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SYMPOSIUM INTRODUCTION

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The HEALTHGRAIN Wheat Diversity Screen: Effects of Genotype and Environment on Phytochemicals and Dietary Fiber Components†

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Analysis of the contents of bioactive components (tocols, sterols, alkylresorcinols, folates, phenolic acids, and fiber components) in 26 wheat cultivars grown in six site × year combinations showed that the extent of variation due to variety and environment differed significantly between components. The total contents of tocols, sterols, and arabinoxylan fiber were highly heritable and hence an appropriate target for plant breeding. However, significant correlations between the contents of bioactive components and environmental factors (precipitation and temperature) during grain development also occurred, with even highly heritable components differing in amount between grain samples grown in different years on different sites.

KEYWORDS: Wheat; tocols; sterols; alkylresorcinols; folates; phenolic acids; fiber

INTRODUCTION

The health benefits of wholegrain cereals are now widely accepted and have formed the basis for health claims in several countries (*1*). In particular, epidemiological studies have shown a protective role against chronic diseases related to the metabolic syndrome, notably, type 2 diabetes and cardiovascular disease (*2*,*3*). However, the whole wheat grain comprises several tissues, which differ in their content and composition of bioactive components: dietary fiber, vitamins, minerals, and phytochemicals (including antioxidants). In particular, the bran (which includes the aleurone layer) is rich in fiber, minerals, and phytochemicals, whereas the germ is rich in protein, oil, and vitamins, notably thiamin and folate (*4*). Removal of the bran and germ during milling therefore results in severe depletion of bioactive compounds, with the white flour derived from the starchy endosperm comprising mainly starch (about 75% dry weight), protein (10−11% dry weight), and fiber (3−4%).

HEALTHGRAIN is an integrated project in the sixth Framework Program of the European Union (2005−2010). The aim is to improve the health and reduce the risk of metabolic diseases in humans by providing a more detailed understanding of the benefits of wholegrain cereals and assisting grain processors to use wholegrain or bioactive components derived from whole grain in products that are healthy and palatable. It therefore includes a range of studies from plant science through processing to human nutrition and consumer expectations and perception (*5*) (www.healthgrain.org).

Wholegrain products are most commonly made from wheat and rye, which have therefore been the focus in HEALTHGRAIN. Because little was then known about the extent of genetic variability for bioactive components in wheat and rye, a "diversity screen" was established in 2005, with 150 wheat lines (130 winter type and 20 spring type) and 10 rye lines being grown together with 40 other cereal lines (oat, barley, spelt, durum wheat, and primitive diploid and tetraploid wheats) on a single site at Martonvásár in Hungary. The wheat lines were selected to represent a range of ages (traditional land races, old and modern varieties), with 107 from Europe and the others from countries ranging from Argentina to New Zealand (*6*). Analyses

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Table 1. Characteristics of the Sites and Heading and Harvesting Data of the Wheat Lines

		Martonvásár (H)		Choryn (P)	Clermont-Ferrand (F)	Saxham (U.K.)
year	2005	2006	2007	2007	2007	2007
longitude	18° 49′ E	18° 49′ E	18° 49′ E	16° 46′ E	3° 04 E	0° 64′ E
latitude	47° 21′ N	47° 21′ N	47° 21′ N	52° 2′ 60 N	45° 46 N	52° 25′ N
altitude	150 m	150 m	150 m	66 m	334 m	70 m
soil type	Chernozem loam	Chernozem loam	Chernozem loam	sandy loam	calcareous loamy clay	Gleyic luvisol
soil pH	7.96 ± 0.05	8.06 ± 0.04	7.6 ± 0.01	6.04 ± 0.06	8.15 ± 0.02	7.98 ± 0.12
soil composition						
Zn ppm	5.5 ± 0.4	6.0 ± 1.0	17.5 ± 1.3	16.7 ± 0.9	9.6 ± 1.1	14.2 ± 0.7
Fe ppm	10.8 ± 1.3	5.6 ± 0.5	772 ± 126	88 ± 9.4	8.2 ± 0.5	96 ± 21.3
S ppm	22.0 ± 2.4	7.2 ± 0.3	25.3 ± 7.3	4.1 ± 1.5	6.6 ± 0.8	5.0 ± 0.3
heading dates	May 21−June 15	May 17−June 9	May 5−May 23	May 17−May 26	April 27−May 15	June 2−June 14
harvest dates	July 20−23	July 18−20	July 5	July 20	July 13	August 22
mean temperature, heading to harvest	19.4 °C	19.3 °C	20.5 °C	17.7 °C	18.4 °C	14.7 °C
total precipitation, heading to harvest	116.0	128.2	126.6	101.4	204.2	232.6

of wholemeal flour of these lines showed wide variation in the amount (from 1.4- to 3.6-fold) and composition of selected groups of phytochemicals (folates, phenolic acids, tocols, sterols, and alkylresorcinols) (*7*−*11*). Similar variation was demonstrated in fiber components, with the content of soluble arabinoxylan in white flour varying by 4.7-fold (*12*).

