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Effects of Genotype and Environment on Steryl Ferulates in Wheat and Rye in the HEALTHGRAIN Diversity Screen†

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The effects of genetic and environmental factors on the content and composition of steryl ferulates in wheat and rye were studied. The wheat and rye genotypes were grown at the same location in Hungary over three consecutive years (28 genotypes) or at four different locations across Europe during a single year (12 genotypes). The steryl ferulates were analyzed using HPLC. The genotype and growing location significantly affected the content and composition of wheat steryl ferulates, whereas the year of growth did not result in considerable variation. Less variation was observed in rye, due to fewer genotypes. Campestanyl and sitostanyl ferulates were the main species in both cereals. Knowledge of the natural variation in steryl ferulates and other bioactive compounds allows cultivators and plant breeders to select genotypes with high, stable levels of beneficial compounds. Thus, it is possible to enhance the intake of health-promoting compounds from natural sources.

KEYWORDS: Steryl ferulate; wheat; winter wheat; spring wheat; rye; wholegrain; genotype; environment; year; location; variation

INTRODUCTION

Cereals are an important dietary source of natural phytosterols and their conjugates, that is, sterol glycosides, acylated sterol glycosides, and sterol esters with phenolic or fatty acids. Steryl ferulates are compounds with a ferulic acid linked to the hydroxyl group of the phytosterol. Ferulic acid conjugates occur in certain grains, including rice, corn, rye, and wheat, and they are concentrated in the bran fraction of the kernel (*1*−*4*). The steryl ferulates separated from rice bran, a mixture called γ-oryzanol, and from corn fiber are more widely studied than the steryl ferulates of other grains (*3*−*5*).

Steryl ferulates are associated with several positive health effects. Diets supplemented with phytostanyl ferulates (0.73%), steryl ferulate-rich corn fiber oil (10%), or γ-oryzanol (0.5−1%) reduced plasma cholesterol levels, cholesterol absorption, and/or formation of aortic fatty streaks in animal studies (*6*, *7*). The decrease in plasma cholesterol levels was also observed in mildly hypercholesterolemic men after consuming low or high γ-oryzanol-containing rice bran oil (*8*). Moreover, various steryl ferulates act as inhibitors of tumor promotion (*9*, *10*) and possess anti-inflammatory activities (*11*, *12*). In addition to cholesterol-lowering effect owing to the phytosterol backbone, steryl ferulates also have antioxidative properties due to the radical scavenging ability of the phenolic hydroxyl group of the ferulic acid moiety. Rice bran oil, synthetic sitostanyl ferulate, or the steryl ferulates extracted from wheat, rye, or rice bran inhibited lipid oxidation or polymerization reactions in vegetable oils (*13*, *14*) and lipid models (*15*−*17*).

Wholegrain wheat and rye were reported to contain 62−123 and 29−70 μg/g of fresh weight (fw) of steryl ferulates, respectively, contributing 6−7 and 4−6% of the total phytosterol content (*1*, *2*, *18*−*20*). The bran fractions of wheat and rye grains are especially rich in steryl ferulates, containing up to 390 μg/g of fw in wheat and 250 μg/g of fw in rye (*1*, *2*, *20*). The major components in wheat and rye are the stanol esters, that is, campestanyl and sitostanyl ferulates, followed by the corresponding steryl esters (*2*). The structures of the compounds are shown in **Figure 1**. Seitz (*18*) considered that the genotype did not have a strong effect on the steryl ferulate content or composition of wheat. However, studies concerning rice have shown considerable natural variation in both content and composition of steryl ferulates. Bergman and Xu (*21*) showed that the variation in total γ-oryzanol levels of seven rice cultivars, grown at four locations in the United States over two consecutive years (1999−2000), resulted from the genotype and, to a greater extent, the growing environment. Later, high genotype-dependent variability was observed in the γ-oryzanol contents of 32 rice cultivars grown in Brazil during a single season (*22*). Miller and Engel (*23*) reported that not only the total content but also the composition of steryl ferulates was affected by genetics and environmental conditions when 11 brown rice cultivars were grown at three locations in Europe during three years in 2000−2002. Furthermore, there were significant variations in the steryl ferulate levels of corn fiber oils, which were extracted either from 16 corn hybrids cultivated at a single location in the United States or from single hybrids cultivated at several locations in the United States

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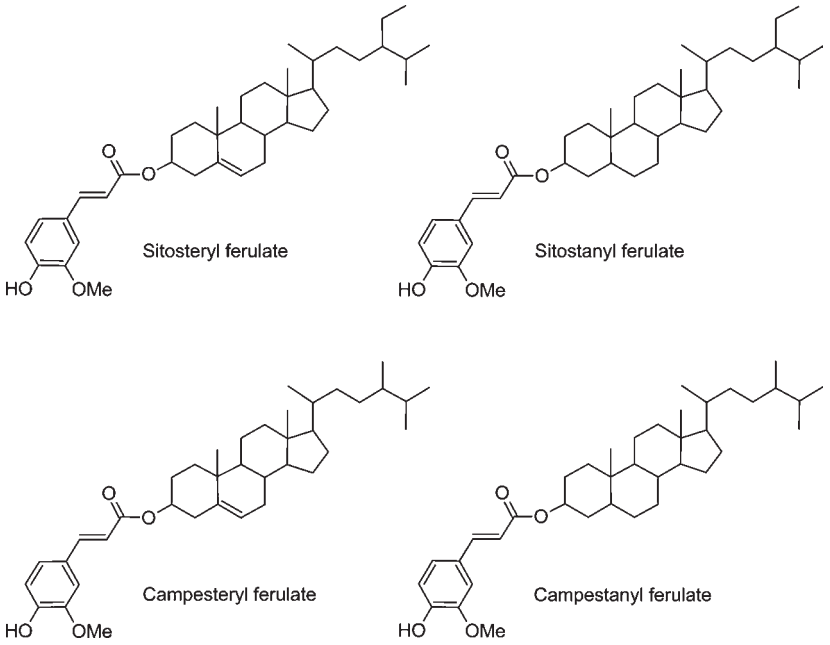

