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Folate in Wheat Genotypes in the HEALTHGRAIN Diversity Screen

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As part of the diversity screen of the HEALTHGRAIN project, the total folate contents of bread wheat (130 winter and 20 spring wheat genotypes), durum wheat (10 genotypes), earlier cultivated diploid einkorn and tetraploid emmer wheat (5 genotypes of each), and spelt (5 genotypes), grown in the same location in a controlled manner, were determined by a microbiological assay. The total folate contents ranged from 364 to 774 ng/g of dm in winter wheat and from 323 to 741 ng/g of dm in spring wheat, thus showing a marked variation. The highest mean for total folate content was measured in the durum wheat genotypes, whereas the earlier cultivated diploid and tetraploid wheat genotypes and spelt were shown to possess comparable or even higher folate contents than bread wheat. HPLC analysis of selected genotypes showed that 5-formyltetrahydrofolate was the major vitamer. The data provide a basis for breeding wheat genotypes with improved folate content.

KEYWORDS: Folate; folate vitamers; wheat; bread wheat; winter wheat; spring wheat; durum wheat; spelt; diploid einkorn; tetraploid emmer; wholegrain

INTRODUCTION

Folate is currently one of the most actively studied vitamins. This is mainly due to its role in the prevention of neural tube defects in the fetus and the association of insufficient or suboptimal folate intakes with risks of several other important diseases (*1, 2*). Folate is thus thought to have impacts beyond preventing megaloblastic anemia, the classical folate deficiency. Therefore, research on folate in foods and the possibility of enhancing folate levels in diets has increased in recent years.

Folate, which is a B vitamin, occurs in foods as reduced derivatives of folic acid (pteroyl-L-glutamic acid), which are largely bound to polyglutamates. It is involved in both mammalian and plant cells as a cofactor in one-carbon transfer reactions, which occur in two important cycles: the DNA biosynthesis cycle and the methylation cycle (*1, 3*). Folate deficiency decreases a cell's capacity to synthesize DNA and thus affects cell division rate. In addition, decreased activity of the methylation cycle lowers activity of methyltransferase enzymes and thus has an influence on many crucial reactions. Folate administration decreases elevated plasma homocysteine, which is regarded as a risk factor for cardiovascular diseases, and suboptimal folate intake is associated with dementia, Alzheimer's disease, and some cancer types (*1, 4*−*6*).

Cereal products are generally regarded as important sources of dietary folate. Folate levels in wheat products depend both on initial grain contents and on separation and selection of the grain fraction that is incorporated into the final product. The importance of cereal products as folate sources was demonstrated by a Finnish study, which reported that bread and cereal products accounted for as much as 36 and 43% of the total dietary folate intake for women and men, respectively (*7*). In Finland, only breakfast cereals are fortified but, on the other hand, consumption of wholegrain products, especially rye bread, is common. Consumption of various cereal products is high worldwide and, therefore, further enhancement of folate contents in cereal products both by increasing folate levels in grain raw materials and by developing improved fractionation and other processes should be encouraged. This is especially important in countries that do not have extensive fortification programs.

Folate levels in wheat grains have been reported to vary over a wide range. Davis et al. (*8*) reviewed studies published up to that time and found a range of 160−810 ng/g of dry matter (dm) in American and Canadian bread wheats. Later studies also found that folate levels vary considerably; total folate contents of 340−1140 ng/g of dm (*9*−*11*) and 910 ng/g of fresh weight (fw) (*12*) were reported. In these studies, 1 or 2 samples of an unspecified origin (*9, 12*) or from 4 (*11*) to a maximum of 12 (*10*) varieties were examined for their folate contents. Comparison of 22 bran samples also revealed differences between different wheats (*13*).

A reliable evaluation of genetic or environmental factors as determinants of folate levels in wheat is not possible on the

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basis of previous studies. The variation in the reported folate contents may be partly explained by real differences in folate contents as a result of different genetic backgrounds or growing conditions (weather or other conditions caused by growing location). However, sampling and analytical methods may have a major impact. There are no comprehensive studies in which a larger number of wheat varieties have been compared in a controlled study. The extent of natural variation in folate content needs to be determined to utilize selection of genotypes or breeding for enhancement of folate levels. Furthermore, to understand the significance of environmental factors, the varieties to compare should be grown over several years or in a controlled manner at several locations. Currently, no published studies on the significance of environmental factors for folate content are available.

