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ARTICLE

Effects of the Environment and Genotype on Tocopherols and Tocotrienols in Wheat in the HEALTHGRAIN Diversity Screen†

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Tocol composition was studied in 26 genotypes of wheat grown in one location for three years and in three other locations for one year. Special emphasis was placed on evaluating the variation of tocols within wheat genotypes and among various growing conditions. In general, both genetic and environmental effects had a strong impact on tocols in wheat genotypes. Because the growing locations and years differed considerably, greater variation due to the environment was found in this study than in earlier ones. Some of the genotypes were more sensitive to the impact of the environment, whereas others were relatively stable. Of the wheat genotypes with an average total tocol content of ≥55 μg/g of dry matter, five genotypes showed relatively low variation: Claire, Cadenza, Lynx, Atlas 66, and Disponent. These genotypes could be potential candidates for the breeding of stable and high-tocol content wheat cultivars.

KEYWORDS: Tocopherols; tocotrienols; tocols; wheat genotypes; environment; location; year

INTRODUCTION

Tocopherols and tocotrienols, collectively known as tocols, are amphipathic and lipid-soluble compounds that consist of a polar chromanol ring and a hydrophobic 16-carbon side chain. Tocopherols have a saturated side chain, whereas that of tocotrienols has three double bonds. Both tocopherols and tocotrienols occur as four forms, α-, β-, γ-, and δ-tocols, which differ in the number and position of methyl groups in the chromanol ring. The most important chemical property of tocols is their ability to act as antioxidants by scavenging lipid peroxyl radicals and quenching or reacting with singlet oxygen and other reactive oxygen and nitrogen species. α-Tocopherol also provides vitamin E activity (see, e.g., refs *1* and *2*).

Tocols are synthesized only in photosynthetic organisms and mainly in plastids: in chloroplast membranes and in the oilbodies of seeds (*3*, *4*). The synthesis and presence of tocols are highly conserved in plants. Tocopherols are widely distributed in higher plants, whereas tocotrienols occur mainly in some nonphotosynthetic tissues, such as seeds and latex, and principally in the endosperm of monocot seeds (*3*). Tocotrienols are not even synthesized in photosynthetic tissues. Photosynthetic tissues have a relatively low level of tocols, but the percentage of α-tocopherol is usually high. Although seeds are rich in tocols, α-tocopherol is the major tocol in only some of them, and other tocols have a greater impact in others (*5*). The main function of tocols in plants is to react with and quench free radicals and singlet oxygen, thus providing protection from oxidative stress. Tocopherols actively protect seeds during storage, germination, and early growth (*6*−*8*). Tocotrienols may also be involved in protecting young seedlings (*5*).

Although current dietary recommendations consider only the natural 2*R*-α-tocopherol vitamer to contribute to vitamin E activity (*9*), other tocols contribute to other biological activities, such as the prevention of neurodegeneration and the lowering of serum cholesterol levels (*10*, *11*). Cereal grains have a relatively low level of α-tocopherol, thus making them only moderate sources of vitamin E. Instead, they contain more of other tocols, and, in fact, cereal grains are the most important sources of tocotrienols in our diet (*2*, *12*). When the large quantities of cereals and cereal products consumed are taken into account, grains and especially whole grains have become acknowledged as valuable and good sources of many phytochemicals and vitamins, including tocols (*13*, *14*). Moreover, the bioavailability of tocols from cereal products has been shown to be high (*15*, *16*). Thus, current dietary guidelines recommend including wholegrain products in a balanced and healthy diet (*17*).

Wheat grains have been reported to contain on average 50 μg/g of tocols, with β-tocotrienol as well as α- and β-tocopherols constituting the major vitamers (*2*). Despite the abundance of data on tocols in individual genotypes, wheat fractions and commercial products (see, e.g., refs *12*, *18*, and *19*), only a few controlled studies have compared the tocol contents of various genotypes or the effects of environmental factors on tocols. In our previous study, we showed that the total tocol content of 150 bread wheat genotypes grown under similar conditions in Hungary ranged from 27.6 to 79.7 μg/g of dry matter (dm) (*19*). Although there was an almost 3-fold difference in total tocol content, the majority of genotypes had a level of 40−60 μg/g of dm. A similar difference in α-tocopherol content was found in eight soft wheats grown in Maryland, with an α-tocopherol content of 3.4−10.1. μg/g of fresh weight (fw) in whole grains (*21*). Studies on other grains, such as einkorn (*22*, *23*), oats (*24*, *25*), rye (*26*), and barley (*27*−*29*) have also found significant differences in

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tocol amounts among genotypes, although the tocol profiles remained relatively stable within each species.

Very little is known about the effects of the environment, for example, soil type, temperature, and precipitation, and the interaction of the environment and genotype on the content and composition of tocols in wheat, as the current knowledge is based mainly on studies of other cereal grains. In a study by Peterson and Qureshi (*24*), 12 oat and 30 barley genotypes were planted in three locations in the United States; they found that the location had a significant effect on the total tocols in oats, but not in barley. Differences in some individual tocols could be found in both species. The authors suggested that the barley genotypes were either more resistant to environmental factors or the locations were more similar to each other in the barley experiment than in those with oats. Another study on hull-less and hulled barley genotypes ($N = 6$) grown in four locations in Italy demonstrated that both the genotype and the location had significant effects on total tocols and most individual tocols (*27*). Neither of these studies, however, attempted to explain the variation in tocols among locations.

