JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Genotype and Environment Effects on the Contents of Vitamins B1, B2, B3, and B6 in Wheat Grain

Peter R. Shewry,[*,†] Frank Van Schaik,[‡] Catherine Ravel,[§] Gilles Charmet,[§] Mariann Rakszegi,[||] Zoltan Bedo,[||] and Jane L. Ward[†]

[†]Department of Plant Science, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom

[‡]TNO Triskelion, P.O. Box 844, 3700 AV Zeist, The Netherlands

[§]INRA-UBP, UMR1095 GDEC, 234 av du Brézet, 63100 Clermont-Ferrand, France

[||]Agricultural Research Institute of the Hungarian Academy of Sciences, Brunszvik u. 2, Martonvásár HU-2462, Hungary

Supporting Information

ABSTRACT: The total contents of thiamine (vitamin B1), riboflavin (B2), and pyridoxine (B6) and the bioavailable forms of niacin (B3) were determined on wholemeal flours of 24 winter wheat varieties grown on four sites (United Kingdom, Poland, France, and Hungary) in 2007 and of two spring varieties grown on the same sites with the exception of Poland. The contents of vitamins B1 (5.53−13.55 μg/g dw), B2 (0.77−1.40 μg/g dw), and B6 (1.27−2.97 μg/g dw) were within the ranges reported previously, while the content of bioavailable vitamin B3 (0.16−1.74 μg/g dw) was about 10−15% of the total contents of vitamin B3 reported in previous studies. Strong correlations were observed between the contents of vitamins B1, B3, and B6, and partitioning of the variance in the contents of these three B vitamins showed that between 48 and 70% was accounted for by the environment. By contrast, the content of vitamin B2 was not correlated with the contents of other B vitamins, and 73% of the variance was ascribed to the error term, which suggests that this trait may be influenced by genotype × environment interactions. Whereas the contents of vitamins B1, B3, and B6 were correlated positively with the mean temperature from heading to harvest ($r > 0.8$), the content of vitamin B2 was positively correlated with precipitation during the 3 months prior to heading. These results are discussed in relation to the development of new wheat varieties with enhanced health benefits.

KEYWORDS: Wheat, B vitamins, thiamine, niacin, riboflavin, pyridoxine

INTRODUCTION

The B vitamin complex comprises eight water-soluble components that often occur together in the same foods and were initially considered to be a single component. Wheat, in particular, wholegrain, is a source of a number of essential and beneficial components including the B vitamins thiamine (B1), riboflavin (B2), niacin (B3), and pyridoxine (B6). Bread and bread products have been reported to account for about 17−18% of the total intake of thiamine, about 11% of the total intake of riboflavin, and 11% of the total intake of niacin in adults in the United States, with fortified ready-to-eat cereals providing an additional 10% in all three cases.[1] Fortified ready-to-eat cereals and other grain products also account for 15% of the dietary intake of pyridoxine.[1]

All B vitamins are concentrated in the bran and/or germ, and commercial milling removes about 68% of the total thiamine, 58−65% of the riboflavin, and 85% of the pyridoxine.[2] Earlier work reviewed by McMasters et al.[3] showed that 32 and 64% of the thiamine is present in the aleurone and embryo (including scutellum), respectively, 37 and 26% of the riboflavin, 82 and 2% of the niacin, and 61 and 21% of the pyridoxine. The contents of these four vitamins in the starchy endosperm were only about 3, 32, 12, and 6%, respectively, of those present in the wholegrain.[3,4] The remaining 1% of the total thiamine, 5% of riboflavin, 4% of niacin, and 12% of pyridoxine were present in the pericarp and testa. Consequently, the consumption of wholegrain cereal products as opposed to products made with white flour results in significant increases in the intakes of all of these important vitamins.

A number of comparative studies of the contents of B vitamins in wheat lines and products have been reported, most notably by Davis et al.,[5,6] who determined thiamine, niacin, riboflavin, and pyridoxine in wholemeal samples of 231 varieties grown on 49 locations over 3 years (406 samples in total); Batfoulier et al.,[7] who determined thiamine, riboflavin, and pyridoxine in flours and reconstituted wholemeals of 49 cultivars; and Sampson et al.,[8] who determined pyridoxine in flour of 22 wheat samples and grain of 15 of these. These studies have therefore provided valuable information on the range of contents of B vitamins in wheat cultivars but have not partitioned this variation between the effects of genotype and environment or explored correlations with other bioactive components.

The HEALTHGRAIN study (2005−2010), supported by the EU under Framework 6, has generated the largest database currently available on the contents of bioactive components in wheat. These include a wide diversity survey of 150 lines grown on a single site[9] and a more detailed study of 26 lines, most of which were analyzed from six different sites or years.[10] The components

Received: February 15, 2011
Accepted: August 24, 2011
Revised: August 15, 2011
Published: August 25, 2011

