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Composition and End-Use Quality of 150 Wheat Lines Selected for the HEALTHGRAIN Diversity Screen

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The HEALTHGRAIN program is focused on developing new healthy food products based on wholegrains of wheat and other cereals, by combining enhanced nutritional quality with good agronomic performance and processing quality. A sample set comprising 130 winter and 20 spring wheat varieties was therefore selected to identify the range of variation in a number of phytochemical and dietary fiber components. These lines were also analyzed for their technological properties (protein and gluten contents, Zeleny sedimentation, bran yield, kernel hardness, etc.), using samples grown on adjacent sites for two successive seasons (2004−2005, 2005−2006). On the basis of the frequency distribution and principal component analysis it was concluded that significant variation for technological quality traits is present in the 150 wheat lines and that it is possible to combine enhanced nutritional quality with good agronomic performance and processing properties.

KEYWORDS: ***T. aestivum*; wheat; HEALTHGRAIN; diversity; quality**

INTRODUCTION

Modern plant breeding has increased the adaptability, productivity, and quality of wheat varieties (*1*). In particular, significant improvements have been made in the breadmaking quality of wheat varieties adapted for growth under European conditions (*2, 3*). Despite these improvements, modern wheat cultivars usually have a restricted geographical range, and consequently breeders tend to exploit adapted rather than exotic germplasm in their programs, restricting the range of variation that may be available.

The HEALTHGRAIN program is supported by the European Union under the sixth Framework Program (see http://www.healthgrain.org) (*4*) and is focused on developing new healthy food products based on wholegrains of wheat and other cereals. Of particular interest are dietary fiber and phytochemical components, and a diversity screen has been carried out to determine the range of variation in these components in 150 wheat lines grown on a single site in 2005 (*5*). These lines were selected to exhibit a wide range of geographical origins and genetic variation, ranging from exotic lines and landraces to modern commercial European cultivars.

It is important that any improvements in the composition of bioactive components should be combined with good agronomic performance and processing quality. Hence, the major types of grain component (starch, protein, and lipids) and a range of quality parameters have been measured on the same material that was used for the analysis of bioactive components, and on a second series of samples grown on a different site within the same experimental farm the following year (2006).

This provides a valuable overview of the range of diversity available to European wheat breeders as well as a unique comparison of a large number of lines grown on a single site. Comparison of these data with the analyses of fiber and phytochemical components demonstrates that it is possible to combine enhanced nutritional quality with good agronomic performance and processing properties (*5*).

MATERIALS AND METHODS

The sample set comprises 130 winter wheats and 20 spring wheat varieties, with full details being provided in Ward et al. (*5*).

The lines were sown in two replicate plots in the field at the Agricultural Research Institute of the Hungarian Academy of Sciences (2004, 2005), Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m). The plots were 2 m long, with six rows spaced at a distance of 20 cm. The soil was of the chernozem type with a loam texture and pH 6.8−7.2. The previous crop was peas. The quantity and distribution of the precipitation was appropriate in both years of the experiment (452 and 409 mm, respectively), although there was a rainy harvest in 2005. The experiments were treated with

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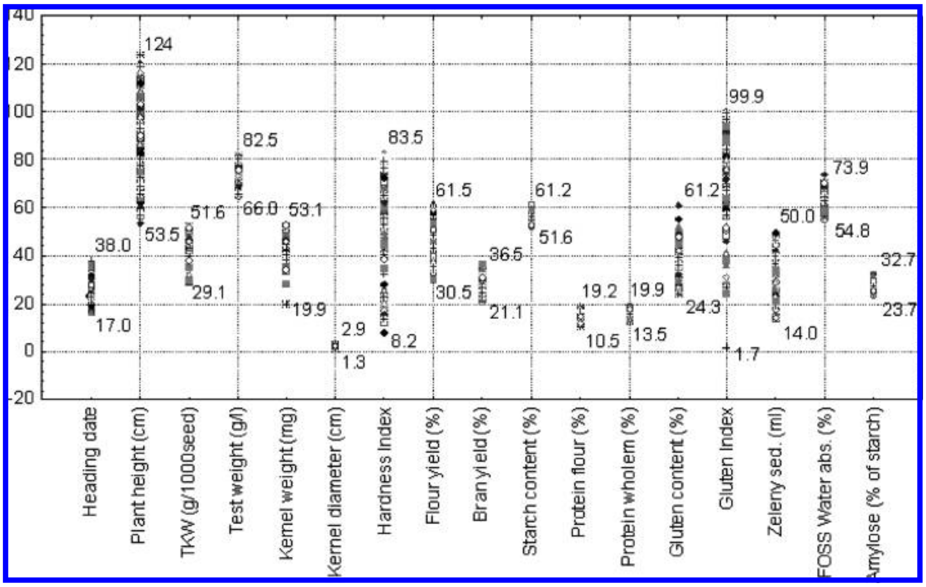