A study performed in the Czech Republic showed significant differences among years when 13 barley genotypes were grown in three years using two different cropping systems (*28*). The effect of each year was highly significant ($p \leq 0.001$), whereas the effects of the genotype and cropping system were smaller. The authors suggested that a colder and drier environment increased tocol levels, whereas hotter and wetter conditions decreased them. Furthermore, the grain sizes were smaller in the cold and dry year, yielding grains with a larger proportion of tocol-rich germ and bran fractions than those of the larger grains. Grain size was thus presumed to be the cause of the variation in tocol contents. A similar result was obtained from a study on brown rice. The authors assumed that increases in the α-tocol content at elevated temperatures could be due to the decreased seed size and that the actual tocol amount in the seed was relatively stable (*30*). However, the study showed no significant effect on other tocols.

Finally, a recent study of one bread wheat cultivar and five einkorn accessions compared the effects of genotype and growing environment (four locations and two years) on tocols (*23*).The study demonstrated that genotype was usually the most important factor explaining the content of several lipid components. The genotype and the year had significant effects on total tocol and tocotrienol contents, whereas only the genotype had a significant effect on the tocopherol content. It should be remembered, however, that all locations were relatively similar to each other (all were in Italy), which could have diminished the possible effects caused by the locations. The two years differed from each other significantly in the amount of precipitation. The authors pointed out that during the year with plentiful rainfall, the einkorn yields were higher, and their total tocol levels were smaller, which is in line with previous findings on high tocol content in small grains. Interestingly, an early-maturing bread wheat genotype showed no differences in yield or tocol composition between the years. The amount of data on the effects of the environment on tocols in grains is still limited, and very little is known on the variation within different wheat genotypes. Thus, there is a need for controlled studies with a variety of wheat genotypes, because the stability of the genotypes under different environments is an important feature in the development of new cultivars for commercial uses.

Because the main focus of the HEALTHGRAIN project is to develop new healthy food products based on whole grains of wheat and rye, the project continued from a large diversity screening study of wheat genotypes (*31*) to an evaluation of the effects of environmental factors on bread wheats. In this study, the aim was to examine the effects of the environment on tocols of selected wheat genotypes. Special emphasis was placed on the variation of tocols within wheat genotypes and across different growing environments. This was done by growing the genotypes for three consecutive years in Hungary and also for one year in three other locations, namely, France, the United Kingdom, and Poland. Thus, there were two types of variation in the growing environments: the years in one location, and the locations in one year.

MATERIALS AND METHODS

Samples. The bread wheats (*Triticum aestivum* var. *aestivum*) included 24 winter wheat and 2 spring wheat genotypes (**Table 1**). The genotypes included land races and breeding lines, modern and older cultivars, of different origins, and were previously characterized in the diversity screen study (*31*). Three of the genotypes (Spartanka, MV Emese, and Gloria) showed a very low total tocol content, $< 40\ \mu g/g$ of dm, and eight (Tommi, Riband, Malacca, Cadenza, Claire, Isengrain, Rialto, and Campari), a very high content, $> 60\ \mu g/g$ of dm, in the diversity screen of the year 2005 harvest (*20*). Two common varieties (Crousty and Tiger) that were used as raw materials in the processing module of the HEALTHGRAIN project were included in the 2007 study. The wheat genotypes were grown in Martonvásár (Hungary, HU) in the years 2005, 2006, and 2007 and also in Echantillon (France, FR), Woolpit (United Kingdom, U.K.), and Choryn (Poland, PL) in the year 2007, representing differences in climatic conditions and soil types (*31*−*33*). In the year 2005, the grains were harvested during a rainy period (*34*). In the year 2007, the temperatures from May to July were highest in Hungary and lowest in the United Kingdom, whereas the precipitation between May and August was in general highest in the United Kingdom. Grains were harvested at crop maturity at each location. In Hungary, the grains were harvested on July 20−23 in the year 2005, on July 18−20 in the year 2006, and on July 5 in the year 2007. In 2007, harvesting dates were later in other locations and were very late in the United Kingdom (August 22) (*33*). Wheat grains were transported to Martonvásár (Hungary), where they were prepared and milled into wholemeal flour of 0.5 mm particle size prior to shipping to the laboratory for tocol analysis.

Two batches of wholemeal flours from the wheat cultivar MV Emese were similarly prepared and used as in-house reference samples. One batch of flour was used with samples harvested in 2005 and 2006 and the other one with samples harvested in 2007. 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) using a previously validated method (*20*).

Performance of the Analytical Method. At the beginning of the study, action limits for the total tocol content of the in-house reference samples (two batches of wholemeal wheat flours and rapeseed oils) were set at mean ±2 × standard deviation (SD). When wholemeal samples were analyzed, if either of the in-house reference samples yielded 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%.

Repeatability of the tocol analysis by HPLC was good, because a rapeseed oil sample analyzed in each batch of HPLC samples yielded stable results. The contents of α-, γ-, and δ-tocopherols (mean ± SD) in the rapeseed oil in-house reference sample analyzed with the samples harvested in 2005 and 2006 were 208 ± 5, 438 ± 12, and 15 ± 2 μg/g ($N = 70$), respectively, and in the second oil analyzed with the samples harvested in 2007 were 204 ± 7, 457 ± 11, and 14 ± 1 μg/g ($N = 29$), respectively. In addition, the repeatability of the whole analytical method on flour samples was good, because the results of the in-house reference samples analyzed in each sample batch had very little variation. Total tocol content of the first flour sample was 30.0 ± 1.7 μg/g of fresh weight (fw) ($N = 73$), and that of the second flour sample was 39.1 ± 1.0 μg/g of fw ($N = 35$). All results for wholemeal samples are given as means of replicate samples on a dry matter basis, if not stated otherwise.

