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Grain hardness of wheat bred in Poland and its relationship to starch damage during milling**

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A b s t r a c t. Four different methods were used to evaluate grain hardness of wheat cultivars and advanced breeding lines bred in Poland. In addition, for genotypes that were highly differentiated in grain hardness the level of starch damage and puroindoline content were analysed. Some differences in results of grain hardness testing indicate that the methods are based on various principles and evaluate different features of grain. It was found that wheat genotypes bred in Poland do not vary considerably in grain hardness and puroindoline content. Regardless of grain hardness, grain of studied wheat genotypes milled under standard conditions yield flour with very similar starch damage levels. It was found that it is easier to change the starch damage degree by changing milling conditions instead of selecting a specific wheat variety.

K e y w o r d s: winter wheat, puroindolines, grain hardness indices, starch damage

INTRODUCTION

Hardness of wheat grain is known to have a significant influence on end-use quality, especially milling and baking traits (Gross *et al*., 2004; Kuchel *et al*., 2006; Obuchowski, 1984). Grain hardness is not a clearly defined characteristic. The complexity of anatomy, structure and mechanical properties of individual parts of grain as well as the different principles on which hardness testing is based result in a considerable variation in obtained results (Gąsiorowski and Poliszko, 1977; Greenwell and Schofield, 1986; Morris and Massa, 2003). A consequence of this fact is the variation in the degree to which hardness testing results are linked with specific indexes of technological quality of wheat grain. However, it is commonly accepted that grain hardness, irrespective of the method applied in its determination, has a significant effect on its technological properties, especially manifested in easy sifting and middlings or semolina sizing, the degree of starch damage during milling, water intake of produced dough, susceptibility to amylolytic enzymes, improved fermentability and, as a consequence, better baking properties of flour and quality of bread (Autron *et al*., 1997; Ceglińska *et al.,* 2007). Recent studies indicate that wheat grain hardness is controlled by the *Ha* locus located at the short arm of chromosome 5D (Appels *et al*., 2001, Wrigley 2002a, 2002b). Two genes, *Pina-D1* and *Pinb-D1*, coding puroindoline a and puroindoline b, respectively, have been found to be closely linked to *Ha* locus (Morris, 2002; Turner *et al*., 2004). Pin-a and Pin-b are dominant components of friabilins, a group of polypeptides of 13-15 kDa, which are found in much bigger amounts on the surface of starch granules of soft grain wheats in comparison to hard grain wheats. Cultivars with soft grains have allele *a* of the wild type *(Pina-D1a)* and they accumulate both puroindolines on the surface of starch granules during endosperm development. On the other hand, medium-hard grain and hard grain cultivars have mutated alleles at *Pinb-D1* and they either have reduced amounts or contain no *Pin-b* on starch granules, whereas most super hard wheats have no Pin-a due to the presence of a null allele at *Pina-D1*. The effect of puroindolines on the structure of bread crumb and rheological properties of dough are ascribed to their affinity to fats (Arbelbide and Bernardo 2006; Autron *et al*., 1997; Bettge *et al*., 1995; Giroux *et al*., 2000).

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Physico-mechanical properties of grain are frequently included in the quality classification of commercial wheat *eg* in the USA wheat grain classification terms Hard Dark Spring Wheat or Soft Wheat are applied (Crop Quality Report 2007, 2008), while in Australia the utilization of wheat is based on two factors *ie* total protein content and grain hardness.

Grain hardness is related not only with its easy milling during the technological process, but also with particle size of obtained products and the degree of starch damage. This is crucial, as the percentage of starch damage in flour produced under standard conditions is one of the attributes characterising wheat grain quality (Crop Quality Report 2007, 2008). Are these properties of wheat grain so essential also when characterising processability of wheat grown in Poland?

The aim of the study was to estimate cultivars and advanced breeding lines bred in Poland in terms of grain hardness measured by different physical methods.

MATERIAL AND METHODS

Grain hardness of wheat and its technological parameters were tested on 50 winter wheat cultivars and advanced breeding lines from the experiment carried out in 2007 at Choryń (DANKO Plant Breeding Ltd.). Two levels of nitrogen fertilization were applied: 90 and 140 kg ha^{-1}. Due to the fact that a considerable percentage of studied genotypes at present are being tested in breeding trials, we decided not to give their code names, but rather describe them with successive numbers.

The grain hardness at uniform moisture content adjusted to 13.5% was tested using several methods:

- in a single-step Brabender hardness testing machine torque (MS) was determined, corresponding to the maximum peak of graph in BU, as well as WHI according to Greenaway (1969);
- using the Quadrumat-Junior mill and a ZBPP sifting machine fitted with 105 μm opening sieves particle size index (PSI) values were determined according to Stenvert (1974) with modification of Obuchowski and Bushuk (1980);
- using the Near Infrared Transmittance (NIT) technique (Infratec^{TM} 1241 Grain Analyzer, FOSS, Denmark) the relative degree of hardness was evaluated;
- starch damage (in %) occurring under standard laboratory milling conditions in a Quadrumat Junior mill was determined in Chopin SD-Matic apparatus, according to the AACC 76-30A method.