Although this study indicated the existence of substantial genetic variation in the amounts and compositions of bioactive components in wheat, the fact that the samples came from single plots did not allow the contributions of environmental factors to the variation to be determined.

To address this point, a second experiment was established to analyze 26 lines grown on the same site in Hungary in 2006 and 2007 and on three additional sites in the United Kingdom, France, and Poland in 2007. Twenty-three of these lines corresponded to those grown in 2005, giving six site × year data sets for analysis. These lines were subjected to the same range of analyses as in the initial diversity screen, with detailed results being reported in the accompanying papers (*13*−*18*).

The present paper therefore focuses on broader aspects of the distribution and relationships of the different groups of components.

MATERIALS AND METHODS

Sites. Four sites were selected to give a wide range of climatic conditions, reflecting that expected in a single season in the European Union (EU) member states. These were the Agricultural Research Institute of the Hungarian Academy of Sciences (Martonvásár, near Budapest, Hungary), Nickerson Seeds U.K. (Saxham, near Bury St Edmunds, U.K.), Danko Plant Breeders Ltd. (Choryn, near Poznań, Poland), and the INRA experimental station at Clermont Ferrand (France).

Soil mineral composition was determined on four random samples taken from each field used for the trials, using extraction with 0.05 M EDTA (pH 7.0) and inductively coupled plasma mass spectrometry (ICP-MS) for most major and minor elements and extraction with 0.016 M KH_2PO_4 and inductively coupled plasma atomic emission (ICP-AES) for Se and S (as described in ref*19*). A brief summary of the site characteristics, soil composition, and heading and harvest dates of the lines is presented in **Table 1**, whereas the temperature and precipitation (as averages of 10 day periods (decades)) between planting and harvest are summarized graphically in **Figure 1**.

There were significant differences between the six site/year combinations in both soil characteristics and weather conditions. For example, EDTA-soluble iron content of the soil ranged from 5.6 to 772 ppm (both sites being at Martonvásár) and the KH_2PO_4-soluble sulfur content from 4.1 ppm (Choryn) to 22 ppm (Martonvásár). The high levels of extractable Fe and Zn in the soil from the 2007 Martonvásár site may have resulted from the effects of waterlogging during several years preceding the field trial. Heading dates ranged from May 5 to June 14 and harvest dates from July 5 to August 22. Precipitation and mean temperatures between heading and harvest ranged from 101.4 to 232.6 mm and between 14.2 and 20.5 °C, respectively (**Table 1**). It is notable that the temperature at the Hungarian site varied more widely than at the other three sites but was consistently hotter during grain filling. In contrast, the U.K. site was cool and wet during the same period.

Lines. The 26 lines selected are listed in **Table 2**. They include 23 lines that were analyzed in the 2005 diversity screen (*6*), and data from these analyses were therefore included in the present study. Most lines were selected because the analyses carried out on the material grown in 2005 showed either low levels of phytochemicals/dietary fiber components or unusually high levels of one or more of these components (**Table 2**). Atlas-66 was selected because it has been used as a source of high protein content in breeding programs (*20*), whereas Chinese Spring is the standard line used in wheat genetic studies worldwide. Avalon and Cadenza were selected as the parents of the standard U.K. doubled haploid mapping population, whereas Valoris and Isengrain are the parents of a doubled haploid population segregating for the content of soluble dietary fiber (*21*). Three additional cultivars were included in 2006 and 2007. MV Emese is the standard cultivar used in variety trials at Martonvásár, whereas Tiger and Crousty were selected as standard lines in HEALTHGRAIN module 3 (Technology and Processing) (*5*).

Agronomic treatments were standard for the individual sites, with 110 kg of N/Ha being applied in Poland, 204 kg of N/Ha in the United Kingdom, 200 kg of N/Ha in France, and 140 kg of N/Ha in Hungary and appropriate use of agrochemicals. Milling (to give wholemeal and flour + bran fractions) and the determination of thousand kernel weight, grain protein, and total lipid were as reported by Rakszegi et al. (*22*).