Statistical Analysis. Statistical differences in total tocol content among genotypes and environments were compared with two-way analysis of variance using a completely randomized block design where either the environmental or the genetic effects were considered fixed. Fisher's least

Table 1. Total Tocol Content among Wheat Genotypes Grown for Three Years and in Four Locations

	total tocol content (μg/g of dm)									
pedigree/genotype	Hungary 2005	Hungary 2006	Hungary 2007	France 2007	United Kingdom 2007	Poland 2007	av	SD	range (μg/g of dm)	groups[b]
Campari	77.2	73.3	86.3	70.5	80.2	81.2	78.1	5.7	70.5−86.3	a
Herzog	59.8	57.3	70.9	53.6	56.1	63.8	60.2	6.3	53.6−70.9	d, e, f
Disponent	58.3	58.2	59.5	47.5	52.0	56.1	55.3	4.6	47.5−59.5	g, h
Tommi	62.1	62.6	70.4	53.6	58.7	63.8	61.9	5.6	53.6−70.4	c, d, e
Tremie	56.9	56.9	67.3	56.2	51.9	62.3	58.6	5.4	51.9−67.3	e, f, g
CF99105	57.4	63.4	70.6	58.2	56.7	61.7	61.3	5.2	56.7−70.6	d, e, f
Valoris	57.1	62.8	72.5	54.7	54.6	67.8	61.6	7.4	54.6−72.5	c, d, e, f
Isengrain	65.6	64.2	68.5	53.6	56.1	62.3	61.7	5.8	53.6−68.5	c, d, e
Claire	64.2	62.7	72.6	62.9	63.3	65.6	65.2	3.8	62.7−72.6	b, c
Maris Huntsman	51.3	53.7	62.9	46.7	50.7	58.1	53.9	5.8	46.7−62.9	h, i
Lynx	56.8	64.6	68.6	56.0	61.4	62.5	61.6	4.8	56.0−68.6	c, d, e, f
Malacca	62.9	71.6	74.6	54.3	58.7	68.4	65.1	7.8	54.3−74.6	b, c
Rialto	73.0	78.0	87.9	73.3	77.6	81.1	78.5	5.5	73.0−87.9	a
Riband	62.6	63.8	69.0	51.1	52.1	57.9	59.4	7.1	51.1−69.0	e, f
Avalon	47.4	50.0	59.4	49.3	46.5	53.6	51.0	4.8	46.5−59.4	i
San Pastore	41.9	47.5	48.2	38.5	41.5	43.8	43.6	3.7	38.5−48.2	j, k
Estica	58.5	59.6	64.8	50.1	56.0	59.1	58.0	4.8	50.1−64.8	f, g
Gloria	36.1	43.7	44.5	38.0	39.5	40.3	40.3	3.3	36.1−44.5	k, l
Spartanka	32.2	58.1	43.4	30.8	35.1	36.3	39.3	10.2	30.8−58.1	l
Obriy	45.8	52.9	49.3	38.4	44.5	46.0	46.1	4.8	38.4−52.9	j
Atlas 66	59.1	63.1	62.9	51.8	52.4	58.4	58.0	4.9	51.8−63.1	f, g
Crousty			76.2	61.5	61.2	69.5	67.1	7.2	61.2−76.2	b
Tiger			68.6	53.5	53.3	60.3	58.9	7.2	53.3−68.6	e, f, g
MV Emese	33.8	46.1	41.3	32.2	34.3	37.0	37.4	5.3	32.2−46.1	l
Chinese Spring[a]	53.8	67.2	68.9	54.3	54.7		59.8	7.6	53.8−68.9	d, e, f
Cadenza[a]	63.2	69.2	68.0	56.4	60.6		63.5	5.3	56.4−69.2	b, c, d
av (μg/g of dm)	55.7	60.4	65.3	51.8	54.2	59.0	57.7			
SD (μg/g of dm)	11.4	8.6	11.9	10.2	10.6	11.8	11.5			
range (μg/g of dm)	32.2−77.2	43.7−78.0	41.3−87.9	30.8−73.3	34.3−80.2	36.3−81.2				

[a] A spring wheat type. [b] Homogenous groups of wheat genotypes are marked with the same letter. Groups were built up by two-way analysis of variance using the environmental effects fixed followed by multiple-range tests ($p < 0.05$).

