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Tocopherols and Tocotrienols in Wheat Genotypes in the HEALTHGRAIN Diversity Screen

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Tocopherol and tocotrienol compositions were studied in 175 genotypes of different wheat types grown under similar conditions to screen for natural diversity. The main focus was on bread wheats, including 130 and 20 winter and spring types, respectively. The average total content of tocopherols and tocotrienols was 49.4 μg/g of dm, with a range of 27.6−79.7 μg/g of dm, indicating a 2.9-fold variation among genotypes. β-Tocotrienol and α-tocopherol were the major vitamers, and in general there were more tocotrienols than tocopherols. In the early cultivated forms of wheat the proportion of tocotrienols was especially high, at ≥62.5%. In conclusion, there was a large variation in total tocopherol and tocotrienol contents in bread wheats and this, along with the high proportions of tocotrienols in other types of wheat, demonstrates the great genetic potential of genotypes to be exploited by plant breeders.

KEYWORDS: Tocopherols; tocotrienols; tocols, wholemeal; bread wheat; durum wheat; spelt; diploid einkorn; tetraploid emmer

INTRODUCTION

Tocopherols and tocotrienols, that is, tocols, are viscous, lipid-soluble liquids that consist of a polar chromanol ring and a hydrophobic 16-carbon side chain. In tocopherols, the side chain is a saturated isoprenoid group, whereas in tocotrienols it has three double bonds (see, e.g., refs *1* and *2*). Tocopherols and tocotrienols both occur as four natural vitamers: α, β, γ, and δ. The vitamers differ from each other by the number and position of methyl groups in the chromanol ring; α-tocol has three methyl groups, β- and γ-tocols have two methyl groups, and δ-tocol has only one methyl group. All natural tocols are 2*R*-stereoisomers, indicating that the side chain is attached to the chromanol ring with the same stereochemistry.

The most important chemical property of tocols is their antioxidant activity. They are primary antioxidants that act by donating hydrogen atoms to lipid peroxyl radicals, thus retarding propagation of lipid oxidation (see, e.g. refs *2*−*4*). α-Tocopherol appears to be the major lipid-soluble chain-breaking antioxidant in biological systems, and it works as part of an antioxidant network, which includes, for example, ascorbic acid, glutathione, and ubiquinol, against oxidative stress. Tocols are also effective quenchers for singlet oxygen and nitrogen oxide radicals. The same antioxidant activities occur in living plant and animal cells and in grains and other foods. In plants, tocols protect lipids from oxidation in photosynthetic membranes and seeds.

Tocols are only synthesized in photosynthetic organisms, in chloroplast membranes and chromoplasts (see, e.g., refs *5*−*7*). The chromanol group is derived from the shikimate pathway, whereas the side chain for tocopherols is derived from phytyl-diphosphate and that for tocotrienols from geranyl-geranyl-diphosphate. The synthetic pathways are fairly well characterized and also include methylation and cyclization steps. Tocopherols may also be built up from free phytol in chloroplasts, which might indicate that during stress and senescence, when chlorophyll breaks down, the phytol residues are being used for tocopherol synthesis and perhaps stored in this form (*6*). During the past decade most of the genes controlling the biosynthesis of tocopherols and tocotrienols have also been revealed, and it has become possible to start trying to modify the amounts and compositions of tocols, which might produce plants with enhanced tocol status. As reviewed in ref *7*, such approaches have already been used in oil seed modification. In general, α-tocopherol is the predominant tocol in leaves, and γ-tocopherol in other plant parts, whereas the distribution of tocotrienols is variable. In a screening study of 80 different plant species, 24 species were found to contain tocotrienols (*5*). No tocotrienols were found in mature photosynthetic tissues; instead, tocotrienols were present in seeds, fruit pericarps, and latex.