Analyses of Bioactive Components. Fractions were then analyzed by the collaborating partners for a range of fiber and phytochemical components selected on the basis of their proposed benefits and the importance of wheat products as dietary sources. Alkylresorcinols are phenolic lipids that are located in the outer layers of the grain and are of interest in nutritional studies as biomarkers for wholegrain intake as well as having potential health benefits (*23*). Phenolic acids are the major group of phytochemicals in the grain and also the major group of antioxidants, their amounts correlating with total antioxidant activity in grain extracts (*24*,*25*). Folates, tocols, and sterols were determined because cereals are important sources of these groups of compounds. Wheat is also a major source of dietary fiber, the major component being arabinoxylan, which accounts for about 1.5−3% of the flour dry weight and about 5−7% of the whole grain (*26*), and the second important fiber component is β-glucan ((1−3,1−4) β-D-glucan) (*27*). The analytical methods are described in detail in the accompanying papers (*13*−*18*).

G × E Analyses. Data sets from the 26 wheat varieties grown in the six environments were used in statistical models with all effects considered as random to estimate variance components with SAS software (proc VARCOMP). For dietary fiber related traits, three technical replicates were used as error terms in the following model: $X = \mu + E + G + G \times E + \varepsilon$, with μ being the grant mean, E the environment main effect (i.e., a single combination location × year), G the genotype main effect, G × E the interaction between the two main effects, and ε the residual error.

For phytochemicals, there were no replicates available, thus in the model $X = \mu + E + G + \varepsilon$, the error term actually contains the G × E components.

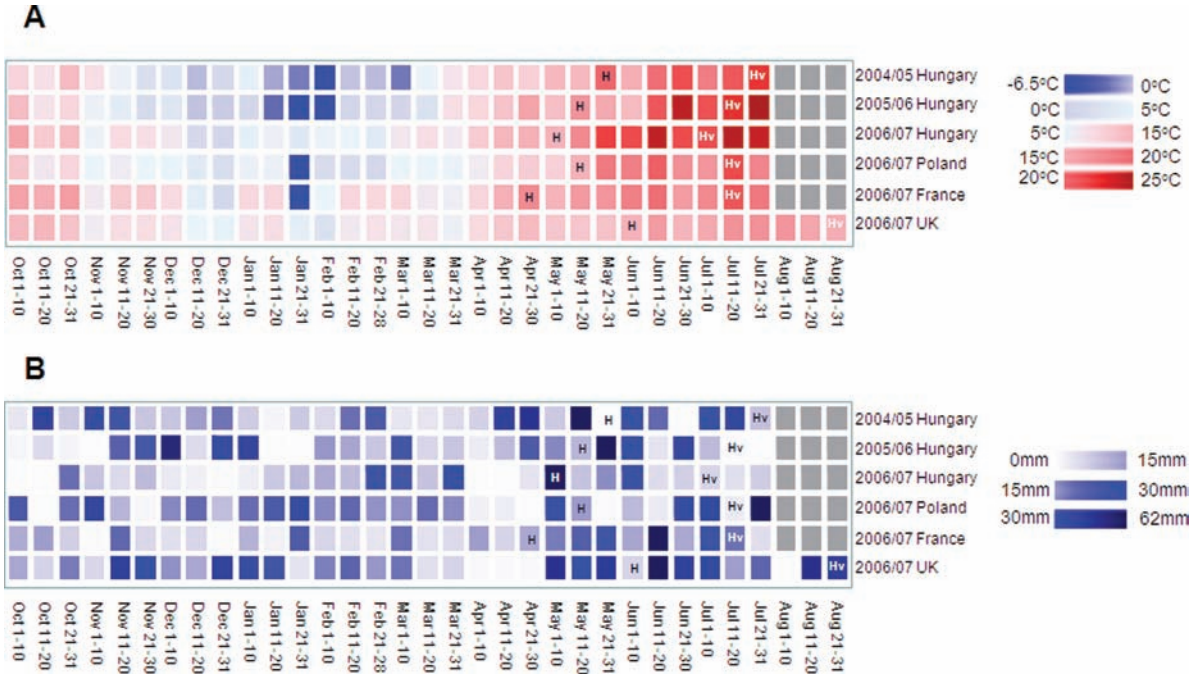

Figure 1. Heat maps showing the temperature (**A**) and precipitation (**B**) recorded for the trial sites in 2005, 2006, and 2007. Values are the means (**A**) and totals (**B**) for 10 day periods (decades). H indicates the start of heading and Hv the harvest date. Data for August are presented only for the U.K. site.

