JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 9699–9709 **9699**

SYMPOSIUM

The HEALTHGRAIN Cereal Diversity Screen: Concept, Results, and Prospects

JANE L. WARD,[†] KAISA POUTANEN,[‡] KURT GEBRUERS,[§] VIENO PIIRONEN,[||]
ANNA-MAIJA LAMPI,[||] LAURA NYSTRÖM,[||] ANNICA A. M. ANDERSSON,[⊥]
PER ÅMAN,[⊥] DANUTA BOROS,[#] MARIANN RAKSZEGI,[∇] ZOLTAN BEDŐ,[∇] AND
PETER R. SHEWRY[*,†]

Centre for Crop Genetic Improvement, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; VTT Technical Research Centre of Finland, Espoo, Finland; Department of Food and Microbial Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, BE-3001 Leuven, Belgium; Department of Applied Chemistry and Microbiology, University of Helsinki, P.O. Box 27, Latokartanonkaari 11, FI-00014 Helsinki, Finland; Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, SE-750 07, Uppsala, Sweden; Laboratory of Nutritional Evaluation of Plant Materials, Institute of Plant Breeding and Acclimatization, 05-870 Radzikow, Blonie, Poland; and Agricultural Research Institute of the Hungarian Academy of Sciences, P.O. Box 19, 2462 Martonvásár, Hungary

One hundred and fifty bread wheat lines and 50 other lines of small-grain cereals (spelt, durum wheat, *Triticum monococcum*, *Triticum dicoccum*, oats, rye, and barley) were selected for diversity in their geographical origin, age, and characteristics. They were grown on a single site in Hungary in 2004–2005, harvested, milled, and analyzed for a range of phytochemicals (tocols, sterols, phenolic acids, folates, alkylresorcinols) and fiber components that are considered to have health benefits. Detailed analyses of these components in the different species are reported in a series of accompanying papers. The present paper discusses the comparative levels of the bioactive components in the different species, showing differences in both ranges and mean amounts. Furthermore, detailed comparisons of the bread wheat lines show that it is possible to identify lines in which high levels of phytochemicals and dietary fiber components are combined with good yield and processing quality. This means that commercially competitive lines with high levels of bioactive components are a realistic goal for plant breeders.

KEYWORDS: Wheat; cereals; phytochemicals; dietary fiber; wholegrain cereals

INTRODUCTION

Wholegrain Cereals and Health. Wholegrain foods have been the focus of significant scientific, governmental, and commercial interest during the past 10 years since epidemiological studies have increasingly shown their protective role against the risk of many chronic diseases, especially those related to metabolic syndrome, that is, type 2 diabetes and cardiovascular diseases. The strength of evidence is already sufficiently convincing to form the basis of health claims in the United States, United Kingdom, and Sweden (*1*) and has been strengthened recently by two meta-analyses. Bringing together data from a total of 286,125 participants in a study of the risk of type 2 diabetes (*2*) and over 149,000 participants in the case of cardiovascular disease (*3*), the studies consistently support the use of wholegrain for the prevention of these chronic diseases. Increasing the consumption by two servings per day was associated with a 21% decrease in the risk of type 2 diabetes (*2*), and 2.5 servings of wholegrain products per day as compared with 0.2 servings per day was also associated with a 21% reduced risk of cardiovascular events (*3*).

The wholegrain has been defined to consist of the intact, ground, cracked, or flaked caryopsis, the principal anatomic components of which, the starchy endosperm, germ, and bran,

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[*] Corresponding author (telephone +44 (0)1582 763133; fax +44 (0)1582 763010; e-mail peter.shewry@bbsrc.ac.uk).
[†] Rothamsted Research.
[‡] VTT Technical Research Centre of Finland.
[§] Katholieke Universiteit Leuven.
[||] University of Helsinki.
[⊥] Swedish University of Agricultural Sciences.
[#] Institute of Plant Breeding and Acclimatization.
[∇] Agricultural Research Institute of the Hungarian Academy of Sciences.

10.1021/jf8009574 CCC: $40.75

Table 1. Details of the Lines Included in the HEALTHGRAIN Diversity Screen

Classification According to Species and Geographical Origin

BREAD WHEAT (150)

European germplasm [according to European Adaptation Map (Feekes, 1978)]

West (34)

France:[a] Ble des Domes, Etoile de Choisy, Camp Remy, Courtot, Isengrain, MagdalenaFR, Recital, Renan, Soisson, Tremie, Apache, Qualital, Ornicar, Thesee, Taldor, Valoris, Caphorn, CF99075, CF99102, CF99105, CF99007

United Kingdom: Maris Huntsman, Avalon, Claire, Galahad, Hereward, Lynx, Malacca, Moulin, Rialto, Spark, Riband, Catbird, Cadenza; **Netherlands:** Estica

West-Central (22)

Germany: Disponent, Herzog, Kanzler, Monopol, Biscay, Cubus, Tommi, Dekan, Akteur, Campari, Ellvis; **Austria:** Agron, Amadeus, Capo, Probstdorfer Perlo; **Switzerland:** Arina, Tamaro, Lona; **Czech Republic:** Hana; **Poland:** Alba, Begra, Korweta

South (27)

Italy: Autonomia, Produttore, San Pastore, Libellula, Ravenna, Sagittario, Blasco, Nomade, Bilancia, Geronimo, Granbel, Guarni, Mieti, Palesio, Manital; **Bulgaria:** Roussalka, Sadovo 1, Momtchil; **Serbia:** Sava, NS Rana1, Balkan, Agrounia, Lasta, Pobeda, Zvezda; **Croatia:** Baranjka; **Israel:** Pan

South-Continental (11)

Hungary: Bankuti 1201, Fleischmann 481, Fertödi 293, GK Tiszataj, Martonvasar 17, Mv Suba, Mv Palotas; **Romania:** Fundulea 29, Carmen, Flamura 85, Gloria

Steppe (13)

Russia: Alabasskaja, Bezostaya 1, Aurora, Skorospelka 3B, Spartanka, Krasnodarskaya 99, Saratov 29; **Ukraine:** Iljicovka, Yubileinayais 50, Obriy, B16, Albatros Odeskaja, Ukrainka

American germplasm (28)

United States: Atlas 66, Scout 66, Nap Hal, TAM 200, Plainsman V, Key, Stephens, Karl 92, Gene, Cardinal, Millenium, Alliance, Vona, Arthur 71, Blue/Agent; **Canada:** Glenlea, Red Fife, Thatcher, Manitoba, Augusta, Fredrick; **Mexico:** Azteca 67, Milan, Pastor, Sultan 95, Mexique 50; **Argentina:** Klein Estrella, Buck Catriel

Asian, Near East, and Australian germplasm (15)

China: Chinese Spring, Yumai 34, Sumai 3; **Korea:** Su 321, Seu Seun 27; **Australia:** Janz, Sunstar, Kukri, Chara, Red River; **Turkey:** Atay-85, Gerek-79, Kirac 66, Kirkpinar 79; **New Zealand:** Kotuku

DURUM WHEAT (10)