Several folate vitamers account for the total folate in wheat. As reviewed by Gregory (*14*), there appears to be little difference in the vitamin activities among the different vitamers consumed at low doses. However, the stability of folate vitamers may differ and, therefore, increasing proportions of the more stable vitamers would be desirable. On the other hand, the stability depends on experimental and food conditions (*14, 15*). There are a few recent papers on folate vitamer distribution, but their results are not fully consistent. 5-Formyltetrahydrofolate (5-HCO-H_4-folate) has been shown to be the main vitamer in wheat grains (*11, 12*). The significance of the other individual vitamers differs to some extent in the published studies. Gujska and Kuncewicz (*11*) reported that 5-HCO-H_4-folate was followed in abundance in grain by 5-methyltetrahydrofolate (5-CH_3-H_4-folate) and 10-formylfolic acid (10-HCO-PGA).

Selection of wheat genotypes with higher folate contents is the first step toward enhancement of folate levels in wheat-based foods. After that, further processing is crucial. Bran and germ fractions are known to be much richer in folate than flours (*10, 13*). Folate in flours correlates with ash content (*16, 17*). Recent studies have given additional evidence on possibilities for further improving wheat products as folate sources. Fenech et al. (*18*) reported that a new milling process allowed isolation of the aleurone cell layer, leading to flour with a high folate content. In addition, both Fenech et al. (*18*) and Vahteristo et al. (*19*) showed that folate in cereal foods was well available and that naturally folate-rich cereal foods could improve folate status. Improvement in folate status can partly result from promotion of intestinal folate biosynthesis induced by dietary fiber (*20*). Thus, folate-rich grain fractions could contribute to folate status both by increasing folate intake and by promoting folate biosynthesis in vivo.

This study is a part of the diversity screen of the integrated project HEALTHGRAIN. The project is supported by the European Union as part of the sixth framework program [see Ward et al. (*21*) and http://www.healthgrain.org/pub]. The diversity screen was established to study variation of phytochemicals and bioactive compounds in the gene pool of cereal grains, aiming at acquiring knowledge that can be utilized in plant breeding. The aim of this substudy was to investigate variation in folate levels in a comprehensive number of wheat genotypes grown at the same location in a controlled manner.

MATERIALS AND METHODS

Samples. The studied genotypes included 150 bread wheat (*Triticum aestivum* var. *aestivum*) genotypes; 130 of them were winter wheat and 20 were spring wheat type. These genotypes (**Table 1**) show wide geographical diversity in origin (from Europe to East Asia, the Americas, and Australia) and include landraces and breeding lines as well as modern and older varieties. They differ in kernel texture (hard or soft endosperm structure), kernel color (red and white), and protein content. In addition, 10 durum wheat (*Triticum turgidum* var. *durum*), 5 early cultivated diploid einkorn (*Triticum monococcum* var. *monococcum*) and tetraploid emmer (*T. turgidum* var. *dicoccum*) genotypes, and 5 varieties of spelt (*T. aestivum* var. *spelta*) were included. Furthermore, the winter wheat variety MV-Emese was provided by the HEALTHGRAIN project to be used as an in-house reference sample. The genotypes were sown in Martonvásár, Hungary, either in fall 2004 (winter types) or spring 2005 (spring types). The origin of the genotypes as well as detailed growing and cultivation conditions are described by Ward et al. (*21*).