Table 2. Characteristics of the Selected Wheat Lines

wheat line	origin	growth habit	year of registration	characteristics
Atlas-66	U.S.A.	winter	1948	high protein
Avalon	U.K.	winter	1980	parent of U.K. mapping population
Cadenza	U.K.	spring	1992	parent of U.K. mapping population
Campari	Germany	winter	2003	high phytochemical content
CF99105	France	winter	*b*	high fiber content
Chinese Spring	China	spring	*c*	standard line used for genetic studies
Claire	U.K.	winter	1999	high phytochemical content
Crousty[a]	France	winter	1994	standard lines used for processing module
Disponent	Germany	winter	1975	high phytochemical and fiber contents
Estica	The Netherlands	winter	1990	high phytochemical content
Gloria	Romania	winter	1977	low fiber and phytochemical contents
Herzog	Germany	winter	1986	high phytochemical content
Isengrain	France	winter	1997	parent of French mapping population, low WE-AX
Lynx	U.K.	winter	1992	high phytochemical content
Malacca	U.K.	winter	1997	high phytochemical content
Maris Huntsman	U.K.	winter	1971	medium contents of phytochemicals, high fiber
MV Emese[a]	Hungary	winter	2001	standard variety used at Martonvásár
Obriy	Ukraine	winter	1983	low fiber and phytochemical contents, high protein
Rialto	U.K.	winter	1993	high phytochemical content
Riband	U.K.	winter	1987	high phytochemical content
San Pastore	Italy	winter	1940	high fiber but low phytochemical content
Spartanka	Russia	winter	1988	low fiber and phytochemical contents
Tiger[a]	Germany	winter	2001	standard line for processing module
Tommi	Germany	winter	2002	high phytochemical content
Tremie	France	winter	1992	high phytochemical content
Valoris	France	winter	1998	parent of French mapping population, high WE-AX

[a] Only grown in 2006, 2007. [b] Breeding line, no release date. [c] Model experimental line dating from about 1900, not released commercially.

Because replicates are not true field replicates, but only technical ones, all terms in the analysis of dietary fiber traits are highly significant (data not shown), but the error term is likely to be an underestimate of the true error. Because of this we did not compute the broad sense heritability of plot means, which would have given overestimates. However, the comparison of variance components and the ratio $\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_{G\times E})$, which is in fact an underestimate of true heritability (h^2), is a suitable parameter for plant breeders, as a high value indicates that genotype behavior is predictable and that the trait is likely amenable to genetic improvement. Further details are provided in Lynch and Walsh (*28*).

RESULTS

Heritability of Bioactive Components. Full accounts of the detailed compositions of the selected groups of bioactive components are presented in the accompanying papers (*13*−*18*).