According to current dietary recommendations (*8*), the recommended dietary allowance (RDA) of vitamin E is 15 mg of 2*R*-α-tocopherol per day, and the estimated average requirement (EAR) is 12 mg. Other naturally occurring tocols are considered not to contribute to vitamin E activity, although they have other biological activities. Because tocols are only biosynthesized in photosynthetic organisms, it is essential for a human being to acquire them from the diet to meet the vitamin

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E requirement and to acquire other tocols to fulfill their respective functions. The main physiological function of α-tocopherol and other tocols is to delay the progress of a variety of degenerative diseases. In addition to functions related to antioxidant activities, tocols have been shown to regulate cellular signaling and gene expression (*9, 10*). Although the current diet in the Western world is considered to have enough α-tocopherol to avoid vitamin E deficiency symptoms, a continuing survey of food intake in the United States showed that only 8% of men and 2.4% of women meet even the current EAR, indicating that there is a need to increase the intake of α-tocopherol (*11*).

Cereal grains are considered to be only moderate sources of tocols, providing 16.8−49 μg of tocols/g (*1*). Although the content in cereals is much less than in vegetable oils and products derived from them, the amount of tocols obtained from cereals is important, because they are consumed at high levels. Very little is known about the bioavailability of tocols from grains and grain products. It has been suggested that it may be difficult to release tocols from grains and seeds, because they are poorly digested, whereas the bioavailability from oils and fats is considered to be much better (*12*). However, bioaccessibility of α-tocopherol from wheat bread was very high, being 99.6 ± 11.3%, and that from wheat germ almost as good, being 53.29 ± 7.85% (*13*), indicating that wheat and wheat products could be important sources of α-tocopherol. In fact, Gao et al. (*14*) suggested increasing the consumption of bread and other cereal servings to six a day to meet the intake recommendations without increasing the amount of oils and fats in the diet. One should, however, bear in mind that many cereal products may have elevated levels of tocols due to inclusion of fats and oils in the products or due to vitamin E supplementation of some ingredients.

It is generally accepted that wheat grains on average contain tocols at levels of 35−59 μg/g (*15*), 39−58 μg/g (*16*), and 50 μg/g (*2*). Commercial wheat grain samples from Finland, Germany, and the Czech Republic contained 40.4, 43.6, and 47.8 μg/g of fw respectively (*17*−*19*). In all of these studies, α- and β-tocols were the major vitamers present, and there were more tocotrienols than tocopherols. In fact, wheat and other cereals are the major sources of tocotrienols in the human diet together with palm oil products (see, e.g., ref *1*). This is very important because tocotrienols have been shown to possess additional positive health effects including prevention of neurodegeneration, induction of immune responses, and anticancer and cholesterol lowering effects (*20, 21*). Although there are plenty of data on average tocol composition in wheat, very little is known about the natural variation within and among different wheat types, cultivars, and genotypes and the effects of agronomic and environmental conditions on tocol composition. A review on several hundred wheat cultivars and varieties grown in the United States showed that there was a 10-fold range in α-tocopherol contents (*22*). No information on other tocols was collected. Factors influencing the variation were varietal differences, crop year, and production site, but fertilization practices, soil type, or class of wheat did not have an influence. In another study, eight selected soft wheat genotypes grown in Maryland had a 3-fold range in α-tocopherol levels (*23*).

The main focus of the HEALTHGRAIN project is to develop new healthy food products based on wholegrains of wheat and rye (*24*). One part of the project is the genetic diversity screen to learn the extent of genetic variation in genotypes to be exploited by plant breeders. The aim of the current study was to examine the genetic diversity of tocopherols and tocotrienols in wheat genotypes harvested in Martonvásár (Hungary) in 2005. Tocopherols and tocotrienols were selected as target phytochemicals because they are known to possess several chemical and physiological activities. In this study, data on tocols in 175 genotypes of different types of wheats grown in controlled and similar conditions were collected, and the variation in tocol contents and profiles within and among wheat types were evaluated.