Hungary: Mv Makaroni; **Austria:** Semperdur, Lajtadur; **Russia:** Parus; **Turkey:** Altin; **Bulgaria:** 1529-91; **Mexico:** Altar 84; **Italy:** Creso; **Germany:** Durabon; **France:** Orjaune

BARLEY (10)

Germany: Igri; **United States:** Dicktoo, Morex; **France:** Plaisant, CFL 93-149, CFL 98-398, CFL 98-404, CFL 98-450; **Poland:** Erhard Frederichen, Rastik; **USA:** Borzymowicki

RYE (10)

Poland: Dankowskie Zlote, Warko, **Germany:** Rekrut, Nikita; **France:** Grandrieu pop., Queyras 72 population, Haute Loire population; **Portugal:** Portugaise 3 population, Portugaise 6 population; **Hungary:** Lovaszpatonai 1

OAT (5)

Poland: Cacko; **Hungary:** Bajka, Mv Pehely; **China:** Fenli; **Austria:** Expander

SPELT (5)

V Oberkulmer Rotkorn, **Germany:** Franckenkorn, **France;** Spy, Ressac, Rouquin

T. monococcum[b] (5)

France: Epeautre-Sault de Vaucluse; **Hungary:** Mv GB04, 08-2004, Mv GB57, 122-2004

T. dicoccum[b] (5)

Hungary: Mv GB304, Mv GB317, Mv GB349, Mv 192-2004, Mv 265-2004

Classification According to Type and Age of the Germplasm

Landraces and old varieties (10): Ble des Domes, Bankuti 1201, Fleischmann 481, Seu Seun 27, Grandrieu population, Queyras 72 population, Haute Loire population, Portugaise 3 population, Portugaise 6 population, Lovaszpatonai 1

Old and transitional varieties (64): Etoile de Choisy, Maris Huntsman, Estica, Disponent, Herzog, Kanzler, Monopol, Agron, Amadeus, Capo, Probstdorfer Perlo, Arina, Tamaro, Hana, Alba, Autonomia, Produttore, San Pastore, Libellula, Roussalka, Sadovo 1, Sava, Fertödi 293, GK Tiszataj, Fundulea 29, Carmen, Flamura 85, Alabasskaja, Bezostaya 1, Aurora, Skorospelka 3B, Saratov 29, Iljicovka, Yubileinayais 50, Obriy, Atlas 66, Scout 66, Plainsman V, Key, Stephens, Glenlea, Red Fife, Thatcher, Manitoba, Augusta, Fredrick, Su 321, Janz, Kukri, Chara, Red River, Atay-85, Gerek-79, Kirac 66, Dankowskie Zlote, Erhard Frederichsen, Borzymowicki, Dicktoo, Creso, Parus, Altin, Oberkulmer Rotkorn, Arthur 71, Kirkpinar 79

Modern varieties (103): Camp Remy, Courtot, Isengrain, MagdalenaFR, Recital, Renan, Soisson, Tremie, Apache, Qualital, Ornicar, Thesee, Taldor, Valoris, Caphorn, Avalon, Claire, Galahad, Hereward, Lynx, Malacca, Moulin, Rialto, Spark, Riband, Cubus, Tommi, Dekan, Akteur, Campari, Ellvis, Lona, Begra, Korweta, Ravenna, Sagittario, Blasco, Nomade, Bilancia, Geronimo, Granbel, Guarni, Mieti, Palesio, Momtchil, NS Rana1, Balkan, Agrounia, Lasta, Pobeda, Baranjka, Martonvasar 17, Mv Suba, Mv Palotas, Spartanka, Krasnodarskaya 99, Albatros Odeskaja, Ukrainka, TAM 200, Karl 92, Gene, Cardinal, Millenium, Alliance, Vona, Azteca 67, Cadenza, Milan, Pastor, Sultan 95, Klein Estrella, Buck Catriel, Yumai 34, Sunstar, Warko, Amilo, Rekrut, Avanti, Fernando, Rasant, Nikita, Igri, Tiffany, Lomerit, Morex, Plaisant, Semperdur, Durabon, Orjaune, Lajtadur, Mv Makaroni, Franckenkorn, Spy, Ressac, Rouquin, Cacko, Zvezda, Manital, Mexique 50, Kotuku, Rastik, Altar 84

Germplasm (23): B16, Nap Hal, Chinese Spring, Sumai 3, Catbird, CF99075, CF99102, CF99105, CF99007, CFL 93-149, CFL 98-398, CFL 98-404, CFL 98-450, 1529-91, MvGB04, 08-2004, MvGB57, 122-2004, MvGB304, MvGB317, MvGB349, 192-2004, 265-2004

Classification According to Growth Habit

Winter types (169) (all except those listed below)

Spring or alternative types (31): Chinese Spring, Sumai 3, Saratov 29, Glenlea, Red Fife, Thatcher, Manitoba, Janz, Kukri, Chara, Red River, Catbird, Erhard Frederichen, Borzymowicki, Creso, Lona, Cadenza, Milan, Pastor, Sultan 95, Sunstar, Morex, Cacko, CFL 93-149, CFL 98-398, CFL 98-404, CFL 98-450, Bajka, Mv Pehely, Fengli, Expander

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[a] Wheat lines prefixed CFL are from INRA (Clermont Ferrand, France). [b] *T. monococcum* and *T. dicoccum* lines prefixed MV are from the germplasm collection at Martonvasar.