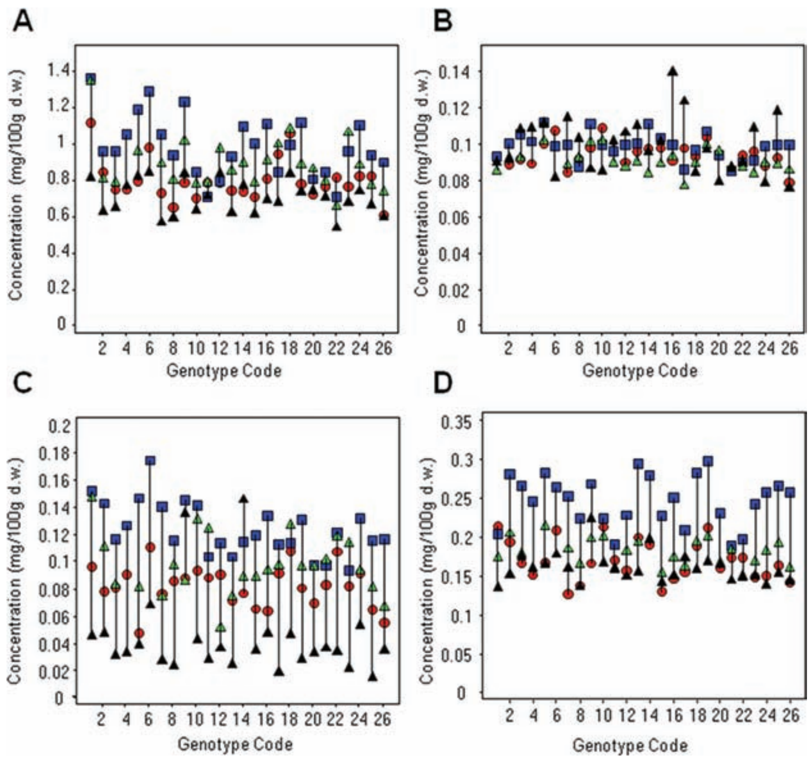

Figure 1. Scatter plots of B vitamin contents of the wheat lines. Concentrations are expressed as mg/100 g dw. (A) Vitamin B1, (B) vitamin B2, (C) vitamin B3, and (D) vitamin B6. Red circle, France; blue square, Hungary; green triangle, Poland; and black triangle, United Kingdom. 1, Atlas 66; 2, Avalon; 3, CF99105; 4, Cadenza; 5, Campari; 6, Chinese Spring; 7, Claire; 8, Crousty; 9, Disponent; 10, Estica; 11, Gloria; 12, Herzog; 13, Isengrain; 14, Lynx; 15, Malacca; 16, Maris-Huntsman; 17, Mv-Emese; 18, Obriy; 19, Rialto; 20, Riband; 21, San-Pastore; 22, Spartanka; 23, Tiger; 24, Tommi; 25, Tremie; and 26, Valoris.

analyzed included dietary fiber components, alkylresorcinols, tocols, sterols, phenolic acids, folates (vitamin B_9),[9,10] and micronutrients (Fe, Zn, and Se).[11] The second series of samples have therefore been analyzed for four other B vitamins (niacin, thiamine, riboflavin, and pyridoxine) to provide data on heritability and relationships with other grain components.

MATERIALS AND METHODS

Materials. Twenty-three lines were selected as representing a range of contents of phytochemicals (sterols, tocols, alkylresorcinols, folates, and phenolic acids) and dietary fiber components based on initial analyses of 150 bread wheat lines grown on a single site at Martonvásár in Hungary in 2005.[9] Three additional lines (Mv Emese, Tiger, and Crousty) were then selected, giving 26 in total, comprising 24 winter type and two spring type (Supporting Information, Table S1). The 26 wheat lines were grown on three sites in Hungary (Martonvásár), the United Kingdom (Nickerson Seeds U.K., Saxham, near Bury St. Edmunds, United Kingdom), and France (INRA experimental station at Clermont Ferrand) in 2006−2007. The winter wheat lines only were also grown at a fourth site in Poland (Danko Plant Breeders Ltd., Choryn) in the same year. Full details of the sites including soil type, pH, and composition and weather conditions during the growth period are reported in ref 10. The agronomic treatments were standard for the individual sites, with 110 kg of N/ha being applied in Poland, 204 kg of N/ha in the United Kingdom, 200 kg of N/ha in France, and 140 kg of N/ha in Hungary and appropriate use of agrochemicals. Grain samples were conditioned to 15.5% moisture content before milling using a Perten Laboratory Mill 3100 (with 0.5 mm sieve) to produce wholemeal. Samples were immediately cooled to −20 °C and stored at the same temperature in sealed bags.

Analysis of Vitamins. Vitamins B1, B2, B3, and B6 were determined using the European standard methods, which are specified below. Only brief details are therefore given. These procedures extract the total contents of vitamins B1, B2, and B6 but only part of the total vitamin B3 content, which is considered to correspond to the biologically available form.[12]

Vitamin B1 (Method NEN-EN 14122:2003). Samples were extracted in duplicate in an autoclave at 119 °C for 15 min, using a 0.1 mol/L hydrochloric acid solution containing sodium ascorbate. The extract was adjusted to pH 4.7 using an acetate buffer and filtered. Part of the filtrate was incubated for at least 2 h at 37 °C with phosphatase (acid) and subsequently analyzed using reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection. This procedure determined thiamine as two forms (thiamineCl and thiamineCl·HCl), which were combined.

Vitamin B2 (Method NEN-EN 14152:2003). Samples were extracted in duplicate in an autoclave at 119 °C for 15 min, using a 0.1 mol/L hydrochloric acid solution containing sodium ascorbate. The extract was adjusted to pH 4.7 using an acetate buffer and filtered. Part of the filtrate is incubated for at least 2 h at 37 °C with phosphatase (acid) and subsequently analyzed for riboflavin using reversed-phase ultraperformance liquid chromatography (UPLC) with fluorescence detection.

Vitamin B3 (Method NEN-EN 15652:2009). Bioavailable vitamin B3 (niacin) was determined as the sum of nicotinamide and nicotinic acid after acid hydrolysis. Samples were extracted in duplicate in an autoclave at 119 °C for 15 min, using a 0.1 mol/L hydrochloric acid solution containing sodium ascorbate. The extract was adjusted to pH 4.7 using acetate buffer and filtered. Part of the filtrate was analyzed for nicotinamide and nicotinic acid using reversed-phase UPLC with postcolumn irradiation and fluorescence detection.

Vitamin B6 (Method NEN-EN 14164:2008). Samples were extracted in duplicate for 30 min, using a 5% (w/v) trichloroacetic acid solution. Part of the extract was adjusted to pH 4.7 using acetate buffer, incubated for at least 12 h at 37 °C with phosphatase (acid), and subsequently analyzed for pyridoxal, pyridoxol, and pyridoxamin using reversed-phase UPLC with fluorescence detection.