Figure 1. Diversity of some agronomic and technological properties in 150 wheat genotypes. Minimum and maximum values are indicated for each property (Martonvásár, 2005−2006).

herbicide (4 L/ha U-46-M Fluid containing 500 g/L MCPA, 15 g/ha Granstar containing 75% tribenuron methyl), insecticide (0.2 L/ha Karate containing 2.5% λ-cyhalothrin), and fungicide (1 L/ha Eminent containing 125 g/L tetraconazole, 1 L/ha Tango Star containing 84 g/L epoxyconazole and 250 g/L fenpropymorph) each year.

Crop nutrition in Martonvásár was normal. Samples received complex chemical fertilizer during autumn (80 kg/ha 34% N, P, K), and they received ammonium nitrate (60 kg/ha 34% N) during spring.

All grain samples were conditioned to 15.5% moisture content before milling using a Perten Laboratory Mill 3100 (with 0.5 mm sieve) to produce wholemeal or a Chopin CD1 Laboratory Mill to produce white flour. All flour samples were immediately cooled and stored at −20 °C.

Agronomical properties, 1000 kernel weight, kernel hardness, protein, starch, amylose, free sugars, lipid, ash and gluten contents, and Zeleny sedimentation of the varieties were measured in the two years of the field experiment. The following standard methods and instruments were used to determine the functional properties of the lines: test weight, 1000 kernel weight (*6*), Perten SKCS 4100 (*7*), Perten Inframatic 8611 (*8, 9*), FOSS Tecator Infratec 1241, Zeleny sedimentation (*10*), Kjeltec 1035 Analyzer (*11*), Perten Falling Number System 1500 (*12*), and Glutomatic System 2200 (*13, 14*).

Total lipid content was determined according to the method of Marchello et al. (*15*). Free sugars were analyzed according to the method of Bach Knudsen and Li (*16*). Digestible starch (*17*) and amylose contents (*18, 19*) were measured with the procedure of Megazyme (Megazyme, Bray, Ireland). Ash content was determined according to AOAC method 923.03 (*20*).

Statistical analysis was carried out using Microsoft Excel and Statistica 6.0 software.

RESULTS AND DISCUSSION

The lines selected included old and modern varieties, landraces, and breeding lines. Of the 150 lines, 108 lines were chosen from various parts of Europe: 34 from the west, 22 from west central, 27 from the south, 12 from the south continental region, and 13 from the steppe region. Twenty-eight lines were selected from North and South America and 15 lines from Australia, Asia, and the Near East. Twenty of the lines were spring type and 130 lines were winter type.

The diversity of the wheat genotypes is clearly illustrated by the differences in morphology and growth habit that were observed in both years of the field experiment. The heading date differed by 20 days between the earliest (Yumai 34) and the latest (Akteur) varieties. Awns were present in 42% of the varieties, whereas 61% were above 80 cm in height.

No evidence of infection by pathogens (such as powdery mildew and leaf rust) was observed, confirming the effectiveness of the crop protection treatments.

A range of traditional milling and baking quality parameters were measured with the means for the two years being summarized in **Figure 1**. The 1000 kernel weight ranged from 29 to 52.5 g, whereas the bran yield also varied widely, from 20.8 to 36.5%. The latter also showed two peaks at 24 and 28%, as shown in **Figure 2a** (histogram). The 1000 kernel weight correlated with bran yield at 0.001 probability level ($r_{0.001}$ = −0.42, r_{crit} = 0.28, FG = 150).

Twenty-three of the lines had white kernel color, and the bran yield from these showed continuous distribution from about 22 to 30% (**Figure 2a**). The only exception was Yumai 34, in which the bran yield was about 34%. This cultivar has also been shown to contain high levels of soluble dietary fiber, in white flour as well as in wholegrain (*21*).

The importance of the white kernel color lies in the light color of the product produced from wholemeal. Using white bran, it is possible to produce healthy food, rich in dietary fiber, whereas the color of the product remains white, which is preferred by many consumers.

Determination of the hardness index (HI) using the Perten SKCS system (**Figure 2b**) showed that 72% of the lines were hard (HI > 50), 22.7% were soft (HI < 50), and 5.3% were intermediate in texture. However, types with extreme hard and soft textures (cv. Blasco, HI = 83.5; cv. Bilancia, HI = 8) were identified, and it is possible that these could be used to extend the range of variation in texture in breeding programs. Seven of the cultivars with white kernels (Gerek-79, Kirkpinar-79, Sultan-95, Kirac-66, Fredrick, Gene, Augusta) had soft endosperm texture.