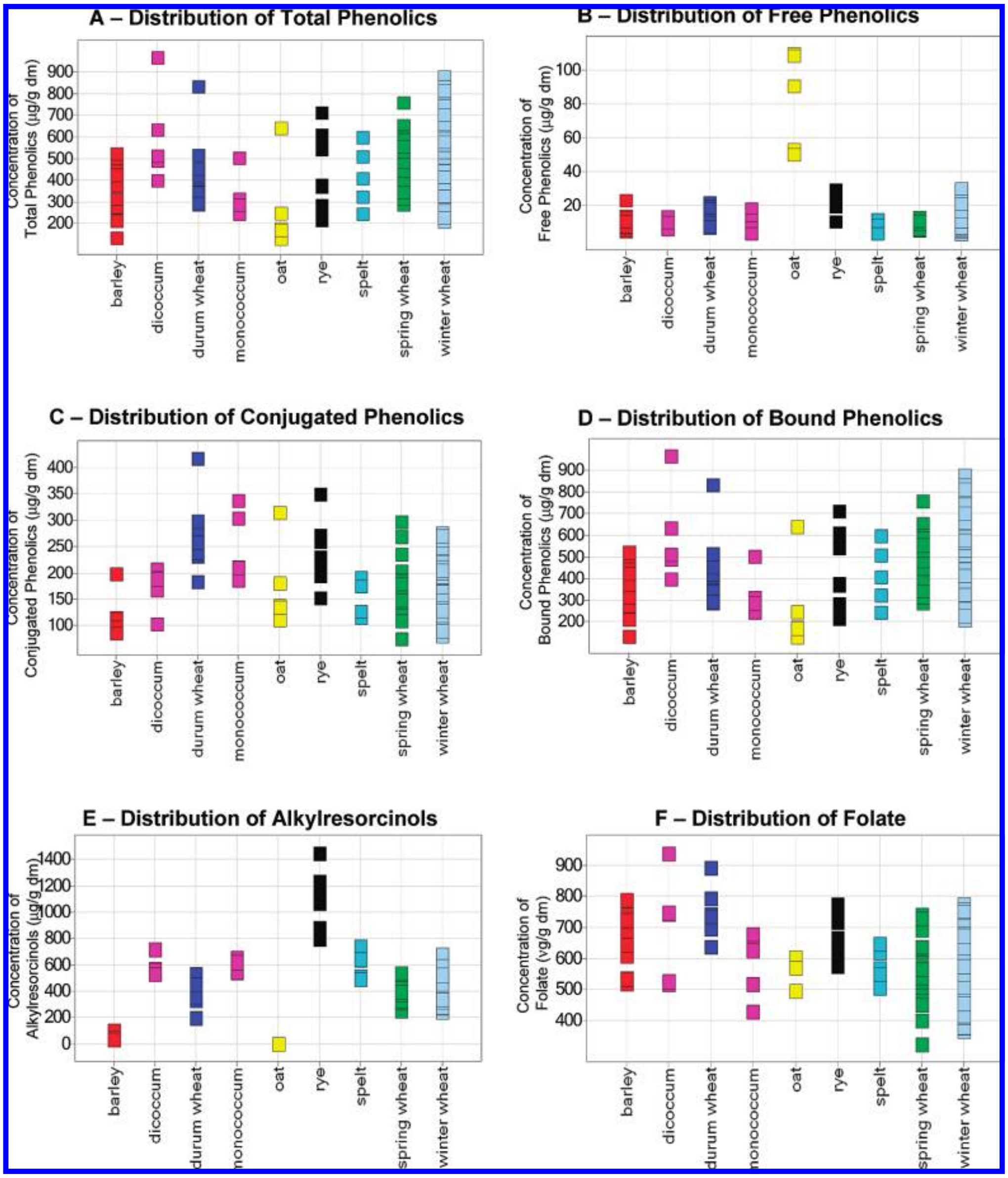


Figure 1. Ranges of concentrations of phenolic compounds (phenolic acids, alkylresorcinols) and folates in wholemeal samples of cereals in the HEALTHGRAIN diversity screen.

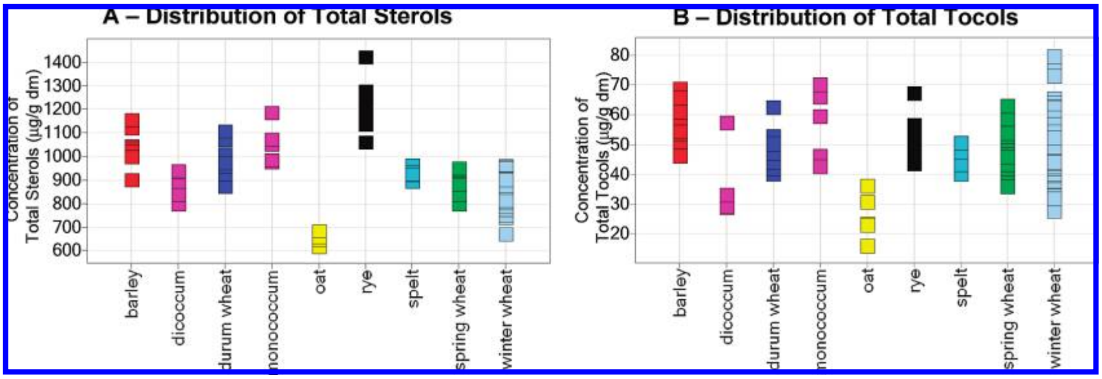


Figure 2. Ranges of concentrations of terpenoids (sterols and tocols) in wholemeal samples of cereals in the HEALTHGRAIN diversity screen.

are present in the same relative proportions as they exist in the intact caryopsis (*4*). Wholegrains are a good source of dietary fiber, vitamins, minerals, and phytochemicals, which have been suggested to contribute to their protective effects as compared to refined grains (*5*). The grain fiber as a single component has also been associated with significant risk reduction. For example, pooled analyses of 10 cohort studies reported a 10% reduced risk of cardiovascular disease with every 10 g/day increment in intake of cereal fiber (*6*).

The outer layers of grains have been shown to contain much higher levels of phytochemicals, such as phenolic compounds, phytosterols, tocols, and folate (*7*−*9*) than the inner parts. The

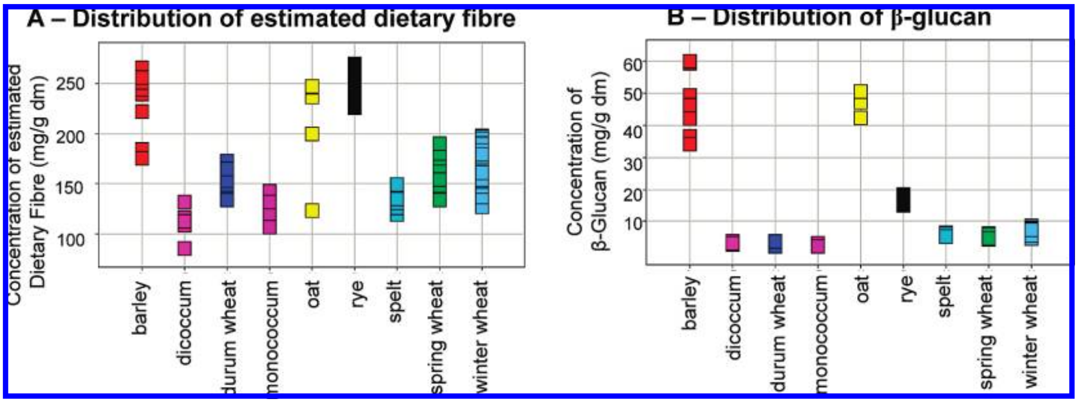


Figure 3. Ranges of concentrations of total dietary fiber and β-glucan in wholemeal samples of cereals in the HEALTHGRAIN diversity screen.

phenolic compounds of wholegrains, including lignans (*10*), alkylresorcinols (*11*), and phenolic acids (*12*), have been shown to be metabolized and absorbed in humans and are among the candidate compounds inducing physiological changes underlying the protective effects. Recent studies have identified many differences in the metabolic profiles of rats fed whole and refined wheat grain (*13*). However, the component(s) in wholegrain that are responsible for these effects on the protection of health and homeostasis and their mechanism(s) of action are still not fully understood. In fact, it is probable that several factors are required and act additively or synergistically to achieve the favorable effects.

The HEALTHGRAIN Project: Selection of Phytochemicals and Fiber Components. HEALTHGRAIN, an integrated project in the Sixth Framework Programme of the European Union, started in 2005 with the aim of improving the well-being and reducing the risk of metabolic diseases of European consumers by increasing the intake of protective grain factors through improved availability of health-promoting and safe cereal-based foods and ingredients of high eating quality. This cross-disciplinary project extends over the wholegrain chain from consumer and nutritional research through development of processing methods to analysis of the variation in the composition of bioactive components in current cereal varieties and the development of new sources of variation and tools for breeding varieties with enhanced properties (*14*).