Table 1. Total Folate Contents in Wheat Types Determined by Microbiological Assay (Nanograms per Gram of Dry Matter)

wheat type	no. of genotypes	mean ± SD	range
winter wheat	130	561 ± 102	364−774
spring wheat	20	551 ± 108	323−741
durum wheat	10	741 ± 66	637−891
spelt	5	577 ± 56	505−647
diploid einkorn	5	577 ± 102	429−678
tetraploid emmer	5	694 ± 176	516−937

The samples were milled to wholemeal flour of 0.5 mm particle size prior to shipment to our laboratory (*21*) and stored at −18 °C until analysis. All of the samples were analyzed for their total folate content using a microbiological assay. Selected samples (seven winter wheat and two spring wheat genotypes and one example each of durum wheat, spelt, and diploid and tetraploid wheat genotypes) were analyzed for their folate vitamer composition by HPLC. The samples were characterized for agronomic and quality parameters including moisture content, 1000 kernel size, and Zeleny sedimentation values (*21, 22*). Bran yield was determined as a percentage of the grain that was milled [(*x* gram bran/*y* gram seed) × 100] with the Chopin CD1 laboratory mill. These data are used to analyze the results in more detail in this paper.

Calibrants. (6*S*)-Tetrahydrofolate (THF), (6*S*)-5-methyltetrahydrofolate (5-CH_3-H_4-folate), (6*S*)-5-formyltetrahydrofolate (5-HCO-H_4-folate), 10-formylfolic acid (10-HCO-PGA), and folic acid (PGA) were obtained from the sources reported by Kariluoto et al. (*23*). 10-Formyldihydrofolate (10-HCO-H_2-folate) was synthesized from (6*R*,*S*)-5,10-methenyltetrahydrofolate hydrochloride (*24*). Calibrants were dissolved, confirmed for their purity, and stored according to the method of Kariluoto et al. (*23*).

Extraction, Trienzyme Treatment, and Purification by Affinity Chromatography. For the determination of total folate content by microbiological assay, samples were prepared using extraction and trienzyme treatment as reported previously (*25*). The main difference was the use of chicken pancreas conjugase, instead of hog kidney conjugase, to deconjugate folate polyglutamates. Briefly, extraction of 1 g samples was performed in duplicate in 15 mL of extraction buffer (50 mM Ches/50 mM Hepes, containing 2% sodium ascorbate and 10 mM 2-mercaptoethanol, pH 7.85). The extraction tubes were flushed with nitrogen and placed in a boiling water bath for 10 min. The tubes were cooled on ice before the pH was adjusted to 7.0 and the addition of α-amylase (EC 3.2.1.1, St. Louis, MO) and chicken pancreas conjugase (5 mg/mL in water, Difco, Sparks, MD). After 3 h of incubation, protease (EC 3.4.24.31) was added, and the incubation was continued at 37 °C for 1 h. The enzymes were then inactivated by heating, and the samples were brought up to exact volumes with 0.5% sodium ascorbate solution at pH 6.1. Two dilutions with approximate folate contents between 0.5 and 1.15 ng/g were prepared for the microbiological assay.

For the determination of the folate vitamer distribution by HPLC, hog kidney conjugase was used to split folate polyglutamates to folate monoglutames. The enzyme preparation was prepared from fresh kidneys and used after its activity had been tested (*23*). Before the conjugase and amylase treatment, the pH was adjusted to 4.9, but for the protease incubation, the pH was raised to 7.0. After protease treatment and enzyme inactivation by heating, samples were centrifuged (*25*). The remaining residue was suspended to 0.1 M K_2HPO_4 and

Table 2. Ranking of the Winter and Spring Wheat Genotypes According to Their Total Folate Content

