DOI:10.1021/jf100053d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Genotype and Environment on the Content and Composition of Phytochemicals and Dietary Fiber Components in Rye in the HEALTHGRAIN Diversity Screen†

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The effects of genotype and environment on the content of bioactive components in rye were determined with four varieties being grown on one site for three years and on three additional sites in the third year and a fourth variety being included in all trials except year 1. Clear differences were observed in the extent to which the contents of dietary fiber components (arabinoxylan, β-glucan, total dietary fiber) and phytochemicals (folates, alkylresorcinols, sterols, tocols, phenolic acids) varied between varieties and between the same varieties grown in different sites (United Kingdom, France, Hungary, Poland) and years (2005−2007 in Hungary), with sterols being the most stable and phenolic acids the least. However, no single variety could be selected as having the highest overall level of bioactive components or as being more stable in comparison across environments.

KEYWORDS: Rye; wholegrain; phytochemicals; dietary fiber; bioactive compounds

INTRODUCTION

Rye (*Secale cereale* L.) is the eighth most important cereal crop in the world with over 17 million tonnes being harvested in 2008 (http://faostat.fao.default.spx). It is traditionally grown and consumed in eastern and northern Europe because of its winter hardiness and adaptation to marginal soils (*1*). However, consumer demand for healthy wholegrain products is leading to expanding markets and increased research interest. Rye grain contains up to twice the level of dietary fiber that is present in wheat grain, with arabinoxylan (AX) being the major type of fiber in both species with lower levels of (1,3)(1,4)-β-D-glucan (β-glucan) (*2*−*4*). β-Glucan has well-established health benefits (reviewed in refs *5* and *6*), which are recognized by approved health claims for barley and oat products in several countries. These benefits appear to be shared by AX (*7*, *8*), although the relative efficacy of the two types of fiber has not been established. Rye is also rich in different types of bioactive components, containing higher levels of alkylresorcinols and sterols than wholegrain wheat and similar levels of phenolic acids, folates, and tocols (*2*, *4*). Tocols (which include forms of vitamin E) and phenolic acids have antioxidant activity, whereas sterols and folates have established benefits in lowering serum cholesterol (*9*) and in fetal development and a number of other conditions (*10*−*13*), respectively. Alkylresorcinols are a group of phenolic lipids located in the outer part of the grain of wheat and rye that may be used as biomarkers for consumption of wholegrain products as well as having potential health benefits (*14*).

The EU FP6 HEALTHGRAIN project focuses on improving the well-being of consumers by reducing risks of metabolic diseases by increasing the consumption of wholegrain products made from wheat and rye (*15*). It included a "diversity screen" to determine the current range of variation in the contents of bioactive components (dietary fiber and phytochemicals), with 150 wheat lines, 10 rye lines, and 40 other cereals being analyzed for their contents and compositions of the groups of bioactive components discussed above (*2*, *4*). Substantial variation in composition was demonstrated among the 10 rye lines, but because these were only grown on one site (Martonvásár,

†Part of the HEALTHGRAIN 2 symposium.

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 Published on Web 05/03/2010

Hungary) for one harvest (2005), it was not possible to separate the contributions of genetic and environmental factors to the observed differences.

A second series of experiments were therefore established, in which 26 selected wheat lines and 5 rye lines were grown at the same site in Martonvásár for two further years (2006, 2007) and at a further three sites in the United Kingdom, France, and Poland for the third year only. Detailed analyses of the wheat lines are reported in the accompanying papers with the present paper focusing on rye.

MATERIALS AND METHODS

Samples. Haute Loire is an old French population that is more genetically heterogeneous than commercial varieties. Dankowskie Zlote is an old Polish cultivar with wide adaptability, whereas Nikita and Rekrut are modern high-yielding German varieties. These four "varieties" were included in the diversity screen in 2005. The fifth variety, Amilo, a widely grown modern variety from Poland, was included in 2006 and 2007 because of its selection as a standard for module 3 (technology and processing) of HEALTHGRAIN. The lines were grown on different sites at Martonvásár (near Budapest, Hungary) in 2006 and 2007 and at Saxham (near Bury St. Edmunds, United Kingdom), Clermont-Ferrand (France), and Choryń (near Poznan, Poland) in 2007. Thus, data from six different environments (i.e., site × year combinations) were analyzed. Agronomy followed conventional practices for the sites. Full details of the sites, their soil type and mineral composition, and the weather conditions are given in Shewry et al. (*16*). All harvested samples were milled at Martonvásár as described in detail by Ward et al. (*4*). Wholemeal samples were milled on a Perten laboratory mill 3100 with a 0.5 mm sieve, after conditioning to 14% moisture content. Flour and bran were produced by milling conditioned samples on a Chopin CD1 laboratory mill (Tripette and Renand, France). All samples were cooled immediately to −20 °C and stored in sealed plastic bags. All analyses are expressed on a dry matter (dm) basis.

Determination of Phytochemicals. Alkylresorcinols were extracted from intact kernels and analyzed according to the method given in ref *17* with only minor modifications (*18*). Briefly, an internal standard was added to wholegrain samples, which were thereafter extracted with ethyl acetate and analyzed by gas chromatography (GC). All samples were analyzed in duplicate with the difference between duplicates being < 10%. An in-house reference (wholemeal rye flour) was used to confirm the accuracy of the analysis.

Tocols (α-, β-, γ-, and δ-tocopherols and tocotrienols) were determined by normal-phase HPLC using fluorescent detection after hot alkaline hydrolysis of the wholemeal flour samples according to the method given in ref *19*.

Sterols were determined by GC after acid and alkaline hydrolyses of the wholemeal flour samples according to the method given in ref *20*.

Total folate contents were determined using a microbiological assay (*21*). Three varieties (Haute Loire, Dankowskie Zlote, and Amilo) were analyzed for folate vitamers by HPLC after affinity chromatographic purification (*22*). The sample preparation procedure included heat extraction followed by trienzyme treatment with α-amylase, hog kidney or chicken pancreas conjugase, and protease (*22*, *23*). A certified reference material, CRM 121 (wholemeal flour), and an in-house reference were used to confirm the accuracy of the analysis (*22*).

Phenolic acids were determined by reversed-phase HPLC according to the method given in ref *24*. Separate duplicate extractions were made for free, conjugated, and bound phenolic acids using 3,5-dichloro-4-hydroxybenzoic acid as internal standard. Free phenolic acids were extracted using a simple aqueous ethanolic extraction, whereas conjugated and bound phenolic acids were released by NaOH hydrolysis and extracted (following acidification with 2 M HCl) using ethyl acetate.

Determination of Nonstarch, Noncellulosic Polysaccharide Content, and Composition. Total (TOT-) and water extractable (WE-) AX were determined by GC of alditol acetates as described by Gebruers et al. (*3*). Nonstarch, noncellulosic polysaccharides were hydrolyzed with trifluoroacetic acid, the resulting monosaccharides were reduced with sodium borohydride and acetylated with acetic anhydride, and the alditol acetates were then separated on a Supelco SP-2380 polar column and detected with a flame ionization detector in an Agilent GC (Agilent 6890 series, Wilmington, DE). The coefficient of variation of the analytical data was typically 3% for triplicate analyses. AX content was defined as 0.88 times the sum of xylose and arabinose. The arabinose content of flour AX was corrected for the presence of arabinogalactan peptide on the basis of an arabinose to galactose ratio of 0.7 and assuming that all of the arabinose of arabinogalactan peptide was water-soluble (*25*).

β-Glucan was determined in wholemeal with the Megazyme Mixed-Linkage β-Glucan Assay Kit (Megazyme, Bray, Ireland) according to the streamlined method that is consistent with methods AOAC 995.16, AACC 32-23, and ICC 166 (*26*). The assay is based on the enzymic degradation of β-glucan with lichenase and β-glucosidase and the quantification of the glucose released using an oxidase/peroxidase reagent. All measurements were in duplicate. The experimental error was below 4% deviation of the mean value.