MATERIALS AND METHODS

Samples. The HEALTHGRAIN wheat diversity screen focused on bread wheats and included a few examples of other forms of wheat. The bread wheats (*Triticum aestivum* var. *aestivum*) included 130 winter and 20 spring wheat genotypes. The other types of wheat included 10 durum wheat (*Triticum turgidum* var. *durum*) cultivars, five spelt (*T. aestivum* var. *spelta*) cultivars, and 5 each of early cultivated diploid einkorns (*Triticum monococcum* var. *monococcum*) and tetraploid emmers (*T. turgidum* var. *dicoccum*). All wheat samples were prepared and milled into wholemeal flour of 0.5 mm particle size in Martonvásár (Hungary) prior to shipment to our laboratory (*24, 25*). Spelt grains were dehulled prior to milling (*24*). In addition, wholemeal flour from wheat cultivar Mv-Emese was similarly prepared and used as an in-house reference sample throughout the study. The flours were stored at −18 °C prior to analysis.

Tocopherol and Tocotrienol Analysis. Wholemeal flours were analyzed for tocopherols and tocotrienols after hot saponification and extraction of nonsaponifiable lipids by normal phase HPLC (NP-HPLC) with fluorescence detection (FLD). The method used was slightly modified from that developed for rye flour (*26*). In brief, a 0.5 g sample of flour was taken for analysis and saponified with KOH in a mixture of ethanol and water under nitrogen at 100 °C for 25 min. Ascorbic acid was used as an antioxidant. Nonsaponifiable lipids, including tocols, were extracted with a heptane/ethyl acetate (8:2) mixture. After washing of the extract and evaporation of the solvent, the residue was dissolved in heptane and filtered through a Millex-LCR filter (0.45 μm, 13 mm) prior to analysis by NP-HPLC-FLD. Separation was performed using an Inertsil silica column (5 μm, 250 mm × 4.6 mm; Varian Chromapack, Middelburg, The Netherlands) with a silica guard column (Guard-Pak Silica, Waters, Milford, MA) and a mobile phase containing 3% of 1,4-dioxane in heptane at a flow rate of 2 mL/min at 30 °C. FLD was set at λ_{ex} = 292 nm and λ_{em} = 325 nm. Each wholemeal sample was worked up in duplicate, and each tocol solution was analyzed twice by HPLC.

Commercial tocopherol vitamers (α-, β-, γ-, and δ-tocopherols, Merck, Darmstadt, Germany) were used for preparing stock solutions in ethanol (purity > 99.5%, for spectroscopy) for identification and quantitation purposes. The stock solutions were checked for concentration and purity by UV spectroscopy and HPLC analysis every 4 weeks and diluted in heptane as working solutions every 2 weeks as described previously (*27*). Calibration curves for α-, β-, γ-, and δ-tocopherols were generated for each batch of HPLC samples using six levels of analytes with a range from 2 to 80 ng/injection. A commercial mixture of tocotrienols and tocopherols (Tocomin 50%, Carotech INC, Edison, NJ) was used to identify tocotrienols in the samples. Individual tocotrienols were quantitated with the respective tocopherols as indicated by the official method of the AOCS (*28*). Selected wheat extracts, Tocomin 50%, and rapeseed oil samples were analyzed by LC-MS for further verification of the identity of tocopherols and tocotrienols (*26, 27*). Liquid chromatography connected to an APCI interfaced ion-trap mass spectrometry (Esquire LC-MS, Bruker Daltonic, Bremen, Germany), working in a positive ion mode, was used to verify the peak identification. Chromatographic separation in LC-MS was performed using a Phenomenex silica column (100 × 2 mm id, 3 μm) with a flow rate of 0.5 mL/min using the same mobile phase as for NP-HPLC-FLD.