Sixty-three percent of the lines had test weights above 78 g/L, the range being 66.0−82.5 g/L.

The protein contents of the wholemeal and white flours ranged from 12.9 to 19.9% and from 10.3−19.0%, respectively, and the wet gluten contents ranged from 24.3 to 61.2% (**Figure 1**). Statistical analysis showed that the protein contents of white and wholemeal flours were highly correlated with each other and with

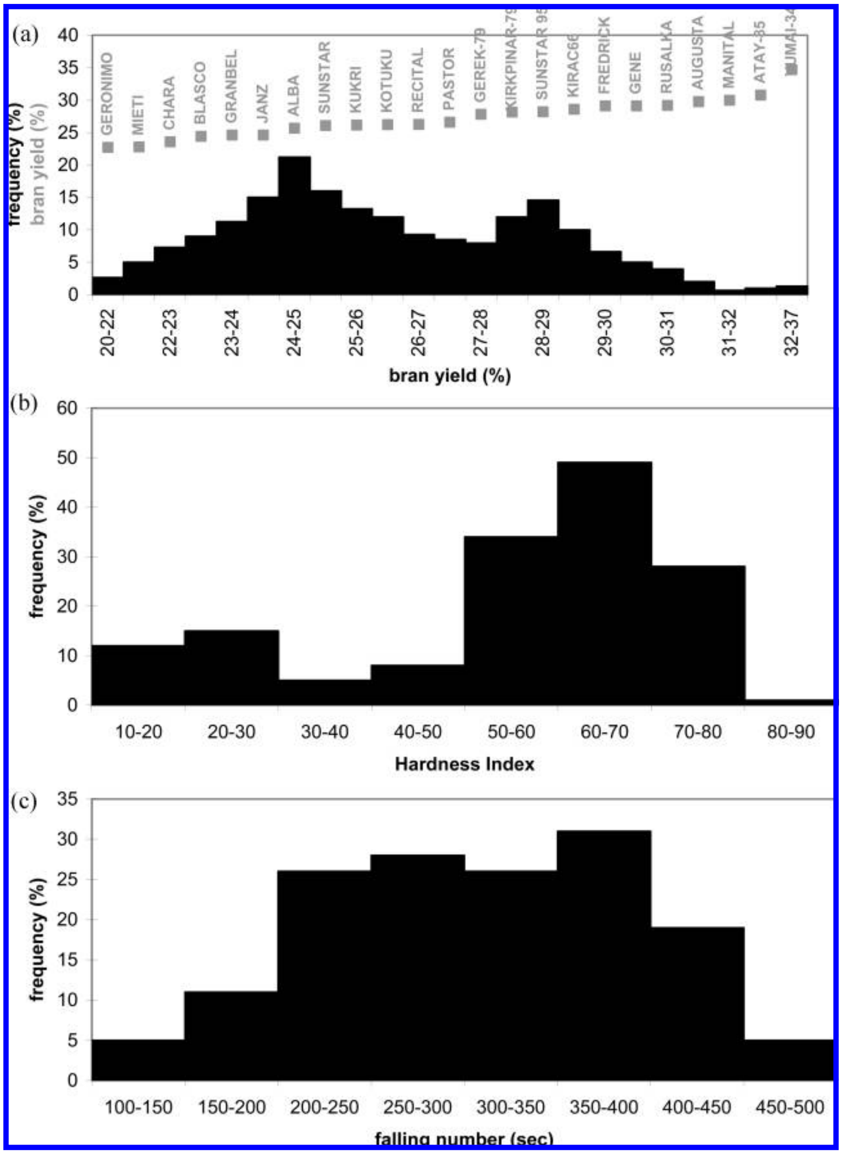

Figure 2. Frequency distribution of kernel characteristics and falling number in the 150 wheat genotypes (Martonvásár 2005−2006): (**a**) bran yield in the 150 genotypes (black) and in 23 wheat genotypes having white kernels (gray); (**b**) grain hardness index (HI); (**c**) falling number.

the gluten content ($r_{0.001\ (\text{protein fl-wm})} = 0.84$, $r_{0.001\ (\text{protein fl-gluten})} = 0.78$, $r_{0.001\ (\text{protein wm-gluten})} = 0.76$, $r_{\text{crit}} = 0.28$, FG = 150). Over half of the lines (55%) had wet gluten contents between 30 and 40%, 27% above 40%, and 9% below 30%.