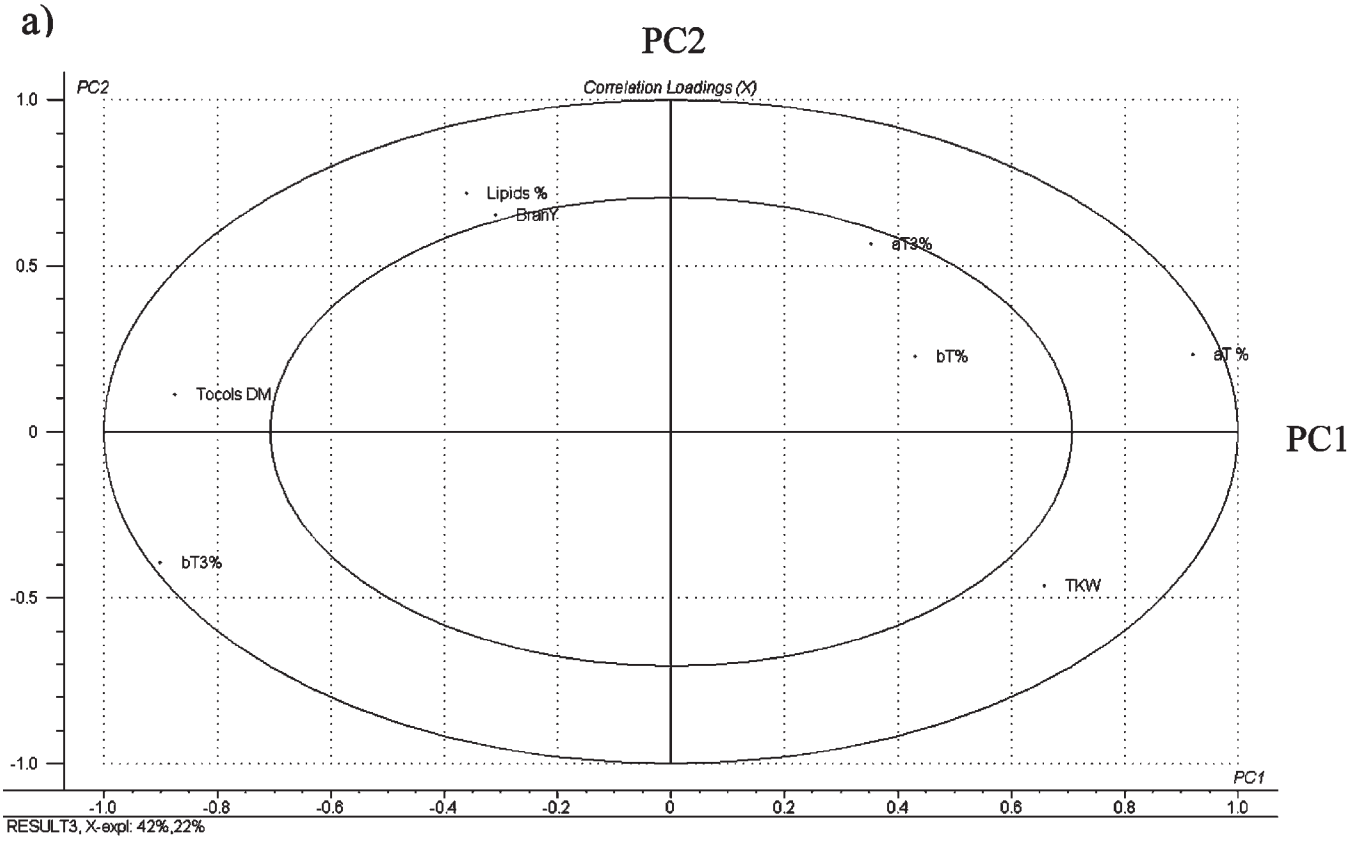
significant difference procedure served as a multiple-range test to separate homogeneous groups from each other.

To relate tocol values to other parameters of the kernels, Pearson correlation coefficients of total tocol content with thousand kernel weights and bran yields were calculated on a fresh weight basis and those of total tocol content with total lipids. Pearson correlation coefficients of total tocol content and the proportions of individual tocols were also calculated. In addition, principal component analysis (PCA) served to demonstrate relationships among the tocol data with other characters of the grains. Dry matter content, thousand kernel weights, bran yields, total lipid content, and data on growing environments were obtained from several partners of the HEALTHGRAIN project (*31*, *33*, *34*). Statgraphics Plus 4.0 software (Manugistics Inc., Rockville, MD) was used for other statistical analyses, except for PCA, which was performed using Unscrambler v. 9.0 software (Camo Software AS, Oslo, Norway).

RESULTS AND DISCUSSION

Overall Variation in Total Tocol Content and Profiles of the Wheat Genotypes. The overall variation in the 150 samples and the relationships among different variables were investigated by PCA. PCA, including normalized values of total tocol content, proportions of individual tocols, indicators of grains (thousand kernel weight, bran yield, and lipid content), environmental factors (year and country), and the geographical areas of origin of the genotypes, produced a loading plot with the two first components accounting for 45 and 22%, respectively, of the total variance in the data set (**Figure 1a**). The loading of total tocols was far from the origin and close to the primary *x*-axis, showing that the first principal component separated the samples by their total tocol content. The loading of the percentage of β-tocotrienol was situated far from those of other tocols, and especially of α-tocopherol, along both the primary and secondary axes, thus indicating that the two principal components were needed to explain the variance in the tocol profiles. The loading plot also showed that in high-tocol grains the percentage of β-tocotrienol was, generally, high, and the percentage of α-tocopherol was relatively small, because these variables correlated with each other. The percentages of α-tocotrienol and β-tocopherol were less significant variables in explaining the variance found in wheat tocols. On the basis of the loading plot, high total tocol content was associated with relatively small grains, because the thousand kernel weight loading was situated far from that of the total tocol. The loading plots of bran yields and lipid content were closely located and clearly separated from total tocols by first and second principal components (**Figure 1b**). Thus, one can assume that lipids were even more concentrated than total tocols in the germ and bran fractions, characterized by high bran yields. The score plot of the wheat grains coded by their growing location (**Figure 1b**) showed that many of the grains from Hungary fell in the upper left quadrant, whereas many of those from France were found in the lower-right quadrant. However, the separation based on growing location was relatively unclear. Grains from the United Kingdom and Poland were scored close to the middle of the plot and overlapped with other grains.

Relationships between Tocol Content and Composition with Other Grain Characteristics. As already evident in the PCA, tocol content and composition of the grains were related with several characteristics of the grains. Considering only correlations with $r > 0.50$, a significant negative correlation was found between