		winter wheat		spring wheat	
group	folate content (ng/g dm)	no. of genotypes	names of genotypes[a]	no. of genotypes	names of genotypes[a]
1	<400	8	Spartanka, Skorospelka-3B, Gerek-79, Sadovo-1, Bilancia, Balkan, Martonvasari-17, Krasnodarskaya-99	2	Chinese-Spring, Pan
2	400−449	9	Momtchil, Autonomia, Nap-Hal, TAM200, GK-Tiszataj, Buck-Catriel, Ravenna, San-Pastore, Agron	1	Lona
3	450−499	23	Scout66, Lasta, Vona, Fertodi-293, Qualital, Gloria, Plainsman-V, Capo, Bezostaja-1, Etoile-De-Choisy, Baranjka, Fundulea-29, Aurora, Palesio, Produttore, Probstdorfer-Perlo, Isengrain, Guarni, Fleischmann-481, Alabasskaja, Sumai-3, Rusalka Cardinal	3	Milan, Sultan95, Catbird
4	500−549	26	Karl-92, Hana, Claire, Yumai-34, Recital, Nomade, Arthur-71, Soissons, Atay-85, Mieti, Amadeus, Jubilejnaja-50, Klein-Estrella, NS-Rana-1, Tamaro, Avalon, Thesee, Kanzler, Obriy, Apache, Alliance, Gene, Blasco, Carmen, MV-Suba, Sava	3	Saratov-29, Sunstar, Azteca67
5	550−599	14	Manital, Libellula, Sagittario, Courtot, Granbel, Ellvis, Arina, Bankuti-1201, Pobeda, MV-Palotas, Tremie, Stephens, Valoris, Renan	5	Red-River, Cadenza, Kukri, Red-Fife, Thatcher
6	600−649	19	Cubus, Iljicsovka, Geronimo, SU321, Zvezda, Dekan, Alba, CF99102, Galahad, Korweta, Albatros-Odesky, Kirac66, B16, Ble-Des-Domes, Biscay, Key, Flamura-85, Blue/AG, Monopol	3	Mexique-50, Pastor, Janz
7	650−699	17	Campari, Ornicar, Augusta, Herzog, Moulin, Disponent, Tommi, Millennium, Ukrainka, Begra, Atlas-66, CF99105, Fredrick, Kotuku, Camp-Remy, Maris-Huntsman, CF99007	1	Chara
8	≥700	14	Malacca, Riband, Taldor, Spark, Hereward, CF99075, Lynx, Caphorn, Kirkpinar-79, Akteur, Rialto, Seu-Seun-27, Estica, Magdalena-FR	2	Manitoba, Glenlea

[a] In each category the genotypes are given in order of increasing folate content.

centrifuged again. Combined supernatants were filtered through 0.45 μm syringe filters before the purification step.

Affinity chromatography was used to purify the extracts before the HPLC analysis. Preparation, testing, and use of affinity columns is reported previously (*25*). Briefly, the columns were prepared by coupling folate binding protein from bovine milk (Scripps Laboratories, San Diego, CA) to agarose (Affi-Gel 10; Bio-Rad Laboratories, Richmond, CA). Columns were equilibrated with 0.1 M potassium phosphate buffer (pH 7.0). After addition of the sample extract, the column was washed with 0.025 M potassium phosphate/1 M NaCl (pH 7.0), followed by 0.025 M potassium phosphate (pH 7.0). Folates were eluted with 0.02 M trifluoroacetic acid/0.01 M dithiothreitol into 5 mL volumetric flasks containing 30 μL of 1 M piperazine, 0.2% sodium ascorbate, and 5 μL of 2-mercaptoethanol.

Microbiological Assay. Total folate contents were determined using 96-well microtiter plates with *Lactobacillus rhamnosus* ATCC 7469 as the test organism (*23*). Sample extract dilutions and the calibrant (6*S*)-5-HCO-H_4-folate (eight levels, 0−80 pg/well) were pipetted into the wells. Inoculated medium (200 μL/well) was then added, and the plates were incubated for 18−20 h at 37 °C before the turbidometric measurements. The certified reference material CRM 121 (wholemeal flour obtained from the Institute for Reference Materials and Measurements, Geel, Belgium) or an in-house wholemeal flour (genotype Mv-Emese) provided by the HEALTHGRAIN project was analyzed in each set of analyses. At the beginning of the study, action limits for the total folate content of the in-house reference were set at average ± 1.5 × standard deviation ($n = 10$). Thus, the action limit was 470 ± 74 ng/g of dm for the in-house reference and 500 ± 70 ng/g of dm for the CRM 121 (the certified value). The results of the set of analyses were rejected if the folate content of the in-house reference sample or the CRM 121 was outside the action limits. In addition, the total folate contents of the duplicated samples were not allowed to differ more than 10%. Throughout the study the average folate content of the CRM 121 was 488 ± 31 ng/g of dm ($n = 21$) and that of the in-house reference, 475 ± 40 ng/g of dm ($n = 40$).