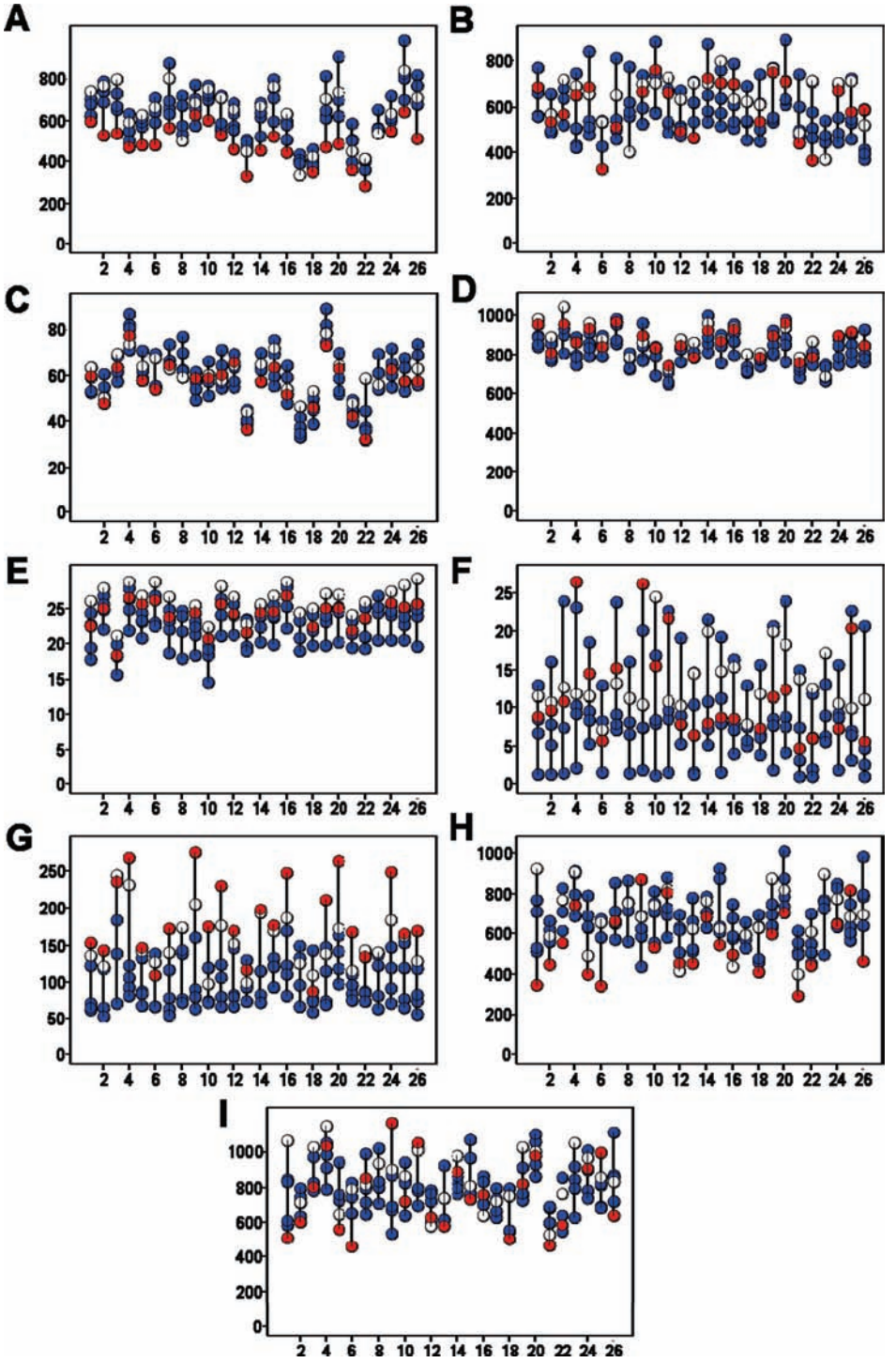

Figure 2. Contents of phytochemicals (**A**, alkylresorcinols; **B**, folates; **C**, tocols; **D**, sterols; **E**, stanols ; **F**, free phenolic acids; **G**, conjugated phenolic acids; **H**, bound phenolic acids; **I**, total phenolic acids) in wholemeal of the 26 wheat lines from the six site × year combinations. Samples are colored by year of growth (red, 2005; white, 2006; blue, 2007). Units are μg/g DW for all except folates (ng/g of DW) and stanols (% total sterols and stanols). Alternate tracks only are labeled. Cultivars: 1, Atlas-66; 2, Avalon; 3, Cadenza; 4, Campari; 5, CF99105; 6, Chinese Spring; 7, Claire; 8, Crousty; 9, Disponent; 10, Estica; 11, Herzog; 12, Isengrain; 13, Gloria; 353 14, Lynx; 15, Malacca; 16, Maris Huntsman; 17, MV Emese; 18, Obriy; 19, Rialto; 20, Riband; 21, San Pastore; 22, Spartanka; 23, Tiger; 24, Tommi; 25, Tremie; 26, Valoris.

The present paper will therefore follow the approach adopted by Ward et al. (*6*) and consider only the amounts of the major groups: phenolic acids (free, bound, conjugated, total), folates, alkylresorcinols, sterols (including % stanols), tocols, and β-glucan (all determined on wholemeal samples), water-extractable arabinoxylan (WE-AX), and water-unextractable arabinoxylan (WU-AX) (both determined on bran and flour fractions). The characteristics of the grain and flour (thousand grain weight, yields of flour and bran on milling, protein contents of flour and wholemeal, falling number, and hardness index) are given in Supplementary Table 1 in the Supporting Information.

The contents of these groups varied in relation to both genotype and environment (i.e., site × year combination), as illustrated in **Figures 2** and **3**. Furthermore, the extent of the