Figure 1. Chemical structures of the steryl ferulates occurring in wheat and rye.

and Canada (*5*). Because there are no corresponding studies on wheat or rye steryl ferulates and previous results related to rice and corn suggest that genetic and environmental factors may have significant influence on the content and composition of steryl ferulates, the natural variation in wheat and rye steryl ferulates needs to be examined.

The aim of this study was to determine whether the genotype and environment affect the content and composition of steryl ferulates in wheat and rye. The wheat and rye samples, produced in a European Union-integrated project, HEALTHGRAIN (2005−2010), included genotypes cultivated in Hungary during three consecutive years and a selection of genotypes also grown at three other locations in Europe during a single year. Both growing years and sites differed considerably in their climatic conditions and soil characteristics, thus providing wide environmental diversity. The corresponding analyses of numerous other phytochemicals and dietary fiber have been accomplished during the HEALTHGRAIN project. Building up a database of variation in the natural content of bioactive compounds in cereals enables the selection of genotypes with high levels of beneficial compounds for cultivation and plant breeding.

MATERIALS AND METHODS

Solvents and Reagents. The HPLC and pro analysi grade solvents and reagents used in the analysis of the steryl ferulates were previously reported by Hakala et al. (*2*). Cycloartenol ferulate (CAF) was applied in the method validation and as an external standard in the steryl ferulate analyses (*2*).

Samples. The samples consisted of 24 winter wheat and 2 spring wheat genotypes (*Triticum aestivum* var. *aestivum*) and 5 rye (*Secale cereale* L.) genotypes. The genotypes are listed in **Table 1**, and their detailed description is given in other publications (*24*, *25*). The 28 cereal genotypes were grown in experimental fields at Martonvásár, Hungary, over three consecutive years in 2005−2007. The two additional winter wheat genotypes (Tiger and Crousty) and one rye genotype (Amilo) were cultivated in Hungary only in 2007. Moreover, steryl ferulates were analyzed from 12 selected genotypes (**Figure 3**) cultivated at three other locations, that is, Enchantillon (France), Woolpit (United Kingdom), and Choryn (Poland), in 2007. However, the spring wheat genotypes were not grown in Poland. The selection was made based on, for example, steryl ferulate levels and stability in Hungary in 2005−2007, and the modernity and geographic origin of the genotypes. The weather and soil conditions and other agronomic characteristics are reported in the HEALTHGRAIN publications (*25*−*27*). The agronomic practices were similar at each location. Two batches of the winter wheat variety MV Emese, cultivated in 2005 or 2007, were used as in-house reference material during the analyses. All cereals were milled at Martonvásár to give flours of 0.5 mm particle size and kept at −18 °C in the dark after milling (*26*). The total sterol contents of the samples are presented elsewhere (*28*−*30*). Other kernel characteristics were provided by the HEALTHGRAIN partners.

Sample Preparation. The procedure for extraction and purification of the steryl ferulates was based on the method of Seitz (*18*) and was previously published (*1*, *2*). The steryl ferulates were first extracted from the cereal sample (2 g) with 80 mL of hot acetone under reflux using a 2050 Soxtec Avanti apparatus (Foss Tecator Ab, Höganäs, Sweden) as described by Nyström et al. (*1*). Each cereal sample was extracted in triplicate. The subsequent base−acid purification of the extract was performed according to the procedure of Hakala et al. (*2*).

HPLC Analysis of Steryl Ferulates. The steryl ferulates (sitosteryl and campesteryl ferulates and sitostanyl and campestanyl ferulates) were analyzed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 325 nm, using an external standard method (*2*). The HPLC apparatus consisted of a pump (Waters 515, Waters Corp., Milford, MA), autosampler (Waters 717 Plus), and tunable absorbance detector (Waters 486) and was equipped with a reversed-phase column (Zorbax ODS, 4.6 × 250 mm, 5 μm, Agilent Technologies, Inc., Santa Clara, CA). The column temperature was set at 50 °C. The mobile phase was a mixture of methanol, water, and acetic acid (97:2:1), and a flow rate of 1.5 mL/min was used. CAF was applied as an external standard for quantification of the steryl ferulates. The concentration of the external standard solution was examined daily prior to HPLC analysis, using a spectrophotometer (Lambda 25, UV−vis spectrometer, Perkin-Elmer, Shelton, CT) at 325 nm (*2*).

Performance of the Analytical Method. The validity of the analytical method was ensured with recovery studies. CAF was added to the in-house reference flour (2 g) at two concentration levels (64 and 129 μg), and the recovery of the standard was 72.6 ± 4.1% (n = 8). The limit of detection for CAF was 0.6 ng per injection (signal-to-noise ratio of 3:1) and the limit of quantification 1.8 ng per injection (3 × limit of detection). The response of the detector was linear over a wide range (2.5 ng−13 μg per injection), as determined with CAF. The range 0.2−2.4 μg per injection was used for quantification.