All measurements were done in two replications.

Variation in the qualitative and quantitative composition of puroindoline proteins was determined in wheat grains using reverse phase high performance liquid chromatography (RP-HPLC). Proteins from ground grain produced after kernel milling in a Mill 3100 Perten Instruments grinding mill were extracted with a tris buffer containing 2% (w/v) Triton X-114 in accordance with the procedure most commonly described in literature (AACC, 2000). Proteins from the detergent-rich phase were precipitated with a triple volume of acetone at -20°C for 16 h. Prior to chromatographic separations the isolated puroindoline proteins were dissolved in a 40% mixture of acetonitrile and water containing 0.1% of trifluoroacetic acid.

RP-HPLC analyses were performed in a Gold Beckman System testing system (two pumps Model 126, a diode detector Model 168). Chromatographic data were analysed using Gold System software ver. 8.1. HMW-GS were separated at a Nucleosil C18 column (4.6 x 250 mm, 5 μm packing diameter) coupled with a Widepore C18 precolumn (4 x 3 mm). Proteins from the column were eluted in a series of solvents: A - water containing 0.1 TFA, B - 95% acetonitrile containing 0.08 TFA, maintaining a linear gradient of successively: 0-24% B for 6 min, 24-60% B for 40 min. Solvent flow rate was 1 ml min^{-1}. Eluent was monitored simultaneously at 214 and 280 nm. The presence of two main protein peaks with retention times of 18.62 and 24.16 min was detected on chromatographs, corresponding to puroindolines PIN-a and PIN-b (*Pina-D1a* and *Pinb-D1b*) obtained for the reference samples from cv. Newana. Quantitative composition of both puroindolines was determined based on the size of areas of individual protein peaks.

RESULTS AND DISCUSSION

The results of grain hardness determinations for analysed wheat cultivars and breeding lines are given in Table 1. No marked differences were found between general means of grain hardness indices measured for the two levels of nitrogen fertilization. Coefficients of variability indicate that the studied genotypes were the most differentiated in terms of WHI index (CV amounted to 34.86 and 24.53% for two levels of N). Relatively high variability was recorded also for NIT (22.76 and 16.95% for 90 and 140 level of N, respectively). The lowest variation (CV below 10%) was found for PSI. It may be seen from Table 1 that, generally, coefficients of variability were lower for grain samples of wheat grown at the higher level of nitrogen fertilization. The values of analyzed indices are, for most samples, very similar and indicate that the studied material does not vary considerably. The only exceptions were two samples, number 41 and 45 (and potentially 42 - grain with the lower fertilization level), which are characterized by markedly softer grain in relation to the other samples. Data presented in Tables 1 and 2 indicate also that the classification of wheat grain hardness based on different methods, although significantly correlated (Table 2), gives different, not always comparable results. For example, samples Nos 3 and 8, classified on the basis of torque as hard grain, were classified as soft grain on the basis of PSI.

The level of nitrogen fertilization has also a varied effect on the characteristic of grain hardness. In the case of several genotypes, the increase of fertilization rate from 90 to 140 kg ha^{-1}

Table 1. Grain hardness of studied wheat genotypes grown at two levels of nitrogen fertilization: I – 90, II - 140 kg ha^{-1} (H – hard grain, M – medium-hard grain, S – soft grain)

