype was found to have a major influence only on grain hardness; for protein content, wet gluten and Zeleny sedimentation value environment prevailed the influence of genotype, and for starch content, alveograph W parameter and hectoliter weight both sources of variation had similar importance. Genotype-by-environment interaction was of smaller size relative to genotype and environment in terms of all the studied quality parameters. Stable genotypes predominate the breeding lines studied. Response of unstable genotypes to environmental conditions was nonlinear in most cases.

Key words: Bread Wheat, Genotype-by-Environment Interaction, Near Infrared Transmittance Technique, Protein Content, Starch Content, Wet Gluten, Zeleny Test.

Bread wheat (Triticum aestivum L.), one of the most important cereal crops in the world, is grown in a wide range of environmental conditions. Several environmental factors influence wheat yield and its quality, such as temperature, precipitation and its distribution during the growing season, sowing time, soil type, and nitrogen fertilization (Peterson et al., 1992; Anderson et al., 1998; Smith and Gooding, 1999). Some genotypes are characterized by a stable performance, while others vary considerably with the environment (Mariani et al., 1995; Peterson et al., 1998; Ames et al., 1999).

The quality of wheat grain depends on several characteristics, among which grain hardness, protein content and composition of high molecular weight glutenin subunits (HMW) are the most important (Obuchowski, 1984; Payne et al., 1987; Shewry et al., 1992; Martin et al., 2001; Gross et al., 2004; Kuchel et al., 2006; Salmanowicz et al., 2008). Grain hardness influences several technological properties, such as easy milling, semolina sizing, degree of starch damage during milling, water intake by dough, and, finally, the quality of produced bread (Autron et al., 1997). Protein quantity and quality evaluated by the Zeleny test and gluten content are key factors in building the structure of dough and next-texture of bread crumbs. On the other hand, wheat lines with a soft grain and relatively low protein content are suitable for cookie and wafer production, and those with an extra hard grain are especially useful for pasta making. One of the basic factors for wheat classification is the alveograph W parameter. In Europe, flour with a W value between 130–160 is classified as useful for bread production, 160–250 as an improver of baking properties.

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Abbreviations: AWP, alveograph W parameter; GEI, genotype-by-environment interaction; GH, grain hardness; HMW, high molecular weight glutenin subunits; HW, hectoliter weight, MS, torque; PC, protein content; PSL, particle size index; NIR, Near Infrared; SC, starch content; WHI, wheat hardness index; WG, wet gluten; ZS, Zeleny sedimentation value.
and above 250 - as a flour with strong gluten. (Faridi et al. 1987). US hard red winter wheat shows a W parameter at the level of 190–285, and soft white for cookie 43–108 (Crop Quality Report, 2008).

Grain hardness is a result of anatomy, structure and mechanical properties of particular parts of grain. It can be evaluated by the determination of torque and wheat hardness index using a single-step Brabender testing machine (Greenaway, 1969) or by the determination of particle size index using a proper mill and a sifting machine (Obuchowski and Bushuk, 1980). Relative degree of hardness may be evaluated using the near-infrared (NIR) spectroscopy technique. The last method is especially interesting for breeders, since it is easy, quick and not destructive for the grain (Martin et al., 2001; Hruškova and Fámiéra, 2003; Osborne, 2006).

Grain hardness in wheat is controlled by the Ha locus on the short arm of chromosome 5D (Appels et al., 2001). This locus is closely linked with two loci encoding puroindolines, Pin-a and Pin-b, which are the main components of polypeptides friabilins. Soft grains have a large amount of both puroindolines on the surface of starch granules. Medium-hard and hard grains are characterized by reduced amounts of both puroindolines or by accumulation of only Pin-a, whereas super hard grains have no puroindoline Pin-a. Besides, some quantitative trait loci (QTLs) associated with grain hardness were detected, e.g., on chromosomes 1A, 3A, 5D, 3BL (Lillemo et al., 2006). Additionally, molecular analyses were performed for identification of Pin-a and Pin-b alleles in the genomes of studied lines.

Grain hardness and protein content are two factors, on which quality classification of wheat genotypes is frequently based. In developing new bread wheat cultivars a key question is the early recognition of genotypes with predicted well bread-making quality, thus properties used to determine grain quality need to be evaluated. In breeding programs, quality characteristics may be evaluated in two stages: (1) during early generation, where grain samples from individuals are analyzed, and (2) in advanced breeding lines examined in the experiments conducted at several places with different soil types, rainfall and temperature. Occurrence of genotype-environment interaction (GEI) may reduce the efficiency of selection of superior individuals or advanced breeding lines. Analysis of GEI in each individual is impossible but in this case, general information on the influence of environment and GEI on performance of quality attributes may be helpful for breeders. Experiments with breeding lines conducted in several environments allow the analysis of GEI and the results—identification of superior genotypes with a desirable response under different environmental conditions, i.e., stable, intensive or extensive, depending on the breeding purpose.

Since evaluation of quality attributes, such as grain hardness, protein content and rheological parameters is costly and time consuming, breeding programs are usually performed only for selected advanced lines. Selection for grain quality in early stages of breeding is, if any, performed by near-infrared reflectance techniques.