As part of the HEALTHGRAIN research program we have initially determined the extent of variation of bioactive compounds in cereals, focusing on wheat but also including rye and other cereals. The target compounds were chosen to represent the different types of bioactive compounds and on the basis of hypotheses in the literature about their protective properties. As dietary fiber is a key factor for the nutritional and health protective effects of grain-based foods, we determined total dietary fiber content and also the major functional components, that is, β-glucan and arabinoxylan. Arabinoxylan is the major dietary fiber and constituent of wheat and rye cell walls and can be fractionated on the basis of its solubility. We therefore also determined the water-extractable fraction, which is considered to be most readily fermented in the human intestine and to show prebiotic potential (*15*).

The phenolic compounds were a clear target for the analysis of variation, as phenolic acids, lignans, and alkylresorcinols not only have been shown to be absorbed and metabolized but also have been suggested to exert various biological activities both in vitro and in vivo. Lignans are phytoestrogens, many of which are known to be converted to enterolactone by the intestinal microbial fermentation and which may be health-protective by many potential mechanisms (*16*). Alkylresorcinols are phenolic lipids typically present in the outer layers of wheat and rye, and in addition to their health effects are of interest as candidate biomarkers of intake of wholegrain wheat and rye (*17*). Phenolic acids are the most abundant phenolic compounds in the grain (*8*).

Folate, sterols, and tocols were also included in the screen because they are considered to have health benefits and because grains are important sources of these components in the diet. Tocols are important lipid-soluble antioxidants in the human body. Sterols are well-known for their cholesterol-lowering effects (*18*). Incorporation of the outer layers of the grain has been suggested as an alternative strategy to fortify cereal foods for increasing folate intake. This is particularly important in countries that have not adopted mandatory folic acid fortification as it appears that the folate in grain is readily bioavailable (*19, 20*).

It is also important that any improvements in nutritional properties are combined with good agronomic performance and processing quality. Detailed analyses of the functional properties of the wheat lines were therefore also carried out. These are described in detail in an accompanying paper (*21*) and discussed briefly below.

THE HEALTHGRAIN DIVERSITY SCREEN

Our ability to improve the content of phytochemicals and other bioactive components in the cereal grain depends on two factors: the extent of variation in these components in the gene pool available for plant breeders and our ability to exploit this variation, using classical breeding technology supported by modern biochemical and molecular tools. The HEALTHGRAIN diversity screen was established to answer the first of these questions, focusing on bread wheat (*Triticum aestivum*), whereas the second is being addressed in a separate part of the program.

Selection of the Lines. The analysis of grain bioactive components requires considerable expertise and resources, and it was therefore decided to focus on bread wheat (*T. aestivum* var. *aestivum*), selecting 150 lines to represent the full range of diversity in the gene pool available for plant breeders. These lines (listed in **Table 1**) show wide geographical diversity in origin (from Europe to East Asia, the Americas, and Australia) and include landraces and breeding lines as well as modern and older cultivars. One hundred and thirty of the bread wheats are winter type, which is the dominant form in world agriculture, whereas 20 are spring type. They also include wheat genotypes representative of the major classes that are recognized in international trade and represent differences in end use quality that have been targeted by plant breeders. The genotypes therefore differ in kernel texture (hard or soft endosperm structure), kernel color (red and white), and protein content.

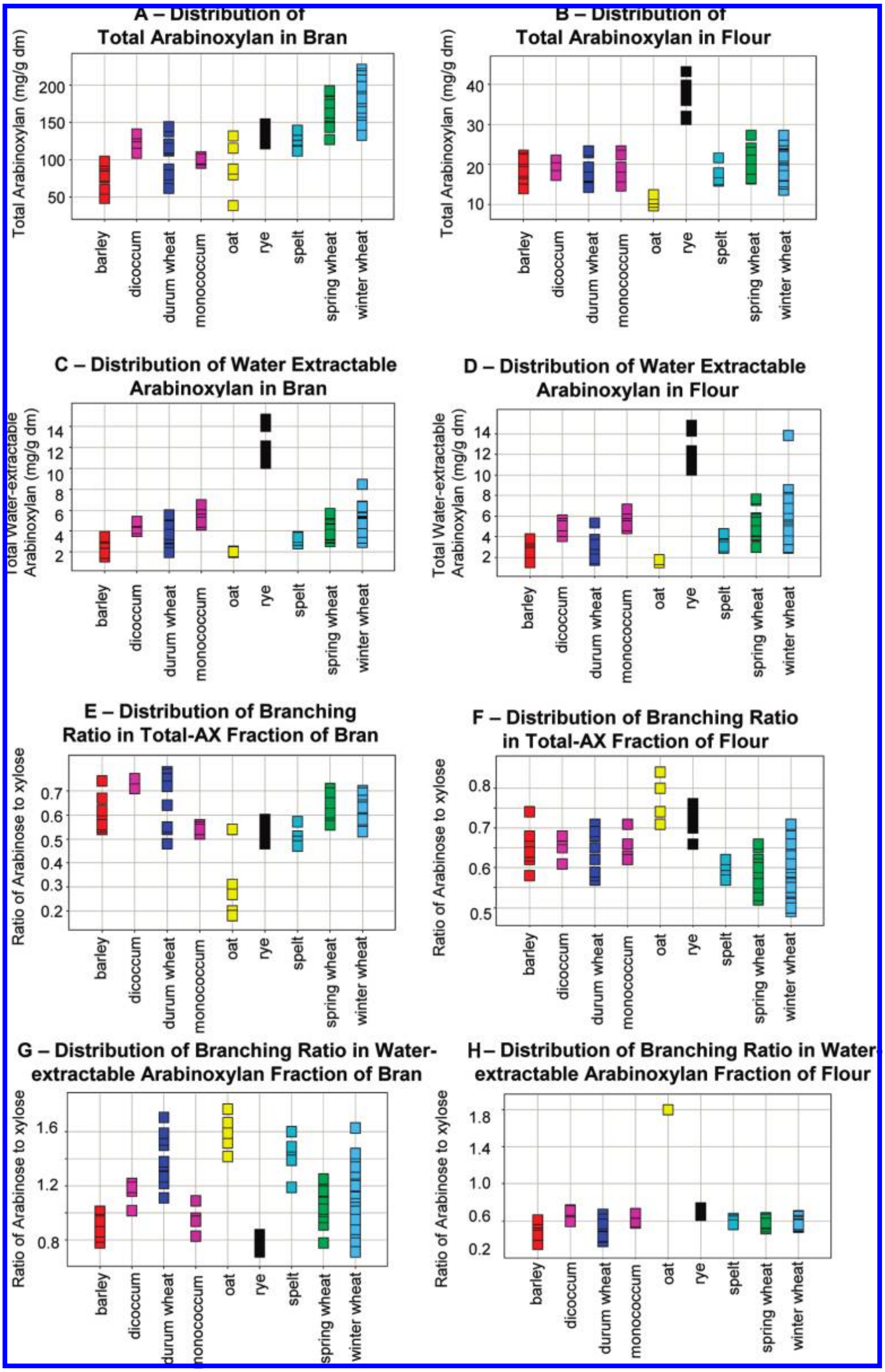


Figure 4. Ranges of variation in the amounts and properties of arabinoxylan fractions in bran and flour fractions from cereals in the HEALTHGRAIN diversity screen.