The repeatability of the method was monitored with the in-house reference, which was analyzed in each extraction series. The two batches of the in-house reference flour, grown and milled in 2005 or 2007, contained 73.2 ± 5.8 (n = 48) and 76.3 ± 5.2 (n = 62) μg/g of fw of steryl ferulates,

Table 1. Steryl Ferulate Contents (Micrograms per Gram of Dry Matter) of the Wheat and Rye Genotypes Grown in Different Years and Locations (*N* = Number of Environments)[a]

		campesteryl ferulate			sitosteryl and campestanyl ferulates			sitostanyl ferulate			total		
genotype	N	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range
wheat													
Campari	3	15	1	14–16	61	7	54–68	34	2	32–36	110 f–i	9	102–120
Herzog	6	10	1	9–11	48	6	41–56	30	4	24–34	88 c–e	9	76–98
Disponent	3	17	3	14–20	62	6	56–69	34	3	31–37	113 h, i	11	101–124
Tommi	3	15	3	13–18	60	8	54–69	37	2	34–38	111 f–i	12	103–125
Tremie	6	15	2	13–18	55	11	43–71	33	7	24–42	103 g–i	18	81–126
CF99105	3	16	1	15–17	60	6	54–65	34	4	30–38	110 f–i	9	102–120
Valoris	6	17	3	14–21	54	10	45–70	30	5	24–36	101 f–i	14	86–125
Isengrain	3	12	2	10–15	60	4	57–64	31	1	30–32	104 e–i	5	101–109
Claire	6	15	2	12–17	54	10	41–67	31	8	22–40	100 f–i	18	79–123
Maris Huntsman	3	12	2	11–15	55	7	47–62	32	6	29–39	99 c–h	12	88–112
Lynx	3	13	2	12–16	56	7	49–62	30	3	28–33	99 c–g	9	90–108
Malacca	6	16	3	12–19	55	4	49–59	35	4	28–38	106 i	6	98–112
Rialto	6	13	2	10–16	47	7	39–55	30	5	22–37	89 c–e	12	76–102
Riband	3	16	5	12–21	59	8	54–68	29	0	28–29	103 d–i	13	94–118
Avalon	3	11	3	9–15	54	8	46–63	33	3	30–35	98 c–g	13	85–111
San Pastore	3	12	1	10–13	40	3	37–42	25	2	23–27	77 a	6	70–81
Estica	6	16	2	15–20	40	5	35–46	18	4	14–23	75 a, b	7	67–84
Gloria	3	13	1	12–14	46	2	43–47	29	2	27–31	87 a–c	5	83–92
Spartanka	3	12	2	10–13	48	3	45–51	30	5	25–34	90 a–d	7	83–97
Obriy	3	14	2	13–16	53	3	49–56	31	3	27–33	98 c–f	8	89–104
Atlas 66	3	15	2	13–16	56	3	54–59	33	1	32–35	104 e–i	3	102–107
Crousty	1	16			62			36			114 e–i		
Tiger	1	15			54			32			101 b–i		
Mv Emese	6	12	1	11–13	44	3	38–47	27	3	23–30	83 b, c	6	73–89
Chinese Spring[b]	5	10	1	8–11	46	8	38–59	32	4	27–37	88 c–e	12	74–104
Cadenza[b]	5	19	3	14–23	42	6	33–48	23	3	19–26	84 b–d	11	66–92
rye													
Haute Loire Pop	3	14	2	13–16	36	6	32–43	19	2	17–21	69 a	10	61–80
Nikita	3	13	1	12–14	40	5	35–45	20	2	18–23	73 a	5	68–78
Rekrut	6	13	2	11–15	40	7	32–49	22	2	19–24	74 a	10	62–86
Dankowskie Zlote	6	11	1	9–12	38	5	32–47	21	3	18–24	69 a	8	59–80
Amilo	1	12			35			17			65 a		

[a] Values with the same letter in a column and subgroup are not significantly different ($p < 0.05$). [b] Spring wheat genotype.

respectively. The corresponding contents of the individual steryl ferulate species were as follows: 10.0 ± 1.0 and 9.9 ± 0.7 μg/g of campesteryl ferulate, 39.1 ± 3.2 and 42.7 ± 2.3 μg/g of sitosteryl ferulate and campestanyl ferulate, and 24.2 ± 2.1 and 23.6 ± 1.6 μg/g of sitostanyl ferulate, in batches processed in 2005 and 2007, respectively.

The steryl ferulate results were first evaluated for the analytical level and then for the repeatability of the replicate samples. In each extraction series, the steryl ferulate content of the in-house reference was not permitted to exceed the action limits (average ± 2 × standard deviation), which were 71.5 ± 12.5 μg/g of fw (n = 10) in 2005 and 78.8 ± 11.2 μg/g of fw (n = 10)