Total starch ranged from 51.6 to 61.2% when measured by NIR, whereas the digestible starch content ranged from 55.4 to 65.7% and the amylose content of the starch (both determined using Megazyme kits) from 23.7 to 32.2% (**Figure 1**). The lower content of total starch than digestible starch results from the use of NIR for measurement. It is nevertheless valid to compare the total starch and digestible starch contents of the lines. The percentage amylose content was not correlated significantly with either the total or digestible starch contents. The free sugar content ranged from 1.04 to 2.47%, total lipids from 2.04 to 3.55%, and ash (mainly minerals) from 1.49 to 2.05%.

Zeleny sedimentation gives an estimate of the bread volume, with values of 30 mL generally being considered as suitable for breadmaking. The values for the lines varied from 13.5 mL (Sumai 3) to 50 mL (Plainsman-V), with 36% of the lines having values above 35 mL, meaning that they have excellent quality according to Hungarian standards. This proportion is perhaps higher than expected because the lines were not selected on the basis of their breadmaking performance and many were grown outside their normal range of adaptation.

Finally, the Hagberg falling number was determined as a measure of α-amylase activity (**Figure 2c**). High α-amylase activity can arise from several syndromes, the most common of which are the production of α-amylase before seed maturity and preharvest sprouting (*22*), and results in reduced quality for breadmaking due to starch damage. In the present study 72% of the lines gave Hagberg scores above 250 s, which is considered to be the cutoff for breadmaking quality.

Principal Components Analysis (PCA). PCA is an ideal way to explore relationships based on multiple traits. For the present analysis the traits were grouped into four classes broadly corresponding to grain milling and composition, kernel characteristics, protein characteristics, and carbohydrates and lipids (**Table 1**). In addition, these characteristics were considered in relation to the age and geographical origin of the lines.

Grain Milling and Composition. The selected traits were bran yield, wholemeal protein content, starch content, and hardness index (**Figure 3a**,**b**). PC1 accounted for 45.5% of the cumulative variance, with starch content having greater positive weight and wholemeal protein content the greatest negative weight. Simi-

Table 1. Factor Coordinates of the Variables and the Contributions of the First Two Factors to the Total Variance

group of characters	properties included in PCA	factor 1	factor 2	Figure
grain milling and composition	starch content	0.91	0.23	**3a**,**b**
	protein content wholemeal	−0.88	−0.32	
	hardness index	−0.41	0.72	
	bran yield	0.25	−0.80	
	% of total variance	45.5	32.9	
kernel characteristics	1000 kernel weight	0.92	−0.21	**3c**,**d**
	SKCS kernel weight	0.98	−0.12	
	kernel diameter	0.94	0.15	
	hardness index	0.17	0.98	
	% of total variance	67.9	26.0	
protein characteristics	Zeleny sedimentation	0.72	0.61	**3e**,**f**
	protein content flour	0.96	−0.002	
	protein content w.meal	0.90	−0.20	
	gluten content	0.83	−0.47	
	gluten index	0.14	0.96	
	% of total variance	59.3	30.9	
carbohydrates and lipids	lipid	−0.28	0.93	**3g**,**h**
	amylose	0.78	0.00	
	free sugars	0.73	0.36	
	% of total variance	40.5	33.3	

larly, PC2 accounted for 32.9% of the total cumulative variance, with hardness and bran yield having the greatest positive and negative effects, respectively. These two PCs therefore together accounted for 78.4% of the cumulative variance, which increased to 93.8% when PC3 (to which hardness and bran yield were again major contributors) was added.

Most of the older varieties, landraces, and germplasm accessions had high protein contents and low starch contents, which is consistent with lower yields. In contrast, most of the modern varieties had hard kernel texture and lower protein content. This is consistent with the modern emphasis on breeding high-yielding breadmaking varieties, which is associated with increased accumulation of starch (+ score in PC1) and hard endosperm texture (+ score in PC2). Consequently, there was a tendency for the modern cultivars to fall into the upper part of the plot and the landraces and germplasm accessions in the left-hand part (**Figure 3a**).

Varieties originating from the west central region of Europe mainly had high protein contents and hardness indices, whereas their starch content and bran yield were low. This is also true for the varieties from the steppe region, but these showed slightly greater diversity. Varieties from southern Europe tended to have low protein contents with soft or hard kernel texture, whereas those from western Europe and the Americas varied widely in their properties and were distributed over the whole of the PCA plot (**Figure 3b**).