All analyses were carried out in duplicate and corrected for moisture content. Relative standard deviation (RSD) values were calculated for a control sample as follows: vitamin B1 concentration, 12.2 μg/g, RSD, 9.2%; vitamin B2 concentration, 11.6 μg/g, RSD, 6%; vitamin B3 concentration, 113 μg/g, RSD, 5.4%; and vitamin B6 concentration, 12.9 μg/g, RSD, 4.2%.

Statistical Analysis. First, for each trait, data sets from the cultivars grown on the four sites were analyzed by variance analysis using the procedure GLM of SAS (SAS Institute Inc., Cary, NC,). In the absence of biological replicates (field plots), the two technical replicates were averaged, and the genotype × site term was used as an error term. Thus, we used the following model, which includes two main fixed effects, genotype and environment:

$$X = \mu + G + E + \varepsilon$$

with μ being the grand mean, G the genotype main effect, E the environment (site) main effect, and ε the error term, which includes G × E, the interaction between the two main effects, and the true residual error. For each main effect, means were compared by Duncan's multirange test.

To calculate the heritability for each trait, the same data sets were used in statistical models with genotype and site effects considered as random to estimate variance components using the procedure VARCOMP of SAS (SAS Institute Inc.). Variance components were used to compute the ratio $Var_G/(Var_G + Var_E + Var_\varepsilon)$, with Var_G, Var_E, and Var_ε being the genotypic, environmental (intersite), and the error variances, respectively. This ratio, which can be considered as the broad sense heritability, is a suitable parameter for plant breeders, as a high value indicates that genotype behavior is predictable and that the trait should be amenable to genetic improvement.

RESULTS

Contents of B Vitamins. The analyses are given in full in the Supporting Information (Table S2) and are summarized in Figure 1, in which data points for the four sites are shown in different colors.

The contents of vitamins B1 (Figure 1A), B3 (Figure 1C), and B6 (Figure 1D) show broadly similar patterns of variation. First, there is a clear site effect, with the contents of all three B vitamins varying between the same lines grown on the four sites. This site effect is confirmed by the variance analysis (Table 1), which also shows significant but lower site effects on vitamin B2. As shown in Figure 2 (calculated from data in Table 1), the site effect explained 42, 56, and 63% of the total variation for vitamins B1, B3, and B6, respectively, but only 9% for vitamin B2. The mean values for all lines per site are given in Table 2. For vitamins B1, B3, and B6, the contents are highest in the samples grown in Hungary (blue points in Figure 1 and Table 2) and lowest in the samples grown in the United Kingdom (black points in Figure 1 and Table 2). However, the content of vitamin B6 is lowest in several lines grown in France (red points in Figure 1D). This suggests G × E interactions, although these cannot be tested statistically. For vitamin B2, the content was significantly higher in Hungary and the United Kingdom than in France or Poland (Table 2). This site effect is also confirmed by the high contributions from the environment (site) to the analysis of variance components (≥48% for vitamins B1, B3, and B6) (see below, Figure 2, and the Supporting Information, Tables S3 and S4). It can be noted that the mean values for vitamins B1, B3, and B6 show the same order between the sites, with Hungary being the highest followed by France and Poland and the United Kingdom site being lowest. Duncan's multirange test shows that all of these sites were significantly different. This variation is also greatest for vitamin B3 (from 0.045 to 0.124 mg/100 g dw) and least for vitamin B1 (0.717−0.994 mg/100 g dw).

Second, there is a clear genotype effect, which is significant for all of the traits studied. This can be seen in Figure 1 but is more clearly shown in Tables 1 and 3, which compare the mean values for the 26 varieties: from 0.69 to 1.17 mg/100 g dw for vitamin B1, from 0.086 to 0.107 mg/100 g dw for vitamin B2, from 0.069 to 0.118 mg/100 g dw for vitamin B3, and from 0.164 to 0.221 mg/100 g dw for vitamin B6. However, some lines are more stable in vitamin content than others, notably San Pastore, Estica, and Gloria (Figure 1, tracks 10, 11, and 21, respectively), and show lower variation in their contents of vitamins B1, B2, and B6 (but not B3) as compared to most other lines (see Table S2 in the Supporting Information). In addition, Duncan's test shows clearly that Atlas 66 has a significantly higher content of vitamin B1.

Heritability of Contents of B Vitamins. The availability of data for the same lines grown in four environments allowed the variation in contents of B vitamins to be partitioned between genotype, environment, and a residual, which includes genotype × environment (G × E) (Figure 2 and the Supporting Information, Tables S3 and S4). When tested against this error term, the two main effects G and E are highly significant for the four traits (Table 1). However, the *distribution* of variance components was not similar. The patterns for vitamins B1, B3, and B6 were again broadly similar, with environment accounting for about 50−70% of the variation and genotype for 5 (vitamin B3), 10 (vitamin B6),

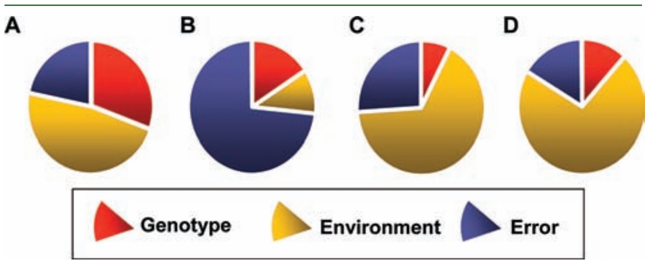

Figure 2. Variance components from heritability calculations on B vitamin data for the wheat lines. (A) Vitamin B1, (B) vitamin B2, (C) vitamin B3, and (D) vitamin B6. Values are calculated from Table 1 using the degrees of freedom.