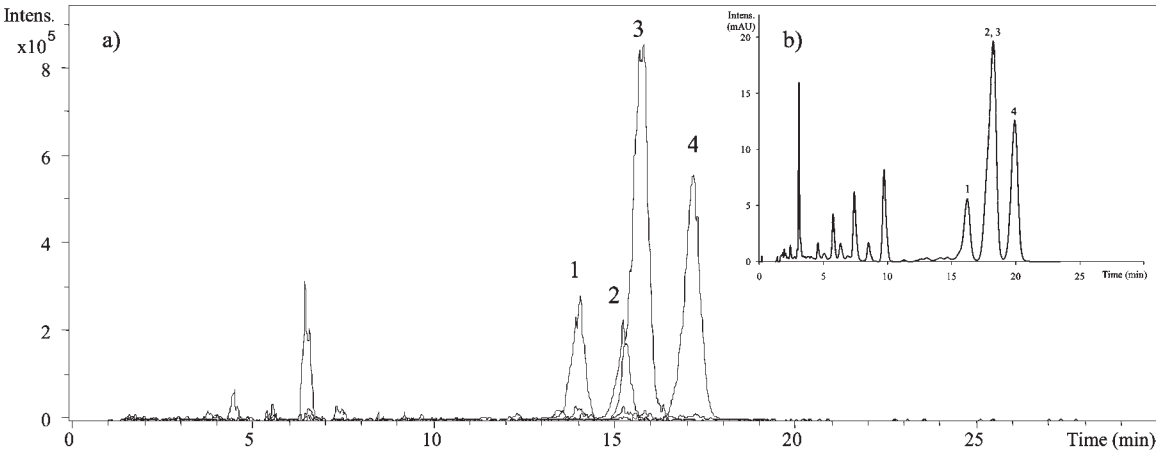

Figure 2. HPLC chromatogram of wholegrain wheat (genotype MV Emese) with (**a**) SIM mass spectroscopy and (**b**) UV detection at 325 nm: 1, campesteryl ferulate; 2, sitosteryl ferulate; 3, campestanyl ferulate; 4, sitostanyl ferulate. For HPLC conditions, detector parameters, and characteristic ions used for SIM, see Materials and Methods. Differences in the retention times result from differences in HPLC systems.

in 2007. Otherwise, the sample series was reanalyzed. Furthermore, analysis of the single sample was repeated if the coefficient of variation (CV) for steryl ferulate contents of the three replicates was above 10%. The possible outliers of all the replicate results were then detected with Dixon's Q test ($p < 0.05$). All results are given as means of the replicate samples on a dry matter (dm) basis. To determine the proportions of the steryl ferulates of total sterols (total phytosterol contents are provided elsewhere (*29*, *30*)), the contents of the steryl ferulates as free sterols were first calculated, using a multiplier of 0.703 (the multiplier is based on the proportion of sterol moiety in the steryl ferulate molecule).

Identification of Steryl Ferulates. The identification of individual steryl ferulates was based on high-performance liquid chromatography−mass spectrometry (HPLC-MS) and literature data (*1*, *2*). The HPLC-MS analysis was performed as described earlier by Hakala et al. (*2*), with minor modifications. The separation of steryl ferulate species was done with a Hewlett-Packard 1100 HPLC (Hewlett-Packard GmbH, Waldbronn, Germany) with conditions similar to those used in the analysis of steryl ferulates by HPLC-UV in the present study. A quadrupole ion-trap mass spectrometer (Esquire-LC, Bruker Daltonik, Bremen, Germany) with atmospheric pressure chemical ionization (APCI) in negative ion mode was used for detection. The characteristic mass-to-charge ratio (m/z) values of the deprotonated ions $[M - H]^-$ for campesteryl ferulate, sitosteryl ferulate, campestanyl ferulate, sitostanyl ferulate, and CAF were 575.4, 589.4, 577.4, 591.4, and 601.4 respectively (*2*). These m/z values were used for selective ion monitoring (SIM). The proportions of the coeluting steryl ferulates, that is, campestanyl and sitosteryl ferulate, were determined on the basis of MS analysis.

Statistical Analyses. The differences in the steryl ferulate contents among the various genotypes, growing years, or locations were examined, using two-way analysis of variance (ANOVA) with genotype as a block factor and Fisher's least significant difference (LSD) procedure. Pearson's correlation coefficients were calculated to determine the relationships between steryl ferulate contents and other kernel characteristics. All statistical analyses were performed, using Statgraphics Centurion XV (Statpoint Technologies, Inc., Warranton, VA), and p values of < 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Steryl Ferulates of Wheat and Rye. The steryl ferulates were analyzed using HPLC. Four steryl ferulate species, that is, campesteryl ferulate, sitosteryl ferulate, and the corresponding stanyl ferulates, were identified in wholegrain wheat and rye. This is in accordance with the findings of Hakala et al. (*2*). A typical HPLC chromatogram, presented in **Figure 2b**, shows that two of the individual steryl ferulates, sitosteryl ferulate and campestanyl ferulate, coeluted with the HPLC method used.

The identification of the compounds was ensured using HPLC-MS, and campestanyl ferulate was the most abundant species in wheat and rye, followed by sitostanyl, campesteryl, and sitosteryl ferulates. The MS analysis of selected samples demonstrated that the peak with two coeluting compounds consisted of sitosteryl ferulate (~20% of the peak area) and campestanyl ferulate (~80%). Thus, sitosteryl ferulate accounted for approximately 10% and campestanyl ferulate 45% of the total steryl ferulate content (**Figure 2a**), and the stanyl esters were the major species, together contributing up to 80% of the total content of steryl ferulates in wheat and rye. Hakala et al. (*2*) and Seitz (*18*) made similar observations and found that the main compound campestanyl ferulate accounted for appoximately 77−85% of the coeluting compounds and thus 42−46% of the total steryl ferulates in wheat and rye. Interestingly, sitosterol and campesterol are the major components of the total phytosterol content (*28*, *29*), whereas the respective stanol species predominate in the steryl ferulate fraction.