The aim of this study was to evaluate the effect of genotype, environment and GEI on quality parameters in winter wheat breeding lines with different grain hardness, examined in a series of trials conducted at different locations.

### Materials and Methods

1. **Plant materials**

   Materials used in the studies covered 23 advanced breeding lines of winter wheat and cultivar Tonacja as a standard. The lines were chosen on the basis of previous experiments, in which grain hardness of 50 breeding lines bred in Poland were evaluated by determination of torque (MS), wheat hardness index (WHI) and particle size index (PSI) (Obuchowski et al., 2010). Out of those 50 breeding lines, 23 lines of different grain hardness were chosen for these experiments (Table 1). Out of 24 genotypes studied in the present experiments, nine were soft (lines no. 2, 7, 17, 41, 42, 44, 45, 48 and cv. Tonacja), 11 hard (lines no. 1, 9, 20, 29, 30, 31, 35, 36, 38, 40, 46) and four “medium” (lines no. 8, 11, 13, 19) (lines were treated as “medium” when evaluation of grain hardness based on different physical methods gave different, not comparable results (Obuchowski et al., 2010). Additionally, molecular analyses were performed for identification of Pin-a and Pin-b alleles in the genomes of studied lines.

2. **Puroindoline analysis**

   DNA extraction: Leaves of 14-day-old wheat plants were used for genomic DNA extraction (Promega Kit). The extracts were diluted to 100 ng mL⁻¹ and stored at −20°C.

   Primer set Pina-D1b was used as a molecular marker for the identification of Pina-D1b alleles (Lillemo et al., 2006). The allele-specific primers Pnb-D1b were used to detect the nucleotide substitution of G to A at the position 223 of Pinb coding region (Pinb-D1b) and Pinb-D1a for the detection of no nucleotide change at the position 223 of Pinb (Li et al. 2008).

   For polymerase chain reaction (PCR) amplifications a gene AMP PCR system 9700 was used. PCR reaction was performed in 25 µL volumes containing 10 pmol of each primer, 250 µM of each of dNTP, 1xPCR buffer, 1.5 mM of MgCl₂, 2.0 unit of Taq DNA polymerase, and 100 ng of genomic DNA. The samples, denatured at 94°C, were submitted to 36 cycles of 45 s denaturation at 94°C, 45 s annealing at 58°C, and 1 min elongation at 72°C, with a final extension of 10 min at 72°C at the end. PCR products were analyzed on 15 g L⁻¹ agarose gels, stained with ethidium bromide, and detected using UV light.
3. Field experiments

Field experiments with 24 chosen genotypes of winter wheat were conducted during 2008/2009 season at four locations: Cerekwica (C) (North-West Poland, near Poznań), Choryń (Ch) (South-West Poland, near Leszno), Laski (L) (Central Poland, near Warsaw), and Dębina (D) (North Poland, near Malbork). The locations differ in soil type: Cerekwica and Laski—podzols, Choryń—light brown soils, Dębina—mud soil. Environments (locations) were additionally differentiated by the use of conventional and high levels of agronomic inputs (designated as 1 and 2, respectively). Fertilization doses of NPK were dependent on soil fertility at given locations; conventional and high inputs differed only in nitrogen doses, which in conventional level at particular locations ranged from 112 to 150 kg ha\(^{-1}\), and in the high level were by 40 kg higher and ranged from 152 to 190 kg ha\(^{-1}\). Seeds were sown on 27−28 September, 2008, in 5 m\(^2\) plots with 8 rows 12.5 cm apart, at a density of 400 seeds m\(^{-2}\). Experiments were carried out in randomized complete block design with two replications. Grain was harvested at maturity in the first decade of August 2009. Total precipitation during vegetation season of 2008/2009 at particular locations ranged in a small degree from 474.3 (Laski) to 548 mm (Choryń). Marked differences between locations were observed in the sum of rainfall during the spring and summer of 2009, i.e., from March to July. In that period, the amount of precipitation was the highest in Choryń (352.6 mm) and the lowest in Laski (284.8 mm). In spring of 2009, an extremely low precipitation occurred in April from 0.0 mm in Dębina to 21 mm in Choryń.

Post harvest, grain yield per plot (at 13% of moisture) was recorded and some quality parameters were evaluated.

4. Grain quality analysis

Grain hardness (GH), hectoliter weight (HW) and some parameters related to bread-making quality: protein (PC), starch (SC) and wet gluten (WG) content, Zeleny sedimentation value (ZS) and alveograph W parameter (AWP) were analyzed using a NIRSystems Infratec 1241 Grain Analyzer (Foss, Hillerod, Denmark) (Hruškova and Famgära, 2003; Osborne, 2006; Silva et al., 2008).