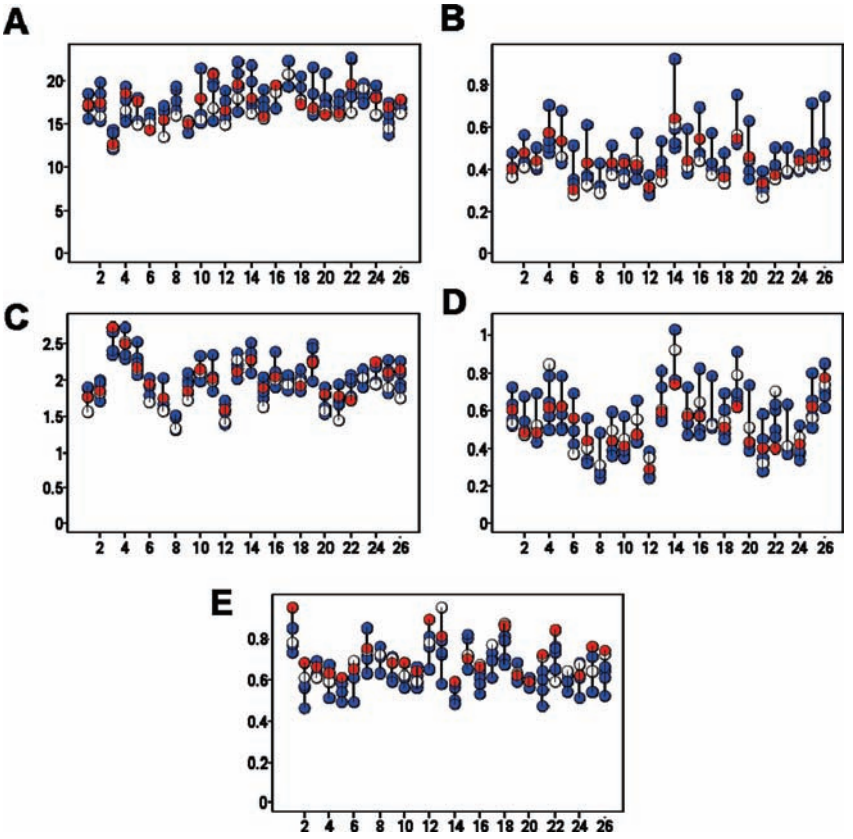

Figure 3. Contents of dietary fiber components (**A**, bran TOT-AX; **B**, bran WE-AX; **C**, flour TOT-AX; **D**, flour WE-AX; **E**, wholemeal β-glucan) in the 26 wheat lines from six site $\times$ year combinations. Samples are colored by year of growth (red, 2005; white, 2006; blue, 2007). The cultivars are listed in the legend to **Figure 2**. Units are % DW.

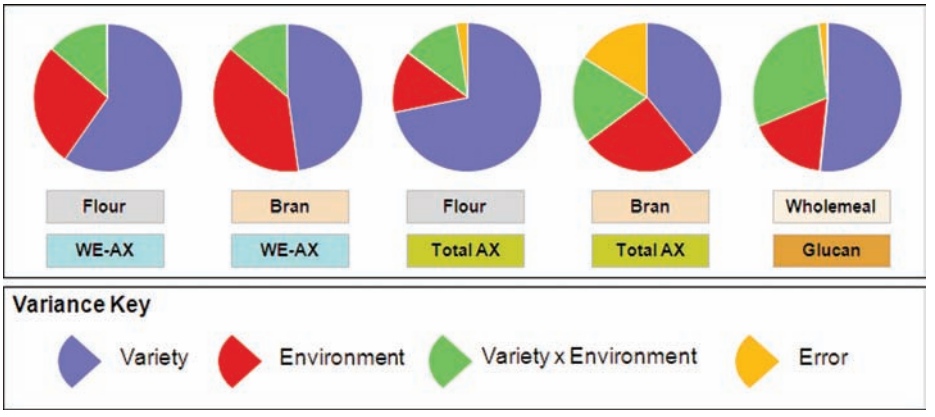

Figure 4. Variance components of dietary fiber components in the 26 wheat lines.

variation also differed between groups. The least variation was in the content of sterols (**Figure 2D**), which is consistent with our previous study (*6*, *8*). In contrast, wide variation was present in the contents of phenolic acids, particularly the free and conjugated fractions (**Figure 2F**,**G**). The individual groups of dietary fiber components also differed in the extent to which they varied between lines and environments (**Figure 3**), with the flour WE-AX fraction showing the highest variability (**Figure 3A**,**D**).

The data in **Figures 2** and **3** show clear differences in the effects of genetic and environmental factors on the contents of various phytochemicals and dietary fiber components. The same data sets were therefore used to calculate their heritability (**Figure 4**). All dietary fiber traits showed high ratios of genetic variance to total variance, ranging from 0.39 (TOT-AX in bran) to 0.71 (TOT-AX in flour) (**Table 3A**; **Figure 4**). WE-AX also had a high ratio in flour (0.59), which is consistent with a previously reported h^2 value of 0.75 (*29*) and a ratio of 0.48 in bran. High genotypic variance values were also shown for total tocols and total sterols (**Table 3B**). Thus, the ratio of genotypic variance to total variance was high (0.77 and 0.57 for tocols and sterols, respectively). The high heritabilities of the contents of dietary fiber components, tocols, and sterols indicate that they are realistic targets for selection in plant breeding. In contrast, the lower ratios of genetic to total variance for folates (0.24) and total phenolic acids (0.05) indicate that stable increases in content will not readily be achieved by breeding.