Effect of Genotype on Steryl Ferulates of Wheat and Rye. The genetic variation in steryl ferulate contents of wheat and rye was studied in 26 wheat and 5 rye genotypes grown in 2005−2007 (**Table 1**). There were up to 2.1-fold differences in the total steryl ferulate contents of various genotypes; the lowest individual content was found in the rye genotype Dankowskie Zlote when grown in France in 2007 (59 μg/g of dm) and the highest content in winter wheat type Tremie grown in Hungary in 2007 (126 μg/g of dm).

The average total steryl ferulate contents of various wheat genotypes differed considerably, ranging from 75 to 114 μg/g of dm. The effect of genetic factors on steryl ferulate content was statistically significant ($F(25, 71) = 5.51$, $p = 0.0000$). The wheat genotypes with the lowest total steryl ferulate contents included both the modern and old European winter types Estica, San Pastore, MV Emese, and Gloria and the spring types Cadenza and Chinese Spring. The most steryl ferulate-rich genotypes, containing on average 110 μg/g of dm or more steryl ferulates, were the winter wheat lines Crousty, Disponent, Tommi, CF99105, and Campari, all of which originated from Germany or France. In accordance with the present results, Seitz (*18*) found that the total steryl ferulate concentration of ground wholegrain wheat varied from 62 to 123 μg/g of fw, when seven different varieties were examined. Other previously reported values, 62−63 μg/g of fw or 74 μg/g of dm, are similar to those of the wheat genotypes with the lowest steryl ferulate contents in the present study (*1*, *2*).

The average total steryl ferulate contents of the five rye genotypes varied from 65 to 74 μg/g of dm and were thus lower than those of wheat genotypes. The differences among the various

Table 2. Pearson Correlation Coefficients among Steryl Ferulate Contents and Other Kernel Characteristics in Wheat (n = 102) and Rye (n = 19)[a]

	campesteryl ferulate	sitosteryl and campestanyl ferulates	sitostanyl ferulate	total sterols	TKW	lipids
wheat						
total steryl ferulates	0.311 (p < 0.01)	0.977 (p < 0.01)	0.843 (p < 0.01)	0.530 (p < 0.01)	−0.556 (p < 0.01)	0.379 (p < 0.01)
campesteryl ferulate		0.252 (p < 0.05)	−0.150 (ns)	0.351 (p < 0.01)	−0.199 (p < 0.05)	0.201 (p < 0.05)
sitosteryl and campestanyl ferulates			0.781 (p < 0.01)	0.508 (p < 0.01)	−0.575 (p < 0.01)	0.330 (p < 0.01)
sitostanyl ferulate				0.361 (p < 0.01)	−0.414 (p < 0.01)	0.339 (p < 0.01)
rye						
total steryl ferulates	0.680 (p < 0.01)	0.958 (p < 0.01)	0.652 (p < 0.01)	0.655 (p < 0.01)	−0.390 (ns)	0.331 (ns)
campesteryl ferulate		0.579 (p < 0.01)	0.152 (ns)	0.646 (p < 0.01)	−0.515 (p < 0.05)	0.604 (p < 0.01)
sitosteryl and campestanyl ferulates			0.491 (p < 0.05)	0.645 (p < 0.01)	−0.486 (p < 0.05)	0.367 (ns)
sitostanyl ferulate				0.244 (ns)	0.209 (ns)	−0.216 (ns)

[a] Significance levels are presented in parentheses. The correlations with TKWs were calculated on a fw basis and among other variables on a dm basis. ns, not significant.

rye genotypes were not statistically significant. In the study of Seitz (*18*), the steryl ferulate content of a single rye variety, 29 μg/g of fw, was lower than the present findings. The steryl ferulate levels of single or mixed varieties of wholegrain rye, 40−64 μg/g of fw (*2*, *20*) or 63−78 μg/g of dm (*1*, *19*), were comparable to those observed in this study. The effect of genotype on steryl ferulate content of rye has not been studied earlier, since only individual samples have been examined. Previously, genetic variation was seen in rice (*21*−*23*) and corn (*5*). The present study gives new data on genotype-dependent natural variation in rye and wheat steryl ferulates. However, greater numbers of rye genotypes should be studied to make accurate conclusions.

The average contents of individual steryl ferulates in various genotypes are shown in **Table 1**. The sum of the sitosteryl and campestanyl ferulates accounted for 50−58% of the total steryl ferulate content in wheat and 52−54% in rye, depending on the genotype. Sitostanyl ferulate contributed 24−36 and 27−30% of the total steryl ferulates in wheat and rye genotypes, respectively. The proportion of campesteryl ferulates also showed wide variation among genotypes, because it was 11−22% of the total content in wheat and 16−20% in rye. Furthermore, statistical analyses showed that the steryl ferulate composition was affected by the genotype in wheat, but not in rye. In wheat, the effects of genetic factors on the proportions of the sum of sitosteryl and campestanyl ferulates, sitostanyl ferulate, and campesteryl ferulate were significant (F values were 8.89, 21.04, and 23.31 (df = 25, 71; p = 0.0000), respectively). In rye, not as wide variation among the genotypes was seen in the distribution of steryl ferulates, probably due to the fewer samples analyzed. The steryl ferulate compositions of wheat and rye were similar to those reported previously, and the composition of rye did not considerably differ from that of wheat genotypes, which is also in agreement with previous studies (*1*, *2*, *18*). Seitz (*18*) stated that there was only slight variation in the distribution of steryl ferulates among genotypes when seven wheat varieties were studied. The wider variation observed in the present study may have resulted from the more extensive set of samples (26 different wheat genotypes were analyzed here).