Total dietary fiber (TDF) was determined using the Uppsala method (AOAC 994.13, AACC 32-25) as the sum of nonstarch polysaccharides (NSP), uronic acids, and Klason lignin. Insoluble and soluble NSP analyses included preparation of a residue after treatment with thermostable α-amylase and amyloglucosidase. Soluble and insoluble NSP components were fractionated and further analyzed separately (*27*, *28*) with soluble components being precipitated with 80% ethanol. After acid hydrolysis, neutral polysaccharide residues were quantified as alditol acetates by GC (*29*) using a Perkin-Elmer AutoSystem XL; uronic acid residues were determined by colorimetry, and Klason lignin was determined gravimetrically after correction for ash content (AACC method 32-25). Total NSP was calculated as the sum of insoluble and soluble polymers. Viscosity of grain water extracts was measured as described earlier (*3*) using the Brookfield Cone/Plate Digital Viscometer, model LVDV-II+ (Stoughton, MA), with an 0.8° cone spindle and a shear rate of 225 s at 25 °C. The replicate errors for these analyses was below 3%, and therefore statistical deviations are not shown.

Determination of Other Grain Components. Moisture, crude protein, and ash contents were determined according to AACC approved methods 44-15A, 46-10, and 08-01, respectively. Total lipids were determined gravimetrically by extraction with an acid solvent consisting of 60:40:1 (v/v/v) chloroform, methanol, and hydrochloric acid as described in ref *30*. Available starch was determined with the procedure of Megazyme without dimethyl sulfoxide, consistent with AACC approved method 76-13, and free sugar by GC as a sum of all mono- and disaccharides, that is, fructose, glucose, maltose, and sucrose (*31*). Amylose content was determined with a Megazyme kit (*32*, *33*).

Statistical Analyses. Statistical analyses were performed with Statistical Analysis Software (SAS version 9.3.1) The general linear model procedure (GLM) was used for variance analysis, whereas the procedure VARCOMP was used to estimate variance components with all effects considered as random.

For variance analysis, data sets from the five rye varieties grown in the six environments were used in the following statistical model: $X = \mu + E + G + \varepsilon$, with μ being the grand 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. The G × E interaction was thus included in the error term, which is likely to be an overestimate of the true error.

The comparison of variance components allowed the calculation of the ratio $\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_{G\times E})$. In absence of replicate, $\sigma^2_{G\times E}$ was replaced by $\sigma^2\varepsilon$. This ratio is in fact an underestimate of broad sense heritability and is a suitable parameter for plant breeders, as a high value indicates that genotype behavior is predictable and that the trait is probably amenable to genetic improvement.

RESULTS AND DISCUSSION

Four rye varieties (Haute Loire, Nikita, Rekrut, Dankowskie Zlote) were grown at Martonvásár over three successive seasons (2004−2005, 2005−2006, 2006−2007) and at three additional sites in 2006−2007 only, providing six environments (ie., site × year combinations). The fifth variety, Amilo, was not grown in 2004−2005, giving only five environments. The sites used in 2006−2007 were selected to give a wide range of environments, from the oceanic climate of the United Kingdom to the

Table 1. Means and Standard Deviations of Fiber Compositions of the Rye Lines Grown in Six Environments

	cultivar means					cultivar standard deviations				
component	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo
thousand kernel weight	36.75	38.02	37.62	38.57	37.93	5.28	4.55	5.03	3.57	4.60
Chopin bran yield (%)	36.53	37.33	36.57	35.90	36.30	6.28	5.93	4.41	4.31	4.04
Chopin flour yield (%)	38.05	39.97	40.45	40.27	38.92	3.77	4.88	5.40	5.08	3.95
protein	13.42	11.30	11.37	11.62	11.20	1.74	1.40	1.60	1.62	1.40
ash	2.07	1.83	1.82	1.80	1.90	0.19	0.26	0.24	0.22	0.25
lipids	2.57	2.40	2.42	2.28	2.26	0.16	0.22	0.19	0.13	0.05
digestible starch	55.05	57.93	57.47	58.77	58.04	1.43	0.97	1.44	1.68	1.57
free sugars	2.95	2.65	2.55	2.52	2.32	1.31	0.94	0.80	0.80	0.49
Klason lignin	2.65	2.62	2.62	2.13	2.50	0.32	0.33	0.20	0.16	0.25
uronic acids	0.23	0.24	0.21	0.21	0.22	0.02	0.02	0.02	0.02	0.02
estimated TDF	22.28	22.35	22.95	21.77	23.12	2.01	1.12	1.28	1.63	2.07
water extract viscosity	51.78	48.20	45.30	35.33	61.42	45.04	42.29	37.38	20.91	23.56
wholemeal										
TDF (%)	16.30	16.19	15.52	15.13	16.30	0.79	0.74	0.47	0.69	0.30
β-glucan (%)	1.83	1.83	1.92	1.89	1.62	0.28	0.29	0.24	0.25	0.45
TOT-AX (%)	8.19	8.02	7.70	7.61	8.13	0.38	0.36	0.36	0.47	0.37
TOT-NSP (%)	13.28	13.14	12.73	12.74	13.58	0.57	0.81	0.60	0.52	0.39
flour										
WE-AX (%)	1.28	1.35	1.38	1.34	1.44	0.09	0.11	0.28	0.31	0.29
A/X ratio	0.63	0.60	0.58	0.58	0.59	0.03	0.03	0.03	0.02	0.02
TOT-AX (%)	3.30	3.27	3.08	2.95	3.01	0.50	0.57	0.48	0.26	0.30
A/X ratio[a]	0.75	0.73	0.71	0.72	0.69	0.01	0.04	0.01	0.06	0.10
bran										
WE-AX (%)	1.21	1.15	1.11	1.04	1.12	0.24	0.20	0.20	0.18	0.12
A/X ratio	0.78	0.73	0.73	0.72	0.73	0.04	0.04	0.03	0.03	0.03
TOT-AX (%)	13.04	13.34	12.42	12.14	13.20	0.95	1.47	1.43	0.53	0.77
A/X ratio	0.56	0.53	0.53	0.54	0.54	0.03	0.03	0.03	0.02	0.03

[a] A/X, ratio of arabinose to xylose in arabinoxylans.

Table 2. Means and Standard Deviations of Contents and Compositions of Tocols, Sterols, and Alkylresorcinols of the Rye Lines Grown in Six Environments