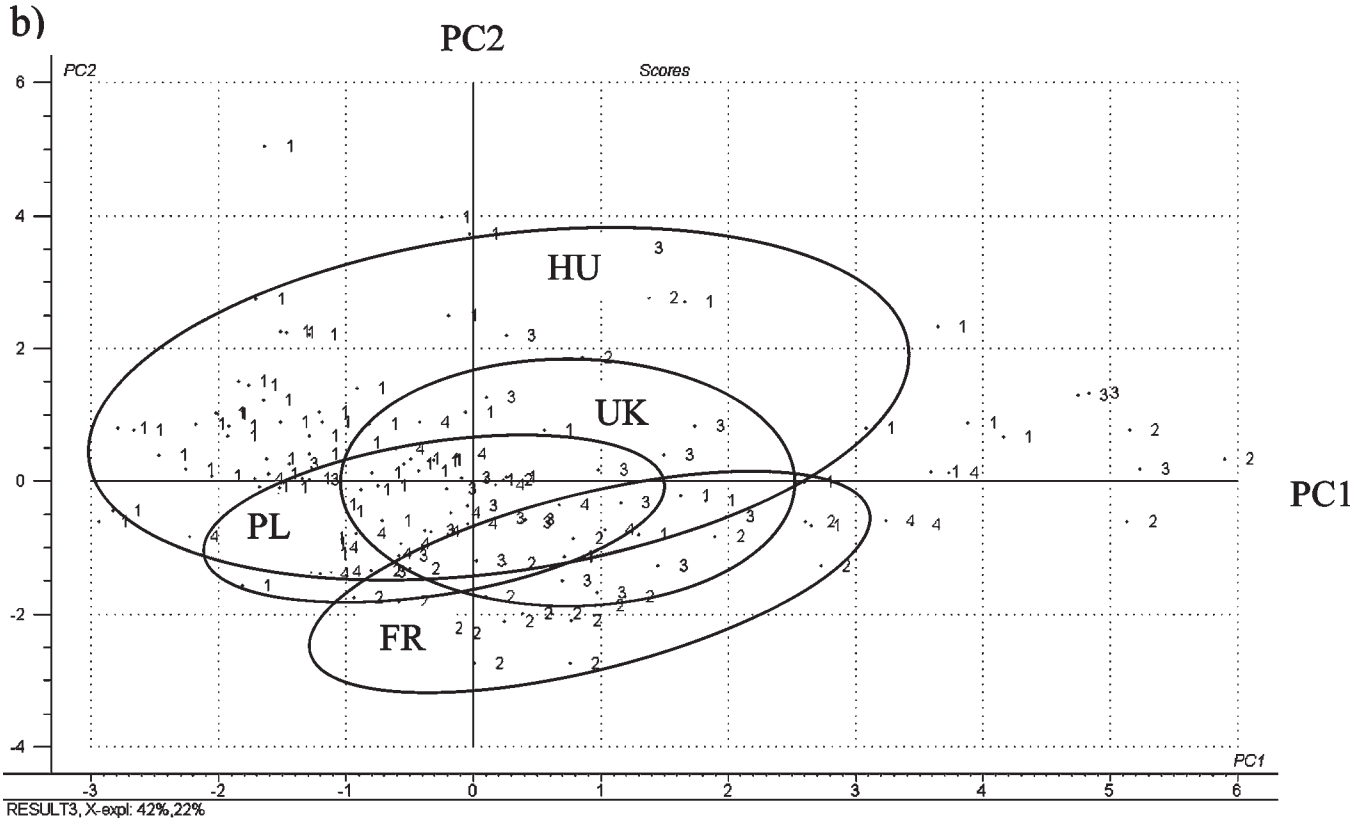


Figure 1. Principal component analysis of tocol content and profiles and other characteristics of the wheat grains grown under six different environments ($N = 150$) (PC, principal component): (**a**) loading plot of the grain characteristic variables (Tocols DM, total tocols; αT%, percentage of α-tocopherol; αT3%, percentage of α-tocotrienol; bT%, percentage of β-tocopherol; bT3%, percentage of β-tocotrienol; BranY, bran yield; TKW, thousand kernel weight); (**b**) score plot of grain objects marked by growing location codes (HU, 1, Hungary; FR, 2, France; UK, 3, United Kingdom; PL, 4, Poland).

Table 2. Variation in α- and β-Tocopherol and Tocotrienol Contents among Wheat Genotypes Grown under Different Environmental Conditions ($N = 6$)