The first principal component therefore allowed genotypes with high starch contents and low wholemeal protein contents, such as the French variety Valoris, the Canadian varieties Augusta and Fredrick, and the Hungarian variety Martonvásári 17, to be distinguished from genotypes with high wholemeal protein contents and low starch, such as the Canadian spring wheat varieties (e.g., Glenlea), the Swiss alternative type Lona, the Ukrainian winter wheat Obriy, the Kansas variety Plainsman-V, and the Hungarian variety Tiszatáj.

Similarly, the second principal component allowed genotypes to be distinguished on the basis of texture and bran yield, for example, soft-grained Mediterranean genotypes such as Autonomia, Libellula, or Baranjka from hard-grained lines such as the Russian Krasnodarskaya 99, the Ukrainian Ukrainka, and the Nebraskan Millennium.

Kernel Characteristics. The selected characteristics were 1000 kernel weight, the kernel weight and diameter, and hardness index determined by the Perten SKCS. In this case PC1 accounted for 67.9% of the cumulative variance with 1000 kernel weight and kernel diameter being the major character determining + values, whereas PC2 accounted for 26.0%, with HI being the main character determining + values (**Figure 3c**,**d**).

In this analysis the soft wheats form a group with scores for PC2 of −1 and below, whereas the hard wheats form a second broad group with PC1 scores between about −2 and +4 and PC2 above −1. No apparent differences can be seen in the distribution of lines according to their age or geographical origin, but the plot clearly demonstrates that none of the selected landraces and lines from the steppe region were soft.

Grain Proteins. This PC analysis combined data on protein content of flour and wholemeal, gluten content, gluten index, and Zeleny sedimentation. PC1 accounted for 59.3% of the cumulative variance, with total protein, gluten content, and Zeleny sedimentation contributing to + scores, whereas PC2 accounted for 30.9% of the variance, with the greatest contribution to + values being gluten index (**Figure 3e**,**f**).

Modern wheat varieties tended to have high gluten indices and low contents of protein and gluten, whereas older varieties tended to have low gluten indices but high contents of protein and gluten and also high Zeleny sedimentation values. All of the selected landraces had low gluten indices and high protein contents.

Comparison of the lines according to their geographical origins (**Figure 3f**) shows that those from west central Europe have the greatest degree of similarity to each other of any geographical group, whereas varieties from the steppe have high gluten indices and high protein contents. The groups of varieties selected from the other geographical regions are more diverse, and no clear relationship was found between their geographical origins and their distributions in the PCA plot.

When we consider PC1, Plainsman-V, Gk-Tiszatáj, Mv-Suba, B16, Klein Estrella, Key, and Bánkúti 1201 have high contents of protein and gluten and high Zeleny sedimentation values. In contrast, Martonvásári-17, Valoris, Soissons, Malacca, Riband, and Isengrain have low values for these traits. PC2 shows that Vona, Jubilejnaja-50, Ukrainka, Kukri, and Monopol have high gluten indices, whereas San-Pastore, Chinese-Spring, Seu-Seun-27, Bánkúti 1201, Sumai-3, and Riband have low gluten indices.

Grain Carbohydrates and Lipids. The contents of lipids, amylose, and free sugars were also used for PCA. PC1 in this analysis accounted for 40.5% of the cumulative variance, with amylose content and free sugars having positive weight. Similarly, PC2 accounted for 33.3% of the total cumulative variance, with lipid content having the greatest positive effects. These two PCs therefore together accounted for 73.8% of the cumulative variance (**Figure 3g**,**h**).

No differences between modern and old varieties were observed on the basis of their amylose and lipid contents, with both tending to have high lipid contents, whereas landraces tended to have low amylose and free sugar contents.

Varieties originating from America had high lipid and low amylose contents, whereas varieties originating from southern Europe showed the opposite trend. The lipid contents of the genotypes from Asia, the Near East, and Australia were high,

