JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effects of Genotype and Environment on the Contents of Betaine, Choline, and Trigonelline in Cereal Grains

Delia-Irina Corol,[†] Catherine Ravel,[§] Mariann Raksegi,[#] Zoltan Bedo,[#] Gilles Charmet,[§] Michael H. Beale,[†] Peter R. Shewry,[†] and Jane L. Ward*[,†]

[†]Plant Science Department, Rothamsted Research, West Common, Harpenden Herts AL5 2JQ, United Kingdom

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

[#]Agricultural Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences, P.O. Box 19, 2462 Martonvásár, Hungary

Supporting Information

ABSTRACT: This study examined the environmental and genetic variation in methyl donor contents and compositions of 200 cereal genotypes. Glycine betaine, choline, and trigonelline contents were determined by ^{1}H NMR, and significant differences were observed between cereal types (G) and across harvesting years and growing locations (E). Glycine betaine was the most abundant methyl donor in all of the 200 lines grown on a single site, and concentrations ranged from 0.43 ± 0.09 mg/g dm in oats to 2.57 ± 0.25 mg/g dm in diploid Einkorn varieties. In bread wheat genotypes there was a 3-fold difference in glycine betaine content. Choline contents, in the same lines, were substantially lower, and mean concentrations ranged from 0.17 mg/g dm in oats to 0.27 mg/g dm in durum wheat. Trigonelline was by far the least abundant of the methyl donors studied. Despite this, however, there were large differences between cereal types. Twenty-six wheat genotypes were grown in additional years at four European locations. The average glycine betaine content was highest in grains grown in Hungary and lowest in those grown in the United Kingdom. Across the six environments, there was a 3.8-fold difference in glycine betaine content. Glycine betaine levels, although moderately heritable (0.36), were found to be the most susceptible to the environmental conditions. Free choline concentrations were less variable across genotypes, but heritability of this component was the lowest of all methyl donor components (0.25) and showed a high G × E interaction. Trigonelline showed the most variation due to genotype. Heritability of this metabolite was the highest (0.59), but given that it is at a very low concentration in wheat, it is probably not attractive to plant breeders.

KEYWORDS: *methyl donors, cultivar, G × E, wheat, whole grain, wholemeal*

INTRODUCTION

It is now widely accepted, largely on the basis of epidemiological studies, that the consumption of whole grain cereals has a range of health benefits including reducing the risk of cardiovascular disease (CVD)[1] and type 2 diabetes.[2] Although the precise components that are responsible for these effects are not known, they could include a number of well-characterized dietary fiber components, vitamins, and phytochemicals that are present in the whole wheat grain (reviewed by Piironen et al.[3]).

A major risk factor in CVD is hyperhomocysteinemia, which is associated with hyperlipidemia.[4] Homocysteine is produced by demethylation of methionine and can either be remethylated to methionine, metabolized to give cysteine, or converted to *S*-adenosylhomocysteine. The remethylation of homocysteine to form methionine requires the addition of a methyl group, which may be provided by betaine.[4,5]

Betaine, more correctly called glycine betaine (*N*,*N*,*N*-trimethylglycine), is produced commercially from sugar beet as a byproduct of sugar production. In human nutrition, it is obtained almost solely from the diet, but can also be produced in animals by conversion of choline. Zeisel et al.[6] surveyed 145 common foods and showed that the highest levels of betaine were present in wheat bran (15 mg/g) and germ (14 mg/g) with about 7 mg/g in spinach and 3 mg/g in canned beets. Whereas betaine occurs only in the free form, choline also occurs in a number of forms, notably phospholipids. Zeisel et al.[6] reported that the levels of free and total choline in wheat germ were 0.69 and 1.52 mg/g, respectively, and those in wheat bran were 0.51 and 0.74 mg/g, respectively. Likes et al.[7] reported similar levels of betaine of 12.93 mg/g in wheat bran and 11.63 mg/g in germ and also reported levels of 2.91 mg/g in whole wheat. Corresponding free choline levels were 0.47 mg/g in bran, 1.15 mg/g germ, and 0.14 mg/g in whole wheat. Total choline (free choline plus phospholipids) were 0.88 mg/g in bran, 1.68 mg/g in germ, and 0.27 mg/g in whole wheat.

Betaine is also known to act as an osmoregulant in many organisms including plants[8,9] and mammals,[10] accumulating in response to drought stress or salinity. Plants may also contain related methyl compounds, which are often also termed betaines, notably proline betaine (derived from the amino acid proline), which is particularly abundant in citrus fruits, and trigonelline (derived from pyridine), which is abundant in

Received: February 28, 2012
Revised: May 1, 2012
Accepted: May 5, 2012
Published: May 5, 2012

dx.doi.org/10.1021/jf3008794 | J. Agric. Food Chem. 2012, 60, 5471−5481

coffee.[11] However, low levels of both compounds are also present in wheat bran and whole grain products.[11] It is possible that these and other betaine analogues can also act as methyl donors in homocysteine metabolism (discussed by De Zwart et al.[12]), whereas trigonelline has also been reported to act as a phytoestrogen[13] and to have anticariogenic activity.[14] However, unlike betaine, the content of trigonelline in plants does not appear to be affected by salinity.[15]

^{1}H NMR spectroscopy of polar solvent extracts can be used to provide rapid high-throughput analyses of soluble components and provides reliable analyses of betaine, free choline, and trigonelline in plant tissues,[16−19] including choline and betaine in wheat grain fractions.[20,21]

HEALTHGRAIN was a 5 year project (2005−2010), supported under the EU sixth framework program, which aimed to improve the health of consumers and reduce the risk of diseases related to the metabolic syndrome by increasing the consumption of protective compounds present in whole grain cereals.[22,23] The project included a "diversity screen", in which 150 lines of bread wheat and 50 lines of other cereals were grown together on a single site in Martonvásár, Hungary, and analyzed for a range of bioactive components (phytochemicals and dietary fiber components) (summarized by Ward et al.[24]). A smaller set of 26 wheat lines and 5 ryes were then grown on the same site in Hungary for two further years and on three additional sites (in the United Kingdom, France, and Poland) in the third year only and subjected to the same analyses. This allowed the effects of genotype and environment to be separated and the heritabilities of the bioactive components calculated.[25] This study provides an unrivaled database of the detailed composition of grain of wheat and other cereals. We have therefore analyzed wholemeal fractions from the same samples by high-field ^{1}H NMR to determine the contents of soluble components including methyl donors (betaine, choline, trigonelline), which are reported here. This is the first study of its kind that examines the methyl donor composition across such a large number of wheat genotypes grown at a single location in the same year. Furthermore, the measurement of the methyl donor components across a range of years and European locations has enabled the trait heritabilities of these compounds to be assessed.

MATERIALS AND METHODS

Chemicals. Authentic standards of glycine betaine, choline, and trigonelline were obtained from Sigma-Aldrich (Gillingham, Dorset, U.K.) and were used without further purification. Deuterated solvents for ^{1}H NMR were purchased from Goss Scientific Instruments (Nantwich, U.K.).

Materials. Full lists of all cultivars described in this paper can be found in Supplementary Table 1 of the Supporting Information. The first field experiment was carried out at Martonvásár (near Budapest, Hungary) in 2004−2005. One hundred and fifty bread wheat lines were selected to represent a wide range of diversity in the gene pool available for plant breeders, including wide geographical diversity in origin (from Europe to East Asia, the Americas, and Australia) and including landraces, breeding lines, and modern and older cultivars. One hundred and thirty were winter type and 20 were spring type. Five modern cultivars of spelt (a hulled form of hexaploid wheat, *Triticum aestivum* var. *spelta*), 10 lines of tetraploid durum wheat (*T. turgidum* var. *durum*), 5 lines each of two early cultivated forms of wheat, diploid einkorn (*T. monococcum* var. *monococcum*) and tetraploid emmer (*T. turgidum* var. *dicoccum*), 10 lines of rye (*Secale cereale*), 5 of oats (*Avena sativa*), and 10 lines of barley (*Hordeum vulgare*) were also included. Full details are given by Ward et al.[24] Twenty-three of the wheat lines and 5 rye lines were selected for further studies, together with 3 additional wheats and 1 additional rye. These were grown at Martonvásár again in 2005−2006 and 2006−2007 and at Nickerson Seeds U.K. (Saxham, near Bury St Edmunds, U.K.), the INRA experimental station at Clermont Ferrand (France), and (with the exception of the three spring wheat lines) Danko Plant Breeders Ltd. (Choryn, near Poznan, Poland) in 2007. 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. Winter, spring, and durum wheats were conditioned to 15.5% moisture content before milling, whereas other species were conditioned to 14% moisture content. Milling was carried out using a Perten Laboratory Mill 3100 (with 0.5 mm sieve) and a Retsch ZM100 (for *T. monococcum* and oats) to produce wholemeal. Samples were immediately cooled to −20 °C and stored at the same temperature in sealed bags.