The correlation between total steryl ferulate and total phytosterol contents was moderately strong and positive in wheat and rye (**Table 2**), and therefore the genotypes with high levels of phytosterols are expected to contain high levels of ferulate conjugates. The steryl ferulates accounted for 6−10 and 4−5% of total sterol content in wheat and rye, respectively, depending on the genotype. The proportion of steryl ferulates of total sterols was thus lower in rye than in wheat genotypes. Although the rye genotypes possessed higher total phytosterol contents than the wheat genotypes (*28*−*30*), the steryl ferulate contents were lower in rye than in wheat. In agreement with our findings, Hakala et al. (*2*), Nyström et al. (*1*), and Lampi et al. (*19*) reported that 6−7% of wheat and 4−6% of rye phytosterols existed in the ferulic acid ester form. The relationships between the contents of total steryl ferulates and the individual steryl ferulate species indicate that in wheat the high total steryl ferulate content is characterized by high contents of campestanyl ferulate (including sitosteryl ferulate) and sitostanyl ferulate, whereas the content of campesteryl ferulate remains more constant, regardless of the total content. In rye, the strongest correlation was found between the total steryl ferulate contents and the contents of campestanyl and sitosteryl ferulate.

The moderately strong and negative relationship between the thousand-kernel weight (TKW) and steryl ferulate content of wheat suggested that small wheat kernels, in which the proportion of bran layers is high, may contain higher levels of steryl ferulates. The TKWs of various genotypes grown in different environments varied considerably, between 26 and 61 g, and smaller wheat kernels tended to develop in growing environments characterized by lower precipitation and higher temperature. Thus, the climatic conditions may in this way be reflected in the steryl ferulate content. The relatively weak positive correlation between the total lipid and steryl ferulate contents results from the fact that steryl ferulates are not accumulated in the lipid-rich germ fraction of the wheat kernel (*1*). Because steryl ferulates are concentrated in the bran (*1*), the high bran yield would be expected to relate to high steryl ferulate content. However, the correlation with the bran yields could not be calculated, due to nonnormally distributed data. In rye, no statistically significant relationships were observed between the steryl ferulate content and the TKW or lipid content, probably due to the small number of samples analyzed.

Effect of Environment on Steryl Ferulates of Wheat and Rye. In general, no considerable year-to-year variation was observed in the total steryl ferulate contents of wheat and rye, when the effect of the growing year was studied in 24 wheat and 4 rye genotypes cultivated at Martonvásár, Hungary, over three consecutive years (**Table 3**). The steryl ferulate contents were 97 ± 11, 100 ± 12, and 102 ± 16 μg/g of dm in wheat and 78 ± 7, 67 ± 1, and 68 ± 6 μg/g of dm in rye genotypes in 2005, 2006, and 2007, respectively. The differences among years were not statistically significant, either in wheat (F(2, 46) = 2.30, p = 0.1116) or in rye (F(2, 6) = 4.78, p = 0.0574). However, the wheat genotypes grown in the rainy year 2005 most often possessed the lowest steryl ferulate contents, whereas the highest levels of steryl ferulates were frequently found in the dry and warm year 2007. In contrast, the steryl ferulate contents of rye genotypes appeared to be highest in 2005. Despite the fact that the weather conditions and soil properties varied considerably among the growing seasons at Martonvásár (*25*, *27*), no significant differences among years were found in the steryl ferulate contents of the wheat or rye.

There were differences in the extent of year-to-year variation in various genotypes. The most stable genotypes, having low

Table 3. Steryl Ferulate Contents (Micrograms per Gram of Dry Matter) of the Wheat and Rye Genotypes Grown in Hungary in 2005−2007 (*N* = Number of Genotypes)[a]

		campesteryl ferulate			sitosteryl and campestanyl ferulates			sitostanyl ferulate			total		
year	N	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range
wheat													
2005	24	14	2	9–20	51	6	41–67	32	4	23–40	97 a	11	79–123
2006	24	12	2	8–18	54	7	42–65	33	5	22–42	100 a	12	81–121
2007	24	15	3	10–21	57	10	37–71	30	5	16–38	102 a	16	70–126
rye													
2005	4	14	3	10–16	44	4	39–49	20	1	18–21	78 a	7	68–86
2006	4	12	1	11–13	35	1	34–36	21	2	19–23	67 a	1	66–69
2007	4	12	1	11–13	36	4	32–40	20	3	17–23	68 a	6	61–74

[a] Values with the same letter in a column and subgroup are not significantly different ($p < 0.05$).