Relationships between Contents of Bioactive Components and Environmental Conditions. Correlations between the groups of

Table 3. Variance Components for Bioactive Compounds

A. Dietary Fiber

variance component	flour WE-AX	bran WE-AX	bran TOT-AX	flour TOT-AX	wholemeal β-glucan
Var (genotype)	0.01471	0.005545	1.94221	0.06582	0.0048443
Var (environment)	0.0065597	0.0044696	1.28428	0.01266	0.001602
Var (variety × environment)	0.0032686	0.0015502	0.94259	0.01157	0.0027719
Var (error)	0.00009743	0.00003078	0.80701	0.0019244	0.000175
$\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_{GxE})$	0.597100228	0.478199452	0.390308455	0.715633916	0.515724141

B. Phytochemicals

variance component	folates	tocols	sterols	stanols (%)	free phenolic acids	conjugated phenolic acids	bound phenolic acids	total phenolic acids	alkylre-sorcinols
Var (genotype)	3572.6	103.85566	3825.2	419.92498	2.78819	286.28961	6578.7	8838.4	11674.6
Var (environment)	6819.2	22.8461	1827.1	591.85083	29.63013	1895.1	5885.4	6449	3910
Var (error)	4288.1	8.6779	1049.8	85.6056	11.1429	746.28899	12477.6	15752.1	2878.8
$\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_\epsilon)$	0.24336678	0.76714375	0.57074648	0.38266092	0.06400624	0.09778724	0.2637631	0.28474685	0.63231041

Table 4. Correlations between the Contents of Bioactive Components and Weather Conditions in 26 Wheat Lines Grown in Six Site × Year Combinations

	average temperature		precipitation heading to harvest	
	R	*p* value	*R*	*p* value
folates	**0.690**	0.129	**-0.514**	0.297
sterols	**0.551**	0.257	−0.199	0.705
% stanols	**0.870**	**0.024**	**-0.589**	0.218
tocols	**0.563**	0.244	−0.067	0.900
alkylresorcinols	0.140	0.792	0.041	0.939
bound phenolic acids	−0.126	0.812	0.181	0.731
conjugated phenolic acids	**0.753**	0.084	**-0.744**	0.090
free phenolic acids	**0.899**	**0.015**	**-0.706**	0.117
total phenolic acids	0.317	0.541	−0.250	0.633
bran Tot-AX	0.060	0.911	0.138	0.795
bran WE-AX	**-0.889**	**0.018**	**0.737**	0.095
flour Tot-AX	−0.516	0.295	0.259	0.620
flour WE-AX	**-0.868**	**0.025**	**0.692**	0.128
wholemeal β-glucan	0.306	0.555	−0.684	0.134

bioactive components and the weather conditions are shown in **Table 4**, with statistically significant correlations being shown in bold. All groups of phytochemicals, except alkylresorcinols and bound phenolic acids, showed strong positive correlations with the mean temperature between heading and harvest, whereas folates and free and bound phenolic acids also showed negative correlations with total precipitation between heading and harvest. Stanols expressed as percent total sterols also showed positive and negative correlations, respectively, with temperature and precipitation during this period.

In contrast, the contents of the WE-AX in bran and white flour were both negatively correlated with temperature and positively correlated with precipitation between heading and harvest, which may be related to high xylanase activities present in grain grown under cool wet conditions (particularly in the material grown in the United Kingdom in 2007 (*30*)).

Correlations between Groups of Bioactive Components. Correlations between the mean contents of dietary fiber and phytochemical components in the six sample sets are given in Supplementary Table 2 in the Supporting Information and illustrated as a heat map in **Figure 5**. Strong positive correlations were observed between the contents of alkylresorcinols, tocols, sterols, and total phenolic acids, although the correlations with individual groups of phenolic acids (free, conjugated, and bound) and the other components varied. In contrast, the content of folates correlated