Five modern cultivars of spelt (a hulled form of hexaploid wheat, *T. aestivum* var. *spelta*), 10 of tetraploid durum wheat (*T. turgidum* var. *durum*), and 5 each of two early cultivated forms of wheat, diploid einkorn (*T. monococcum* var. *monococcum*) and tetraploid emmer (*T. turgidum* var. *dicoccum*), were also included. Finally, 10 lines of rye (*Secale cereale*), 5 of oats (*Avena sativa*), and 10 of barley (*Hordeum vulgare*) were selected, using similar criteria to those used to select the wheats. It should be noted that all of these species with the exception of oats are closely related, being classified in the grass tribe Triticeae. Oats is classified in a separate tribe, the Aveneae, which is in the same subfamily as the Triticeae (the Festucoideae).

Cultivation and Processing of Lines. The lines were sown in two replicate blocks in the field at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m) in 2004 (winter types) or 2005 (spring types). The plots

Table 2. Pearson Correlation Coefficients (r) between Bran Yield, 1000 Grain Weight, and the Contents of Bioactive Components in the Wheat Lines[a]

	bran yield	1000 grain wt
bran yield		**$r = -0.34$** **$P < 0.001$**
1000 grain wt	**$r = -0.34$** **$P < 0.001$**	
total sterols	$r = 0.256$ $P = 0.0015$	**$r = -0.332$** **$P < 0.001$**
total folates	$r = 0.137$ $P = 0.095$	$r = -0.127$ $P = 0.121$
total alkylresorcinols	**$r = 0.344$** **$P < 0.001$**	**$r = -0.324$** **$P < 0.001$**
total tocols	**$r = 0.472$** **$P < 0.001$**	$r = -0.489$ **$P < 0.001$**
total phenolic acids	$r = 0.196$ $P = 0.016$	$r = -0.029$ $P = 0.726$
flour TOT-AX	$r = 0.047$ $P = 0.596$	$r = 0.135$ $P = 0.098$
bran TOT-AX	**$r = -0.514$** **$P < 0.001$**	$r = 0.302$ **$P < 0.001$**
flour WE-AX	$r = 0.109$ $P = 0.186$	$r = 0.167$ $P = 0.0406$
bran WE-AX	$r = -0.011$ $P = 0.892$	$r = 0.043$ $P = 0.598$

[a] Values in bold are statistically significant (P value < 0.01).

were 2.5 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 pea, and there was a rainy period before harvest in 2005. The wheat lines were similarly grown in 2005/2006 when the weather was more typical. The plots were treated with herbicide (4 L/ha U-46-M fluid containing 500 g/L 2-methyl-4-chlorophenoxyacetic acid, 15 g/ha Granstar containing 75% tribenuron methyl), insecticide (0.2 L/ha Karate containing 2.5% λ-cihalotrin), and fungicide (1 L/ha Eminent containing 125 g/L tetrakonazol, 1 L/ha Tango Star containing 84 g/L epoxykonazol, and 250 g/L fenpropimorf) each year.

Winter, spring, and durum wheats were conditioned to 15.5% moisture content before milling, whereas other species were conditioned to 14% moisture content. Milling was carried out using a Perten Laboratory Mill 3100 (with 0.5 mm sieve) and Retsch ZM100 (for *T. monococcum* and oats) to produce wholemeal. The grains of spelt were dehulled before milling. White flour was produced with a Chopin CD1 laboratory mill. Samples were carefully milled and then immediately cooled to −20 °C to protect bioactive components from heat damage. Wholegrain samples were used to determine alkylresorcinols, whereas wholemeal samples were used to determine the other groups of phytochemicals, β-glucans, and estimated total dietary fiber. Bran and flour samples were used to determine arabinoxylan fractions.

The contents of these bioactive components are described in detail in the accompanying papers. These papers also report details of the experimental procedures used to analyze the grain for sterols (*22*), tocols (*23*), alkylresorcinols (*24*), folates (*25*), phenolic acids (*26*), and dietary fiber components (*27*).

All concentrations are expressed on a dry matter basis.

COMPARATIVE LEVELS OF PHYTOCHEMICALS AND DIETARY FIBER COMPONENTS IN CEREAL SPECIES

The ranges of concentrations of phytochemical and dietary fiber components in the different species are summarized in **Figure 1** with data points corresponding to individual lines. Because 130 winter bread wheat lines were analyzed compared with only 20 spring bread wheats and 5 or 10 of the other species, it is not surprising that greater diversity is apparent in the former. Nevertheless, some clear trends can be noted.

Phenolic Compounds. Phenolic components are the most diverse and complex group of phytochemicals in cereal grain, including phenolic acids, lignans, alkylresorcinols, and flavonoids and are known to be concentrated in the outer layers of the grain.

The most complex group of phenolic compounds in the small-grain cereals are the phenolic acids. These have an aromatic ring with one or more hydroxyl groups and are divided into two groups based on hydroxycinnamic acid and hydroxybenzoic acid. The major forms in wheat grain include ferulic acid, *p*-coumaric acid, sinapic acid (all based on cinnamic acid), syringic acid, vanillic acid, and forms of hydroxybenzoic acid (all based on benzoic acid). Although some phenolic acids exist in free form, the majority are linked via a range of bonds to either insoluble polymeric components (notably arabinoxylans, proteins, and lignin) (defined as bound phenolics) or low molecular weight components such as sugars, sterols, and terpenes (conjugated phenolics). It is therefore usual to isolate grain phenolics in three fractions corresponding to the free, bound, and conjugated forms.