Table 1. Mean Squares of Variance Analysis for B Vitamins in the Wheat Lines

source of variation	degree of freedom	vitamin B1	vitamin B2	vitamin B3	vitamin B6
genotype	25	0.072[a]	17.3×10^{-4}[b]	1.2×10^{-3}[a]	2.0×10^{-3}[a]
site	3	0.654[a]	54.6×10^{-4}[a]	46.1×10^{-3}[a]	6.4×10^{-2}[a]
error	175	0.006	6.5×10^{-5}	3.6×10^{-4}	3.1×10^{-4}

[a] P value <0.001. [b] P value <0.01.

Table 2. Mean Contents (dw) of B Vitamins in the Wheat Lines[a]

description	vitamin B1				vitamin B2			
	Hungary	France	Poland	United Kingdom	Hungary	France	Poland	United Kingdom
mean concn (mg/100 g dw)	0.994 A	0.804 C	0.900 B	0.717 D	0.099 A	0.094 B	0.092 B	0.100 A
SD	0.167	0.115	0.144	0.0915	0.007	0.007	0.006	0.016
CV (%)	16.84	14.31	15.95	12.75	7.31	7.11	6.58	15.46

description	vitamin B3				vitamin B6			
	Hungary	France	Poland	United Kingdom	Hungary	France	Poland	United Kingdom
mean concn (mg/100 g dw)	0.124 A	0.082 C	0.098 B	0.045 D	0.247 A	0.170 C	0.183 B	0.162 D
SD	0.019	0.016	0.022	0.031	0.032	0.026	0.018	0.019
CV (%)	15.54	18.87	22.60	68.72	12.89	15.32	9.62	11.96

[a] On the basis of Duncan's multiple range test, means with the same letter are not significantly different.

Table 3. Mean Contents (dw) of B Vitamins in the Wheat Lines[a]

description	mg/100 g dw			
	vitamin B1	vitamin B2	vitamin B3	vitamin B6
Campari	0.9481 ± 0.1776 CDE	0.1071 ± 0.0066	0.0790 ± 0.0485	0.2085 ± 0.0543
Herzog	0.8549 ± 0.0906 EFGHI	0.0966 ± 0.0090	0.0737 ± 0.0351	0.1806 ± 0.0349
Disponent	0.9764 ± 0.2025 BCD	0.0998 ± 0.0099	0.1142 ± 0.0311	0.2155 ± 0.0429
Tommi	0.8955 ± 0.1522 DEF	0.0896 ± 0.0079	0.0931 ± 0.0320	0.1838 ± 0.0530
Tremie	0.8085 ± 0.1115 FGHIJ	0.1004 ± 0.0134	0.0695 ± 0.0416	0.1956 ± 0.0507
CF99105	0.7930 ± 0.1260 GHIJK	0.1001 ± 0.0084	0.0781 ± 0.0347	0.1982 ± 0.0464
Valoris	0.7184 ± 0.1381 KL	0.0857 ± 0.0105	0.0690 ± 0.0342	0.1769 ± 0.0543
Isengrain	0.7943 ± 0.1297 FGHIJ	0.0999 ± 0.0087	0.0692 ± 0.0324	0.2121 ± 0.0581
Claire	0.8188 ± 0.2064 FGHIJ	0.0975 ± 0.0137	0.0802 ± 0.0456	0.1822 ± 0.0530
Maris-Huntsman	0.8883 ± 0.1757 DEFG	0.1066 ± 0.0229	0.0854 ± 0.0378	0.1817 ± 0.0478
Lynx	0.8844 ± 0.1599 DEFG	0.0979 ± 0.0108	0.1069 ± 0.0307	0.2174 ± 0.0423
Malacca	0.7860 ± 0.1609 HIJK	0.0987 ± 0.0062	0.0778 ± 0.0354	0.1643 ± 0.0433
Rialto	0.8884 ± 0.1665 DEFG	0.1025 ± 0.0042	0.0844 ± 0.0422	0.2212 ± 0.0536
Riband	0.7914 ± 0.0662 GHIJK	0.0914 ± 0.0072	0.0746 ± 0.0298	0.1819 ± 0.0330
Avalon	0.8179 ± 0.1321 FGHIJ	0.0938 ± 0.0048	0.0954 ± 0.0408	0.2093 ± 0.0528
San-Pastore	0.7855 ± 0.0548 HIJK	0.0870 ± 0.0014	0.0802 ± 0.0296	0.1745 ± 0.0194
Estica	0.7478 ± 0.0892 JKL	0.0995 ± 0.0098	0.1026 ± 0.0442	0.2031 ± 0.0241
Gloria	0.7551 ± 0.0421 IJKL	0.0967 ± 0.0048	0.0867 ± 0.0406	0.1712 ± 0.0144
Spartanka	0.6900 ± 0.1107 L	0.0910 ± 0.0025	0.0958 ± 0.0409	0.1691 ± 0.0225
Obriy	1.0025 ± 0.1096 BC	0.0917 ± 0.0048	0.0994 ± 0.0355	0.2073 ± 0.0526
Atlas 66	1.1689 ± 0.2518 A	0.0913 ± 0.0035	0.1107 ± 0.0497	0.1830 ± 0.0345
Crousty	0.7552 ± 0.1548 IJKL	0.0944 ± 0.0070	0.0811 ± 0.0396	0.1668 ± 0.0404
Tiger	0.8751 ± 0.1776 DEFG	0.0956 ± 0.0105	0.0784 ± 0.0393	0.1791 ± 0.0435
Mv-Emese	0.8753 ± 0.1397 DEFGH	0.0968 ± 0.0203	0.0809 ± 0.0414	0.1766 ± 0.0233
Chinese Spring*	1.0425 ± 0.2242 B	0.0967 ± 0.0125	0.1182 ± 0.0528	0.2179 ± 0.0422
Cadenza*	0.8632 ± 0.1671 EFG	0.1004 ± 0.0102	0.0839 ± 0.0464	0.1869 ± 0.0512

[a] The letters following the means for vitamin B1 indicate the Duncan's group (means with the same letter are not significantly different). These letters are not quoted for the other B vitamins, which showed lower genotypic variance.

and 27% (vitamin B1). Atlas 66 (Figure 1, track 1) had a particularly high and stable content of vitamin B1, while Spartanka had the lowest content of vitamin B1, although the content was also stable across the four sites (Figure 1, track 22). A completely different pattern was observed for vitamin B2, with 70% of the variation ascribed to error (including G × E) and only 9% to environment and 6% to genotype. Accordingly, the ratio of genotype variance to the sum of all variance components (phenotypic variance) ranged from 0.07 (vitamin B3) to 0.30 (vitamin B1).