| Line no. | Code            | Pedigree               | Grain hardness\(^a\) | Puroinedolone |
|---------|-----------------|------------------------|----------------------|--------------|
| 1       | CHD 169/04      | (Zyta × Ritmo) × Tarso | H                    | a b          |
| 2       | CHD 329/04      | Clever × C461/96      | S                    | a a          |
| 7       | DED 5854/03     | Clever × NAD93/92     | S                    | a a          |
| 8       | DED 6240/03     | Sw45422 × Nutka       | M                    | a b          |
| 9       | DED 4152/03     | Clever × SMH4118-7    | H                    | a b          |
| 11      | DED 6658/03     | Clever × Ludwig       | M                    | a a          |
| 13      | LAD 166/05      | (Soraja × M3511/90) × Fineza | M                  | a a          |
| 17      | LAD 180/05      | Batis × CRT9          | S                    | a a          |
| 19      | LAD 245/04      | (MIB3511/90 × CRT9) × Tonacja | M                  | a b          |
| 20      | LAD 252/04      | (MIB3511/90 × CRT9) × Tonacja | H                  | a b          |
| 29      | CHD 760/04      | (SW4510 × C1091/95) × Tonacja | H                  | a b          |
| 30      | CHD 65/04       | (DAD338/91 × CRT9) × Tarso     | H                    | a a          |
| 31      | CHD 114/04      | (Tarso × G57/93) × Sukces | H                  | a b          |
| 35      | CHD 382/04      | P822 × OCH898         | H                    | b a          |
| 36      | CHD 565/04      | (Ludwig × Sw44320) × Nutka | H                  | a a          |
| 38      | CHD 737/04      | (S9635A × Kris) × Mewa | H                    | a a          |
| 40      | DED 3481/03     | SW13030 × STH504      | H                    | a b          |
| 41      | DED 5845/03     | Clever × NAD93/92     | S                    | a a          |
| 42      | DED 5897/03     | Clever × NAD93/92     | S                    | a a          |
| 44      | DED 6986/03     | Mewa × Clever         | S                    | a a          |
| 45      | LAD 125/04      | BOA × CRT9            | S                    | a a          |
| 46      | LAD 319/04      | Herzog × Charger      | H                    | a a          |
| 48      | LAD 355/04      | (Bentos × Soraja) × Charger | S                  | a a          |
| 49      | Tonacja         | Jubilataka × SMH2182  | S                    | a a          |

\(^a\)According to Obuchowski et al. (2010): H—hard, S—soft, M—“medium”.

Table 1. Code, pedigree and grain hardness of studied wheat breeding lines.
alveograph W parameter is a combination of dough strength and extensibility and is expressed in joules.) Five independent analyses were recorded from each 600-g sample of whole grains using apparatus fitted with a sample transport module and standard sample cups. Sample were scanned from 570 to 1050 nm, and data were collected every 2 nm.

5. Statistical analysis

One-way analysis of variance was performed for each experiment, from which information on significance of genotype differentiation was obtained and experimental error was estimated. In the next step, the data from a series of experiments were processed using the computer program SERGEN (Calinski et al. 1998) based on the methods developed by Kaczmarek (1986), and Calinski et al. (1997, 2005, 2009). In these methods, GEI effect related to each genotype is measured by the value of the relevant F-statistic; a given genotype is considered as stable, if F-statistic value for GEI is not significant at P=0.05. The regression of GEI effects on the observed environmental means (measured by the relevant F-statistic) is the measure of genotype adaptability. The main effect of each genotype is estimated as the difference between genotype mean and general mean. Besides, the coefficients of regression and determination (in percentage), and also the F-statistic values for testing the significance of the regression and of the deviations from regression are calculated. Unstable genotypes are classified according to the results of regression analysis as intensive, when the regression coefficient is significantly positive, and as extensive, when the regression coefficient is negative and significant.

Mean values over environments for soft, medium and hard genotypes were compared by testing contrasts using F-statistic. Correlation coefficients were computed to analyze the relationships between yield and studied grain characteristics.

Results

Molecular analyses revealed that all the lines with soft
grains had wild \textit{Pina} and \textit{Pinb} alleles (Table 1). Lines classified as hard on the basis of physical analyses had mutated alleles of \textit{Pina} (line no. 35) or \textit{Pinb} (lines no. 1, 9, 20, 29, 31, 40) genes, but in four hard lines (nos. 30, 36, 38, 46) only wild alleles were identified. Breeding lines designated as “medium” had \textit{Pina-D1a} and \textit{Pinb-D1b} (lines no. 8, 19) or \textit{Pinb-D1a} (lines no. 11, 13) allele.

Mean values of characteristics studied in particular environments are presented in Table 2. The difference with the environment was small in starch content and hectoliter weight, but large in grain hardness and Zeleny sedimentation value. Among the environments, Ch2, was the most favorable for quality parameters of the wheat genotypes studied because in that environment the mean values of grain hardness, protein content, wet gluten content and alveograph parameter were the highest. In contrast, in that environment grain yield was very low.

Initial one-way analysis of variance performed for each environment indicated a significant differentiation of genotypes regarding all the analyzed traits. Participation of individual environments in GEI was the lowest in D2 and relatively high in C1 and Ch2 (Table 2).