	cultivar means					cultivar standard deviations				
component	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo
tocols (μg/g)	56.22	59.92	58.86	55.05	58.99	5.96	9.38	7.66	7.46	8.42
α-tocopherol	21.53	19.63	18.72	18.08	19.24	1.59	2.28	2.57	2.03	1.69
β-tocopherol	5.35	4.30	4.19	4.10	4.39	0.56	0.69	0.56	0.57	0.65
α-tocotrienol	19.12	22.06	21.52	19.32	20.22	3.03	4.92	4.02	3.49	4.61
β-tocotrienol	10.16	13.93	14.44	13.89	14.73	2.76	3.13	3.00	3.30	2.91
folate (ng/g)	1018.33	880.67	889.67	864.67	935.60	176.03	171.38	153.70	142.86	181.23
total sterols (μg/g)	1233.33	1110.17	1116.17	1118.00	1083.40	99.78	96.84	77.60	58.45	72.18
sitosterol	620.50	526.33	526.33	519.50	512.20	46.49	39.94	35.01	19.85	28.20
campesterol	221.50	198.67	197.00	191.17	180.00	20.66	25.96	26.71	15.89	14.53
stigmasterol	33.83	40.67	38.33	34.83	36.60	5.19	8.55	8.43	4.22	6.69
stanols	186.33	196.50	201.67	211.83	193.60	24.43	27.62	20.21	19.45	17.71
other sterols	171.00	148.17	152.83	160.83	161.40	10.83	9.64	18.82	9.62	13.39
total AR[a] (μg/g)	905.67	1030.00	901.33	919.83	957.60	123.57	223.28	168.43	130.36	63.33
C17:0	212.30	237.75	217.05	232.37	230.86	27.48	54.32	43.75	41.43	14.96
C19:0	289.72	327.63	289.93	298.87	314.90	42.38	71.46	51.88	47.97	19.64
C21:0	213.52	254.88	208.30	206.12	220.30	32.10	55.50	42.09	28.13	23.45
C23:0	98.47	112.40	93.97	92.12	98.20	15.84	27.56	19.96	12.22	10.55
C25:0	91.58	98.72	92.15	90.32	93.36	9.18	16.44	17.66	11.66	13.12
ratio C17:0/C21:0	1.00	0.93	1.04	1.12	1.05	0.04	0.07	0.07	0.10	0.10

[a] AR, alkylresorcinols.

continental climate of Central Europe. In fact, the range of weather conditions experienced over this year (summarized in the accompanying paper (*16*)) was greater than expected on the basis of previous weather records, with the Martonvásár site (Hungary) experiencing particularly high temperatures during grain filling and the U.K. site (Saxham) unusually high levels of precipitation over the same period.

The varieties grown in 2005−2006 and 2006−2007 were harvested, milled, and analyzed for a range of dietary fiber and bioactive components as described previously for the 2004−2005 harvest (*2*). Full analyses of the samples are provided in the Supporting Information (Tables S1−S3).

The mean contents of the selected components in the five varieties are given in **Tables 1−3**, based on analyses of all environments, whereas **Figure 1** shows the mean values for the major groups of components for the four sites in 2007 (left panels), the Martonvásár site (H) for three years (2005, 2006, 2007) (middle panels), and all six sites and years (right panels). This shows clear differences in both the extent to which the components vary between varieties and between sites and years

Table 3. Means and Standard Deviations of Contents and Compositions of Free, Conjugated, and Bound Phenolic Acid Fractions of the Rye Lines Grown in Six Environments

	cultivar means					cultivar standard deviations				
component	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo	Haute Loire	Nikita	Rekrut	Dankowskie Zlote	Amilo
total phenolic acids (μg/g)	1181.17	1242.00	1119.17	1116.67	1243.60	388.68	385.35	266.17	309.17	201.05
total free phenolic acids (μg/g)	22.17	22.33	21.67	17.17	22.00	6.11	7.42	7.31	6.91	10.51
4-hydroxybenzoic acid (μg/g)	3.02	1.50	2.10	1.71	1.76	1.20	0.26	0.22	0.87	1.17
vanillic acid (μg/g)	6.38	6.56	6.49	5.55	8.00	3.14	3.74	3.42	2.76	3.11
syringic acid (μg/g)	1.49	1.37	1.40	1.15	1.56	0.47	0.52	0.45	0.40	0.87
syringic aldehyde (μg/g)	3.03	2.77	3.72	3.65	3.70	2.18	2.41	2.05	2.16	2.25
caffeic acid (μg/g)	2.12	1.85	1.67	1.01	1.06	1.44	1.10	1.39	0.34	0.50
2,4-dihydroxybenzoic acid (μg/g)	1.33	1.47	1.24	1.48	1.32	0.51	0.64	0.76	0.78	0.74
sinapic acid (μg/g)	5.54	5.77	5.66	3.70	4.99	2.99	3.50	4.10	2.99	3.88
ferulic acid (μg/g)	5.51	5.48	5.31	4.92	5.47	0.94	1.43	1.29	1.30	2.24
total conjugated phenolic acids (μg/g)	226.17	189.00	182.83	166.33	170.20	51.83	66.97	39.21	38.00	67.67
4-hydroxybenzoic acid (μg/g)	7.72	5.43	5.31	4.81	4.67	6.99	4.13	4.73	3.91	4.04
vanillic acid (μg/g)	3.64	1.52	1.71	1.84	0.81	4.19	2.49	2.22	1.85	1.10
syringic acid (μg/g)	2.47	1.85	2.38	1.96	1.82	1.67	1.16	1.06	0.96	1.11
syringic aldehyde (μg/g)	4.24	3.60	4.88	4.68	4.67	2.88	2.83	2.47	2.52	2.92
caffeic acid (μg/g)	2.37	2.58	2.79	2.57	2.79	2.98	2.81	2.59	2.39	2.83
2,4-dihydroxybenzoic acid (μg/g)	22.07	19.52	19.63	15.28	7.93	32.08	26.66	27.52	20.15	13.24
sinapic acid (μg/g)	93.54	83.78	83.44	77.02	88.58	22.60	27.84	13.08	18.36	32.40
ferulic acid (μg/g)	87.53	69.39	61.74	56.31	58.60	28.41	39.06	22.06	24.52	35.33
total bound phenolic acids (μg/g)	932.83	1030.67	915.00	933.00	1051.40	389.03	409.05	289.76	316.39	201.78
4-hydroxybenzoic acid (μg/g)	4.01	3.74	3.35	3.33	3.44	1.25	1.63	1.16	1.07	1.55
vanillic acid (μg/g)	4.86	3.22	3.07	2.97	3.27	2.24	0.85	0.54	0.97	0.51
syringic acid (μg/g)	2.89	1.81	1.54	1.43	1.31	2.99	1.37	1.12	1.12	1.10
syringic aldehyde (μg/g)	1.97	1.95	1.68	1.78	1.61	0.55	0.34	0.22	0.24	0.24
caffeic acid (μg/g)	256.20	344.80	275.00	296.80	285.60	155.52	79.62	59.73	57.89	65.66
2,4-dihydroxybenzoic acid (μg/g)	70.40	50.28	46.42	49.12	41.60	86.49	48.63	45.72	39.95	22.56
sinapic acid (μg/g)	157.69	166.89	151.36	143.00	189.20	69.70	79.71	59.24	80.55	51.19
ferulic acid (μg/g)	483.30	511.94	473.35	482.85	523.80	186.85	197.16	135.26	125.89	140.77

(indicated by the SDs). However, although the means calculated for the different cultivars differed for some components, the effect of genotype on the overall composition was generally not significant. However, highly significant effects of environment were observed (**Table 4**). Significant values for genotype effects were also observed for some individual groups of components, bran TOT-AX, Klason lignin, wholemeal β-glucan, sterols, and total folates, with p values of 0.0162, 0.0142, 0.007, 0.0050, and 0.0432, respectively.

The five varieties also showed some differences in the extent to which their compositions varied across the environments, which is illustrated by comparing the standard deviations shown in **Figure 1**. However, these differences were not consistent between groups of components, and it was not possible to conclude that any individual varieties were more intrinsically stable than others. For most components, low values were observed for genotypic variance (estimated from only five cultivars), giving unsurprisingly low values (< 0.10) for the ratio $\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_{G\times E})$. Variance components for traits showing a significant genotype effect are given in Supplementary Table 4 of the Supporting Information. Detailed discussions of the individual groups of components are presented below.

Variation in Individual Groups of Components between Varieties, Sites, and Years. **Figure 2** provides details of the differences between the lines grown at individual sites and years; details of precipitation and temperature for the sites are provided by Shewry et al. (*16*). It is therefore possible to draw some broad comparisons between the local environment and grain composition.