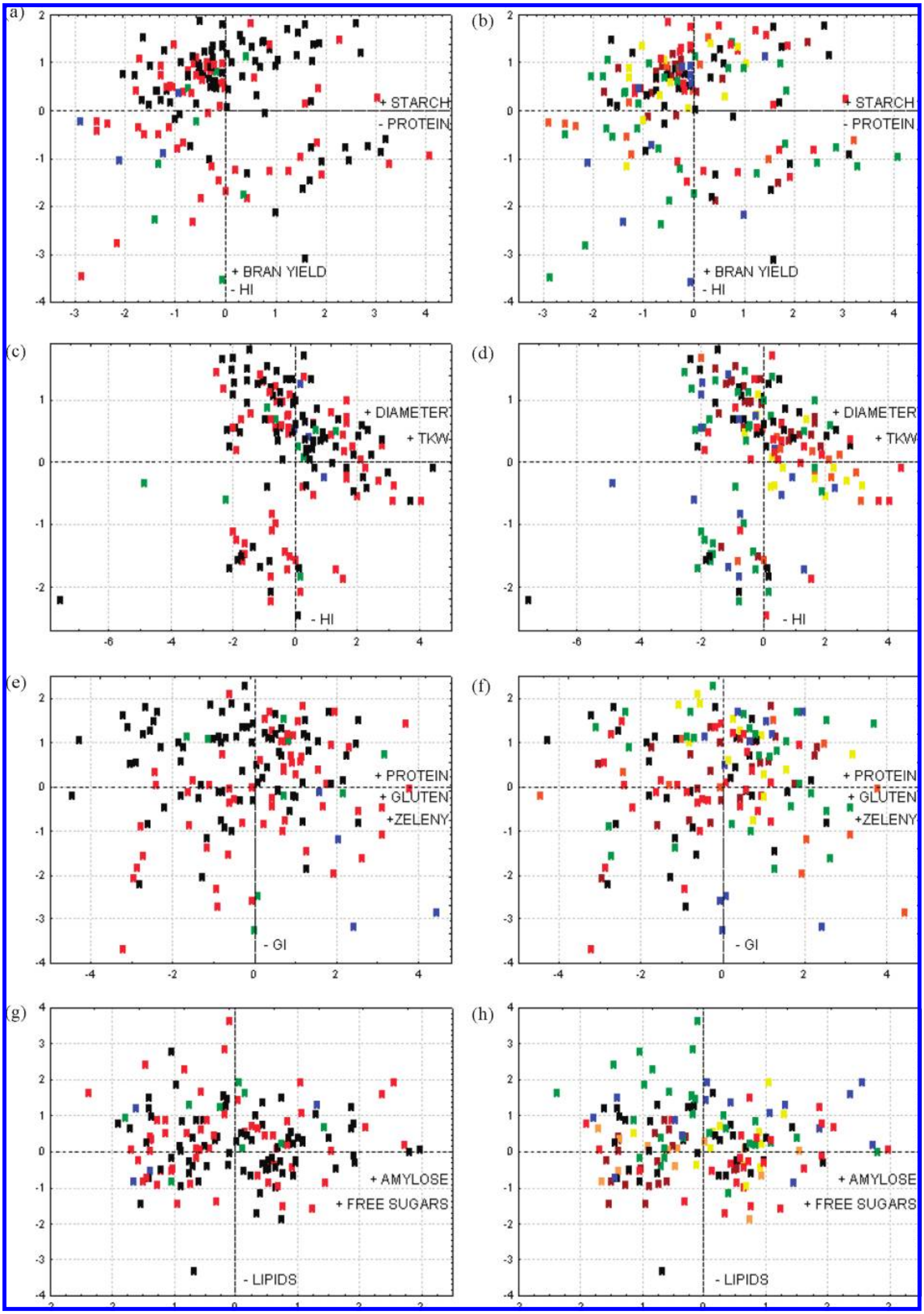

Figure 3. Principal component analysis for the 150 genotypes based on four types of trait: (**a**, **b**) milling and composition; (**c**, **d**) grain characteristics; (**e**, **f**) protein characteristics; (**g**, **h**) lipid and amylose content. Panels **a**, **c**, **e**, and **g** are colored to show the age of the lines (black, modern; red, old; blue, landrace; green, germplasm); panels **b**, **d**, **f**, and **h** show their geographical origins (black, west; dark red, west central; red, south; orange, south continental; yellow, steppe; green, America; blue, Asia, Near East, Australia).

Table 2. Correlations of the Compositional Properties of the 150 Wheat Genotypes with All of the Parameters Studied[a]