Analysis of variance for a series of experiments calculated according to SERGEN software revealed a significant influence of the environment and GEI on variation of all the analyzed traits. Deviations from regression appeared to be important for grain hardness, protein and starch content, Zeleny sedimentation value, alveograph W parameter and grain yield, and not significant for wet gluten and hectoliter weight (Table 3). (In the analysis of variance, the mean squares for genotypes and regression were not tested, since the appropriate $T^2$-statistic would require that the number of environments be higher than the number of genotypes, which was not the case here.) The share of variation of genotype, environment and GEI in total variation indicates that variation of grain hardness was influenced mainly by genotype, whereas in variation of protein content, wet gluten and Zeleny sedimentation value, the influence of environment prevailed the influence of genotype. For starch content, alveograph W parameter, hectoliter weight, and grain yield both the sources of variation were similarly important. The influence of GEI was the highest for grain yield and considerably lower for all the quality traits, and the lowest was for grain hardness and test weight.

1. Grain hardness

The mean value of GH was 52.4 and in particular genotypes ranged from 37.2 to 66.5 (Table 4). Mean values in soft, medium and hard grain genotypes were 42.3, 52.9 and 60.0, respectively. The differences between means in these groups of genotypes, tested by $F$-statistic, were highly significant ($P<0.01$).

Main effects were significantly negative in all the soft grain genotypes with an exception for cv. Tonača, whereas in all the hard genotypes those effects were significantly positive and non-significant in medium lines. Out of 24 genotypes studied, 7 appeared to be unstable ($F$-statistic for GEI significant at $P=0.05$). Among unstable lines, 5 belonged to soft and 2 to hard genotypes. Generally, response of unstable genotypes to various environmental conditions could not be explained by regression – regression coefficient was significant only in line no. 2 with a determination coefficient of 51.4% (Table 4).

2. Protein content

Protein content of the grains of studied genotypes ranged from 12.3 (line no. 48) to 14.7% (line no. 30) (Table 5). Mean values of PC in hard, medium and soft groups of lines were equal to 13.4, 13.2 and 13.0%,
respectively, and only the difference between soft and hard groups was significant ($P<0.05$). Main effects were significant in 9 genotypes; among them negative effects prevailed. Three lines (no. 29, 30 and 40) were characterized by significantly positive main effects and all of them were of hard grain.

Out of 24 studied genotypes, 6 appeared to be unstable ($F$-statistic for interaction $> F_{0.05}$). However, only in 3 lines, instability could be explained by regression analysis (lines no. 17, 35, 40). Hard lines no. 35 and 40 were characterized by significant positive regression coefficient ($b=0.432$ and $b=3.38$, respectively, $P<0.05$), which means that these lines are intensive, i.e., more responsible in favorable conditions. In contrast, soft line no. 17 had significantly negative coefficient of regression ($b=-0.261$, $P<0.05$) and relatively high determination coefficient equal to 63.7%, which indicates that this line is an extensive.

### Table 4. Mean values, genotype main effects, $F$-statistic values for GE interaction, coefficients of regression and determination, and $F$-statistic for deviation from regression for wheat grain hardness.

| Line no. | Mean | Main effect | $F$-statistic for GEI | Regression coefficient | Determination coefficient (%) | $F$-statistic for deviations from regression |
|----------|------|-------------|-----------------------|-------------------------|--------------------------------|--------------------------------------------|
| 1-H      | 64.5 | 12.12**     | 1.40                  | -0.257                  | 45.3                           | 0.92                                       |
| 2-S      | 45.0 | -7.38**     | 3.35**                | 0.433*                  | 51.4                           | 1.90                                       |
| 7-S      | 45.9 | -6.52**     | 2.72*                 | 0.316                   | 33.6                           | 2.91**                                     |
| 8-M      | 57.2 | 4.84*       | 1.63                  | 0.065                   | 2.4                            | 1.85                                       |
| 9-H      | 56.3 | 3.90*       | 1.46                  | -0.184                  | 21.1                           | 1.34                                       |
| 11-M     | 50.9 | -1.47       | 1.26                  | -0.228                  | 37.9                           | 0.91                                       |
| 13-M     | 53.2 | 0.82        | 1.77                  | -0.004                  | 0.1                            | 2.06                                       |
| 17-S     | 40.8 | -11.61**    | 0.74                  | 0.121                   | 18.1                           | 0.71                                       |
| 19-M     | 54.7 | 2.50        | 1.73                  | -0.300                  | 46.3                           | 1.09                                       |
| 20-H     | 55.4 | 3.06*       | 0.67                  | -0.081                  | 9.0                            | 0.71                                       |
| 29-H     | 66.5 | 14.15**     | 1.92                  | -0.241                  | 27.8                           | 1.62                                       |
| 30-H     | 57.9 | 5.55*       | 0.85                  | -0.167                  | 29.8                           | 0.70                                       |
| 31-H     | 57.5 | 5.12*       | 1.02                  | -0.154                  | 21.2                           | 0.94                                       |
| 35-H     | 55.8 | 3.46**      | 6.49**                | 0.490                   | 35.9                           | 5.00**                                     |
| 36-H     | 62.6 | 10.22**     | 1.69                  | -0.040                  | 0.9                            | 1.96                                       |
| 38-H     | 58.9 | 6.56**      | 1.59                  | 0.218                   | 27.4                           | 1.35                                       |
| 40-H     | 66.3 | 13.90**     | 0.87                  | -0.135                  | 19.1                           | 0.82                                       |
| 41-S     | 37.7 | -14.68**    | 1.91                  | 0.252                   | 30.5                           | 1.55                                       |
| 42-S     | 41.3 | -11.11**    | 1.41                  | -0.082                  | 4.4                            | 1.57                                       |
| 44-S     | 38.8 | -13.60**    | 3.23**                | -0.290                  | 23.9                           | 2.83*                                      |
| 45-S     | 37.2 | -15.15**    | 2.56*                 | 0.121                   | 5.3                            | 2.83*                                      |
| 46-H     | 58.1 | 5.69*       | 2.44*                 | -0.136                  | 7.0                            | 2.65*                                      |
| 48-S     | 40.3 | -12.11**    | 5.80**                | 0.257                   | 10.4                           | 6.06**                                     |
| Tonacja-S | 54.3 | 1.97        | 1.23                  | 0.021                   | 0.2                            | 2.60*                                      |