Alkylresorcinols. The mean total alkylresorcinol contents of the five varieties grown in 2006 and 2007 varied between 725 and 1118 μg/g of dm, which is within the range reported for varieties grown in 2005 (821−1444 μg/g of dm for the 10 varieties, 881−1444 μg/g of dm for the 4 varieties also grown in 2006 and 2007) (*2*). The contents were, however, higher than in 30 Swedish rye samples grown in 1998, which had contents of 568−1022 μg/g of dm (*17*). The average contents in the different cultivars grown at the six environments were 906, 1030, 901, 920, and 958 μg/g dm for Haute Loire, Nikita, Rekrut, Dankowskie Zlote, and Amilo, respectively, which are not significantly different from each other. The relative homologue compositions were also similar for all varieties with 22−26% C17:0, 31−34% C19:0, 22−25% C21:0, 10−11% C23:0, and 8−12% C25:0. The ratio of C17:0 to C21:0 was 0.9−1.1, which is typical for rye (*17*, *34*).

The total alkylresorcinol content varied more between environments than between varieties, being highest in lines grown in Hungary in 2005 (881−1444 μg/g of dm) and lowest in those grown in Hungary in 2006 (724−1017 μg/g of dm). This contrasts with analyses of wheat lines grown in the same sites and years, where the lowest content was in lines grown in Hungary in 2005, the second lowest in those grown in Hungary in 2006, and the highest in Hungary in 2007 (*35*).

A significant correlation was observed between total AR content and the relative content of AR homologues when data for 26 wheat lines grown in the same environments were considered (*35*). A similar correlation was not observed for rye except for C25:0, which decreased with increasing AR content ($p = 0.014$, $R^2 = 0.21$), in accordance with wheat. The absence of correlation for the other homologues may be due to the low number of rye samples analyzed (5 varieties) compared to the wheat samples (26 varieties).

Tocols. The mean total tocol contents (55.0−59.9 μg/g of dm) of the 5 varieties grown in 2006 and 2007 were similar to those reported for the 10 cultivars grown in 2005 (*2*) and for 10 varieties grown in 1999 in Finland (*36*). Haute Loire had the lowest range (17.9 μg/g of dm) and Nikita the highest (22.8 μg/g of dm), but the

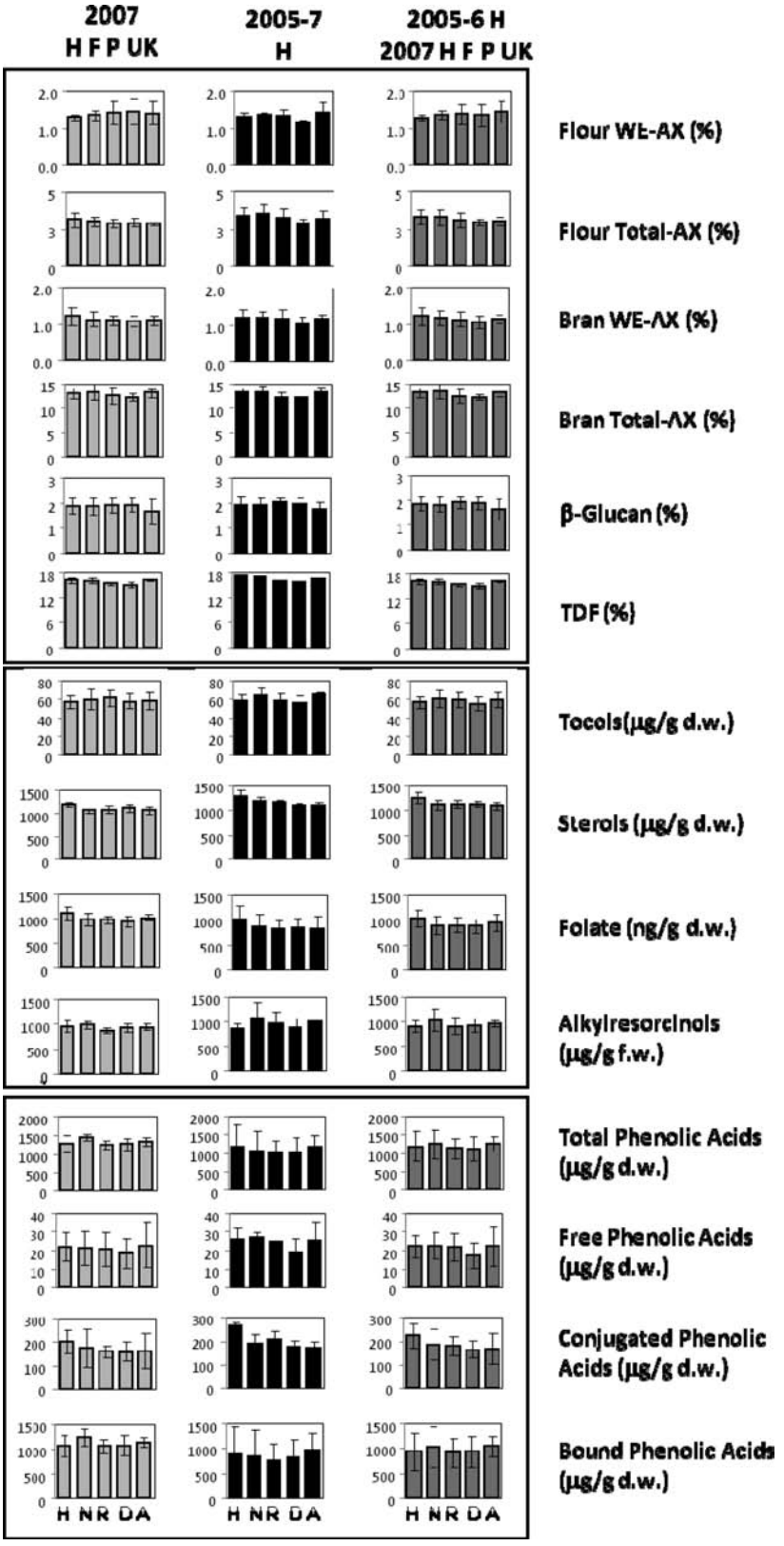

Figure 1. Mean contents of fiber and phytochemical components in the rye lines grown on four sites (in the United Kingdom, France, Poland, and Hungary) in 2007 (left-hand panels), in Hungary in 2005, 2006, and 2007 (middle panels), and in the six environments (right-hand panels). H, Haute Loire; N, Nikita; R, Rekrut; D, Dankowskie Zlote; A, Amilo.

mean values and standard deviations of the five varieties showed no major differences in total tocol contents. The range of total tocol contents was greater among the four sites in 2007 (19.6 ± 1.9 μg/g of dm) than in the three years at Martonvásár (11.7 ± 6.0 μg/g of dm).

Among growing years, the mean tocol content was highest in 2007 (66.3 ± 1.8 μg/g of dm) and lowest in 2006 (55.3 ± 4.3 μg/g of dm). When growing locations were taken into account, the mean tocol content was highest in the samples grown in Hungary (66.3 ± 1.8 μg/g of dm) and lowest in those grown in France

Table 4. *p* Values of the Contents of Bioactive Components with Genotype and Environment (i.e., Each Site × Year Combination)[a]

	main effect	
	genotype ($n = 5$)	environment ($n = 6$)
tocols (μg/g)	0.174	<0.001
folate (ng/g)	0.0432	<0.001
total sterols (μg/g)	0.0050	<0.001
total AR (μg/g)	0.207	0.003
total phenolic acids	0.427	<0.001
total free phenolic acids	0.121	<0.001
total conjugated phenolic acids	0.0597	0.003
total bound phenolic acids	0.452	<0.001
Klason lignin	0.0142	0.366
wholemeal β-glucan	0.0071	<0.001
flour WE-AX (%)	0.749	0.0140
flour TOT-AX (%)	0.155	<0.001
bran WE-AX	0.0865	<0.001
bran TOT-AX (%)	0.0162	<0.001

[a] Results were deemed to be significant at $p < 0.05$.