High-Performance Liquid Chromatography (HPLC). Folate monoglutamates were separated on a Shandon (ThermoQuest, Cheshire, U.K.) Hypersil ODS column (150 mm × 4.6 mm; 3 μm particle size) (*26*). Column temperature was kept at 30 °C. Gradient elution was performed with acetonitrile and 30 mM potassium phosphate buffer, pH 2.2, at a flow rate of 0.9 mL/min. The detection was carried out by a UV detector set at 290 nm and by a fluorescence detector set at 290 nm excitation and 356 nm emission wavelengths for reduced folates and at 360 nm/460 nm for 10-HCO-PGA. Peaks were identified and their identities confirmed as reported previously (*23, 26*). Quantification of folate vitamers was based on an external standard method with peak areas plotted against concentrations. Calibrants (eight levels) were purified with affinity chromatography.

Expression of the Results and Statistical Analysis. The total folate contents are given on a dry matter basis, calculated using moisture contents (*21, 22*). The relationship between total folate content and kernel size and bran yield was analyzed using correlation analysis. The Pearson correlation coefficients were calculated using Statgraphics Plus 4.0. In these analyses, total folate contents on a fresh weight basis were used. The proportions of individual vitamers were calculated against the sum of vitamers determined by HPLC.

RESULTS AND DISCUSSION

Total Folate in Winter and Spring Wheat. In the 130 winter wheat genotypes, the total folate contents ranged from 364 to 774 ng/g of dm, the mean content being 561 ng/g of dm (**Table 1**). The variation between the genotypes was thus significant; the best folate sources contained in excess of 2-fold more folate than the poorest sources. When the range in the total folate contents was divided into intervals of 50 ng/g of dm, giving eight groups, the results showed that all of the groups included a considerable number (i.e., 8−26) of genotypes (**Table 2**). This comparison thus showed that the genotypes were quite evenly distributed over the entire range. However, the highest number of genotypes (26 genotypes) fell within the interval 500−549 ng/g of dm and the second highest (23 genotypes) within the interval 450−499 ng/g of dm, which means that 37% of the genotypes had folate contents in the range of 450−549 ng/g of dm.

The mean total folate content of the 20 spring wheat genotypes, 551 ng/g of dm, was close to that of the winter wheat genotypes, as was the total range (323−741 ng/g of dm) (**Table 1**). Thus, as in the case of winter wheat, the best spring wheat genotypes contained in excess of 2-fold more folate than the poorest spring wheat genotypes. Fourteen of the 20 genotypes fell into the four intervals covering the total range 450−649

ng/g of dm when the genotypes were divided into groups similar to the winter wheat genotype groups (**Table 2**).

The highest folate content among the winter wheat genotypes was measured in Magdalena-FR, which is an old French variety. Most of the other genotypes in the top group were modern European varieties of English (Malacca, Riband, Spark, Hereward, Lynx, Caphorn, Rialto), German (Akteur), Dutch (Estica), and French (Taldor) origin. However, five old genotypes of various origins and a germplasm also belonged to the top group. Genotypes in the bottom group, on the other hand, originated from Russia, Italy, France, Turkey, Bulgaria, Serbia, and Hungary and included both old and modern genotypes. Overall comparison of the total folate contents of the winter and spring wheat genotypes with information on their origin and other general properties (old vs modern, winter vs spring types, hard vs soft wheat) (*21*) thus showed that good and poor folate sources were found in all wheat types.