*: $P<0.05$, **: $P<0.01$; #According to Obuchowski et al. (2010): H—hard, S—soft, M—“medium”.

# Genotype main effect was calculated as a difference between each genotype GH and overall mean across environments.

### 3. Starch content

The general mean of starch content amounted to 66.8%. Comparison of the means for hard, medium and soft genotypes revealed that hard genotypes were characterized by lower SC (66.1%) than medium and soft (67.4 and 67.2%, respectively), but these differences were statistically non-significant. Main effects were markedly important for 15 genotypes; in the group of hard grain genotypes prevailed negative effects and of soft grain – positive (Table 5).

Among 24 genotypes studied, 7 revealed significant GEI; 4 were of soft, 2 hard and 1 medium grain. In 4 breeding lines, GEI could be explained by regression of interaction effects on environmental main effects (significant regression coefficients for lines no. 7, 19, 35 and 45); in these lines determination coefficients were relatively high and ranged from 55.0 (line no. 19) to 64.0% (line no. 35) (Table 5).
Table 5. Mean values over environments and summarised results of GEI analyses of studied wheat breeding lines for protein, starch and wet gluten content, Zeleny sedimentation value, alveograph W parameter and hectoliter weight.

| Group of genotypes       | Mean (range) | Significant^1 main effect−line no. | Significant^1 GEI−line no. | Significant^1 regression coefficient−line no. | Significant^1 F-statistic for deviations from regression−line no. |
|--------------------------|--------------|-----------------------------------|---------------------------|---------------------------------------------|---------------------------------------------------------------|
|                          |              | positive                          | negative                  |                                             |                                                               |
|                          |              |                                   |                           |                                             |                                                               |
| **Protein content (%)**  |              |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 13.4 (12.4−14.7) | 29, 30, 40                         | 1, 9, 20                  | 35, 40, 46                                  | 35, 40                                                        |
| “Medium”-grained^3 lines| 13.2 (13.1−13.3) |                                     |                           |                                             |                                                               |
| Soft-grained lines^4     | 13.0 (12.5−13.5) | 44, 48, T                          | 7, 17, 48                 |                                             | 17, 7, 48                                                      |
| **Starch content (%)**   |              |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 66.1 (64.5−67.9) | 20                                 | 1, 9, 29, 30, 38          | 35, 46                                      | 35, 46                                                        |
| “Medium”-grained^3 lines| 67.4 (66.6−67.9) | 8, 13, 19                          |                           | 19                                          | 19                                                            |
| Soft-grained lines^4     | 67.2 (66.2−68.0) | 2, 44, 45, 48, T                   | 7, 44, 45, 48, T          |                                             | 7, 45, 44, 48                                                 |
| **Wet gluten (%)**       |              |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 27.7 (25.5−31.2) | 29, 30, 36, 40                     | 1, 9, 20                  | 20, 35                                      | 20, 35                                                        |
| “Medium”-grained^3 lines| 27.4 (27.1−27.6) |                                     |                           | 19                                          | 19                                                            |
| Soft-grained lines^4     | 26.3 (24.6−27.7) | 41, 42, 44, 48                     | 7, 48                     |                                             | 7, 48, 48                                                     |
| **Zeleny sedimentation value (mL)** |          |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 44.1 (35.5−53.6) | 29, 30, 36, 40                     | 20, 30, 35, 40, 46        | 20, 30, 35, 40, 46                          | 20, 30, 35, 40, 46                                            |
| “Medium”-grained^3 lines| 40.6 (38.7−41.6) | 8                                  |                           | 19                                          | 19                                                            |
| Soft-grained lines^4     | 37.9 (34.1−41.7) | 17, 41, 42, 44, 45, 48, T          | 7, 48                     |                                             | 7, 48, 48                                                     |
| **Alveograph W parameter (J)** |          |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 252.5 (216.8−301.7) | 29, 30, 35, 36, 40                | 20, 38, 46                | 20, 30, 35, 40, 46                          | 20, 30, 35, 40, 46                                            |
| “Medium”-grained^3 lines| 245.2 (238.2−255.4) | 13                                |                           | 8                                           | 8                                                            |
| Soft-grained lines^4     | 227.4 (213.4−252.3) | 17, 41, 42, 44, 45, 48, T          | 2, 7, 48                  |                                             | 2, 7, 48                                                      |
| **Hectoliter weight (kg)** |            |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 76.1 (73.0−78.9) | 20, 31, 40                         | 9, 29, 38                 | 1, 46                                       | 46                                                            |
| “Medium”-grained^3 lines| 77.0 (73.5−79.1) | 8, 13, 19                          | 11                         | 19                                          | 19                                                            |
| Soft-grained lines^4     | 75.2 (72.5−78.4) | 2, T                               | 7, 41, 42, 44, 45         |                                             | 44, 44                                                        |
| **Grain yield per plot (kg)** |          |                                   |                           |                                             |                                                               |
| Hard-grained lines^2     | 4.56 (4.06−5.11) | 9, 20                              | 29, 30                    | 35, 40, 46                                  | 35, 40, 46                                                    |
| “Medium”-grained^3 lines| 4.85 (4.65−5.01) | 13, 19                             |                           | 19                                          | 19                                                            |
| Soft-grained lines^4     | 4.64 (4.46−4.85) | 17, 42                             |                           | 7, 44                                       | 7, 44                                                        |