ENVIRONMENTAL CONDITIONS

The field experiments were a part of the integrated project HEALTHGRAIN and are described in more detail by Shewry et al.,[25] which includes data on precipitation, temperature, soil characteristics, and dates of heading and harvest. Briefly, the total precipitation between heading and harvest varied from 116 to 128 mm in Hungary, whereas the average temperature during the same period varied from 19.3 to 20.5 °C. When the four locations in 2007 were compared, the mean temperature between heading and harvest was highest in Hungary (20.5 °C) and lowest in the United Kingdom (14.7 °C), whereas the accumulated precipitation was highest in the Untied Kingdom (232 mm) and lowest in Poland (101 mm). Of note is the fact that the temperature at the Hungarian site varied more widely than at the other three sites, experiencing both lower minimum temperatures together with higher maximum temperatures. It was also consistently hotter during the grain-filling period. By contrast, the U.K. site was cool and wet during the same period.

NMR Profiling. Glycine betaine, choline, and trigonelline were analyzed by ^{1}H NMR according to previously published methods.[26,27] Triplicate aliquots of wholemeal (50 mg) were extracted at 50 °C using 1 mL of D_2O/CD_3OD (80:20) containing d_4-TSP (0.05% w/v) as internal standard. After centrifugation (5 min), the resulting supernatant was heated for 2 min at 90 °C to remove any residual enzyme activity, before transfer to a 5 mm NMR tube for analysis. NMR spectra were collected at 300 K on an Avance spectrometer (Bruker Biospin, Coventry, U.K.) equipped with a 5 mm selective inverse probe, operating at 600.0528 MHz. Data were collected using a water suppression pulse sequence with a relaxation delay of 5 s. Each spectrum was acquired using 128 scans of 64000 data points with a spectral width of 7309.99 Hz. Spectra were automatically Fourier transformed using an exponential window with a line broadening value of 0.5 Hz. Phasing and baseline correction were carried out within the instrument software. ^{1}H chemical shifts were referenced to d_4-TSP at δ 0.00.

Spectra were automatically reduced, using Amix (Analysis of MIXtures software, Bruker Biospin), to ASCII files containing integrated regions or "buckets" of equal width (0.001 ppm). Spectral intensities were scaled to the d_4-TSP region (δ 0.05 to −0.05). The ASCII file was imported into Excel for the addition of sampling/treatment details.

Regions for methyl donors were identified via comparison to a library of known standards. Regions used for the quantitation were δ 3.2615−3.2745 for glycine betaine, δ 3.2005−3.2085 for choline, and δ 8.8035−8.8825 for trigonelline. These identified

Table 1. Comparison of Mean Concentrations of Methyl Donor Compounds in Wholemeal Samples of Different Cereals[a]

		glycine betaine (mg/g dm)			choline (mg/g dm)			trigonelline (μg/g dm)		
cereal	*n*	mean ± SD	min	max	mean ± SD	min	max	nean ± SD	min	max
winter wheat	130	1.59 ± 0.35	0.97	2.94	0.22 ± 0.02	0.18	0.28	3.18 ± 1.50	0.53	8.55
spring wheat	20	1.62 ± 0.32	1.18	2.25	0.23 ± 0.02	0.21	0.27	2.56 ± 1.54	0.64	6.36
durum wheat	10	2.32 ± 0.41	1.66	2.77	0.27 ± 0.02	0.24	0.31	5.12 ± 2.46	1.78	9.34
spelt	5	2.31 ± 0.33	1.83	2.77	0.21 ± 0.01	0.20	0.22	2.32 ± 0.94	1.24	3.35
einkorn	5	2.57 ± 0.25	2.22	2.83	0.25 ± 0.04	0.21	0.30	1.08 ± 0.63	0.31	1.91
emmer	5	2.05 ± 0.34	1.51	2.45	0.22 ± 0.02	0.18	0.24	2.21 ± 3.31	0.15	7.94
rye	10	2.27 ± 0.48	1.76	2.98	0.26 ± 0.02	0.22	0.29	31.13 ± 12.5	15.73	50.15
barley	10	1.02 ± 0.23	0.71	1.36	0.32 ± 0.03	0.27	0.37	0.25 ± 0.24	0.01	0.83
oats	5	0.43 ± 0.09	0.29	0.55	0.17 ± 0.01	0.15	0.18	111.7 ± 10.1	97.23	123.86

[a]Concentrations are the mean value from three replicates.

spectral regions for methyl donors were integrated against the known concentration of TSP in the sample (0.05% w/v).

Calculations of mean, standard deviations, and coefficients of variation were carried out using Microsoft Excel. Methyl donor concentrations across genotypes and environments were compared by analysis of variance (ANOVA) also in Microsoft Excel. To relate methyl donor values to the physical parameters of the kernels and to weather conditions, Pearson correlation coefficients were calculated on a dry weight basis using Spotfire Decision Site (v. 9.1.2., TIBCO, Somerville, MA, USA).

G × E Analyses. Data sets from the 26 wheat varieties grown in the six different environments were used in statistical models with all effects considered as random to estimate variance components with SAS software (proc VARCOMP). Three technical replicates were used as error terms in the following model:

$$X = \mu + E + G + (G\times E) + \varepsilon$$

Because replicates were technical and not true field replicates, the error term is likely to be an underestimate of the true error, and it is actually very low, except for trigonelline. Therefore, we used the ratio $\sigma^2_g/(\sigma^2_g + \sigma^2_E + \sigma^2_{G\times E})$ as a surrogate to heritability h^2. Indeed, this parameter, although likely to be an underestimate of h^2, is a suitable parameter for plant breeders, as a high value indicates that the trait is mostly affected by the genotype.

RESULTS AND DISCUSSION

Comparison of Cereals. Integration of binned NMR spectra (ca. 10000 bins of 0.001 ppm each) allowed batch processing of the large number of spectra and accurate quantitation of the methyl donors. For betaine and choline the sharp *N*-trimethyl singlets (located at 3.268 and 3.204 ppm, respectively) were utilized for quantitation, whereas for trigonelline, the aromatic multiplet, corresponding to two hydrogens, at 8.88 ppm was most convenient. Signals for proline betaine were only evident at trace levels in the NMR data of wholemeal flour, and therefore this metabolite could not be quantified. Glycine betaine was the most abundant of the three components. For the lines grown at a single site in 2005, the average contents of glycine betaine in wholemeal varied from 0.43 ± 0.09 mg/g dm in oats (n = 5) to 2.57 ± 0.25 mg/g dm in einkorn (diploid wheat) (n = 5) (Table 1; Supporting Information, Supplementary Figure 1). The average contents in winter (n = 130) and spring (n = 20) wheat genotypes were 1.59 ± 0.39 and 1.62 ± 0.32 mg/g dm, respectively, with the contents in the 150 lines ranging from 0.97 to 2.97 mg/g dm.

The free choline levels in the same samples were also determined. The levels of this metabolite, a biosynthetic precursor of glycine betaine, were considerably lower than those observed for glycine betaine and ranged from 0.17 ± 0.01 mg/g dm in oat to 0.32 ± 0.03 mg/g dm in barley (n = 10). The average contents in winter (n = 130) and spring (n = 30) wheat genotypes were again similar at 0.22 ± 0.02 and 0.23 ± 0.02 mg/g dm, respectively. The concentrations of free choline across the 150 bread wheat genotypes analyzed ranged from 0.18 to 0.28 mg/g dm. The contents of both glycine betaine and free choline in the wholemeal samples agreed with those reported by Likes et al.[7] for whole wheat flour derived from a Kansas hard red winter wheat.

Trigonelline was the least abundant of the compounds analyzed in wholemeal samples of cereal grains. In the majority of samples, concentrations were only a few micrograms per gram. Exceptions were rye (n = 10) and oats (n = 5), in which the levels were higher, with mean values of 31.15 ± 12.5 and 111.7 ± 10.1 μg/g dm, respectively. In the remaining cereals, the mean levels were highest in durum wheat (5.12 ± 2.46 μg/g dm). Across bread wheats, the lowest content of trigonelline was 0.53 μg/g dm, whereas the highest was 8.55 μg/g dm. Barley genotypes (n = 10) typically contained the lowest levels of trigonelline with a mean concentration of only 0.25 ± 0.24 μg/g dm.