The total folate contents of all the winter and spring wheat genotypes fall within the wide range reported in the previous studies [160−1140 ng/g of dm (*8*−*12*)] for different wheat grain samples of various origins. However, they were below the range reported by Arcot et al. (*10*) for 12 Australian wheats (799−1140 ng/g of dm). They found a trend toward higher folate content in lower protein wheats. Our results do not show a similar trend, as Glenlea, Manitoba, and Atlas 66 are well-known wheat varieties with high protein content and also belonged to the high folate content groups. In four Polish wheat cultivars, the total folate contents ranged from 340 to 400 ng/g of dm (*11*) when the folate contents were measured by HPLC, generally known to give lower contents than the microbiological assay, which was used in this study and by Arcot et al. (*10*). Comparison of bran samples of seven winter wheats from two locations gave a range of 3310−4140 ng/g of dm, whereas the range for total folate in bran of nine spring wheats was wider, 1820−3500 ng/g of dm (*13*). The previous studies thus indicate that there is variation in folate levels of wheat genotypes. However, the number of genotypes analyzed in all of the previous studies is small, and there are no previous studies with such a marked number of genotypes as examined in this study. In addition, all of the genotypes were grown in similar conditions, as required for true comparisons of genotypes. Confirmation of the analytical method is also crucial; analysis of reference samples is needed to rule out methodological differences and to provide for calibration of the method over longer analysis periods. Thus, this study resulted in valuable new information on the extent of the variation in folate levels of bread wheats.

Total Folate in Other Genotypes. When different wheat classes were compared, the highest mean for total folate content, 741 ng/g of dm, was measured in the durum wheat genotypes, and the range (637−891 ng/g of dm) was narrower than for winter and spring wheat samples (**Table 1**). Thus, only one durum genotype fell into both the sixth and seventh categories used in the respective groupings of the winter and spring wheat genotypes, and all of the other durum wheats contained >700 ng/g of dm of folate. Previously, Mullin and Jui (*13*) compared six durum wheat genotypes by measuring total folate in their bran samples and found less variation than in this study; the total folate contents ranged from 2200 to 2810 ng/g of dm.

The number of genotypes of spelt and the early cultivated diploid einkorn and tetraploid emmer genotypes studied was small: only five for each of them. Therefore, the results give only some indication about these cereals as folate sources. The mean total folate content of both spelt and diploid wheat genotypes was 577 ng/g of dm, which is close to the mean contents for winter and spring wheat. A somewhat higher mean folate content of 694 ng/g of dm was measured in tetraploid wheats (**Table 1**). The ranges were again marked; they were especially wide for diploid and tetraploid wheats. The highest folate content of the study, 975 ng/g of dm, was measured in one of the tetraploid genotypes. To our knowledge, there are no previously published data on folate in these early cultivated cereals. The wide range of variation in this small set of samples studied suggests that wild diploid and tetraploid relatives should be further studied for their folate content.

Table 3. Proportion (Percent) of the Different Folate Vitamers in the Studied Genotypes

vitamer	winter wheat	spring wheat	durum wheat	spelt	diploid and tetraploid
H_4	6 ± 3	6	7	7	9
5-CH_3-H_4	11 ± 4	10	11	23	21
10-HCO-H_2	12 ± 9	8	10	12	11
10-HCO-PGA	23 ± 9	24	16	18	20
5-HCO-H_4	41 ± 11	47	46	34	34
PGA	6 ± 1	5	10	5	5

Folate Vitamer Distribution. Folate vitamer distribution was analyzed in a few selected samples of each wheat type (**Table 3**). The folate vitamer distributions of winter, spring, and durum wheats were similar. 5-HCO-H_4-folate was the most abundant vitamer, contributing on average >40% of all the analyzed vitamers, whereas in spelt and diploid and tetraploid genotypes, its contribution was somewhat lower (34%), and correspondingly the contribution of 5-CH_3-H_4-folate was higher. 5-HCO-H_4-folate, which is one of the more stable vitamers, was also reported to be the main vitamer in wheat grains in previous studies (*11, 12*). It was also observed in this study that 5-HCO-H_4-folate and 5-CH_3-H_4-folate were coupled with each other in all of the cereals: when the content of 5-HCO-H_4-folate was lower, the content of 5-CH_3-H_4-folate was higher, and thus the sum of these two vitamers was about 50% of all the vitamers. Only a few previous studies on the folate vitamers in wheat grains are available, and their results on proportions of the individual vitamers differ. This may be largely explained by differences in the methods and differing stabilities of the vitamers during storage and analysis of the samples; both conversion of some of the vitamers to another vitamer and decomposition of the vitamers may occur.