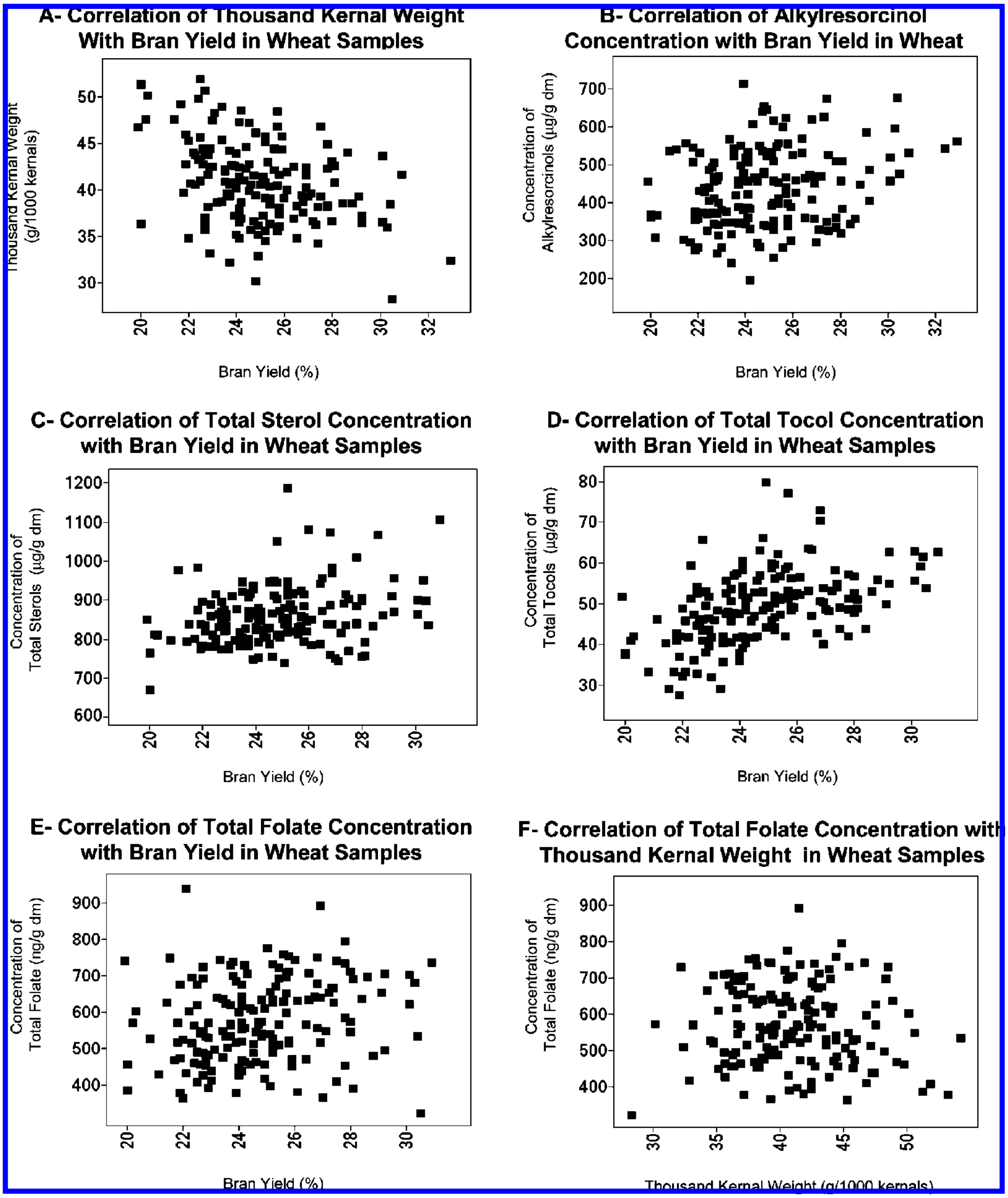
Details of the amounts and compositions of these three phenolic acid fractions are reported in the relevant papers in this issue (*26, 28−30*), and only the total amounts of the three groups are compared in **Figure 1A−D**. The total amounts of all phenolic acids (free, conjugated, and bound) show similar ranges but tend to be lower for barley (**Figure 1A**), and this difference is also observed for the three separate fractions (**Figure 1B−D**). In addition, the content of free phenolic acids is clearly greater in oats, accounting for between 5 and 30% of the total phenolic acids compared with an average of 1.6% in other cereals. However, in most cases the amounts of these components fall within the range shown by the 130 winter wheat lines.

Alkylresorcinols are a group of phenolic lipids located exclusively in the outer (bran) layer of the grain. They are clearly present in higher levels in rye than in wheat, and higher levels are also present in the “primitive” wheat lines (*Triticum monococcum*, *Triticum dicoccum*, and spelt) than in the more advanced durum and bread wheat lines (**Figure 1E**). Within the latter the range is greater in winter than in spring wheats, but this may reflect the larger number of lines that were analyzed (130 compared with 20). Lower contents of alkylresorcinols were present in the barley varieties, and they were absent from oats.

In contrast, oat contains a group of phenolic compounds that are not present in the other cereals: the avenanthramides (*31*). These are in fact *N*-cinnamoylanthranilate alkaloids derived from *p*-coumaric, caffeic, or ferulic acids with a number of components being present in groats and/or hulls (*31, 32*).

The total levels in the five oat lines ranged from about 42 to 91 μg/g (*28*).

Folates. Folates exist in several forms, with 5-formyltetrahydrofolate being the most abundant vitamer in cereals (*25, 28−30*).


A- Correlation of Thousand Kernal Weight With Bran Yield in Wheat Samples

B- Correlation of Alkylresorcinol Concentration with Bran Yield in Wheat

C- Correlation of Total Sterol Concentration with Bran Yield in Wheat Samples

D- Correlation of Total Tocol Concentration with Bran Yield in Wheat Samples

E- Correlation of Total Folate Concentration with Bran Yield in Wheat Samples

F- Correlation of Total Folate Concentration wit Thousand Kernal Weight in Wheat Samples

Figure 5. Correlations between (**A**) 1000 grain weight and bran yield, (**B**—**E**) concentrations of phytochemicals and bran yield, and (**F**) folate and 1000 grain weight in wheat varieties in the HEALTHGRAIN diversity screen.

The range of concentrations of total folates (**Figure 1F**) was widest in *T. monococcum* and *T. dicoccum* and narrowest in oats, with the spring and winter wheats showing almost identical ranges.

Terpenoids. Two major groups of terpenoids were determined: the phytosterols and tocols (**Figure 2**).

The total sterols (**Figure 2A**) comprise sterols and stanols, a total of 15 individual components in the different species (*22, 28—30*). The individual species have defined concentration ranges that vary between species and type. The concentrations were highest in rye, with all 10 lines having higher levels than in the hexaploid wheats (bread wheats and spelt), and lowest in oats (except for one winter wheat line). The tetraploid (*T. dicoccum*, durum) and diploid (*T. monococcum*) wheats contained higher levels than the bread wheats with *T. monococcum*, in particular extending into the concentration range of the rye lines. Thus, the highest levels were present in the three diploid species of the Triticeae: rye, *T. monococcum*, and barley.

The tocols comprise tocophenols and tocotrienols, with each group occurring in four forms (α, β, γ, and δ). α- and β-tocols were important vitamers in all grains, whereas γ- and δ-tocols were present in considerable amounts only in barley. The distribution of total tocols was broadly similar to that of total sterols (compare **Figure 2B** with **Figure 2A**) with some notable differences. First, the ranges within all species were greater than those for total sterols. Second, the range in bread wheats was much greater than those in the other species with winter wheats containing the highest levels and, with the exception of oats, also the lowest levels, of total tocols. As with total sterols, all oat varieties tended to have low levels of tocols.