^1 at \( P=0.05 \).
^2 Hard-grained lines, no. 1, 9, 20, 29, 30, 31, 35, 36, 38, 40, 46.
^3 “Medium”-grained lines, no. 8, 11, 13, 19.
^4 Soft-grained lines, no. 2, 7, 17, 41, 42, 44, 45, 48, Tonacja (T).
4. **Wet gluten content**

Wet gluten obtained from the grain of studied genotypes ranged between 24.6 and 31.2% (Table 5). Mean values for hard, soft and medium lines were similar and equal to 27.7, 27.4 and 26.3%, respectively. Differences between group of hard, soft and medium grain were non-significant. Main effects were significant in 11 cases, from which 7 (3 negative and 4 positive) concerned hard and 4 (all negative) soft lines. F-statistic for GEI appeared to be significant ($P<0.05$) for 4 genotypes, but only for one line (no.35) GEI could be described by regression ($b=0.550$, $P<0.01$, coefficient of determination 72.2%).

5. **Zeleny sedimentation value**

Zeleny sedimentation values varied on a large scale: from 34.1 (line no. 48) to 53.6 mL (line no. 30) (Table 5). Genotypes with hard grain were characterized by significantly higher ZS values (44.1 mL on an average) than medium (40.6 mL) and soft (37.9 mL) lines. Out of 24 studied lines, 12 had significant main effects, of which only 4 were positive and all of them were observed for hard genotypes.

GEI was important for 8 studied genotypes among which hard grain lines prevailed (5 out of 8), but reaction of unstable lines on different environmental conditions could not be explained by regression - in all cases only deviations from regression were significant (Table 5).

6. **Alveograph W parameter**

The mean values of alveograph W parameter for particular genotypes ranged from 213.4 (line no. 48) to 301.7 J (line no. 30) (Table 5). Generally, mean AWP values for hard and medium lines (252.5 and 245.2 J, respectively) were significantly higher than that for soft lines (227.4 J). Within the group of hard grain lines, a wide range of AWP was observed: from 216.8 to 301.7 J, while in soft grain group this parameter ranged on a narrow scale - from 213.4 to 252.3 J. Main effects were significant for 15 studied lines and positive values were observed mainly for hard grain lines (5 out of 6).

Among the genotypes studied, 8 appeared to be unstable (F-statistic for GEI significant at least at $P=0.05$), but regression coefficient was significant only for line no. 35 ($b=0.432$, $P<0.05$). Reaction of that line on various environmental conditions may be expected as an intensive, whereas the reaction of the rest of unstable lines on environments is unpredictable, because for these lines only deviations from regression were significant (Table 5).

7. **Hectoliter weight**

Variation of studied genotypes in hectoliter grain weight was relatively narrow: mean value of HW was equal to 75.9 kg and for particular genotypes ranged from 72.3 (line no. 44) to 79.1 kg (line no. 13) (Table 5). Breeding lines of soft grains were observed to have somewhat lower values of HW (75.2 kg) in comparison to lines of hard (76.1 kg) and medium grain (77.0 kg); however the differences between these groups of lines were non-significant. Main effects were statistically important for 17 lines with a similar proportion of positive and negative values. The greater part of lines was stable over environments; only for 4 lines, F-statistic for GEI was significant (no. 1, 19, 44, 46). Unstable genotypes did not have a clear tendency in their response to environmental conditions because only for line no. 44 regression coefficient ($b=0.673$, $P<0.05$) was significant with the coefficient of determination equal to 63.5%. (Table 5). Positive regression coefficient for this line indicates its intensive reaction on various environments.