Validation and Performance of the Analytical Method. The limit of determination of all tocols was 2 ng/injection, representing 0.7 μg of tocols/g of flour using a 30 μL injection, and the HPLC response was linear over the whole calibration range from 2 to 80 ng/injection. Recoveries of added tocopherols to wholemeal wheat flour at 4 and 10 μg/g levels were good, being 91, 93, 87, and 92% (N = 11) for α-, β-,

Table 1. Variation of Total Tocol Contents in Wheat Types

wheat type	no. of genotypes	total tocol content (μg/g of dm)		
		av	SD	range
winter wheat	130	49.9	8.7	27.6−79.7
spring wheat	20	49.6	6.4	35.9−63.2
durum wheat	10	48.1	7.0	40.1−62.7
spelt	5	46.2	4.4	40.2−50.6
diploid einkorn	5	57.0	12.1	42.7−70.2
tetraploid emmer	5	36.4	12.0	29.0−57.5
total	175	49.4	8.8	27.6−79.7

γ-, and δ-tocopherols, respectively. At the beginning of the study, action limits for the total tocol contents of two in-house reference samples, rapeseed oil and wholemeal wheat flour, were set at average ± 2 × standard deviation ($N = 10$). The rapeseed oil and flour samples were analyzed in each batch of samples. When diversity screen samples were analyzed, if either of the in-house reference samples gave values outside the action limits, the whole analysis batch was rejected. In wholemeal samples, the difference in total tocol content between the duplicate samples did not exceed 10%. If the difference was greater, the sample was reanalyzed, and the mean of all the replicates was calculated and reported. Repeatability of the tocol analysis by HPLC was good, because a rapeseed oil sample analyzed in each batch of HPLC samples gave stable results. Contents of α-, γ-, and δ-tocopherols in the rapeseed oil samples were 207 ± 4, 437 ± 12, and 15 ± 1 μg/g ($N = 64$), respectively. In addition, the repeatability of the whole analytical method on flour samples was good, because the results of the in-house reference sample analyzed in each sample batch had very little variation. Total tocol content was 33.8 ± 2.0 μg/g of fw ($N = 63$). All results on wholemeal samples are given as means of replicate samples on a dry matter basis if not stated otherwise. The proper peak identification was verified by measurement of protonated molecular ions and their fragment ions. Mass charge ratios (m/z) of $[M + H]^+$ ions were 431, 417, 417, and 403 for α-, β-, δ-, and δ-tocopherols, and 425, 411, 411, and 397 for tocotrienols, respectively. Characteristic fragment ions were m/z 205 and 165 for α-tocopherol and α-tocotrienol, m/z 191 and 151 for β-tocopherol and β-tocotrienol and for γ-tocopherol and γ-tocotrienol, and m/z 177 and 137 for δ-tocopherol and δ-tocotrienol.

Statistical Analysis. Statistical differences between medians of total tocol contents of different wheat species were measured with a Kruskal−Wallis test. To relate tocol values to other parameters of the kernels, Pearson correlation coefficients of total tocol contents with 1000 kernel weights and bran yields were calculated on a fresh weight basis, and those of total tocol contents with total lipids, individual tocols, and total phytosterols were calculated on a dry matter basis. Dry matter contents, 1000 kernel weights, bran yields, and total lipid and total phytosterol contents were obtained from several partners of the HEALTHGRAIN project (*24, 25, 29*). Statgraphics Plus 4.0 software (Manugistics, Inc., Rockville, MD) was used for statistical analyses.

RESULTS AND DISCUSSION

Genetic Variation and Levels of Total Tocol Contents in Wheat Species. The average total tocol contents of winter, spring, and durum wheat and spelt genotypes were comparable, being 49.9, 49.6, 48.1, and 46.2 μg/g of dm, respectively, whereas those of the early cultivated wheat types were different (**Table 1**). The average level in diploid einkorn genotypes, 57.0 μg/g of dm, was greatest, and that of tetraploid emmer genotypes, 36.4 μg/g of dm, was smallest. There was not, however, a statistically significant difference ($p < 0.05$) in total tocol contents among wheat types according to the Kruskal−Wallis test. The average total tocol content among all genotypes was 49.4 μg/g of dm. Despite relatively constant average values among wheat types, there was a large variation within each wheat type, as indicated by ranges of 1.3−2.9-fold. The range was the greatest in winter wheat species, which included the genotype with the lowest level, 27.6 μg/g of dm, and that with the highest level, 79.7 μg/g of dm. The variation in all genotypes can also be described by noting that 50% of them had values of <43.4 μg/g of dm or >54.3 μg/g of dm. The large ranges, especially in the bread wheat species, showed the great potential for exploitation of this variation for plant breeding purposes.