(47.1 ± 1.6 μg/g of dm). α-Tocotrienol was the major component in most samples, but α-tocopherol was the major tocol in the samples grown in France in 2007. In quantitative terms, the α-tocopherol levels in the French samples were similar to those from the other sites, with the increased proportion of α-tocopherol resulting from much lower α-tocotrienol contents.

The total tocol content and percentage of α-tocopherol showed a moderately strong negative correlation ($r = -0.609$; $p < 0.001$) with a similar relationship being found for the 26 wheat varieties grown in the same environments (*19*). This indicates that both wheat and rye have relatively constant levels of α-tocopherol, whereas the amounts of other tocols vary considerably.

Sterols. The mean total sterol contents of the five varieties grown in 2006 and 2007 (1083−1233 μg/g of dm) were similar to those reported for the 10 cultivars grown in 2005 (*2*) but higher than in two previously reported studies (700−1000 μg/g of fw) (*37*, *38*). Sitosterol was the major sterol in all samples with contents ranging from 474 to 712 μg/g of dm, and the content increased in line with that of total sterols. The total sterol content of Haute Loire (1233 ± 100 μg/g of dm) was significantly higher and clearly separated from the total sterol contents of the other varieties.

There were differences in total sterol content among growing years with the total contents in 2005 being the highest. In addition, the growing locations had an influence on total sterol contents and two separate groups could be defined: a high sterol group containing the Polish samples and a low sterol group containing the samples from Hungary and France. The mean values and standard deviations of total sterol contents of the five varieties were relatively stable in the six environments.

The mean total sterol content of the varieties grown in Hungary was higher in 2005 (1256 ± 136 μg/g of dm) and lower in 2006 and 2007 (1125 ± 48 and 1081 ± 49 μg/g of dm, respectively). This is consistent with a previous study (*37*), which showed that grain of six rye varieties harvested during dry and warm conditions had the lowest sterol contents. However, this relationship did not apply to the multisite trial in 2007, with the samples grown in Poland having the highest mean levels (1186 ± 42 μg/g of dm) and those grown in France the lowest (1057 ± 71 μg/g of dm).

Within individual varieties, Haute Loire and Nikita had smaller ranges in total sterol content between locations, whereas Rekrut, Dankowskie Zlote, and Amilo had smaller ranges between the three growing years in Hungary. This indicates that the varieties responded differently to the environmental factors and, thus, had different stabilities.

Total Folates. The mean total folate contents of the 5 cultivars grown in 2006 and 2007 (904−1070 ng/g of dm with an average of 973 ng/g of dm) were higher than the range of 574−775 ng/g of dm (average of 693 ng/g of dm) reported for the 10 cultivars grown in 2005 (*2*). For individual varieties, the difference between the highest and lowest folate contents were on average 1.7-fold. The ranges reported here are in accordance with previously published data. Hegedüs et al. (*39*) reported 650 ng/g of fw in rye flour, but higher values have been reported for grain: 920 ng/g of fw (*40*), 1226 and 1349 ng/g of dm for two Polish cultivars (*41*), and 1430 ng/g of fw (*42*). Ranges of 689−853 and 643−934 ng/g of dm were reported for grain of 10 rye varieties grown at the same location in 1999 (*43*) and 2000 (*44*), respectively.

The mean values and standard deviations of total folate contents of the five varieties were rather stable. Analysis of variance showed weakly significant differences in average total folate contents among the varieties grown in the six environments (p value = 0.0432) and no significant difference among the four growing locations. However, there was a stronger significant difference in total folate content between years ($p = 0.0006$) with higher folate contents in 2007 than in 2006 or 2005. The average total folate contents of the selected genotypes were 666 ± 66 ng/g of dm in 2005, 806 ± 125 ng/g of dm in 2006, and 1090 ± 138 ng/g of dm in 2007.

Haute Loire had the highest total folate content of the lines grown in Hungary over all three years and in Poland in 2007 and the widest overall range (548 ng/g of dm), whereas Dankowskie Zlote had the narrowest range (381 ng/g of dm). Within each variety, the ranges in total folate content among the three years were greater than those among the four growing locations, which differed from the analysis of wheat lines grown at the same sites (*22*).

Folate Vitamers. Whereas total folates were determined by microbiological assay, the individual vitamers were determined by HPLC (full analyses of vitamers in three varieties are presented in Supplementary Table 5 of the Supporting Information). HPLC gave lower total values, with the sum of the vitamers being about 60% of the total values by microbiological assay. Most of the folate was formylated, with 5-HCO-H_4folate accounting for 10−32% of the total (varying among growing locations) and 10-HCO-H_2folate and 10-CHO-PGA together for 17−36%. These two vitamers reflect the amount of 10-HCO-H_4folate that is readily oxidized. 10-HCO-H_4folate cannot be quantified in our HPLC system because it is rapidly converted to 5,10-CH^+-H_4-folate in the acidic mobile phase. Although 5,10-CH^+-H_4folate is an endogenous folate vitamer in plants, it may also be formed from 10-HCO-H_4folate and to a lesser extent from 5-HCO-H_4folate during the analysis (*45*). The proportion of 5,10-CH^+-H_4-folate ranged from 18 to 29% and that of 5-CH_3-H_4folate from 7 to 30%. H_4folate could not be quantified reliably, but on the basis of analyses of a small number of samples, its proportion was estimated at about 7%. Folic acid was present at low levels (4−6%).

There is considerable variation in the proportions of vitamers reported in the literature. Müller (*42*) reported that 5-HCO-H_4folate was the dominant vitamer (~55%) in rye grains with about 5% 5-CH_3-H_4folate, 22% folic acid, 12% 10-CHO-PGA, and 5% H_4folate. Gujska and Kuncewicz (*41*) reported 86% 5-HCO-H_4folate in rye grains with 9% of 5-CH_3-H_4folate and 4% of 10-CHO-PGA. Patring et al. (*46*) reported 26−42% of 5-HCO-H_4folate in wholegrain rye with 6−17% 5-CH_3-H_4folate, 36−63% 10-CHO-PGA, and 0−12% PGA. However, not all vitamers were determined in all studies. For example, had 5,10-CH^+H_4folate not been determined in the present study, the proportions of other vitamers would have "increased" by about