8. **Grain yield**

The lines were characterized by relatively broad variation in grain yield. Mean value was equal to 4.64 kg and for particular breeding lines ranged from 4.06 (line no. 30) to 5.11 kg per plot (line no. 20) (Table 5). Comparison of means in hard, medium and soft genotypes revealed that hard genotypes were characterized by significant lower grain yield (4.56 kg) than medium lines (4.85 kg); non-significant difference (0.08 kg) was revealed between hard and soft genotypes. Positive main effects were markedly important for two hard (no. 9 and 20), two soft (no. 17

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**Table 6. Correlation coefficients between quality parameters, hectoliter weight and grain yield**

| Trait                  | Protein content | Starch content | Wet gluten | Zeleny sediment. value | Alveograph W parameter | Hectol. weight | Grain yield |
|------------------------|-----------------|----------------|------------|------------------------|------------------------|----------------|-------------|
| Grain hardness         | 0.508**         | −0.525**       | 0.556**    | 0.676**                | 0.605**                | 0.390**        | −0.155      |
| Protein content        | −0.620**        | 0.061**        | 0.932**    | −0.455**               | 0.459**                | 0.567**        | −0.037      |
| Starch content         | 0.695**         | 0.746**        | 0.695**    | 0.173                  | −0.433**               | 0.746**        | 0.051       |
| Wet gluten             | 0.921**         | 0.746**        | 0.173      | 0.471**                |                         |                |             |
| Zeleny sediment. value | 0.746**         | 0.051          | 0.471**    |                         | 0.471**                |                |             |
| Alveograph W parameter | 0.053           |                |            | 0.471**                |                        |                |             |
| Hectol. weight         |                 |                |            |                        | 0.471**                |                |             |

*: $P<0.05$, **: $P<0.01$
and 42) and two medium (no. 13 and 19) lines. GEI was significant in six lines, among which three were hard- (no. 20, 35, 40) and two soft- (no. 7, 44) and one medium-grained (no. 19). The response of unstable lines to different environmental conditions could not be explained by regression—in all cases deviations from regression were significant (Table 5).

9. Correlation between yield and quality parameters

Correlations among grain hardness, quality parameters and grain yield are summarized in Table 6. Grain hardness showed a significantly positive correlation with all the studied quality properties, but a negative correlation with starch content and non-significant with grain yield. Strong correlations were observed between quality parameters, i.e., protein content, wet gluten, sedimentation value and alveograph W parameter. Starch content was negatively associated with wet gluten, sedimentation value and W parameter, and positively with hectoliter weight and grain yield. Correlation between hectoliter weight and quality parameters was generally low and non-significant. Correlation coefficients between yield and protein content, wet gluten, sedimentation value and alveograph W parameter were significantly negative.

Discussion

In this study, the response of wheat breeding lines with different grain hardness to various environmental conditions was examined in terms of technological characteristics measured by NIR. In the present study, breeding lines were classified into hard-, soft- and medium-grains based on physical analyses (Obuchowski et al., 2010). The results of molecular analysis performed for identification of \( Pina-D1 \) and \( Pinb-D1 \) alleles were consistent with those obtained by physical methods only in soft-grained lines. In medium- and hard-grained lines, some genotypes with the same set of \( Pina-D1 \) and \( Pinb-D1 \) alleles as in soft-grained lines were identified. This indicates that grain hardness of those lines was a result of genetic factors other than \( Pina-D1 \) and \( Pinb-D1 \). That is in agreement with the findings that locus \( Ha \) alone does not explain all the variation in grain hardness. Several studies have indicated that other regions of wheat genome are also responsible for grain hardness (Galant et al., 2001; Martin et al., 2001; Igrejas et al., 2002; Arbelbide and Bernardo, 2006; Narasimhamoorothy et al., 2006; Tsilo et al., 2011).

Among the seven traits analyzed, the most stable trait in all environments was grain hardness, whereas the most affected by environment were protein and gluten content and Zeleny sedimentation value (Table 3). Analysis of grain hardness by NIR gave results consistent with those obtained previously by physical methods, i.e., by determination of torque (MS), wheat hardness index (WHI) and particle size index (PSI). Significant correlation was observed between GH and MS, and between WHI and PSI (Obuchowski et al., 2010). Breeding lines classified in those studies as hard or soft, were characterized, respectively, by high or low values of GH, respectively, in the present study. However, lines no. 8, 11, 13 and 19, for which earlier classification was not consistent, for example line no. 8 on the basis of torque was distinguished as hard and on the basis of PSI as soft (Obuchowski et al., 2010). In the present study, lines no. 8, 11, 13 and 19 were treated as “medium”-grained lines, although a relatively high value of GH, similar to values for hard-grained genotypes, indicates that these lines tend to have a hard grain. It should be noticed that in the studies of Obuchowski et al. (2010) grain samples originated from experiment conducted during season 2006/2007 under different environmental conditions, and nevertheless results of hardness estimation were similar.