	tocol content (μg/g of dm)											
	α-tocopherol			β-tocopherol			α-tocotrienol			β-tocotrienol		
pedigree/genotype	av	SD	range	av	SD	range	av	SD	range	av	SD	range
Campari	17.6	1.4	16.2−19.9	9.0	1.0	7.7−10.1	7.7	1.2	6.4−9.2	43.9	3.4	40.2−49.3
Herzog	14.6	0.9	13.1−15.6	7.3	1.1	5.6−8.1	5.7	1.0	4.8−7.3	32.6	4.3	28.1−39.9
Disponent	12.9	0.6	11.8−13.4	6.5	1.2	4.9−8.1	5.6	1.0	4.4−6.9	30.3	2.7	26.3−33.0
Tommi	13.9	1.0	12.7−15.5	6.3	1.5	4.3−8.7	6.3	1.2	4.6−7.9	35.4	3.1	31.3−40.2
Tremie	13.3	1.2	11.5−14.8	5.2	0.8	3.9−6.1	6.6	1.0	5.4−7.9	32.8	3.6	26.4−37.0
CF99105	14.6	0.5	14.1−15.5	7.5	0.8	6.3−8.5	6.5	0.8	5.5−7.4	31.9	3.8	27.6−38.0
Valoris	14.2	0.8	13.5−15.5	5.1	1.0	4.0−6.1	6.2	0.7	5.1−6.9	36.1	5.7	29.6−44.5
Isengrain	16.9	0.9	15.6−17.9	7.4	1.0	6.0−8.3	6.1	0.7	4.7−6.7	31.3	4.7	25.4−36.4
Claire	14.7	1.2	13.5−16.7	7.2	0.9	5.7−8.0	8.0	1.2	6.7−9.7	35.3	2.4	32.3−39.7
Maris Huntsman	11.4	1.2	10.1−13.2	4.4	0.6	3.6−5.1	5.8	1.0	4.7−6.9	32.2	4.7	26.7−39.1
Lynx	14.2	1.0	12.9−16.4	6.4	1.1	4.9−8.1	6.7	1.1	5.5−8.0	34.3	2.5	31.4−38.1
Malacca	15.2	1.0	14.0−16.4	6.2	1.4	4.2−8.1	6.7	1.0	5.0−7.6	37.1	5.5	30.0−43.4
Rialto	18.2	1.1	16.5−19.5	9.2	1.2	7.8−11.0	7.0	1.2	5.7−8.4	44.1	3.3	41.2−50.2
Riband	13.2	0.9	12.2−14.1	5.7	1.2	3.9−7.2	6.2	0.8	4.9−7.4	34.4	5.1	27.7−40.9
Avalon	12.6	0.7	11.8−13.5	5.5	1.2	3.7−7.3	5.8	0.9	5.0−6.9	27.1	3.4	23.8−33.0
San Pastore	13.0	0.6	12.2−15.3	4.9	0.7	4.2−6.2	4.0	0.6	3.4−4.8	21.6	2.5	18.1−25.2
Estica	13.4	1.0	12.8−15.3	6.0	0.9	4.9−7.4	5.4	0.9	4.0−6.5	33.3	3.8	27.9−38.5
Gloria	14.4	1.2	12.8−15.9	5.3	0.8	4.5−6.7	5.2	0.7	4.1−5.9	15.4	2.6	12.9−19.9
Spartanka	14.1	2.7	11.7−19.4	5.2	2.0	4.2−9.2	4.3	0.9	3.2−5.7	15.8	5.4	9.8−23.8
Obriy	12.4	0.8	11.6−13.4	4.7	0.6	4.3−5.9	6.0	0.8	4.5−7.0	23.1	3.4	17.9−26.7
Atlas 66	15.6	0.8	14.4−16.4	6.6	1.3	5.0−8.8	6.7	0.8	5.4−7.5	29.0	3.7	24.5−34.3
Crousty	14.4	0.4	13.8−14.8	6.6	0.9	5.6−7.4	5.8	1.0	4.3−6.5	40.3	5.8	34.6−47.5
Tiger	13.6	0.6	13.0−14.1	47.3	0.9	6.6−8.3	5.6	0.8	4.5−6.2	32.4	5.8	26.8−40.0
MV Emese	12.0	1.4	10.4−14.5	5.0	0.8	4.4−6.6	4.8	0.8	3.7−6.1	15.6	3.1	12.2−19.2
Chinese Spring[a]	17.6	1.0	15.9−18.3	8.0	0.6	7.3−9.0	6.4	0.7	5.6−7.0	27.8	6.0	22.5−35.4
Cadenza[a]	15.4	1.2	14.0−17	8.1	1.0	7.2−9.8	5.4	0.9	4.0−6.3	34.6	4.3	30.0−39.1
mean (μg/g of dm)	14.4			6.4			6.0			31.1		
SD	1.7			1.3			0.9			7.7		

[a] A spring wheat type.

total tocols (of fw) and thousand kernel weights (−0.646, $p < 0.01$, df = 148), which indicates that small grains tended to have a higher content of tocols than did larger grains. This can be explained by the fact that the proportions of high-tocol germ and bran fractions are greater in small grains than in large ones, whereas the reverse is true for low-tocol endosperm as indicated previously (*20*, *28*, *30*).

There were significant correlations between the content of total tocols and the percentages of α-tocopherol ($r = -0.733$, $p < 0.01$, df = 148) and β-tocotrienol ($r = 0.675$, $p < 0.01$, df = 148). The correlation with α-tocopherol was negative, and the one with β-tocotrienol, positive, which indicates that the proportion of α-tocopherol decreased and that of β-tocotrienol increased in grains having a high content of total tocols. Finally, a moderately strong positive relationship was found between total tocols and the percentage of the sum of tocotrienols ($r = 0.646$, $p < 0.01$, df = 148). The same trend in tocol content and composition was found in our previous study on variation among 150 bread wheat genotypes grown under similar environmental conditions (*20*).

Variation in Tocols Caused by Genetic Factors. Total tocol content of the genotypes grown under the six environments ranged from 30.8 to 87.9 μg/g of dm, with 57.7 μg/g of dm as the average value (**Table 1**). Two-way analysis of variance showed that both the genotype and the environment had almost equal and highly significant effects on total tocol content ($F(25, 149) = 59.4$, $p = 0.0000$; $F(5, 149) = 58.6$, $p = 0.0000$). Multiple-range tests showed that the 26 genotypes built up 12 homogeneous groups based on their average total tocol content ($p < 0.05$). Four genotypes (i.e., MV Emese, Spartanka, Gloria, and San Pastore) showed average values below 45 μg/g of dm, and five genotypes (i.e., Rialto, Campari, Crousty, Claire, and Malacca) showed values above 65 μg/g of dm, indicating that both low- and high-tocol genotypes were included in the study. The four low-tocol genotypes were among the genotypes with the largest grains, and the five high-tocol ones were small or medium in size (*33*). All of the high-tocol genotypes were modern ones originating from the United Kingdom, France, or Germany (*31*). Environmental factors had great effects on the variation in tocol content in some genotypes, whereas in some genotypes the effects were only minor, as was evident in the ranges of individual genotypes (**Table 1**). Three genotypes (Claire, San Pastore, and Gloria) had a range of total tocols of < 10 μg/g of dm, and two (Malacca and Spartanka) had one > 20 μg/g of dm. Susceptibility to variation in environmental conditions could not be attributed to geographical origin (*33*). Moreover, correlations between total tocol content (of fw) and thousand kernel weights within each genotype were even more negative and moderately stronger (−0.614 to −0.933, df = 4 − 5) than among all genotypes, indicating a strong genetic impact on these two characteristics of each genotype. The only exception was Chinese Spring, which had the smallest thousand kernel weight (*33*).