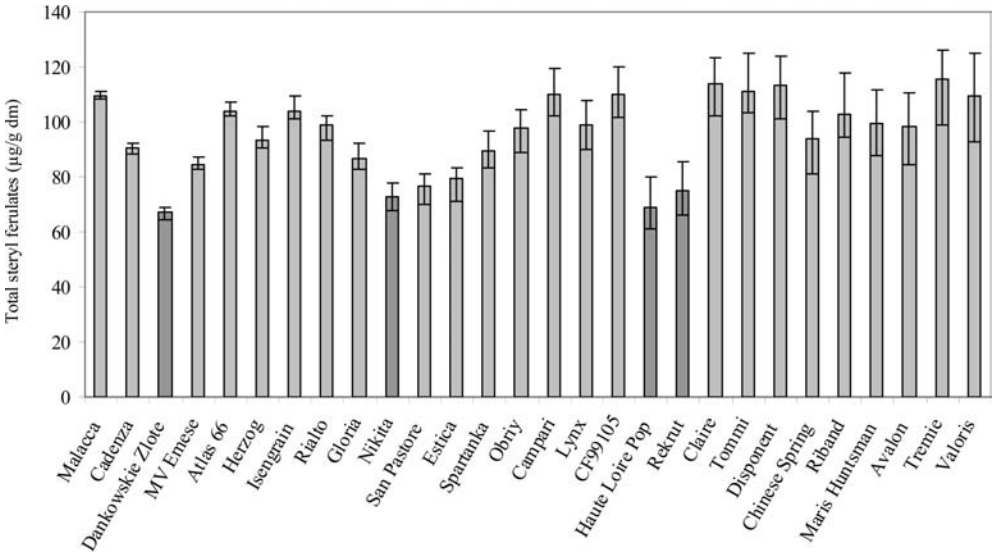

Figure 3. Average total steryl ferulate contents of the 28 genotypes grown in Hungary in 2005−2007 in order of increasing variation among years. The error bars represent the range in three years. The rye genotypes are marked with darker shading.

variation in their steryl ferulate contents over the growing years, were the winter wheat types Malacca, MV Emese, and Atlas 66, the spring wheat type Cadenza, and the rye genotype Dankowskie Zlote (**Figure 3**). Moreover, two of the preceding genotypes, namely, the modern English variety Malacca and the old American variety Atlas 66, were characterized by high steryl ferulate contents (>100 μg/g of dm). Individual genotypes with the widest variation among years included the winter wheat types Valoris (range >30 μg/g), Tremie, and Avalon (ranges >25 μg/g).

The distribution of wheat steryl ferulates was only moderately influenced by the growing year (**Table 3**), whereas no year-to-year variation was observed in the total contents. Although the variation was low, there were statistically significant differences in the steryl ferulate compositions among years (*F* values for the proportions of campesteryl ferulate, sum of sitosteryl and campestanyl ferulates, and sitostanyl ferulate were 39.87, 40.87, and 66.82 (df = 2, 46; $p = 0.0000$), respectively). The proportion of sitosteryl and campestanyl ferulates was slightly higher in 2007 (56 ± 2%), when the total content was highest, than in other years of growth (53 ± 2% in 2005 and 55 ± 2% in 2006). Similarly, the distribution of rye steryl ferulates was not strongly affected by the growing year. However, statistical differences were found in the proportions of sitosteryl and campestanyl ferulates ($F(2, 6) = 14.07$, $p = 0.0054$) and sitostanyl ferulate ($F(2, 6) = 6.74$, $p = 0.0318$). An exceptionally high proportion of sitosteryl and campestanyl ferulate was observed in 2005 (57 ± 2%), when the total content was highest, compared with other growing seasons (51 ± 1% in 2006 and 53 ± 2% in 2007). The year of growth is known to affect the distribution of rice steryl ferulates (*23*), whereas the effects on wheat and rye grains have not been studied earlier.

Variation among locations was statistically significant in the steryl ferulate contents of 10 wheat genotypes ($F(3, 25) = 19.09$, $p = 0.0000$) grown at four sites in Europe in 2007. The smaller *F* value of the block factor, genotype ($F(9, 25) = 7.24$, $p = 0.0000$), indicates that variation caused by the growing location was greater than that caused by genetic factors. The lowest average contents, 79 ± 10 and 81 ± 9 μg/g of dm, were observed in the genotypes cultivated in France or the United Kingdom, respectively, whereas somewhat higher contents (94 ± 10 μg/g of dm) were found in Poland (**Table 4**). The wheat genotypes grown

Table 4. Steryl Ferulate Contents (Micrograms per Gram of Dry Matter) of the Wheat and Rye Genotypes Grown at Four Locations in 2007 (N = Number of Genotypes)[a]

		campesteryl ferulate			sitosteryl and campestanyl ferulates			sitostanyl ferulate			total		
location	N	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range
wheat													
Hungary	10	16	4	10–21	56	10	39–71	30	6	16–37	101 c	17	71–126
France	10	13	3	8–16	41	5	33–49	25	5	16–33	79 a	10	66–98
United Kingdom	10	16	4	11–23	42	5	35–50	23	4	14–29	81 a	9	69–98
Poland	8	15	2	11–18	49	5	41–56	31	5	20–38	94 b	10	78–112
rye													
Hungary	2	12		11–12	37		34–40	20		18–21	69		65–73
France	2	10		9–11	32		32–32	18		18–19	60		59–62
United Kingdom	2	12		11–13	39		38–40	24		23–24	75		75–75
Poland	2	13		12–15	46		46–47	23		22–24	83		80–85

[a] Values with the same letter in a column and subgroup are not significantly different ($p < 0.05$).