properties	lipids (%)	starch (%)	digestible starch (%)	amylose (% of starch)	free sugars (%)	protein flour (%)	protein wholemeal (%)	gluten (%)
compositional								
lipids (%)	1.00							
starch (%)	−0.22	1.00						
digestible starch (%)	−0.24	0.48	1.00					
amylose (% of starch)	ns	0.003	0.04	1.00				
free sugars (%)	ns	0.37	ns	0.20	1.00			
protein flour (%)	ns	−0.66	−0.41	ns	ns	1.00		
protein wholemeal (%)	0.27	−0.75	−0.59	ns	ns	0.84	1.00	
gluten (%)	ns	−0.63	−0.44	ns	ns	0.78	0.77	1.00
breadmaking								
gluten index	ns	ns	ns	ns	ns	ns	ns	−0.32
Zeleny sed (mL)	ns	−0.33	ns	ns	ns	0.65	0.46	0.34
FOSS water abs (%)	ns	−0.57	−0.31	ns	ns	0.77	0.72	0.75
flour yield (%)	ns	ns	ns	ns	ns	−0.45	ns	−0.29
bran yield (%)	0.21	ns	ns	ns	ns	−0.28	ns	ns
agronomical								
heading date	0.19	ns	ns	−0.28	−0.15	ns	ns	ns
plant height (cm)	0.28	−0.27	ns	ns	ns	0.28	0.33	0.31
TKW (g/1000 seeds)	−0.25	ns	ns	ns	ns	ns	ns	ns
test wt (g/L)	ns	ns	ns	ns	ns	0.31	0.21	ns
kernel wt (mg)	−0.29	ns	ns	ns	ns	0.20	ns	0.22
kernel diameter (cm)	−0.29	ns	ns	ns	ns	0.32	ns	0.33
hardness index	ns	ns	ns	ns	ns	0.44	ns	0.24

[a] $r_{0.05}$= 0.20, $r_{0.01}$= 0.25, $r_{0.001\%}$= 0.32. ns, not significant.

whereas genotypes from the south continental region of Europe had low lipid contents. Varieties from the west central region of Europe tended to have low amylose contents, and varieties from the steppe region had high amylose contents.

Genotypes with high lipid content included Atlas-66, Arthur-71, Cadenza, Thatcher, Red-Fife, and Chinese Spring, whereas Apache, Mv-Emese, Herzog, Libellula, Produttore, San-Pastore, and Ellvis had low lipid contents. The amylose content was high in Manital, Pastor, Kirkpinar-79, Atay-85, and Kirac66 and low in Glenlea, Martonvásári 17, Baranjka, Autonomia, Sumai-3, and Ble-Des-Domes.

It can be concluded that significant variation for technological quality traits is present in the 150 wheat lines selected for the HEALTHGRAIN diversity screen, with modern varieties generally showing similar or better technological quality properties than landraces or old varieties: this has presumably resulted from continuous breeding efforts over the past century.

Other studies carried out as part of the HEALTHGRAIN project support the widely accepted view that the bran components are particularly rich in dietary fiber and bioactive compounds. The aim of wheat breeders should therefore be to develop genotypes in which the white flour as well as the bran contains higher levels of these components. However, it is essential that these properies should be combined with high yield and good properties for conventional processing (as measured here) if such varieties are to become widely grown and utilized.

Correlation Analyses. Correlations between the compositional data and some baking and agronomic properties of the studied wheat varieties were calculated (**Table 2**). As expected, the protein and gluten contents of the flour showed strong negative correlations with the total and digestible starch contents of the flour. The content of proteins and, in particular, the content of gluten proteins, are major determinants of breadmaking quality, and both of these were positively correlated with Zeleny sedimentation. The contents of proteins and gluten were also positively correlated with flour water absorption, which probably reflects the fact that most high-protein breadmaking wheats also have hard endosperm texture: this leads to greater starch damage on milling and hence high water absorption. The amylose content of the starch was positively correlated with the quantity of the free sugars, whereas the content of lipids was negatively correlated with starch content and positively correlated with protein content.

The heading date was positively correlated with the contents of amylose and free sugars and negatively correlated with lipids. These correlations may result from the fact that varieties with late heading dates may be affected by heat and drought during grain filling, leading to reduced accumulation of carbohydrates in the grain and hence smaller grain and lower yields. Such grain would also be expected to have higher lipid contents resulting from the proportionally higher contributions of the lipid-rich embryo and aleurone layer. Similarly, kernel weight and size were negatively correlated with lipid content but positively correlated with protein and the gluten contents.

Impact of Environmental Factors. The results discussed above are based on the means of analyses carried out on material harvested in successive years, 2005 and 2006. The varieties were grown on adjacent sites on the same experimental farm. However, the two years differed in the environmental conditions during the period of grain development (June−July), most notably in the quantity of precipitation (**Figure 4**). The average temperature in Hungary was about 10−12 °C in October in both years after sowing. Precipitation during this period was low in 2005 but greater in 2004. In both years the temperature fell below zero at the beginning of February, when the precipitation was 10−20 mm. The temperature increased above 10 °C in April and May, but precipitation was very low in 2006. Harvest was rainy in 2005, whereas a higher amount of precipitation fell before harvest in 2006 (**Figure 4**).