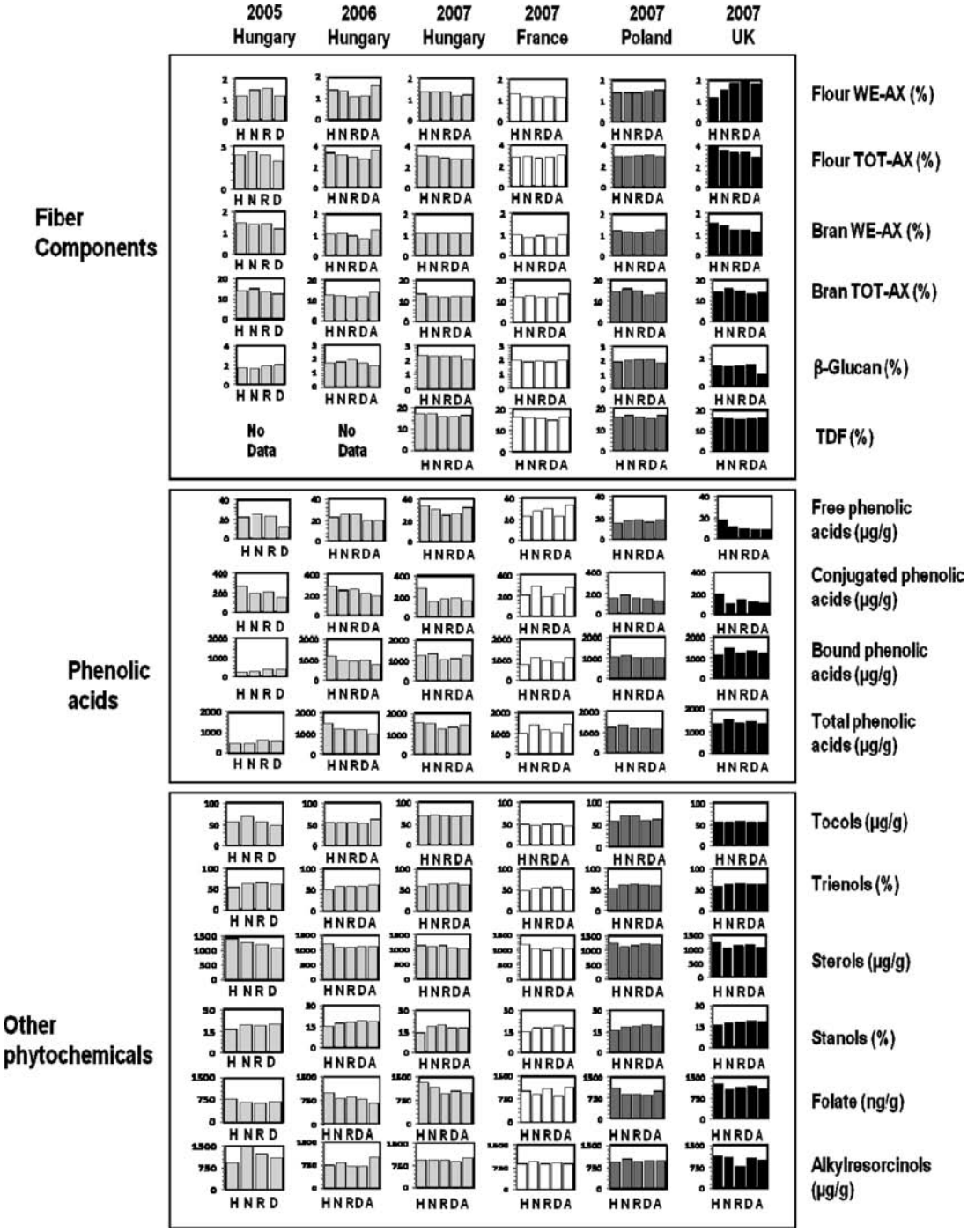

Figure 2. Mean contents of fiber and phytochemical components in the rye lines grown in the six environments. H, Haute Loire; N, Nikita; R, Rekrut; D, Dankowskie Zlote; A, Amilo.

6 percentage units. Nevertheless, it can be concluded that vitamer distribution in rye is characterized by a high proportion of formylated folates.

Rye contained more 5-CH_3-H_4folate and less 5-HCO-H_4folate compared to wheat lines grown in adjacent plots (*22*). However, in both wheat and rye high proportions of 5-CH_3-H_4folate were present in varieties with high total folate contents and of 5-HCO-H_4folate in varieties with low total folate contents.

Phenolic Acids. Phenolic acids showed the greatest range of differences between environments with the level of bound phenolic acids being particularly low in the samples grown at Martonvásár in 2005 (310 ± 79 μg/g of dm), compared with those grown in 2006 (942 ± 155 μg/g of dm) and 2007 (1109 ± 165 μg/g of dm) and at the other sites. The mean total phenolic acid contents (936−1551 μg/g of dm) of the five varieties grown in 2006 and 2007 were significantly higher than those reported for the 10 cultivars grown in 2005 (*2*). This does not appear to relate to the temperature profile, which was similar to that in France in 2007 and lower than those in Martonvásár in 2006 and 2007, or to the precipitation, which was slightly higher than that in Martonvásár in 2006 but lower than that in the United Kingdom in 2007. However, free phenolic acids were lowest and bound phenolic acids highest in the samples grown in the United Kingdom in 2007, where the weather conditions were the coolest and wettest of any of the environments, and highest in the samples grown in Martonvásár and Poland in 2007, both sites

having hot dry summers. This indicates that a combination of temperature and precipitation may be important.

The mean total phenolic acid content was highest in 2007 (1303 ± 154 μg/g of dm) and lowest in 2005 (533 ± 51 μg/g of dm). The lowest values were observed in the material grown in Hungary in 2005 and resulted from very low values for bound phenolic acids in material grown at this site. These levels are consistent with previous reports of total phenolic acid content in rye (*47*, *48*). Comparison of the material grown in Martonvásár across three years showed that the mean free phenolic acid content did not vary significantly, with concentrations of 20, 22, and 21 μg/g of dm in 2005, 2006, and 2007, respectively. The total soluble conjugated phenolic acid concentration was highest in 2006 (232 ± 36 μg/g of dm) and lowest in 2007 (173 ± 55 μg/g of dm), with the 2007 samples containing lower concentrations of 2,4-dihydroxybenzoic acid (2.3 ± 1.1 μg/g of dm) than in 2005 and 2006. The levels of other soluble conjugated phenolic acids (e.g., sinapic and ferulic acid) were more comparable between 2005 and 2007. The composition of bound phenolic acids varied greatly among the three years, with greatest variation being observed in caffeic acid, 2,4-dihdroxybenzoic acid, sinapic acid, and ferulic acid. However, the contents of bound phenolic acids did not vary to such a great extent when wheat cultivars were grown under the same conditions (*49*), suggesting that programming of phenolic acid content varies between cereal species.

Variation between locations in 2007 was lower than that observed for the single location in Hungary across three years. The mean total phenolic acid content was generally highest in the samples grown in Hungary (1385 ± 140 μg/g of dm) and the United Kingdom (1412 ± 87 μg/g of dm) and lowest in those grown in France (1201 ± 186 μg/g of dm) and Poland (1213 ± 67 μg/g of dm). The contributions of different classes of phenolic acids also varied. Samples grown in the United Kingdom had higher proportions of bound phenolic acids (90%) at the expense of free (0.8%) and soluble conjugated forms (9.2%). This contrasts with material grown in France, which had higher proportions of free (2.3%) and soluble conjugated (19.4%) and lower contents of bound fractions (78%). The mean content of free phenolic acids varied from 10.6 to 29 μg/g of dm and was higher in samples grown in France and Hungary compared to those grown in Poland and the United Kingdom. These differences were mainly due to changes in the levels of free vanillic and syringic acids. The content of soluble conjugated phenolic acids was significantly higher ($p = 0.003$) for samples grown in France, with average levels of 233 μg/g of dm. This difference was mainly due to higher amounts of ferulic acid conjugates: 112 μg/g of dm compared to 48 μg/g of dm from samples grown in Poland and the United Kingdom. Bound phenolic acids were typically higher in the samples grown in Hungary (1172 μg/g of dm) and the United Kingdom (1273 μg/g of dm) compared to those grown in France (941 μg/g of dm). Within this fraction the concentrations of some phenolic acids were relatively constant, for example, sinapic acid (mean = 182−210 μg/g of dm), whereas others such as 2,4- and 4-hydroxybenzoic acids and ferulic acid varied more. For example, the levels of ferulic acid were highest in samples grown in the United Kingdom (668 μg/g of dm) and lowest in those grown in France (550 μg/g of dm).