Range of Contents in 150 Bread Wheat Genotypes. By far the largest numbers of samples analyzed in this study were bread wheats, with all genotypes having been grown at the same location in a single year. Hence, it can be assumed that most of the variation in composition of these samples could be ascribed to the genotype rather than the environment, allowing a comparison of genotypes to be made. The concentrations of glycine betaine, choline, and trigonelline in bread wheats typically followed a normal distribution (Figure 1). A full listing of genotypes grouped into eight concentration ranges for each component is given in Supplementary Table 2 in the Supporting Information. The majority of genotypes had glycine betaine levels between 1.16 and 2.20 mg/g dm. Those genotypes that did not fall within this range included 12 winter wheat cultivars with low glycine betaine concentrations, between 0.95 and 1.16 mg/g dm, and 11 genotypes with high concentrations, between 2.20 and 2.98 mg/g dm (Table 2). Of these, 9 were winter wheats (Tiszataj, Alba, TAM 200, Claire, Kanzler, Gerek 79, Arthur 71, and Malacca) and 2 were spring wheat cultivars (Cadenza and Red Fife). The winter wheat Malacca had the highest glycine betaine content (2.98 mg/g dm) of all the bread wheats.

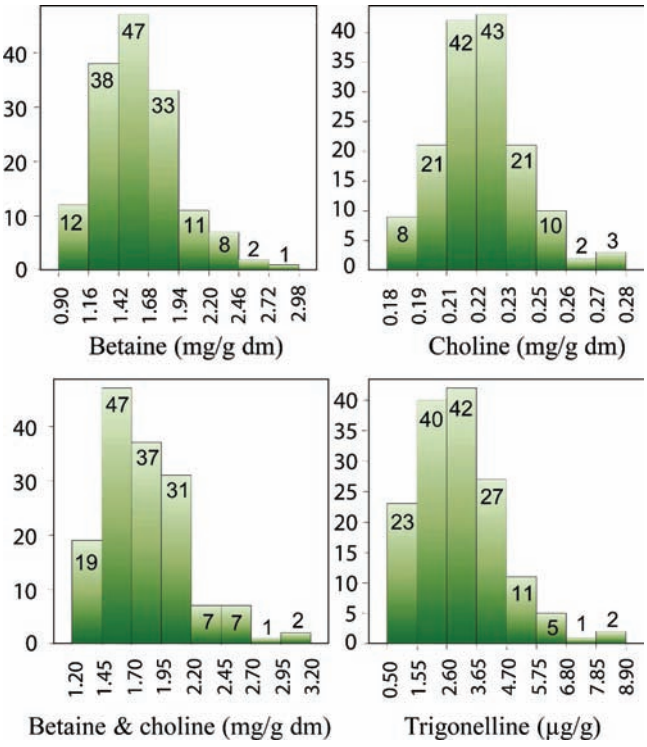

Figure 1. Frequency distributions of 150 bread wheat genotypes based on methyl donor concentration.

Choline levels varied from 0.18 to 0.28 mg/g dm, with the majority of the lines having contents between 0.193 and 0.245 mg/g dm. Those that did not fall within this range included 9 winter wheat genotypes with low contents, between 0.18 and 0.193 mg/g dm, and 15 genotypes with high contents, between 0.245 and 0.284 mg/g dm (Table 2). Of the latter, 11 were winter wheats (SU321, Yumai 34, Atay 85, Mv-Suba, Sumai 3, Seu Seun 27, Frederick, Spark, Kirkpinar 79, Kirac 66, Klein Estrella) and 4 were spring wheat cultivars (Chara, Red Fife, Sunstar, Kukri). The combined contents of choline and glycine betaine in the bread wheat genotypes are also given in Table 2 and in the Supporting Information (Supplementary Table 2). They ranged from 1.2 to 3.2 mg/g dm, with the trends reflecting the betaine levels as the concentrations of betaine were generally some 10 times higher than the free choline levels.

Trigonelline was the least abundant of the three compounds in bread wheats, with concentrations some 1000 times lower than the other two, ranging from 0.5 to 8.55 μg/g dm. The majority of genotypes had contents between 1.55 and 5.75 μg/g dm. Those that did not fall within this range included 18 winter wheat genotypes with low contents, between 0.5 and 0.1.55 μg/g dm, and 8 genotypes with high contents, between 5.75 and 8.55 μg/g dm. Of these, 7 were winter wheats (Caphorn, Cardinal, Roussalka, Lynx, Produttore, Claire, Bankuti 1201) and just 1 was a spring wheat cultivar (Milan) (Table 2; Supplementary Table 2 in the Supporting Information).

Effect of Environmental Conditions and Growing Location on Glycine Betaine. Twenty-six genotypes were selected and grown for two further years to determine the effects of environment, genotype, and genotype × environment interactions on the contents of the three components (Table 3). When grown at Martonvásár (Hungary) for three years (2005, 2006, and 2007), the concentration of glycine betaine ranged from 1.00 mg/g dm (Chinese-Spring, 2006) to 2.94 mg/g dm (Malacca, 2005). Values of the means across the 26 genotypes ranged from 1.5 to 1.8 mg/g dm and showed a high coefficient of variation (CV = 18.55−26.4%). The variance of individual genotypes due to year of growth ranged from 1.97 to 25.9%, with Malacca again having the highest content of glycine betaine (2.40 ± 0.48 mg/g dm) when the data were averaged over the three years. Similarly, San-Pastore had the lowest mean concentration (1.16 ± 0.14 mg/g dm) over the three year period.

The effects of environment were studied further by analysis of the 26 selected lines grown in 2007 in the United Kingdom, France, and Poland as well as Hungary. Comparison of these samples (grown at four locations in a single year) showed a similar pattern in variation in glycine betaine content (Table 3), from 0.78 mg/g dm (Chinese-Spring, U.K.) to 2.51 mg/g dm (Estica, Poland). The mean values for the 26 lines across the growing locations ranged from 1.13 (U.K.) to 1.80 (Hungary) mg/g dm and showed a high coefficient of variation (CV = 12.8−20.5%). The variance of individual genotypes due to location was typically higher than that observed for the single-site comparison over three growing years and ranged from 7.68% (Mv-Emese) to 33.8% (Estica). The contents of glycine

Table 2. Groupings of Bread Wheat Genotypes Showing the Highest and Lowest Concentrations of Glycine Betaine, Choline, and Trigonelline[a]

metabolite	concentration range	genotypes
		High Concentration Groupings
glycine betaine	2.20−2.98 mg/g dm	Tiszataj, Alba, TAM 200, Claire, Kanzler, Gerek 79, Arthur 71, Malacca, Cadenza (S), Red Fife (S)
choline	0.245−0.28 mg/g dm	SU321, Yumai 34, Atay 85, Mv-Suba, Sumai 3, Seu Seun 27, Frederick, Spark, Kirkpinar 79, Kirac 66, Klein Estrella, Chara (S), Red Fife (S), Sunstar (S), Kukri (S)
glycine betaine and choline	2.45−3.20 mg/g dm	Tiszataj, Alba, TAM 200, Claire, Kanzler, Gerek 79, Arthur 71, Malacca, Cadenza (S), Red Fife (S)
trigonelline	5.75−8.55 μg/g dm	Caphorn, Cardinal, Roussalka, Lynx, Produttore, Claire, Bankuti, Milan (S)
		Low Concentration Groupings
glycine betaine	0.90−1.16 mg/g dm	Carmen, Apache, Mv-Suba, San-Pastore, Qualital, NS Rana 1, B16, Nap Hal, Geronimo, Thesee, Valoris, Sumai 3
choline	0.18−0.19 mg/g dm	Spartanka, Pobeda, Valoris, Thesee, Taldor, Ornicar, Martonvasari 17, Lasta, Baranjka
glycine betaine and choline	1.20−1.45 mg/g dm	Apache, Carmen, San-Pastore, Qualital, Mv-Suba, NS Rana 1, Sumai 3, Thesee, Valoris, Nap Hal, B16, Ornicar, Mv-Emese, Produttore, Geronimo, Gloria, Red River 68 (S), Catbird (S), Chinese-Spring (S)
trigonelline	0.50−1.55 μg/g dm	Soissons, Sumai 3, Granbel, Kirkpinar 79, Korweta, Stephens, Begra, Albatros Odeskii, Etoile de Choisy, Isengrain, Klein Estrella, Ravenna, Herzog, Alba, Nomade, SU321, Ornicar, Sultan 95 (S), Kukri (S), Chara (S), Sunstar (S), Catbird (S)