Genotype	NIT		MS (BU)		WHI (BU)		PSI (%)		Grain type
	I	II	I	II	I	II	I	II	
1	69.8	65.9	465	445	41.9	39.4	47.7	45.4	H
2	31.3	54.1	385	385	26.2	31.8	52.2	52.7	S
3	73.3	59.7	450	475	32.1	39.5	55.0	53.3	S
4	59.9	60.0	355	410	29.6	31.5	50.4	50.5	M
5	44.7	58.1	485	500	40.7	37.6	51.0	52.5	M
6	35.4	49.8	430	510	35.2	40.8	49.8	50.5	M
7	33.9	52.0	355	385	21.9	23.0	58.4	55.2	S
8	68.4	61.5	460	455	27.1	27.9	50.3	52.2	S
9	40.6	51.7	450	505	42.0	47.6	49.9	48.3	H
10	34.7	62.0	395	440	30.9	37.9	48.1	49.1	M
11	55.3	53.8	450	400	28.7	36.5	48.7	49.0	M
12	54.0	54.4	430	445	32.1	36.6	51.4	51.8	S
13	54.2	55.5	385	415	26.4	32.9	47.7	49.3	M
14	45.5	43.5	405	385	30.7	32.2	49.6	50.4	S
15	51.7	48.6	425	420	41.7	33.2	49.1	49.0	M
16	60.5	57.7	430	465	34.7	37.2	49.7	50.0	M
17	42.5	48.9	390	380	27.9	28.4	52.0	52.7	S
18	60.0	48.2	400	370	33.6	26.7	48.9	50.0	M
19	49.6	44.2	485	450	30.3	29.1	46.9	48.8	M
20	55.3	58.5	470	475	35.1	40.9	45.8	45.6	H
21	60.5	60.6	435	475	39.5	38.0	50.0	51.4	M
22	57.6	43.2	490	465	34.0	33.6	51.9	52.6	M
23	40.5	33.6	440	355	35.2	29.8	48.5	47.8	M
24	48.1	53.6	375	430	29.3	35.2	48.3	49.4	M
25	59.8	57.4	430	405	40.2	37.7	48.4	47.9	M
26	52.6	55.1	440	455	40.4	44.2	51.5	51.9	M
27	62.2	53.6	440	420	29.7	27.7	52.3	53.6	M
28	51.6	45.7	450	415	34.3	29.3	49.2	51.6	M
29	65.6	63.7	520	490	47.3	41.2	45.2	46.4	H
30	58.5	59.2	480	430	35.8	33.9	44.6	47.4	H
31	51.0	56.8	450	445	36.9	38.9	43.3	47.9	H
32	42.9	42.0	395	450	31.6	40.2	48.9	49.8	M
33	60.7	58.2	395	475	33.8	39.3	43.7	46.3	M
34	50.8	51.8	450	455	42.4	47.1	48.6	49.9	M
35	67.2	69.1	495	495	52.7	52.9	46.1	45.2	H
36	64.1	64.0	510	510	50.0	46.8	46.7	47.3	H
37	62.7	57.4	445	445	30.3	34.0	47.9	48.3	M
38	64.9	64.3	590	550	57.3	51.9	42.3	42.8	H

T a b l e 1. Continuation

Genotype	NIT		MS (BU)		WHI (BU)		PSI (%)		Grain type
	I	II	I	II	I	II	I	II	
39	59.1	62.6	445	395	35.0	30.4	47.2	48.2	H
40	68.8	66.5	450	465	37.5	44.1	40.9	41.3	H
41	34.2	40.5	320	375	16.0	20.4	54.5	54.4	S
42	18.8	34.5	320	380	21.0	26.4	54.7	52	S
43	61.7	61.6	470	405	37.0	30.1	46.6	48.9	M
44	43.1	47.4	375	375	21.1	31.8	50.7	50.1	S
45	28.4	33.1	350	360	11.6	12.1	60.0	59.9	S
46	61.9	67.6	505	490	42.1	40.3	49.0	48.2	H
47	52.9	48.1	485	470	38.8	38.5	50.6	51.8	M
48	46.4	35.4	395	365	28.6	26.4	52.5	52.9	S
49	58.1	57.7	490	500	37.7	38.8	49.8	48.4	M
50	54.1	47.7	400	420	25.2	24.6	51.0	53.7	S
Mean ± SD	52.6± 11.97	53.6± 9.09	435± 53.0	437± 47.0	33.6± 11.70	31.7± 7.77	52.57± 3.78	50.0± 1.22	
Range	50.00	34.50	270	195	45.70	40.80	19.10	18.60	
CV (%)	22.76	16.95	12.18	10.76	34.86	24.53	7.19	2.45	

caused a slight increase in indexes indicating grain hardness; however, in most cases this effect was negligible, but there were also cases when grain hardness decreased.

The puroindoline proteins content were determined for 14 soft wheat genotypes distinguished on the basis of physico-mechanical tests (Table 1) as well as 12 genotypes classified as hard wheat. Table 3 presents means as well as maximum and minimum values determined on the basis of the size of peak areas for both puroindolines assayed by RP-HPLC. It results from chromatographic data presented in Table 3 show that both soft and hard wheats bred in Poland contain both puroindolines PIN-a and PIN-b. The contents of these proteins in wheat grains are determined primarily by genotype. On average, soft wheats contain more PIN-a proteins than hard wheats do. Significant correlation was found between the amount of both puroindolines and PSI values (Table 3).

The level of starch damage during milling of grain under standard conditions in a laboratory mill is shown in Table 4. Comparisons were made on selected wheat grain samples, representing extreme hardness values – from soft grain (samples Nos 41 and 42) to hard grain, as *eg* samples Nos 38 or 46. Starch damage in relation to all analysed samples was very similar and ranged from 6.9% for soft grain to 8.0% for samples of harder grain. However, flour from grain characterized by higher hardness not always had a higher starch damage degree (Tables 1 and 4).