Dietary Fiber Components. The mean contents of TDF of the five varieties grown in four locations in 2007 varied between 15.1 and 16.3% dm, including 12.7−13.6% total NSP, 0.21−0.24 uronic acids, 2.1−2.7% lignin, and 1.6−1.9% β-glucan. Haute Loire, Amilo, and Nikita had the highest contents of TDF and Dankowskie Zlote the lowest. The differences in total NSP content were not significant between rye varieties. The TDF content also varied between locations, being significantly higher in varieties grown in Hungary (16.6% dm) and lower in the three other sites (15.5−15.8% dm). The range of variation in the content of TDF was similar to that reported for 19 rye varieties grown in Denmark in 1997 (*50*) in one location and also to those reported by Bach Knudsen (*51*) and Åman et al. (*52*). However, when the same 19 rye samples were harvested the following year, the contents of TDF were about 13% higher, the range being 15.5−20.9% dm (*50*). Nyström et al. (*2*) reported lignin and β-glucan levels of 2.0−2.9 and 1.7−2.0% dm, respectively, for the 10 rye varieties grown as part of the HEALTHGRAIN diversity screen (including 4 of the varieties studied here).

The major fiber component is AX, which accounted for 7.6−8.2% dm in wholemeal, 12.1−13.3% dm in bran, and 2.9−3.3% dm in flour. The WE-AX content was 1.0−1.2% dm in bran and 1.3−1.4% dm in flour. The A/X ratio was 0.6 in flour WE-AX, 0.7−0.8 in bran WE-AX, 0.7 in flour TOT-AX, and 0.5−0.6 in bran TOT-AX. These values for AX fall in the ranges described for 10 rye cultivars grown on the Martonvásár site in 2004−2005 (*2*). Vinckx and Delcour (*53*) reported TOT-AX contents in rye wholemeal ranging from 6.5 to 12.2% dm, whereas levels of 14.9−39.5% dm and 2.1−4.9% dm were reported by others for rye bran and flour, respectively (*54*−*57*). The higher AX levels reported for the bran in the literature are due to differences in milling parameters and genetic differences. A/X ratios of 0.5−0.8, 0.6, and 0.5 for TOT-AX in wholemeal, flour, and bran, respectively, were reported in refs *53* and *57*. WE-AX levels ranging from 1.5 to 3.0% dm were reported for wholemeal, flour, and bran with A/X ratios varying between 0.5 and 0.7 (*53*).

For most varieties the greatest variation in flour WE-AX content was observed between the different sites, with lower variation between the different harvest years in Hungary. In contrast, greater variation in flour TOT-AX was observed between the different harvest years in Hungary than between the different sites. For bran WE-AX and TOT-AX, the variations in content between the growing sites and between the different years were similar.

When calculated on a site basis, the average content of WE-AX in flour was highest in the samples grown in the United Kingdom (1.6% dm) and lowest in those grown in France (1.2% dm). The samples grown in the United Kingdom in 2007 (with cold and wet weather between heading and harvest), and Hungary in 2005 (with warm and dry conditions) had the highest levels of bran WE-AX (1.3 and 1.4% dm) with the lowest levels being present in the samples grown in France (0.9% dm) (with intermediate levels of temperature and precipitation). In the case of the U.K. samples, the higher WE-AX levels may be attributed to the cold wet weather conditions between heading and harvest in 2007, favoring microbial growth and/or preharvest sprouting. These may result in high activity of xylanases (*58*), which digest water-unextractable AX to increase the level of WE-AX. In line with our results, Dornez et al. (*59*) and Gebruers et al. (*58*) also reported higher levels of WE-AX in wheat grown under cold wet conditions. Similarly, high TOT-AX levels were present in flour and bran samples from rye grown in the United Kingdom (2007) (3.3 and 14.3% dm, respectively) and Hungary (2005) (3.9 and 13.7% dm, respectively) and low levels in those grown in France (2.8 and 12.2% dm, respectively). Hence, as for bran WE-AX, there was no straightforward relationship between weather conditions and TOT-AX content. However, Saastamoinen et al. (*60*) showed an increase in the TOT-AX content of rye with increasing precipitation. An opposite effect was reported for wheat by Coles et al. (*61*), whereas no significant year effects were observed by Dornez et al. (*59*).

Table 5. Correlations between the Contents of Bioactive Components in Rye Grain and the Mean Temperature and Precipitation during the Period from Heading to Harvest and Precipitation from 3 Months before Heading to Harvest (Dates Based on Heading Times for Wheat Varieties Planted in Adjacent Plots)[a]

	mean temperature		precipitation		precipitation 3 months before heading to harvest	
	R	*p* value	*R*	*p* value	*R*	*p* value
folates (ng/g of dw)	−0.183	0.729	0.252	0.630	−0.243	0.642
sterols (μg/g of dw)	0.074	0.889	0.045	0.933	**0.564**	0.243
% stanols	−0.222	0.672	**0.449**	0.372	**0.749**	0.087
tocols (μg/g of dw)	0.243	0.643	0.302	0.561	0.303	0.559
total alkylresorcinols (mg/g of dw)	−0.127	0.810	0.140	0.791	**0.702**	0.120
bound phenolic acids (mg/g of dw)	**−0.441**	0.382	**0.550**	0.258	−0.041	0.939
total conjugated phenolic acids (mg/g of dw)	**0.690**	0.129	**−0.934**	**0.006**	**−0.675**	0.141
total free phenolic acids (mg/g of dw)	**0.906**	**0.013**	**-0.927**	**0.008**	**-0.801**	0.055
total phenolic acids (mg/g of dw)	−0.361	0.482	**0.445**	0.376	−0.156	0.768
bran TOT-AX (%)	**−0.574**	0.234	**0.410**	0.419	**0.888**	**0.018**
bran WE-AX (%)	−0.270	0.605	0.355	0.490	**0.873**	**0.023**
flour TOT-AX (%)	−0.073	0.891	−0.023	0.965	**0.640**	0.171
flour WE-AX (%)	**−0.811**	**0.050**	**0.883**	**0.020**	**0.931**	**0.007**
β-glucan (%)	**0.797**	0.058	**-0.492**	0.321	**−0.635**	0.175

[a] Results in bold describe correlation coefficients of 0.4 or higher. Associated *p* values highlighted in bold type illustrate correlations that are not considered to have occurred by chance and that have a *p* value of <0.05. For significant correlations, values should have both a correlation coefficient above 0.4 and an associated *p* value of <0.05.

The β-glucan contents of wholemeal were highest in the rye samples grown in Hungary in 2007 (2.2% dm) and lowest in those grown in the United Kingdom in 2007 (1.38% dm). This difference may be accounted for by the different weather conditions at the two sites, with warm and dry conditions (as occurred in Hungary in 2007) promoting high β-glucan levels (*61*, *62*).

Genotype and Environment Effects on Bioactive Components. The availability of six data sets for different environments allowed the relative contributions of genotype and environment to the various groups of bioactive components to be calculated. Full data for the major groups of components are presented in Supplementary Table 4 of the Supporting Information, whereas significant associations between bioactive compounds, genotype, and environment are given in **Table 4**. No significant effects of genotype on the total contents of alkylresorcinols, tocols, or phenolic acids were found. However, significant effects of genotype on the contents of some individual tocol, sterol, and alkylresorcinol components were observed (data not shown). Genotype showed weak but significant effects on total folates, bran TOT-AX, Klason lignin, TDF, and wholemeal β-glucan. In contrast, environment showed moderate to strong significant effects on almost all components. This led to low heritability values estimated as the ratio $\sigma^2_G/(\sigma^2_G + \sigma^2_E + \sigma^2_{G\times E})$. The genotypic variance (σ^2_G) calculated for the five outbreeding varieties is likely to be underestimated as intravariety variation could not be estimated. Moreover, no replicates were available for some traits. In this case, we used $\sigma^2\varepsilon$ to estimate $\sigma^2_{G\times E}$. Thus, the values obtained give underestimated values for heritability. The weak effects of genotype on most groups of bioactive components contrast with wheat, in which AX components in particular were highly heritable (*16*). This may be explained by the fact that data were available for 26 pure inbred lines of wheat, giving better estimates of variance components for traits compared with data for only five outbreeding varieties of rye. For sterols, with a similar model, the high values of $\sigma^2_G/\sigma^2_G + \sigma^2_E + \sigma^2_\varepsilon$ in both species confirm that sterols are realistic targets for selection in plant breeding.