Dietary Fiber. Dietary fiber comprises the grain components that are not digested by the time that they enter the colon. It comprises mainly nonstarch polysaccharides and oligosaccharides, lignin, undigestable (or resistant) starch, and substances associated with nonstarch polysaccharides and lignin. In the

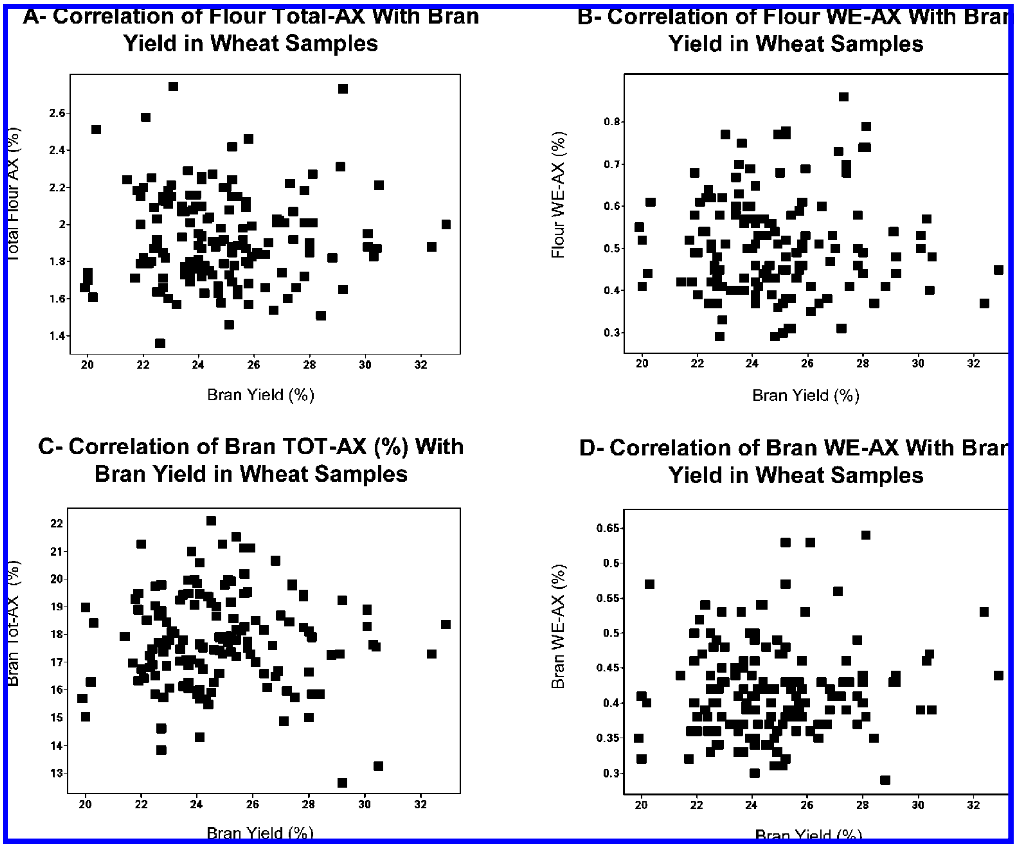


Figure 6. Correlation between bran yield and (**A**) flour T-AX, (**B**) flour WE-AX, (**C**) bran T-AX, and (**D**) bran WE-AX in wheat varieties in the HEALTHGRAIN diversity screen.

present study, dietary fiber was estimated by difference, subtracting the contents of crude protein, crude ash, total lipids, free sugars, and enzyme digestible starch from the dry weight of the flour (*27*).

The estimated dietary fiber contents ranged from about 85 to 270 mg/g of dm, being greatest in the three cereals that are husked: barley, rye, and oats (**Figure 3A**). However, the single naked line of oats (Cacko) contained a substantially lower level of fiber than the others (123 mg/g of dm compared with 200−248 mg/g of dm) (*29*). Similarly, the naked barley lines (Rastik and CFL98-450) were low in fiber content compared to the hulled ones (*28*). The ranges of estimated fiber levels in the spring and winter bread wheat lines extended to higher values than in the diploid and tetraploid *Triticum* species. The estimated fiber contents of the spelt lines were at the lower end of those for bread wheats. At first glance this is surprising as the main difference between spelt and bread wheats is the presence of a hull, which would result in higher fiber contents. However, the spelt samples were dehulled before milling (*27*).

The major components of the cereal endosperm cell walls are (1−3)(1−4)-β-D-glucans (mixed-linkage β-glucans) and arabinoxylans (AX), and these constitute the major dietary fiber components in flour and bran fractions. Mixed-linkage β-glucans were determined on wholemeal flour samples, showing much higher contents in barley and oats than in the other species (**Figure 3B**). This is consistent with the fact that the starchy endosperm cell walls of these species are known to be rich in β-glucans, whereas those of wheat and rye are richer in AX (see refs *33*−*35*).

Arabinoxylans can be divided into two fractions based on their extractability or nonextractability in water. The former, called water-extractable (WE)-AX, are of particular interest as they constitute part of the soluble dietary fiber fraction (with β-glucans), which is considered to have health benefits (*36*−*39*).

The contents of WE-AX and total AX (TOT-AX) were determined on flour and bran fractions derived from the different cereal lines (**Figure 4A**,**B**). The TOT-AX levels in flour fractions were similar for all wheat species and barley, but were substantially higher for rye and lower for oat (**Figure 4B**). For WE-AX in flour, the highest concentrations were observed in rye, whereas lower levels were measured in barley, oat, spelt, and *dicoccum* compared to the other wheat types (**Figure 4D**). Similar analyses of the bran fractions are shown in **Figure 4A**,**C**. The WE-AX levels in the bran samples were similar to those in the flour samples. The highest contents of TOT-AX were present in bran of the spring and winter types of wheat, whereas lines of barley and oats had the lowest TOT-AX. In addition to the total levels of AX and WE-AX in flour and bran, measurements were also made of the arabinose/xylose ratios in each fraction, the results being shown in **Figure 4E**−**H**. In the TOT-AX fraction, the oat and rye flour samples contain higher proportions of arabinose. The bran fractions of the oat samples contained the lowest proportions of arabinose, whereas the durum wheat bran samples had the highest arabinose/xylose ratios. The same measurements were also made on the WE-AX fraction (**Figure 4G**,**H**). The proportions of arabinose were similar in the water-soluble fractions of flours of all of the cereals except for the oat samples, for which the arabinose/xylose ratios were considerably higher. The arabinose/xylose ratios of the WE-AX bran fractions varied more between the different cereals, with oat and spelt containing higher proportions of arabinose. Conversely, the

Table 3. Breakdown of Lines with Regard to Contents

(**A**) Lines with High Contents of Phytochemicals, Dietary Fiber Components, and Phytochemicals + Fiber Components

lines with high phytochemical content (total phytochemical ranking 6−9)		lines with high fiber content (total fiber ranking 6−11)		lines with high phytochemical and high fiber content
Akteur	Atlas 66	Agron	Amadeus	CF99105
Augusta	Azteca 67	Cardinal	Catbird	Disponent
Biscay	Cadenza	CF9907	CF99105	Hereward
Campari	CF99105	Dekan	Disponent	Moulin
Claire	Disponent	Etoile de Choisy	Fundulea 29	
Ellvis	Estica	Galahad	Gerek-79	
Hereward	Herzog	Geronimo	Granbel	
Kanzler	Lynx	Hereward	Iljicsovka	
Malacca	Monopol	Jubilejnaja-50	Karl 92	
Moulin	Rialto	Kirac66	Kirkpinar 79	
Riband	Thatcher	Klein Estrella	Magdalena FR	
Tommi	Tremie	Martonvasari 17	Milan	
		Momtchil	Moulin	
		Nap-Hal	Nomade	
		Pastor	Ravenna	
		Red Fife	Renan	
		Rusalka	Sadovo 1	
		San Pastore	Sava	
		Spark	Ukrainka	
		Zvezda		

(**B**) Lines with High Contents of Phytochemicals, Dietary Fiber Components, and Phytochemicals + Fiber Components, Combined with Bran Yields below 26% and 1000 Grain Weights above 35 g (dw)

lines with high phytochemical content (total phytochemical ranking 6−9)		lines with high fiber content (total fiber ranking 6−11)		lines with high phytochemical and high fiber content
Akteur	Campari	Agron	Amadeus	CF99105
CF99105	Estica	Cardinal	CF99105	
Cadenza	Lynx	Iljicsovka	Magdalena FR	
Azteca 67	Tommi	Klein Estrella	Sadovo 1	
		Momtchil	Ukrainka	
		San Pastore	Yubileinayais 50	
		Zvezda		

WE-AX fractions of barley and rye had the lowest proportions of arabinose.