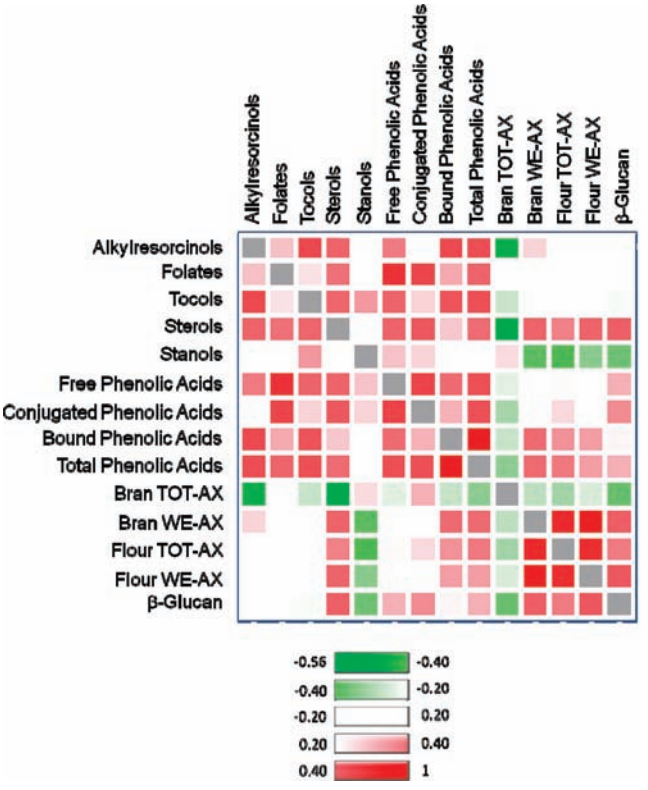

Figure 5. Heat map illustrating correlations between the contents of phytochemicals and dietary fiber components in the 26 wheat lines.

only with the contents of free and conjugated phenolic acids (and hence also total phenolic acids). No significant correlations were observed between the percentage of stanols and the other groups of phytochemicals. Positive correlations with fiber components were observed for sterols (bran WE-AX, flour WE-AX, and wholemeal β-glucan) and for bound phenolic acids (bran WE-AX), but not for the other groups of phytochemicals.

These correlations between groups of components do not necessarily imply a direct relationship and in most cases they are likely to result from indirect effects. First, it is known that most of the components studied here are concentrated in the bran fraction, and any factors affecting the proportion of the bran (including grain size) will affect their concentrations in the whole grain. This was demonstrated in our previous study (*6*), and similar

correlations were found with some of the components studied here (not shown). Second, the fact that the contents of tocols, sterols, folates, stanols, and free and conjugated phenolic acids show similar positive correlations with temperature and negative correlations with precipitation means that correlations between these groups of components will also be observed when grain samples grown under various environmental conditions are compared.

The analyses reported here have significant implications for plant breeders and for grain and food processors. First, the high heritability of several major groups of bioactive components (arabinoxylan fiber, tocols, and sterols) means that these are realistic targets for plant breeders to produce novel cultivars with enhanced health benefits. For example, for a trait with $h^2 = 0.7$, the expected genetic advance per selection cycle for a 10% selection intensity (i.e., the 10% best lines are selected to produce the next generation) is $0.7 \times 1.715 = 1.2$ times the phenotypic standard variation. This means, for example, that a doubling of the content of WE-AX can be achieved in two selection cycles (10−16 years). However, more rapid progress can be achieved by marker-assisted transfer of the major QTL for WE-AX (see ref *21*). However, the amounts of even the most highly heritable components are affected by environmental conditions, meaning that their precise contents will vary from region to region, from year to year, and probably even within a single field. This poses a challenge to food processors who wish to provide products with enhanced health benefits as it may not only be necessary to source specific varieties but also to routinely determine the contents of bioactive components in specific grain samples. However, it may also provide opportunities to source material grown in environments that result in higher levels of specific bioactive components, for example, to exploit the higher levels of folates, tocols, sterols, and phenolic acids (free and conjugated fractions) present in grain grown at higher temperatures. The development of simple and economical methods for determining composition is therefore also crucial to monitor intake and facilitate product development.

It is also necessary to ensure that increases in the contents of bioactive components are combined with good agronomic performance yield and with high quality for processing. Our previous study (*6*) showed that the content of bioactive components was not related to the age or origin of the genotypes, with some of the lines which contained the highest contents being modern high-yielding cultivars. The results of the present study are consistent with this and demonstrate that heritable variation in the content of bioactive components can be exploited by breeders to develop new wheat cultivars with enhanced health benefits. Nevertheless, the development of cereal products with enhanced nutritional benefits will clearly be facilitated by exploitation of the genetic variation in the content of bioactive components described here.

ABBREVIATIONS USED

A, arabinose; AX, arabinoxylan; DW, dry weight; EDTA, ethylenediaminetetraacetic acid; ICP-AES, inductively coupled plasma atomic absorption spectroscopy; ICP-MS inductively coupled plasma mass spectrometry, TOT-AX, total arabinoxylan; WE-AX, water-extractable arabinoxylan; X, xylose.

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Supporting Information Available: Supplementary tables of characteristics of grain and flour and correlations between contents of bioactive components in grain of 26 wheat lines grown in six site × year combinations. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

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