[a]Data were selected from a comparison of 150 bread wheats grown on a single site in the same year (2005). (S) denotes spring wheat.

dx.doi.org/10.1021/jf3008794 | J. Agric. Food Chem. 2012, 60, 5471−5481

Table 3. Glycine Betaine Concentrations (Milligrams per Gram Dry Matter) of 26 Wheat Cultivars Grown in 6 Environments

	2005	2006	2007				statistics for 3 years at one site (Hungary, 2005–2007)			statistics for 1 year (2007) across four locations (Hungary, France, Poland, U.K.)			statistics for total data across six environments (Hungary, 2005–2007; Poland, France, U.K., 2007)		
genotype	H	H	H	F	P	U.K.	mean	SD	CV (%)	mean	SD	CV (%)	mean	SD	CV (%)
Atlas-66	1.59	1.72	1.51	1.20	1.42	1.05	1.61	0.11	6.58	1.30	0.21	16.17	1.42	0.25	17.68
Avalon	1.37	1.28	1.51	1.39	2.03	1.06	1.39	0.12	8.55	1.50	0.40	26.90	1.44	0.33	22.69
Cadenza	2.23	2.04	2.05	1.66		1.23	2.11	0.10	4.93	1.65	0.41	25.13	1.84	0.40	21.79
Campari	1.74	1.63	1.87	1.30	1.58	1.18	1.75	0.12	6.80	1.48	0.31	20.77	1.55	0.26	16.96
CF99105	1.42	1.41	1.80	1.20	1.88	1.16	1.54	0.22	14.57	1.51	0.38	25.46	1.48	0.30	20.42
Chinese-Spring	1.20	1.00	1.34	0.99		0.78	1.18	0.17	14.46	1.03	0.28	27.32	1.06	0.22	20.30
Claire	2.34	1.79	2.13	1.59	2.12	1.23	2.08	0.28	13.28	1.77	0.44	24.82	1.87	0.41	22.03
Crousty		1.07	1.51	0.98	1.45	0.96	1.29	0.31	24.23	1.22	0.29	24.08	1.19	0.26	22.22
Disponent	1.84	1.47	2.45	1.35	2.00	1.27	1.92	0.50	25.90	1.77	0.56	31.75	1.73	0.45	26.24
Estica	1.63	1.39	1.94	1.42	2.51	1.17	1.65	0.28	16.73	1.76	0.59	33.80	1.68	0.48	28.91
Gloria	1.21	1.24	1.25	1.12	1.16	1.02	1.23	0.02	1.97	1.14	0.10	8.54	1.16	0.09	7.52
Herzog	1.62	1.47	2.10	1.26	1.70	1.16	1.73	0.33	19.18	1.55	0.43	27.97	1.55	0.34	21.95
Isengrain	1.58	1.43	1.64	1.29	1.53	1.08	1.55	0.11	6.90	1.39	0.25	18.05	1.43	0.21	14.64
Lynx	1.47	1.64	1.85	1.52	1.55	1.27	1.65	0.19	11.48	1.55	0.24	15.42	1.55	0.19	12.43
Malacca	2.94	2.01	2.25	1.58	2.35	1.25	2.40	0.48	20.21	1.86	0.53	28.53	2.06	0.60	28.96
Maris-Huntsman	1.95	1.81	2.12	1.58	2.20	1.30	1.96	0.16	8.01	1.80	0.43	23.93	1.83	0.34	18.57
Mv-Emese	1.16	1.60	1.35	1.24	1.30	1.12	1.37	0.22	16.25	1.25	0.10	7.68	1.30	0.17	13.29
Obrii	1.65	1.73	1.60	1.28	1.40	1.17	1.66	0.07	3.93	1.36	0.18	13.45	1.47	0.22	15.06
Rialto	1.46	1.50	1.80	1.22	1.60	1.10	1.59	0.19	11.82	1.43	0.33	22.95	1.45	0.26	17.68
Riband	1.95	1.90	2.38	1.68	2.04	1.27	2.08	0.26	12.61	1.84	0.48	25.92	1.87	0.37	19.92
San-Pastore	1.06	1.12	1.32	1.06	1.38	0.94	1.16	0.14	11.93	1.18	0.21	17.87	1.15	0.17	14.86
Spartanka	1.33	1.43	1.85	1.40	1.75	1.49	1.54	0.28	17.98	1.63	0.21	13.06	1.54	0.21	13.56
Tiger		1.34	1.78	1.11	1.49	0.97	1.56	0.31	19.97	1.34	0.37	27.45	1.34	0.32	23.77
Tommi	1.67	1.49	2.10	1.32	1.71	1.13	1.75	0.31	17.70	1.56	0.43	27.42	1.57	0.34	21.47
Tremie	1.54	1.19	1.60	1.34	1.66	1.10	1.44	0.22	15.30	1.42	0.26	18.16	1.40	0.23	16.43
Valoris	1.15	1.33	1.62	1.00	1.37	0.96	1.37	0.24	17.34	1.24	0.32	25.54	1.24	0.25	20.27
av	1.63	1.50	1.80	1.31	1.72	1.13	1.64			1.48			1.51		
SD	0.43	0.28	0.33	0.21	0.35	0.15	0.31			0.23			0.25		
min	1.06	1.00	1.25	0.98	1.16	0.78	1.16			1.03			1.06		
max	2.94	2.04	2.45	1.68	2.51	1.49	2.40			1.86			2.06		
CV (%)	26.36	18.56	18.55	15.70	20.53	12.82	18.98			15.69			16.78		

dx.doi.org/10.1021/jf3008794 | J. Agric. Food Chem. 2012, 60, 5471−5481

betaine were generally highest in the samples grown in Hungary and Poland and significantly ($p < 0.001$) lower in those grown in the United Kingdom and France.

Taking all six environments (i.e., multiple sites and years) into account, the genotype with the highest mean glycine betaine concentration was Malacca (2.06 ± 0.6 mg/g dm). Conversely, Chinese-Spring contained the lowest glycine betaine concentration over the six environments (1.06 ± 0.22 mg/g dm) Despite the large variation (CV ranged from 7.5 to 28.96%) observed between environments, some genotypes appeared to be more "stable" than others. Figure 2 illustrates the mean glycine betaine contents across the six environmental conditions years for each genotype. Plots are ordered by the observed concentration ranges across the 3 year, multienvironment study. The most stable lines (exhibiting the lowest concentration range across six conditions) were Gloria, San-Pastore, Mv-Emese, and Spartanka, whereas those showing the highest variation included Riband, Disponent, Estica, and Malacca. Thus, despite Malacca having the highest mean concentration of glycine betaine across the six environments, it is also clear that this genotype is very susceptible to effects of the environment. Spartanka has a somewhat lower mean concentration of glycine betaine (1.54 ± 0.21 mg/g dm) yet appears to be more stable to the effects of environment, displaying less variation in concentration across the six environments. It also had the highest "minimum concentration" of any genotype (1.33 mg/g dm). Thus, when genotypes are selected for high contents of glycine betaine, it is necessary to consider not only the mean concentrations observed but also the range of concentrations observed when grown under different environmental conditions.