Correlations Between Contents of B Vitamins. Correlations between the contents of the B vitamins were calculated within each location to remove the influence of the environment. The strongest and most consistent correlations were observed

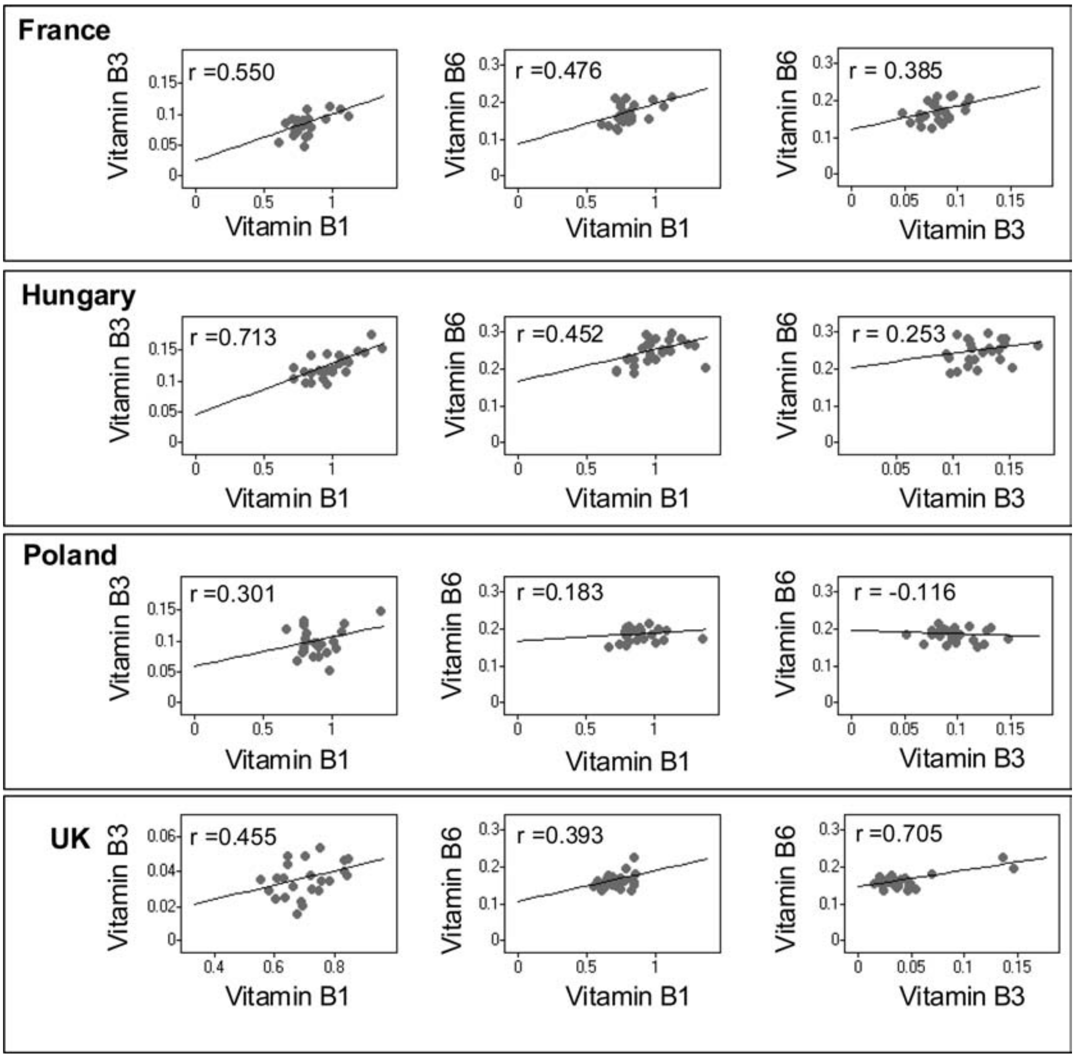

Figure 3. Correlations between the contents of vitamins B1, B3, and B6 in the wheat lines.

between the contents of vitamins B1 and B3, which were positively correlated with each other at each location as shown in Figure 3 and Table 4. The contents of B1 and B6 showed strong correlations in the samples from three locations (United Kingdom, France, and Hungary) but only a weak (and statistically insignificant) correlation when the samples from Poland were analyzed. Similarly, although strong correlations between vitamins B2 and B6 were observed in samples grown in Hungary, France, and Poland, no correlations were observed when the United Kingdom samples were analyzed. By contrast, the contents of vitamin B2 showed no statistically significant correlations with the contents of vitamins B1 or B3.

The availability of data for a range of other grain characteristics including the contents of phytochemicals in wholemeal and of dietary fiber components in white flour and bran allowed wider correlations to be explored. No consistent, statistically significant correlations between B vitamins and these other components were observed when data from individual locations were analyzed separately.

Correlations of B Vitamins with Weather Conditions. The contents of vitamins B1, B3, and B6 were correlated positively with the mean temperature from heading to harvest ($r > 0.8$) (Table 5) but not with precipitation during grain development. Conversely, vitamin B2 content was not correlated with temperature but was positively correlated with precipitation during the 3 months prior to heading ($r = 0.982$, $p = 0.018$).