Association of Total Folate Contents with Kernel Characteristics. The number of genotypes compared in this highly controlled study was large, especially in the case of winter wheat, and was also substantial for spring wheat. Furthermore, various agronomical and quality parameters were determined in all of the genotypes to further characterize the samples analyzed for bioactive compounds and phytochemicals (*22*). Therefore, we were able to study the correlation of folate levels with other parameters. Interaction of the various attributes is more generally evaluated by Ward et al. (*21*). In this paper, we compared the total folate contents with, in particular, kernel size and bran yield, as well as with Zeleny values, which is a widely accepted approximate measure of baking quality.

The results showed that the total folate contents of the winter wheat genotypes were higher the smaller the kernel size (**Figure 1a**). For this comparison, the average kernel sizes (as mg) were calculated on the basis of the 1000 kernel weights. The negative correlation between these parameters was statistically significant ($p < 0.05$). Correspondingly, the total folate content was higher when bran yield was higher (**Figure 2a**). Correlation of the kernel size and bran yield with the total tocol contents was

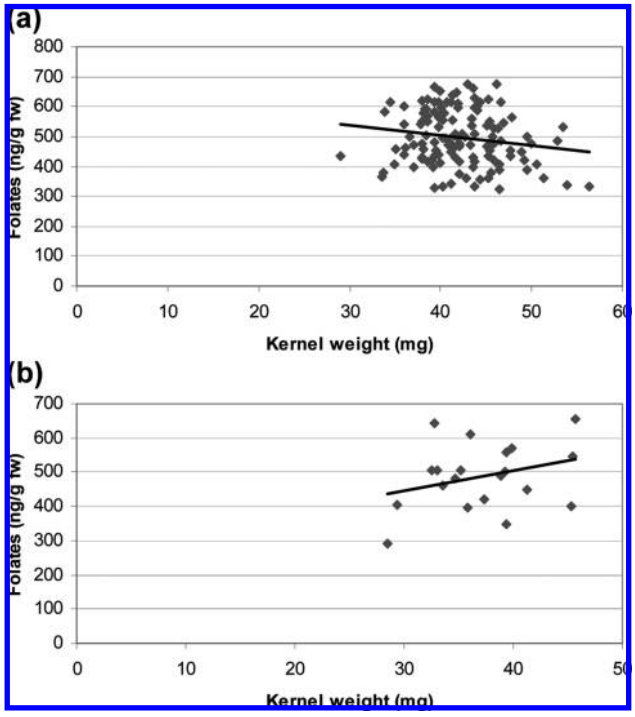

Figure 1. Correlation of total folate contents and kernel weight (**a**) in winter wheat genotypes (correlation significant, $p < 0.05$) and (**b**) in spring wheat genotypes (not significant).

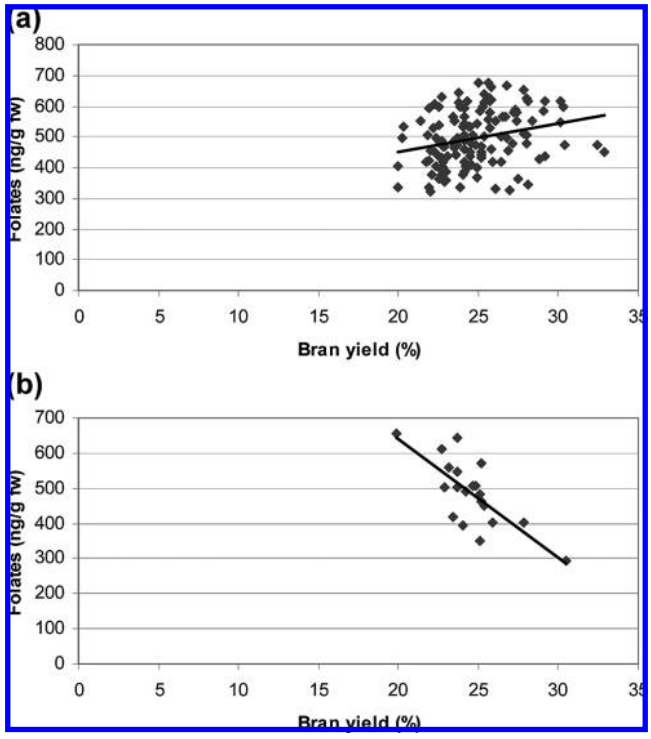

Figure 2. Correlation of total folate contents and bran yield (**a**) in winter wheat genotypes ($p < 0.05$) and (**b**) in spring wheat genotypes ($p < 0.01$).