These differences in climatic conditions resulted in significant effects of year on agronomic and quality parameters, including heading date, plant height, contents of protein and starch, Zeleny sedimentation, and yields of bran and flour. Statistical analysis

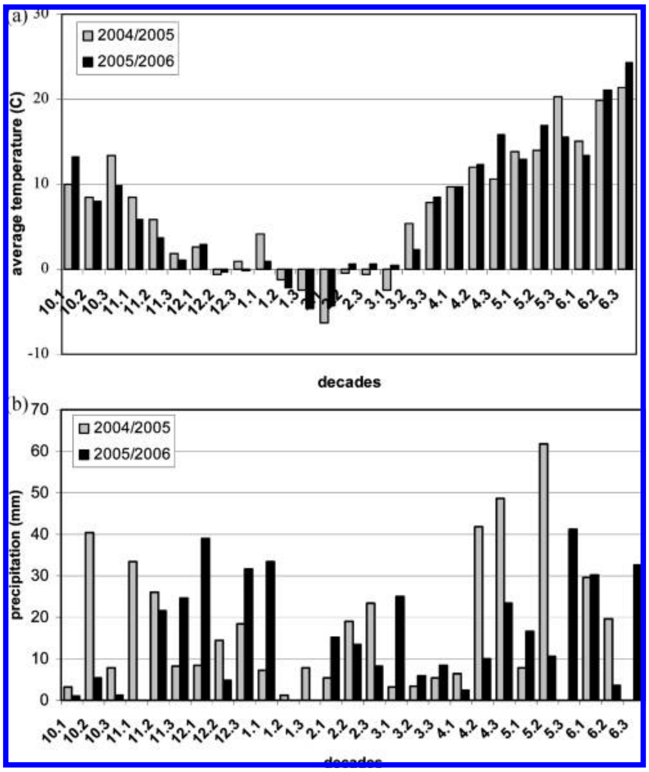

Figure 4. Weather conditions in the two years of the field experiment presented as an average of 10 day periods (10 days = one decade) from October (10) to June (6): (**a**) average temperature; (**b**) quantity of precipitation.

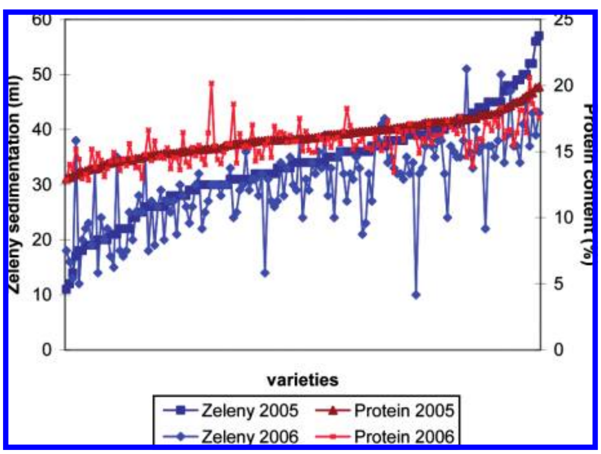

Figure 5. Relative ranking of the cultivars (*x*-axis) in two years (2005−2006) according to Zeleny sedimentation and protein content of the wholemeal.

showed that the genotype significantly affected all of the studied parameters at the 0.001 probability level except for grain yield, which was significant at the 0.05 probability level. At the same time the year significantly affected the heading date, the plant height, the starch content, the protein content of wholemeal, the Zeleny index, the kernel diameter and hardness, the falling number, and the yield of flour and bran at the 0.001 probability level. Other parameters such as the seed yield, the 1000 kernel weight, the water absorption, the test weight, the protein content of flour, the SKCS kernel weight, the gluten content, and the gluten index were not affected significantly by the year. Nevertheless, despite these effects, the relative rankings of the cultivars based on the different properties remained remarkably consistent over the two years (**Figure 5**).

The results of these two field trials show that substantial diversity is present in the selected genotypes for both agronomic and technological quality traits and that the differences are largely maintained over the two years despite significant differences in the weather. Most of this variability has resulted from breeding, involving 100 years of conscious selection. Although the varieties were not selected for processing quality and many were grown outside their region of origin, the number showing good breadmaking performance was surprisingly high. Furthermore, comparison of the results with the analyses of fiber and phytochemical components (as discussed in ref *5*) indicates that it should be possible to combine enhanced nutritional quality with high yield and processing quality for traditional end uses.

ABBREVIATIONS USED

FG, degrees of freedom, number of samples minus one; HI, hardness index; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NIR, near-infrared spectroscopy; PC, principal component; PCA, principal component analysis; r_p, coefficient of correlation where p is the probability level; r_{crit}, the limit of the r value, from which higher values mean significant correlations.

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