DISCUSSION

Piironen et al.[13] recently reviewed the contents of B vitamins in whole wheat flour, based on analyses reported by refs 7 and 14−21. The ranges were 2.2−6.3 μg/g dw of vitamin B1 as compared with 5.53−13.55 μg/g dw in the present study, 0.8−2.2 μg/g dw of vitamin B2 as compared with 0.77−1.40 μg/g dw in the present study, 19−64 μg/g dw of vitamin B3 as compared with 0.16−1.74 μg/g dw in the present study, and 1.3−7.5 μg/g dw of vitamin B6 as compared with 1.27−2.97 μg/g dw in the present study. The comprehensive study of ref 6 similarly reported mean contents of 4.6, 1.3, 55.1, and 4.6 μg/g dw of vitamins B1, B2, B3, and B6, respectively, in a set of 406 samples comprising 231 varieties, three crop years, and 49 growing locations. The contents of vitamins B1, B2, and B6 determined in the current study are therefore in line with these previous reports. However, the contents of vitamin B3 were substantially lower than in these previous studies as only the nutritionally available form was determined. On the basis of the

Table 4. Significant Correlations between the Contents of B Vitamins at Each Location and Across All Sites[a]

	r (p)				
	France	Hungary	Poland	United Kingdom	all sites
			(A) vitamin B1		
vitamin B2	0.22 (0.279)	**0.47** (**0.014**)	0.14 (0.505)	0.18 (0.378)	0.014 (0.890)
vitamin B3	**0.55** (**0.004**)	**0.71** (**<0.001**)	**0.301** (0.153)	**0.46** (**0.0195**)	**0.71** (**<0.001**)
vitamin B6	**0.48** (**0.014**)	**0.45** (**0.02**)	0.18 (0.391)	**0.39** (**0.047**)	**0.64** (**<0.001**)
			(B) vitamin B2		
vitamin B1	0.22 (0.279)	**0.47** (**0.014**)	0.14 (0.505)	0.18 (0.378)	0.014 (0.890)
vitamin B3	0.27 (0.178)	**0.39** (**0.048**)	0.01 (0.962)	**0.42** (**0.047**)	−0.12 (0.239)
vitamin B6	**0.57** (**0.003**)	**0.71** (**<0.001**)	**0.53** (**0.008**)	0.26 (0.235)	**0.24** (**0.014**)
			(C) vitamin B3		
vitamin B1	**0.55** (**0.004**)	**0.71** (**<0.001**)	**0.301** (0.153)	**0.46** (**0.0195**)	**0.708** (**<0.001**)
vitamin B2	0.27 (0.178)	**0.39** (**0.048**)	0.01 (0.962)	**0.42** (**0.047**)	−0.118 (0.239)
vitamin B6	0.38 (0.052)	0.202 (0.212)	0.12 (0.589)	**0.7** (**<0.001**)	**0.67** (**<0.001**)
			(D) vitamin B6		
vitamin B1	**0.48** (**0.014**)	**0.45** (**0.02**)	0.18 (0.391)	**0.39** (**0.047**)	**0.639** (**<0.001**)
vitamin B2	**0.57** (**0.003**)	**0.71** (**<0.001**)	**0.53** (**0.008**)	0.26 (0.235)	**0.242** (**0.014**)
vitamin B3	0.38 (0.052)	0.202 (0.212)	0.12 (0.589)	**0.7** (**<0.001**)	**0.67** (**<0.001**)

[a] Figures depicted in bold represent a significant (p <0.05) correlation.

Table 5. Correlations between the Mean Contents of B Vitamins and the Weather Conditions at the Four Sites

	mean temperature heading to harvest	precipitation 3 months before heading to harvest (mm)	precipitation heading to harvest (mm)	precipitation 3 months before heading (mm)
		vitamin B content		
vitamin B1	r = 0.888 (p = 0.112)	r = −0.495 (p = 0.505)	r = −0.429 (p = 0.571)	r = −0.346 (p = 0.654)
vitamin B2	r = −0.242 (p = 0.758)	r = −0.395 (p = 0.605)	r = 0.136 (p = 0.864)	r = 0.982 (p = 0.018)
vitamin B3	r = 0.954 (p = 0.0463)	r = −0.647 (p = 0.353)	r = −0.560 (p = 0.424)	r = −0.399 (p = 0.601)
vitamin B6	r = 0.803 (p = 0.197)	r = −0.374 (p = 0.626)	r = −0.428 (p = 0.572)	r = −0.095 (p = 0.905)
		weather conditions		
Hungary	20.5	243.7	126.6	117.1
France	18.4	205.5	101.4	104.1
Poland	17.7	289.7	204.2	85.5
United Kingdom	14.2	360.2	232.6	127.6

comparison between the values reported here and the total values in the literature, the biologically available vitamin B3 only accounted for about 10−20% of the total.

However, the design of the experiment allowed several novel aspects of B vitamin content to be studied. First, the availability of analyses for the same lines grown on four sites allowed the variation in B vitamin content to be partitioned between the effects of genotype (G), environment (E). This showed that the contents of vitamins B2, B3, and B6 were only poorly heritable, ranging from 7% for vitamin B3 to 16% for vitamin B2. The higher heritability of vitamin B1 (30%) indicates that increases in content may be possible by breeding. This analysis also showed that vitamin B2 differed from the other three B vitamins in that most of the variation in content was ascribed to the error term, suggesting that the content of vitamin B2 may be affected by G × E interactions as opposed to the environment alone. This could indicate a fundamental difference between the effects of genotype and environment on vitamin B2 synthesis as compared to the other B vitamins.

The four B vitamins showed similar effects of site, with the contents being highest in the samples grown in Hungary and lowest in those grown in the United Kingdom. The samples grown in France and Poland had intermediate contents of B vitamins, with the Polish samples having higher contents of vitamins B1, B3, and B6 and the contents of vitamin B2 being similar in the samples from the two sites.