As in our study, diploid einkorn genotypes were found to be richest in total tocols among the wheat types studied (*30*). Fifty-four accessions of einkorn originating from several areas in Europe, as well as Morocco and Turkey, were cultivated in Italy and compared with a few examples of other types of wheat. The levels of tocols in einkorn accessions were in general greater than for other wheat types, with an average of 78.0 μg/g of dm. The average total tocol contents for bread wheat, durum, spelt, and tetraploid emmer cultivars were 61.5 μg/g of dm (five cultivars), 50.5 μg/g of dm (five cultivars), 68.3 μg/g of dm (two cultivars), and 65.3 μg/g of dm (two cultivars), respectively (*30*). In general, tocol contents of all the wheat types were higher than in our study. Total tocols were also, to some extent, higher in another study performed in Italy, where the average values for three spelt and durum wheats and two soft wheats were 56.5, 60.6, and 74.3 μg/g of dm, respectively (*31*). Lower tocol levels of spelt found in our study could be partly explained by dehulling of the grains in addition to differences caused by genetic and environmental factors.

There was a 1.9-fold difference between maximum and minimum values for total tocol contents for 54 accessions of einkorn, ranging from 61.5 to 115.9 μg/g of dm, and differences for bread wheat and durum cultivars were 1.4- and 1.5-fold, respectively (*30*). Similarly, in the Belgian study, a 1.6-fold range in total tocopherol contents of five varieties of soft winter wheats and a 1.4-fold range in nine dehulled spelt varieties were found (*32*). These values indicate that in most studies, variations within wheat types and among genotypes have been found to be at comparable levels. One should be especially careful in drawing general conclusions from the extent of variation found in total tocol contents in durum wheat, spelt, and early cultivated wheat types, because the number of genotypes studied in these wheat types were limited and, thus, could show only a part of the genetic diversity. However, the overall data on 175 genotypes show that there is a lot of genetic variation that provides a great potential to be exploited by plant breeders.

Genetic Variation of Tocol Profiles in Wheat Species. Tocol profiles of the wheat types were relatively stable. In all genotypes, α- and β-tocopherols and tocotrienols were the major tocols present (**Table 2**). Of the other tocols, only γ-tocopherol was found, and it contributed only a minor amount, <1.3 μg/g, in five winter wheat genotypes. β-Tocotrienol was the most abundant tocol in all genotypes except for one winter wheat, which also had the second lowest level of total tocols. β-Tocotrienol contributed from 10.0 to 44.9 μg/g of dm to total tocols in the genotypes, and its proportion was, on average, 50.8% of total tocols, with a range of 31.3−68.5%. The second most abundant tocol was α-tocopherol, the content of which in the genotypes varied from 6.4 to 19.9 μg/g of dm, representing on average 26.8% of total tocols and having a range of 12.2−40.8%. The ranges within wheat types were as great as those for total tocols. The average profiles found were typical of wheats in general (*15, 16*). In a recent study, a similar tocol profile was observed in a *Triticum* sp. sample, in which the amounts of α- and β-tocopherols were 15.4 and 5 μg/g of dm, respectively, and those of α- and β-tocotrienols were 5.0 and 19.6 μg/g of dm, respectively, yielding a total content of tocols