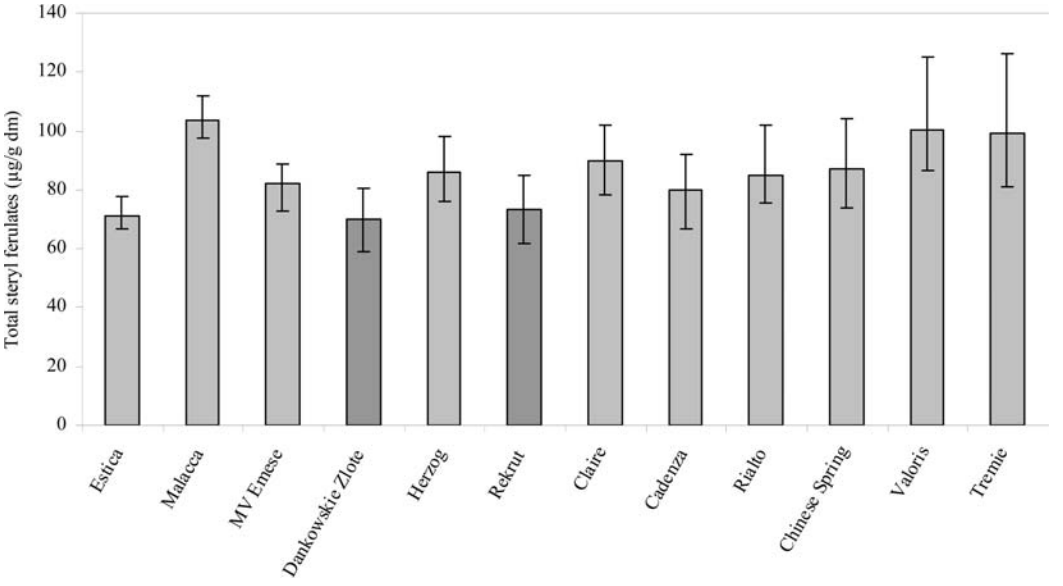

Figure 4. Average total steryl ferulate contents of the 12 genotypes grown in Hungary, France, the United Kingdom, and Poland in 2007 in order of increasing variation among locations. The error bars represent the range at four locations. The rye genotypes are marked with darker shading.

in Hungary possessed the highest levels of steryl ferulates, 101 ± 17 μg/g of dm. During the growing season in 2007, the weather was dry and warm in Hungary and at the same time very wet and cool in the United Kingdom (*25*). Thus, it appears that elevated temperature and low precipitation may result in higher steryl ferulate content. There were also differences in the soil characteristics of the growing locations (*27*), which may have affected the steryl ferulate levels of the genotypes. Nevertheless, the differences in weather and soil conditions in Hungary during the three growing years did not cause significant variation. In two rye genotypes, the steryl ferulate contents decreased as follows: Poland > United Kingdom > Hungary > France, thus differing from wheat. However, greater numbers of rye genotypes should be analyzed to confirm the effect of the growing location.

The locational variation of individual genotypes is demonstrated in **Figure 4**. The variation among locations was widest in the wheat genotypes Tremie, Valoris, and Chinese Spring (range ≥30 μg/g), and the most stable genotypes, with ranges lower than 16 μg/g, were the winter wheat types Estica, Malacca, and MV Emese. In the genotype Malacca the low variation among locations was combined with considerably high steryl ferulate content. In addition, Malacca and MV Emese showed high stability over the growing years, as well. The variation was wide, among both years and locations in Tremie and Valoris. Generally, the growing location caused more variation in the steryl ferulate contents than the year of growth. In a few genotypes, that is, Estica, Claire, and Rekrut, the magnitude of variation among locations was similar to that of the year-to-year variation.

The growing location caused variation in the distribution of steryl ferulates in wheat. The average steryl ferulate compositions of the genotypes cultivated at four locations in 2007 are

listed in **Table 4**. The differences among locations in the proportions of steryl ferulate species were statistically significant in 10 wheat genotypes; the F values were 52.68, 46.47, and 55.00 (df = 3, 25; $p = 0.0000$) for campesteryl ferulate, the sum of sitosteryl and campestanyl ferulates, and sitostanyl ferulate, respectively. The highest proportion of sitosteryl and campestanyl ferulates ($55 \pm 2\%$) was found when the genotypes were grown in Hungary and the total content of steryl ferulates was highest, whereas in the other locations the proportion did not vary, despite the differences in total contents. Equally, the proportion of these steryl ferulates in rye was higher ($56 \pm 2\%$) when the two genotypes were grown in Poland and the highest total content was observed, compared with other locations. The variation in steryl ferulate composition due to the growing location has not been studied before. In rice cultivars, however, the location of growth affected the distribution of steryl ferulates (*23*). Moreover, Britz et al. (*31*) stated that elevated growth temperature resulted in increased levels of total γ-oryzanol and its major components in rice cultivars, and the various steryl ferulate species of γ-oryzanol were not evenly affected. In the present study, the same observation was made in wheat; the highest levels of total steryl ferulates and the main compound were found in Hungary, which was the warmest of the growing locations during the season 2007.

The growing year, location, or temperature affected the steryl ferulate contents of rice (*21*, *23*, *31*) and corn (*5*). To our knowledge, however, the effects of environmental factors on the steryl ferulate contents of wheat and rye have not been studied before. The present study thus provides valuable information on the environmental variation in wheat and rye steryl ferulates, showing the effect of growing location to be greater than that of genetic factors, whereas the year of growth is suggested not to cause such wide variation.

Conclusions. The present study is the first to demonstrate the effects of genetic and environmental factors on wheat and rye steryl ferulates. We showed that genotype and growing location have a strong impact on the content and composition of wheat steryl ferulates, whereas the year of growth does not cause significant variation. In rye either no or a small variation was observed compared to wheat, due to fewer genotypes analyzed. The knowledge of the natural diversity of wheat and rye steryl ferulates provided by this study will be used as part of an extensive database for cultivators and plant breeders. This diversity database will allow the selection of genotypes with high and stable levels of bioactive, health-promoting compounds.

ABBREVIATIONS USED

ANOVA, analysis of variance; APCI, atmospheric pressure chemical ionization; CAF, cycloartenol ferulate; CV, coefficient of variation; dm, dry matter; fw, fresh weight; HPLC, high-performance liquid chromatography; LSD, least significant difference; MS, mass spectrometry; m/z, mass-to-charge ratio; SIM, selective ion monitoring; TKW, 1000 kernel weight; UV, ultraviolet.

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