Protein content, wet gluten content and Zeleny sedimentation value were greatly influenced by environment, although they were also significantly influenced by genotype and GEI (Table 3). Generally, the higher dose of nitrogen fertilization caused larger values of these traits in all locations. The most favorable for the expression of these quality characters appeared to be environment Ch2, in which N fertilization was applied to medium-fertility soil. In addition, at that location the highest sum of rainfall was observed during spring and summer 2009. In contrast, the lowest protein content, wet gluten and Zeleny sedimentation value were noted in environment L1 (light soil and standard N fertilization), in which a small amount of precipitation occurred during the vegetation period. Similarly, many other studies demonstrated that environmental conditions have a larger effect on protein content than the genotype (e.g. Baenziger et al., 1985; Uhlen et al., 1998; Rharrabti et al., 2001; Mut et al., 2010). Protein quality, measured by alveograph W parameter, appeared to be influenced in a similar degree by genotype and environment. In the present study, the importance of GEI varied among the analyzed quality parameters; GEI variation, although significant, was several times lower than that of genotype and environment and the lowest in relation to genotype was for grain hardness and hectoliter weight but in relation to environment for wet gluten content and also hectoliter weight (Table 3). Small effect of GEI on sedimentation value (SDS) and other quality characteristics was revealed among others by Lukow and McVetty (1991), Boggini et al. (1997), Peterson et al. (1998), Ames et al. (1999), and Budak et al. (2003). The results obtained in our experiments conducted in eight environments were similar to the results presented by Peterson et al. (1998), which are based on experiments performed in replicated trials at 10 locations and two years (totally in 17 environments).

Since GEI variation can make selection of valuable
genotypes difficult, information on the response of particular lines to different environmental conditions is of value for breeders. In these studies, GEI was significant for all the analyzed traits but number of unstable lines, i.e., lines for which F statistic for GEI was significant at $P=0.05$, was relatively low and ranged from 4 out of 24 genotypes examined for wet gluten content and hectoliter weight to 6-8 for the remaining traits (Table 5). Among unstable lines four lines, no. 7 (soft), 35 (hard), 46 (hard) and 48 (soft), appeared to be unstable in the case at least five out of seven analyzed traits; lines no. 7, 35 and 48 were unstable for all the traits with an exception for hectoliter weight, whereas hard line no. 46 was unstable regarding grain hardness, protein and starch content, alveograph W parameter and hectoliter weight (Tables 4, 5). Unfortunately, the response of lines no. 7, 46 and 48 to environmental conditions was not simple and cannot be explained by linear regression. This means that the response of these lines to various environments is unpredictable. Compared to these lines, line no. 35 showed a significantly positive regression coefficient in protein, starch and gluten content and alveograph W parameter (Table 5), which indicates that this line is intensive, i.e., more responsive in favorable conditions.

The small number of unstable lines detected in the presented studies may also be caused by the few types of environments and the short period of the experiment (one year). However, based on such trials, breeders have chosen the best lines to develop as cultivars. Williams et al. (2008) showed that experiments assessing wheat quality characteristics were most often conducted in two years (above 35%) or in one year (above 15%); multi-year trials are rare because they are costly and time-consuming.

It would be interesting for breeders to distinguish the location(s), in which the differences between genotypes are clearly manifested. In the present study, environment D2 was from an agronomical point of view the richest because of the good physical structure, fertility of soil (mud soil) and high level of nitrogen fertilization applied. Participation of that environment in GEI was very small (Table 2) which means that the response of all the studied genotypes on environmental conditions at this location was similar. Moreover, the mean values of quality parameters were not the highest in that environment. This was probably caused by drought occurring at Dębina during the spring of 2009. The most favorable environment for grain quality was Ch2 (light brown soil, increased dose of N fertilization), and its participation in GEI was relatively high, which means that the response of all the genotypes studied on environmental conditions at this location was differentiated. This suggests that this environment could be distinguished as the most conducive for the selection of bread genotypes in early generations, when grain samples are too small for multilocation trials.

We evaluated the relationships between grain hardness, quality parameters and grain yield, and found a significant correlation between hardness, protein content, Zeleny sedimentation value and alveograph W parameter. Similar relationships have been reported by Martin et al. (2001), Hruškova and Famera (2003), and Jirs et al. (2008). In present study, a negative correlation was found between grain yield and quality characteristics; this confirms the association between those traits (see, e.g., Keijer et al. 2007, Oury and Godin 2007, Bordes et al. 2008).

The negative relationship between yield and quality parameters (Table 6) was the reason why no line with good quality was chosen for the next step of breeding experiments. Breeding lines were selected mainly on the basis of grain yield. Out of 23 breeding lines evaluated in the present study, only two lines were subjected to pre-official trials: no. 2 and 17, both soft-grained. Line no. 2 was characterized by positive, although insignificant, main effect for grain yield and resistance to diseases (data not shown), and line no. 17 by high and stable yield. Interesting was also medium-grained and high-yielding line no. 19, but it was not selected for further experiments, because its yield was unstable over environments and the reaction of this line on environmental conditions was unpredictable.

Conclusions

The present study showed that grain hardness is determined mainly by wheat genotype, whereas the influence of environment is of less importance. In protein content, wet gluten and Zeleny sedimentation value, environment prevailed the influence of genotype, and for starch content, alveograph W parameter and hectoliter weight both sources of variation have similar importance. Genotype-environment interaction was of smaller size relative to genotype and environment in terms of all the studied quality parameters. Majority of advanced breeding lines had stable quality parameters across environments. The response of unstable genotypes to different environments was in major cases, nonlinear and unpredictable. Negative association between high yield and good quality should be an important target for the next breeding efforts.

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