Table 2. Characterization of the Tocol Profiles of Wheat Types

	tocol content (μg/g of dm)											
	α-tocopherol			β-tocopherol			α-tocotrienol			β-tocotrienol		
wheat type	av	SD	range	av	SD	range	av	SD	range	av	SD	range
winter wheat	13.5	1.7	9.1−19.9	6.3	1.5	3.2−13.3	4.8	1.0	2.5−7.6	25.3	6.5	10.0−44.0
spring wheat	13.9	1.4	11.3−16.0	6.3	1.2	4.3−9.2	4.6	0.7	3.5−6.0	24.9	4.7	14.0−36.1
durum wheat	10.7	1.3	8.3−13.1	4.6	1.4	3.9−8.6	6.1	0.9	4.7−8.1	26.7	5.5	19.4−34.8
spelt	11.0	1.1	9.9−12.5	6.2	1.0	5.0−7.1	4.8	0.9	3.5−5.8	24.2	3.0	20.3−26.9
diploid einkorn	9.1	2.0	7.0−12.1	2.8	1.2	1.6−4.9	9.8	1.8	8.1−12.4	35.3	9.5	24.6−44.9
tetraploid emmer	7.7	1.0	6.4−8.6	3.1	0.7	2.4−3.8	4.7	0.6	4.0−5.6	20.9	10.4	15.3−39.4
total	13.0	2.1	6.4−19.9	6.0	1.6	1.6−13.3	5.0	1.3	2.5−12.4	25.4	6.6	10.0−44.9

Table 3. Characterization of Wheat Types According to the Proportion of Tocotrienols in Total Tocols

	percentage of sum of α- and β-tocotrienols from total tocol contents		
wheat type	av	SD	range
winter wheat	59.7	5.7	40.3−71.4
spring wheat	59.0	4.5	49.5−65.3
durum wheat	68.0	4.6	62.2−75.2
spelt	62.7	3.6	58.3−68.0
diploid einkorn	79.2	2.3	75.8−81.3
tetraploid emmer	69.1	5.8	62.5−78.3
total	61.0	6.6	40.3−81.3

of 45.0 μg/g of dm (*5*). In a comparison of tocol profiles of different wheat types, it was observed that the profiles of early cultivated wheat types, that is, diploid einkorns and tetraploid emmers, differed to some extent from those of the other wheat types. The early cultivated types contained less tocopherols, and especially diploid einkorns contained more tocotrienols, than the other wheat types. The smallest amount of α-tocotrienol found in a diploid einkorn genotype was the same as the greatest amount found in any other wheat types.

Statistically significant relationships were found when the proportions of individual tocols were compared to total tocol contents. There was a moderately strong positive correlation between the percentage of β-tocotrienol and total tocol amount ($r = 0.573$, df = 173, $p < 0.01$) and a moderately strong negative correlation between the percentage of α-tocopherol and total tocol amount ($r = -0.559$, df = 173, $p < 0.01$). This may indicate that in genotypes rich in tocols, there was usually a high proportion of β-tocotrienols, whereas the proportion of α-tocopherol was low.

When tocol profiles were examined, it was found that the percentage of α- and β-tocotrienols, when taken together, ranged from 59.0% of the total tocols for spring wheat genotypes to 79.2% of total tocols in diploid einkorn genotypes (**Table 3**). Average values for the other wheat types ranged from 59.7 to 69.1%. The ranges within wheat types varied from 1.1-fold in diploid einkorn to 1.8-fold in winter wheat genotypes. Four of the five diploid einkorns had the highest percentages of tocotrienols of the 175 genotypes, indicating that these genotypes might be valuable to plant breeders for elevating tocotrienol contents in wheats.

As for our studies, diploid einkorn accessions grown in Italy were found to have very high proportions of tocotrienols, with an average value of 78.6%; however, somewhat lower values for bread wheat and spelt genotypes were found, being 64.5 and 63%, respectively (*30*). In our study, the average values for durum wheat and tetraploid emmer genotypes were also high, being 73.2 and 78.6%, respectively. Also in line with our results, another Italian study showed that the proportion of tocotrienols in durum wheat, at 77%, was greater than that of spring wheat or spelt, at 66 and 68%, respectively (*31*).