clearly stronger than their correlation with total folate content (*27*). These associations can be explained by higher proportions of the outer layers in the smaller kernels. Bran and germ fractions are the richest milling fractions in terms of folate contents. Arcot et al. (*10*) reported that the folate levels of wheat bran were 4-fold higher than those in flour and 2-fold higher than levels in grain. Little is known about the detailed distribution of folate in wheat kernel. Pomeranz (*28*) concluded, on the basis of the published studies, that the aleurone layer provides about 60% of vitamin B_6 and that folate is probably distributed in a manner similar to that of vitamin B_6. An aleurone flour isolated by a new technique contained 5150 ng/g of folate, whereas cereal made from wheat bran contained only 940 ng/g of folate (*18*). Previous studies thus indicate that folate is concentrated in the aleurone layer. In our previous study (unpublished data) we also showed that the folate level increased with increasing ash and fiber contents until it leveled out or decreased when the fiber and ash contents increased further. Obviously the outmost layers are thus less rich in folate than the aleurone layer.

A similar correlation was not seen in spring wheat with a smaller number of genotypes; in contrast, there seemed to be an opposite trend (**Figures 1b** and **2b**). The total folate content was lower when bran yield was higher ($p < 0.01$). Furthermore, the folate content was higher the higher was the kernel size. This association was, however, not statistically significant. We do not have any clear reason for this difference in the winter and spring wheat genotypes. Obviously the number of genotypes needs to be high to show significant correlation when the genotypes are from various origins. Because of the partly opposite results for spring wheat compared with winter wheat, correlation of the folate contents with kernel size or bran yield was not significant when calculated for all the bread wheats together.

Folate-rich varieties could be found in all categories when the genotypes were ranked according to the Zeleny values into five groups. The Zeleny method is a microsedimentation rapid test for rheological properties, which characterizes wheat varieties according to breadmaking quality. Twelve genotypes (B16, Monopol, CF99105, Akteur, Caphorn, Zvezda, CF99102, Albatros-Odessky, Ukrainka, Key, Pastor, Chara) belonged to the top group for both of the attributes. They included both germplasms, old and modern varieties originating from different ecological regions of Europe, and also varieties from the U.S. Midwest region (Key), Mexico (Pastor), and Australia (Chara) (*21*). B16, Monopol, Key, and Chara are important genotypes for protein content and quality improvement in breeding. Akteur is a modern German variety known to have an excellent breadmaking quality, and Caphorn is a new and promising modern variety with high grain yield. However, the results showed that the bread making quality, expressed as Zeleny values, and folate contents were independently inherited traits as we could also find several genotypes with low folate contents and high Zeleny values, that is, Plainsman-V, Buck-Catriel, GK-Tiszataj, Capo, Cardinal, Probstdorfer-Perlo, Qualital, Vona, Alabasskaja, TAM200, Pan, and Lona. This will help further breeding efforts to develop modern wheat varieties with high folate content and good breadmaking quality for the cereal-based processing industry.

In conclusion, this study provides new data on variation in folate contents of bread wheat, durum wheat, diploid and tetraploid wheat, and spelt. The large number of genotypes and controlled experimental conditions provided a unique opportunity to obtain reliable information on folate in bread wheat genotypes. The results showed significant variations in the folate contents of both spring and winter wheats. The folate content of the winter wheat genotypes was higher the smaller the kernel size and the higher the bran yield. Durum wheat, the earlier cultivated diploid and tetraploid wheat genotypes, and spelt genotypes were compared with bread wheats for the first time and were shown to possess comparable or even higher folate

contents. The data provide a basis for breeding wheat genotypes with improved folate contents.

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