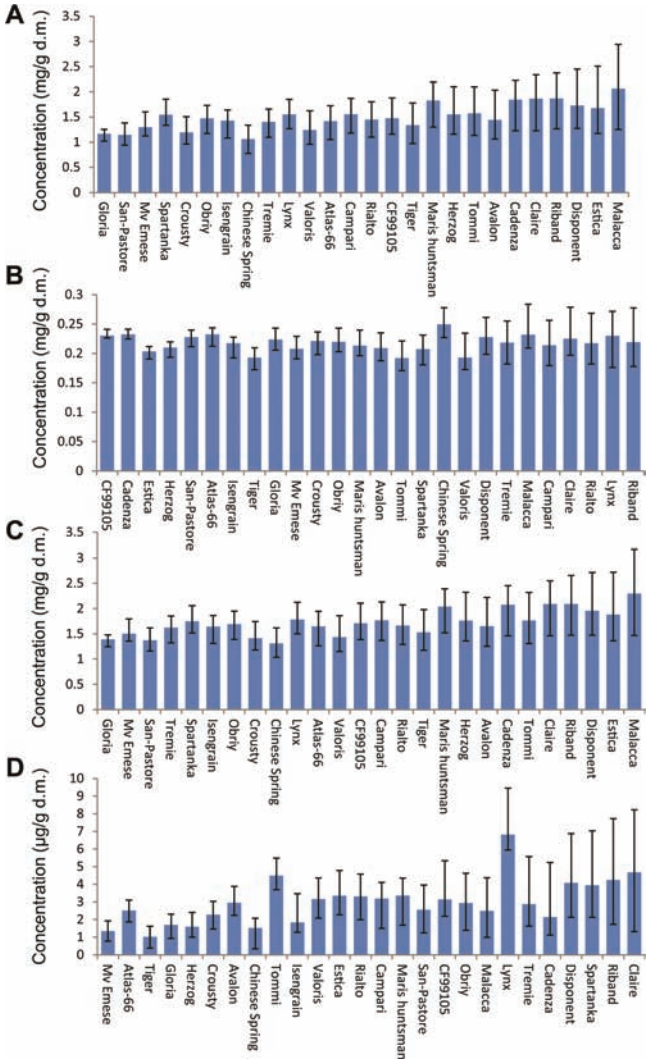

Figure 2. Methyl donor concentrations across six environmental conditions and four locations (Hungary, France, Poland, U.K.) in 2007 and Hungary in 2005 and 2006: (A) glycine betaine; (B) choline (B); (C) betaine plus choline; (D) trigonelline. Error bars represent measured range across six environments. Ordered in order of those showing least to highest variation.

Effect of Environmental Conditions and Growing Location on Choline. Free choline concentrations were less variable for the 26 genotypes grown under different environmental conditions compared to the betaine contents (Table 4). When grown in Hungary in 2005, 2006 and 2007, the concentrations of choline ranged from 0.18 mg/g dm (Spartanka, 2005) to 0.28 mg/g dm (Chinese-Spring, Claire, Malacca, and Riband, 2007). Values of the means across the 26 genotypes ranged from 0.22 to 0.24 mg/g dm and showed a lower coefficient of variation (CV = 6.6−10.7%) than observed for betaine. The variance of individual genotypes due to year of growth ranged from 0.52 to 14.65%. Chinese-Spring and Disponent had the highest mean concentrations of choline over three years (0.25 mg/g dm). Similarly, Mv-Emese and Valoris had the lowest mean concentration (0.20 mg/g dm) over the three year period.

Comparison of samples grown at four locations in a single year (2007) showed wider variation in choline concentration (Table 4), with contents ranging from 0.17 to 0.28 mg/g dm (Riband, Malacca, Claire, and Chinese-Spring, grown in Hungary). The mean values for the 26 genotypes across the growing locations ranged from 0.20 (Poland) to 0.24 (Hungary) mg/g dm and showed a slightly higher coefficient of variation (CV = 9.07−10.7%). Some genotypes showed an increased coefficient of variation compared to the analysis of data from a single site over three years, which probably related to the wider difference between sites.

Taking all six environments (i.e., multiple sites and years) into account, the genotype with the highest mean choline concentration was Chinese-Spring (0.25 ± 0.02 mg/g dm). Conversely, Tiger, Tommi, and Valoris had the lowest choline concentrations over the six environments (0.19 ± 0.02 mg/g dm). Despite the variation (CV ranged from 2.99 to 15.3%) observed between environments, some genotypes appeared to be more "stable" than others. Figure 2 illustrates the mean choline contents across the six environmental conditions for each genotype. Plots are ordered on the basis of the concentration ranges across the 3 year, multienvironment study. The most stable lines (exhibiting the lowest concentration range across six conditions) were CF99105 and Cadenza, whereas those showing the highest variation included Riband, Claire, Lynx, and Rialto. Significant differences due to genotype are clearly seen between some lines (e.g., Cadenza vs Estica) but not between other lines that are more susceptible to the environmental conditions.

Summation of the contents of choline and glycine betaine did not significantly alter the groupings of the highest or lowest genotypes (Figure 2), reflecting the fact that the concentration of betaine in most of the samples was an order of magnitude greater than that of choline.

Table 4. Free Choline Concentrations (Milligrams per Gram Dry Matter) of 26 Wheat Cultivars Grown in 6 Environments

	2005	2006	2007				statistics for 3 years at one site (Hungary, 2005−2007)			statistics for 1 year (2007) across four locations (Hungary, France, Poland, U.K.)			statistics for total data across six environments (Hungary, 2005−2007; Poland, France, U.K., 2007)		
genotype	H	H	H	F	P	U.K.	mean	SD	CV (%)	mean	SD	CV (%)	mean	SD	CV (%)
Atlas-66	0.24	0.22	0.24	0.24	0.24	0.21	0.23	0.01	4.54	0.23	0.01	5.73	0.23	0.01	5.26
Avalon	0.21	0.22	0.23	0.21	0.19	0.19	0.22	0.01	5.53	0.21	0.02	10.63	0.21	0.02	8.41
Cadenza	0.22	0.24	0.23	0.23		0.24	0.23	0.01	3.97	0.23	0.01	2.33	0.23	0.01	2.99
Campari	0.23	0.24	0.26	0.19	0.18	0.19	0.24	0.01	5.95	0.20	0.04	17.50	0.21	0.03	15.12
CF99105	0.23	0.23	0.24	0.23	0.23	0.23	0.23	0.01	2.93	0.23	0.01	3.02	0.23	0.01	2.38
Chinese-Spring	0.23	0.24	0.28	0.24		0.26	0.25	0.03	10.49	0.26	0.02	6.78	0.25	0.02	7.75
Claire	0.21	0.23	0.28	0.20	0.20	0.23	0.24	0.04	14.65	0.23	0.04	16.68	0.23	0.03	13.44
Crousty		0.22	0.24	0.23	0.20	0.22	0.23	0.01	4.66	0.22	0.02	8.06	0.22	0.02	6.98
Disponent	0.24	0.23	0.26	0.22	0.21	0.20	0.25	0.01	5.79	0.22	0.03	12.12	0.23	0.02	9.84
Estica	0.20	0.21	0.21	0.20	0.21	0.19	0.21	0.01	3.64	0.20	0.01	4.39	0.20	0.01	4.08
Gloria	0.21	0.22	0.22	0.24	0.22	0.22	0.22	0.01	5.00	0.23	0.01	4.64	0.22	0.01	5.41
Herzog	0.22	0.22	0.22	0.21	0.19	0.20	0.22	0.00	0.94	0.21	0.01	5.82	0.21	0.01	5.21
Isengrain	0.22	0.22	0.22	0.22	0.19	0.23	0.22	0.00	0.52	0.22	0.02	7.34	0.22	0.01	5.81
Lynx	0.23	0.26	0.27	0.22	0.18	0.23	0.25	0.02	8.36	0.22	0.04	17.59	0.23	0.03	14.33
Malacca	0.23	0.24	0.28	0.21	0.21	0.22	0.25	0.03	11.78	0.23	0.04	15.38	0.23	0.03	11.95
Maris-Huntsman	0.21	0.24	0.21	0.20	0.20	0.22	0.22	0.02	7.39	0.21	0.01	5.53	0.21	0.02	7.43
Mv-Emese	0.20	0.19	0.20	0.22	0.20	0.23	0.20	0.01	3.82	0.21	0.01	6.66	0.21	0.01	6.90
Obrii	0.20	0.22	0.24	0.23	0.21	0.22	0.22	0.02	9.15	0.22	0.01	6.29	0.22	0.01	6.34
Rialto	0.23	0.23	0.27	0.20	0.18	0.19	0.24	0.02	8.92	0.21	0.04	18.55	0.22	0.03	14.71
Riband	0.23	0.22	0.28	0.18	0.20	0.21	0.24	0.03	12.42	0.22	0.04	19.78	0.22	0.03	15.26
San-Pastore	0.21	0.23	0.23	0.24	0.23	0.22	0.22	0.01	4.97	0.23	0.01	3.51	0.23	0.01	4.46
Spartanka	0.18	0.23	0.21	0.22	0.20	0.21	0.21	0.03	12.31	0.21	0.01	5.87	0.21	0.02	9.00
Tiger		0.21	0.20	0.18	0.17	0.20	0.20	0.01	3.60	0.19	0.01	6.77	0.19	0.01	7.57
Tommi	0.20	0.21	0.22	0.18	0.17	0.17	0.21	0.01	5.91	0.19	0.02	12.55	0.19	0.02	10.71
Tremie	0.21	0.23	0.26	0.20	0.18	0.23	0.23	0.02	9.59	0.22	0.03	14.45	0.22	0.03	11.57
Valoris	0.19	0.19	0.23	0.17	0.18	0.19	0.20	0.03	13.01	0.19	0.03	14.01	0.19	0.02	11.13
av	0.22	0.23	0.24	0.21	0.20	0.21	0.23			0.22			0.22		
SD	0.02	0.01	0.03	0.02	0.02	0.02	0.02			0.02			0.01		
min	0.18	0.19	0.20	0.17	0.17	0.17	0.20			0.19			0.19		
max	0.24	0.26	0.28	0.24	0.24	0.26	0.25			0.26			0.25		
CV (%)	7.51	6.63	10.70	9.86	9.45	9.07	7.01			7.33			6.31		

dx.doi.org/10.1021/jf3008794 | J. Agric. Food Chem. 2012, 60, 5471−5481

Table 5. Trigonelline Concentrations (Micrograms per Gram Dry Matter) of 26 Wheat Cultivars Grown in 6 Environments