Genetic Variation among Individual Winter and Spring Wheat Genotypes. Because the main focus in the HEALTHGRAIN project is bread wheats, a more detailed evaluation of tocols was done for them. As presented in **Tables 1** and **2**, there was a 2.9-fold difference in winter wheat genotypes and a 1.8-fold difference in spring wheat genotypes in total tocol contents, and the average contents of individual tocols were similar with comparable ranges. Bread wheat genotypes listed according to their total tocol contents into eight classes showed that 47% of winter wheat and 60% of spring wheat genotypes had 45−55 μg/g of dm of total tocols (**Table 4**), indicating that a lot of variation was present. Of the 15 poorest sources of tocols, 1 was from the United States (Nap-Hal) and 1 from Switzerland (Lona), and the others were from eastern parts of Europe. Of the 13 best tocol sources, 1 genotype originated from Poland (Alba), 1 from the United States (TAM200), and 1 from Mexico (Cadenza), whereas the others were from western parts of Europe. This may suggest that the geographical origin could influence the tocol levels in wheat genotypes, and the trend might be that lower levels occur in genotypes from the eastern parts of Europe. This possibility and the reasons behind it should, however, be studied further. A similar trend was also found in a study on einkorn accessions, where the lowest levels of total tocols were found in einkorn from eastern Europe (*30*). The 15 genotypes with the lowest levels of tocols included old and transitional varieties, modern varieties, and germplasms (*24*), whereas 11 of the 13 genotypes with the highest levels of tocols were modern varieties.

Because a few of the genotypes of this study had been studied earlier, it was possible to compare the results among studies. Two winter wheat varieties, namely, Mieti and Sagittario, were also cultivated in Italy and gave total tocol values of 63.7 and 55.5 μg/g of dm, respectively (*30*). In our study the contents were clearly lower, being 48.5 and 47.4 μg/g of dm, respectively. In addition, the proportions of tocotrienols of the two varieties were greater when grown in Italy, being 67.1 and 61.5%, than when grown in Hungary, where proportions of tocotrienols were 60.5 and 49.5%. Thus, cultivation and environmental factors have important effects on the tocol contents and profiles. On the other hand, in both studies the two genotypes were among those with average levels of tocols, indicating a similar character in both environments. Similarly, the winter wheat variety Rialto, when grown in Belgium, had the greatest amount of total tocopherols among five varieties (*32*), and in our study it was the genotype with the third highest level of total tocols.

Table 4. Total Tocol Contents of Winter and Spring Wheat Genotypes (Genotypes Are Listed According to Increasing Tocol Content)

total tocol content (μg/g of dm)	no. of varieties	winter wheat varieties	no. of varieties	spring wheat varieties
<35	5	Sadovo-1, Nap-Hal, Spartanka, Momtchil, Bezostaja-1		
35−39.9	9	Flamura-85, Gloria, Aurora, Qualital, GK-Tiszataj, Balkan, NS-Rana-1, Manital, Krasnodarskaya-99	1	Lona
40−44.9	22	Carmen, Albatros-Odesky, MV-Palotas, MV-Suba, Pobeda, Yumai-34, Fertodi-293, Gerek-79, Agron, Geronimo, Ukrainka, San-Pastore, B16, Iljicsovka, Blasco, Blue/AG, Bankuti-1201, Prosbstdorfer-Perlo, Autonomia, Key, Vona, Alabasskaja	4	Mexique-50, Catbird, Chara, Pastor
45−49.9	30	Lasta, Zvezda, Ravenna, Obriy, Granbel, Cardinal, Buck-Catriel, Jubilejnaja-50, Hana, SU321, Kotuku, Sagittario, Avalon, Cubus, CF99075, CF99007, Karl-92, Sava, Atay-85, Tamaro, Skorospelka-3B, Kirkpinar-79, Martonvasari-17, Mieti, Seu-Seun-27, Magdalena-FR, Recital, Scout66, Dekan, Augusta	4	Pan, Janz, Red-River, Milan
50−54.9	31	Amadeus, Plainsman-V, Libellula, Fredrick, Camp-Remy, Maris-Huntsman, Taldor, Arina, Fundulea-29, Rusalka, Bilancia, Soissons, Begra, Stephens, Korweta, Thesee, Fleischmann-481, Caphorn, Apache, Palesio, Alliance, Renan, Ornicar, Baranjka, Etoile-De-Choisy, Arthur-71, CF99102, Kirac66, Millennium, Sumai-3, Moulin	8	Kukri, Saratov-29, Sultan95, Red-Fife, Glenlea, Thatcher, Manitoba, Chinese-Spring
55−59.9	21	Capo, Galahad, Klein-Estrella, Produttore, Guarni, Hereward, Ble-Des-Domes, Gene, Lynx, Akteur, Tremie, Valoris, CF99105, Disponent, Monopol, Estica, Spark, Atlas-66, Nomade, Courtot, Herzog	2	Sunstar, Azteca67
60−64.9	8	Alba, Kanzler, Tommi, Riband, Malacca, Biscay, Ellvis, Claire	1	Cadenza
≥65	4	Isengrain, Rialto, Campari, TAM200		