The amount of variation in individual tocols among the growing locations and years differed from one tocol to another (**Table 2**). β-Tocotrienol was the major tocol with a mean of 31.1 μg/g of dm, followed by α-tocopherol with a mean of 14.4 μg/g of dm. When the mean coefficients of variation (CV) of individual tocols were considered, α-tocopherol showed the smallest variation (12%) followed by α-tocotrienol, β-tocopherol, and β-tocotrienol (15, 20, and 25%, respectively), suggesting that α-tocopherol content seems to be the most stable one and that of β-tocotrienol to vary the most. Six genotypes had an α-tocopherol range of < 2 μg/g of dm, and only one genotype had a β-tocotrienol range

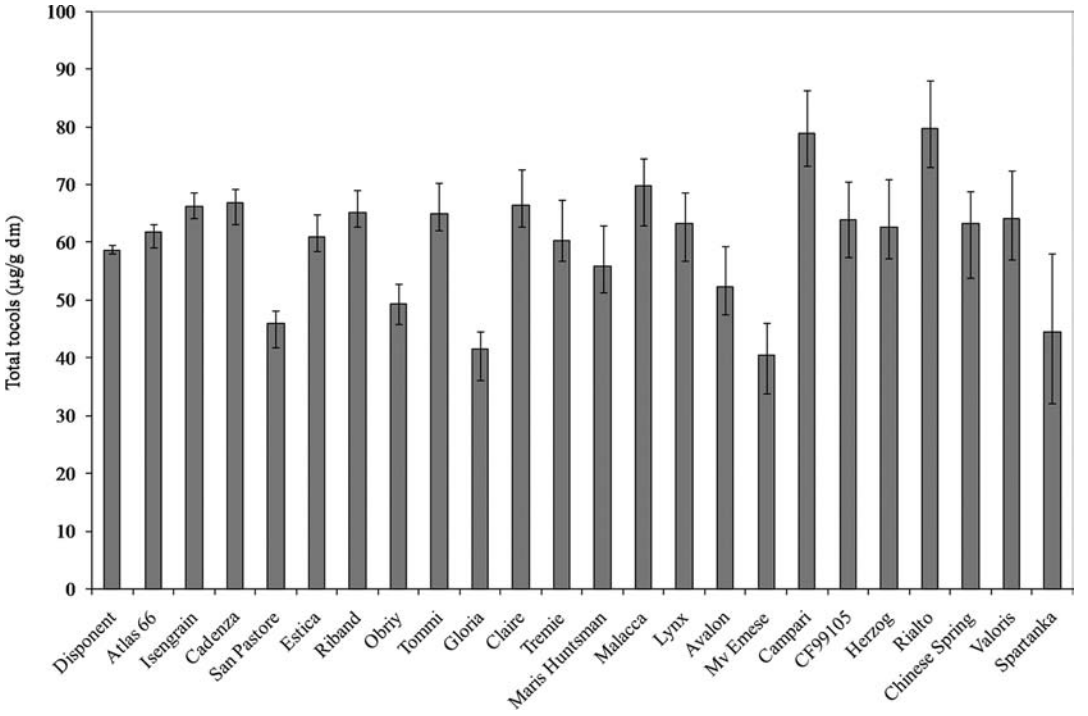


Figure 2. Average total tocol content of different genotypes grown in Hungary in 2005−2007 in order of increasing variation among years. Bars represent the range in three years.

of $< 7\ \mu$g/g of dm among the different environments. Greater stability in α-tocopherol contents compared to other tocols was also found in 54 accessions of einkorn (*22*) and in five accessions of einkorn and one of bread wheat, all grown in Italy (*23*). Because α-tocopherol is known to be the main constituent of the wheat germ fraction, whereas tocotrienols are abundant in the bran and endosperm fractions (see, e.g., refs *18* and *19*), Hidalgo et al. (*23*) concluded that in einkorn accessions, the year-to-year variation of compounds accumulated in the endosperm (e.g., tocotrienols) was important, whereas the variation in compounds occurring mainly in the germ (e.g., tocopherols) was limited. The same was true in this study.

Variation in Tocols Caused by Environmental Factors. There was only a 1.2-fold difference in the average total tocol content of the 26 genotypes grown in Hungary in three years, and the two-way analysis of variance showed highly significant differences among the years as well as among the genotypes ($F(2, 73) = 29.4$, $p = 0.0000$; $F(25, 73) = 18.2$, $p = 0.0000$) (**Table 1**). The mean total tocol content increased during the study, because the contents in the years 2005, 2006, and 2007 were 55.7 ± 11.4, 60.4 ± 8.6, and $65.3 \pm 12.2\ \mu$g/g of dm, respectively. Multiple-range tests showed that there was a significant difference among each of the years ($p < 0.05$). Except for five genotypes (Spartanka, Obriy, Atlas 66, MV Emese, and Cadenza), all of the others had the highest level of tocols in 2007. Differences in total tocol content can partly be explained by differences in grain sizes. The thousand kernel weights were decreased (40.7 ± 5.7, 38.9 ± 4.2, and 35.0 ± 6.1 g in 2005, 2006, and 2007, respectively), and aligned to the increase in total tocol contents, which might reflect the rainy harvesting period in 2005 and a warm growing season in 2007 (*33*). Similarly, five accessions of einkorn grown in Italy in two years gave higher yields and lower total tocol contents after a rainy period during growing and ripening with similar temperatures, indicating the importance of rain on the quality of the seeds (*23*).