	2005	2006	2007				statistics for 3 years at one site (Hungary, 2005−2007)			statistics for 1 year (2007) across four locations (Hungary, France, Poland, U.K.)			statistics for total data across six environments (Hungary, 2005−2007; Poland, France, U.K., 2007)		
genotype	H	H	H	F	P	U.K.	av	SD	CV (%)	av	SD	CV (%)	av	SD	CV (%)
Atlas-66	3.10	2.26	2.49	2.63	2.72	1.87	2.62	0.44	16.68	2.43	0.39	15.87	2.51	0.42	16.76
Avalon	3.62	2.24	2.63	2.51	2.84	3.88	2.83	0.71	25.17	2.97	0.62	21.03	2.95	0.65	22.08
Cadenza	1.93	1.12	1.19	1.21		5.24	1.41	0.45	31.93	2.55	2.33	91.46	2.14	1.77	82.54
Campari	3.64	2.73	4.11	1.50	3.33	3.83	3.49	0.70	20.03	3.19	1.17	36.68	3.19	0.95	29.82
CF99105	5.34	2.39	2.61	2.19	3.29	3.06	3.44	1.64	47.66	2.79	0.49	17.56	3.14	1.15	36.55
Chinese-Spring	1.85	0.34	1.52	1.84		2.08	1.24	0.79	64.10	1.81	0.28	15.38	1.52	0.69	45.31
Claire	8.23	3.45	3.98	1.32	5.17	5.91	5.22	2.62	50.22	4.09	2.02	49.25	4.68	2.35	50.32
Crousty		1.46	2.38	1.74	3.03	2.77	1.92	0.65	33.87	2.48	0.56	22.63	2.28	0.67	29.30
Disponent	3.20	2.13	4.84	2.48	6.88	4.93	3.39	1.37	40.27	4.78	1.80	37.63	4.08	1.80	44.23
Estica	3.67	2.88	2.27	3.20	3.29	4.78	2.94	0.70	23.91	3.39	1.04	30.69	3.35	0.85	25.25
Gloria	2.11	1.25	0.93	2.31	2.25	1.35	1.43	0.61	42.60	1.71	0.68	39.91	1.70	0.59	34.92
Herzog	1.37	1.00	1.32	1.46	2.01	2.41	1.23	0.20	16.35	1.80	0.50	28.03	1.59	0.52	32.42
Isengrain	1.28	2.02	1.58	1.27	1.42	3.47	1.63	0.37	22.78	1.93	1.03	53.33	1.84	0.85	45.96
Lynx	6.79	6.27	6.08	5.95	6.37	9.46	6.38	0.37	5.73	6.96	1.67	23.98	6.82	1.32	19.41
Malacca	4.37	1.75	2.03	0.99	2.25	3.59	2.71	1.44	53.05	2.21	1.07	48.27	2.49	1.25	50.10
Maris-Huntsman	3.55	2.52	1.68	4.32	3.75	4.35	2.58	0.94	36.41	3.52	1.26	35.82	3.36	1.06	31.56
Mv-Emese	1.32	1.87	1.39	1.93	0.80	0.77	1.53	0.30	19.48	1.22	0.55	44.91	1.35	0.50	36.96
Obrii	4.63	1.39	1.83	3.59	3.19	2.98	2.62	1.76	67.07	2.90	0.75	26.02	2.94	1.18	40.20
Rialto	3.64	1.99	3.27	3.11	3.29	4.58	2.97	0.86	29.12	3.56	0.68	19.16	3.31	0.84	25.23
Riband	3.84	3.01	4.74	1.72	4.41	7.72	3.87	0.86	22.34	4.65	2.45	52.77	4.24	2.02	47.61
San-Pastore	1.94	1.25	2.17	3.96	2.31	3.70	1.79	0.48	26.84	3.03	0.92	30.45	2.55	1.05	41.29
Spartanka	3.54	2.13	2.88	7.04	3.54	4.49	2.85	0.70	24.70	4.49	1.83	40.69	3.94	1.71	43.43
Tiger		1.26	0.78	0.38	1.62	1.07	1.02	0.34	33.58	0.96	0.52	54.22	1.02	0.47	46.11
Tommi	5.49	4.08	3.69	4.05	4.80	4.83	4.42	0.95	21.40	4.34	0.56	13.00	4.49	0.66	14.81
Tremie	2.34	2.73	2.14	1.63	5.57	2.87	2.40	0.30	12.43	3.06	1.75	57.43	2.88	1.39	48.27
Valoris	2.49	2.83	2.08	4.16	3.03	4.36	2.47	0.38	15.23	3.41	1.06	31.18	3.16	0.92	28.97
av	3.47	2.24	2.56	2.63	3.38	3.86	2.71			3.09			2.98		
SD	1.73	1.17	1.32	1.56	1.51	1.93	1.27			1.30			1.26		
min	1.28	0.34	0.78	0.38	0.80	0.77	1.02			0.96			1.02		
max	8.23	6.27	6.08	7.04	6.88	9.46	6.38			6.96			6.82		
CV (%)	49.95	52.08	51.55	59.25	44.72	50.10	47.05			42.00			42.11		

dx.doi.org/10.1021/jf3008794 | J. Agric. Food Chem. 2012, 60, 5471−5481

Effect of Environmental Conditions and Growing Location on Trigonelline. The contents of trigonelline in bread wheats are much lower than those of glycine betaine and choline. However, despite this low concentration, large differences between genotypes could be observed. The trigonelline concentrations, from wheat grown in Hungary in 2005, 2006, and 2007, ranged from 0.34 μg/g dm (Chinese-Spring, 2006) to 8.23 μg/g dm (Claire, 2005) (Table 5). Values of the means across the 26 genotypes ranged from 2.24 ± 1.17 to 3.47 ± 1.73 μg/g dm and showed a much higher coefficient of variation (CV = 49.95−52.08%) than observed for glycine betaine or choline. The variance of individual genotypes due to year of growth ranged from 5.73 to 67.07%. Lynx had the highest mean trigonelline concentration over three years (6.38 ± 0.37 μg/g dm) and Tiger the lowest mean concentration (1.02 ± 0.34 μg/g dm).

Comparison of samples grown at four locations in a single year (2007) showed variation in trigonelline concentration similar to tost in glycine betaine and choline (Table 5), with contents ranging from 0.38 μg/g dm (Tiger, France) to 9.46 μg/g dm (Lynx, U.K.). The mean values for the 26 genotypes across the growing locations ranged from 2.56 ± 1.32 μg/g dm (Hungary) to 3.86 ± 1.93 μg/g dm (U.K.). The coefficients of variation were similar to those of the samples grown in successive years at a single site and ranged from 44.72 to 59.25%. The variance of individual genotypes ranged from 15.4% (Chinese-Spring) to 91.5% (Cadenza). The contents of trigonelline were generally highest in the samples grown in Poland and the United Kingdom.

Taking all six environments (i.e., multiple sites and years) into account, Lynx had the highest mean trigonelline concentration (6.82 ± 1.32 μg/g dm) and Tiger contained the lowest (1.02 ± 0.47 μg/g dm). All of the genotypes studied showed high variation due to genotype across the six environments (CV = 14.8 −82.5%), but despite this variation significant genotypic differences could be observed between some lines. Figure 2 illustrates the mean trigonelline content across the six environments for each genotype. Plots are ordered on the basis of the concentration ranges across the 3 year, multienvironment study. The most stable lines (exhibiting the lowest concentration range across six conditions) were Mv-Emese, Atlas-66, Tiger, and Gloria, whereas those showing the highest variation included Riband and Claire. Of note is the trigonelline concentration in Lynx. This genotype consistently showed higher trigonelline levels compared to the other genotypes, and although the coefficient of variation across six environments was larger (19.41%) than for other genotypes, the lowest concentration of trigonelline detected for any location (5.95 μg/g dm) was greater than the highest values observed for many of the genotypes studied.