Correlations of Contents of Bioactive Components. *Correlations with Weather Conditions*. Correlations between the contents of bioactive components and weather conditions (mean temperature and precipitation measured over decades (i.e., 10 day periods)) as described by Shewry et al. (*16*) are presented in **Table 5**. Total conjugated and total free phenolic acids both show positive correlations with temperature and negative correlations with precipitation, whereas total bound phenolic acids show opposite effects (negative and positive correlations, respectively). Similar differences between free/conjugated and bound phenolic acids and weather conditions were observed in wheat lines grown on the same sites, with the conjugated/free phenolic acid fractions showing strong positive and negative correlations with temperature and precipitation, respectively, and the bound phenolic acid fraction weak negative and positive correlations, which were not statistically significant (*16*). The free, bound, and conjugated phenolic acid fractions each comprise complex mixtures of components, and because of this it is not possible to speculate on the significance of these differences in their responses to environmental factors. Nevertheless, it does indicate that they may differ in their biological roles.

As in wheat, the content of flour WE-AX was strongly positively correlated with precipitation and negatively correlated with temperature, although, unlike in wheat, similar correlations were not observed with the bran WE-AX (*16*). In wheat these effects were ascribed to the presence of high endoxylanase activity in grain grown under cool, damp conditions, leading to breakdown of insoluble AX to give soluble components (*58*), and this may also apply to rye.

Bran TOT-AX was negatively correlated with temperature and positively correlated with precipitation, whereas wholemeal β-glucan showed positive and negative correlations with these parameters, respectively.

No correlations between total folates, sterols, and tocols and weather conditions were observed, whereas in wheat all of these groups were positively correlated with temperature and total folates were negatively correlated with precipitation (*16*). These differences may indicate that the contents of these phytochemicals are more stable in rye, but could also reflect the smaller data set (only 5 rye lines compared with 26 wheat lines) giving less significant correlations.

Correlations between Bioactives and Other Factors. Correlations between groups of bioactive components, bran yield, and thousand grain weight are given in Supplementary Tables 6 and 7 of the Supporting Information. Total alkylresorcinols and total tocols both showed strong negative correlations with thousand grain weight ($r = -0.638$ and -0.713, respectively, $p < 0.001$) (Supplementary Table 7 of the Supporting Information). This is consistent with their concentration in the bran, smaller grains having a greater proportion of bran. It is therefore surprising that they did not also correlate negatively with bran yield; this

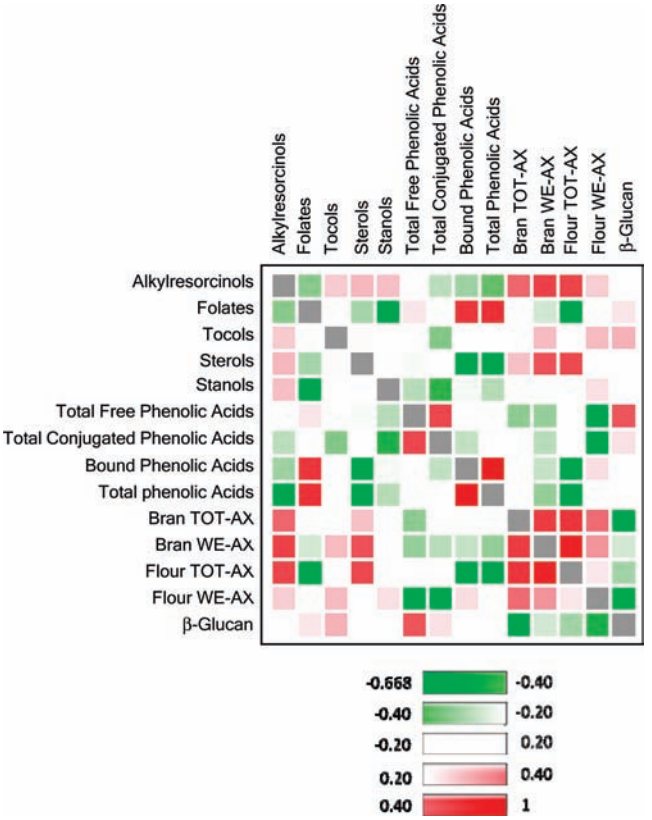

Figure 3. Heat map showing correlations between the mean total contents of phytochemicals and fiber components in the rye lines grown in the six environments.

probably resulted from the fact that the milling procedure did not give reproducible separation of bran from white flour, with yields varying between 25 and 41%. The only other statistically significant correlation with thousand grain weight was for bran WE-AX ($r = -0.589$, $p < 0.001$) (Supplementary Table 6 of the Supporting Information). Folate content did not correlate with thousand kernel weight or bran yield when all of the environments were considered, although correlations with these parameters were reported by Nyström et al. (*2*) on the basis of 10 lines grown in 2005 only. It also contrasts with the analysis of wheat lines grown at the same sites (*22*), which showed that total folate contents were positively correlated with bran yield and negatively correlated with thousand grain weight.

Correlations between bioactive components are summarized graphically in **Figure 3**. Particularly notable are strong correlations between all four AX fractions (WE-AX and TOT-AX in bran and flour) and between bran AX factions and alkylresorcinols (which presumably reflect the colocation of these components in bran). Other correlations are between total folates and total/bound phenolic acids (positive), between sterols and bran AX (TOT-AX and WE-AX) (positive), and between sterols and conjugated/bound phenolic acids (negative). Total sterols were also strongly positively correlated with total grain lipids ($r = 0.713$, $p = 0.001$) (Supplementary Table 4 of the Supporting Information).

It is also interesting to compare the correlations in **Figure 3** with the equivalent display of correlations for the wheat varieties grown at the same sites (see Figure 5 in ref *16*). It is notable that stronger correlations are observed in wheat than in rye, which may reflect the smaller data set available for rye (analyses of 5 rye varieties compared with 26 wheats).

The analyses reported here, based on six environments, show that the extent of genetic variation in the contents of fiber and phytochemical components in five rye lines is very limited compared with the strong impact of environmental factors. In particular, the contents of phenolic acids are particularly variable between sites, years, and lines, whereas the contents of total sterols are the least variable, which agrees with our previous study of 10 lines grown at Martonvásár in 2005 (including 4 lines that are included in the current paper) (*2*). Although it is necessary to exercise caution in drawing conclusions because only five rye varieties were studied, it is nevertheless striking that the genetic variation in composition was less than observed in wheat lines grown in adjacent plots (*16*), which may reflect the fact that rye is an outbreeding species, whereas wheat is inbreeding. Commercial rye lines are therefore mixtures of many genotypes (which may differ in composition), whereas wheat cultivars are pure homozygous lines.

ABBREVIATIONS USED

A, arabinose; AX, arabinoxylan; β-glucan, (1,3)(1,4)-β-D-glucan; dm, dry matter; GC, gas chromatography; HPLC, high-performance liquid chromatography; NSP, nonstarch polysaccharide; SD, standard deviation; TDF, total dietary fiber; TOT-AX, total AX; WE-AX, water-extractable AX.

Supporting Information Available: Supplementary Tables 1−7. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review January 6, 2010. Revised manuscript received March 31, 2010. Accepted April 17, 2010. This publication is financially supported by the European Commission in the Communities Sixth Framework Program, 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 herein. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. The Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium) is acknowledged for the postdoctoral fellowship of K.G.