Analysis of the relationships between the weather conditions at each of the growing sites and the mean concentration of B vitamins demonstrated that vitamins B1, B3, and B6 showed similar positive correlations in mean temperature between heading and harvest. Hungary had the highest mean temperature over the growth period, and the United Kingdom had the lowest, with France and Poland having similar mean temperatures. High temperature during grain filling may also result in smaller grains, which have higher proportions of outer layers and embryo relative to starchy endosperm. Because B vitamins are concentrated in these tissues, their content in whole grain would be expected to be higher if grain size is reduced.

The content of vitamin B2 showed less response to temperature, and partitioning of the variation showed that it was strongly influenced by the interaction of the genotype with the environment. It also showed a greater response to the rainfall in the period before heading than the concentrations of the other B vitamins.

It is difficult to select for components with low heritabilities in plant breeding programs; hence, it is unlikely that the contents of B vitamins in wheat, with the possible exception of vitamin B1, could be substantially increased by breeding. An alternative is to use GM technology to up-regulate the synthesis of B vitamins, as described for a number of health-related components in model and crop plant species (reviewed in ref 22). This approach has been used most successfully to increase the content of provitamin A (β-carotene) in "Golden Rice"[23] and maize,[24] but success has also been achieved with engineering increases in the content of folates (vitamin B9) in tomato fruit[25,26] and rice[27] and vitamin E (tocopherols) in soybean.[28] Naqvi et al.[29] have also reported the production of transgenic maize overexpressing genes involved in the biosynthetic pathways of three different vitamins: provitamin A, folate, and vitamin C. This resulted in increases in the contents of the three vitamins by 169-, 2-, and 6-fold, respectively, as compared with untransformed seeds. However, this approach is unlikely to be used for the B vitamins discussed here, at least in the short term, as their biosynthetic pathways are complex and their genetic control and regulation are not well understood.[30,31]

The most surprising aspect of the results was the demonstration of correlations between the contents of vitamins B1 and B3 and B1 and B6. The B vitamins are initially defined as a water-soluble fraction, and subsequent identification and characterization of the individual components have shown that they have little in common except solubility. Although thiamine (B1), niacin (B3), and pyridoxine (B6) are all aromatic compounds with at least one nitrogen atom present in their ring structures, the closest structural relationship is between niacin and pyridoxine (which showed no consistent correlation), which are both small molecules containing substituted pyridine rings. There are also no known relationships between the biosynthetic pathways for these three B vitamins.

■ ASSOCIATED CONTENT

S Supporting Information. Origins and growth habits of the selected wheat lines (Table S1); contents of vitamins B1, B2, B3, and B6 in the wheat lines grown on the four sites (Table S2); partitioning of variance in the contents of vitamins B1, B2, B3, and B6 between genotype (G), environment (E), G × E, and error components (Table S3); and *F* and *P* values of variance analysis for B vitamins (Table S4). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +44(0)1582 763133. Fax: +44(0)1582 763010. E-mail: peter.shewry@bbsrc.ac.uk.

Funding Sources

This publication 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 is funded by the Biotechnology and Biological Sciences Research Council (BBSRC).

■ ABBREVIATIONS USED

dw, dry weight; HPLC, high-performance liquid chromatography; RSD, relative standard deviation; TOT-AX, total arabinoxylan; UPLC, ultraperformance liquid chromatography; WE-AX, water-extractable arabinoxylan

■ REFERENCES

(1) National Research Council. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*; Institute of Medicine USA; The National Academies Press, Washington D.C., USA, 1998.

(2) Keagy, P. M.; Borenstein, B.; Ranum, P.; Connor, M. A.; Lorenz, K.; Hobbs, W. E.; Hill, G.; Bachman, A. L.; Boyd, W. A.; Kulp, K. Natural levels of nutrients in commercially milled wheat flours. II. Vitamin analysis. *Cereal Chem.* **1980**, *57*, 59–65.

(3) MacMasters, M. M.; Hinton, J. J. C.; Bradbury, D. Microscopic structure and composition of the wheat kernel. In *Wheat: Chemistry and Technology*, 2nd ed.; Pomeranz, Y., Ed.; AACC: St. Paul, MN, 1971; pp 51−113.

(4) Betschart, A. A. Nutritional quality of wheat and wheat foods. In *Wheat: Chemistry and Technology*, 3rd ed.; Pomeranz, Y., Ed.; AACC: St. Paul, MN, 1988; Vol. 2, pp 91−130.

(5) Davis, K. R.; Litteneker, N.; Le Tourneau, D.; Cain, R. F.; Peters, L. J.; McGinnis, J. Evaluation of the nutrient composition of wheat. I. Lipid constituents. *Cereal Chem.* **1980**, *57*, 178–184.

(6) Davis, K. R.; Cain, R. F.; Peters, L. J.; Le Tourneau, D.; McGinnis, J. Evaluation of the nutritent composition of wheat. II. Proximate analysis, thiamine, riboflavin, niacin and pyridoxine. *Cereal Chem.* **1981**, *58*, 116–120.

(7) Batifoulier, F.; Verny, M.-A.; Chanliaud, E.; Remesy, C.; Demigne, C. Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. *Eur. J. Agron.* **2006**, *25*, 163–169.

(8) Sampson, D. A.; Wen, Q.-B.; Lorenz, K. Vitamin B6 and pyridoxine glucoside content of wheat and wheat flours. *Cereal Chem.* **1996**, *73*, 770–774.

(9) Ward, J.; Poutanen, K.; Gebruers, K.; Piironen, V.; Lampi, A.-M.; Nystrom, L.; Anderson, A. A. M.; Åman, P.; Boros, D.; Rakszegi, M.; Bedo, Z.; Shewry, P. R. The HEALTHGRAIN cereal diversity screen: Concept, results and prospects. *J. Agric. Food Chem.* **2008**, *56*, 9699–9709.