Heritability of Glycine Betaine, Choline, and Trigonelline Contents. The 26 lines were grown under a wide range of conditions including different soil types, rainfall and soil water availability, temperature, and agricultural practices. These variables can be described collectively as the environment and may have significant effects on crop performance and composition. Genotypes may also show specific interactions with the environment (G × E interactions),[28] which may affect the ability of plant breeders to develop new cultivars with high stability in terms of agronomic performance, yield, and quality. The availability of data sets for six site × year combinations allowed the variation in the contents of glycine betaine, choline, and trigonelline to be apportioned between the effects of genotype, environment, G × E interactions and that which cannot be explained by these factors (termed error) (Supporting Information, Supplementary Table 4).

Glycine betaine showed a ratio of genetic variance to total variance of 0.36, which indicates that the content of this component is moderately heritable (Figure 3). The heritability

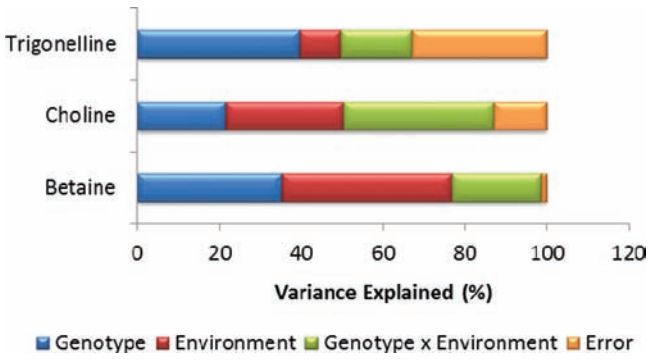

Figure 3. Bar charts showing variance components for methyl donors.

of trigonelline was higher (0.59), but the proportion of variance ascribed to error was large, which probably reflected the low abundance and difficulty in quantification of this component. By contrast, choline showed the lowest heritability of 0.25. The content of glycine betaine levels showed the highest contribution of the environment, whereas the content of choline showed the greatest G × E effect.

Correlation with Agronomic Properties. Methyl donors analyzed in this study did not show any strong correlations with other properties of the grain reported by Rakszegi et al.,[29] Ward et al.,[24] and Shewry et al.[25] Weak correlations were observed between betaine and choline concentrations and thousand-kernel weight (Table 6), whereas betaine and trigonelline concentrations showed weak correlations with bran yield. This is consistent with the fact that all three components are concentrated in the outer layers of the grain and the embryo, which are recovered in the bran fraction on milling. Hence, any factors that affect the ratio of the bran to white flour, including the grain size, will affect their concentrations in wholemeal.

Correlations with Weather. The mean concentrations of glycine betaine, choline, and trigonelline in the 26 wheat genotypes were used to explore correlations with weather conditions over the growth period at each location (Figure 4 and Supplementary Table 3 in the Supporting Information), with statistically significant correlations being shown in bold in Table 7. The concentration of glycine betaine showed a strong positive correlation with mean temperature between heading and harvest and weak negative correlations with precipitation both before heading and also between heading and harvest. The concentration of choline was also correlated with temperature between heading and harvest, although the p value indicated a lower level of significance. As for glycine betaine, the concentration of choline concentration was typically lower in grain from environments that received higher rainfall. By contrast, the concentration of trigonelline showed strong positive correlations with precipitation between heading and harvest date. The content of trigonelline was also negatively correlated with temperature from heading to harvest and also showed a weakly positive correlation with the minimum temperature observed in a 10 day period between heading and harvest. This latter correlation indicated that trigonelline levels

Table 6. Correlations of Methyl Donor Concentrations with Physical Properties of the Grain

	thousand-kernel weight (g/1000 kernals)	bran yield (%)	flour yield (%)	protein content in flour (%)	protein content in wholemeal (%)
betaine	$r = -0.225$	$r = 0.200$	$r = -0.076$	$r = -0.073$	$r = -0.043$
	($p = 0.006$)	**($p = 0.014$)**	($p = 0.354$)	($p = 0.376$)	($p = 0.604$)
choline	$r = -0.284$	$r = 0.083$	$r = -0.034$	$r = 0.298$	$r = 0.296$
	($p = 0.0005$)	($p = 0.3116$)	($p = 0.680$)	**($p = 0.0002$)**	**($p = 0.0002$)**
trigonelline	$r = 0.057$	$r = 0.168$	$r = -0.124$	$r = 0.187$	$r = 0.247$
	($p = 0.497$)	($p = 0.0397$)	($p = 0.1317$)	**($p = 0.021$)**	**($p = 0.002$)**

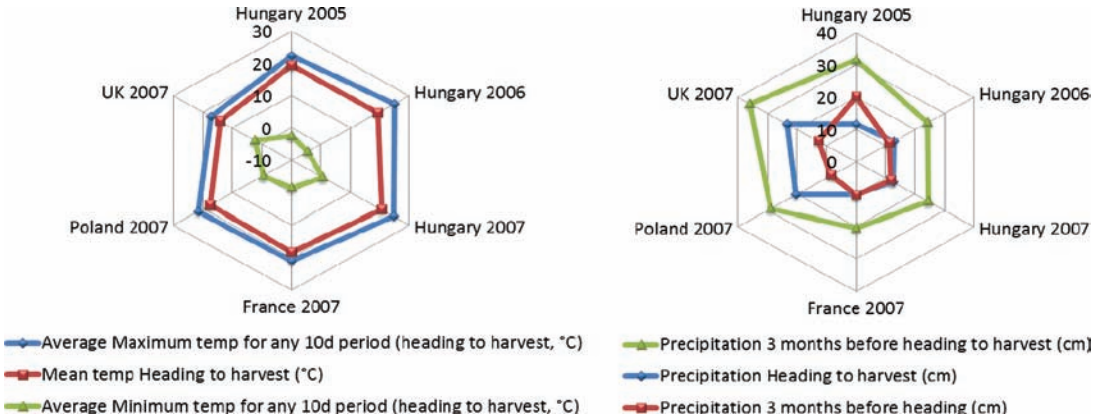

Figure 4. Radar plots illustrating variation in temperature (°C) and precipitation (cm) for each year × location combination.

Table 7. Correlations of Methyl Donor Concentrations with Weather Parameters[a]

	av min temperature for any 10 day period from heading to harvest	av max temperature for any 10 day period from heading to harvest	mean temperature from heading to harvest	precipitation 3 months before heading	precipitation from heading to harvest	precipitation 3 months before heading to harvest
betaine	$r = -0.232$	**$r = 0.750$**	**$r = 0.774$**	$r = -0.039$	$r = -0.309$	$r = -0.262$
	($p = 0.658$)	($p = 0.09$)	($p = 0.07$)	($p = 0.941$)	($p = 0.551$)	($p = 0.616$)
choline	$r = -0.066$	**$r = 0.581$**	**$r = 0.560$**	$r = 0.147$	**$r = -0.484$**	$r = -0.350$
	($p = 0.901$)	($p = 0.227$)	($p = 0.248$)	($p = 0.781$)	($p = 0.331$)	($p = 0.497$)
trigonelline	**$r = 0.585$**	**$r = -0.799$**	**$r = -0.716$**	$r = 0.340$	**$r = 0.710$**	**$r = 0.906$**
	($p = 0.222$)	($p = 0.057$)	($p = 0.109$)	($p = 0.510$)	($p = 0.114$)	($p = 0.013$)

[a]Values in bold represent correlations with a correlation coefficient (r) above 0.4.

may have been affected more by a high minimum temperature between heading and harvest rather than the mean temperature during this period.

The studies reported here show significant variation in the contents of glycine betaine, choline, and trigonelline in wheat samples, which could be ascribed to the effects of genotype, environment, and G × E interactions. Although the heritability of trigonelline was high (0.59), the contents of glycine betaine showed only moderate (0.36) and low (0.25) heritability and are therefore not attractive targets for plant breeders who wish to produce new types of wheat with enhanced health benefits. Furthermore, the high variation from sample to sample means that it is necessary to monitor individual grain samples if the benefits of these components are to be exploited in food products. The high-throughput NMR method used here provides a rapid system that could be used to monitor the content of glycine betaine, choline, trigonelline, and a range of other soluble polar components in wheat and a range of other raw materials and food products.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary Tables 1−4 and Supplementary Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +44 1582 763133. Fax: +44 1582 763010. E-mail: jane.ward@rothamsted.ac.uk.