There were clear differences in the ranges of the genotypes grown in Hungary (**Figure 2**). Some genotypes (i.e., Disponent, Atlas 66, and Isengrain) showed ranges of total tocols of $< 5\ \mu$g/g of dm, indicating that these genotypes were relatively stable under different climatic conditions, whereas three genotypes (i.e., Spartanka, Valoris, and Chinese Spring) showed ranges $> 15\ \mu$g/g of dm. As stated previously, the genotype Spartanka had a much higher level of tocols in 2006 than under any other environmental conditions; similarly, Spartanka also yielded very high values for total folate (*35*).

There was a 1.3-fold difference in the average total tocol content of the 26 genotypes grown in the four locations in 2007, and the two-way analysis of variance showed highly significant differences among the locations as well as among the genotypes ($F(3, 101) = 184$, $p = 0.0000$; $F(25, 101) = 93.7$, $p = 0.0000$) (**Table 1**). The average total tocol contents of the grains grown in Hungary, Poland, the United Kingdom, and France were 65.3 ± 11.9, 59.0 ± 11.8, 54.2 ± 10.6, and $51.8 \pm 10.2\ \mu$g/g of dm, respectively. Multiple range tests showed that each of the locations gave significantly different levels of total tocols in the genotypes studied ($p < 0.05$). The ranges among growing locations were, in general, greater than among years (**Figures 2** and **3**), which reveals the importance of soil and climatic factors to tocol content. None of the genotypes showed ranges in total tocol content of $< 5\ \mu$g/g among locations, and only four (i.e., Gloria, MV Emese, Claire, and San Pastore) had $< 10\ \mu$g/g of dm. As many as four genotypes (i.e., Malacca, Riband, Valoris, and Herzog) had ranges $> 17\ \mu$g/g of dm.

As with differences found among harvesting years, differences in total tocol contents among locations can partly be attributed to differences in grain sizes. The mean thousand kernel weights of genotypes increased parallel to the decrease in total tocol content, being 35.0 ± 6.1 g in grains grown in Hungary and 50.9 ± 5.2 g in grains grown in France, respectively (*33*).

In general, both genetic and environmental factors had a strong and important impacts on tocopherols and tocotrienols in wheat genotypes. There were large differences in environmental conditions with respect to geographical, soil, and climatic conditions, which further challenged the stability of the genotypes. In addition, greater variation resulting from the environmental factors

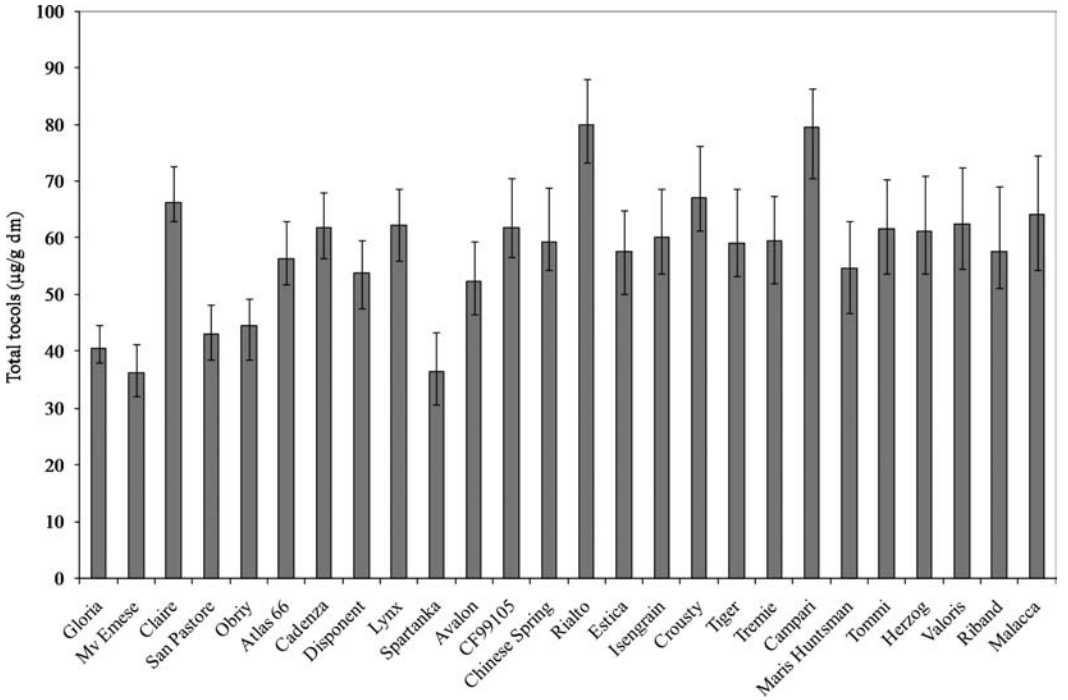


Figure 3. Average total tocol content of different genotypes grown in Hungary, France, United Kingdom, and Poland in 2007 in order of increasing variation among locations. Bars represent the range at four locations.

was found in this study than in earlier ones. Some of the genotypes were more sensitive than the others to the impact of the environment. Only one genotype, Valoris, had one of the largest ranges in both growing years and locations, thus reflecting a high sensitivity to both environmental factors studied, whereas most genotypes showed instability in either year or location. There were three genotypes that showed very low overall variation (< 10 μg/g of dm): Gloria, San Pastore, and Claire. The high-variation genotype was a modern one, whereas two of the three low-variation genotypes represented old and transitional varieties, indicating that at least some of these varieties were more stable than the modern varieties. Of the high- and mid-tocol genotypes, with a total tocol content of ≥ 55 μg/g of dm, five genotypes showed relatively low variation (< 13 μg/g of dm): Claire, Cadenza, Lynx, Atlas 66, and Disponent. These genotypes could be potential candidates for the breeding of stable and high-tocol wheat cultivars.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; NP, normal phase; FLD, fluorescence detection; PCA, principal component analysis; dm, dry matter; fw, fresh weight; av, average; SD, standard deviation.

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