T a b l e 2. Correlation coefficients between grain hardness indices (n=100)

Indices	MS	WHI	PSI
NIT	0.619**	0.668**	-0.402**
MS	-	0.912**	-0.443**
WHI	-	-	-0.667**

** P<0.01.

T a b l e 3. Puroindoline protein content in soft and hard grain of wheat genotypes (% d.m.) and correlation coefficient (r) between puroindoline content and PSI values

Puro-indolines	Mean ± SD	Min	Max	r
Soft grain genotypes (n=14)				
PIN-a	0.046 ± 0.006	0.034	0.069	0.648**
PIN-b	0.018 ± 0.004	0.008	0.029	0.860**
Hard grain genotypes (n=12)				
PIN-a	0.038 ± 0.005	0.028	0.052	0.624*
PIN-b	0.015 ± 0.004	0.004	0.026	0.883**

*P<0.05, **P<0.01.

The results concerning starch damage are higher than those for soft American wheats (Soft Red Winter), where these values fell within the 4.0-4.8% range; however, they are comparable to values recorded for Hard Red Spring wheat (starch damage range of 6.0 to 8.3%) (Crop Quality Report, 2007). It needs to be assumed that this is not so much a consequence of varietal traits, but rather of different milling conditions, as American wheats were milled in a MLU-202 Buhler laboratory mill, while Polish wheats in a Quadrumat Junior mill. This assumption was confirmed by the results of a study by Górniak (2006), who showed a close dependence between particle size of flour and contents of damaged starch. However, such a low variation in starch damage found in Polish wheats indicates that in terms of their mechanical properties they constitute very similar material, possibly being a consequence of selection resulting from breeding works aimed at the generation of superior wheats solely in terms of their processability (higher protein content, grain vitreosity), resulting most frequently in the elimination of soft wheat cultivars, considered inferior in terms of their technological value.

T a b l e 4. Starch damage of flour (AACC method) from grain samples of wheat grown at two levels of nitrogen fertilization: I – 90, II – 140 kg ha^{-1}

Genotype No.	Grain type	Starch damage (%)	
		I	II
1	H	7.7	7.7
2	S	7.2	7.3
3	S	7.4	7.4
7	S	7.4	6.9
8	S	7.6	7.3
10	M	8.0	nd
11	M	7.3	nd
13	M	7.0	7.4
19	M	7.6	7.5
21	M	7.2	7.3
22	M	7.5	7.5
27	M	7.5	7.9
28	M	7.3	7.1
29	H	7.8	7.7
30	H	7.0	7.0
31	H	7.4	7.3
37	M	7.1	7.4
38	H	7.8	7.7
39	H	7.9	7.6
40	H	7.6	7.4
41	S	7.3	7.0
42	S	7.1	7.3
46	H	7.6	7.8
48	S	7.1	7.3
50	S	7.1	7.0

nd – not determined.

Another factor, in the opinion of the authors of this study resulting in such a low variation in grain hardness and thus starch damage, concerns highly uniform, mild climatic conditions in Poland. This is also connected with a lower standard of agronomy than in the neighbouring countries, manifested in the level of nitrogen fertilization. It also needs to be mentioned that starch damage is not always advantageous – although it results in an increazed water intake of flour, it deteriorates the volume index of produced bread (Ooglińska *et al.*, 2007). In order to verify to what degree the technological process affects the flour starch damage, the degree of starch damage was compared in flours from different stages of milling process obtained from commercial milling of a batch of domestic wheat grain (Table 5). The results of this comparison clearly show that milling conditions had a markedly bigger effect on the degree of starch damage than cultivar selection of domestic wheat.

T a b l e 5. Starch damage and water intake of flour streams obtained from various stages of grain milling in industrial mill

Stage of milling	Starch damage (%)	Water intake (farinograph) (%)
	Breaking Rolls:	
B1	3.8	49.3
B3 flour 1+2	5.5	54.9
B4/B5	8.3	63.9
	Reducing Rolls:	
C1	5.2	54.4
C2/C3, flour 1+2	5.1	56.8
C5/C6	7.1	66.3
C9/C10	8.2	71.7
C11 , flour 1	9.0	70.8
C11, flour 2	8.8	74.1

CONCLUSIONS

1. Common wheat cultivars and breeding lines bred in Poland are characterized by narrow variation in mechanical properties of grain manifested in its hardness – genotypes of soft or medium-hard grain predominated.

2. There is a significant correlation between the amount of both PIN-a and PIN-b puroindolines in wheat grain and particle size index values.

3. The relatively slight variation of grain hardness is reflected in the very slight variation in the degree of starch damage resulting from commodity traits. This suggests that, if necessary, the degree of starch damage in flour may be more readily regulated by altering grain milling conditions than by cultivar breeding.

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