Funding

Generation of cereal tissues was carried out during the HEALTHGRAIN project (FOOD-CT-2005-514008) and was a project funded by the European Commission in the Communities 6th Framework Programme.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Analyses of cereal tissues were carried out by MeT-RO staff at Rothamsted Research. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the U.K.

■ ABBREVIATIONS USED

CVD, cardiovascular disease; NMR, nuclear magnetic resonance; D_2O, deuterium oxide; CD_3OD, deuteron methanol; TSP, trimethylsilyl propionate; dm, dry matter; CV, coefficient of variation; av, average; min, minimum; max, maximum; SD, standard deviation.

■ REFERENCES

(1) Mellen, B. P.; Walsh, T. F.; Herrington, D. M. Whole grain intake and cardiovascular disease: a meta-analysis. *Nutr., Metab. Cardiovasc. Dis.* **2008**, *18*, 283−290.

(2) De Munter, J. S. L.; Hu, F. B.; Spiegelman, D.; Franz, M.; van Dam, R. M. Whole grain, bran and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. *PLoS Med.* **2007**, *4*, 1389−1395.

(3) 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 International: St. Paul, MN, 2009; pp 179−222.

(4) Obeid, R.; Herrmann, W. Homocysteine and lipids: *S*-adenosyl methionine as a key intermediate. *FEBS Lett.* **2009**, *583*, 1215−1225.

(5) Lever, M.; Slow, S. The clinical significance of betaine, an osomolyte with a key role in methyl group metabolism. *Clin. Biochem.* **2010**, *43*, 732−744.

(6) Zeisel, S. H.; Mar, M.-H.; Howe, R. C.; Holden, J. M. Concentrations of choline-containing compounds and betaine in common foods. *J. Nutr.* **2003**, *133*, 1302−1307 ; (erratum) *133*, 2918−2919.

(7) Likes, R.; Madl, R. L.; Zeisel, S. H.; Craig, S. A. S. The betaine and choline content of a whole wheat flour compared to other mill streams. *J. Cereal Sci.* **2007**, *46*, 93−95.

(8) Storey, R.; Wyn Jones, R. G. Betaine and choline levels in plants and their relationship to NaCl stress. *Plant Sci. Lett.* **1975**, *4*, 161−168.

(9) Sakamoto, A.; Murata, N. Genetic engineering of glycine betaine synthesis in plants: current status and implications for enhancement of stress tolerance. *J. Exp. Bot.* **2000**, *51*, 81−88.

(10) Lever, M.; Slow, S. The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clin. Biochem.* **2010**, *43*, 732−744.

(11) Slow, S.; Donaggio, M.; Cressey, P. J.; Lever, M.; George, P. M.; Chambers, S. T. The betaine content of New Zealand foods and estimated intake in the New Zealand diet. *J. Food Compos. Anal.* **2005**, *18*, 473−485.

(12) De Zwart, F. J.; Slow, S.; Payne, R. J.; Lever, M.; George, P. M.; Gerrard, J. A.; Chambers, S. T. Glycine betaine and glycine betaine analogues in common foods. *Food Chem.* **2003**, *83*, 197−204.

(13) Allred, K. F.; Yackley, K. M.; Vanamala, J.; Allred, C. D. Trigonelline is a novel phytoestrogen in coffee beans. *J. Nutr.* **2009**, *139*, 1833−1838.

(14) Ferrazzano, G. F.; Amato, I.; Ingenito, A.; De Natale, A.; Pollio, A. Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). *Fitoterapia* **2009**, *80*, 255−262.

(15) Storey, R.; Wyn Jones, R. G. Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry* **1977**, *16*, 447−453.

(16) Defernez, M.; Gunning, Y. M.; Parr, A. J.; Shepherd, L. V. T.; Davies, H. V.; Colquhoun, I. J. NMR and HPLC-UV profiling of potatoes with genetic modifications to metabolic pathways. *J. Agric. Food Chem.* **2004**, *52*, 6075−6085.

(17) Le Gall, G.; Colquhoun, I. J.; Davis, A. L.; Collins, G. J.; Verhoeyen, M. E. Metabolite profiling of tomato (*Lycopersicon esculentum*) using ^{1}H NMR spectroscopy as a tool to detect potential unintended effects following a genetic modification. *J. Agric. Food Chem.* **2003**, *51*, 2447−2456.

(18) Piccioni, F.; Capitani, D.; Zolla, L.; Mannina, L. NMR metabolic profiling of transgenic maize with the *Cry1A(b)* gene. *J. Agric. Food Chem.* **2009**, *57*, 6041−6049.

(19) Del Campo, G.; Berregi, I.; Caracena, R.; Zuriarrain, J. Quantitative determination of caffeine, formic acid, trigonelline and 5-(hydromethyl)furfural in soluble coffees by ^{1}H NMR spectrometry. *Talanta* **2010**, *81*, 367−371.

(20) Graham, S. F.; Hollis, J. H.; Migaud, M.; Browne, R. A. Analysis of betaine and choline contents of aleurone, bran and flour fractions of wheat (*Triticum aestivum* L.) using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. *J. Agric. Food Chem.* **2009**, *57*, 1948−1951.

(21) Ward, J. L.; Shewry, P. R. Future prospects for the analysis of bioactive components in cereal grain. In *HEALTHGRAIN Methods. Analysis of Bioactive Components in Small Grain Cereals*; Shewry, P. R., Ward, J. L., Eds.; AACC: St Paul, MN, 2010; pp 273−280.

(22) Poutanen, K.; Shepherd, R.; Shewry, P. R.; Delcour, J. A.; Björck, I.; van der Kamp, J. W. Beyond whole grain: the European HEALTHGRAIN project aims at healthier cereal foods. *Cereal Foods World* **2008**, *53*, 32−35.

(23) Poutanen, K.; Shepherd, R.; Shewry, P. R.; Delcour, J. A.; Björck, I.; van der Kamp, J. W.; Ranieri, R. More of the grain − progress in the HEALTHGRAIN project for healthy cereal foods. *Cereal Foods World* **2010**, *55*, 79−84.

(24) Ward, J. L.; Poutanen, K.; Gebruers, K.; Piironen, V.; Lampi, A.-M.; Nyström, L.; Andersson, A. A. M.; Åman, P.; Boros, D.; Rakszegi, M.; Bedő, Z.; Shewry, P. R. The HEALTHGRAIN cereal diversity screen: concept, results and prospects. *J. Agric. Food Chem.* **2008**, *56*, 9699−9709.

(25) Shewry, P. R.; Piironen, V.; Lampi, A.-M.; Edelmann, M.; Kariluoto, S.; Nurmi, T.; Fernandez-Orozco, R.; Ravel, C.; Charmet, G.; Andersson, A. A. M.; Åman, P.; Boros, D.; Gebruers, K.; Dornez, E.; Courtin, C. M.; Delcour, J. A.; Rakszegi, M.; Bedő, Z.; Ward, J. L. The HEALTHGRAIN wheat diversity screen, effects of genotype and environment on phytochemicals and dietary fiber components. *J. Agric. Food Chem.* **2010**, *58*, 9291−9298.

(26) Ward, J. L.; Harris, C.; Lewis, J.; Beale, M. H. Assessment of ^{1}H NMR spectroscopy and multivariate analysis as a technique for metabolite fingerprinting of *Arabidopsis thaliana*. *Phytochemistry* **2003**, *62*, 949−957.

(27) Baker, J. M.; Hawkins, N. D.; Ward, J. L.; Lovegrove, A.; Napier, J. A.; Shewry, P. R.; Beale, M. H. A metabolomic study of substantial equivalence of field-grown genetically modified wheat. *Plant Biotechnol. J.* **2006**, *4*, 381−392.

(28) Fehr, W. R. *Principles of Cultivar Development: Theory and Technique*; Macmillan Publishing: New York, 1987; Vol. *1*, pp 536.

(29) Rakszegi, M.; Boros, D.; Kuti, C.; Lang, L.; Bedo, Z.; Shewry, P. R. Composition and end-use quality of 150 wheat lines selected for the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9750−9757.