COMPARATIVE ANALYSIS OF THE BREAD WHEATS

Agronomic and Quality Parameters. The agronomic properties, 1000 kernel weight, kernel hardness, protein content, gluten content, and Zeleny sedimentation of the wheats were determined on grain from two field experiments harvested in 2005 (which were used for the analyses discussed above) and 2006. They are described in detail elsewhere (*21*) and summarized only briefly here.

The diversity of the wheat genotypes is exemplified by their grain yields, which differed by >4-fold in the field trials. Clear variation was also observed in morphology, growth habit, and resistance to fungal diseases. There was a 20 day difference between the heading dates of the earliest (Yumai 34) and the latest (Akteur) varieties. Variation was also present in traditional milling and baking quality parameters, with the 1000 kernel weight ranging between 28.3 and 53.9 g. The test weight was >78 g/L in 63% of the lines, the remaining lines having test weights ranging down to 68 g/L. Seventy-two percent of the kernels were hard, 22.7% were soft, and 5.3% were of mixed hardness type. The hardness index ranged between 60 and 70 in 32% of the genotypes, with the extreme values being 10 and 86. The bran yield varied between 20 and 32%.

The protein content of white flour determined using a Kjeltec 1035 Analyzer varied from 10.5 to 19%. The protein content of the wholemeal was also measured, showing that the difference between the protein contents of the white and wholemeal flours was greatest for the samples with relatively low protein contents. The wet gluten content ranged from 24 to 48%, with 9% of the genotypes having wet gluten contents below 30%, 55% of the genotypes having wet gluten contents of 30−40%, and 27% of the genotypes having wet gluten contents of >40%.

Zeleny sedimentation provides an estimate of the bread volume, with values above 30 mL indicating that the variety is suitable for breadmaking. The Zeleny sedimentation values for the bread wheat genotypes ranged between 11 mL (Sumai 3) and 57 mL (Plainsman V), indicating wide variation in breadmaking performance.

Association of Bioactive Components with Grain Characteristics. It is well established that the outer parts of the grain (the aleurone, pericarp, and testa) are enriched in many phytochemicals (e.g., phenolics, alkylresorcinols, and phytosterols) and dietary fiber components (*40−43*). Hence, higher proportions of these components would be expected in lines in which the outer layers account for a higher proportion of the total grain. This can be confirmed by comparison of the proportion of bioactive components with 1000 grain weight (smaller grains having a greater ratio of outer layers to starchy endosperm) and bran yield. These two parameters were negatively correlated (**Table 2**; **Figure 5**A).

The correlations between bran yield, 1000 grain weight, and bioactive components are illustrated in **Table 2** and **Figures 5** and **6**. Significant positive correlations were observed between total alkylresorcinols, total tocols, TOT-AX in bran, and bran yield. Because bran yield was also inversely correlated with 1000 grain weight, these components also showed similar, but negative, correlations with this parameter. Total sterols also showed a significant negative correlation with 1000 grain weight, but the correlation with bran yield was not statistically significant. With the exception of TOT-AX in bran, none of the fiber components showed statistically significant correlations with bran yield or 1000 grain weight.

The content of total folates did not clearly correlate with either 1000 grain weight or bran yield when all wheat lines were considered (**Table 2**; **Figure 5E**,**F**). However, Piironen et al. (*25*) showed that when the lines were separated into winter and spring types, contrasting trends were observed, with the total folate content correlating positively with bran yield in the winter lines but negatively in the spring lines.

Selection of Wheat Varieties with High Contents of Bioactive Components. The availability of an extensive database of grain composition and other properties means that it is relatively easy to select lines containing high levels of individual bioactive components or combinations of these. To do this the lines were grouped into five classes for each phytochemical and dietary fiber component, with group 1 comprising the 30 lines with the highest contents and group 5 the 30 lines with the lowest contents. Addition of the scores for the individual phytochemicals means that lines with the highest levels of all components (tocols, sterols, alkylresorcinols, phenolics, and folates) would have a score of 5. Similarly, lines with the highest levels of all dietary fiber (DF) components (β-glucan %, flour WE-AX %, flour total AX%, bran WE-AX %, and bran total AX %) would also have a score of 5. In fact, the total phytochemical scores of the lines ranged from 6 to 24, the DF scores from 6 to 24, and the total bioactive scores (phytochemical score + DF score) from 13 to 43. Lines with the lowest scores (i.e., highest amounts) for these compoments are listed in **Table 3A**. The availability of data for seed weight and bran yield also allows us to restrict the selection to lines that have high seeds weights and low bran yields, as shown in **Table 3B**.

The data in **Table 3** are, of course, based only on material grown on a single site and for one growing season. Consequently, they will be biased toward the selection of varieties that are particularly well adapted to the geographical location and local weather conditions during growth. Nevertheless, they do demonstrate that it is possible to combine high yield and good milling performance with high levels of bioactive components

Potential for Exploitation. The HEALTHGRAIN diversity screen has generated the most extensive database currently available on bioactive components in wheat and other small-grain cereals. It includes data on several groups of phytochemicals that are considered to have potential health benefits and on dietary fiber components. Although most of the analyses were carried out on wholemeal fractions, determination of a range of other grain traits and processing properties allows the selection of lines in which high levels of one or more groups of bioactive components are present throughout the grain (rather than concentrated in the bran) and are combined with acceptable yield and processing quality.

Nevertheless, it must be borne in mind that the data originate from a single series of samples grown on one site in one year. Hence, the extents of environmental impacts on the amounts and compositions of bioactive components and of genotype × environment interactions are not known. Although the literature on this topic is limited, available papers indicate that it certainly occurs. For example, multisite trials have shown variation in phenolic acid content and composition in hard spring wheats (*45*). However, these data also indicate that although the absolute amounts of the components may vary from site to site and year by year, the ranking order of the lines remains fairly constant. It is therefore concluded that further studies on a range of sites over several years should lead to the identification of lines which are high in bioactives and suitable for cultivation in a range of climatic zones or for incorporation into breeding programs as sources of high levels of bioactive components.

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Received for review March 27, 2008. Revised manuscript received June 24, 2008. Accepted September 5, 2008. This work is financially supported by the European Commission in the Communities 6th Framework Programme, Project HEALTHGRAIN (FOOD-CT-2005-514008). It reflects the authors' views, and the Community is not liable for any use that may be made of the information contained in this publication. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the U.K. The Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium) is acknowledged for the postdoctoral fellowship of K.G.

JF8009574