Relating Total Tocol Contents of Bread Wheats to Other Kernel Characteristics. The HEALTHGRAIN diversity screen material and the collaboration among partners allowed statistical analyses of kernel characteristics. In winter wheat genotypes, there was a moderately strong positive correlation between total tocol contents (fw) and bran yields ($r = 0.499$, df = 128, $p < 0.01$) and a moderately strong negative correlation with kernel size ($r = -0.543$, df = 128, $p < 0.01$). The respective correlations were not statistically significant in spring wheat samples, which may be explained by the smaller number of samples, but had a similar direction. Thus, among all bread genotypes, the moderately strong positive correlation between total tocol contents (fw) and bran yields ($r = 0.477$, df = 148, $p < 0.01$) was positive and that between total tocol contents (fw) and kernel size ($r = -0.488$, df = 148, $p < 0.01$) was negative, indicating that kernels with greater amounts of outer layers and kernels smaller in size tended to have greater levels of total tocols. This finding can easily be explained, because it is generally acknowledged that, as for many other bioactive compounds, the germ, the pericarp, the testa, and the aleurone fraction are the richest sources of tocols (*33*).

There was a relatively weak but statistically significant positive correlation between total tocol and total lipid contents (dm) in bread wheats ($r = 0.192$, df = 148, $p < 0.05$), and although the correlation in winter wheat genotypes was not significant, that in spring wheat genotypes was moderately strong ($r = 0.685$, df = 18, $p < 0.01$). These values indicate that with higher lipid contents, the tocol contents also tended to be higher. Correlations between total tocol contents and those of phytosterols (dm) were moderately strong and positive in winter, spring, and all bread wheat genotypes ($r = 0.481$, df = 148, $p < 0.01$; $r = 0.595$, df = 18, $p < 0.01$; $r = 482$, df = 148, $p < 0.01$, respectively). Thus, the levels of the two classes of lipid-soluble bioactive compounds seem to be strongly related, which further supports the observation that many bioactive compounds are similarly localized in the kernel (*24*).

In conclusion, this study shows that there is a large variation in tocopherols and tocotrienols in the genetic pools of different types of wheat. Total tocopherol contents ranged from 27.6 to 79.7 μg/g of dm. The 130 genotypes of winter wheat included the one with the lowest and the one with the highest levels of tocols, indicating a great potential for improvement in tocol content even in this type of wheat. Other types of wheats, especially the early cultivated forms, increase further the potential for improving the proportion of tocotrienols, which could be of great importance because cereals and cereal products are already the major sources of these bioactive compounds in the human diet.

ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; dm, dry matter; fw, fresh weight; HPLC, high-performance liquid chromatography; NP, normal phase; *m/z*, mass to charge ratio; FLD, fluorescence detection; αT, α-tocopherol; βT, β-tocopherol; γT, γ-tocopherol; δT, δ-tocopherol; αT3, α-tocotrienol; βT3, β-tocotrienol; γT3, γ-tocotrienol; δT3, δ-tocotrienol; SD, standard deviation.

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