(10) Shewry, P. R.; Piironen, V.; Lampi, A.-M.; Edelmann, M.; Kariluoto, S.; Nurmi, T.; Fernandez-Orozco, R.; Andersson, A. A. M.; Åman, P.; Fras, A.; Boros, D.; Gebruers, K.; Dornez, E.; Courtin, C. M.; Delcour, J. A.; Ravel, C.; Charmet, G.; Rakszegi, M.; Bedo, Z.; Ward, J. L. Effects of genotype and environment on the content and composition of phytochemicals and dietary fiber components in rye in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2010**, *58*, 9291–9298.

(11) Zhao, F. J.; Su, Y. H.; Dunham, S. J.; Rakszegi, M.; Bedo, Z.; McGrath, S. P.; Shewry, P. R. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* **2009**, *49*, 290–295.

(12) Carter, E. G. A.; Carpenter, K. J. The bioavailability for humans of bound niacin from wheat bran. *Am. J. Clin. Nutr.* **1982**, *36*, 855–861.

(13) Piironen, V.; Lampi, A.-M.; Ekholm, P. Micronutrients and phytochemicals in wheat grain. In *Wheat: Chemistry and Technology*, 4th ed.; Khan, K., Shewry, P. R., Eds.; AACC: St. Paul, MN, 2009; pp 179−222.

(14) Butterfield, S.; Calloway, D. H. Folacin in wheat and selected foods. *J. Am. Diet. Assoc.* **1972**, *60*, 310–314.

(15) Ranum, P. M.; Barrett, F. F.; Loewe, R. J.; Kulp, K. Nutrient levels in internationally milled wheat flours. *Cereal Chem.* **1980**, *57*, 361–366.

(16) Kabir, H.; Leklem, J.; Miller, L. T. Measurement of glycosylated vitamin B6 in foods. *J. Food Sci.* **1983**, *48*, 1422–1425.

(17) Hegedüs, M.; Pedersen, B.; Eggum, B. O. The influence of milling on the nutritive value of flour from cereal grains. 7. Vitamins and tryptophan. *Plant Foods Hum. Nutr.* **1985**, *35*, 175–180.

(18) Jägerstad, M.; Häkansson, B. Vitamin changes during processing of cereals. In *Proc. Congr. Cereal Sci. Technol. in Sweden*; Asp, N., Ed.; Lund University: Lund, Sweden, 1988; pp 207−219.

(19) Pomeranz, Y. Chemical composition of kernel structures. In *Wheat: Chemistry and Technology*, 3rd ed.; Pomeranz, Y., Ed.; AACC: St. Paul, MN, 1988; Vol. 1, pp 97−158.

(20) Klopfenstein, C. F. Nutritional quality of cereal-based foods. In *Handbook of Cereal Science and Technology*, 2nd ed.; Kulp, K., Ponte, J. G. J., Eds.; Marcel Dekker: New York, 2000; pp 705−723.

(21) Batifoulier, F.; Verny, M.-A.; Chanliaud, E.; Remesy, C.; Demigne, C. Effect of different breadmaking methods on thiamine, riboflavin and pyridoxine contents of wheat bread. *J. Cereal Sci.* **2005**, *42*, 101–108.

(22) Davies, K. M. Genetic modifications of plant metabolism for human health benefits. *Mutat. Res.* **2007**, *622*, 122–137.

(23) Paine, J. A.; Shipton, C. A.; Chaggar, S.; Howells, R. M.; Kennedy, M. J.; Vernon, G.; Wright, S. Y.; Hinchliffe, E.; Adams, J. L.; Silverstone, A. L.; Drake, R. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat. Biotechnol.* **2005**, *23*, 482–487.

(24) Zhu, C. F.; Naqvi, S.; Breitenbach, J.; Sandmann, G.; Christou, P.; Capell, T. Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18232–18237.

(25) de la Garza, R. D.; Quinlivan, E. P.; Klaus, S. M. J.; Basset, G. J. C.; Gregory, J. F.; Hanson, A. D. Folate biofortification in tomatoes by engineering the pteridine branch of folate synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13720–13725.

(26) de la Garza, R. I. D.; Gregory, J. F.; Hanson, A. D. Folate biofortification of tomato fruit. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4218–4222.

(27) Storozhenko, S.; De Brouwer, V.; Volckaert, M.; Navarrete, O.; Blancquaert, D.; Zhang, G. F.; Lambert, W.; Van der Straeten, D. Folate fortification of rice by metabolic engineering. *Nat. Biotechnol.* **2007**, *25*, 1277–1279.

(28) Karunanandaa, B.; Qi, Q. G.; Hao, M.; Baszis, S. R.; Jensen, P. K.; Wong, Y. H. H.; Jiang, J.; Venkatramesh, M.; Gruys, K. J.; Moshiri, F.; Post-Beittermiller, D.; Weiss, J. D.; Valentin, H. E. Metabolically engineered oilseed crops with enhanced seed tocopherol. *Metab. Eng.* **2005**, *7*, 384–400.

(29) Naqvi, S.; Zhu, C. F.; Farre, G.; Ramessar, K.; Bassie, L.; Breitenbach, J.; Conesa, D. P.; Ros, G.; Sandmann, G.; Capell, T.; Christou, P. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 7762–7767.

(30) Webb, M. E.; Marquet, A.; Mendel, R. R.; Rébeillé, F.; Smith, A. G. Elucidating biosynthetic pathways for vitamins and cofactors. *Nat. Prod. Rep.* **2007**, *24*, 988–1008.

(31) Begley, T. P.; Chatterjee, A.; Hanes, J. W.; Hazra, A.; Ealick, S. E. Cofactor biosynthesis—Still yielding fascinating new biological chemistry. *Curr. Opin. Chem. Biol.* **2008**, *12*, 118–125.